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Sustainable Fruit Growing

From Orchard to Table

Edited by

Boris Duralija

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About the Editors

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Boris Duralija is a distinguished Croatian researcher and full professor with international recognition and influence in the field of fruit production. He is a professor of pomology and related disciplines at the University of Zagreb Faculty of Agriculture (Zagreb, Croatia), where he is responsible for undergraduate and graduate courses, as well as other lifelong learning courses. His research interests within the broad field of pomology include several interdisciplinary topics, such as biodiversity of edible wild fruits, fruit growing systems, climate change, and the effects of different horticultural practices on fruit yield and quality. He has been a guest lecturer at several international universities and institutes (PR China, Czech Republic, Germany, Malaysia, Northern Macedonia, Slovenia, South Korea, Turkey, etc.). He is strongly committed to popularizing science through various activities to promote fruit science and more, such as consumer evaluation of strawberry quality, lectures, TV and radio broadcasts, and writing for popular magazines and journals. He also organized several educational programs for fruit growers and people from the horticultural industry. From 2012 to 2021, he was the national coordinator for Croatia for the Fascination of Plants Day organized by ESPO. He convened the meeting Plant Quality and Sustainable Productionand was guest editor of the Special Issue of the journal *Agriculturae Conspectus Scientificus* (ACS, Vol. 71, No. 4, 2006), in which 14 articles were published. He is currently an editorial board member and reviewer for many indexed journals and author of more than 100 articles published in international journals and at national and international symposia and conferences.

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Editorial

Sustainable Fruit Growing: From Orchard to Table-Editorial Commentary

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Fruit production has faced many challenges in recent years as society seeks to increase fruit consumption while increasing safety and reducing the harmful effects of intensive farming practices (e.g., pesticides and fertilizers) [1]. In the last 50 years, the population has more than doubled and is expected to grow to 9 billion people by 2050 [2]. Per capita consumption of fruit is also increasing during this time and the global fruit industry is facing a major challenge to produce enough fruit in quantity and quality. The need for sustainable production of nutritious food is critical for human and environmental health.

Apple trees are grown on an area of about 4.6 million hectares and are the leading temperate fruits, producing more than 85 million tonnes in 2020, which is more than 10 kg for every single person in the world [3]. In the last 50 years (from 1970 to 2020), apple fruit production has increased threefold (220%) compared to the area under cultivation (66%) [3]. This is mainly due to intensification through the use of high-yielding varieties on suitable rootstocks, modern mechanization, synthetic fertilizers and pesticides. To be sustainable, an apple farm must produce adequate yields of high quality, be profitable, protect the environment, conserve resources, and be socially responsible in the long term [4,5]. For example, the use of plastic in apple orchards can be reduced by using the material several times in the same year or years, by using it in every other row, or by replacing it with another reflective material-an important step toward environmentally friendly, sustainable horticulture [6]. Thanks to the changes that have taken place in recent years in the field of image analysis methods and computational performance, it is possible to develop solutions for automatic fruit counting based on registered digital images and predict yield, which contributes to better planning of harvest [7].

The results of the life cycle assessment (LCA) of three farms growing six types of fruit showed that several production activities have a high impact on the environment: In descending order of absolute value, fruit refrigeration, agronomic operations, irrigation, and fertilizer use were found to have the greatest impact [8]. Other activities, such as the use of agrochemicals, planting and the use of plastic in harvesting and packaging, had lower overall impacts. The high environmental impacts associated with most production activities underscore the need to make primary food production cleaner, more resource-efficient, and less energy-intensive [8].

Climate change is more than ever an important issue for agriculture and fruit growers. Many new threats arising from it are present in the area of existing orchards, such as the accumulation of enough chill units, extreme low and high temperatures, droughts, storms, floods, stronger winds, frost damage, hail, the appearance of new pests, etc. Temperature is the most important environmental factor affecting fruit and nut trees and productivity because it affects tree physiology and the susceptibility of flower buds, flowers, and young fruits and nuts to low temperatures or spring frosts. Fruit and nut trees are at high risk of having buds, flowers and young fruits and nuts vulnerable to low temperatures in early spring because temperatures can still drop below 0 °C when ecodormancy ends and flowering and young fruit formation begin. Severe weather conditions, especially frost in winter and early spring, may pose a significant threat to sustainable nut and fruit

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production in some regions, while fruit and nut trees with high chilling requirements may not be able to meet chilling requirements in some areas [9].

Some new pests, such as the brown marmorated stink bug (*Halyomorpha halys* Stål, 1855), an invasive polyphagous species, are emerging in traditional fruit growing areas in the United States and Europe and threatening production. New safe insecticides should be introduced into practice for sustainable fruit growing, which includes reducing chemical pollution of the environment. Some new green insecticides whose use could increase safety in fruit production need to be explored. Potential candidates with insecticidal activity against the marmorated stink bug are microparticles loaded with leaves of *Stevia rebaudiana* (Bertoni) [10].

Wild edible fruits have a much greater diversity of species and are sometimes extremely resistant to adverse environmental conditions [11]. If we look at wild fruit plants that have grown without the help of humans, they can sustain themselves for centuries in the same area. For example, wild Himalayan figs (*Ficus palmata* Forssk.) are well adapted to local pedoclimatic conditions and, combined with easy propagation and production, can contribute to the local economy and have a significant impact on the socioeconomic and ecological balance. The results showed high variability in some of the studied traits of 35 accessions from different parts of northeast Pakistan, indicating their good potential for further improvement and use in sustainable agricultural production [12]. Results from the relatively limited number of sea buckthorn (*Hippophae rhamnoides* L. ssp. *Caucasica* Rousi) genotypes from Turkey show promise traits for further improvement in both horticultural and nutritional traits, indicating potentially even greater variability if more genotypes are to be considered in the future [13].

Based on the research mentioned in this Special Issue, it can be concluded that edible wild fruits may be an ideal model for how to approach modern intensive production and the principle of sustainability.

Some fruit species are still in the early stages of domestication, such as Cornelian cherry (*Cornus mas* L.), which has been grown wild in the Balkan Peninsula for centuries with no apparent problems with disease or pests. For these reasons, it is necessary to highlight the importance of local selection of cornelian cherries for sustainable cornelian cherry production in areas where new orchards are planned [14].

For reasons of mixing genes in sexual propagation in breeding programmes or in the wild, fruit plants are mainly propagated asexually in modern industrial production. When apricot cultivars are propagated asexually over a long period of time, they can produce interclonal variations that sometimes have better fruit characteristics than the original plant. The results of the study in this Special Issue have shown that clones of the apricot cultivar Sekerpare have a wide diversity of morphological characteristics and nutritional and nutraceutical compositions even at a small single site [15]. The concept of sustainable apricot production can be described as a "three-legged stool" whose legs are economic viability, environmental sustainability and social acceptability. Communicating the health benefits of apricots to consumers is essential for sustained demand for apricot products, which is a prerequisite for sustainable apricot production [15].

The quality of fruits for fresh consumption is mainly determined by their flavour, aroma, attractive appearance, and high content of bioactive compounds that play an important role in human nutrition. Peaches can serve as a source of sugars, mainly sucrose, as well as phenols, carotenoids and anthocyanins, and also provide valuable antioxidants. In the study from the Czech Republic, the Czech cultivar 'Krasava' was found to have a very high content of titratable acids, phenols, flavonoids and antioxidant capacity [16]. Pomological studies of fruit quality and consumer acceptance can give farmers clues as to which species and cultivars they can grow to be more successful in the market.

People are now increasingly concerned about the sustainability of fruit production and the fruit industry as a whole. Therefore, new studies that can take this into account will be needed in the future.

More than 15 years ago, I convened the meeting "Berry Plant Quality and Sustainable Production" and was the guest editor of the Special Issue of the journal Agriculturae Conspectus Scientificus (ACS, Vol. 71, No. 4, 2006), in which 14 articles were published. Berries, as one of the most intensively produced fruits in Europe, were discussed from different aspects, from the quality of the starting material to alternative production strategies towards sustainability [17–19].

This time the Special Issue (SI) focused on different fruit species and aspects that can promote more sustainable fruit growing in the future. The SI "Sustainable Fruit Growing: From Orchard to Table" contains the following 11 commentary and research papers by 45 scientists from Australia, Bosnia and Herzegovina, Croatia, Czech Republic, Germany, Italy, Montenegro, Pakistan, Poland, Turkey and the United States:

1. Sustainable Fruit Growing: From Orchard to Table-Editorial Comentary
2. Innovative Strategies for the Use of Reflective Foils for Fruit Colouration to Reduce Plastic Use in Orchards [6]
3. Detecting Apples in the Wild: Potential for Harvest Quantity Estimation [7]
4. Interpreting Environmental Impacts Resulting from Fruit Cultivation in a Business Innovation Perspective [8]
5. Chilling and Heat Accumulation of Fruit and Nut Trees and Flower Bud Vulnerability to Early Spring Low Temperatures in New Mexico: Meteorological Approach [9]
6. Polyphenol-Based Microencapsulated Extracts as Novel Green Insecticides for Sustainable Management of Polyphagous Brown Marmorated Stink Bug (*Halyomorpha halys* Stål, 1855) [10]
7. Evaluation of the Characteristics of the Native Wild Himalayan Fig (*Ficus palmata* Forsk.) from Pakistan as a Potential Species for Sustainable Fruit Production [12]
8. Main Agro-Morphological and Biochemical Berry Characteristics of Wild-Grown Sea Buckthorn (*Hippophae rhamnoides* L. ssp. *caucasica* Rousi) Genotypes in Turkey [13]
9. Sustainable Cornelian Cherry Production in Montenegro: Importance of Local Genetic Resources [14]
10. Assessment of Morphological Traits, Nutritional and Nutraceutical Composition in Fruits of 18 Apricot cv. Sekerpare Clones [15]
11. Determination of Selected Beneficial Substances in Peach Fruits [16]

For this valuable collection of data, the editor would like to thank all the authors who submitted their articles for this Special Issue and congratulate them on the publication of their articles with *Sustainability*. This would not have been possible without the active support of all the Academic Editors and the dedicated reviewers with their constructive comments, for which I am very grateful. Last but not least, I would like to thank Prof. Dr. Marc A. Rosen, the Editor-in-Chief of *Sustainability*, Ms. Zero Xing, the Managing Editor, and the entire MDPI team for their support of this Special Issue.

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References

1. Bursać Kovačević, D.; Brdar, D.; Fabečić, P.; Barba, F.J.; Lorenzo, J.M.; Putnik, P. Strategies to achieve a healthy and balanced diet: Fruits and vegetables as a natural source of bioactive compounds. In *Agri-Food Industry Strategies for Healthy Diets and Sustainability*; Academic Press: Cambridge, MA, USA, 2020; pp. 51–88.
2. Lam, D. How the World Survived the Population Bomb: Lessons From 50 Years of Extraordinary Demographic History. *Demography* **2011**, *48*, 1231–1262. [CrossRef]
3. Food and Agriculture Organization of the United Nations (FAO). Available online: http://www.fao.org/faostat/en/ (accessed on 11 January 2022).

4. Reganold, J.P.; Glover, J.D.; Andrews, P.K.; Hinman, H.R. Sustainability of three apple production systems. *Nature* **2001**, *410*, 926–930. [CrossRef]
5. Duralija, B.; Putnik, P.; Brdar, D.; Bebek Markovinović, A.; Zavadlav, S.; Pateiro, M.; Domínguez, R.; Lorenzo, J.M.; Bursać Kovačević, D. The Perspective of Croatian Old Apple Cultivars in Extensive Farming for the Production of Functional Foods. *Foods* **2021**, *10*, 708. [CrossRef] [PubMed]
6. Hess, P.; Kunz, A.; Blanke, M.M. Innovative Strategies for the Use of Reflective Foils for Fruit Colouration to Reduce Plastic Use in Orchards. *Sustainability* **2020**, *13*, 73. [CrossRef]
7. Janowski, A.; Kaźmierczak, R.; Kowalczyk, C.; Szulwic, J. Detecting Apples in the Wild: Potential for Harvest Quantity Estimation. *Sustainability* **2021**, *13*, 8054. [CrossRef]
8. Medici, M.; Canavari, M.; Toselli, M. Interpreting Environmental Impacts Resulting from Fruit Cultivation in a Business Innovation Perspective. *Sustainability* **2020**, *12*, 9793. [CrossRef]
9. Djaman, K.; Koudahe, K.; Darapuneni, M.; Irmak, S. Chilling and Heat Accumulation of Fruit and Nut Trees and Flower Bud Vulnerability to Early Spring Low Temperatures in New Mexico: Meteorological Approach. *Sustainability* **2021**, *13*, 2524. [CrossRef]
10. Živković, I.P.; Jurić, S.; Vinceković, M.; Galešić, M.A.; Marijan, M.; Vlahoviček-Kahlina, K.; Mikac, K.M.; Lemic, D. Polyphenol-Based Microencapsulated Extracts as Novel Green Insecticides for Sustainable Management of Polyphagous Brown Marmorated Stink Bug (*Halyomorpha halys* Stål, 1855). *Sustainability* **2020**, *12*, 10079. [CrossRef]
11. Li, H.; Bolarić, S.; Vokurka, A.; He, J.; Wang, D.; Li, X.; Mešić, A.; Wang, J.; Duralija, B. Genetic Variability and Structure of Fragaria nilgerrensis Schlecht. Germplasm in Sichuan Province. *Horticulturae* **2021**, *7*, 353. [CrossRef]
12. Khan, M.R.; Khan, M.A.; Habib, U.; Maqbool, M.; Rana, R.M.; Awan, S.I.; Duralija, B. Evaluation of the Characteristics of Native Wild Himalayan Fig (Ficus palmata Forsk.) from Pakistan as a Potential Species for Sustainable Fruit Production. *Sustainability* **2022**, *14*, 468. [CrossRef]
13. Ilhan, G.; Gundogdu, M.; Karlović, K.; Židovec, V.; Vokurka, A.; Ercişli, S. Main Agro-Morphological and Biochemical Berry Characteristics of Wild-Grown Sea Buckthorn (*Hippophae rhamnoides* L. ssp. *caucasica* Rousi) Genotypes in Turkey. *Sustainability* **2021**, *13*, 1198. [CrossRef]
14. Jaćimović, V.; Božović, D.; Ercisli, S.; Bosančić, B.; Necas, T. Sustainable Cornelian Cherry Production in Montenegro: Importance of Local Genetic Resources. *Sustainability* **2020**, *12*, 8651. [CrossRef]
15. Karatas, N.; Ercisli, S.; Bozhuyuk, M.R. Assessment of Morphological Traits, Nutritional and Nutraceutical Composition in Fruits of 18 Apricot cv. Sekerpare Clones. *Sustainability* **2021**, *13*, 11385. [CrossRef]
16. Mrázová, M.; Rampáčková, E.; Šnurkovič, P.; Ondrášek, I.; Nečas, T.; Ercisli, S. Determination of Selected Beneficial Substances in Peach Fruits. *Sustainability* **2021**, *13*, 14028. [CrossRef]
17. Ondrašek, G.; Romić, D.; Romić, M.; Duralija, B.; Mustač, I. Strawberry growth and fruit yield in a saline environment. *Agric. Conspec. Sci.* **2006**, *71*, 155–158.
18. Klamkowski, K.; Treder, W. Morphological and physiological responses of strawberry plants to water stress. *Agric. Conspec. Sci.* **2006**, *71*, 159–165.
19. Miličević, T.; Ivić, D.; Cvjetković, B.; Duralija, B. Possibilities of Strawberry Integrated Disease Management in Different Cultivation Systems. *Agric. Conspec. Sci.* **2006**, *71*, 129–134.

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Article

Innovative Strategies for the Use of Reflective Foils for Fruit Colouration to Reduce Plastic Use in Orchards

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Abstract: (1) *Background*: Plastic in fruit orchards represents an environmental issue due to large CO_{2eq} emissions associated with its production from fossil fuel and disposal (often incineration). (2) *Materials and methods*: Apple cv. "Braeburn Hillwell" trees on M9 rootstocks under a hail net were used at Campus Klein-Altendorf (CKA), Germany (50 °N) in 2018. In order to reduce the use of plastics to improve the red colouration of fruit particularly under hail nets, three alternatives to the current use of reflective mulch in each alleyway between the tree rows were explored, with uncovered grass alleyways as control. About 2800 colour measurements were done in the four weeks prior to harvest on 720 attached fruit below and above 1 m height in the field, and ca. 6900 additional colour measurements were conducted at harvest. (3) *Results*: The underlying regulatory mechanisms contrasted between the diffusive reflection of the white woven ground cover (such as Lumilys™ or Extenday™) in the alleyways and aluminium foil under the trees with regular (straight) light reflection. Good fruit colouring and a plastic reduction were achieved (a) through spreading the white woven ground cover in every other row, and (b) through substituting the white ground cover with aluminium foil (80% recycled). Both methods can reduce greenhouse gas (GHG) emissions (75–110 kg CO_{2e}q/ha for the first option a). (4) *Conclusion*: Plastic use in fruit orchards can be reduced by multiple use of the material in the same or several years, spreading it in every other row or substituting it by another reflective material, a relevant step towards an environment-friendly sustainable horticulture.

Keywords: anthocyanin; Extenday™; fruit colouration; light reflection; Lumilys™; plastic recycling; reflective films; resource conservation; sustainable horticulture

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1. Introduction

Criticism concerning food and fruit production at all stages of the supply chain includes unnecessary waste from the field to wholesale, retail, restaurants and the consumer [1–4]. The excessive use of plastic is criticised due to its fossil-fuel based, non-renewable nature, which can constitute a major source of GHG emissions due to production, pollution, waste and possibly micro plastics as important aspects for a sustainable, environment-friendly horticulture.

As a result of climate change and associated changes in the weather system, such as an increase in hailstorms, protective hail nets have become more widespread [3]; the combination of the resultant lack of light under the hail net [5] and warmer autumn temperatures [6] prevents the (red) colouration in many fruits and in many locations worldwide, from Brazil to Chile, Washington DC to Ontario and Bonn to Bologna [3]. The consumer (and trade), however, demands red-coloured fruit. In the consumer's perception, the red colour is associated with a ripe fruit, sweetness and good taste [3,7].

Hence, the objective of the present study was to investigate possible alternatives or modifications to the use of reflective mulch for colouration of fruit such as apricot, apple, (Anjou) pear, (red) grape berry, peach, persimmon (kaki), etc. In other words, the stimulation of the anthocyanin synthesis [8–10] for sustainable cultivation of these fruit

crops. Apple was chosen here due to its widespread cultivation in many countries with temperate climate zones throughout the world [4]. With ca. 80 million metric tons annual production (FAOSTAT [11]), it counts as the third largest fruit crop worldwide.

2. Materials and Methods

2.1. Location, Fruit Trees, Colour Measurement and Maturity Indexing

Nine-year-old cv. 'Braeburn Hillwell' apple trees on M9 rootstocks were cultivated at Campus Klein-Altendorf (CKA), University of Bonn, Germany (50 °N). The trees were trained to slender spindles and planted N-S to optimise light conditions (Jackson and Palmer, 1972) [12]. Fruit colour was measured with a portable i1 Pro (Xrite, Michigan, USA) in the lower third (<1 m tree height) and middle third (<1 m) of the trees, since fruit in the shaded lower parts of the tree canopy (Figures 1 and 2) are particularly prone to poor colouration. In these lower and middle parts of the tree, five fruits were marked in the west and five in the east tree periphery. Colour was repeatedly measured four times per fruit, i.e., every 90° around the fruit equator, on these still attached fruits and marked spots, resulting in 2800 values, and averaged for Figure 3, while colour values measured on the down-facing side of the fruit under 1 m height are presented in Figure 4. The second round of colour measurements was on harvested fruit using automated machine grading in an MSE 2000 (Greefa, Geldermalsen, Holland) with dedicated single fruit evaluation [13]. The 100 fruit samples for maturity indexing at two dates comprised ten apples per plot and treatment, i.e., five fruits from the east side and five fruits from the west side of the tree, and included the assessment of starch degradation, sugar content and fruit firmness, using standard methods [5].

(**a**) (**b**)

Figure 1. Different approaches using (**a**) (recycled) aluminium foil directly under the trees (left), or (**b**) Lumilys woven textile both after 6 weeks exposure, both at the end of the experiment before harvest.

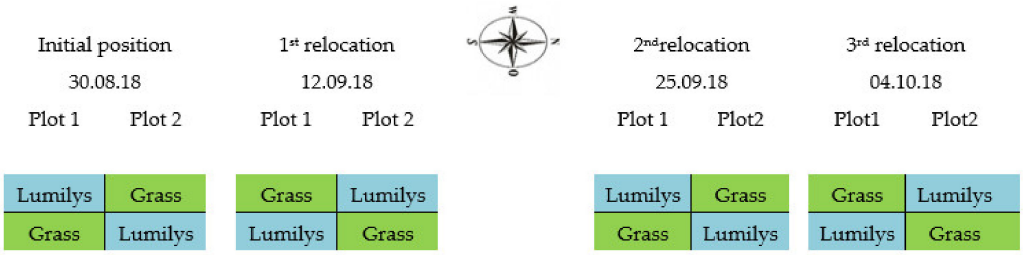

Figure 2. Pictorial representation of moving Lumilys mulch within the same row ("relocation") in autumn 2018.

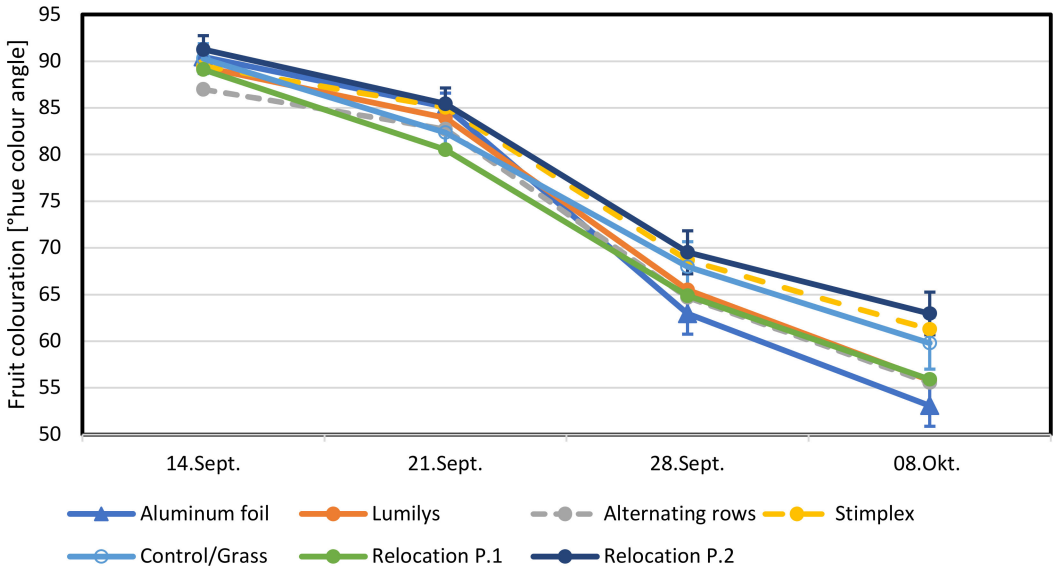

Figure 3. Effect of plastic (closed circles) and aluminium (triangles) reflective mulches or chemical measures (Biostimulant Stimplex^{TM}) relative to the control (open circles) **on red colour formation (anthocyanin synthesis) at the fruit equator** as a decline in °hue colour angles for apple cv. 'Braeburn Hillwell' at Campus Klein-Altendorf in 2018 (n = 100 colour measurements per treatment and date plus SEs at the 5% error level).

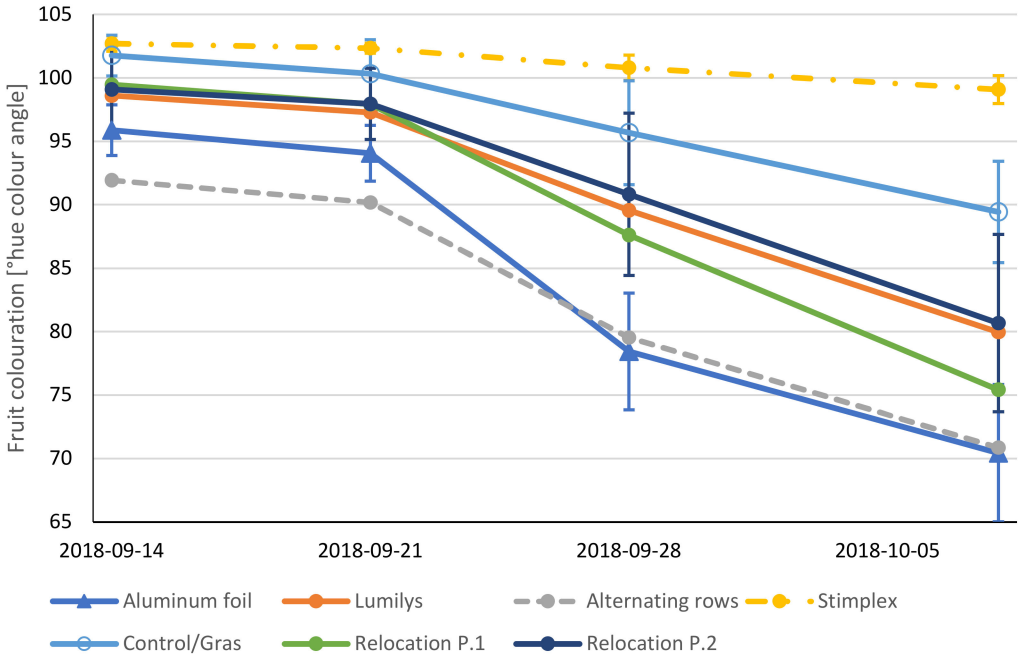

Figure 4. Hue colour angle of the **bottom side of apples below 1 m height** exposed to aluminium foil (triangle), Lumilys^{R} reflective (plastic) mulch, Lumilys^{R} in alternating tree rows (closed circles), the biostimulant Stimplex^{R}, control/grass (open circle) and relocation P.1 and P.2 for the four measurement dates (n = 10/per treatment and measurement day plus SEs at the 5% error level for Stimplex^{TM} control, relocation P2 and P1 and aluminium foil).

2.2. Reflective Mulches and Biostimulants

Reflective white woven textile mulch type Lumilys^{TM} (100 g/m^2; Beaulieu Textiles, Belgium) [14] was spread on 30 August 2018, i.e., ca. five weeks prior to the anticipated harvest (traditional way of using reflectors), with grassed alleyways without ground cover as control. The 2.6 m wide white polypropylene mulch cloth leaves a ca. 50 cm wide gap for soil respiration/aeration and water uptake close to the tree trunk.

The innovative alternatives to the white reflective groundcover in every row included (a) spreading only every other row (thereby saving 50% of the plastic) (Figure 2) and (b) the substitution of the (diffuse) reflective mulch by aluminium foil (type Profissimo®, dm pharmacies, Karlsruhe, Germany) (regular reflection) under the trees, leaving a 2.3 m wide gap on the grassed alleyway 1; the foil used was made from 80% recycled aluminium. The biostimulant Stimplex®/Acadian®, based on extracts of the brown algae (*Ascophyllum nodorum*; Acadian Seaplants Co., Dartmouth, NS, Canada), was our third alternative (Table 1).

Table 1. Experimental design to stimulate fruit colour at Klein-Altendorf in 2018.

Treatment	Spreading Time or Treatment Date	Dosage/Relocation
Aluminium		
Aluminium foil	30 August 2018	n.a.
Plastic		
Lumilys every row (current standard)	30 August 2018	n.a.
Lumilys in alternating rows	30 August 2018	n.a.
Lumilys relocation 1	30 August 2018	12 Sept.; 25 Sept.; 4 Oct. 2018
Lumilys relocation 2	30 August 2018	12 Sept.; 25 Sept.; 4 Oct. 2018
Control (untreated; grass, without groundcover)		
Control 1 with uncovered grass	n.a.	n.a.
Control 2 with uncovered grass	n.a.	n.a.
Chemical alternative		
Biostimulant Stimplex 1	14 Sept. and 1 Oct. 2018	4 L/ha
Biostimulant Stimplex 2	14 Sept. and 1 Oct. 2018	4 L/ha

Notes: n.a-not applicable.

The white woven reflective mulch (Lumilys^{TM}) was moved ca. every ten days in the fruit colouration period of six weeks before the harvest. In this relocation trial, the reflective mulch was spread in the alleyway either on the west side or the east side of the apple trees.

2.3. Statistics

The experiment comprised 80 apple trees. Each treatment consisted of ten trees plus one border tree, and the plots were replicated. A hundred and forty maturity tests were conducted at two dates on 10 apples per plot and treatment, i.e., five fruits from the east and five fruits from the west side of the tree, and averaged. A total of 2800 colour angle measurements were performed in the orchard on 560 attached fruits below and above 1 m height (Figure 3). All 5,515 apple fruits in the experiment were subjected to automated grading for colour (Figure 4), ca. 700 apples per treatment. Data were checked for normal distribution and homogeneity of variance, and then subjected to an analysis of variance (ANOVA) using Excel's statistical function and SEs at the 5% error level presented in Figures 3 and 4.

3. Results and Discussion

Repeated non-destructive colour measurements on the attached fruit (Figure 3) show the decline in the hue colour angle during fruit maturation, i.e., progressive development of red (anthocyanin) colour formation of the apple cv. 'Braeburn Hillwell' fruit. With about 63 °hue, the least red colouration was observed with the relocation of the reflective mulch within the same row and the uncovered grassed as control (60 °hue; Figure 3).

3.1. Development of Fruit Colouration on the Tree and Effect of Fruit Position in the Canopy on Colouration

The best fruit colouration (55 °hue) was observed with reflective mulch every other row/alleyway ("alternating rows"; Figures 2 and 3) and the aluminium foil under the trees (53 °hue; values averaged over the east and west side and over <1 m and >1 m).

Both polypropylene mulch relocations in plot 1 and plot 2 (Figure 2) and their use in every other row ("alternating") provide a plastic reduction of 50%. The colour of the apple fruit from relocation plot 1 (56 °hue) and relocation plot 2 (62 °hue) differed drastically, particularly in the period during 28 September to 8 October, with an apparent discrepancy between the decrease in the °hue colour angle of 9 °hue for apple in plot 1 and the 3 °hue for relocation plot 2 (Figure 3). In plot 1 the relocation of the reflective sheets within the same row had the strongest colouration effect, and the fruit were exposed longer to Lumilys reflective mulch in the alleyway on the east side than in relocation plot 2. ***The reflective mulch (Lumilys) appeared more effective on the less sunny east side of the apple tree rows with north-south planting, especially in late autumn with a decreasing solar angle,*** which has not been shown before, to our knowledge. Fruit on the ***west*** side of the trees benefited from sufficient PAR and UVB radiation for colouration (anthocyanin synthesis).

The lower, more shaded and green side of the apple fruit, particularly in the bottom of the tree canopy below 1 m, is severely affected by the lack of light. ***This bottom side of the fruit below 1 m in the tree canopy benefited most, in terms of the best red colour, from the aluminium foil directly under the tree (70.4 °hue) and Lumilys^R in every other ("alternating") alleyway (70.8 °hue)*** (Figure 4).

When the chemical biostimulant Stimplex^{TM} was applied, however, the colouration on the lower side of apple fruits was not improved (99 °hue), relative to the untreated control (grass; 89.4 °hue; Figure 4), and hence appeared unsuitable for this purpose.

The use of aluminium foil under the tree would mean a 100% reduction of plastic for fruit colouration in the orchard. Other (organic) alternatives like fresh wheat straw, which conveniently becomes available concomitantly, and bio-degradable white sports field paint failed in previous studies in our location in the Northern hemisphere [8], as well as evaporative cooling [15] and other biostimulants [15,16].

3.2. Colour Assessment—Automated Grading of Harvested Fruit

The colour threshold for the machine grading of all ca. 6900 harvested apple fruits was set to >50% red peel colouration for the classification as premium fruit. Reflective mulch every in other row ("alternating") had the largest share, with 81% in this sorting category (>50% surface red), followed by the aluminium foil, with 80% in relation to the traditional design of the reflective mulch in every row (75%). Where Lumilys mulch was relocated three times within the same row to save 50% of plastic, fruit from relocation plot 1 gained a similar hue value as Lumilys, with 73%, but that from relocation plot 2 exhibited a much lower value, with only 59%, i.e., less red colour (Figure 2). This discrepancy between fruit colour in plot 1 and plot 2 is in line with the results of the field colour measurements with attached fruit (Figure 1a). The control with uncovered grassed alleyways showed only 65% fruit in this premium colour category, as with the biostimulant Stimplex (Figure 5). The results of the colour sorting (Figure 5) correspond to the results of the colour angle measurements in the field (Figure 3), which also showed the best red fruit colour in both the application of the reflective mulch (Lumilys^R in alternating tree rows and the aluminium foil directly underneath the trees). The results of the colour measurements of attached apple fruits in the field (n = 3600 measurements) and of the automated sorting machine of ca. 6900 fruits show that the aluminium foil directly underneath the trees leads to apples with a better red colouring, thereby substituting 100% of plastic mulch by (recycled) aluminium. The reflective mulch (Lumilys) in the alleyway of every other row ("alternating") provided an equally good red colour, thereby reducing the plastic mulch input in the orchard by 50%.

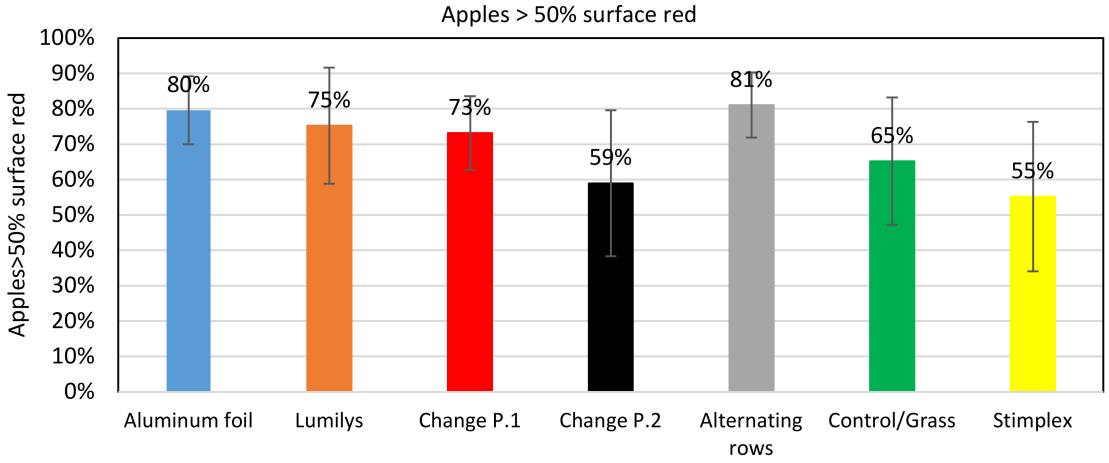

Figure 5. Effect of colour enhancing measures on the portion of apples with more than 50% red colour on their surface, with SDs at the 5% error level from machine sorting results (n = 6900).

3.3. Maturity Indexing of Apple Fruit

The maturity indexing showed that the biostimulant Stimplex^{TM} accelerated ripening (Table 2) and advanced fruit maturation by one week, causing it to ripen as early as 4 October 2018. The red colour of the apples was bright red, and they retained their firmness (Table 4). An early harvest could lead to an increase in the sales price for apples of an early variety (such as "Gala") at an early location [13] (Table 2). All other treatments with reflective mulches had no influence on fruit maturity and, above all, maintained firmness as a pre-requisite for storability.

Table 2. Maturity indexing (Streif index) of apples on 4 October 2018 (n = 10 per treatment).

Treatment	Streif Maturity Index		Streif Expectation	
	Average	SD	Min	Max
Control 1 + 2/grass	0.32	0.18	0.14	0.25
Aluminium foil	0.3	0.19	0.14	0.25
Lumilys every row	0.3	0.05	0.14	0.25
Lumilys relocation P. 1	0.26	0.08	0.14	0.25
Lumilys relocation P. 2	0.26	0.07	0.14	0.25
Lumilys alternating rows	0.39	0.22	0.14	0.25
Stimplex biostimulant	0.2	0.06	0.14	0.25

In Table 2, grey colour background represents metal, blue plastic and green a chemical alternative.

3.4. Economics

The additional proceeds, as a result of the colour improvement, were calculated by taking the percentage change of the apples with more than 50% red colour as compared to the control. The price difference between fruits with > 50% red colour, which sell at an average of 0.45 €/kg (€ 0.35–0.55), and those with less than 50% red colour, which sell at an average of 0.08 €/kg, was multiplied by yields of 30 t/ha, 40 t/ha and 50 t/ha. The results show that the largest financial gain was from well-coloured fruit from trees with reflective mulch in every other alleyway (row), followed by aluminium foil (under the tree) and then

Lumilys in every alleyway/row ("traditional" approach) and change P. 1. These results confirm that Lumilys in alternating alleyways/rows and aluminium foil represent possible alternatives to spreading reflective mulches like Lumilys^R in every row (Figure 1a).

The calculation is based on the cost for Lumilys^R reflective mulches (2700 m/ha), at 50 cent/m^2 (plus 240 €/ha hooks), and the recycled aluminium foil, at 51 cent/m^2 (plus 328 €/ha sandbags), which hardly differed. The biostimulant Stimplex^R was more economical due to the lower cost of 151 €/ha for two product applications (2 × 4 L/ha) and a low workload. Annual costs were based on a 10-year lifespan for Lumilys and a single or double use of the (recycled) aluminium foil.

A financial gain as a difference between gross income and cost was seen in the reflective mulch in every other alleyway/row ("alternating"), aluminium foil (under the tree), Lumilys and change P. 1, as compared to control/grass. Based on a yield of 50 t/ha, a financial gain was achieved with (i) Lumilys^R in every row 1091 €/ha), (ii) Lumilys^R in every other row ("alternating") (2899 €/ha), and (iii) aluminium foil (1388 €/ha), while the triple re-location of the Lumilys^R foil saves 50% in material costs but is not profitable due to the insufficient red colour of the apples for marketing as premium fruit (Table 3).

Table 3. Financial gain of employing the four alternatives for fruit colouration.

Parameter	Additional Revenue Per Hectare			Cost/ha	Financial Gain		
Yield:	**30 t/ha**	**40 t/ha**	**50 t/ha**		**30 t/ha**	**40 t/ha**	**50 t/ha**
Control	0	0	0	0	0	0	0
Aluminium foil	2171 €/ha	2895 €/ha	3619 €/ha	2231 €/ha	−60 €/ha	664 €/ha	1388 €/ha
Lumilys	1689 €/ha	2253 €/ha	2816 €/ha	1725 €/ha	−36 €/ha	528 €/ha	1091 €/ha
Relocation P.1	1457 €/ha	1943 €/ha	2429 €/ha	1733 €/ha	−276 €/ha	210 €/ha	696 €/ha
Relocation P.2	−118 €/ha	/	/	1733 €/ha	/	/	/
Alternating rows	2335 €/ha	3114 €/ha	3892 €/ha	993 €/ha	1342 €/ha	2121 €/ha	2899 €/ha
Stimplex	−534 €/ha	/	/	139 €/ha	/	/	/

/ no colour improvement relative to the untreated control, red background indicates financial losses, green financial gains.

3.5. Sustainable Apple Colouration—Economic and Ecological Aspects

Current criticism in the public and media targets the excessive use of plastic in the fruit supply chain [4,17–20]. The present study has shown that the use of plastics for fruit colouration could be decreased in three ways. Both the plastic mulch (Lumilys) in every other row and aluminium produced apple fruit with sufficient fruit colouration for economic marketing as premium fruit in class I and saved 50% or 100%, respectively, of plastic input in the orchard. The traditional use of plastic mulch in the alleyway in every row [21,22] is associated with 2700 m rolls × 2.6 m width × 0.1 kg/m^2 ⇒ 700 kg polypropylene/ha. Using the emission factor of 2.2–3.2 kg CO_2eq/kg PP (PlasticsEurope, Brussels, Belgium, 2005) [23], this is equivalent to GHGs of 1.5–2.2 t CO_{2eq}/ha for full orchard coverage (100%). A reduction in the use of plastic mulch in every other row (or translocation) would hence save 0.75–1.1 t CO_2 eq/ha in the material alone, with a multiple use, for ca. 10 years, of 75–110 kg CO_2 eq/ha/year.

For one hectare, 108 kg of aluminium foil is required to substitute the plastic mulch in every row. Aluminium production requires a high energy input of 13–16 kWh/kg aluminium (Hydro Inc., Chicago, IL, USA, 2016) [24]. Hence, it often takes place where energy is cheaply available, adding long-distance transport to the final validation. We used aluminium for the first time, since recycled material became available, which contains 80% recyclat and requires only 20% of new aluminium for its production. Based on the German electricity grid emission factor of 0.6 kg CO_2 eq/kWh, the GHG emissions associated with the production of primary aluminium are equivalent to 7.8–9.6 kg CO_2 eq/kg aluminium. Per acreage, with a need for 108 kg aluminium per hectare, this amounts

to 821–1037 kg CO_{2eq}/ha. Since the employed aluminium foil was made of 80% recycled aluminium with ca. 10% GHG of the primary aluminium (Hydro.com 2016), this results in 223–290 kg CO_2eq/ha. However, using aluminium foil made from 100% recycled aluminium would reduce the value to 82–104 kg CO_{2eq}/ha (Table 4). Even using the aluminium foil twice could significantly reduce the CO_2 load to between 112 and 145 kg CO_2 eq/ha. A combination of 100% recycled aluminium foil with a 2-year use would reduce the CO_2 burden to 41–52 kg CO_2 eq/ha. The decisive factor is that the use of the 100% recycled aluminium foil is associated with less CO_2 emissions, which makes its use more sustainable.

Table 4. Greenhouse gas (GHG) emissions associated with different scenarios of employing mulches for fruit colouration.

Material	Material Input/ha	Lifespan (years)	GHG Emissions (kg CO_2eq/ha & year)
Lumilys (polypropylene) every row	700 kg PP/ha	10	150–220
Lumilys change and alternating rows	350 kg PP/ha	10	75–110
Aluminium foil 80% recycled aluminium	108 kg alu/ha	1	223–290 *
Aluminium foil 80% recycled aluminium	108 kg alu/ha	2	112–145 *
Aluminium foil 100% recycled aluminium	108 kg alu/ha	1	82–104 *
Aluminium foil 100% recycled aluminium	108 kg alu/ha	2	41–52 *

* range originates from 7.8–9.6 CO_2eq/kg aluminium (Hydro.com, 2016)]; plastic in blue, metal in green background colour.

Both results of the colour measurements in the field (Figure 3) and machine grading (Figure 5) show the large potential for colour improvement in situations with colouration problems on the East side (morning sun, weak and short) and in the lower part (below 1 m) of the tree canopy (Figure 3).

This environmental benefit is largely caused by the fact that the aluminium foil was not laid out over the entire alleyway, due to its regular reflection property [25]. At present, the single use of the aluminium foil in an orchard protected by a hail net may be extended to two years, looking at the clean stage of the material at the end of its use in the orchard under our conditions (Figure 1a). The aluminium foil employed consisted of 80% recycled aluminium and may be used for another year, depending on its contamination. Meanwhile, there are also aluminium foils made of 100% recycled aluminium, which is sustainable. The possibility of recycling the aluminium foil used depends on the degree of contamination, and it may even be recycled again.

Despite its origin from fossil fuel, the use of reflective polypropylene (PP) mulches such as Lumilys^{TM} is sustainable, when used repeatedly (7-years warranty—estimated 10-year lifespan) and properly disposed of. At the waste collection points, the plastic material is washed, shredded and then melted—the PP pellets/granules are the source of recycled plastics such as boxes or flower pots [17].

4. Conclusions

The sustainability aspect is judged here on the basis of a sufficient red colour of the apples, which determines their sales price and orchard (bio-)economy. Short-lived biodegradable plastic mulches [4] are not suitable for this purpose, if fossil-fuel based. The use of aluminium foil made of 80–100% recycled aluminium is associated with considerably lower CO_2 emissions than new aluminium, with its high energy demand. Both the recycled aluminium foil with single (or duplicate) use, and the Lumilys plastic mulch,

a material with a long lifespan, used twice a year over several years and/or in every other row, results in ecologically and economically sound horticulture and could be further improved by recycling the fairly clean white single pure plastic polypropylene.

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References

1. Blanke, M.M. Reducing ethylene levels along the food supply chain–a potential key to reducing waste? Invited "Perspective". *J. Sci. Food Agric.* **2014**, *94*, 2357–2361. [CrossRef] [PubMed]
2. Gierling, F.; Blanke, M.M. Carbon reduction strategies for regionally grown and locally consumed white and red wine. *J. Environ. Manag.* **2021**, *278*. [CrossRef] [PubMed]
3. Bosančić, B.; Mićić, N.; Blanke, M.M.; Pecina, M. Effects of netting on fruit quality-a main effects meta principal components analysis (PCA) using apple as a model. *Plant Growth Regul.* **2018**, *86*, 455–465. [CrossRef]
4. Yang, Y.; Puwang, L.; Jing, J.; Minghze, L.; Yonghzen, L.; Chuang, Z.; Chao, W.; Zuyu, H.; Liu, Y.; Song, S. Renewable sourced biodegradable mulches and their environment impact. *Sci. Hortic.* **2020**, *268*, 109375. [CrossRef]
5. Solomakhin, A.A.; Blanke, M.M. Overcoming adverse effects of hailnets on fruit quality and microclimate in an apple orchard. *J. Sci. Food Agric.* **2007**, *87*, 2625–2637. [CrossRef]
6. Blanke, M.M.; Kunz, A. Effect of climate change on pome fruit phenology at Klein-Altendorf-based on 50 years of meteorological and phenological records [Einfluß rezenter Klimaveränderungen auf die Phänologie bei Kernobst am Standort Klein-Altendorf]. *Erwerbs-Obstbau* **2009**, *51*, 101–113. [CrossRef]
7. Hamadziripi, E.; Muller, M.; Theron, K.J.; Steyn, W.J. Consumer preference for apple eating quality and taste in relation to canopy position. Proc. ISHS Stellenbosch congress Dec 2012 (K. Theron, ed). *Acta Hort.* **2014**, *1058*, 254–260.
8. Blanke, M.M. Alternatives to reflective mulch cloth (Extenday^{TM}) for apple fruit colouration under hail nets? *Sci. Hort.* **2008**, *116*, 223–226. [CrossRef]
9. Weber, S.; Damerow, L.; Kunz, A.; Blanke, M.M. Anthocyanin synthesis and light utilization can be enhanced by reflective mulch-visualisation of light penetration into a tree canopy. *J. Plant Physiol.* **2019**, *233*, 52–57. [CrossRef]
10. Smrke, T.; Persic, M.; Verberic, R.; Sircelj, H.; Jakopic, J. Influence of reflective foil on persimmon (*Diospyros kaki* Thumb.) fruit peel colour and selected bioactive compounds. *Sci. Rep.* **2019**, *9*, 19069. [CrossRef]
11. FAOSTAT. Crops, Data. Available online: http://www.fao.org/faostat/en/#data/QC/visualize (accessed on 20 June 2020).
12. Jackson, J.E.; Palmer, J.W. Interception of light by model hedgerow orchards in relation to latitude, time of year and hedgerow configuration and orientation. *J. Appl. Ecol.* **1972**, 341–357. [CrossRef]
13. Meinhold, T.; Damerow, L.; Blanke, M.M. Reflective materials under hailnet improve orchard light utilisation, fruit quality and particularly fruit colouration. *Sci. Hort.* **2011**, *127*, 447–451. [CrossRef]
14. Beaulieu Technical Textiles, 2018. Lumilys–Smart reflective ground cover. Available online: http://www.lumilys.com (accessed on 22 September 2020).
15. Blanke, M.M. Possibilities of improving apple fruit colouration [Möglichkeiten zur Verbesserung der Rotfärbung bei Äpfeln—A review]. *Erwerbs-Obstbau* **2015**, *57*, 47–62. [CrossRef]
16. Blanke, M.M.; Kunz, A. Alternatives to phosphonates for fruit colouration. *Sci. Hort.* **2016**, *198*, 434–437. [CrossRef]
17. Schuhknecht, H.; Damerow, L.; Kunz, A.; Blanke, M.M. Colour development of apple with reflective mulches and biostimulants. *Acta Hort.* **2018**, *1228*, 433–439. [CrossRef]
18. Blanke, M.M. Biostimulants in horticulture-a review Biostimulantien im Gartenbau-eine Branche (er-)findet sich—A review. *Erwerbs-Obstbau* **2016**, *58*, 82–87. [CrossRef]
19. Blanke, M.M. Circular economy of (micro-)plastic in horticulture. GKL-Tagung zur Bestandesaufnahme von Mikro- und Makroplastik im Obst- und Gartenbau. *Erwerbs-Obstbau* **2020**. [CrossRef]
20. Blanke, M.M. Bessere Farbe und Fruchtqualität durch Reflexionsfolien. *Besseres Obst.* **2018**, *65*, 19–23.
21. Andris, H.L.; Crisoto, C.H. Reflective materials enhance 'Fuji' apple color. *Calif. Agric.* **1996**, *50*, 27–30. [CrossRef]
22. Prive, J.P.; Russell, L.; Leblanc, A. Use of Extenday ground cover on production of 'Gala' apples (*Malus domestica*) in New Brunswick, Canada. *N. Z. J. Crop Hortic. Sci.* **2008**, *36*, 221–230. [CrossRef]
23. PlasticsEurope, 2005. Free download from homepage or access via Simapro or GaBi Plastics Europe was earlier called APME. Available online: www.plasticseurope.org/en/resources/eco-profiles (accessed on 30 September 2020).

24. Hydro.com. 2016. Available online: https://www.hydro.com/de/hydro-in-deutschland/uber-aluminium/aluminium-lebenszyklus/recycling/ (accessed on 1 October 2020).
25. Meinhold, T.; Damerow, L.; Richters, J.P.; Blanke, M.M. Optical properties of reflective ground covers with potential for enhancing fruit colouration. *Biosyst. Eng.* **2010**, *107*, 155–160. [CrossRef]

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Detecting Apples in the Wild: Potential for Harvest Quantity Estimation

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Abstract: Knowing the exact number of fruits and trees helps farmers to make better decisions in their orchard production management. The current practice of crop estimation practice often involves manual counting of fruits (before harvesting), which is an extremely time-consuming and costly process. Additionally, this is not practicable for large orchards. Thanks to the changes that have taken place in recent years in the field of image analysis methods and computational performance, it is possible to create solutions for automatic fruit counting based on registered digital images. The pilot study aims to confirm the state of knowledge in the use of three methods (You Only Look Once—YOLO, Viola–Jones—a method based on the synergy of morphological operations of digital imagesand Hough transformation) of image recognition for apple detecting and counting. The study compared the results of three image analysis methods that can be used for counting apple fruits. They were validated, and their results allowed the recommendation of a method based on the YOLO algorithm for the proposed solution. It was based on the use of mass accessible devices (smartphones equipped with a camera with the required accuracy of image acquisition and accurate Global Navigation Satellite System (GNSS) positioning) for orchard owners to count growing apples. In our pilot study, three methods of counting apples were tested to create an automatic system for estimating apple yields in orchards. The test orchard is located at the University of Warmia and Mazury in Olsztyn. The tests were carried out on four trees located in different parts of the orchard. For the tests used, the dataset contained 1102 apple images and 3800 background images without fruits.

Keywords: computing image analysis; deep learning; yield mapping in an orchard; fruit counting; computer vision

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1. Introduction

The yield forecasting process can start in two stages. The first estimation may take place during the flowering of trees, which is particularly important for the estimation of future harvest [1,2]. The second stage, which was analyzed in the article, is counting the fruit on the tree [3,4]. Naturally, the future income is correlated with the number, size and quality of apples [5–7]. The fruit supply chain is long and complex, and numerous stakeholders are involved, including farm input suppliers, orchardists, collectors, packing stations, transporters/shipping companies, retailers/food service providers and the government and authorities, among others [8]. Several steps are included moving from upstream (production) to downstream (trade, storage, processing). In practice, the question of harvest size is revealed at several production stages, which is necessary for the preparation of the harvest itself and further affords the fruit commercial campaign. At this moment, the

producer has to estimate the harvest size to contract the receipt of the fruit [7]. According to data from the Central Statistical Office of Poland, in 2017, the area of agricultural land was 16,414,831 hectares, including 361,965 hectares of orchards (2.2%); a total of 156,995 farms had orchards. Taking into account the existence of statistical systems for forecasting fruit yields in individual countries and at the European level, counting fruit in orchards and sending it to the central data exchange center would be an extremely good complement to the data. Storage and packing stations and processing companies that sign contracts require a forecast about the quantity of received fruit. For distribution planning, it is a requirement to determine the number of transported products and their recipients early enough. The optimization of fruit distribution will allow a change in the communication model of fruit producers and consumers (distribution companies, refrigerators, supermarkets, etc.) [8,9]. The main problem is the current information about the yield forecast [10,11], and this can lead to the conduction of transactions without mutual knowledge, which leads to asymmetry in the decision-making process [12,13].

On a national scale, it is also important to forecast the quantities of apples produced on the market. Estimating yields based on previous harvests is not particularly accurate. We propose a solution called Fruit Calculation System (FCS). The first task is determining the number of apples. In the next stages of the study, the possibility of forecasting yields based on flowers and qualitative evaluation of apples (size, color, spots) should be examined. It should be added, at flowering time, that yield forecast is strongly impaired by the uncertainty of flower pollination, fruit set and further June drop.

Due to the possibilities for technical devices to be used by orchardists themselves, in this research, a system based on independent digital image material acquisition and transmission to a server was considered, where calculations will be carried out or the photo materials will be collected by a trained local representative. The end-user will have access to the final reports based on an application that communicates with the server that stores estimated results. The article tests three methods of counting apples for use in FCS.

Estimating the number of fruits before harvesting provides useful information for logistics. Although significant progress has been made in fruit detection, it is difficult to estimate the actual number of fruits on a tree. In practice, fruits often overlap in the image and are partially or completely hidden by leaves. Therefore, methods that detect fruits do not offer a general solution for estimating the exact number of fruits [14]. In the typical image classification process, the task is to specify the presence or absence of an object; however, the counting problem requires one to reason how many instances of an object are present in the scene. The counting problem arises in several real-world applications such as cell counting in microscopic images [15], wildlife counting in aerial images [16], fish counting [17] and crowd monitoring [18,19] in surveillance systems. Most modern research focuses on one of the components of the proposed system, i.e., fruit counting on the registered image. A non-destructive method was proposed to count the number of fruits on a coffee branch by using information from digital images of a single side of the branch and its growing fruits [20]. Recent years have seen significant advances in computer vision research based on deep learning. The algorithm efficiently counts fruits even if they are in the shade, occluded by foliage or branches, or if there is some degree of overlap amongst fruits [21–23], fruit diseases or damage [24–26].

Taking into account the rapid technological development related primarily to the miniaturization of measuring devices and the increase in computing power in mobile devices, it is possible to undertake the task of creating an apple-counting system based on a smartphone or an image obtained from a drone camera. To realize this hypothesis, preliminary studies were carried out in the natural environment. To verify the hypothesis, in our pilot study, was tested three methods of counting apples to create an automatic system for estimating apple yields in orchards.

2. Methods and Methodology

Detection of apple fruit by reference to color [27,28], shape [29], visibility [30] and size requires the use of appropriate computer vision techniques [31–33]. The selection of appropriate techniques depends on the goal to be achieved through the digital acquisition of an apple image [9,34,35]. The goal may be to assess the number of fruits or estimate their condition or their size [31,32]. Therefore, it is not easy to separate the obtained image of an apple on sub-surfaces with pixels unequivocally (uniformly) connected with fruit and other pixels (so-called background). Variable observation and environmental conditions were indicated as the main reason. Unfortunately, none of the classic methods offer direct high (satisfying) efficiency. The goal can be defined as two main tasks:

- fruit counting [36–41],
- information about chosen fruits, such as color and quality rate, resulting from the counting.

Despite the impressive results achieved by these approaches, all of them need strong supervision information during the training phase. Based on literature research, the following groups of methods can be distinguished [27–33,38–41]:

- Convolutional Neural Networks (CNN)—This method is more accurate than the latest one based on Gaussian Mixture Models (GMMs). The multi-class classification approach used in this method provides an accuracy of 80% to 94% without the need for any pre-or post-processing steps. The deep learning network reacts to different fruit colors and lighting conditions. To check the suitability of the method for yield estimation, tests were conducted. The described method allows the achievement of approximately 96% accuracy concerning the actual number of apples [14].
- Deep Simulated Learning (DSL)—Automatic number of fruits alone estimation based on robotic agriculture provides a real solution in this area. The network is fully trained on synthetic data and tested on real data. To capture functions on multiple scales, a modified version of the Inception-ResNet architecture was used. The algorithm counts effectively even if the fruits are hidden in the shade, obscured by leaves or branches, or if the fruits overlap to some extent. Experimental results show 91% average test accuracy in real images and 93% in synthetic images. The proposed methodology works effectively even if the variant has a lighting deviation [23].
- Mixed method—This method combines deep segmentation, frame-to-fruit tracking and 3D location to accurately count the visible fruits in the image sequence. Segmentation is performed on the monocular camera image stream, both in natural light and under controlled night-time lighting. The first step is to train a fully revolutionary network (FCN) and to divide the video frame images into fruit and fruit pixels. Then, frame-by-frame fruit is tracked using a Hungarian algorithm, in which an objective result is determined based on the improved Kalman filter, i.e., Kanade–Lucas–Tomasi (KLT) [42].

Detection using the general descriptor YOLOv3-608 COCO TRAINVAL, although effective, can be improved by creating a customized set of weights and classes based on a specific spectrum of possible detection images. The Training Dataset contained 1102 apple images and 3800 background images without fruit. Each picture, named after the source, was pre-processed manually. Non-apple elements have been removed. The size of the image was then changed to the box of the fruit in the image. Thanks to this, the parameters for the proper scaling of the source image and its background were known. This allowed for more flexible preparation of images for machine learning, which was performed by overlaying the source images on any background—here, in the form of pictures of leaves, branches, etc. As a result of such overlapping combined with the changing of the scales of the vertical and horizontal axes, rotation, adding noise and blur, 16,530 images were created.

Despite the high performance of object detection using YOLO, it has been decided to use it as a parallel solution—dividing the main image into smaller sections processed by separate Central Processing Units or Graphics Processing Units.

The actual number of apples on the tree was determined manually. This approach has made it possible to establish a clear reference level. Each result obtained by the tested methods was visually verified in the image. As part of the verification, it was checked whether the counted objects are apples (which groups of pixels on the tested objects qualified as apples).

Three methods of counting objects in photos were tested in the research.

2.1. The Use of Image Filtration and Hough Transform—Solution A

In this solution, several steps were taken to move from a simple picture of the fruit to counting its shapes (Figure 1).

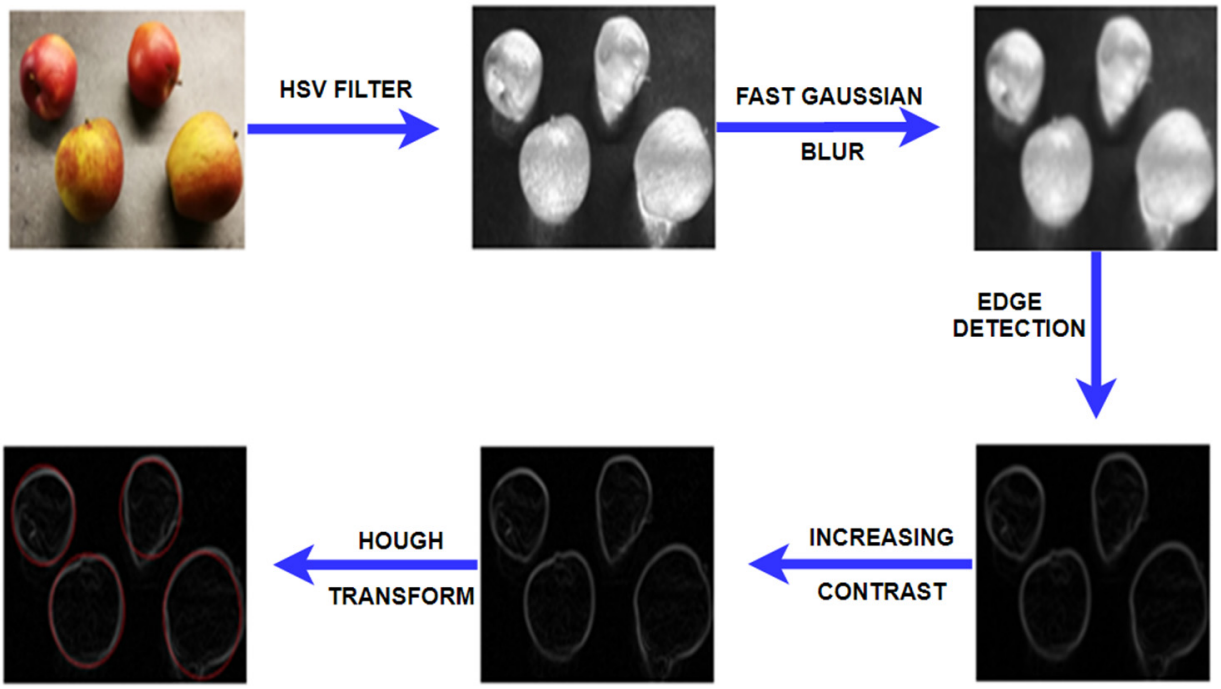

Figure 1. Steps are required to detect a number of fruit shapes from a digital image. Source: own study.

In this case, the color image (stored in RGB—Red, Green, Blue components) is transformed to the HSV representation model (H—hue, S—saturation, V—value) [43–45].

The use of the HSV model makes it easier to indicate where the fruit pixels are by using the HSV value (after blurring the images with a Gauss filter; Figure 2). Work began in the autumn and these were the first attempts to acquire and process images.

Appropriately selected filter edge parameters narrow the search area even more. It is possible to fit in circles (an approximation of apple shapes) by using the Hough transform method, for example.

Previous research has made comparisons of edge detection and Hough transformation techniques for the extraction of geologic features [46] or Msplit estimation [47,48].

Figure 2. HSV (hue, saturation, value) filtration mode for mockups of an apple fruit image efficiency ratio of 41% with many selection errors (Tree no. 1). Source: own study.

2.2. Viola–Jones Object Detection—Solution B

Another approach involves using an object detection framework and finding objects by using a dataset of positive image objects (Figure 3) for training it. This process requires training the classifier on thousands of images and searching these images for target objects.

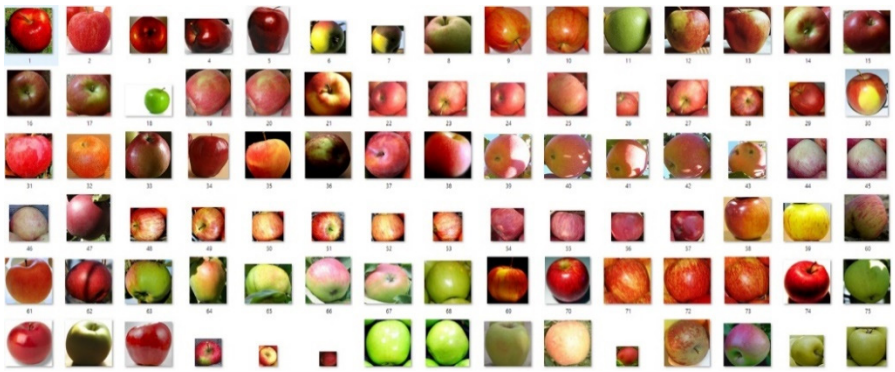

Figure 3. Positive image samples for database training. Source: own study.

The Viola–Jones algorithm was used because it has several advantages, such as a sophisticated feature selection and an invariant detector that determines scales. This results in scaled functions instead of scaling the image itself [49].

The use of the Viola–Jones algorithm [50] is based on the description of features rather than the pixels of the image directly. The analysis of the features proposed by Viola and Jones is performed in random rectangles, as in Figure 4.

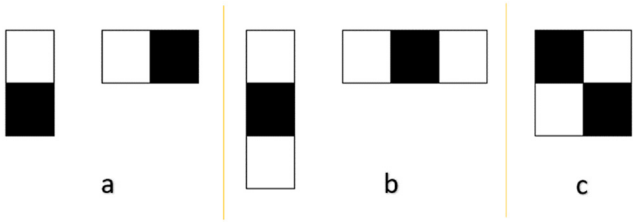

Figure 4. Example rectangle features (based on the original article in [49]) are shown relative to the enclosing detection window. The sum of the pixels that lie within the white rectangles is subtracted from the sum of the pixels in the grey rectangles. Rectangle features can contain two sub-rectangles (**a**), three rectangles (**b**), or four rectangles (**c**), and their size can be changed cascadingly.

Each feature result is a single value, which is calculated by subtracting the sum of the values of the pixels under white rectangles from the sum of the pixels under black rectangles.

Thanks to using such a generalization, it is possible to cascadingly increase the size of black and white rectangles, thus allowing for studying and comparing images with different scales.

Unfortunately, despite the promising initial assumptions, it turned out that the algorithm (the Viola–Jones algorithm) is not suitable for generalizing the classification of objects (creating classes)—it is used primarily to detect specific objects, which, in the case of apples, turned out to be an erroneous assumption. Additionally, even when detecting specific objects (not classes), it has a problem with torsion tilt and different lighting conditions. Fruit count tests were also performed for the selected apple tree (Tree no. 1). An efficiency of 55% was achieved. The result is presented in Figure 5.

Figure 5. Effective detection of apple objects using the Viola–Jones algorithm (Tree no. 1). Source: own study.

2.3. YOLO: Real-Time Object Detection—Solution C

The use of the modern real-time object detection system YOLO (You Only Look Once) is the third solution assessed. YOLO uses a single ConvNet (or CNN, convolution neural network) for classification and localizing by using bounding boxes. The advantage of this solution, as the authors indicated, is the reconstruction of object detection to a single regression problem, directly from image pixels to coordinates defining rectangular envelopes, and the probability of the occurrence of appropriate classes of objects [51].

The YOLO algorithm can be described in a few steps. The input image is divided into an SxS grid (Figure 6). Each cell in this grid is designed to predict the existence of only one object in it.

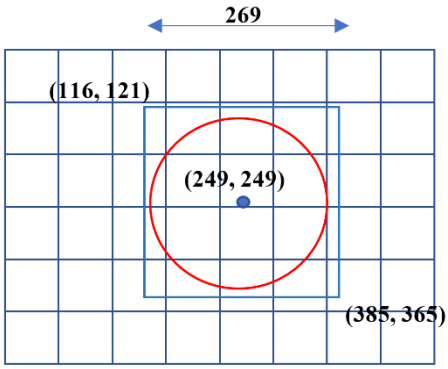

Figure 6. An example of the division of an image into a grid in the YOLO algorithm. Source: own study.

The blue line is the bounding box (bbox), which must be described by 5 components related to the selected cell of the grid, and these coordinates must be normalized, i.e., defined within the range of 0–1. The following parameters describe each field:

- x blue box = $(385 - 116)/2 = 135$ but normalized and related to the corresponding grid cell, here: $(135 - 100)/100 = 0.35$
- y blue box = $(365 - 121)/2 = 122$ but normalized and related to the relevant grid cell, here: $(122 - 100)/100 = 0.22$
- w width blue box = $(385 - 116)/500 = 0.54$ (normalization in relation to the width of the whole image)
- h height blue box = $(365 - 121)/500 = 0.49$ (normalization relative to the height of the whole image)
- c confidence, which is the Intersection over Union (IoU) between the predicted box and the ground truth (c = area of overlap/area of union).

It is assumed that only one type of class is assigned to one cell. The output vector is in the form of a tensor SxSx(C + B $\times$ 5), where B stands for the number of blue boxes. The rest looks like a normal CNN, with convolutional and max-pooling layers. All details can be found in the source document [52].

The main goal of our solution was to use real-time detection with image acquisition by a mobile device in practical implementation. Moreover, it was important to choose a neural network dedicated to the performance of mobile devices. Each image was divided into 4 or 16 parts depending on the resolution of the image, and each analyzed fragment was analyzed separately. This increased the digital detection of objects and reduced the memory load of the algorithm. This gives an insight into the future possibilities of analyzing images acquired in the form of video recordings as parallel, multithread computing.

3. Results

The use of YOLO allowed us to obtain better results than with other classifiers. At the same time, the working time was much shorter with YOLO. To evaluate the work and

results, a set of YOLOv3-608 scales trained at the COCO was used http://cocodataset.org/ accessed on 10 January 2021.

With confidence threshold = 10% and assuming a search only for 47 classes (apples in the weighing file) and excluding overlapping of objects more than 30%, 66 objects were found for the above image, which represents 67% of detectable objects in such an image (Figure 7). This illustrates the multi-threaded detection of apple objects based on the numerically corrected image with:

- change of the clarity in the arbitrary range from −18 to 18 levels,
- noise removal using the non-local means algorithm,
- Gamma correction from 1.1 to 1.6,
- Increase in the number of detectable items from 27% to 80% (Figure 8).

Figure 7. Example of YOLO (You Only Look Once) operation on the selected tree (**a**—original image, **b**—counted apples). Source: own study.

Figure 8. Results of apple object detection based on the numerically corrected image (Tree no. 1). Source: own study.

Apple fruit detection, regarding their specific color (problem 1), shape (problem 2) visibility and size (problem 3), requires the use of appropriate computer vision techniques. The research carried out has led to the following conclusions:

1. The impact of the first problem can be significantly reduced by:

- Anti-noise filters: non-local means filters are suggested but it is possible to experiment with local ones,
- Edge detecting algorithms, for example, operators based on the first derivative (Prewitt, Sobel, Canny, Scharr or Roberts) or second derivative (Marr–Hildreth algorithms [10]),
- Use of a thermal imaging camera. The literature related to the subject of study includes an effective attempt to use a thermal infrared camera; however, its cost is extremely high, which limits the scale of the task, and the achievable resolution is still not satisfactory.

2. The solution to the second problem is to adopt the circular shape of a standard apple and use Hough transform or Msplit estimation to complement the incomplete shape of the circle.

3. The different distances of apple fruits from the camera during the acquisition of a digital image results in different sizes (numbers of pixels) of their digital representation. While this is not important when assessing the number of fruits, it is of high importance when it comes to interpreting fruit size or belonging to the examined apple tree. Hence, it is important to know the size of the expected single apple in the picture. The goal can be reached by using one of two methods separately or by compiling them. Using a fixed focal length camera, the known location of the camera and the apple tree allows for the approximation of the size of the fruit, and its assessment in terms of dimensions. The stability of the focal length and the positions of the camera and the tree guarantee a differential analysis of the development of inflorescence and, later, fruit; however, the parameters of the camera should be selected individually. A synthetic comparison of the three methods is presented in Table 1.

Table 1. Comparison of the systems used for object identification.

	Method A	**Method B**	**Method C**
	image filtration and Hough transformers	Viola–Jones object detection	YOLO: Real-Time Object Detection
Color	The color of this assumption is important. Initial image filtration is based on the ranges of individual color components.	Does not matter	Does not matter
Shape	Only shapes were similar to circles.	A well-prepared descriptor works properly on different shapes but you should put them yourself in the training set.	Using a well-trained network or having trained it with new images, there is no need to place fragments of the image of the fruit if its detection is desired. The algorithm does it.
Size	Relaxation of the radius causes considerable elongation of the object search operation.	Does not matter	Does not matter
Processing time	Very long	Medium (not in real-time)	In real-time

Table 2 presents the results of fruit counting efficiency using three methods on four test trees.

Table 2. Results comparison of the systems used for object identification.

Tree No.	The Real Number of Apples	Hough Transform		Viola–Jones		YOLO	
		Detected Apples	Effectiveness [%]	Detected Apples	Effectiveness [%]	Detected Apples	Effectiveness [%]
1	220	90	41	121	55	176	80
2	82	25	30	40	49	73	89
3	52	15	29	29	55	43	83
4	30	14	46	19	63	26	84
Average			37		55		84

The reference number of apples on the tree was determined manually. The results indicate the use of YOLO as an effective solution for counting the number of fruits on the objects presented in the article. Limitations in detecting more apples resulted from physical (partially overshadowing objects) and environmental conditions.

4. Discussion

Research work on issues related to fruit detection based on digital images has become extremely popular in recent years. This is primarily related to the development of innovative agricultural robots using modern image processing algorithms [52]. Concerning the effects of research work on various approaches of automatic apple counting based on images, the proposed approach has given satisfactory results. In terms of fruit detection, the obtained accuracy ranges between 80% and 96%. Naturally, such an accuracy range is related to the adopted method and the characteristics of the plants on which the fruits grow. Linker et al. in their approach reached the estimation accuracy of 85% [53]. They based their calculations on information about color and texture [1,54]. In the works of Wei et al. and Payne et al., among others, the results are also influenced by sunlight and color saturation [52,55]. Zhao et al. used a feature image fusion method to recognize mature tomatoes obtained, with 93% detection [56]. A similar level (92.4%) was reached by Qiang et al. [57]. Kelman et al. based their calculations on the shape of the detected objects, which resulted in 94.4% fruit detection in the pictures [58]. Similar results to those presented in the article were achieved by Kurtulmus et al. (84.6%) [59] and Yamamoto et al. (80% and 88%) [60]. The apple-counting method based on YOLO has limitations due to the operating algorithm. An erroneous definition of the detection bounding box causes a small error in interpretation for a large box to be insignificant, but for a small box to increase in insignificance. The biggest problem, regardless of the method, is that the fruit is covered by leaves and two fruits are in close proximity, therefore the system can interpret them as one object.

The process of forecasting the number of apples in the future harvest can be divided into two basic stages. The first one is related to monitoring the condition of trees and counting the number of flowers on the trees [1,2], and counting the ripening apples. From the orchardists' point of view, a special role is played by the possibility to determine the size of the harvest [61], hence a large number of emerging scientific studies in this area [3–8]. In this study, several approaches to fruit (apple) number evaluation were analyzed in a practical way, which allowed for the compilation of the results presented below.

Fruit images, including apples, are characterized by a high degree of texture irregularities. The lack of surface uniformity results from the differences in fruit exposition and is a natural consequence of the fruit location within the tree crown, occultation by branches, leaves and others. Although the optimistic assumption of apple shape observation from any position and camera angle indicates the approximation of the circular shape, the overlapping of fruit images and the mentioned covering of fruits with other elements recorded in the images and with the shadows cast by them can also cause an unpredictable change in the shape of a single fruit in an image [2]. A single fruit can also be interpreted as two apples or more when the image of an apple is divided by a view of a branch.

The whole process of fruit counting, when it comes to one tree, is based on taking a series of pictures with the center of the projection shifted to a small longitudinal parallax. This allows the obtainment of a smudged image of a single tree. In this way, a full picture of the tree crown was obtained. A similar solution can be applied to the proposed schemes of the material image acquisition from a drone or mobile device for the whole orchard.

From the technical side of the image processing system, it is necessary to collect an appropriate number of apple images, on the basis of which the system can start its calculations. Häni et al. adopted 1000 high-resolution images acquired in orchards, together with human annotations of the fruit on trees. The fruits were marked with polygonal masks for each object, which helped to precisely detect, locate and segment the object [61]. For their research, Gao et al. authors acquired 800 images, which after processing gave a total input of 12,800 images [6]. An analogous number of images (800 images) was used by Fu et al. in their research using low-cost Kinect V2 sensors [7]. In this research, a similar number of input photos were taken as taken by other researchers. Our input base was 1102 images. In the field, three photos were taken for each tree on one side.

After choosing the method of counting the apples in digital images, it is necessary to propose the structure of the system for taking images in the orchard. The key assumption of the proposed solution was to minimize the costs of its creation and use of the system. Hence, it assumes the use of generally available mobile devices as a component of digital image acquisition—georeferenced images (determined on the basis of Global Navigation Satellite Systems (GNSS) technology). Such a solution offers the possibility of mass use in horticulture.

5. Conclusions

The main objective of this study was to verify the optimal method for identifying and counting apples on trees from photographs taken in the orchard. Based on the tests performed, it can be concluded that the best results are obtained using the YOLO method.

The reduced number of trees accepted for the test allowed manual counting of the number of fruits on each tree. With a larger test sample, without the ability to count and determine a reliable number of reference fruits, the tests would have low reliability. Therefore, for validation of individual object recognition methods, in the authors' opinion, the presented sample is sufficient. The adopted approach provided an unambiguous reference number of counted fruits. It allowed to unequivocally determine the level of counting accuracy. The obtained accuracy of individual methods was confirmed by literature review and achievements of other researchers. After carrying out the pilot experiments according to the assumptions presented above, the decision was made to implement the task using smartphones equipped with a camera with the required image acquisition accuracy and accurate positioning by GNSS (Figure 9).

Initially, a solution can be proposed based on the measurements with the mobile device, because of its advantage over the classical methods, used mainly by mass users.

Regarding the considerations related to fruit counting, YOLO was chosen for its:

- efficiency,
- possibility of implementation on mobile devices,
- effectiveness,
- ability to increase the effectiveness by constantly supplementing the YOLO training patterns requiring time for specific apple cultivars.

The main component obtaining the data is an orchardist or a person indicated by him/her. The measurement is made according to the assumptions that were initially set for the given orchard (depending on the way the trees are planted, density, number of rows, etc.).

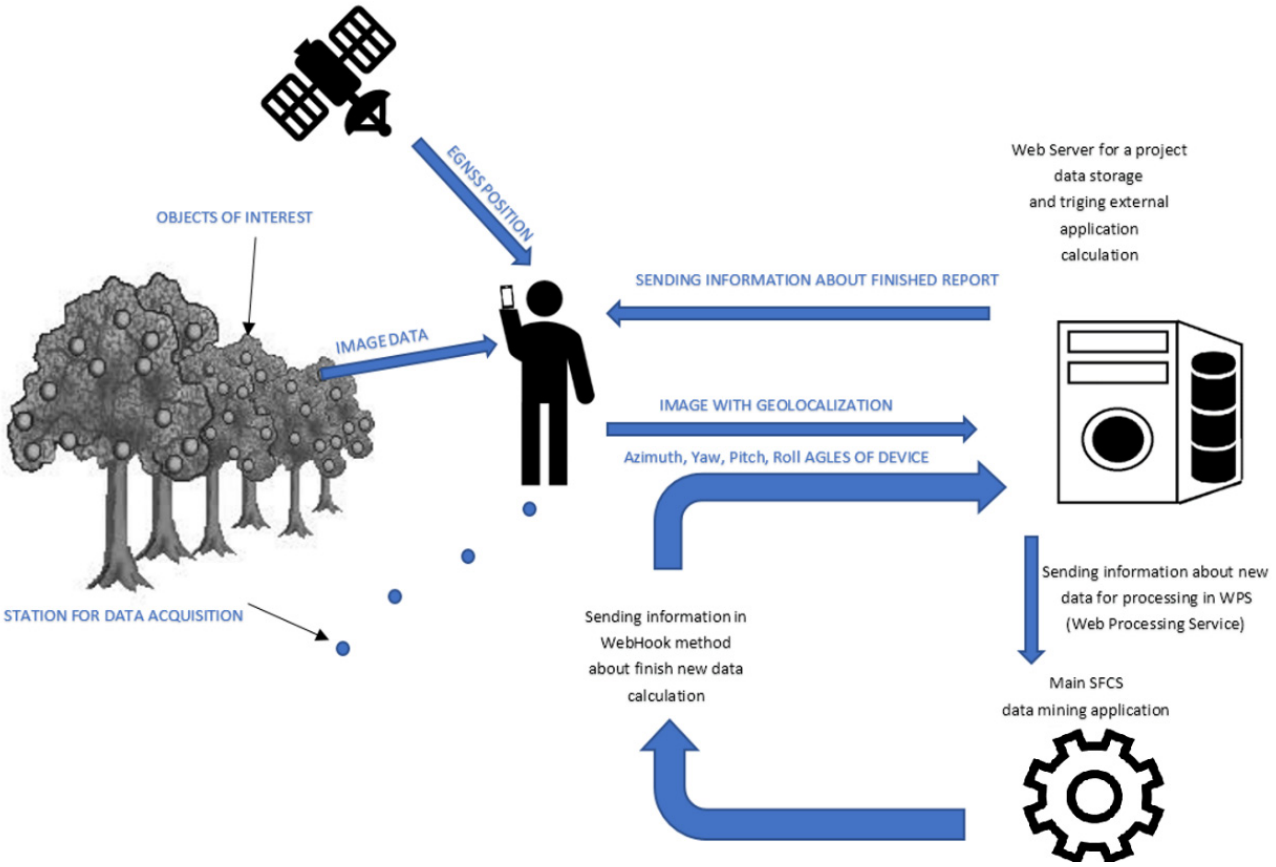

Figure 9. The global scheme of the system functioning. Source: own study.

The mobile application made available to the orchardist allows the user to take images with initial control.

The proposed solution preliminarily assesses the images in terms of chromatics, a histogram and its alignment and width, which makes it possible to reject completely incorrect photos (at the stage of acquiring them).

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References

1. Wu, D.; Lv, S.; Jiang, M.; Song, H. Using channel pruning-based YOLO v4 deep learning algorithm for the real-time and accurate detection of apple flowers in natural environments. *Comput. Electron. Agric.* **2020**, *178*, 105742. [CrossRef]
2. Wang, X.A.; Tang, J.; Whitty, M. Side-view apple flower mapping using edge-based fully convolutional networks for variable-rate chemical thinning. *Comput. Electron. Agric.* **2020**, *178*, 105673. [CrossRef]

3. Vasconez, J.P.; Delpiano, J.; Vougioukas, S.; Cheein, F.A. Comparison of convolutional neural networks in fruit detection and counting: A comprehensive evaluation. *Comput. Electron. Agric.* **2020**, *173*, 105348. [CrossRef]
4. Samiei, S.; Rasti, P.; Richard, P.; Galopin, G.; Rousseau, D. Toward joint acquisition-annotation of images with egocentric devices for a lower-cost machine learning application to apple detection. *Sensors* **2020**, *20*, 4173. [CrossRef] [PubMed]
5. Gao, F.; Fu, L.; Zhang, X.; Majeed, Y.; Li, R.; Karkee, M.; Zhang, Q. Multi-class fruit-on-plant detection for an apple in SNAP system using Faster R-CNN. *Comput. Electron. Agric.* **2020**, *176*, 105634. [CrossRef]
6. Fu, L.; Majeed, Y.; Zhang, X.; Karkee, M.; Zhang, Q. Faster R–CNN–based apple detection in dense-foliage fruiting-wall trees using RGB and depth features for robotic harvesting. *Biosyst. Eng.* **2020**, *197*, 245–256. [CrossRef]
7. Przyborski, M.; Szczechowski, B.; Szubiak, W.; Szulwic, J.; Widerski, T. Photogrammetric Development of the Threshold Water at the Dam on the Vistula River in Wloclawek from Unmanned Aerial Vehicles (UAV). In Proceedings of the 15th International Multidisciplinary Scientific GeoConference SGEM 2015, Albena, Bulgaria, 18–24 June 2015; Volume I, pp. 493–500. [CrossRef]
8. Zhang, X.; He, L.; Karkee, M.; Whiting, M.D.; Zhang, Q. Field evaluation of targeted shake-and-catch harvesting technologies for fresh market apple. *Trans. ASABE* **2020**, *63*. [CrossRef]
9. Jidong, L.; De-An, Z.; Wei, J.; Shihong, D. Recognition of apple fruit in natural environment. *Optik* **2016**, *127*, 1354–1632. [CrossRef]
10. Maldonado, W., Jr.; Barbosa, J.C. Automatic green fruit counting in orange trees using digital images. *Comput. Electron. Agric.* **2016**, *127*, 572–581. [CrossRef]
11. Liu, J.; Yuan, Y.; Zhou, Y.; Zhu, X.; Syed, T.N. Experiments and analysis of close-shot identification of on-branch citrus fruit with realsense. *Sensors* **2018**, *18*, 1510. [CrossRef]
12. Kowalczyk, C.; Nowak, M.; Źróbek, S. The concept of studying the impact of legal changes on the agricultural real estate market. *Land Use Policy* **2019**, *86*, 229–237. [CrossRef]
13. Renigier-Bilozor, M.; Wisniewski, R.; Bilozor, A. Rating attributes toolkit for the residential property market. *Int. J. Strateg. Prop. Manag.* **2017**, *21*, 307–317. [CrossRef]
14. Hani, N.; Pravakar, R.; Volkan, I. Apple counting using convolutional neural networks. *Int. Conf. Intell. Robot. Syst.* **2018**. [CrossRef]
15. Ren, S.; He, K.; Girshick, R.; Sun, J. Faster r-cnn: Towards real-time object detection with region proposal networks. In *Advances in Neural Information Processing Systems, Proceedings of the Neural Information Processing Systems Conference, Montreal, QC, Canada, 7–12 December 2015*; Neural Information Processing Systems Foundation, Inc.: Ljubljana, Slovenia, 2015; pp. 91–99.
16. Laliberte, A.S.; Ripple, W.J. Automated wildlife counts from remotely sensed imagery. *Wildl. Soc. Bull.* **2003**, *31*, 362–371.
17. Del Río, J.; Aguzzi, J.; Costa, C.; Menesatti, P.; Sbragaglia, V.; Nogueras, M.; Sarda, F.; Manuèl, A. A new colorimetrically-calibrated automated video-imaging protocol for day-night fish counting at the OBSEA coastal cabled observatory. *Sensors* **2013**, *13*, 14740–14753. [CrossRef]
18. Ryan, D.; Denman, S.; Fookes, C.; Sridharan, S. Crowd counting using multiple local features. In Proceedings of the Digital Image Computing: Techniques and Applications, Melbourne, Australia, 1–3 December 2009; pp. 81–88.
19. Ma, Y.; Wu, X.; Yu, G.; Xu, Y.; Wang, Y. Pedestrian detection and tracking from low-resolution unmanned aerial vehicle thermal imagery. *Sensors* **2016**, *16*, 446. [CrossRef] [PubMed]
20. Ramos, F.A.; Prieto, E.C.; Montoya, C.E. Oliveros, Automatic fruit count on coffee branches using computer vision. *Comput. Electron. Agric.* **2017**, *137*, 9–22. [CrossRef]
21. Liu, X.; Chen, S.W.; Aditya, S.; Sivakumar, N.; Dcunha, S.; Qu, C.; Taylor, C.J.; Das, J.; Kumar, V. Robust fruit counting: Combining deep learning, tracking, and structure from motion. In Proceedings of the 2018 IEEE/RSJ International Conference on Intelligent Robots and Systems (IROS), Madrid, Spain, 1–5 October 2018; pp. 1045–1052. [CrossRef]
22. Patel, H.N.; Jain, R.K.; Joshi, M.V. Fruit detection using improved multiple features based algorithm. *Int. J. Comput. Appl.* **2011**, *13*, 1–5. [CrossRef]
23. Rahnemoonfar, M.; Sheppard, C. Deep count: Fruit counting based on deep simulated learning. *Sensors* **2017**, *17*, 905. [CrossRef] [PubMed]
24. Bagheri, N.; Mohamadi-Monavar, H.; Azizi, A.; Ghasemi, A. Detection of Fire Blight disease in pear trees by hyperspectral data. *Eur. J. Remote Sens.* **2018**, *51*. [CrossRef]
25. Martinelli, F.; Scalenghe, R.; Davino, S.; Panno, S.; Scuderi, G.; Ruisi, P.; Villa, P.; Stroppiana, D.; Boschetti, M.; Goulart, L.R.; et al. Advanced methods of plant disease detection. A review. *Agron. Sustain. Dev.* **2015**, *35*, 1–25. [CrossRef]
26. Yang, Y.; Zhang, Y.; Deng, Y.; Yi, X. Endozoochory by granivorous rodents in seed dispersal of green fruits. *Can. J. Zool.* **2018**. [CrossRef]
27. Bhargava, A.; Bansal, A. Fruits and vegetables quality evaluation using computer vision: A Review. *J. King Saud Univ. Comput. Inf. Sci.* **2018**. [CrossRef]
28. Al Ohali, Y. Computer vision based date fruit grading system: Design and implementation. *J. King Saud Univ. Comput. Inf. Sci.* **2011**, *23*, 29–36. [CrossRef]
29. Blasco, J.; Aleixos, N.; Molto, E. Computer vision detection of peel defects in citrus by means of a region oriented segmentation algorithm. *J. Food Eng.* **2007**, *81*, 535–543. [CrossRef]
30. Lak, M.B.; Minaei, S.; Amiriparian, J.; Beheshti, B. Apple fruits recognition under natural luminance using machine vision. *Adv. J. Food Sci. Technol.* **2010**, *2*, 325–327.
31. Lingua, A.; Marenchino, D.; Nex, F. Performance analysis of the SIFT operator for automatic feature extraction and matching in photogrammetric applications. *Sensors* **2009**, *9*, 3745–3766. [CrossRef]

32. Luna, E.; San Miguel, J.C.; Ortego, D.; Martínez, J.M. Abandoned object detection in video-surveillance: Survey and comparison. *Sensors* **2018**, *18*, 4290. [CrossRef]
33. Zhou, R.; Zhong, D.; Han, J. Fingerprint identification using SIFT-based minutia descriptors and improved all descriptor-pair matching. *Sensors* **2013**, *13*, 3142–3156. [CrossRef]
34. Jiang, N.; Song, W.; Wang, H.; Guo, G.; Liu, Y. Differentiation between organic and non-organic apples using diffraction grating and image processing—A cost-effective approach. *Sensors* **2018**, *18*, 1667. [CrossRef]
35. Wang, Z.; Koirala, A.; Walsh, K.; Anderson, N.; Verma, B. In field fruit sizing using a smart phone application. *Sensors* **2018**, *18*, 3331. [CrossRef] [PubMed]
36. Akin, C.; Kirci, M.; Gunes, E.O.; Cakir, Y. Detection of the pomegranate fruits on tree using image processing. In Proceedings of the 2012 First International Conference on Agro-Geoinformatics (Agro-Geoinformatics), Shanghai, China, 2–4 August 2012; IEEE: New York, NY, USA; pp. 1–4. [CrossRef]
37. Sahu, D.; Potdar, R.M. Defect identification and maturity detection of mango fruits using image analysis. *Am. J. Artif. Intell.* **2017**, *1*, 5–14. [CrossRef]
38. Blazek, M.; Janowski, A.; Kazmierczak, M.; Przyborski, M.; Szulwic, J. Web-cam as a means of information about emotional attempt of students in the process of distant learning. In Proceedings of the 7th International Conference of Education, Research and Innovation, Seville, Spain, 17–19 November 2014; pp. 3787–3796. [CrossRef]
39. Nagrodzka-Godycka, K.; Szulwic, J.; Ziolkowski, P. State-of-the-art framework for high-speed camera and photogrammetric use in geometry evaluation of prestressed concrete failure process. In Proceedings of the 16th International Multidisciplinary Scientific Geoconference (SGEM 2016), Albena, Bulgaria, 30 June–6 July 2016.
40. Ziolkowski, P.; Niedostatkiewicz, M. Machine learning techniques in concrete mix design. *Materials* **2019**, *12*, 1256. [CrossRef]
41. Bellocchio, E.; Ciarfuglia, T.A.; Costante, G.; Valigi, P. Weakly supervised fruit counting for yield estimation using spatial consistency. *IEEE Robot. Autom. Lett.* **2019**, *4*, 2348–2355. [CrossRef]
42. Xu, Y.; Yu, G.; Wang, Y.; Wu, X.; Ma, Y. A hybrid vehicle detection method based on Viola-Jones and HOG+ SVM from UAV images. *Sensors* **2016**, *16*, 1325. [CrossRef] [PubMed]
43. Dorj, U.O.; Lee, M.; Yun, S.S. An yield estimation in citrus orchards via fruit detection and counting using image processing. *Comput. Electron. Agric.* **2017**, *140*, 103–112. [CrossRef]
44. Al-Saddik, H.; Laybros, A.; Billiot, B.; Cointault, F. Using image texture and spectral reflectance analysis to detect yellowness and esca in grapevines at leaf-level. *Remote Sens.* **2018**, *10*, 618. [CrossRef]
45. Yamamoto, K.; Guo, W.; Yoshioka, Y.; Ninomiya, S. On plant detection of intact tomato fruits using image analysis and machine learning methods. *Sensors* **2014**, *14*, 12191–12206. [CrossRef]
46. Argialas, D.P.; Mavrantza, O.D. Comparison of Edge Detection and Hough Transform Techniques for the Extraction of Geologic features. International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences, 2004, Volume 34 (Part XXX). Available online: https://www.cartesia.org/geodoc/isprs2004/comm3/papers/376.pdf (accessed on 17 December 2020).
47. Janowski, A. The circle object detection with the use of M split estimation. *E3S Web Conf.* **2018**, *26*, 00014. [CrossRef]
48. Janowski, A.; Bobkowska, K.; Szulwic, J. 3D modelling of cylindrical-shaped objects from lidar data—An assessment based on theoretical modelling and experimental data. *Metrol. Meas. Syst.* **2018**, *25*, 47–56. [CrossRef]
49. Aashish, K.; Vijayalakshmi, A. Comparison of Viola-Jones and Kanade-Lucas-Tomasi face detection algorithms. *Orient. J. Comput. Sci. Technol.* **2017**. [CrossRef]
50. Viola, P.; Jones, M.J. Robust real-time face detection. *Int. J. Comput. Vis.* **2004**, *57*, 137–154. [CrossRef]
51. Jarolmasjed, S.; Khot, L.R.; Sankaran, S. Hyperspectral imaging and spectrometry-derived spectral features for bitter pit detection in storage apples. *Sensors* **2018**, *18*, 1561. [CrossRef]
52. Redmon, J.; Divvala, S.; Grishik, R.; Farhadi, A. You Only Look Once: Unified, Real-Time Object Detection. *arXiv* **2018**, arXiv:1506.02640. Available online: https://pjreddie.com/media/files/papers/yolo.pdf (accessed on 17 December 2020).
53. Wei, X.; Jia, K.; Lan, J.; Li, Y.; Zeng, Y.; Wang, C. Automatic method of fruit object extraction under complex agricultural background for vision system of fruit picking robot. *Optik* **2014**, *125*, 5684–5689. [CrossRef]
54. Linker, R.; Cohen, O.; Naor, A. Determination of the number of green apples in RGB images recorded in orchards. *Comput. Electron. Agric.* **2012**, *81*, 45–57. [CrossRef]
55. Kuznetsova, A.; Maleva, T.; Soloviev, V. Using YOLOv3 algorithm with pre-and post-processing for apple detection in fruit-harvesting robot. *Agronomy* **2020**, *10*, 1016. [CrossRef]
56. Payne, A.; Walsh, K.; Subedi, P.; Jarvis, D. Estimating mango crop yield using image analysis using fruit at 'stone hardening'stage and night time imaging. *Comput. Electron. Agric.* **2014**, *100*, 160–167. [CrossRef]
57. Zhao, Y.; Gong, L.; Huang, Y.; Liu, C. Robust tomato recognition for robotic harvesting using feature images fusion. *Sensors* **2016**, *16*, 173. [CrossRef] [PubMed]
58. Qiang, L.; Jianrong, C.; Bin, L.; Lie, D.; Yajing, Z. Identification of fruit and branch in natural scenes for citrus harvesting robot using machine vision and support vector machine. *Int. J. Agric. Biol. Eng.* **2014**, *7*, 115–121.
59. Kelman, E.E.; Linker, R. Vision-based localisation of mature apples in tree images using convexity. *Biosyst. Eng.* **2014**, *118*, 174–185. [CrossRef]
60. Kurtulmus, F.; Lee, W.S.; Vardar, A. Immature peach detection in colour images acquired in natural illumination conditions using statistical classifiers and neural network. *Precis. Agric.* **2014**, *15*, 57–79. [CrossRef]

61. Häni, N.; Roy, P.; Isler, V. A comparative study of fruit detection and counting methods for yield mapping in apple orchards. *J. Field Robot.* **2020**, *37*, 263–282. [CrossRef]
62. Database of Images Used to This Article—Multicolour. Available online: https://mostwiedzy.pl/en/open-research-data/images-of-apples-for-the-use-of-the-viola-jones-method-data-set-no-1-multicolor,614102311611642-0 (accessed on 17 December 2020).
63. Database of Images Used to This Article—Greyscale. Available online: https://mostwiedzy.pl/en/open-research-data/images-of-apples-for-the-use-of-the-viola-jones-method-data-set-no-2-grey-scale,10310105381038599-0 (accessed on 17 December 2020).

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Interpreting Environmental Impacts Resulting from Fruit Cultivation in a Business Innovation Perspective

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Abstract: Sustainability of food production is a major concern today. This study assessed the environmental impact of fruit production and discussed business implications for sustainability. Data were collected from three agricultural enterprises growing six species of fruit, extending over a total of 34 hectares, and producing roughly one thousand tons of fruit per year. The results of the life-cycle assessment (LCA) showed that several production activities heavily impact the environment: in descending order of absolute terms, fruit refrigeration, agronomic operations, irrigation, and fertilizer use were recognized as the most impacting. Other activities, including agrochemical applications, planting, and plastic use for harvesting and packaging, showed overall lower impacts. The high environmental impact associated with most of the production activities emphasizes the need to make the primary food production cleaner, more resource-efficient, and less energy-intensive. Affordable incremental innovations able to reshape the way business is conducted in the context of primary food production are proposed, mainly relying on process rationalization and digital switchover. The analysis of the business path toward increased sustainability involves strategic issues, ranging from the reshaping of production processes to relationships with consumers, affecting value proposition, creation, and capture.

Keywords: business models; innovation; resource efficiency; sustainability; environmental assessment; fruit production; cleaner production; life cycle assessment (LCA); carbon footprint

1. Introduction

The agriculture and food sector is of primary importance to human health and well-being. On the other hand, it generates several adverse environmental effects that harm natural resources, constituting the input of food production. These effects are the by-products of food production, which are largely subjected to inefficiencies in processes, energy, and input use. The importance of integrating sustainability into the production process has caught the attention of practitioners and researchers, in several fields, over the last few decades [1]. The need for sustainable practices has been recognized in the face of challenges, such as achieving cost-effective production and reducing energy consumption [2].

This kind of issue was recently addressed in the context of a sustainable business model innovation, in which scholars, practitioners, and, generally, food system stakeholders, started to pay attention in the last decade [3]. This concept concerns the way business is performed by generating competitive advantages while contributing positively to enterprises, the environment, and society [4]. Within such a framework, it is widely recognized that positive business changes can be stimulated by adopting various tools for sustainable business thinking. These tools include life-cycle assessment (LCA), as defined by the International Organization for Standardization (ISO) [5,6], eco-design [7], eco-ideation [8], the business model canvas, as readapted by Bocken et al. [9], and stakeholder analysis [10]. The adoption

of these practices, especially in large manufacturing firms, has so far encompassed the elimination of pollution from processes, the minimization of waste material, waste treatment, the reduction of energy consumption, as well as the attraction of customers, and cost savings [11].

One of the peculiarities distinguishing primary food production from industrial processing is the limited, but significant, scope related to sustainability innovations, as value proposition is essentially based on natural products, with limited to no presence of industrial manufacturing. For this reason, tools, such as LCA, designed to quantify the environmental burden associated to material and energy consumption of a product across its entire life, and can provide exhaustive information to assess current business environmental footprints, as well as production inefficiencies [5]. Besides giving an overview of the existing production processes, LCA can be used to draw out possible solutions to mitigate production impacts, offering a holistic perspective to conduct business model analysis for innovation and pave the way for environmental and economic improvements. This use is especially relevant for small and medium enterprises (SMEs), which generally lag behind large companies in the implementation of sustainability management tools [12]. Facing this problem, we formulated the following research question: which solutions may be implemented at the strategic level to mitigate the environmental impact of production activities and achieve a higher level of sustainability?

In this work, we applied an LCA to fruit production to give a measure of the environmental impact characterizing fruit production activities, while identifying inefficiencies, and finding room for possible business model innovations. We selected a case study in the Emilia-Romagna region (northeast Italy) represented by three large agricultural enterprises growing six species of fruit, extending over 34 hectares, and producing a total of roughly one thousand tons of fruit per year. The study is relevant because, economically, fruit growing is one of the most important agricultural activities in Emilia-Romagna, a region that is considered Italy's main orchard. Fruit growing is also an intensive crop in the area, and it is socially important because of its impact on job opportunities and cultural elements. Its reach goes beyond the basic agricultural activity: once harvested, fruit produce is directly available for consumption or can be further processed to obtain products, such as juices and fruit preserves, an industry that is deeply rooted in the Emilia-Romagna region. The paper is organized as follows. In Section 2, we describe the case study and apply the LCA methodology, reporting the goal and scope definition, and the inventory analysis; LCA results are reported in Section 3. In Section 4, we propose possible technological and process improvements, also discussing business strategy implications. In Section 5, concluding remarks and indications for future research are outlined.

2. Materials and Methods

2.1. Description of the Case Study

Data were collected in 2019 from three agricultural enterprises in which the following fruit species are cultivated: apricot, nectarine, pear, plum, apple, and kiwi. The orchards are located between Forlì and Faenza, in the Emilia-Romagna region (northeast Italy). Telephone interviews and on-site meetings with farm technicians during fruit cultivation were administered to collect qualitative information and quantitative data. Table 1 summarizes the main characteristics of the orchards. The LCA was performed to assess the environmental impact associated with the fruit production originating from these orchards, measured according to the dedicated technical standard ISO 14040:2006 (principles and framework) [5] and 14044:2006 (requirements and guidelines) [6], following the specified phases: goal and scope definition, inventory analysis (life cycle inventory (LCI) phase), impact assessment (life cycle impact assessment (LCIA) phase), and result interpretation.

2.2. Goal and Scope Definition

LCA was performed to measure the environmental impact characterizing the fruit produced in the three agricultural enterprises, with the aim of identifying process inefficiencies and finding room for possible ad-hoc innovations able to make production more efficient, less energy-intensive,

and less dependent from non-renewable energy sources. In addition to this, LCA results will be used for learning and educational purposes. The identification of inefficiencies throughout the production process can make enterprise decision-makers aware of existing environmental burdens, with the LCA itself representing a step towards the comprehension of sustainability management tools by technicians. LCA results can also serve as decision support for individuals, both as citizens or consumers, potentially directly entering in eco-labels or other consumer information from producers (e.g., printed on the packaging) or indirectly through reporting research findings [13]. In this respect, LCA can effectively support consumer decisions in choosing products with a lower environmental impact.

Table 1. Main characteristics of the orchards.

	Apricot	**Nectarine**	**Plum**	**Pear**	**Apple**	**Kiwi**
Variety	Faralia	Romagna Red	September Yummy	Abate Fetel	Rosy Glow	Hayward
Training system	Vase	Slender spindle	Slender spindle	Slender spindle	Solax	Pergola
Growing surface (ha)	0.35	1.20	7.70	1.16	1.04	22.95
Layout (m)	4.0 × 1.5	3.5 × 0.6	4.2 × 1.5	4.5 × 2.0	3.5 × 2.0	5.0 × 2.0
Plant density (trees/ha)	1666	4762	1587	1111	1428	1000
Year of establishment	2011	2012	2013	2010	2010	2017
Orchard life (years)	15	15	15	15	15	20
Yearly yield (t/ha)	21.6	27.5	59.9	22.9	58.6	16.1

The approach adopted for the estimation was cradle-to-farm gate, evaluating the impact of the fruit produced, i.e., immediately available for direct sale or distribution. All of the input used for production, particularly mass and energy flows, have been tracked, considering the following four fruit life cycle phases: fruit cultivation, use of agricultural and energy inputs in agronomic activities, fruit harvest, and fruit storage in cold rooms. Data were collected during 2019 until last harvests were completed, concerning the season 2018–2019.

2.3. Functional Unit and System Boundaries

The functional unit (FU) relates to all inputs and outputs in the LCI and consequently, the LCIA profile, defining what is being studied [6]. In this study, 1 kg of each fruit, packaged in a 14 g polyethylene package, was chosen as FU, considering the yields in the season 2018–2019, as such, unit lends itself to possible downstream consumer behavior analysis. Therefore, data provided by enterprise technicians, expressed as unit per hectare of the orchard, were traced back to a yield expression (kg per hectare) to make it consistent with FU. The system boundaries were defined, as shown in Figure 1. In addition to the processes outlined in Figure 1, planting of fruit trees was included within the system boundaries and weighted, considering a 15-year (20 years for kiwi) orchard lifetime. The orchard end-of-life was modeled in the same way, considering the use of machinery for tree pruning. The mass of tree by-products was assumed equal to 50 kg for apricot and plum, 70 kg for nectarine, 20 kg for pear, 40 kg for kiwi, and 13 for apple, which is consistent with the tree mass assessment reported in [14].

2.4. Inventory Analysis

The inventory analysis defines in detail data describing the production process and the flows of materials and energy. In LCA, it is useful to distinguish between two types of data: foreground data and background data. Foreground data refer in large part to primary data collected at the orchard level, based upon information provided by enterprise technicians. The LCA software SimaPro v.9.0 accounted for the definition of background data and changes to the ecosphere, as a result of the production activities performed within the system boundary (Figure 1).

2.4.1. Foreground Data

Foreground data are reported in Table 2. Information regarding fertilization and application of agrochemicals were made available in orchard registers in which all cultivation measures along the

production season were noted. Water use was registered both as cultivation measure (fertigation) and as irrigation, monitored via dripper meters within drip irrigation systems at each sixth plant. Boxes with a tare weight of 2 kg (gross approximately 16 kg) and bins with a tare weight of 33 kg (gross approximately 230 kg) were used to collect fruit during the harvests. Both items are made of high-density polyethylene, with an estimated useful life of 15 years.

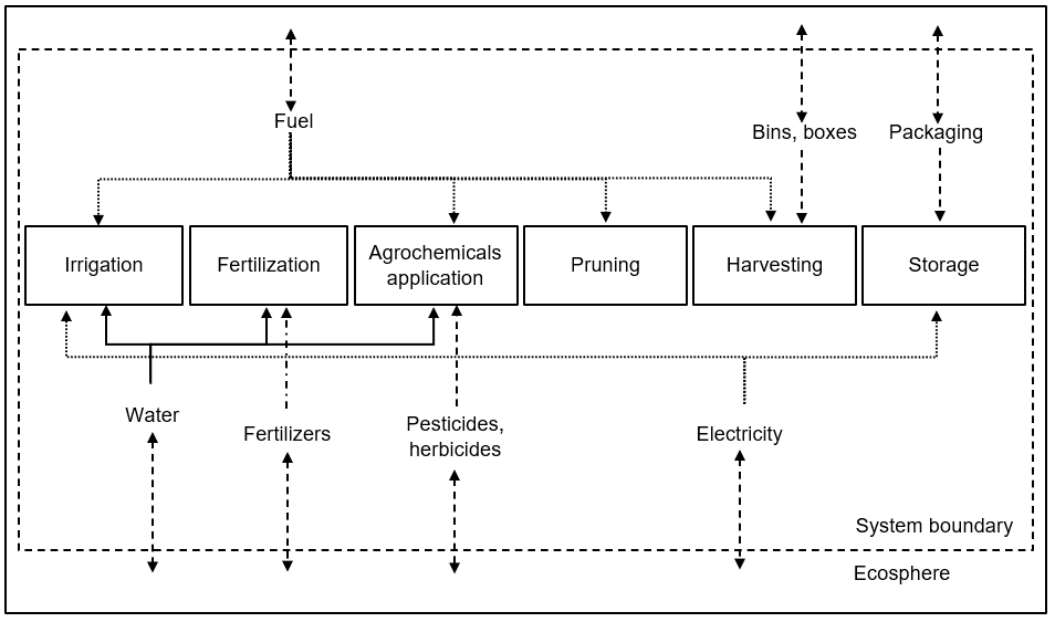

Figure 1. Main processes, input, and elementary flows included in the foreground system.

Enterprise technicians provided data regarding average fuel consumptions for agricultural machines, and the number of operations occurred in the orchards during the season. This information is summarized in Table 3. Pruning was carried out in a closed-loop cycle with biomass reintegrated, with positive contributions to soil organic matter improvement. Yearly pruning residues were quantified in the measure of 2 tons per hectare for every orchard and modeled as organic input to soil.

Electrical energy supplied from the national grid was used for irrigation and refrigeration; the main obstacle in reporting information consisted of the fact that there was no explicit data on energy consumption for the different production stages. For this reason, secondary data were estimated based on the following information provided by technicians: electrical energy for irrigation was estimated considering the water pump available of nominal power equal to 10.5 kW and average water mass flow equal to 640 liters per minute. Once harvested, fruits were stored in cold rooms. Since it was not possible to split energy data required for refrigeration by checking invoices, it was assumed that a weekly demand of 0.1 kWh per kilogram of fruit stored from a similar context [15], over the following average refrigeration time set out by farm technicians: 12.5 days for apricots, 4 for nectarines, 35 for plums, 90 for pears and kiwifruit, and 65 for apples. The refrigeration plants were ammonia (NH_3) based.

2.4.2. Foreground Data

Background data account for the production of input, energy, and waste management. They were taken from the international databases Ecoinvent v.3.6 and Agri-footprint v4.0, which include inventories of crop cultivation, data on transport, and agricultural input production for life cycle assessments. Background data collection was enabled within SimaPro v.9.1.

Table 2. Primary data collected.

	Unit	Apricot	Nectarine	Plum	Pear	Apple	Kiwi
- Fertilizers							
Growth regulators	(kg ha^{-1})	7.01	3.65	11.35	28.80	2.26	8.20
N-based	(kg ha^{-1})	331.19	-	137.40	-	148.97	1.50
N-P-K-based	(kg ha^{-1})	534.49	0.55	119.48	-	-	201.75
N-K-based	(kg ha^{-1})	14.83	-	-	17.10	-	7.02
Mg-N-based	(kg ha^{-1})	19.65	-	107.53	-	-	-
Microelements	(kg ha^{-1})	0.57	-	28.97	46.96	8.80	54.86
Compost	(t ha^{-1})	20	20	20	20	20	20
- Agrochemicals							
Insecticides	(kg ha^{-1})	19.35	14.54	65.6	60.44	13.27	21.54
Fungicides	(kg ha^{-1})	16.01	23.12	59.6	57.87	23.23	6.00
Herbicides	(kg ha^{-1})	5.03	2.47	2.17	11.80	4.40	2.66
Other agrochemicals	(kg ha^{-1})	-	29.87	-	-	14.00	-
- Water use							
Irrigation	(m^3 ha^{-1})	1633.33	2666.67	3266.67	3266.67	3984.00	4166.67
Treatments	(m^3 ha^{-1})	9.17	18.54	63.60	78.39	168.16	97.00
- Collection							
Boxes	(ha^{-1})	1413	-	3744	15	-	
Bins	(ha^{-1})	-	120	-	99	303	89

Table 3. Use of agricultural machines in orchards.

	Chopping	Fertilization	Pruning [1]	Agrochemical Application	Harvest
- Fuel consumption (L ha^{-1})					
Tractor	6	3	3	8	3
+ harvester wagon	-	-	2.5	-	2.5
+ bin wagon	-	-	-	-	2.5
- No. orchard operations ($year^{-1}$)					
Apricot	12	10	1	10	1
Nectarine	12	3	3	16	1
Plum	7	13	3	21	1
Pear	7	22	1	30	1
Apple	10	9	3	35	1
Kiwi	10	14	4	10	1

[1] 1/15 operation was considered to model plant eradication at the end of life (1/20 for kiwi).

3. Results

The elementary flows emerged within the inventory analysis were assigned in SimaPro v.9.1 to the following relevant impact categories: "Human health", "Ecosystem quality", "Climate change" and "Resources" according to the substances" ability to contribute to different environmental impacts. Particularly, the way environmental impact affects human health had been quantified with the metric Disability-Adjusted Life Years (DALY), expressed as the number of years lost due to illness, disability, or premature death [16]. "Ecosystem quality" encompasses multiple independent impact categories such as eutrophication, acidification, ecotoxicity, land use and water use, and it is measured in Potential Damage Fraction (PDF), defined as the fraction of species that have a high probability of not surviving in the affected area due to unfavorable living conditions [17]. "Climate change" describes the potential impact of different greenhouse gaseous emissions and it is measured in terms of carbon footprint (kg CO_2 eq.), while "Resources" models the primary energy needed (MJ) to made inputs available at

the technosphere. All these categories had been modeled in SimaPro v.9.1 with the life cycle impact methodology "IMPACT 2002+" [18].

3.1. Impact Assessment

The impact assessment was reported in terms of total environmental damage for humans according to the most known impact categories, as reported within Section 3.1.1. Then, it was allocated to each production activity considered (Section 3.1.2). The last Section 3.1.3, summarizes the impact for each production activity regarding the category "Climate Change".

3.1.1. Total Damage

The environmental impact of the four impact categories was normalized to depict a significant overview of the LCA results. Normalization is helpful to understand the relative weight of each category, making all units of measure compatible. As a result, total damage was split among impact categories as follows: 34.7% human health, 26.8% resources, 26.6% climate change, and 12.0% ecosystem quality (Figure 2). Human health was found to be the most impacting category for each type of fruit. The impact for resources was slightly higher than climate change for nectarines, pear, apple, and kiwi, but was lower, even in absolute terms, for apricots and plums. Ecosystem quality was the least impacting category. This result can be reasonably associated with the fact that fruit cultivation remains mostly a natural process characterized by relatively low industrialization. Overall, winter fruit is characterized by a higher impact compared to autumn and summer fruit, mainly because of the longer conservation time.

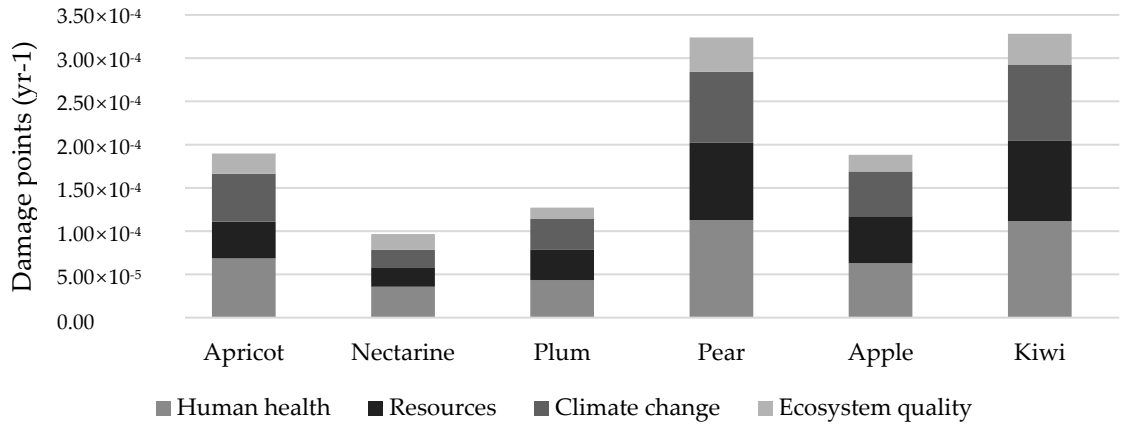

Figure 2. Normalized impact categories of fruit production.

The impact categories considered in this LCA were further divided into relevant subcategories (Figure 3). Other subcategories generally considered in environmental assessments, such as carcinogens, ozone layer depletion, respiratory organics, aquatic ecotoxicity, terrestrial acidification, land occupation and mineral extraction, resulted as not relevant.

3.1.2. Impact of the Production Processes

The processes considered in this LCA for the various fruits cultivated are characterized by different environmental impacts (Table 4). Planting activity is below 5%, with the highest value for nectarines. The impact of fertilizers is particularly high for apricots, while relevant for plums and kiwifruit; in this regard, the impact of organic fertilizer (compost) is negligible. Irrigation has a noticeable impact, with varying contribution between water and pumping energy, although energy impact is generally lower than water. Regarding agrochemicals, the overall impact is low, except for nectarines (6.41%) and apples (5.37%); insecticides and fungicides are likely to affect the environment jointly much more relevantly than herbicides, whose contribution is generally negligible. The impacts

associated with agronomic operations, due to consumption of fossil fuel, accounted for the highest contributions, between 1/5 and 2/5 of the total damage. Contribution from plastic use (harvesting boxed and packaging) was considered negligible, as roughly below 3%. It is evident that, for all fruit stored for a long time in cold rooms, refrigeration is the most impacting activity, with a relative impact up to half of the total damage for pears (53.55%).

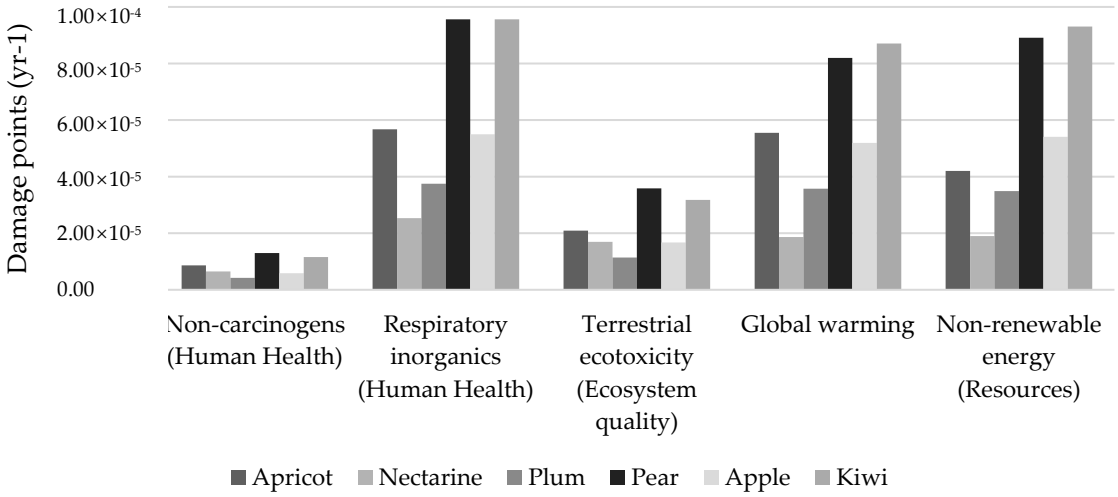

Figure 3. Normalized impact subcategories of fruit production.

Figure 4 shows the most impacting activities, thereby making it possible to identify the different contribution by each fruit. The highest impact for winter fruit (pears, apples, and kiwifruit) was due to refrigeration, while fertilizers for apricots and agronomic operations for nectarines, which is the least impacting fruit.

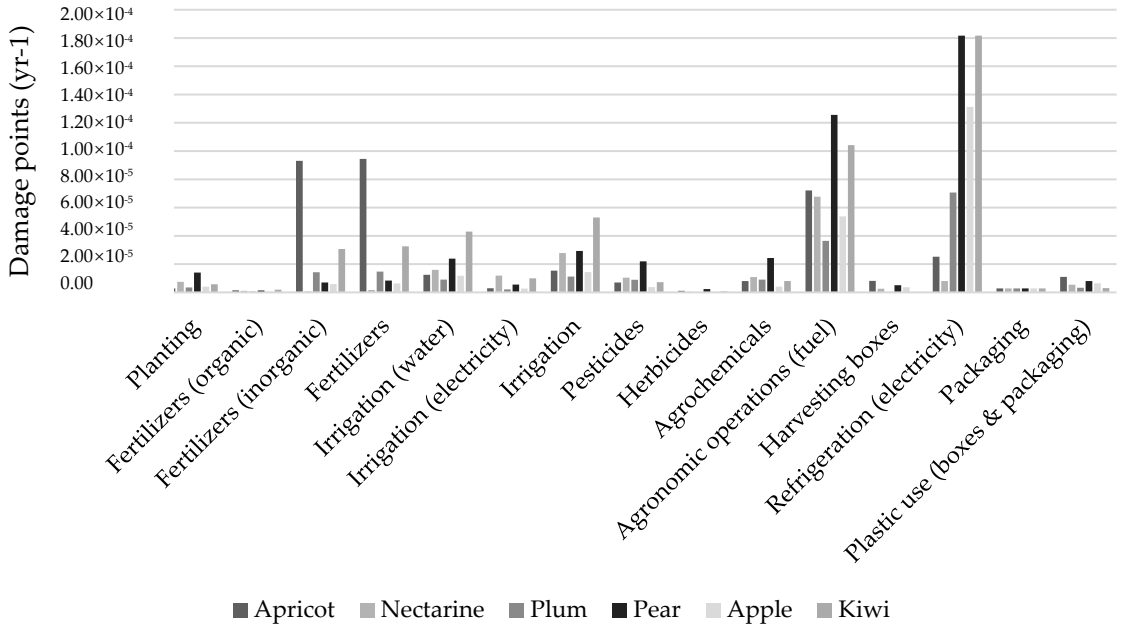

Figure 4. Most impacting processes for fruit production.

Table 4. Normalized impact of fruit production processes (yr^{-1}).

	Apricot	Nectarine	Plum	Pear	Apple	Kiwi
- Planting	**0.82%**	**4.42%**	**1.87%**	**3.09%**	**1.66%**	**1.19%**
Fertilizers (organic)	0.40%	0.65%	0.27%	0.29%	0.21%	0.39%
Fertilizers (inorganic)	26.83%	0.36%	7.73%	1.55%	2.38%	6.38%
- Fertilizers	**27.23%**	**1.01%**	**8.01%**	**1.84%**	**2.59%**	**6.76%**
Irrigation (water)	3.58%	9.43%	4.95%	5.26%	4.78%	8.93%
Irrigation (electricity)	0.84%	6.97%	1.15%	1.21%	1.08%	2.06%
- Irrigation	**4.43%**	**16.40%**	**6.10%**	**6.48%**	**5.86%**	**10.99%**
Fungicides, insecticides, herbicides	2.00%	6.16%	4.84%	4.84%	1.51%	1.50%
	0.31%	0.25%	0.09%	0.53%	0.14%	0.16%
- Agrochemicals	**2.32%**	**6.41%**	**4.93%**	**5.37%**	**1.66%**	**1.66%**
- Agronomic operations (fuel)	**20.80%**	**39.97%**	**19.82%**	**27.71%**	**21.95%**	**21.64%**
- Harvesting boxes	**2.33%**	**1.53%**	**0.19%**	**1.13%**	**1.46%**	**0.04%**
- Refrigeration (electricity)	**7.27%**	**4.76%**	**38.48%**	**40.09%**	**53.55%**	**37.72%**
- Packaging	**0.82%**	**1.69%**	**1.56%**	**0.63%**	**1.17%**	**0.59%**

3.1.3. Carbon Footprint

As highlighted within Section 2.3, the type of functional unit chosen (1 kg of fruit) is suitable for consumer value interpretations, potentially supporting consumer decisions in choosing products with lower environmental impact. Table 5 shows the carbon footprint associated with fruits considered.

Table 5. Carbon footprint ($gCO_{2eq.}$)—functional unit (FU): 1 kg of fruit.

	Apricot	Nectarine	Plum	Pear	Apple	Kiwi
Planting	3	7	3	13	4	6
Fertilizers	322	5	50	29	21	110
Irrigation	27	57	19	51	25	91
Agrochemicals	17	23	19	52	9	17
Agronomic operations	62	59	31	109	46	90
Plastic use	46	34	29	40	36	29
Refrigeration	72	23	201	516	373	516
Remaining processes	1	0	1	3	0	4
Total	550	208	354	811	514	862

4. Discussion

In this section, we discuss the results in terms of possible innovations regarding the production process (Section 4.1). Thus, we analyze the multiple ways in which value can be addressed under the lens of business model innovation (Section 4.2).

4.1. Incremental Innovation at the Process Level

4.1.1. Mitigating the Impacts Associated with Refrigeration

The high impact accounting for fruit refrigeration emphasized the need to make the refrigeration process more efficient, less energy-intensive, and less dependent on non-renewable energy sources. Fruit depos in the case study were supplied by ammonia-based refrigeration plants, which constitute the majority of cooling units in developed countries. Ammonia is a common choice of refrigerant in large systems thanks to its thermo-dynamical properties that make it 3–10% more efficient than other refrigerants [19], and plants based on ammonia are some of the most diffused [20]. This natural refrigerant is toxic and flammable in large concentrations. However, it causes no ozone

depletion or global warming per se, unlike other refrigerants, such as chlorofluorocarbons (CFCs) and hydrofluorocarbons (HFCs).

The issue of refrigeration can be handled within an agricultural enterprise in different ways. One of the challenges in the refrigeration process is to use the already installed cooling capacity more optimally. In the last years, significant efforts in making refrigeration units more efficient are acknowledged. As an example, it was demonstrated that using closed coupled components in a compact refrigeration package, and electronic refrigerant injection control technology, facility ammonia charge could be reduced by more than 98%, with a related 7% reduction in energy and 3% reduction in water usage [19]. Compressors' optimal control was important to reduce refrigeration energy demand. Optimizing compressors' operation has led to minimizing power consumption while preserving the total refrigeration load requirement. A yearly monetary saving between €30,000 and €50,000 euros for eight refrigerating units, ranging in cooling capacity size from 180 kW to 795 kW, was estimated in [21]. Possible strategies for limiting the supply of refrigeration energy from fossil fuels is utilizing a combination of renewable energy sources, even limited to a portion of the refrigerating unit. Thermally driven absorption chillers within ammonia-based refrigeration units can be powered by low-grade heat or renewable energy resources. Such a practice can effectively represent a possibility to recover and re-use amounts of waste heat deriving from other industrial processes, improving in turn process efficiencies, and mitigating the associated environmental impacts. Significant industrial opportunities for the utilization of lower temperature heat from various industrial processes and energy sources are today available [22].

Another opportunity concerning sustainable energy concerns the integration of sustainable sources like solar photovoltaic (PV). PV systems are attractive because of their simplicity and low maintenance requirements [23]. They can be integrated directly into the unit they are providing power for, and can be mounted on roofs, without taking away agricultural land.

All the aforementioned strategies implicate a re-design of the refrigeration process, taking action on plants or plant portions, requiring business planning and investments over a low- to mid-term period. Other aspects involve a socio-cultural perspective, dealing with consumer preferences and choices. In the food sector, cold rooms are indispensable tools to ensure consumers with safe and genuine products: temperature is an essential requirement for the conservation of fruit (but also vegetables) and the preservation of nutritional properties. However, especially in case of extended supply chains, this mechanism seems to be heavily overused since producers are forced to anticipate the harvest, making fruit ripen in cold rooms. Furthermore, on the demand side, consumers are rather accustomed to the almost constant availability of the most widespread fruit varieties during the year. Mature consumers could perceive and evaluate seasonal fruit as more valuable of fruit ripened within cold rooms for a long time, being aware of a reduced environmental impact characterizing short food supply chain, in which production stage is more temporally and spatially close to consumption stage. A change in the demand side, even driven by virtuous food producers, would be desirable. In this regard, promising signs have been acknowledged in [24], in which it was observed that adopters of sustainability practices generally rate the benefits (measured as satisfaction level) higher than the investments in labor and financial resources.

4.1.2. Mitigating the Impacts Associated with Agricultural Operations

The high environmental impact of farming operations, from planting to harvesting poses a call for rationalizing farm operations. Enhanced connectivity and advanced data management can support substantial changes to increase efficiency. Since fuel consumption is proportional to in-field travelled distance, the use of technologies like auto-steering and section control can help in reducing unnecessary input applications, including fertilizers and agrochemicals, and thus fuel. Overlap reduction for auto-guidance systems may fall within 3–7%, with a proportional amount of agricultural inputs saved to the un-overlapped area [25]. In general, precision agriculture also encompasses a set of other novel technologies (e.g., remote sensing, variable-rate technologies) able to make agriculture more efficient,

thus mitigating the negative impacts of farming [26]. Information and Communication Technology applied to agriculture has enabled rapid, precise, and localized collection and processing of large pieces of data, generating suitable information for the daily operations of agricultural machinery. Auto-steering and section control on fertilizer spreaders appear to be viable solutions for many large-scale farms, and relevant economic benefits can arise from combining the use of the several Precision Agriculture technologies [27]. Process rationalization and digital switchover open numerous opportunities because they allow for process flexibility and manage complexity through data science. Digitization has become a fundamental path also for agricultural enterprises facing the growing complexity and uncertainty in their context. However, they represent changes in work culture rather than just technology, as they affect every worker, from enterprise managers to operators.

A new frontier in farm mobility is represented by agricultural machines powered with electric or hybrid motors. A wide number of agricultural machine manufactures are putting efforts on e-mobility development, and on the normation side, a new ISO technical standard is currently in progress and could be ready within the next three years. The adoption of electrical agricultural machinery will contribute to switching the environmental burden from the agricultural fuel supply chain to electricity generation and battery management. In this context, the possible advantages are twofold: on the one hand, energy supply can be delegated to renewable sources, and on the other hand, energy efficiency will be likely to increase, as the most diffused steel diesel engines show low overall energy efficiency (about 20%) [28].

4.1.3. Water Use: Room for Reduction?

Despite impacts due to irrigation were not associated with relevant environmental damage, the considerable amounts of irrigation water used is a relevant issue. The unit cost of water is low, and this may lead to the opportunistic behavior of irrigating much more than necessary. A possibility to come up with effective actions is to adopt comprehensive, integrated approaches for environmental management of water, improving water control capability and enhancing water supply predictability. Moreover, it would be appropriate to increase transparency and accountability in the context of agricultural enterprise, with water pricing based on measured deliveries [29].

4.1.4. Reducing Inorganic Fertilizers Use

The overall impact of fertilizers in orchards can vary from season to season. For the case study considered in this work, a particularly high impact was registered for the apricot orchard, while it is lower but still substantial for plums and kiwifruit. On the contrary, the impact of organic fertilizer (compost) was negligible for all orchards considered.

Farmers determine the amounts of nitrogen, phosphorous, and potassium fertilizers to be applied over the fields based on both their experience and the amounts applied the previous season, with maximum threshold levels based on worst environmental and climate situations delineated by regional agricultural policy administration. Since fertilizers are relatively cheap, often farmers prefer applying much more than required by fruit trees. Several negative effects on the environment are the consequence: to name but the most acknowledged, air pollution due to nitrogen emissions, water and soil degradation because of nitrogen leaching, eutrophication [26,30]. Precision agriculture can mitigate those negative effects through site-specific mapping and variable fertilizer application rates within plots. Satellite imaging or monitoring Unmanned Aerial Vehicles (UAVs) mounting N-sensors or multi-spectral cameras can sense cultivated fields and crops, and the deriving information can be processed to obtain fertilizer prescription maps, with the orchard divided into several areas characterized by a precise amount of fertilizer to be applied (kg/ha). Monitoring technologies are relatively cheap compared to traditional agricultural machines, and the information they make available can help reduce the impact of agronomic operations.

Other eco-sustainable solutions refer to conservation agriculture. The reduction of tillage and other farming operations can increase soil organic matter, while reducing costs, and can be applied to fruit growing, too.

4.2. Business Model Innovation

The use of the LCA framework has allowed highlighting several opportunities to make production processes cleaner and to reduce input use. Moreover, these opportunities can reflect in the way the agricultural enterprise addresses its own business canvas. All incremental innovations above described contributing to enhancing the value proposition, creation, and capture. Concerning value proposition, the proposed innovations for making production cleaner and more efficient can translate in a superior value that is offered to customers, with positive impacts for the society and the environment that can benefit from a reduced energy and input demand. In this way, the enterprise has the chance to strengthen consumer relationship, and address consumer decisions in choosing products characterized by a mitigated social and environmental burden.

Considering the definition provided in [31], meeting consumer expectations while reducing the environmental impact of the organization can be viewed as green marketing [32,33]. This approach fits particularly well with the context of fruit production, especially if the use of resources is reduced and if the enterprises adopt plans to reduce waste and pollution. Moreover, market communication of socio-economical aspects arising from LCA is viewed as part of sustainable marketing programs [11] and is associated with competitive advantage [34]. The benefits of green marketing can even go beyond the single company and embrace the whole value chain thanks to the reverse information flow and the importance of added-value services provided by intermediaries [35].

Value creation is concerned with making production activities and resource use more efficient. The call for agricultural inputs characterized by reduced impacts can, in turn, let suppliers and other partners of the supply chain be more aligned with the proposed innovative business model. Improvements in technical sustainability standards encourage quantification by the internal enterprise accounting for the impacts on ecosystems [36,37]. Apparently, this sounds like a virtuous cycle: sustainability assessments promote the internalization of ecosystems costs by embracing the growing focus on quantification of product sustainability, thereby influencing the sustainability standards of tomorrow. Value capture affects the enterprise cost structure and revenues. Economic benefits can result from monetary savings due to reduced agricultural inputs and by higher prices that customers would be willing to pay for more environmentally friendly products. These benefits must be counter-balanced by investments to improve technical efficiency. The economic impact of innovation uptake is a current research topic, with many promising findings highlighting the financial convenience, both on the cost reduction [27,38], and increased revenues [39] sides. Regarding revenues, the success of more environmental-friendly products depends on the adopted marketing policy able to satisfy and meet consumers' demand more effectively than competitors [39,40]. On the other hand, enterprises are required to deliver clear and pertinent product information through labeling and packaging; thus, increasing the perceived value and consumers' interest in buying products characterized by a mitigated environmental burden [11].

The above discussion shows that incremental innovations enhancing the environmental performance can benefit not just the enterprise that can make production more efficient and adopt green marketing strategies opening the possibility to increase in sales, but also the consumers themselves and the surrounding environment.

5. Conclusions

One of the urgent changes required to make agriculture more sustainable is to make production processes more efficient. The agricultural activities analyzed have highlighted several opportunities to reduce the environmental impact associated with fruit production in terms of efficiency improvement and input use reduction. Apparently, changes and improvements in production processes are

significantly relevant for those producers that utilize large amounts of natural inputs like land and freshwater, artificial inputs like inorganic fertilizers, and non-renewable energy sources.

Fresh food refrigeration is an urgent issue, especially for natural goods out of season. In addition, it would be important to invest in more efficient machinery and processes. On the other hand, enterprise communication strategies should encompass the lower environmental burden characterizing seasonal products, educating the public to more responsible consumption.

At the same time, there is room to reduce agricultural inputs such as water and fertilizers, as well as the energy required by in-field operations. However, we experienced in this case study that successful technology adoption also passes from the development of workers and employees, who are in the best position to identify the potential of technologies and direct them towards the optimization of the enterprise. People's skills, innovative capacity, and willingness to contribute are fundamental elements of a successful technology uptake process [40]. Enterprise managers, on the other side, have the delicate task of guiding technological change processes, triggering cultural transformation and management models.

Quantifying the environmental profile of production processes represent the first step for agricultural enterprises to reshape the business model and anticipate strategic business planning compared to competitors. A cleaner and more efficient production process can translate in a superior created value that is offered to customers, as well as a higher value captured by the enterprise. In most cases, costs related to the implementation of innovative practices can be economically affordable even for SMEs, and this can positively reflect into shared advantages with other food supply chain actors. Tools, such as LCA, can pave the way to bridge the gap between producers and consumers, building a strong relationship based on transparency and trust, and address consumer decisions in choosing products, with lower environmental impacts.

Future studies may focus on extending the analysis to other crops with similar farming and distribution characteristics, as well as on broader applications of environmental assessments, covering impacts on the whole supply chain, business innovation practices and consumer relationships. Another promising area of research is the analysis of other dimensions of sustainability, including economic impact categories (not just costing) and social aspects.

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References

1. Carter, C.R.; Rogers, D.S. A framework of sustainable supply chain management: Moving toward new theory. *Int. J. Phys. Distrib. Logist. Manag.* **2008**. [CrossRef]
2. Boiral, O. Global warming: Should companies adopt a proactive strategy? *Long Range Plan.* **2006**, *39*, 315–330. [CrossRef]
3. Massa, L.; Tucci, C.L. Business model innovation. In *The Oxford Handbook of Innovation Management*; Oxford University Press: Oxford, UK, 2013. [CrossRef]

4. Bocken, N.M.P.; Rana, P.; Short, S.W. Value mapping for sustainable business thinking. *J. Ind. Prod. Eng.* **2015**, *32*, 67–81. [CrossRef]
5. International Organization for Standardization (ISO). *Environmental Management e Life-Cycle Assessment e Principles and Framework ISO 14040*; ISO: Geneva, Switzerland, 2006.
6. International Organization for Standardization (ISO). *Environmental Management e Life-Cycle Assessment e Requirements and Guidelines ISO 14044*; ISO: Geneva, Switzerland, 2006.
7. Baumann, H.; Boons, F.; Bragd, A. Mapping the green product development field: Engineering, policy, and business perspectives. *J. Clean. Prod.* **2002**, *10*, 409–425. [CrossRef]
8. Bocken, N.M.P.; Allwood, J.M.; Willey, A.R.; King, J.M.H. Development of an eco-ideation tool to identify stepwise greenhouse gas emissions reduction options for consumer goods. *J. Clean. Prod.* **2011**, *19*, 1279–1287. [CrossRef]
9. Bocken, N.M.P.; Schuit, C.S.C.; Kraaijenhagen, C. Experimenting with a circular business model: Lessons from eight cases. *Environ. Innov. Soc. Transit.* **2018**, *28*, 79–95. [CrossRef]
10. Reed, M.S.; Graves, A.; Dandy, N.; Posthumus, H.; Hubacek, K.; Morris, J.; Prell, C.; Quinn, C.H.; Stringer, L.C. Who's in and why? A typology of stakeholder analysis methods for natural resource management. *J. Environ. Manag.* **2009**, *90*, 1933–1949. [CrossRef] [PubMed]
11. Awan, U.; Kraslawski, A.; Huiskonen, J. Progress from blue to the green world: Multilevel governance for pollution prevention planning and sustainability. In *Handbook of Environmental Materials Management*; Springer International Publishing: New York, NY, USA, 2020; pp. 1–22. [CrossRef]
12. Johnson, M.P.; Schaltegger, S. Two decades of sustainability management tools for SMEs: How far have we come? *J. Small Bus. Manag.* **2016**, *54*, 481–505. [CrossRef]
13. Dijkman, T.J.; Basset-Mens, C.; Antón, A.; Núñez, M. LCA of food and agriculture. In *Life Cycle Assessment: Theory and Practice*; Springer International Publishing: New York, NY, USA, 2017; pp. 723–754. [CrossRef]
14. Scandellari, F.; Ventura, M.; Malaguti, D.; Ceccon, C.; Menarbin, G.; Tagliavini, M. Net primary productivity and partitioning of absorbed nutrients in field-grown apple trees. *Acta Hortic.* **2010**. [CrossRef]
15. Balduzzo, M. Analisi Energetica di un Impianto di Conservazione Ipogeo. Master's Thesis, University of Padova, Padova, Italy, 2014. (In Italian)
16. Hofstetter, P. DALYs—An index for human health assessment. In *Perspectives in Life Cycle Impact Assessment*; Springer: New York, NY, USA, 1998; pp. 155–193. [CrossRef]
17. Goedkoop, M.; Spriensma, R. The Eco-Indicator 99—A Damage Oriented Method for Life Cycle Assessment, Methodology Report. In *PRé Consultant*, 3rd ed.; 2001; Available online: http://www.pre-sustainability.com/legacy/download/EI99_annexe_v3.pdf (accessed on 28 September 2020).
18. Jolliet, O.; Margni, M.; Charles, R.; Humbert, S.; Payet, J.; Rebitzer, G.; Rosenbaum, R. IMPACT 2002+: A new life cycle impact assessment methodology. *Int. J. Life Cycle Assess.* **2003**, *8*, 324–330. [CrossRef]
19. Shanmugam, S.K.G.; Mital, M. An ultra-low ammonia charge system for industrial refrigeration. *Int. J. Refrig.* **2019**, *107*, 344–354. [CrossRef]
20. Benhadid-Dib, S.; Benzaoui, A. Refrigerants and their impact in the environment. Use of the solar energy as the source of energy. *Energy Procedia* **2011**, *6*, 347–352. [CrossRef]
21. Widell, K.N.; Eikevik, T. Reducing power consumption in multi-compressor refrigeration systems. *Int. J. Refrig.* **2010**, *33*, 88–94. [CrossRef]
22. Anand, G.; Erickson, D.C.; Makar, E. Characterization of ammonia-water absorption chiller and application. *Int. J. Air-Cond. Refrig.* **2018**, *26*. [CrossRef]
23. Tester, J.W.; Drake, E.M.; Driscoll, M.J.; Golay, M.W.; Peters, W.A. *Sustainable Energy: Choosing among Options*, 2nd ed.; MIT Press: Cambridge, MA, USA, 2012; p. 1066. ISBN 978-0-262-01747-3.
24. De Steur, H.; Temmerman, H.; Gellynck, X.; Canavari, M. Drivers, adoption, and evaluation of sustainability practices in Italian wine SMEs. *Bus. Strategy Environ.* **2020**, *29*, 744–762. [CrossRef]
25. Batte, M.T.; Ehsani, M.R. The economics of precision guidance with auto-boom control for farmer-owned agricultural sprayers. *Comput. Electron. Agric.* **2006**, *53*, 28–44. [CrossRef]
26. Medici, M.; Pedersen, S.M.; Carli, G.; Tagliaventi, M.R. Environmental benefits of precision agriculture adoption. *Econ. Agro-Aliment.* **2019**, *21*, 637-565. [CrossRef]

27. Pedersen, S.M.; Medici, M.; Anken, T.; Tohidloo, G.; Pedersen, M.F.; Carli, G.; Canavari, M.; Tsiropoulos, Z.; Fountas, S. Financial and environmental performance of integrated precision farming systems. In *Precision Agriculture 2019, Proceedings of the 12th European Conference on Precision Agriculture, ECPA, Montpellier, France, 8–11 July 2019*; Wageningen Academic Publishers: Wageningen, The Netherlands, 2019; pp. 833–839. [CrossRef]
28. Biggs, L.; Giles, D. *Current and Future Agricultural Practices and Technologies Which Affect Fuel Efficiency*; Intelligent Energy Europe for a Sustainable Future: 2011. Available online: https://ec.europa.eu/energy/intelligent/projects/sites/iee-projects/files/projects/documents/efficient20_review_of_agricultural_practices_and_technologies_en.pdf (accessed on 28 September 2020).
29. De Wrachien, D.; Medici, M. The future of irrigated agriculture between opportunities and threats the role played by irrigated agriculture. *Irrig. Drain. Syst. Eng.* **2020**, *9*, 1–2. [CrossRef]
30. Snyder, C.S.; Bruulsema, T.W.; Jensen, T.L.; Fixen, P.E. Review of greenhouse gas emissions from crop production systems and fertilizer management effects. *Agric. Ecosyst. Environ.* **2009**. [CrossRef]
31. Menon, A.; Menon, A. Enviropreneurial marketing strategy: The emergence of corporate environmentalism as market strategy. *J. Mark.* **1997**. [CrossRef]
32. Prakash, A. Green marketing, public policy and managerial strategies. *Bus. Strategy Environ.* **2002**, *11*, 285–297. [CrossRef]
33. Canavari, M.; Coderoni, S. Green marketing strategies in the dairy sector: Consumer-stated preferences for carbon footprint labels. *Strateg. Chang.* **2019**, *28*, 233–240. [CrossRef]
34. Macombe, C.; Feschet, P.; Garrabé, M.; Loeillet, D. 2nd International Seminar in Social Life Cycle Assessment—Recent developments in assessing the social impacts of product life cycles. *Int. J. Life Cycle Assess.* **2011**, *16*, 940–943. [CrossRef]
35. Brindley, C.; Oxborrow, L. Aligning the sustainable supply chain to green marketing needs: A case study. *Ind. Mark. Manag.* **2014**, *43*, 45–55. [CrossRef]
36. Bakshi, B.; Small, M.J. Incorporating ecosystem services into life cycle assessment. *J. Ind. Ecol.* **2011**, *15*, 477–478. [CrossRef]
37. Chouinard, Y.; Ellison, J.; Ridgeway, R. The big idea: The sustainable economy. *Harv. Bus. Rev.* **2011**, *89*, 52–62.
38. Aggelopoulou, K.D.; Pateras, D.; Fountas, S.; Gemtos, T.A.; Nanos, G.D. Soil spatial variability and site-specific fertilization maps in an apple orchard. *Precis. Agric.* **2011**. [CrossRef]
39. Canavari, M.; Coderoni, S. Consumer stated preferences for dairy products with carbon footprint labels in Italy. *Agric. Food Econ.* **2020**, *8*, 4. [CrossRef]
40. Peattie, K.; Belz, F.-M. Sustainability marketing—An innovative conception of marketing. *Mark. Rev. St. Gallen* **2010**. [CrossRef]

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Chilling and Heat Accumulation of Fruit and Nut Trees and Flower Bud Vulnerability to Early Spring Low Temperatures in New Mexico: Meteorological Approach

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Abstract: Fruit and nut trees production is an important activity across the southwest United States and this production is greatly impacted by the local climate. Temperature is the main environmental factor influencing the growth and the productivity of the fruit and nut trees as it affects the trees' physiology and the vulnerability of flower bud, flowers, and young fruit and nut to the low temperatures or spring frost. The objective of the present study is to estimate the chilling and heat accumulation of fruit and nut trees across New Mexico. Three study sites as Fabian Garcia, Los Lunas, and Farmington were considered and climate variables were collected at hourly time step. The Utah model and the Dynamic model were used to estimate the accumulated chilling while the Forcing model was used for the heat accumulation. The possible fruit and nut trees endodormancy and ecodormancy periods were also determined at the study sites. The results obtained chilling hours of 715 ± 86.60 h at Fabian Garcia, 729.53 ± 41.71 h at Los Lunas, and 828.95 ± 83.73 h at Farmington using the Utah model. The accumulated chill portions during trees' endodormancy was 3.12 ± 3.05 CP at Fabian Garcia, 42.23 ± 5.08 CP at Los Lunas, and 56.14 ± 1.84 CP at Farmington. The accumulated heat was 8735.52 ± 1650.91 GDH at Fabian Garcia, 7695.43 ± 212.90 GDH at Los Lunas, and 5984.69 ± 2353.20 GDH at Farmington. The fruit and nut trees are at no risk of bud flowers vulnerability at Fabian Garcia while they are under high risk of bud flowers and or young fruit and nut vulnerability to low temperatures early spring as hourly temperature can still drop below 0 °C in April at the end of ecodormancy and flower blooming and young fruits and nuts development stage at Los Lunas and Farmington. Severe weather, especially frost conditions during winter and early spring, can be a significant threat to sustainable nut and fruit production in the northern New Mexico while high chilling requirement fruit and nut trees might not meet chill requirements in the southern New Mexico.

Keywords: fruit and nut trees; chilling; heat accumulation; modeling; New Mexico

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1. Introduction

Temperate fruit trees production is an important activity which heavily depends on the prevailing climatic conditions in the production area and air temperature is the main factor controlling fruit trees phenology [1]. Under cold winter conditions, the temperate plants go dormant to protect the fragile growing tissue from frost and preserve accumulated nutrients. When chilling requirements are not met for a fruit tree, crop growth, bud initiation, flowering and fruit set are delayed in the following growing season [2,3]. On the other hand, increasing air temperature may accelerate winter chill accumulation and advances

crop growth and flowering dates [4–7]. In contrast, spring phenology of fruit trees is delayed by warm winter [8–11]. Plants are affected by the winter temperature causing chilling effects and spring temperature causing forcing effects and these two mechanisms are determinant to spring phenology of the fruit trees [12]. Each fruit tree has accumulated chilling requirements and goes dormant by releasing winter endodormancy and ecodormancy [13,14]. Endodormancy is initiated by low temperature, whereas ecodormancy is driven by heat [15,16]. There is also accumulated heat requirement for the fruit trees for plant growth, leaf unfolding, bud burst initiation, and flowering [17]. The chilling and heat requirements are considered as the driving factors in breaking endodormancy and ecodormancy [16]. It is widely adopted that chilling period covers 1 November–30 April [12,18]. However, Guo et al. [19] found that chestnut chilling period covered from 14 September to 24 March, while the forcing period started on 4 January and ended on 23 May in Beijing, China. On the 1963–2008 period average, the forcing period therefore overlapped with the chilling period indicating that heat accumulation started when only about 50% of the chilling requirement had been fulfilled [19]. Guo et al. [19] reported that for jujube, the forcing phase begins when 55% of chilling requirements were fulfilled. While the occurrence of freezing conditions induces variation in a possible chill effectiveness in Beijing [19], that variation was also reported by Luedeling and Gassner [20] and Luedeling et al. [21] in studies on walnuts in California where temperatures rarely drop below 0 °C.

The chilling and heat requirements of the fruit trees have been quantified and estimated by horticulturist using modeling tools: Chilling Hours Model [22], the Utah Model [23], the Dynamic Model [24,25], Richardson-based Models, the North Carolina Model [26], the Melo-Abreu Model [27], the Positive Utah model [28] and the Low Chill Model [29], the Modified Utah Model (MUM) [30]. The North Carolina Model is an adaptation of the Utah model with adjusted temperature ranges for apple trees. The Melo-Abreu et al. [27] model is the generalization and simplification of the Utah Model which was applied to olive trees with good performance. Among these models, the Chilling Hours Model, the Utah Model, and the Dynamic Model are worldwide used with reasonably good performance [2,4,16,31–44]. These models showed different performance across the globe and the responses also vary with years, observation sites, and the growing regions [39,40]. Among the different chilling models, the dynamic model appears the most robust chilling model [20,34,36,41,45]. Heat requirement of the trees is modelled by the Forcing model also called the Growing Degree Hour model [46] which assumes heat accumulation starting when air temperature falls between a base temperature and the critical temperature with a maximum heat accumulation at the optimum temperature. The base temperature of a plant species is the minimum threshold temperature at which plant growth starts and the critical temperature is the upper threshold temperature at which all crop physiological activities cease [47].

Chill requirements vary with tree species. In Alabama, chill requirements are 800–1100 for standard apple, 400–750 for Japanese plum, about 1000 for cherry, and 50–800 for blackberry [48]. Luedeling et al. [43] found chill and heat requirements for cherry trees to be 68.6 $\pm$ 5.7 chill portions (CP) equivalent to 1375 $\pm$ 178 chilling hours or 1410 $\pm$ 238 Utah chill units and a heat requirement of 3473 $\pm$ 1236 growing degree hours in Bonn, Germany. Chilling and heat requirements were 79.8 $\pm$ 5.3 CP and 13,466 $\pm$ 1918 GDH for chestnut bloom in Beijing, 104.2 $\pm$ 8.9 CP and 2698 $\pm$ 1183 GDH for cherry bloom in Germany, and 37.5 $\pm$ 5.0 CP and 11,245 $\pm$ 1697 GDH for walnut leaf emergence in California [43], respectively. Chill portion and heat requirements of cherry cultivars varied from 30.4 to 57.6 CP and from 7326 to 9450 GDH, in Spain, respectively, [49]. Chilling requirements of 15 peach cultivars ranged between 263 and 2123 chill hour (CH), 377 and 1134 chill unit (CU), and 21.3 and 74.8 CP and the heat requirements varied from 4824 to 5506 GDH in Korea for the 1919–2018 period [50]. Local almond cultivars chilling and heat requirements ranged from 3.4 to 15.5 CP and between 3962 and 8873 GDH, respectively, while the corresponding values for foreign cultivars varied from 6.7 to 22.6 CP and from 2894 to 10,504 GDH, respectively, in Tunisia [51].

Fruit and nut trees are a rewarding addition to backyard landscapes beside the medium to large commercial fruit and nut trees fields across New Mexico where the climatic conditions are variable. Different fruit crops are grown over 1206.37 ha and nut trees grown over 20,769.28 ha across New Mexico with the market value of $210.253 million [52]. Fruit and nut trees such as Apricots, plums, apples, cherries, peaches, pears, grapes, persimmons, figs, pecans, pistachios, almonds, jujubes, blueberries, and different berries are produced across New Mexico. The late-blooming and non-uniform varieties with some late flowers have a better chance to produce than uniform and early blooming varieties [53]. Apples (466.60 ha), grapes (518 ha), peaches (62 ha), apricots (41.68 ha), pears (40.47 ha), and sweet cherries (33.18 ha) are the main fruit trees while pecan trees are the main nut trees grown in New Mexico [52]. The State of New Mexico is characterized by recurrent occurring late spring frosts across the state, injuring the flowers and young fruits of early flowering species [53]. The recurrent question growers ask the extension agents is related to fruit and nut tree species and cultivars choice with reference to their local environmental or climatic conditions. While the answer is not straightforward, very limited information exists on the chill and heat requirements of different fruit and nut trees across the main agroecological zone in New Mexico. Therefore, it is critical to estimate the chill portions and chill units in different parts of the state to assist growers with tree species and cultivars selection for each location for sustainable and profitable fruit production. The objective of this study is to help growers in decision-making regarding the fruit trees choice depending on their chill and heat requirement at different parts of the state of New Mexico, USA.

2. Materials and Methods

2.1. Study Sites and Data Collection

This study is focused on the State of New Mexico. Three locations were chosen as Fabian Garcia (32.28°, −106.77°, elevation 1186.0 m), Los Lunas (34.77°, −106.76°, elevation 1476.0 m), and Farmington (36.69°, −108.31°, elevation 1720.0 m) and are situated in the southern, central, and northwestern New Mexico, respectively, and where fruit and nut production is the predominant agricultural activity. New Mexico is characterized by a semiarid climate at the eastern and southern regions while the central, the northern, and western regions are characterized by arid climate. All climatic variables were monitored on an hourly basis at each site using an automated weather station. For the present study, only hourly and daily average temperatures were used.

2.2. Data Management

In this study, the following calendar was adopted: for the fruit trees across New Mexico. The dormancy season is assumed to start on 1 July and ends on 30 June of the following year. The period of July 2015 to June 2020 at Fabian Garcia, July 2017 to June 2020 at Los Lunas, and July 2015 to June 2020 at Farmington were considered under the present study due to data availability with no gap or periodic missing data. The time series data were checked for quality control following the methodology described by Allen et al. [54] and the abnormal data point were removed before the analysis.

2.3. Estimation of the Heat and Chilling Accumulation: Heat and Chilling Model

Among the numerous models developed and evaluated for chill and heat accumulation by fruit trees, the Forcing model [46], the Utah Model [23], and the Dynamic Model [24,25] are the most used across the globe. The Forcing model revealed to be accurate with high precision. Similarly, the Utah model and the Dynamic model are the most used and the most precise chill accumulation models. For the present study we consider the three models to estimate the potential heat and chill accumulation at the three research stations. The chilling requirement was calculated each year as the sum of hourly chilling units (CU) from endodormancy onset until the transition from endodormancy to

ecodormancy. Similarly, the heat requirement is the sum of the growing degree hours from the onset to the end of the ecodormancy.

- Forcing model: Growing Degree Hour (GDH) Model

The Forcing model is the Growing Degree Hour (GDH) Model [46]. The GDH Model equivalent to the thermal unit accumulation model assumes heat accumulation when the actual hourly temperature (Ti) is between the fruit trees base temperature (Tb) which is the lowest temperature threshold and the critical temperature (Tc) which is the upper threshold temperature, and the optimum temperature (Tu) at which the maximum heat accumulations occurs. The GDH function is described below:

$$\mathrm{GDH} = \begin{cases} F\left(\frac{\mathrm{Tu}-\mathrm{Tb}}{2}\right)\left(1+\cos\left(\pi+\frac{\pi(\mathrm{Ti}-\mathrm{Tb})}{\mathrm{Tu}-\mathrm{Tb}}\right)\right); \mathrm{Tu} \geq \mathrm{Ti} \geq \mathrm{Tb} \\ F(\mathrm{Tu}-\mathrm{Tb})\left(1+\cos\left(\frac{\pi}{2}+\frac{\pi(\mathrm{Ti}-\mathrm{Tu})}{2(\mathrm{Tc}-\mathrm{Tu})}\right)\right); \mathrm{Tc} \geq \mathrm{Ti} \geq \mathrm{Tu} \\ 0; \mathrm{Ti} > \mathrm{Tc}\ \textit{or}\ \mathrm{Ti} < \mathrm{Tb} \end{cases} \quad (1)$$

F is a plant stress factor that is commonly set to 1, if no particular stress exists. Tb, Tu, and Tc were set to 4, 25, and 36 °C, respectively, as suggested by Anderson et al. [46] for fruit trees.

- Chilling requirement of fruit trees: The Utah Model [23]

The Utah Model attributes the weighing parameter as function of actual temperature to determine the effectiveness of chilling. The Utah Model introduces the concept of negative chill accumulation and the chilling effectiveness. Thus, the temperature ranging between 0 and 16 °C promotes the breaking rest while all temperature greater than 16 °C accounts for negative chill. The chilling accumulation is effective at 7 °C. The distribution of chill accumulation as function of temperature is presented in Table 1.

Table 1. Temperature conversion to chill unit factor.

Temperature (°C)	Chill Unit Accumulated
<1.4	0.0
1.5–2.4	0.5
2.5–9.1	1.0
9.2–12.4	0.5
12.5–15.9	0.0
16–18	−0.5
>18	−1.0

- The dynamic model [24,25]

The dynamic model developed in Israel assumes winter chill accumulation as a result of two-step process: production of intermediate chilling products which are destructible by the high temperatures, and the conversion of the intermediate products into permanent chill portions under moderate temperatures. The chill portions are accumulated and summed throughout winter. The detailed description of the dynamic model with all the used equations is presented in Fishman et al. [24,25], Luedeling et al. [2], Darbyshire et al. [55], and Luedeling and Brown [4].

The chilling portions, chilling hours, and heat requirement were estimated using the diagram presented in Figure 1 while the bud and flowers and or young fruit or nut vulnerability to low temperatures early spring was computed through the frequency of occurrence and or exposure to the below zero degree Celsius and below the killing freezing temperature of −2 °C using the daily average temperature and the hourly temperature from January to the end of April of each year.

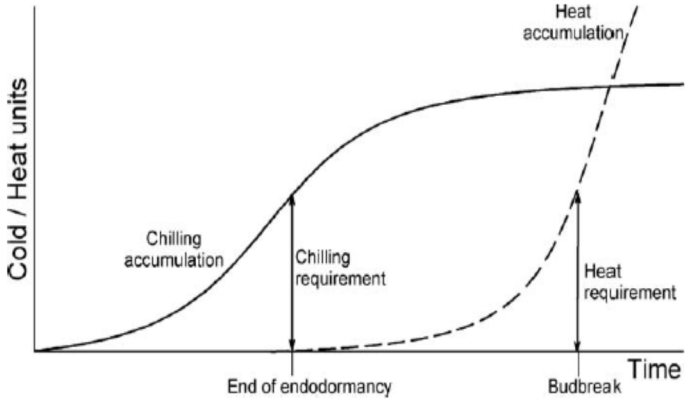

Figure 1. Schematic illustration of chilling and heat accumulation during the dormancy period as a function of time, under the assumption that chilling and heat are accumulated sequentially [2].

3. Results

3.1. Variation in Air Temperature at Fabian Garcia, Los Lunas, and Farmington

Air temperature increased at Fabian Garcia from 1.6 °C early January to the maximum daily average temperature of 30 °C late June–early July and decreased thereafter (Figure 2a). On average, chilling accumulation period by the Utah Model (1.5 < temperature < 12.4) started from 8 November and ended late February. Daily average temperature was never negative according to our study period. For the period from October to March, average daily temperature was 11.3 ± 3.0 °C while the average temperature for the rest of the year was 24.7 ± 2.2 °C. Average annual temperature was 18.0 ± 2.6 °C.

At the Los Lunas site, daily average temperature was maximal at mid-July and minimal late December–early January and varied from −3.4 to 28.6 °C (Figure 2b). Annual daily temperature was 8.0 ± 1.3 °C. Daily average temperature fell within the range [1.5, 12.4] during the period from 8 October to 14 December and from 6 January to 23 March on average. For the period from October to March, average daily temperature was 6.2 ± 2.8 °C while the average temperature for the rest of the year was 21.7 ± 2.0 °C.

Daily air temperature at Farmington varied from −4.3 ± 6.4 °C early January to 25.6 ± 1.6 °C late June and the annual average air temperature was 11.7 ± 3.1 °C for the period of 2015 to 2020 (Figure 2c). Seasonal average air temperature for the period of October–March was 6.5 ± 3.6 °C while the average temperature from April to September was 19.2 ± 2.6 °C. The appropriate period for chilling accumulation at Farmington (1.5 < temperature < 12.4) varied from 4 October to 27 November and from 30 January to 9 May.

On annual basis, annual air temperature is 10.0 °C and 6.3 °C lower at Los Lunas and Farmington than the air temperature at Fabian Garcia, respectively. In the October–March period average air temperate was 5.1 °C and 4.8 °C lower at Los Lunas and Farmington than the air temperature at Fabian Garcia and there is more chance for greater chilling accumulation at Farmington and Los Lunas than at Fabian Garcia. In other words, greater chilling requiring fruit and nut trees are recommended at Los Lunas and Farmington than at Fabian Garcia.

Long-term monthly absolute minimum and maximum temperatures and average monthly minimum and maximum temperatures at Fabian Garcia, Los Lunas, and Farmington for the 1985–2020 period are presented in Table 2. The long-term February absolute minimum temperature was −13.9 °C at Fabian Garcia, −18.4 °C at Los Lunas, and −19.3 °C at Farmington. March and April absolute minimum temperatures were −7.4 and −2.7 °C at Fabian Garcia, −7.7 and −5.4 °C at Los Lunas, and −11.5 and −7.4 °C at Farmington, respectively, (Table 2). These early spring absolute minimum temperatures demonstrate the high probability of bud, flowers, and young fruit and nut vulnerability and exposure to early spring frost mostly at the Farmington area. Long-term summer period June, July, and August absolute maximum temperatures were quite high as 43.4, 42.5, and 40.8 °C at

Fabian Garcia, 40.5, 40.3, and 37.6 °C at Los Lunas and 37.9, 39.0, and 37.0 °C at Farmington (Table 2) and which might expose the fruit and nut trees to heat stress.

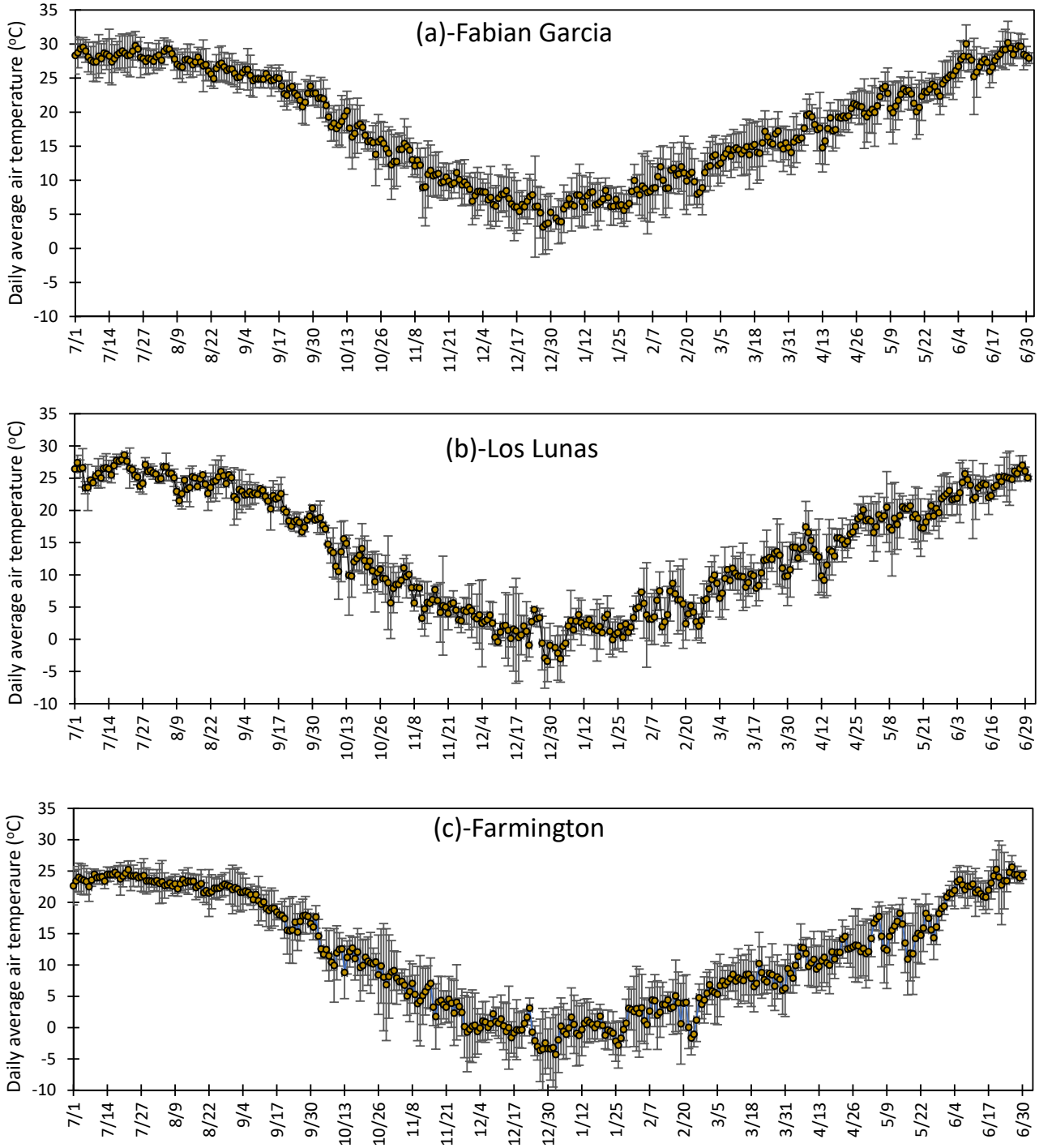

Figure 2. Daily average air temperature with standard deviation from 1 July to 30 June for the 1 July 2015 to 30 June 2020 period at (**a**) Fabian Garcia and (**b**) Los Lunas, and (**c**) Farmington.

3.2. Variation in Daily Chill Portion and Seasonal Total Chill Hours for the Period of 2015 to 2020 at Fabian Garcia, Los Lunas, and Farmington

Chill portion estimated by the Utah model at all three study sites is presented in Figure 3. Daily chill portion was null or negative from the beginning of June to the end of September at Fabian Garcia (Figure 3a). Chill portion varied with years and became positive early October, increased and was equal to unity during few days mostly in December and

January, and decreased toward the end of April. The 2005–2020 period chill portion increased from early October, reached a maximum average chill portion value of 0.55 from early December to mid-January, and it decreased and became null by the end of April.

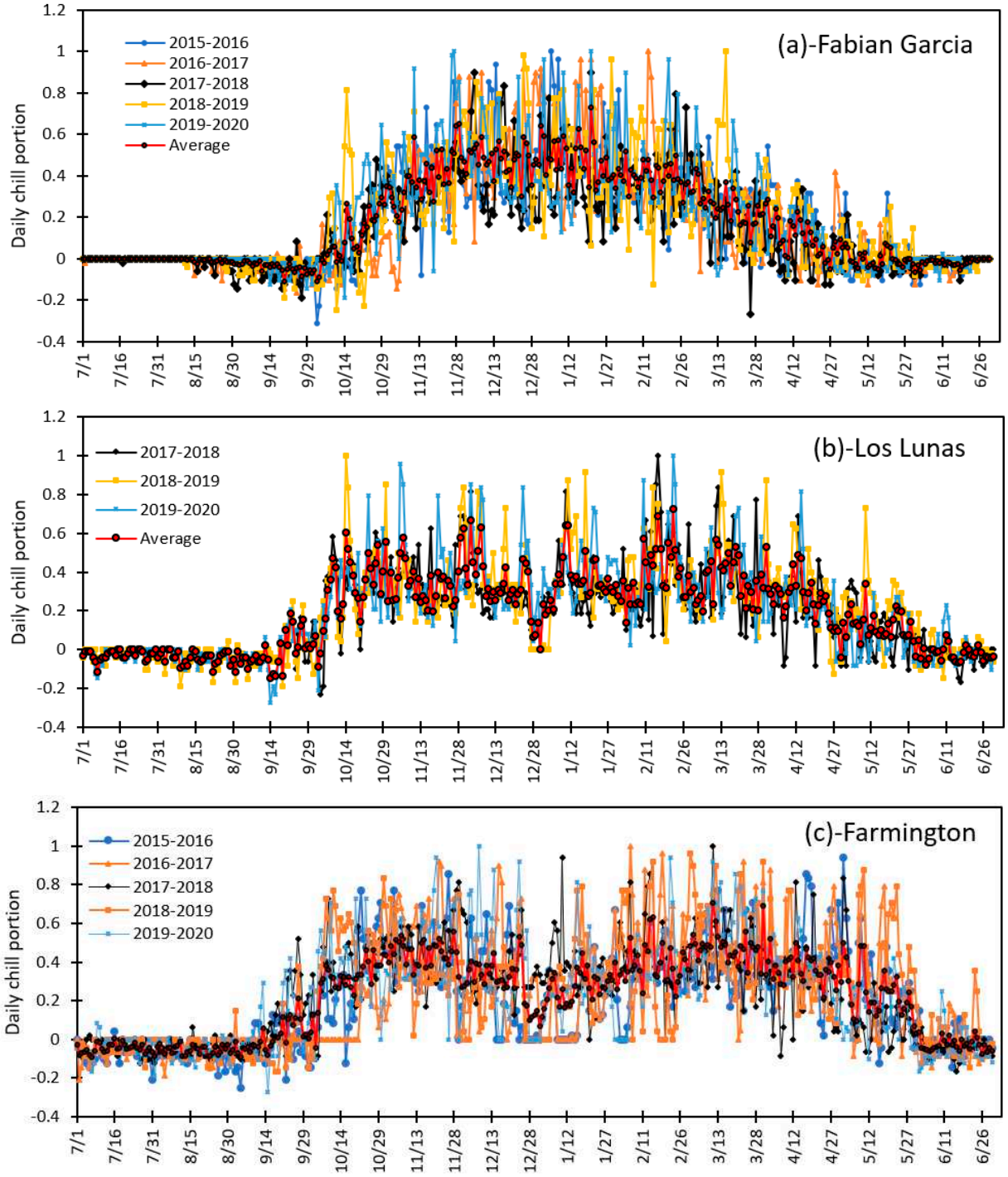

Figure 3. Daily chill portion for the 2015–2020 period at (**a**) Fabian Garcia, (**b**) Los Lunas, and (**c**) Farmington (Utah model).

At Los Lunas, chill accumulation was effective from the beginning of October to the end of May. While it averaged 0.4 on the daily time step, it varied from 0.1 to 1 with few days' chill portion of 0.9 (Figure 3b). There was abrupt drop in chill portion to null at the end of December with the lower daily average temperature of −3.5 °C. Daily chill portion was null or negative from the beginning to mid-August. The variation in the chill portion

during the effective period is due to the variation in hourly and daily air temperature at Los Lunas.

Chill portion by the Utah model at Farmington was slightly negative from 1 June to 15 September. Chill portion varied between 0 and 1 during the period of 15 September to 30 May, and averaged 0.4 during the favorable chilling accumulation period (Figure 3c). Overall, chill portion increased from mid-September and reached an average maximum of 0.5 early November and decreased thereafter up to 0.1 at the end of December. It increased again up to 0.7 on average at mid-March and decreased again until late May.

With reference to Figure 3, the chill hours' values recorded at Fabian Garcia were 689.06, 660.84, 617.82, 791.10, and 820.64 h for the consecutive seasons from 2015–2016 to 2019–2020 seasons, respectively (Table 3). The recorded seasonal chill hours were 686.08, 733.26, and 760.24 h at Los Lunas for the 2017–2018, 2018–2019 and 2019–2020 seasons, respectively. In Farmington, the chill hours were higher than that at Los Lunas and Fabian Garcia and varied from 750.50 to 964.04 h. The endodormancy accumulated chill hours averaged 715 $\pm$ 86.60, 729.53 $\pm$ 41.71, and 828.95 $\pm$ 83.73 h at Fabian Garcia, Los Lunas, and Farmington, respectively. There was 1.9 and 16% more chill hours at Los Lunas and Farmington compared to Fabian Garcia. In other words, chill hour increased with latitude across the state of New Mexico.

3.3. Variation in Daily Growing Degree Hours and Seasonal Total Heat Accumulated from 2015 to 2020 at Fabian Garcia, Los Lunas, and Farmington

The variation in the daily growing degree hours by the Forcing model at Fabian Garcia, Los Lunas, and Farmington is presented in Figure 4. Considering the fruit and nut trees growing season that covers the period from 1 July to 30 June, the daily growing hours at Fabian Garcia increased from 1 July to 30 September and decreased until its minimum value was close to zero at the end of December, increased again up to the end of April and decreased toward the end of June (Figure 4a). Daily growing degree hours ranged from 0 to 480 GDH and showed two peaks at the end of September and at the end of April. The most important part of the growing degree hour curve is the increasing section from January to April which represents the accumulated heat during the ecodormancy phase of the fruit and nut trees after the endodormancy had been accomplished. Therefore, daily heat accumulation increased from January to April with an abrupt decrease during the second half of February (Figure 4a). With the overall ecodormancy period covering early January to 18 March, the accumulated heat values at Fabian Garcia varied from 6947.58 to 11,352.27 GDH and averaged 8735.52 $\pm$ 1650.91 GDH (Table 3).

At Los Lunas, the daily heat varied from 0 to 480 GDH (Figure 4b). It slightly increased during July and August and decreased from the beginning of September to early December and stayed at its lowest level in December and January and increased thereafter. There were some regular drops in the growing degree hours in February, March, and April (Figure 4b) due to the changes in the hourly average temperature during the period of February-April at Los Lunas. While the ecodormancy period covers the period from early January to 8 April, the possible total accumulated heat by fruit and nut trees varied from 7544.88 to 10,748.86 GDH and averaged 7695.43 $\pm$ 212.90 GDH (Table 3).

Daily growing degree hours varied from 0 to 450 GDH with few days when the daily heat was 580 GDH at Farmington (Figure 4c). Overall, daily heat decreased from an average of 350 GDH late August to 0 by the end of November and remained stable at the minimum value during December and January. Heat started to accumulate from the beginning of February to end May at 360 GDH a day. The possible total accumulated heat varied from 4007.80 to 9894.20 GDH and averaged 5984.69 $\pm$ 2353.20 GDH (Table 3) during the ecodormancy period that covers early January to late April at Farmington.

The accumulated heat during trees ecodormancy varied greatly with location and decreased from the southern to the northern New Mexico. This change in the heat is due to the severity of the winter weather with latitude. There were 11.9 and 31.5% less accumulated heat at Los Lunas and Farmington, compared to the accumulated heat at Fabian Garcia during the ecodormancy respectively.

Table 2. Long-term monthly absolute minimum and maximum temperatures and average monthly minimum and maximum temperatures at Fabian Garcia, Los Lunas, and Farmington for the 1985–2020 period.

Months	Fabian Garcia				Los Lunas				Farmington			
	Abs. Tmin	Ave. Tmin	Abs. Tmax	Ave. Tmax	Abs. Tmin	Ave. Tmin	Abs. Tmax	Ave. Tmax	Abs. Tmin	Ave. Tmin	Abs. Tmax	Ave. Tmax
January	−7.7	−0.7 ± 1.0	24.0	13.1 ± 1.9	−15.2	−3.7 ± 1.1	21.1	9.2 ± 2.0	−19.4	−5.7 ± 1.8	15.9	5.2 ± 1.8
February	−13.9	1.2 ± 1.4	27.1	16.2 ± 1.8	−18.4	−2.3 ± 1.1	24.1	12.3 ± 1.9	−19.3	−4.1 ± 1.3	20.7	7.9 ± 1.8
March	−7.4	4.3 ± 1.5	30.9	20.6 ± 1.7	−7.7	0.4 ± 1.3	27.6	17.0 ± 1.7	−11.5	−1.4 ± 1.2	25.5	12.9 ± 1.8
April	−2.7	8.1 ± 1.5	35.3	25.4 ± 1.6	−5.4	3.9 ± 1.4	31.8	21.6 ± 1.7	−7.4	1.7 ± 1.4	28.5	17.6 ± 1.6
May	3.1	13.2 ± 1.1	39.3	30.4 ± 1.6	−1.4	9.2 ± 1.3	37.9	26.8 ± 1.9	−4.0	6.9 ± 1.5	36.2	23.3 ± 2.0
June	10.7	18.9 ± 1.3	43.4	35.5 ± 1.6	5.4	14.9 ± 1.3	40.5	32.8 ± 1.6	2.3	12.8 ± 1.4	37.9	30.1 ± 1.7
July	14.9	21.1 ± 1.0	42.5	34.5 ± 1.6	10.6	18.1 ± 1.0	40.3	32.7 ± 1.5	7.9	16.5 ± 1.0	39	31.7 ± 1.5
August	14.1	20.2 ± 1.1	40.8	33.3 ± 1.7	9.8	17.1 ± 1.1	37.6	31.3 ± 1.6	5.9	15.5 ± 1.1	37.0	29.9 ± 1.6
September	6.7	16.5 ± 1.4	37.9	30.5 ± 1.7	0.7	12.8 ± 1.4	36.2	28.1 ± 1.5	−1.1	10.8 ± 1.4	35.4	26.0 ± 1.5
October	−3.5	10.2 ± 1.3	35.1	25.4 ± 1.5	−7.1	6.1 ± 1.4	31.9	22.1 ± 1.6	−9.8	4.1 ± 1.5	29.7	19.1 ± 1.7
November	−7.0	3.6 ± 1.7	28.7	18.2 ± 1.9	−8.4	−0.2 ± 1.5	24.7	14.6 ± 2.0	−12.5	−1.9 ± 1.4	22.6	11.2 ± 2.2
December	−8.3	−0.5 ± 1.1	24.3	12.8 ± 1.5	−17.6	−3.6 ± 1.1	20.3	8.9 ± 1.6	−23.2	−5.5 ± 1.4	17	5.1 ± 1.5

Abs. Tmin is absolute minimum temperature; Abs. Tmax is absolute maximum temperature; Ave. Tmin is average minimum temperature; Ave. Tmax is average maximum temperature.

Table 3. Estimated chill portion, chilling hours and heat accumulation at Fabian Garcia, Los Lunas, and Farmington for the 2015–2020 period.

Sites	Fruit Tree Growing Seasons	Endodormancy Period	Ecodormancy Period	Chill Portions	Chilling Hours	Accumulated Heat (GDH)
Fabian Garcia	2015–2016	20 October–31 December	1 January–10 March	33.8	689.06	8855.81
	2016–2017	6 November–30 December	31 December–27 February	0	660.84	6947.58
	2017–2018	21 October–31 December	1 January–18 March	1.6	617.82	11,352.27
	2018–2019	24 October–31 December	1 January–8 March	5.9	791.1	7817.70
	2019–2020	18 October–31 December	1 January–14 March	3	820.64	8702.91
Los Lunas	2017–2018	6 October–01 January	2 January–8 April	36.4	686.08	10,748.86
	2018–2019	5 October–03 January	4 January–7 April	45.7	733.26	7845.97
	2019–2020	4 October–01 January	2 January–1 April	44.6	760.24	7544.88
Farmington	2015–2016	7 October–17 January	18 January–13 April	53.5	750.50	6316.67
	2016–2017	26 October–29 January	30 January–29 March	57.9	781.46	4475.90
	2017–2018	24 October–11 January	12 January–28 April	57.9	964.04	9894.20
	2018–2019	1 October–6 January	7 January–9 April	55.1	798.50	4007.80
	2019–2020	5 October–6 January	7 January–15 April	55.7	850.24	5228.87

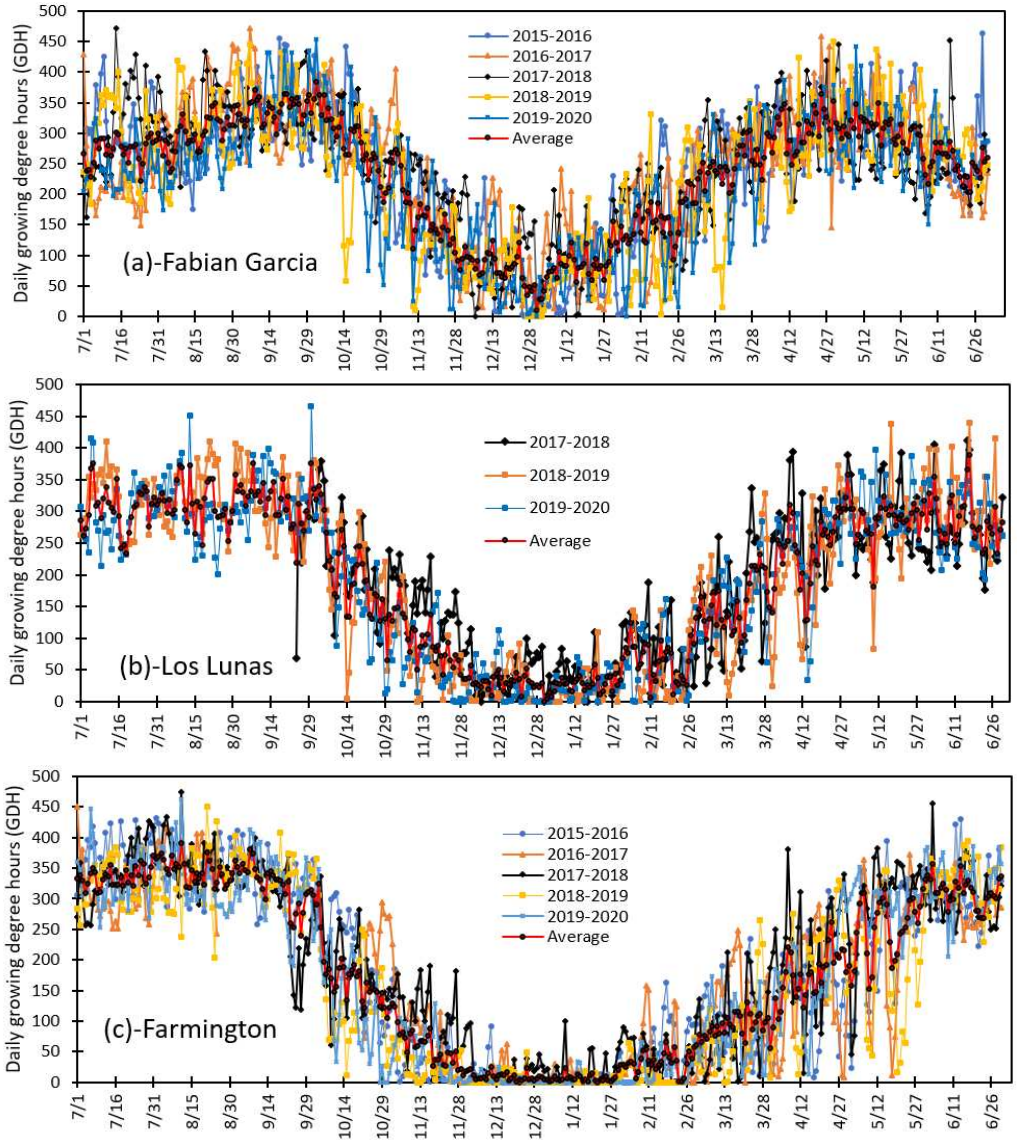

Figure 4. Daily growing degree hours for the 2015–2020 period at (**a**) Fabian Garcia, and (**c**) Farmington and for the 2017–2020 period at (**b**) Los Lunas.

3.4. Variation in Daily Chill Portion and Seasonal Total Chill Portion during Fruit and Nut Trees Endodormancy at Fabian Garcia, Los Lunas and Farmington during the 2015 to 2020 Period

The Dynamic model estimated chill portion during the fruit and nut trees growing season is presented in Figure 5. On average according to the Dynamic model, the chill portion started accumulating as late as the end of December at Fabian Garcia while it started accumulating by early to mid-October at Los Lunas and Farmington (Figure 5). The total growth season chill portion varied from 58 to 85 chill portions and averaged 68 chill portions at Fabian Garcia (Figure 5a). It varied from 87 to 107 chill portions and averaged 98 chill portions at Los Lunas (Figure 5b) while it varied from 108 to 128 chill portions and averaged 118 chill portions at Farmington (Figure 5c). However, the magnitude of the total chill portions of the trees during the growing season are not ergonomically and physiologically important for fruit and nut trees production at the study sites. In contrast, the accumulated chill portions during trees endodormancy are physiologically most important for tree production. As the endodormancy period of the fruit and nut trees varied with the study site, the physiologically possible chill portions at Fabian Garcia, Los Lunas, and Farmington

are presented in Table 3 and varied from 0 to 33.8 CP and averaging 9.26 ± 13.9 CP at Fabian Garcia, from 36.4 to 45.7 CP and averaging 42.23 ± 5.08 CP at Los Lunas, and from 53.5 to 57.9 CP and averaging 56.14 ± 1.84 CP at Farmington. It is important to indicate that the effective chill portions at Fabian Garcia were as low as 5.9 CP during four growing seasons out of five (80% of seasons) and averaged 3.12 ± 3.05 CP and should be considered for decision-making regarding tree species and cultivars choice by fruit and nut producers to minimize the risk of non-productive fruit trees which will never meet their chill requirement in Fabian Garcia region.

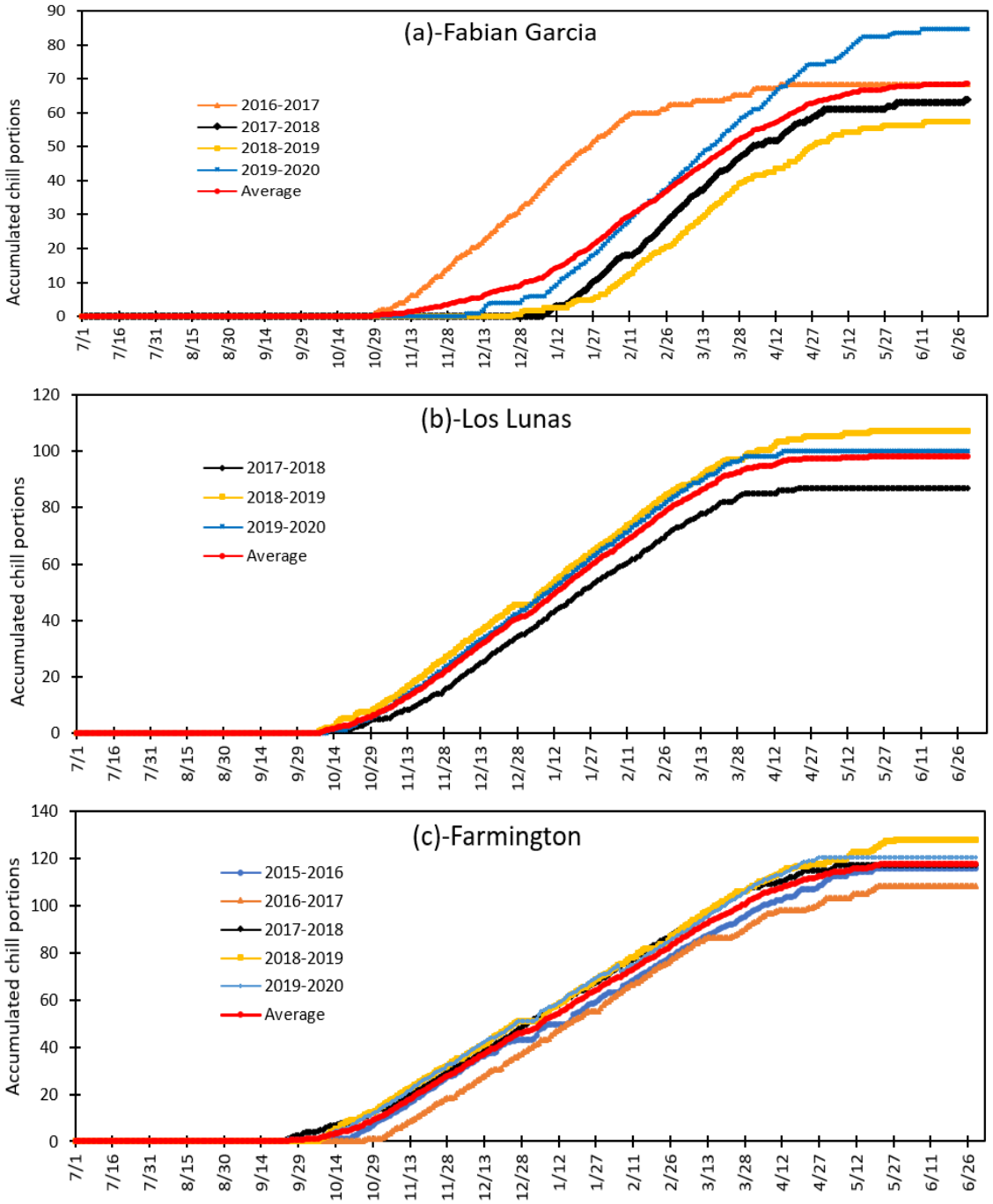

Figure 5. Accumulated chill portions estimated by the Dynamic model for fruit trees growing seasons and the average accumulated chill portions at (**a**) Fabian Garcia, (**b**) Los Lunas, and (**c**) Farmington for the 2015–2020 period (dynamic model).

3.5. Vulnerability of Flower Bud to Early Spring Low Temperatures at Fabian Garcia, Los Lunas, and Farmington

The ecodormancy basically ends at the latest by mid-March with flower blooming and fruit formation and development in Fabian Garcia area. The daily average air temperature never reached the kill freezing temperature of −2 °C at Fabian Garcia. It increased and remained above 4 °C at the beginning of February, above 9 °C from 16 February toward the end of April (Figure 6c). Therefore, there is no risk of frost damage on the flower bud of fruit and nut trees in the Fabian Garcia area. However, due to the variability of climate and hazardous abrupt change in climate variables, we considered looking into the hourly air temperature during early spring period. Figure 7 presents the changes in the hourly air temperature at Fabian Garcia. Hourly air temperature fell below 0 °C the latest around the last week of February during the 2015–2020 period at Fabian Garcia and there is a minimal risk of frost damage on the bud and flower and young fruits and nuts.

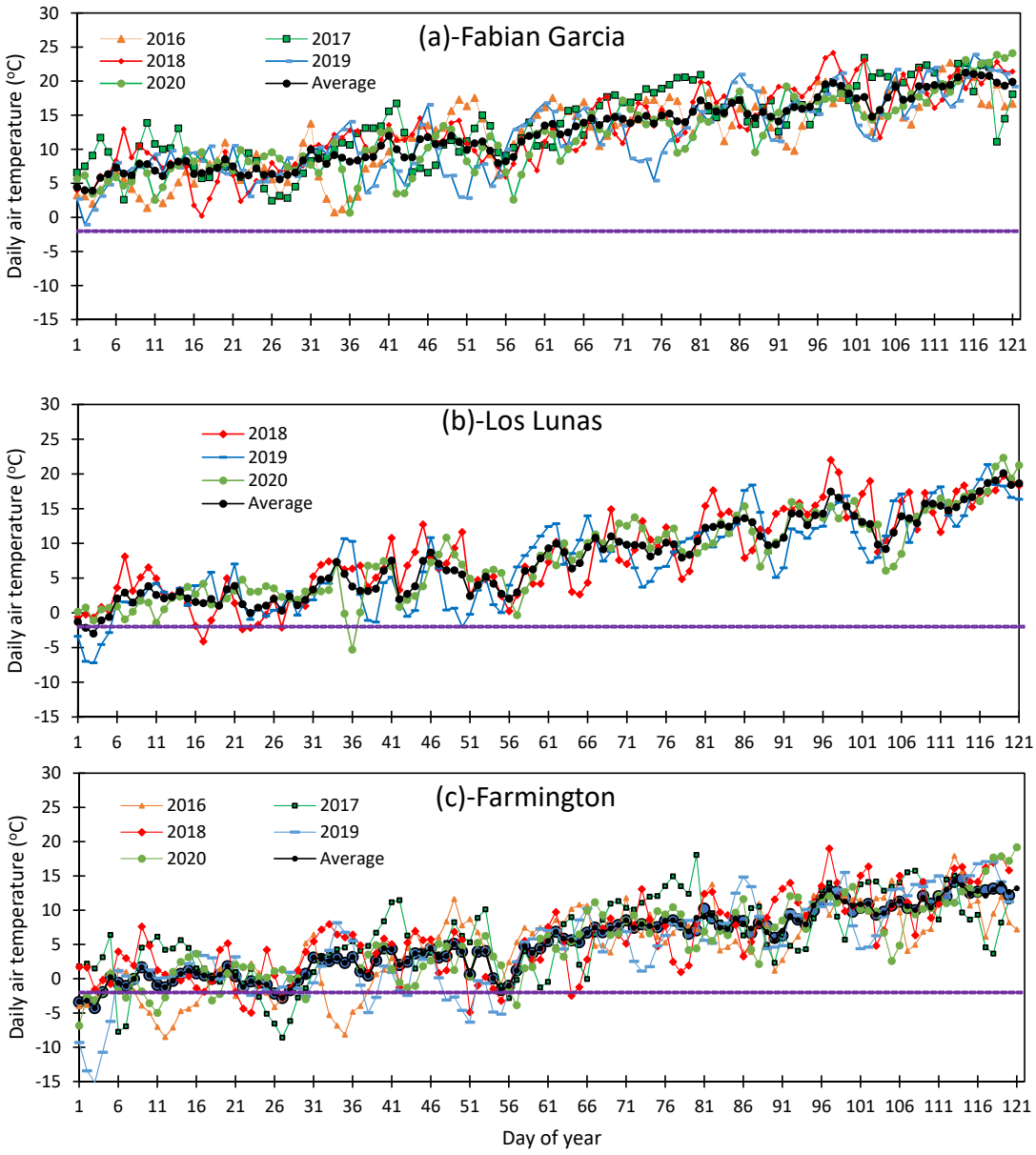

Figure 6. Variation in the daily air temperature from 1 January to 30 April during the 2016–2020 period at (**a**) Fabian Garcia, (**b**) Los Lunas, and (**c**) Farmington.

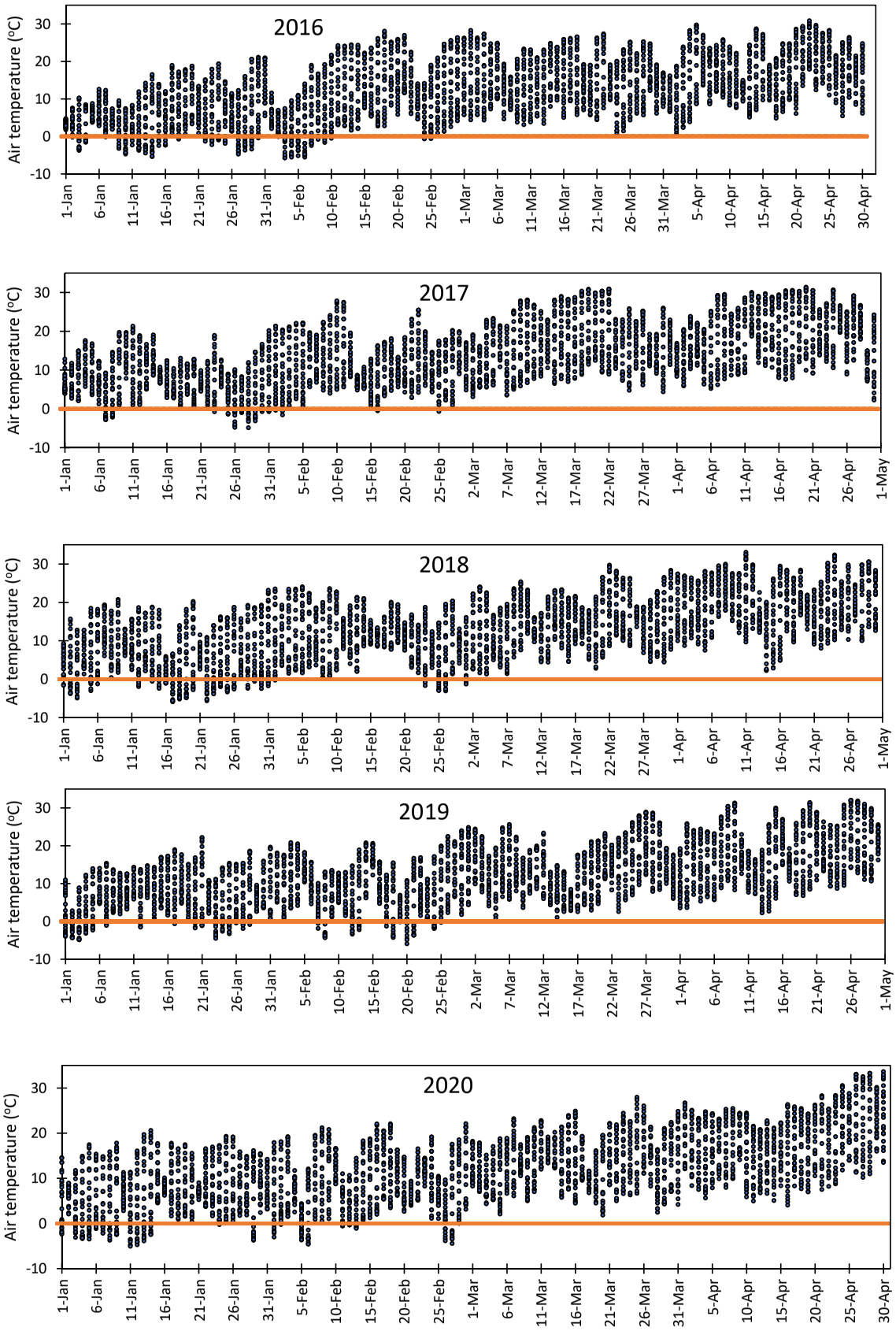

Figure 7. Variation in the hourly air temperature from 1 January to 30 April during the 2016–2020 period at Fabian Garcia.

Daily air temperature was below −2 °C mostly in January in 2018 and only once early 2020 February at Los Lunas. Air temperature reached −2 °C late 2019 February. Daily air temperature was above 0 °C by the end of February and steadily increased as in March and April, (Figure 6b) minimizing the risk of vulnerability of trees flower bud as the ecodormancy ends early April at Los Lunas. The close look at the hourly air temperature at Los Lunas (Figure 8), in opposite to Fabian Garcia, hourly temperature was below 0 °C several times in January and February 2018, 2019, and 2020. Hourly air temperature was below 0 °C few times in March each season and as late as 15 April 2018, 14 April 2019, and 15 April 2020. The occurrence of the 0 and or negative temperature mid-April remains a recurrent risk for fruit and nut trees production at Los Lunas as the ecodormancy might end the latest in early April and exposing the flowers and or young fruits or nuts to frost damage. The damage might be more important up to the loss of the whole annual production in the case of early flowering trees species with low chilling and heat requirements.

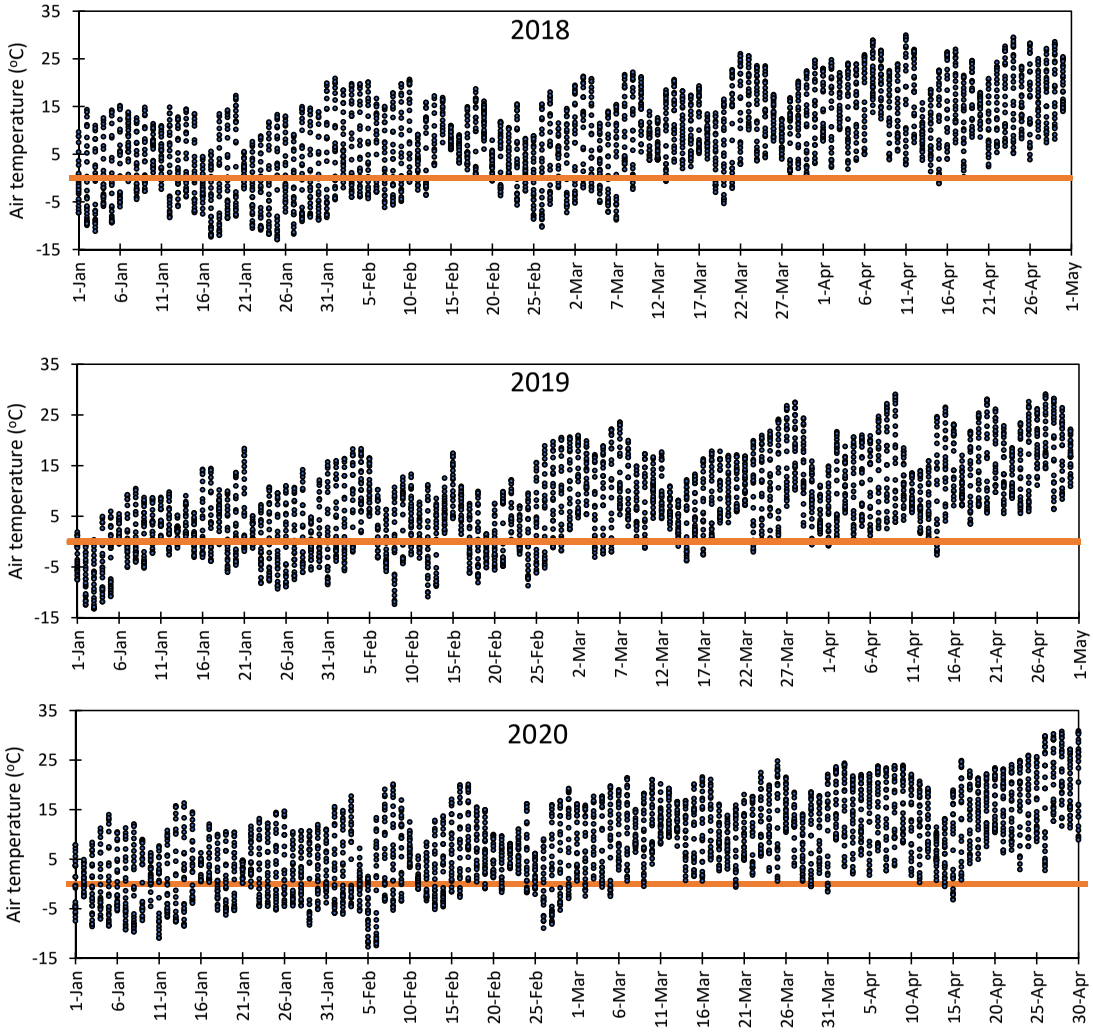

Figure 8. Variation in the hourly air temperature from 1 January to 30 April during the 2018–2020 period at Los Lunas.

At Farmington, daily air temperature was below the kill freezing temperature of −2 °C several days every spring and the latest was early March and it increased thereafter (Figure 6c). However, the daily temperature was 0 °C on very few days even at the beginning of April, implying higher risk of bud flower, flower, and young fruit and nuts exposure to frost at Farmington. The hourly air temperature shown in Figure 9 was null and negative instantaneously until the end of 2016 April 2017, mid-April 2019, and 2020. As the

ecodormancy theoretically ends from mid to end April, there is a very high probability for the bud flower to be under frost damage risk and the young fruits and nuts of the early flowering and low heat requirements trees to be exposed to frost damage at Farmington where a very late frost usually occurs during the third week of May [56].

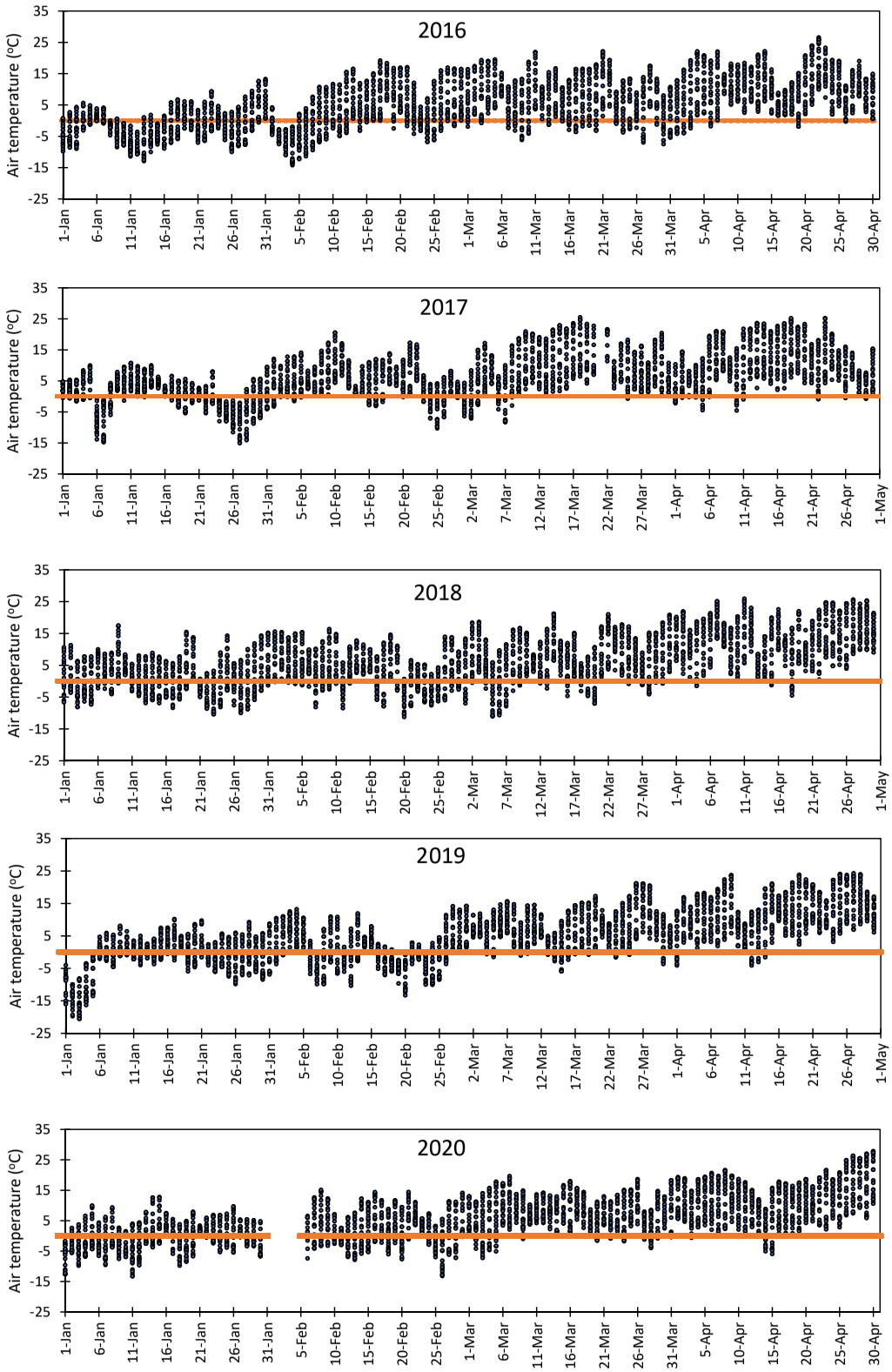

Figure 9. Variation in the hourly air temperature from 1 January to 30 April during the 2016–2020 period at Farmington.

4. Discussion

The present study simulated the chilling and heat that the fruit and nut trees could possibly accumulate at Fabian Garcia, Los Lunas, and Farmington during the 2015–2020 period. As summarized in Table 3, chill accumulation was very low as 3.12 CP at Fabian Garcia and 42.23 and 56.14 CP at Los Lunas and Farmington, respectively. This study provided the small and commercial fruit trees and nuts producers as well as urban back yard gardeners with the basic information for fruit trees choice with reference to their locations and the chill and heat requirements as compared to the values in Table 3. As an example, the Table 4 gives some fruit and nut trees chill and heat requirements across the globe and we strongly recommend reader, decision-makers, and fruit trees growers to refer to a larger study record across the literature as the heat and chilling requirements vary greatly within the same tree species.

While other sources of fruit and nut trees phenology data could be used in the present study to validate the selected models, fruit and nut trees orchards create a microclimate specific plant growth environment and the climatic conditions in the leaf canopy is usually influenced by light intensity, temperature, and relative humidity. Several satellites are used for terrestrial vegetation phenology monitoring, however, a strong deviation of ground base phenology data from the satellite-based data is observed and the relationship is stage dependent [57,58]. Fan et al. [59] pointed that remote sensing observations are at the plant population or plant community level and ground observations are at the level of individual species and differences in phenological observations may result from the difference in observation scales. Therefore, ground-observed phenology might offer a better and accurate data set with high temporal resolution and detailed information about individual fruit and nut tree species [60]. Schwartz et al. [61] indicated that satellite remote sensing allows exploration of landscape phenological events while ground phenological performance observations are plant species specific during the vegetation period. Nezval et al. [62] demonstrated that the modern method of phenological observation by phenocameras is suitable for mixed forests, but classical ground-based observations by a phenologist are still crucial in order to verify the simulated results using the meteorological approach.

In New Mexico, the timing of fruit and nut trees flowering is critical for the yield and the profitability and early emergence is risky and put crop growth and reproduction under early spring frost conditions. There is greater risk or flower bud vulnerability to low temperature in early spring at Farmington and Los Lunas than at Fabian Garcia as shown by Figures 7–9 and the long-term absolute minimum temperature and average temperature data presented in Table 2. Rodriido [63] reported that the buds lose their cold tolerance ability at the onset of swelling. Frost is the main cause to economic losses in fruit production across the United States [64]. Frost has multiple effects as it can physically damage plant tissue [65,66], cause cellular deshydratation with development of ice crystals [67], diseases infestation through cellular lesions and loss of buds and shoots from 60 to 100% during seven seasons out of eight [66]. Salazar-Gutiérrez et al. [68,69] reported that early spring frosts cause significant injury to floral tissue of sweet cherry and apples and is a resinous problem for fruit trees growers and commercial producers in the Pacific Northwest and other regions of the United States. The risk reported in the present study might be greater for the fruit trees planted in the valley and flood plains in New Mexico. In fact, air temperature is usually lower in the valley and flood plains than on the plateaus during winter time. The research sites under this study are located on the plateaus. Personal communication with fruit trees growers in Farmington area indicated 75% of production loss due to the impact of spring frost on buds, flowers, and young fruits such as apples, sweet cherries, plums, apricots, and peaches.

While this study might provide some guidelines for fruit and nut trees production across the state of New Mexico, it is lacking real field data to confirm the models' performance. Across New Mexico, apples are much more grown in Bernalillo, Rio Amba, Santa Fe, Sandoval, Taos, San Miguel, Dona Ana, and San Juan counties; apricots in Bernalillo, Rio Amba, Otero, and Santa Fe, Taos, and Valencia counties; cherries in Bernalillo,

Rio Amba, Otero, Mora Taos, Valencia counties; grapes in Cibola, Dona Ana, Lincoln, Otero, Rio Amba, Sandoval, San Juan, San Migel, and Socorro counties; pomegranates in Otero, Sierra, and Dona Ana counties; and the nuts (pecans, almonds, chestnuts, walnuts, hazelnuts, and pistachios) are grown in Dona Ana, Eddy, Chaves, Otera, Bernalillo, Lea, Grant, Luna, Sandoval, Sierra, and Valencia counties [52]. It is therefore critical to estimate the chilling and heat requirements for the grapes, cherries, apples, apricots, plums, almonds, peaches, pecans, and other fruit and nuts trees grown across New Mexico for fruit and nut tree production viability and for the profitability of the production system for increasing the revenue of fruit and nut tree growers.

With the global combined ocean and air temperature increase of 0.65–1.06 °C during the 1880–2012 period [70] in general and with the overall increase in annual maximum temperature at the rates that varied from 0.6 to 3.1 °C per century and the increase in the minimum temperatures at the rates that varied from 0.1 to 8 °C over the last century across the southwest Unites States [71], there could be a shift from plant vegetative to reproductive phase in response to the elevated temperature [72]. Campoy et al. [3] indicated that climate change may significantly alter fruit and nut trees growth and reproduction with a reducing impact of the production. Santos et al. [73] projected increase in heat accumulation and decrease in chilling accumulation in Portugal. Lagave et al. [74] and Guo et al. [75] reported anticipated plant phenological timing in fruit trees. Projections showed advancing trends in bloom dates of different fruit and nut trees with changing dormancy breaking processes [76,77]. The shift in air temperature could be beneficial or detrimental to the fruit quality depending on the chilling and heat requirements of the fruit tree species [74,78] which affect budburst, flowering, and fruit maturation [79,80]. With the elevated temperature conditions, chilling accumulation is expected to start later and end earlier with lengthening effect on the endodormancy but shortening chilling accumulation period [81] and this phenomenon will be more pronounced in the regions with mild winter [82,83]. At Fabian Garcia where chill portion is almost null, chilling accumulation risks do not exist and some fruit and nut trees species and cultivars may not be viable at Fabian Garcia while the bud or flower or young fruit vulnerability may be increased at Farmington and Los Lunas. This aligned with Wang et al. [84] who suggested that with the global warming, southward planting has become difficult than the northward planting in the northern hemisphere. Luedeling [16] reported that projections of future chill indicate substantial losses for the warmest growing regions, while temperate regions will experience relatively little change, and cold regions may even see chill increases. Benmoussa et al. [85] reported a decline in the projected winter chilling accumulation by almond, pistachio, and peach cultivars in Tunisia and by the year 2100, pistachios and peaches may experience alarming chill shortfalls and only almonds may remain viable. However, the major source of variation and inaccuracy in chilling assessments is the choice of the chill model used to make the assessment [16,86,87].

Table 4. Some examples of estimated chill portions, chilling hours, and heat requirement by different fruit trees across the globe.

References	Locations	Tree Species	Chill Portions	Chilling Hours	Heat Requirement (GDH)
Bailey et al. [88]	New Jersey, USA	Apricots		873–1343	
Anderson et al. [46]		Sour cherry		954	6130
Linvill [30]	South Carolina, USA		65		
Kuden et al. [89]	California, USA	Pistacho			600–1050
Dokoozlian [90]	California, USA			50–400	
Egea et al. [91]	Santomera, Spain	Almond		266–996	5942–7577
Alonso et al. [92]	Spain	Almond		400–600	5500–9300
Ruiz et al. [34]	Calasparra, Spain	Apricots		596–1266	4078–5879
Okie et al. [93]		Plums		450	
Alburquerque et al. [49]	Murcia, Spain	Sweet cherry	30.4–57.6	335 ± 38–1323 ± 68	7326–9450
Luedeling et al. [40]		Walnuts	53.3–79.5	700	
Rahemi and Pakkish [94]	Iran	Pistachio		750–1400	8 852–15 420
Schalau [95]	Yavapai, Arizona	Fruit trees		500–1000	
Chaar and Astorga [96]	Junín, Argentina	Peach			2177–6490
Luedeling [16]	Klein-Altendorf, Germany	Cherry	68.6 ± 5.7	1375 ± 178	3473 ± 1236

Table 4. *Cont.*

References	Locations	Tree Species	Chill Portions	Chilling Hours	Heat Requirement (GDH)
Campoy et al. [97]	Western Cape, South Africa	Apricot	26.6–57.2	312–1022	
Campoy et al. [97]	Murcia, Spain	Apricot	31–51.8		4605–6247
Luedeling et al. [21]	Beijing, China	Chestnu	79.8 ± 5.3		13466 ± 1918
Luedeling et al. [21]	Klein-Altendorf, Germany	Cherry	104.2 ± 8.9		2698 ± 1183
Prudencio et al. [98]	Spain	Almonds	49–66	308–843	32,225–36,087
Raminrez et al. [99]	Chile	Almonds	23–32	220–440	5814 ± 669–12,341 ± 637
Elloumi et al. [100]	Tunisia	Pistachio	36	206	
Ikinci et al. [101]	Tuekey	Pomegranate			25,000–88,052
Guo et al. [19]	Beijing, China	Jujube	89 ± 6		13,619 ± 2033
Guo et al. [19]	Beijing, China	Chestnut	93 ± 6		17,418 ± 1983
Scott Clark [102]		Pecans	17–83	200–1000	
Funes et al. [103]	Girona, Spain	Apple	62.5–68.4		7416.2 ± 687–10,272.5 ± 1032
Yaacoubi et al. [104]	Palmas, Brazil	Apple	20.3–30.8		6893
Yaacoubi et al. [104]	Marsillargues, France	Apple	39.1–70.8		9443
Yaacoubi et al. [104]	Ain Taoujdate, Morocco	Apple	64.2–67.2		5985
Yaacoubi et al. [104]	Ain Taoujdate, Morocco	Almonds	12.4–16.4		
Zhuang et al. [105]	Nanjing, China	Japanese apricot	24–82		691.9–2634.7
Benmoussa et al. [51]	SfaxEl-Maou, Tunisia	Local almond	3.4–15.5		3962–8873
Benmoussa et al. [51]	SfaxEl-Maou, Tunisia	Foreign almond	6.7–22.6		2894–10,504
Measham et al. [106]	Western Australia	Cherries	30.4–61.7		
Thomppson [107]	Georgia, USA	Peach			800
Montazeran et al. [108]	Iran	Barberry		1400	2904–3432
Gaeta et al. [109]	Italy	Almond	24–62		3263–6699
Chavez et al. [110]	Georgia, USA	Japanese plums			500–900
Chavez et al. [110]	Georgia, USA	Europian plums			700–1000
Díez-Palet et al. [111]	Egypt	Almond	8.40 ± 3.8–52.85 ± 6		6232 ± 1221–10,201 ± 1834
Díez-Palet et al. [111]	Egypt	Apple	37.8 ± 2.7–54.4 ± 3		7471 ± 1191–9501 ± 1556
Kaufmann and Blanke [112]		Sweet cherries		400–750	4000–13,000
Parkes et al. [113]	Australia	Apple	57 ± 2.9–77 ± 1.5	662 ± 44.5–908 ± 23.3	
Yang et al. [114]	Heilongjiang province, China	Ulmus pumila	86		5853
Yang et al. [114]	Heilongjiang province, China	Populus simonii	86		5853
Yang et al. [114]	Heilongjiang province, China	Syringa oblata	86		5853
Nasrabadi et al. [115]	Iran	Pomegranate		605.56–700	7750–9000
Kwon et al. [50]	Republic of Korea	Peach	21.3–74.8	377–1134	4824–5149
Camargo-Alvarez et al. [116]	Washington State, USA	Grapevines		947–1162	

5. Conclusions

The Utah model and the Dynamic model were used to estimate the accumulated chill portions while the Forcing model was used for the potential heat accumulation by fruit and nut trees at Fabian Garcia, Los Lunas, and Farmington in the State of New Mexico (USA). Reasonable chill portions might have been accumulated by the fruit and trees at Los Lunas and Farmington and negligible to very low chill portions occur to be accumulated at Fabian Garcia in the Southern New Mexico. The accumulated heat during trees ecodormancy varied with locations and is not a constraint for fruit and nut production across the study area. While the results of the present study are from the meteorological approach, they could help tree growers, crop consultants, and university researchers in understanding the present trend in fruit and nut trees phenology and production across the study areas. These findings may help in the choice of fruit and/or nut trees species and cultivars across New Mexico with regards to the chilling and heat requirements of the tree species or cultivars for endodormancy and ecodormancy versus the potential accumulation of chilling and heat by the trees and reducing the risk of nonproductive fruit and nut trees under certain environment. They might also be a valuable resource for the adoption of adaptation and mitigation strategies under global warming and improve the resilience of fruit and nut trees production in New Mexico and cope with climate change. With the high probability of bud, flower, and young fruit and nut vulnerability to low temperatures early spring in Farmington and Los Lunas areas, more cold-tolerant cultivars should be adopted for the production sustainability and profitability in those areas. For the future research, in situ observation data should be collected and used to validate the applied models and to develop decision-making tools for fruit and nut trees phenology prediction across New Mexico and the neighboring regions.

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References

1. Delpierre, N.; Vitasse, Y.; Chuine, I.; Guillemot, J.; Bazot, S.; Rutishauser, T.; Rathgeber, B.K.C. Temperate and boreal forest tree phenology: From organ-scale processes to terrestrial ecosystem models. *Ann. For. Sci.* **2016**, *73*, 5–25. [CrossRef]
2. Luedeling, E.; Gebauer, J.; Buerkert, A. Climate change effects on winter chill for tree crops with chilling requirements on the Arabian Peninsula. *Clim. Chang.* **2009**, *96*, 219–237. [CrossRef]
3. Campoy, J.A.; Ruiz, D.; Cook, N.; Allderman, L.; Egea, J. Clinal variation of dormancy progression in apricot. *S. Afr. J. Bot.* **2011**, *77*, 618–630. [CrossRef]
4. Luedeling, E.; Brown, P.H. A global analysis of the comparability of winter chill models for fruit and nut trees. *Int. J. Biometeorol.* **2011**, *55*, 411–421. [CrossRef]
5. Vitasse, Y.; Signarbieux, C.; Fu, Y.H. Global warming leads to more uniform spring phenology across elevations. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 1004–1008. [CrossRef]
6. Fu, Y.H.; Piao, S.; Vitasse, Y.; Zhao, H.; DeBoeck, H.J.; Liu, Q.; Yang, H.; Weber, U.; Hänninen, H.; Janssens, I.A. Increased heat requirement for leaf flushing in temperate woody species over 1980–2012: Effects of chilling, precipitation and insolation. *Glob. Chang. Biol.* **2015**. [CrossRef] [PubMed]
7. Fu, Y.H.; Piao, S.; Zhou, X.; Geng, X.; Hao, F.; Vitasse, Y.; Janssens, I.A. Short photoperiod reduces the temperature sensitivity of leaf-out in saplings of Fagus sylvatica but not in horse chestnut. *Glob. Chang. Biol.* **2019**, *25*, 1696–1703. [CrossRef]
8. Hart, R.; Salick, J.; Ranjitkar, S.; Xu, J. Herbarium specimens show contrasting phenological responses to Himalayan climate. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 10615–10619. [CrossRef]
9. Schieber, B.; Kubov, M.; Janík, R. Effects of climate warming on vegetative phenology of the common beech Fagus sylvatica in a submontane Forest of the Western Carpathians: Two decades analysis. *Pol. J. Ecol.* **2015**, *65*, 339–351. [CrossRef]
10. Signarbieux, C.; Toledano, E.; Sanginés de Carcer, P.; Fu, Y.H.; Schlaepfer, R.; Buttler, A.; Vitasse, Y. Asymmetric effects of cooler and warmer winters on beech phenology last beyond spring. *Glob. Chang. Biol.* **2017**, *23*, 4569–4580. [CrossRef]
11. Yu, L.; Han, Y.; Jiang, Y.; Dong, T.; Lei, Y. Sex-specific responses of bud burst and early development to nongrowing season warming and drought in Populus cathayana. *Can. J. For. Res.* **2018**, *48*, 68–76. [CrossRef]
12. Fu, Y.H.; Zhao, H.; Piao, S.; Peaucelle, M.; Peng, S.; Zhou, G.; Ciais, P.; Huang, M.; Menzel, A.; Peñuelas, J.; et al. Declining global warming effects on the phenology of spring leaf unfolding. *Nature* **2015**, *526*, 104–107. [CrossRef]
13. Lang, G.A.; Early, J.D.; Martin, G.C.; Darnell, R.L. Endo, para-, and ecodormancy: Physiological terminology and classification for dormancy research. *HortScience* **1957**, *22*, 371–377.
14. Basler, D.; Körner, C. Photoperiod and temperature responses of bud swelling and bud burst in four temperate forest tree species. *Tree Physiol.* **2014**, *34*, 377–388. [CrossRef]
15. Harrington, R.; Anton, C.; Dawson, T.P.; De Bello, F.; Feld, C.K.; Haslett, J.R.; Kluvánková, T.; Kontogianni, A.; Lavorel, S.; Luck, G.W.; et al. Ecosystem services and biodiversity conservation: Concepts and a glossary. *Biodivers. Conserv.* **2010**, *19*, 2773–2790. [CrossRef]
16. Luedeling, E. Climate change impacts on winter chill for temperate fruit and nut production: A review. *Sci. Hortic.* **2012**, *144*, 218–229. [CrossRef]
17. Guo, L.; Xu, J.C.; Dai, J.H.; Cheng, J.M.; Luedeling, E. Statistical identification of chilling and heat requirements for apricot flower buds in Beijing China. *Sci. Hortic.* **2015**, *195*, 138–144. [CrossRef]
18. Chen, L.; Huang, J.G.; Ma, Q.; Hänninen, H.; Rossi, S.; Piao, S.; Bergeron, Y. Spring phenology at different altitudes is becoming more uniform under global warming in Europe. *Glob. Chang. Biol.* **2018**, *24*, 3969–3975. [CrossRef] [PubMed]
19. Guo, L.; Dai, J.; Ranjitkar, S.; Yu, H.; Xu, J.; Luedeling, E. Chilling and heat requirements for flowering in temperate fruit trees. *Int. J. Biometeorol.* **2014**, *58*, 1195–1206. [CrossRef] [PubMed]
20. Luedeling, E.; Gassner, A. Partial Least Squares regression foranalyzing walnut phenology in California. *Agric. For. Meteorol.* **2012**, *158*, 43–52.

21. Luedeling, E.; Guo, L.; Dai, J.; Leslie, C.; Blanke, M.M. Differential responses of trees to temperature variation during the chilling and forcing phases. *Agric. For. Meteorol.* **2013**, *181*, 33–42. [CrossRef]
22. Weinberger, J.H. Chilling requirements of peach varieties. *Proc. Am. Soc. Hortic. Sci.* **1950**, *56*, 122–128.
23. Richardson, E.A.; Seeley, S.D.; Walker, D.R. A model for estimating the completion of rest for Redhaven and Elberta peach trees. *HortScience* **1974**, *9*, 331–332.
24. Fishman, S.; Erez, A.; Couvillon, G.A. The temperature dependence of dormancy breaking in plants: Computer simulation of processes studied under controlled temperatures. *J. Theor. Biol.* **1987**, *126*, 309–321. [CrossRef]
25. Fishman, S.; Erez, A.; Couvillon, G.A. The temperature dependence of dormancy breaking in plants: Mathematical analysis of a two-step model involving a cooperative transition. *J. Theor. Biol.* **1987**, *124*, 473–483. [CrossRef]
26. Shaltout, A.D.; Unrath, C.R. Rest completion prediction model for starkrimson delicious apples. *J. Am. Soc. Hortic. Sci.* **1983**, *108*, 957–961.
27. Melo-Abreu, J.P.; Barranco, D.; Cordeiro, A.M.; Tous, J.; Rogadoe, B.M.; Villalobos, F.J. Modelling olive flowering date using chilling for dormancy release and thermal time. *Agric. For. Meteorol.* **2004**, *125*, 117–127.
28. Linsley-Noakes, G.C.; Allan, P. Comparison of 2 models for the prediction of rest completion in peaches. *Sci. Hortic.* **1994**, *59*, 107–113. [CrossRef]
29. Gilreath, P.R.; Buchanan, D.W. Rest prediction model for low-chilling *Sungold* nectarine. *J. Am. Soc. Hort. Sci.* **1981**, *106*, 426–429.
30. Linvill, D.E. Calculating chilling hours and chill units from daily maximum and minimum temperature observations. *HortScience* **1990**, *25*, 14–16. [CrossRef]
31. Erez, A.; Fishman, S.; Linsley-Noakes, G.C.; Allan, P. The dynamic model for rest completion in peach buds. *Acta Hortic.* **1990**, *276*, 165–174. [CrossRef]
32. Erez, A. Bud dormancy; phenomenon, problems and solutions in the tropics and subtropics. In *Temperate Fruit Crops in Warm Climates*; Erez, A., Ed.; Kluwer Academic: Dordrecht, The Netherlands, 2000; pp. 17–48.
33. Cesaraccio, C.; Spano, D.; Snyder, R.L.; Duce, P. Chilling and Forcing model to predict bud-burst of crop and forest species. *Agric. For. Meteorol.* **2004**, *126*, 1–13. [CrossRef]
34. Ruiz, D.; Campoy, J.A.; Egea, J. Chilling and heat requirements of apricot cultivars for flowering. *Environ. Exp. Bot.* **2007**, *61*, 254–263. [CrossRef]
35. Perez, F.J.; Vergara, R.; Or, E. On the mechanism of dormancy release in grapevine buds: A comparative study between hydrogen cyanamide and sodium azide. *Plant Growth Regul.* **2009**, *59*, 145–152. [CrossRef]
36. Luedeling, E.; Zhang, M.H.; Girvetz, E.H. Climatic changes lead to declining winter chill for fruit and nut trees in California during 1950–2099. *PLoS ONE* **2009**, *4*, e6166. [CrossRef]
37. Luedeling, E.; Hale, A.; Zhang, M.; Bentley, W.J.; Dharmasri, L.C. Remote sensing of spider mite damage in California peachorchards. *Int. J. Appl. Earth Obs.* **2009**, *11*, 244–255. [CrossRef]
38. Luedeling, E.; Blanke, M.; Gebauer, J. Climate change effects on winter chill for fruit crops in Germany-Auswirkungen des Klimawandels auf die Verfügbarkeit von Kältewirkung (Chilling) für Obstgehölze in Deutschland. *Erwerbs-Obstbau* **2009**, *51*, 81–94. [CrossRef]
39. Luedeling, E.; Zhang, M.H.; Luedeling, V.; Girvetz, E.H. Sensitivity of winter chill models for fruit and nut trees to climatic changes expected in California's central Valley. *Agric. Ecosyst. Environ.* **2009**, *133*, 23–31. [CrossRef]
40. Luedeling, E.; Zhang, M.; McGranahan, G.; Leslie, C. Validation of winter chill models using historic records of walnut phenology. *Agric. For. Meteorol.* **2009**, *149*, 1854–1864. [CrossRef]
41. Zhang, J.L.; Taylor, C. The dynamic model provides the best description of the chill process on' Sirora' pistachio trees in Australia. *HortScience* **2011**, *46*, 420–425. [CrossRef]
42. Luedeling, E.; Girvetz, H.; Semenov, M.A.; Brown, P.H. Climate change affects winter chill for temperate fruit and nut trees. *PLoS ONE* **2011**, *6*, 13. [CrossRef]
43. Luedeling, E.; Kunz, A.; Blanke, M.M. Identification of chilling and heat requirements of cherry trees a statistical approach. *Int. J. Biometeorol.* **2013**, *57*, 679–689. [CrossRef]
44. Fernandez, E.; Whitney, C.; Cuneo, I.F.; Luedeling, E. Prospects of decreasing winter chill for deciduous fruit production in Chile throughout the 21st century. *Clim. Chang.* **2020**, *159*, 423–439. [CrossRef]
45. Campoy, J.A.; Ruiz, D.; Egea, J. Dormancy in temperate fruit trees in a global warming context: A review. *Sci. Hortic.* **2011**, *130*, 357–372. [CrossRef]
46. Anderson, J.L.; Richardson, E.A.; Kesner, C.D. Validation of chill unit and flower bud phenology models for "Montmorency" sour cherry. *Acta Hortic.* **1986**, *184*, 71–78. [CrossRef]
47. Djaman, K.; O'Neill, M.; Owen, C.K.; Smeal, D.; Koudahe, K.; West, M.; Allen, S.; Lombard, K.; Irmak, S. Crop Evapotranspiration, Irrigation Water Requirement and Water Productivity of Maize from Meteorological Data under Semiarid Climate. *Water* **2018**, *10*, 405. [CrossRef]
48. Powel, A.; Dozier, W.; Williams, D.; Himelrick, D. *Fruit Culture in Alabama. Winter Cgilling Requirements*; The Alabama Cooperative Extension System (Alabama A&M University and Auburn University): Auburn, AL, USA, 2018.
49. Alburquerque, N.; García-Montiel, F.; Carrillo, A.; Burgos, L. Chilling and heat requirements of sweet cherry cultivars and the relationship between altitude and the probability of satisfying the chill requirements. *Environ. Exp. Bot.* **2008**, *64*, 162–170. [CrossRef]

50. Kwon, J.H.; Nam, E.Y.; Yun, S.K.; Kim, S.J.; Song, S.Y.; Lee, J.H.; Hwang, K.D. Chilling and heat requirement of peach cultivars and changes in chilling accumulation spectrums based on 100-year records in Republic of Korea. *Agric. For. Meteorol.* **2020**, *288*, 108009. [CrossRef]
51. Benmoussa, H.; Ghrab, M.; Mimoun, M.B.; Luedeling, E. Chilling and heat requirement for local and foreign almond (*Prunus dulcis* Mill.) cultivars in a Mediterranean location based on 30 years of phenology records. *Agric. For. Meteorol.* **2017**, *239*, 34–46. [CrossRef]
52. USDA-NASS. *Census of Agriculture USDA*; National Agricultural Statistics Service: Washington, DC, USA, 2019; p. 820.
53. Yao, S.; Heerema, R. *Fruits and Nuts for New Mexico Orchards*; NMSU, Cooperative Extension Service, College of Agricultural, Consumer and Environmental Sciences: Las Cruces, NM, USA, 2014; Available online: http://aces.nmsu.edu/pubs/_h/H310.pdf (accessed on 15 December 2020).
54. Allen, R.G.; Pereira, L.S.; Raes, D.; Smith, M. Crop evapotranspiration: Guide-lines for computing crop water requirements–FAO Irrigation and Drainage Paper 56. *FAO Rome* **1998**, *300*, D05109.
55. Darbyshire, R.; Webb, L.; Goodwin, I.; Barlow, S. Winter chilling trends for deciduous fruit trees in Australia. *Agric. For. Meteorol.* **2011**, *151*, 1074–1085. [CrossRef]
56. Djaman, K.; Owen, C.; West, M.M.; Allen, S.; Koudahe, K.; Darapuneni, M.; O'Neill, M. Relationship between Relative Maturity and Grain Yield of Maize (*Zea mays* L.) Hybrids in Northwest New Mexico for the 2003–2019 Period. *Agriculture* **2020**, *10*, 290. [CrossRef]
57. Studer, S.; Stöckli, R.; Appenzeller, C. A comparative study of satellite and ground-based phenology. *Int. J. Biometeorol.* **2007**, *51*, 405–414. [CrossRef]
58. Cui, L.; Shi, J. Evaluation and comparison of growing season metrics in arid and semi-arid areas of northern China under climate change. *Ecol. Indic.* **2021**, *121*, 107055. [CrossRef]
59. Fan, D.; Zhao, X.; Zhu, W.; Sun, W.; Qiu, Y. An improved phenology model for monitoring green-up date variation in Leymus chinensis steppe in Inner Mongolia during 1962–2017. *Agric. For. Meteorol.* **2020**, *291*, 108091. [CrossRef]
60. White, M.A.; Hoffman, F.; Hargrove, W.W. A global framework formonitoring phenologcal responses to climate change. *Geophys. Res. Lett.* **2005**, *32*. [CrossRef]
61. Schwartz, M.D.; Reed, B.C.; White, M.A. Assessing satellite-derived start-of-season (SOS) measures in the conterminous USA. *Int. J. Climatol.* **2002**, *22*, 1793–1805. [CrossRef]
62. Nezval, O.; Krejza, J.; Světlík, J.; Šigut, L.; Horáček, T. Comparison of traditional ground-based observations and digital remote sensing of phenological transitions in a floodplain forest. *Agric. For. Meteorol.* **2020**, *291*, 108079. [CrossRef]
63. Rodrigo, J. Spring frost in deciduous fruit trees morphological damage and flower hardiness. *Sci. Hortic.* **2000**, *85*, 155–173. [CrossRef]
64. Chmielewski, F.M.; Götz, K.P.; Weber, K.C.; Moryson, S. Climate change and spring frost damages for sweet cherries in Germany. *Int. J. Biometeorol.* **2018**, *62*, 217–228. [CrossRef]
65. Cittadini, E.D.; de Ridder, N.; Peri, P.L.; Keulen, H.V. A method for assessing frost damage risk in sweet cherry orchards of South Patagonia. *Agric. For. Meteorol.* **2006**, *141*, 235–243. [CrossRef]
66. Inouye, D.W. Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology* **2008**, *89*, 353–362. [CrossRef] [PubMed]
67. Pearce, R.S. Plant freezing and damage. *Ann. Bot.* **2001**, *87*, 417–424. [CrossRef]
68. Salazar-Gutierrez, M.R.; Chaves, B.; Anothai, J.; Whiting, M.; Hoogenboom, G. Variation in cold hardiness of sweet cherry flower buds through different phenological stages. *Sci. Hortic.* **2014**, *172*, 161–167. [CrossRef]
69. Salazar-Gutierrez, M.R.; Chaves, B.; Hoogenboom, G. Freezing tolerance of apple flower buds. *Sci. Hortic.* **2016**, *198*, 344–351. [CrossRef]
70. IPCC. *Climate Change 2013: The Physical Science Basis. Contribution of Working Group 1 to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Stocker, T.F., Qin, D., Plattner, G.K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, C., Midgley, P.M., Eds.; Cambridge University Press: Cambridge, UK, 2016; p. 1535.
71. Djaman, K.; Koudahe, K.; Bodian, A.; Diop, L.; Ndiaye, P.M. Long-Term Trend Analysis in Annual and Seasonal Precipitation, Maximum and Minimum Temperatures in the Southwest United States. *Climate* **2020**, *8*, 142. [CrossRef]
72. Balasubramanian, S.; Sureshkumar, S.; Lempe, J.; Weigel, D. Potent induction of *Arabidopsis thaliana* flowering by elevated growth temperature. *PLoS Genet.* **2006**, *2*, 0980–0989. [CrossRef]
73. Santos, J.A.; Costa, R.; Fraga, H. Climate change impacts on thermal growing conditions of main fruit species in Portugal. *Clim. Chang.* **2017**, *140*, 273–286. [CrossRef]
74. Legave, J.M.; Guedon, Y.; Malagi, G.; El Yaacoubi, A.; Bonhomme, M. Differentiated responses of apple tree floral phenology to global warming in contrasting climatic regions front. *Plant Sci.* **2015**, *6*, 13. [CrossRef]
75. Guo, L.; Dai, J.; Ranjitkar, S.; Xu, J.; Luedeling, E. Response of chestnut phenology in China to climate variation and change. *Agric. For. Meteorol.* **2013**, *180*, 164–172. [CrossRef]
76. Guédon, Y.; Legave, J.M. Analyzing the time-course variation of apple and pear tree dates of flowering stages in the global warming context. *Ecol. Model.* **2008**, *219*, 189–199. [CrossRef]
77. Legave, J.M.; Giovannini, D.; Christen, D.; Oger, R. Global warming in Europe and its impacts on floral bud phenology in fruit species. *Acta Hortic.* **2009**, *838*, 21–22. [CrossRef]

78. Santesteban, L.G.; Miranda, C.; Royo, J.B. Average dates and accumulated thermal requirements for different phenophases of peach as influenced by climate. In *VII International Peach Symposium, Acta Horticulturae*; Girona, J., Marsal, J., Eds.; International Society for Horticultural Science: Leuven, Belgium, 2012; Volume 962, pp. 277–284.
79. Fraga, H.; Costa, R.; Moutinho-Pereira, J.; Correia, C.M.; Dinis, L.T.; Gonçalves, I.; Silvestre, J.; Eiras-Dias, J.; Malheiro, A.C.; Santos, J.A. Modeling phenology, water status, and yield components of three Portuguese grapevines using the STICS crop model. *Am. J. Enol. Viticult.* **2015**, *66*, 482–491. [CrossRef]
80. Moriondo, M.; Ferrise, R.; Trombi, G.; Brilli, L.; Dibari, C.; Bindi, M. Modelling olive trees and grapevines in a changing climate. *Environ. Model. Softw.* **2015**, *72*, 387–401. [CrossRef]
81. Park, Y.; Lee, B.; Park, H. Predicted effects of climate change on winter chill accumulation by temperate trees in South Korea. *Hortic. J. Preview* **2018**, *87*, 166–173. [CrossRef]
82. Atkinson, C.J.; Brennan, R.M.; Jones, H.G. Declining chilling and its impact on temperate perennial crops. *Environ. Exp. Bot.* **2013**, *91*, 48–62. [CrossRef]
83. Baldocchi, D.; Wong, S. Accumulated winter chill is decreasing in the fruit growing regions of California. *Clim. Chang.* **2008**, *87*, S153–S166. [CrossRef]
84. Wang, S.; Huang, C.; Tao, J.; Zhong, M.; Qu, X.; Wu, H.; Xu, X. Evaluation of chilling requirement of kiwifruit (Actinidia spp.) in south China. *N. Z. J. Crop Hortic. Sci.* **2017**, *45*, 289–298. [CrossRef]
85. Benmoussa, H.; Luedeling, E.; Ghrab, M.; Mimoun, M.B. Severe winter chill decline impacts Tunisian fruit and nut orchards. *Clim. Chang.* **2020**, *162*, 1249–1267. [CrossRef]
86. Wang, X.; Li, D.; Zhang, D.; Shi, X.; Wu, Y.; Qi, Z.; Ding, H.; Zhu, K.; Xia, Y.; Zhang, J. Improving crucial details and selecting the optimal model for evaluating the chilling requirement of *Paeonia lactiflora* Pall. at low latitudes during four winters. *Sci. Hortic.* **2020**, *265*, 109175. [CrossRef]
87. Fernandez, E.; Whitney, C.; Luedeling, E. The importance of chill model selection-a multi-site analysis. *Eur. J. Agron.* **2020**, *119*, 126103. [CrossRef]
88. Bailey, C.H.; Kotowski, S.; Hough, L.F. Estimate of chilling requirements of apricot selections II. *Acta Hortic.* **1982**, *121*, 99–102. [CrossRef]
89. Kuden, A.B.; Kasaka, M.; Tanriver, E. Determining the chilling requirements and growing degree hours of some pistachionut cultivars and regions. *Acta Hortic.* **1995**, *419*, 85–90. [CrossRef]
90. Dokoozlian, N.K. Chilling temperature and duration interact on the budbreak of 'perlette' grapevine cutting. *HortScience* **1999**, *34*, 1054–1056. [CrossRef]
91. Egea, J.; Ortega, E.; Martínez–Gómez, P.; Dicenta, F. Chilling and heat requirements of almond cultivars for flowering. *Environ. Exp. Bot.* **2003**, *50*, 79–85. [CrossRef]
92. Alono, J.M.; Anson, J.M.; Espiau, M.T.; Sociasi Company, R. Determination of endodormancy break in almond flower buds by a correlation model using the average temperature of different day intervals and its application to the estimation of chill and heat requirements and blooming date. *J. Am. Soc. Hortic. Sci.* **2005**, *130*, 308–318. [CrossRef]
93. Okie, W.R.; Hancock, J.F. *Plums. Temperate Fruit Crop Breeding*; Hancock, J.F., Ed.; Springer: Amsterdam, The Netherlands, 2008; pp. 337–357.
94. Rahemi, M.; Pakkish, Z. Determination of chilling and heat requirements of pistachio (Pistacia vera L.) cultivars. *Agric. Sci. China* **2009**, *8*, 803–807. [CrossRef]
95. Schalau, J. Deciduous Fruit Tree Pruning. University of Arizona Cooperative Extension. 2009. Available online: https://cals.arizona.edu/yavapai/anr/hort/byg/archive/fruittreepruning2009.html (accessed on 15 June 2020).
96. Chaar, J.; Astorga, D. Determination of the requirement of cold and heat in peach [*Prunus persica* (L.) Batsch.] through a correlation model. *RIA Rev. Investig. Agropecu.* **2012**, *38*, 289–298.
97. Campoy, J.A.; Ruiz, D.; Allderman, L.; Cook, N.; Egea, J. The fulfillment of chilling requirements and the adaptation of apricot (*Prunus armeniaca* L.) in warm winter climates: An approach in Murcia (Spain) and the Western Cape (South Africa). *Eur. J. Agron.* **2012**, *37*, 43–55. [CrossRef]
98. Prudencio, A.S.; Martínez-Gómez, P.; Dicenta, F. Evaluation of breaking dormancy, flowering and productivity of extra-late and ultra-late flowering almond cultivars during cold and warm seasons in south-east of Spain. *Sci. Hortic.* **2018**, *235*, 39–46. [CrossRef]
99. Ramirez, L.; Sagredo, G.H.; Reginato, G.H. Prediction models for chilling and heat requirement to estimate full bloom of almond cultivars in the Central Valley of Chile. *Acta Hortic.* **2020**, *872*, 107–112. [CrossRef]
100. Elloumi, O.; Ghrab, M.; Mimoun, M.B. Chilling accumulation effects on performance of pistachio trees cv. Mateur in dry and warm area climate. *Sci. Hortic.* **2013**, *159*, 80–87. [CrossRef]
101. Ikinci, A.; Mamay, M.; Unlu, L.; Bolat, I.; Ercisli, S. Determination of heat requirements and effective heat summations of some pomegranate cultivars grown in Southern Anatolia. *Erwerbs-Obstbau* **2014**, *56*, 131–138. [CrossRef]
102. Scott, C. Chill Requirement of Pecans. 2015. Available online: http://www.pecangrowers.org.au/here/wp-content/uploads/Pecan-Growing-Chill-Requirement-of-Pecans.pdf (accessed on 12 October 2020).
103. Funes, I.; Aranda, X.; Biel, C.; Carbo, J.; Camps, F.; Molina, A.J.; Herralde, F.D.; Grau, B.R. Save Future climate change impacts on apple flowering date in a Mediterranean subbasin. *Agric. Water Manag.* **2016**, *164*, 19–27. [CrossRef]

104. Yaacoubi, A.E.; Malagi, G.; Oukabli, A.; Citadin, I.; Hafidi, M.; Bonhomme, M.; Legave, J.M. Differentiated dynamics of bud dormancy and growth in temperate fruittrees relating to bud phenology adaption, the case of apple and almond trees. *Int. J. Biometeorol.* **2016**, *60*, 1695–1710. [CrossRef] [PubMed]
105. Zhuang, W.; Cai, B.; Gao, Z.; Zhang, Z. Determination of chilling and heat requirements of 69 Japanese apricot cultivars. *Eur. J. Agron.* **2016**, *74*, 68–74. [CrossRef]
106. Measham, P.F.; Darbyshire, R.; Turpin, S.R.; Murphy White, S. Complexity in chill calculations: A case study in cherries. *Sci. Hortic.* **2017**, *216*, 134–140. [CrossRef]
107. Thompson, C. Georgia's Peach Farmers Hoping for Colder Winter This Year. 2017. Available online: https://newswire.caes.uga.edu/story.html?storyid=6416secure.caes.uga.edu/extension/publications/files/pdf/B%201518_2.PDF (accessed on 21 June 2020).
108. Montazeran, A.; Khadivi, A.; Khaleghi, A. The first report: Chilling and heat requirements of seedless barberry (*Berberis vulgaris* L. var. asperma). *Sci. Hortic.* **2018**, *231*, 188–193. [CrossRef]
109. Gaeta, L.; Stellacci, A.M.; Losciale, P. Evaluation of three modelling approaches for almond blooming in Mediterranean climate conditions. *Eur. J. Agron.* **2018**, *97*, 1–10. [CrossRef]
110. Chavez, D.; Florkowska, M.; Chen, C.; Cook, J. Home Garden Plums. University of Georgia Extension Bulletin 1518. 2019. Available online: https://secure.caes.uga.edu/extension/publications/files/pdf/B%201518_2.PDF (accessed on 12 October 2020).
111. Díez-Palet, I.; Funes, I.; Savé, R.; Biel, C.; de Herralde, F.; Miarnau, X.; Vargas, F.; Àvila, G.; Carbó, J.; Aranda, X. Blooming under mediterranean climate: Estimating cultivar-specific chill and heat requirements of almond and apple trees using a statistical approach. *Agronomy* **2019**, *9*, 760. [CrossRef]
112. Kaufmann, H.; Blanke, M. Substitution of winter chilling by spring forcing for flowering using sweet cherry as model crop. *Sci. Hortic.* **2019**, *244*, 75–81. [CrossRef]
113. Parkes, H.; Darbyshire, R.; White, N. Chilling requirements of apple cultivars grown in mild Australian winter conditions. *Sci. Hortic.* **2020**, *260*, 108858. [CrossRef]
114. Yang, Y.; Wu, Z.F.; Guo, L.; He, H.S.; Ling, Y.H.; Wang, L.; Zong, S.W.; Na, R.; Du, H.B.; Li, M.H. Effects of winter chilling vs. spring forcing on the spring phenology of trees in a cold region and a warmer reference region. *Sci. Total Environ.* **2020**, *725*, 138323. [CrossRef]
115. Nasrabadi, M.; Ramezanian, A.; Eshghi, S.; Sarkhosh, A. Chilling and heat requirement of pomegranate (*Punica granatum* L.) trees grown under sustained deficit irrigation. *Sci. Hortic.* **2020**, *263*, 109–117. [CrossRef]
116. Camargo-Alvarez, H.; Salazar-gutiérrez, M.; Keller, M.; Hoogenboom, G. Modeling the effect of temperature on bud dormancy of grapevines. *Agric. For. Meteorol.* **2020**, *280*, 107782. [CrossRef]

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Polyphenol-Based Microencapsulated Extracts as Novel Green Insecticides for Sustainable Management of Polyphagous Brown Marmorated Stink Bug (*Halyomorpha halys* Stål, 1855)

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Abstract: The brown marmorated stink bug (*Halyomorpha halys* Stål, 1855) is an invasive polyphagous species that threatens fruit growing both in the United States and Europe. Many pesticide active ingredients have been studied in *H. halys* management, but for sustainable fruit growing, which implies the reduction of chemical harm to the environment, new safe insecticides should be implemented into the practice. For this purpose, novel green insecticide based on natural polyphenols of species *Stevia rebaudiana* (Bertoni) Bertoni and *Aronia melanocarpa* (Michx.) Elliott 1821 was developed. Stevia leaves (SLE) and Aronia pomace (APE) aqueous extracts were prepared using the ultrasound-assisted extraction method. Optimal extraction conditions for bioactive compounds (total polyphenols, flavonoids, anthocyanins, and flavan-3-ols, respectively) and antioxidant activity were determined using response surface methodology. Bioactive compounds rich SLE and APE were encapsulated in calcium alginate microparticles by the ionic gelation method. Physicochemical characteristics (morphology, size, encapsulation efficiency, loading capacity, and swelling) of microparticles showed very good properties with especially high encapsulation efficiency. Fitting to simple Korsmeyer–Peppa's empirical model revealed that the underlying release mechanism of polyphenols is Fickian diffusion. SLE loaded microparticles showed very good pesticidal efficiency against *Halyomorpha halys*, especially on younger larval stages after both contact and digestive treatment. Microparticles loaded with APE did not achieve satisfactory digestive efficiency, but a certain toxic impact has been observed at contact application on all *H. halys* growth stages. Microparticles loaded with SLS exhibited prolonged insecticidal action against *H. halys* and could be a potential candidate as a green insecticide whose application could increase fruit growing safety.

Keywords: stevia leaves; aronia pomace; encapsulation; polyphenols; *Halyomorpha halys*; green insecticides

1. Introduction

An insect that has caught a lot of attention from many scientists in the last several years is the brown marmorated stink bug (*Halyomorpha halys* Stål, 1855; Hemiptera: Pentatomidae). Native to

Eastern Asia, it was introduced to North America [1] and most recently Chile [2]. On the European continent, it was first recorded in Switzerland in 2007 and soon after in Germany, France, Italy, Greece, and Hungary. More recently, it had spread to the rest of Europe, including Croatia [1,3,4]. Pathways of movement of *H. halys* are human-mediated and adults have been found as stowaways in cargo, packing crates, aircraft, machinery, vehicles, and personal luggage [5,6].

Halyomorpha halys is polyphagous and feeds on a wide range of plant species (>170 plant species), including economically important plants and crops [4,7]. In its native and newly invaded range, *H. halys* can cause 100% crop loss in fruit and corn production [1,8,9]. Damage on plants caused by *H. halys* is made by inserting their feeding stylets into fruits, seeds, or pods, which leads to scarring, pitting, faded sunken areas, and deformation [5,9,10]. *Halyomorpha halys* can also transmit different pathogenic bacteria and yeasts to the plants they infest [11]. Moreover, this insect is a human nuisance pest, as adults are known to overwinter inside protected environments (houses) and disturb people in their daily activities [6]. Although *H. halys* does not attack humans, adults release chemical defense compounds that are classified as a clinically significant indoor allergen, which can induce allergic sensitization like rhinitis or conjunctivitis in humans [12].

In the last decade, effectiveness of many pesticide active ingredients in the management of *H. halys* has been studied [7,13–17]. The best effect has been established for several pyrethroids, neonicotinoids, carbamates, some organophosphates, and organochlorines [16]. Till today, no resistance to insecticides has been detected in the management of *H. halys* [17,18]. However, chemical substances often used in the suppression of *H. halys* can harm beneficial arthropods and could cause increasing in pest outbreaks. Moreover, it is the unclear genetic structure of *H. halys* and possible resistance response to this extensive chemical control.

Natural sources of polyphenolic compounds are used in a wide range of industrial applications, as well as traditional medicine and a healthy diet. Polyphenols encompass several classes of structurally diverse natural products biogenetically arising from the shikimate-phenylpropanoids-flavonoids pathways. These compounds are necessary for plant growth, pigmentation, reproduction, resistance to pathogens, and other functions. These adaptive characteristics are a result of natural selection during evolution. Plants can respond this way to diverse enemies (e.g., pests) and stressors, thus making them more resistant [19–21].

Stevia (*Stevia rebaudiana* (Bertoni) Bertoni) [22] and Aronia (*Aronia melanocarpa* (Michx.) Elliott 1821) [23] are mass cultivated plants with relatively high total polyphenolic compound contents. As such, they represent a convenient and economical source of polyphenolic compounds and could be used in the form of simple extract to control insects. It is well documented that polyphenols are used as a repellent to reduce insect infestation through their deterrent properties or anti-feeding effects [24]. A large range of insects belonging to different orders appears to have a sensitivity to polyphenols including, Hemiptera (Homoptera) [25], Lepidoptera [26], Orthoptera [27], and Diptera [28]. While chemical composition and potential beneficial effects on human health of *A. melanocarpa* are well-known [29], the influence of *S. rebaudiana* extract on various insects is not well documented in the literature.

Polyphenols are very sensitive to heat and light, so it is very important to preserve their effectiveness during storage and application. Encapsulation in biopolymeric matrices via the ionic gelation method has been recognized as an effective method in preserving functionality, stability, and bioavailability of polyphenols allowing their controlled release [30]. Furthermore, this method is sustainable, economical, and uses nontoxic biodegradable natural materials, like sodium alginate [31–35]. To suppress the initial repellent properties of polyphenolic compounds, the encapsulation method represents a convenient way of targeted delivery to invasive pests.

Here, we test the potential of natural extracts on the invasive *H. halls*. Our objectives were to (1) optimize the extraction procedure of polyphenols from stevia leaves and black chokeberry pomace with only water as a solvent, (2) formulate microparticles loaded with extracts rich in bioactive compounds, (3) evaluate contact and digestive toxicity of the encapsulated natural extracts on *H. halys*.

2. Materials and Methods

All chemicals used for the experimental procedures were of analytical grade.

2.1. Preparation of Stevia Leaves Extract (SLE)

Optimization of the extraction procedure was performed using DesignExpert 7.0 program (Response surface methodology design, Box–Behnken design) and was used to determine optimal conditions for stevia leaves extraction in terms of total polyphenolic compounds, total flavonoids, and antioxidant activity. Commercially available dry stevia leaves were powdered using FOSS homogenizer 2094 (Hillerød, Denmark) to a mesh size <450 μm, and were weighed out and mixed with 100 mL of distilled water. The extraction of polyphenols from stevia leaves was performed using an ultrasound-assisted extraction (UAE) technique (Hielscher UP200St-G-Ultrasonic generator, Sonotrode S26d14). Optimization of the extraction procedure was based on the following parameters: (i) concentration: 2–6 g/L; (ii) amplitude: 25%–75%; and (iii) time: 3–9 min (Table A1).

2.2. Preparation of Aronia Pomace Extract (APE)

Aronia (Aronia melanocarpa, cv. 'Nero') pomace was obtained from field-collected samples. After the processing of the Aronia sample to produce juice, the dried "spent" pomace was used for further extraction. The extraction procedure was optimized using the DesignExpert 7.0 program (Response surface methodology design, Box–Behnken design) to maximize the yield of polyphenolics and anthocyanins and obtain the highest antioxidant activity for the plant extract. Herein, based on our pre-trials and the literature, the temperature of extraction was taken into consideration since anthocyanins are susceptible to thermal degradation at temperatures above 60 °C and are more stable below this threshold [36]. APE was milled into the powder using FOSS homogenizer 2094 (Hillerød, Denmark) to a mesh size <450 μm and was subjected to UAE in distilled water (100 mL). The extraction of polyphenols from Aronia pomace was performed using an ultrasound-assisted extraction (UAE) technique (Hielscher UP200St-G-Ultrasonic generator, Sonotrode S26d14). Optimization of the extraction procedure was based on the following parameters: (i) concentration: 10–30 g/L; (ii) amplitude: 25%–75%; and (iii) time: 1–3 min (Table A3).

2.3. Determination of Total Polyphenolic Content (TPC)

The modified Folin Ciocalteu's method [37] was used for the determination of TPC. A mixture of 0.1 mL extract (SLE or APE) with 7.9 mL distilled water and 0.5 mL Folin Ciocalteu reagent (diluted with distilled water in 1:2 ratio) and 1.5 mL 20% Na_2CO_3 was left for 2 h to react. The intense blue color was developed and the optical absorbance was measured at 765 nm using a UV−vis spectrophotometer (UV-1700, Shimadzu, Japan) [38]. The calibration curve was plotted using standard gallic acid and the data are expressed as mg gallic acid equivalents (GAE) per L of extract.

2.4. Determination of Total Flavonoids (TF)

The total flavonoids (TF) were determined as reported by Ivanova et al. [39]. One mL of extract was added in a 10 mL volumetric flask containing 4 mL of distilled water. The volume of 300 μL of $NaNO_2$ (0.5 g/L) solution was added to the suspension and after 5 min, 300 μL of $AlCl_3$ (1 g/L), respectively. After 6 min, 2 mL of NaOH (1 mol/L) was added to the mixture. The final volume was set to 10 mL with the addition of distilled water. The optical absorbance was measured at 360 nm against the blank (distilled water) using a UV−vis spectrophotometer (UV-1700, Shimadzu, Japan). The calibration curve was plotted using the quercetin standard and the data are expressed as mg quercetin equivalents (QE) per L of extract.

2.5. Radical Scavenging Assays (ABTS and DPPH)

The antioxidant activity (AA) of the extracts was determined via 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) methods, according to the well-known procedures [40,41], respectively. Briefly, for the DPPH method, a volume of 3.9 mL of methanolic DPPH solution was added to 100 μL of a sample. The free radical-scavenging capacity of the sample was determined by measuring the absorbance decrease at 517 nm after 30 min of incubation against the blank sample. For the ABTS method, an amount of 40 μL of the extract was added to 4 mL of the ABTS radical solution, and the absorbance readings were taken after exactly 6 min against the appropriate reagent blank instead of the sample. Measurements were performed using a UV−vis spectrophotometer (UV-1700, Shimadzu, Japan). For both methods, a water-soluble vitamin E analog, Trolox (100–1000 μM) was used to plot the calibration curve, and the data obtained are expressed as mmol Trolox equivalents (TE) per L of extract.

2.6. Determination of Total Anthocyanins (TA)

TA was determined using a modified method with 1% (*v*/*v*) hydrochloric acid in 70% EtOH solution [42]. Juice samples were diluted, added to the extraction solution and absorbance was measured at 525 nm. Results were calculated as per the equation:

$$Total\ anthocyanins = \frac{A_{525} \times Mr_{malvidin\ 3-glucoside} \times 1000}{\varepsilon \times 5} \quad (1)$$

Measurements were performed using a UV−vis spectrophotometer (UV-1700, Shimadzu, Japan). Results are expressed as mg malvidin 3-glucoside equivalents (M3GE) per L of APE.

2.7. Encapsulation of Bioactive Compounds Using Ionic Gelation Method

Obtained extracts (SLE or APE) were loaded into biopolymeric microparticles. Sodium alginate (1.5% *w*/*v*) and $CaCl_2$ (2% *w*/*v*) solutions were prepared by dissolving the latter, separately in the extracts obtained (SLE or APE). Encapsulation was performed with Büchi—Encapsulator B-390 (Switzerland) via ionic gelation method by dropwise addition of sodium alginate (carrier) enriched with bioactive compounds into the calcium-containing extract solution (cross-linking solution). Conditions of the encapsulations were: Nozzle size 300 μm, frequency of 600 Hz (amplitude 3), and the pressure of 0.4 bar. Microparticles (MPs) were stored overnight in the extract containing Ca^{2+} ions to harden. Two types of microparticles were obtained: (1) MPs containing SLE (Ca-Alg/SLE) and (2) MPs containing APE (Ca-Alg/APE). MPs were air-dried for 24 h until a constant mass was achieved and stored in a sealed container.

2.8. Physicochemical Characterization of Microparticles and Total Polyphenols Release Kinetics

The size of MPs (μm) was determined using optical microscopy (OM) (Leica MZ16a stereomicroscope, Leica Microsystems Ltd., Switzerland). For the determination of encapsulation efficiency, loading capacity, and swelling degree of dry MPs, detailed methods are described in our previous publications [31,33]. The loading capacity of TPC in MPs was determined by dissolving 10 mg of dry microparticles in 5 mL of a mixture of 0.2 M $NaHCO_3$ and 0.06 M $Na_3C_6H_5O_7 \times 2H_2O$ at pH 8 [31]. Results are presented as mg GAE g^{-1} of dry MPs. Release kinetics were observed as a cumulative release (%) of TPC from prepared MPs. To observe the release profile of TPC from MPs loaded with SLE or APE for up to 120 h, 5 g of dry microparticles Ca-Alg/SLE, or 6 g of dry microparticles Ca-Alg/APE was put into 100 mL distilled water [41].

2.9. Preparation of Viscous Solution for the Application of Microparticles

MPs were added to the sodium alginate (0.2% *w*/*v*) solution (5 g/100 mL SLE or 6 g/100 mL APE). The suspension was stirred for 5 min and used as a dipping medium for soybean leaves and pods [43]. Dipping was performed with submersion of leaves/pods and transfer to the Petri dishes.

A laboratory trial was set up in autumn 2019 with adults and third and fourth larval stages of *H. halys* collected in a soybean field in the vicinity of Šašinovec (middle Croatia, 45°50′13.9″ N 16°11′38.9″ E). Collected insects were kept in entomological cages to recover overnight before testing, without additional feeding and previous contact with insecticides. From the same soybean field, leaves and pods have been collected for a digestive experiment.

In two experiments, the contact and digestive efficacy of two encapsulated extracts (SLE or APE) were evaluated. Investigated ingredients and doses are shown in Table 1. Each type of prepared microparticles was evaluated for contact and digestive action.

Table 1. Treatment labels, insect stages, investigated actions, and applied dosage.

Treatment	Stage of Insect	Action Investigated	A Dose of a.i. Applied per Repetition (mg)
Ca-ALG/SLE	adults	contact	150
		digestive	
	3rd larval stage	contact	150
		digestive	
	4th larval stage	contact	150
		digestive	
Ca-ALG/APE	adults	contact	180
		digestive	
	4th larval stage	contact	180
		digestive	
Untreated	adults	contact	water
		digestive	
	3rd larval stage	contact	
		digestive	
	4th larval stage	contact	
		digestive	

For all treatments, ten adult or larvae (depending on variant) of *H. halys* were placed in a Petri dish ($r = 90$ mm). Contact action was evaluated by applying encapsulated ingredients on the bugs in the Petri dishes ($r = 90$ mm) by spraying 0.2% sodium alginate solution containing MPs using a laboratory sprayer in a volume of 3 mL per Petri dish. One Petri dish represented one replicate. Digestive action was evaluated by placing *H. halys* into Petri dishes in which treated soybean leaves and pods were placed. The untreated control for all experiments included a treatment in which bugs were placed into Petri dishes treated with water or, in case of digestive action, they were fed with soybean leaves and pods treated with water. Each application and the investigated action of tested ingredients occurred in four replicates. Each replicate had ten individuals of a specific life stage of *H. halys.* In total 16 different variants were tested on 640 *H. hyalis* individuals.

2.10. Efficacy Assessment and Data Analyses

The number of dead *H. halys* in each Petri dish was determined every 24 h for three days. Based on the number of dead *H. halys* found in the treatment and the untreated control, the efficacy of the ingredients

was determined according to Abbott's formula. Statistical data analysis (one-way ANOVA, Kruskal Wallis test) was performed using ARM 2019® GDM software (Gylling Data Management, 2019) [44].

3. Results and Discussion

3.1. Optimal Extraction Parameters for SLE and APE

Optimization of ultrasound-assisted extraction was performed to achieve an economical method of extraction using only water as a solvent. For SLE, optimal values were found to be at maximum investigated values concentration: 6 g/L, amplitude: 75%, and time: 9 min. (Tables 2 and A2). In this case, increased temperatures did not significantly decrease polyphenols extraction (Figure A1). APE optimal values were found to be at 10.23 g/L, 74.13% amplitude, with 2 min and 55 s of extraction time (Tables 3 and A4). For APE, in our preliminary trials (data not shown), we observed degradation of some polyphenolic compounds (anthocyanins) at higher temperatures, so the maximum temperature threshold was set to ~55 °C (Figure A2) and this was also reported in the literature [36]. The end time of the extraction procedure was significantly lower compared to the optimal SLE. Thus, we observe higher values in terms of total polyphenols in SLE (approx. 2.7×), respectively to the APE, but with significantly higher input in energy and time. Lower TPC in APE may also be ascribed to the fact that "spent" pomace was used in the extraction procedure, where most of the compounds were released from the cells in the process of the juice production. Results for TF/TA and AA were in high correlation (above 0.94). Predicted values were following experimental values, with a relative error for TPC of 0.0694%–0.589%. In the case of TA, a higher relative error value (7.9%) can be observed, which may be ascribed to the relative instability of these compounds during and after the processing (Table 3).

Table 2. Optimal variable setup with predicted-optimal vs. actual-experimental responses for SLE.

Variables	Amplitude (%)	Concentration (g/L)	Time (min)	Desirability
Optimal values	75	6	9	0.984
Responses	TPC (mg GAE/L)	TF (mg QE/L)	ABTS (mmol TE/L)	DPPH (mmol TE/L)
Predicted (Opt.)	465.162	49.061	2.369	3.104
Actual (Exp.)	467.926	49.639	2.416	3.036
Relative error %	0.589	1.164	1.957	2.229

Table 3. Optimal variable setup with predicted-optimal vs. actual-experimental responses for APE.

Variables	Amplitude (%)	Concentration (g/L)	Time (min)	Desirability
Optimal values	70.18	11.52	2 min 55 s	1.000
Responses	TPC (mg GAE/L)	Anthocyanins (mg M3GE/L)	ABTS (mmol TE/L)	DPPH (mmol TE/L)
Predicted (Opt.)	174.3529	12.4934	0.9170	0.8018
Actual (Exp.)	174.2319	11.5050	0.9055	0.7672
Relative error %	0.0694	7.9112	1.2571	4.3113

3.2. Physicochemical Characteristics of Microparticles Loaded with SLE or APE

Morphological characteristics of microparticles are important parameters that should be considered in the formulation and subsequent application of it to pest control, as spherical beads with uniform size distributions are preferable in the delivery of active functional ingredients [45]. The size and shape analysis of microparticles Ca-Alg/SLE and Ca-Alg/APE was undertaken after the encapsulation procedure. In general, both prepared wet microparticles showed similar narrow size distributions (Table 4). There were visible dry extract particles entrapped into the Ca-alginate matrix. The color of prepared formulations was either slightly green (Ca-Alg/SLE) or red (Ca-Alg/APE). This occurred because of the natural colors present in the stevia leaves (chlorophylls) and Aronia pomace (anthocyanins). The prepared MPs were almost spherical, but after drying to constant mass (approximately four weeks on air at room temperature), their sphericity was lost (Figure 1). Stabilization was achieved since we have not observed the degradation of the bioactive compounds

for six months when samples were kept under room temperature in the dark chamber (data not shown). The surface of dried MPs is not smooth and rounded anymore. During the drying process, Ca-Alg/SLE and Ca-Alg/APE MPs significantly decreased in size. The decrease in size was ~45%, resulting from the high-water content in microparticles. There is a loss of irregularity on the surface and shape, which could be explained as a consequence of the water and gel network collapsing during the drying process [46].

Table 4. Physicochemical characteristics of microparticle formulations, Ca-Alg/SLE, and Ca-Alg/APE.

Sample	Size of Dry Microparticles (μm)	Encapsulation Efficiency (%)—TPC	Loading Capacity (mg TPC/g Dry Microparticles)	Swelling Degree (%) of Dry Microparticles
Ca-Alg/SLE *	220.121 ± 48.235	112.229 ± 1.315	2.586 ± 0.178	123.225 ± 1.385
Ca-Alg/APE **	218.657 ± 62.147	111.021 ± 4.521	1.017 ± 0.068	60.111 ± 18.018

* SLE—stevia leaves extract; ** APE—Aronia pomace extract.

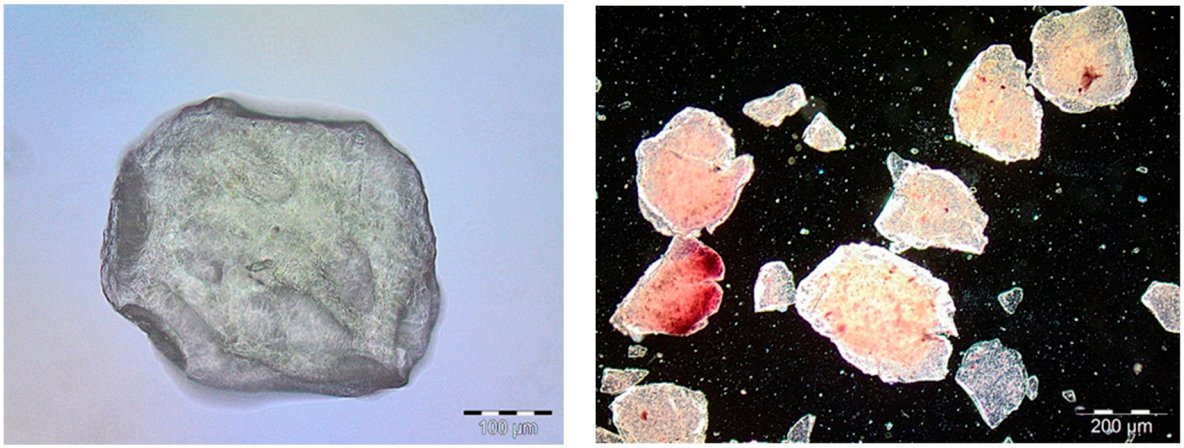

Figure 1. Dry Ca-Alg/SLE microparticle under the light microscope (**left**) and Ca-Alg/APE microparticles under the phase-contrast light microscope (**right**). Scale bars are indicated.

Encapsulation efficiency (EE%) of TPC in Ca-alginate microparticles tends to depend on the physical properties of particles (i.e., structure), and the type of encapsulation process. To overcome polyphenolic losses by diffusion and to maximize encapsulation efficiency during the encapsulation process, extracts were also added to the cross-linking solution. Because of the significant diffusional migration of polyphenolic compounds in and out of the microparticles during the ionic gelation process, the same concentration of solutions (extracts) was prepared both for the carrier (sodium-alginate) and cross-linking-solution ($CaCl_2$). This was undertaken to overcome polyphenolic losses by diffusion and to increase the EE% [45]. High EE% values (over 110%) were then observed (Table 4). Furthermore, others have shown that the drying process also has a significant impact on quality parameters and the content of bioactive compounds encapsulated in microparticles [47].

Swelling of microparticles occurred by water absorption, which loosens the networks of gel structure, resulting in the accelerated release of polyphenol from prepared microparticles [48]. When particles are exposed to continuous water absorption, gel particles erode through excessive swelling. For Ca-alginate microparticles, alginate influenced the density of the gel particle structure, while Ca^{2+} influenced the cross-linking strength [49]. The swelling degree of Ca-Alg/SLE microparticles was almost 50% higher than the swelling degree of Ca-Alg/APE microparticles (Table 4). This could be explained by the variety of present polyphenolic compounds influencing the gel structure firmness. The penetration of water into the denser network with high cross-linking density is difficult, that is, swelling is limited by cross-links and the swelling can be used as a measure of the extent of

cross-linking [50]. Accordingly, loading with SLE indicated a lower cross-linking degree of Ca-Alg/SLE in comparison with Ca-Alg/APE.

Physicochemical characterization revealed good morphological properties, loading capacity, and swelling degree, especially EE% of microparticles loaded with either SLE or APE.

3.3. In Vitro Release of Total Polyphenols from Microparticles Loaded with SLE and APE

Microparticles loaded with SLE or APE prepared by the ionic gelation method through extrusion dripping are reservoirs of bioactive components surrounded by a wall that can control the release. The release profiles for SLE and APE are presented in Figure 2. Both release profiles are characterized by rapid initial release followed by a slower release (obeying the power law equation). A plateau was reached after about 20 min, which leads to quick initial efficiency. SLE release fraction is higher than APE which is following a higher degree of swelling. Moreover, the loading capacity of polyphenols in Ca-Alg/SLE microparticles was relatively higher than in the Ca-Alg/APE microparticles, so the higher fraction of release in the latter could also be explained by this result.

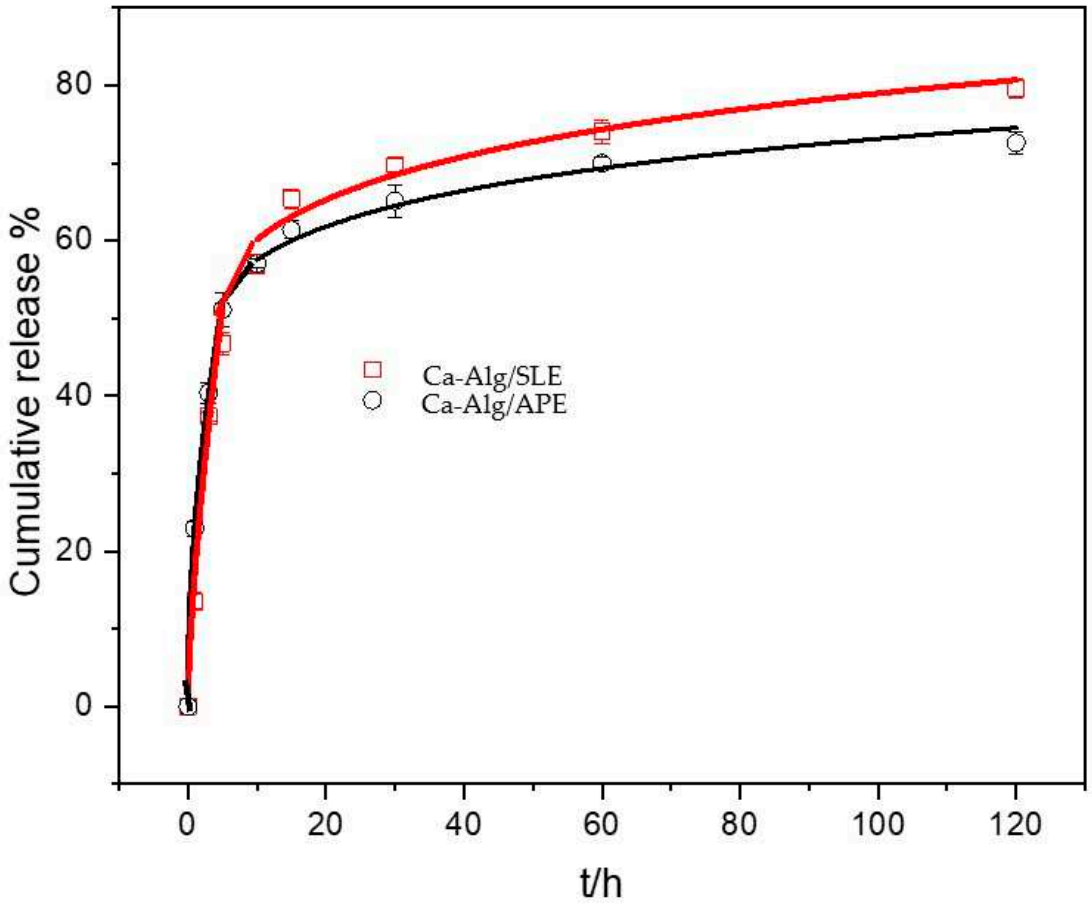

Figure 2. The release profile of total polyphenols from dry microparticles loaded with SLE or APE as the cumulative release (%) over time (min).

To explain and understand the kinetics and type of mechanism involved in the release of total polyphenolic compounds from microparticles, the Korsmeyer–Peppas model was applied [51]. According to this model, different controlling mechanisms may be distinguished by a simple empirical equation:

$$f = kt^n \quad (2)$$

where f is cumulative release at time t, k is a kinetic constant characteristic for a particular system considering structural and geometrical aspects, n is the release exponent representing the release mechanism.

The values of the release constants k, and exponents n obtained by fitting the release curves are listed in Table 5. Lower n values than 0.43 indicate that the release process of polyphenols is controlled by diffusion through microparticles. Differences in microparticle composition (i.e., type of extract) do not affect the controlling release mechanism. The values of kinetic constant (Table 5) indicated that the release rate of APE is slower than of SLE. Slower APE diffusion through the alginate matrix could be ascribed to the denser Ca-Alg/APE structure in comparison Ca-Alg/SLE.

Table 5. Variation of the release constant (k/h), exponent (n), and correlation coefficient (R^2) of polyphenols released from microparticles loaded with SLE and APE.

Dry Microparticles	k/h	n	R^2
Ca-Alg/SLE	50.560	0.0944	0.9989
Ca-Alg/APE	45.127	0.1044	0.9981

Generally, it has been shown that of primary importance in the release of loaded compounds are (i) the calcium alginate network structure, (ii) steric reasons due to the existence of physical entanglements of cross-linked calcium alginate, and (iii) specific interactions between loaded compounds and calcium ions [52]. Polyphenols structure and size of molecules also play a role in possible interactions with alginate residues, all of which are factors that affect release kinetics. This may also explain the possible soldering of specific polyphenols [53].

The underlying mechanism of polyphenols release is diffusion through the alginate gel matrix. This has also been reported for other low-molecular-weight compounds [54]. It means that the rate of polyphenols diffusion is much less than that of polymer swelling and relaxation. Equilibrium of absorption in the surface exposure of the polymeric system takes place rapidly, leading to conditions of time-dependent links. The kinetics of this phenomenon are characterized by diffusivity. The values of kinetic constant (Table 5) exhibited that the release rate of APE is slower than of SLE. Slower APE diffusion through the alginate matrix could be ascribed to the interaction's denser Ca-Alg/APE structure in comparison to Ca-Alg/SLE. From these results, it could be concluded that Ca-alginate microparticles act as a barrier for polyphenol release [46,55,56].

3.4. The Efficiency of Plant-Based Microparticles on H. halys

Results in Table 6 show that stevia-based microparticles have certain efficiency against *H. halys*, especially on younger larval stages (third stage) and that the main efficiency is achieved after contact (73%) but also very good efficiency after digestive (60%) treatment. Older *H. halys* larvae (fourth stage) and adults showed some efficiency only when microparticles have been applied for contact action. Application based on microparticles loaded with APE did not reach the level expected, however, some efficiency has been observed at contact application on both investigated *H. halys* growth stages.

Table 6. Mortality (%) of encapsulated natural extracts in the experiment.

Treatment	A Dose of a.i. Applied per Repetition (mg)	Growth Stage	Investigated Action	Days After the Treatment		
				1	2	3
Ca-Alg/SLE	150	adults	contact	20 $^{ab+}$	20 ^{bc}	20 ^{c}
			digestive	0 ^{c}	0 ^{d}	0 ^{c}
	150	3rd larval stage	contact	25 ^{a}	75 ^{a}	75 ^{a}
			digestive	0 ^{c}	60 ^{a}	60 ^{ab}
	150	4th larval stage	contact	0 ^{c}	30 ^{b}	47.5 ^{b}
			digestive	0 ^{c}	5 ^{cd}	5 ^{c}
Ca-Alg/APE	180	adults	contact	10 ^{bc}	10 ^{cd}	10 ^{c}
			digestive	0 ^{c}	0 ^{d}	0 ^{c}
	180	4th larval stage	contact	5 ^{c}	5 ^{cd}	5 ^{c}
			digestive	0 ^{c}	0 ^{d}	0 ^{c}
Bartlett's X2				5.85	9.14	12.55
P(Bartlett's X2)				0.016 *	0.027 *	0.006 *
P(Kruskal–Wallis X2)				0.006	0.001	0.001

$^{+}$ Mean values of the same column followed by the same letter are not significantly different (* $p \geq 0.05$; LSD test).

It is interesting that in all variants, except the third larval stage in the SLE microparticles variant, significant efficiency occurred on the first day after application and did not increase over time. Considering polyphenolic compound release (Figure 2), it is obvious that SLE and APE both had quick initial insecticidal activity, with prolonged SLE insecticidal action in the following days, and without the further insecticidal activity of APE compounds. In the untreated control, all tested individuals survived.

The insecticidal effect of non-nutritive sweetener Truvia and other sweeteners like mannose has already been proven for some insects. Baudier et al. [57] investigated the effect of Erythritol, non-nutritive sugar alcohol, on species *Drosophila melanogaster* and noted its toxic effect on its movement and life span. Erythritol is the mortality-causing agent within Truvia. The same authors have also emphasized that consumption of Erythritol is safe for humans, even when consumed at high levels, suggesting its use as a novel, human-safe insecticide, which could be applied in urban pest control. Although mannose is toxic to a very important representative of Hymenoptera species (e.g., honeybee), the same effect was not observed for some Dipteran species (e.g., *D. melanogaster* and *Ceratitis capitata*). Erythritol toxicity needs to be inspected in other insect species [56]. Ahmad et al. [58] investigated ethanolic and methanolic extracts and dichloromethane extract of *S. rebaudiana*, *Ginkgo biloba*, and *Parthenium hysterophorous* against insect species *Anopheles stephensi*. It was observed that *S. rebaudiana* methanolic extract caused high larvicidal activity. *S. rebaudiana* can also be used as a repellent plant as its repulsive effect was observed at coffee plantations on coffee berry borer, *Hypothenemus hampei* [59]. Results from our study also demonstrated a potentially good insecticidal effect of *S. rebaudiana* against the third larval stage of invasive *H. halys*. This nuisance pest causes discomfort in humans and causes some health risks, so its suppression needs to be carefully planned to avoid additional side effects on human health. Based on this study SLE loaded microparticles could be promising candidates for urban control of this pest. The insecticidal effect of *A. melanocarpa* on insect species is not known in scientific research, therefore, these results provide the first insights into the effect of APE on insects.

Further tests using SLE- or APE-loaded microparticles, other insects, and other methods of application are needed to determine the full capacity of these potential "green" insecticides. For field applications, phototoxicity, photodegradation, and dispersal ease (among many others) of polyphenolic compounds are necessary to determine the effectiveness of the compounds as a potential bio-pesticide. Additionally, determining the exact mode of action may better facilitate the reduction of the lethal concentration to be comparable to some of the other (bio) insecticides on the market.

4. Conclusions

Ultrasound-assisted extraction presents an economical and effective extraction method for bioactive compounds from stevia leaves and black chokeberry pomace using only water as a solvent. Furthermore, to stabilize natural extracts and make application easier, we have prepared Ca-alginate microparticles loaded with SLE or APE. In the end, a sufficient number of polyphenolic compounds to achieve the desired insecticidal effect was obtained. SLE microparticles showed pesticidal efficiency against *H. halys*, especially on younger larval stages. Very good efficiency was achieved after contact, while lower but still satisfactory efficiency was achieved after digestive treatment. Microparticles loaded with APE did not achieve satisfactory efficiency, probably due to the significantly lower total polyphenolic content, but a certain toxic impact has been observed at contact application on all *H. halys* growth stages. These results provide the first insights into the effect of microencapsulated SLE and APE in insect pest suppression and could be a promising tool in the sustainable management of this invasive species, especially in urban areas and home applications.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A. (Stevia Leaves Extract)

Table A1. Response surface methodology design (Box–Behnken design) for stevia (*Stevia rebaudiana* (Bertoni) Bertoni) leaves extract preparation (random generation).

Std	Run	Amplitude (%)	Concentration (g/L)	Time (min)
10	1	75	4	3
6	2	50	6	3
12	3	75	4	9
13	4	50	4	6
16	5	50	4	6
5	6	50	2	3
7	7	50	2	9
3	8	75	2	6
11	9	25	4	9
9	10	25	4	3
1	11	25	2	6
17	12	50	4	6
2	13	25	6	6
14	14	50	4	6
15	15	50	4	6
4	16	75	6	6
8	17	50	6	9

Table A2. Sequential Model Sum of Squares [Type I].

Response	Source	Sum of Squares	df	Mean Square	F Value	*p*-Value Prob > F
TPC	Quadratic vs. 2FI	1575.52	3	525.17	5.52	0.0291
ABTS	2FI vs. Linear	0.30	3	0.10	4.17	0.0372
DPPH	Linear vs. Mean	5.03	3	1.68	10.05	0.0011
Flavon.	Linear vs. Mean	1456.94	3	485.65	77.31	<0.0001

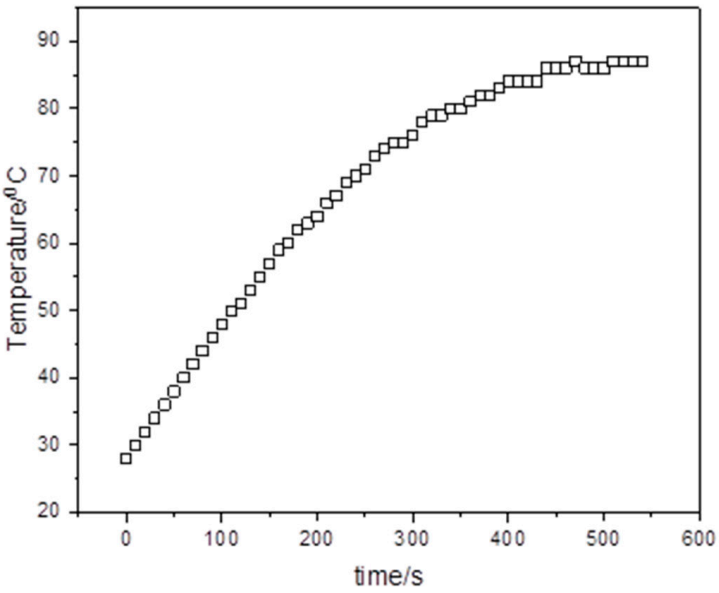

Figure A1. The temperature profile of ultrasound-assisted extraction at optimal variables.

Appendix B. (Black Chokeberry Pomace Extract)

Table A3. Response surface methodology design (Box–Behnken design) for Aronia (*Aronia melanocarpa* (Michx.) Elliott) pomace extract preparation (random generation).

Std	Run	Amplitude (%)	Concentration (g/L)	Time (min)
8	1	75	20	3
17	2	50	20	2
7	3	25	20	3
11	4	50	10	3
16	5	50	20	2
12	6	50	30	3
2	7	75	10	2
13	8	50	20	2
9	9	50	10	1
10	10	50	30	1
15	11	50	20	2
6	12	75	20	1
14	13	50	20	2
3	14	25	30	2
1	15	25	10	2
4	16	75	30	2
5	17	25	20	1

Table A4. Sequential Model Sum of Squares [Type I].

Response	Source	Sum of Squares	df	Mean Square	F Value	*p*-Value Prob > F
TPC	2FI vs. Linear	18.09	3	6.03	12.75	0.0009
ABTS	2FI vs. Linear	3.264×10^{-4}	3	1.088×10^{-4}	6.33	0.0111
DPPH	2FI vs. Linear	2.463×10^{-4}	3	8.210×10^{-4}	5.46	0.0175
Anthocy.	2FI vs. Linear	0.11	3	0.036	65.83	<0.0001

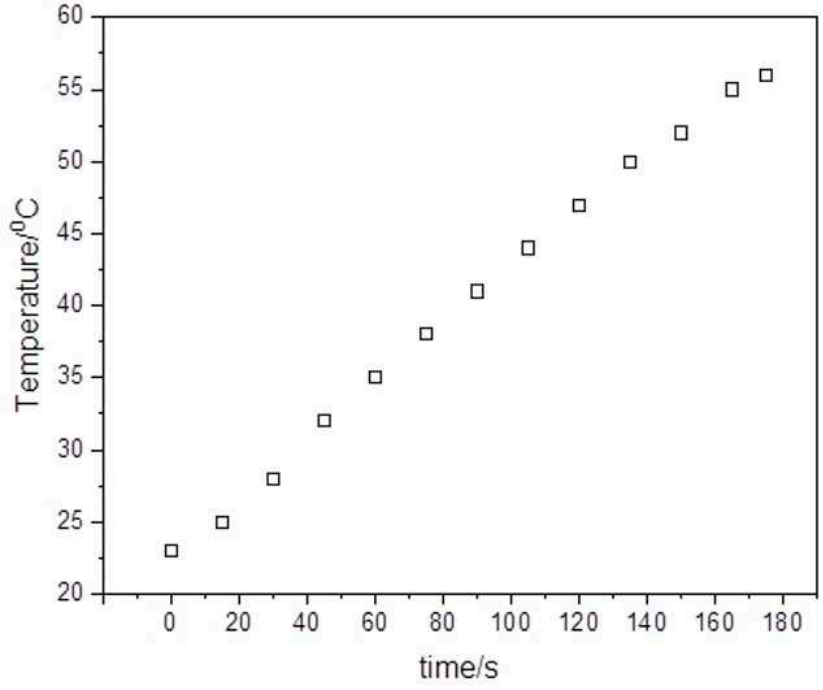

Figure A2. The temperature profile of ultrasound-assisted extraction at optimal variables.

References

1. Bariselli, M.; Bugiani, R.; Maistrello, L. Distribution and damage caused by *Halyomorpha halys* in Italy. *EPPO Bull.* **2016**, *46*, 332–334. [CrossRef]
2. Leskey, T.C.; Nielsen, A.L. Impact of the Invasive Brown Marmorated Stink Bug in North America and Europe: History, Biology, Ecology, and Management. *Annu. Rev. Entomol.* **2018**, *63*, 599–618. [CrossRef]
3. Rot, M.; Devetak, M.; Carlevaris, B.; Žežlina, J.; Žežlina, I. First record of Brown Marmorated Stink Bug (*Halyomorpha hayis* (Stål, 1855)) in Slovenia. *Acta Entomol. Slov.* **2018**, *26*, 5–12.
4. Vetek, G.; Koranyi, D. Severe damage to vegetables by the invasive brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae), in Hungary. *Period. Biol.* **2017**, *119*, 131–135. [CrossRef]
5. Gariepy, T.D.; Bruin, A.; Haye, T.; Milonas, P.; Vétek, G. Occurrence and genetic diversity of new populations of *Halyomorpha halys* in Europe. *J. Pest Sci.* **2015**, *88*, 451–460. [CrossRef]
6. Haye, T.; Gariepy, T.; Hoelmer, K.; Rossi, J.-P.; Streito, J.-C.; Tassus, X.; Desneux, N. Range expansion of the invasive brown marmorated stinkbug, *Halyomorpha halys*: An increasing threat to field, fruit and vegetable crops worldwide. *J. Pest Sci.* **2015**, *88*, 665–673. [CrossRef]
7. Lee, D.-H.; Short, B.D.; Joseph, S.V.; Bergh, J.C.; Leskey, T.C. Review of the Biology, Ecology, and Management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea. *Environ. Entomol.* **2013**, *42*, 627–641. [CrossRef]
8. Nielsen, A.L.; Hamilton, G.C.; Matadha, D. Developmental Rate Estimation and Life Table Analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae). *Environ. Entomol.* **2008**, *37*, 348–355. [CrossRef]
9. Candian, V.; Pansa, M.G.; Briano, R.; Peano, C.; Tedeschi, R.; Tavella, L. Exclusion nets: A promising tool to prevent *Halyomorpha halys* from damaging nectarines and apples in NW Italy. *Bull. Insectol.* **2018**, *71*, 21–30.

10. Maistrello, L.; Vaccari, G.; Caruso, S.; Costi, E.; Bortolini, S.; Macavei, L.; Foca, G.; Ulrici, A.; Bortolotti, P.P.; Nannini, R.; et al. Monitoring of the *invasive Halyomorpha halys*, a new key pest of fruit orchards in northern Italy. *J. Pest Sci.* **2017**, *90*, 1231–1244. [CrossRef]
11. Rice, K.B.; Bergh, C.J.; Bergmann, E.J.; Biddinger, D.J.; Dieckhoff, C.; Dively, G.; Fraser, H.; Gariepy, T.; Hamilton, G.; Haye, T.; et al. Biology, Ecology, and Management of Brown Marmorated Stink Bug (Hemiptera: Pentatomidae). *J. Integr. Pest Manag.* **2014**, *5*, 1–13. [CrossRef]
12. Mertz, T.L.; Jacobs, S.B.; Craig, T.J.; Ishmael, F.T. The brown marmorated stinkbug as a new aeroallergen. *J. Allergy Clin. Immunol.* **2012**, *130*, 999–1001.e1. [CrossRef]
13. Nielsen, A.L.; Hamilton, G.C. Seasonal Occurrence and Impact of *Halyomorpha halys* (Hemiptera: Pentatomidae) in Tree Fruit. *J. Econ. Entomol.* **2009**, *102*, 1133–1140. [CrossRef] [PubMed]
14. Nielsen, A.L.; Hamilton, G.C.; Shearer, P.W. Seasonal Phenology and Monitoring of the Non-Native *Halyomorpha halys* (Hemiptera: Pentatomidae) in Soybean. *Environ. Entomol.* **2011**, *40*, 231–238. [CrossRef]
15. Lee, D.-H.; Cullum, J.P.; Anderson, J.L.; Daugherty, J.L.; Beckett, L.M.; Leskey, T.C. Characterization of Overwintering Sites of the Invasive Brown Marmorated Stink Bug in Natural Landscapes Using Human Surveyors and Detector Canines. *PLoS ONE* **2014**, *9*, e91575. [CrossRef]
16. Leskey, T.C.; Short, B.D.; Butler, B.R.; Wright, S.E. Impact of the Invasive Brown Marmorated Stink Bug, *Halyomorpha halys* (Stål), in Mid-Atlantic Tree Fruit Orchards in the United States: Case Studies of Commercial Management. *Psyche A J. Entomol.* **2012**, *2012*, 1–14. [CrossRef]
17. Lee, D.-H.; Leskey, T.C. Flight behavior of foraging and overwintering brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae). *Bull. Entomol. Res.* **2015**, *105*, 566–573. [CrossRef]
18. Zobel, E.S.; Hooks, C.R.R.; Dively, G.P. Seasonal Abundance, Host Suitability, and Feeding Injury of the Brown Marmorated Stink Bug, *Halyomorpha halys* (Heteroptera: Penatomidae), in Selected Vegetables. *J. Econ. Entomol.* **2016**, *109*, 1289–1302. [CrossRef]
19. Lattanzio, V.; Lattanzio, V.M.T.; Cardinali, A. Role of Polyphenols in the Resistance Mechanisms of Plants against Fungal Pathogens and Insects. In *Phytochemistry, Advances in Research*; Imperato, F., Ed.; Research Signpost: Trivandrum, Kerala, India, 2006; pp. 23–67.
20. Vinceković, M.; Maslov Bandić, L.; Jurić, S.; Jalšenjak, N.; Čaić, A.; Živičnjak, I.; Đermić, E.; Karoglan, M.; Osrečak, M.; Topolovec-Pintarić, S. The enhancement of bioactive potential in Vitis vinifera leaves by application of microspheres loaded with biological and chemical agents. *J. Plant Nutr.* **2019**, *42*, 543–558. [CrossRef]
21. Jurić, S.; Sopko Stracenski, K.; Król-Kilińska, Ż.; Žutić, I.; Fabek Uher, S.; Đermić, E.; Topolovec-Pintarić, S.; Vinceković, M. The enhancement of plant secondary metabolites content in Lactuca sativa L. by encapsulated bioactive agents. *Sci. Rep.* **2020**, *10*, 3737. [CrossRef]
22. Myint, K.; Wu, K.; Xia, Y.; Fan, Y.; Shen, J.; Zhang, P.; Gu, J. Polyphenols from Stevia rebaudiana (Bertoni) leaves and their functional properties. *J. Food Sci.* **2020**, *85*, 240–248. [CrossRef]
23. Lee, J.E.; Kim, G.-S.; Park, S.; Kim, Y.-H.; Kim, M.-B.; Lee, W.S.; Jeong, S.W.; Lee, S.J.; Jin, J.S.; Shin, S.C. Determination of chokeberry (*Aronia melanocarpa*) polyphenol components using liquid chromatography–tandem mass spectrometry: Overall contribution to antioxidant activity. *Food Chem.* **2014**, *146*, 1–5. [CrossRef]
24. Simmonds, M.S.J.; Blaney, W.M.; Delle Monache, F.; Marini Bettolo, G.B. Insect antifeedant activity associated with compounds isolated from species ofLonchocarpus andTephrosia. *J. Chem. Ecol.* **1990**, *16*, 365–380. [CrossRef]
25. Dreyer, D.L.; Jones, K.C. Feeding deterrency of flavonoids and related phenolics towards Schizaphis graminum and Myzus persicae: Aphid feeding deterrents in wheat. *Phytochemistry* **1981**, *20*, 2489–2493. [CrossRef]
26. De Paula, J.R.; Vieira, I.J.C.; Das, M.F.; Da Silva, M.F.; Fo, E.R.; Fernandes, J.B.; Vieira, P.C.; Pinheiro, A.; Vilela, E.F. Sesquiterpenes, triterpenoids, limonoids and flavonoids of Cedrela odorata graft and speculations on the induced resistance against Hypsipyla grandella. *Phytochemistry* **1997**, *44*, 1449–1454. [CrossRef]
27. Dini, J.; Owen-Smith, N. Condensed tannin in Eragrostis chloromelas leaves deters feeding by a generalist grasshopper. *Afr. J. Range Forage Sci.* **1995**, *12*, 49–52. [CrossRef]
28. Capasso, R.; Evidente, A.; Tremblay, E.; Sala, A.; Santoro, C.; Cristinzio, G.; Scognamiglio, F. Direct and mediated effects on Bactrocera oleae (Gmelin) (Diptera; Tephritidae) of natural polyphenols and some of related synthetic compounds: Structure-activity relationships. *J. Chem. Ecol.* **1994**, *20*, 1189–1199. [CrossRef]

29. Kulling, S.; Rawel, H. Chokeberry (*Aronia melanocarpa*)—A Review on the Characteristic Components and Potential Health Effects. *Planta Med.* **2008**, *74*, 1625–1634. [CrossRef]
30. Belščak-Cvitanović, A.; Jurić, S.; Đorđević, V.; Barišić, L.; Komes, D.; Ježek, D.; Bugarski, B.; Nedović, V. Chemometric evaluation of binary mixtures of alginate and polysaccharide biopolymers as carriers for microencapsulation of green tea polyphenols. *Int. J. Food Prop.* **2017**, *20*, 1971–1986. [CrossRef]
31. Vinceković, M.; Jalšenjak, N.; Topolovec-Pintarić, S.; Đermić, E.; Bujan, M.; Jurić, S. Encapsulation of Biological and Chemical Agents for Plant Nutrition and Protection: Chitosan/Alginate Microcapsules Loaded with Copper Cations and Trichoderma viride. *J. Agric. Food Chem.* **2016**, *64*, 8073–8083. [CrossRef]
32. Vinceković, M.; Topolovec-Pintarić, S.; Jurić, S.; Viskić, M.; Bujan, M.; Đermić, E.; Žutić, J.; Fabek Uher, S. Release of Trichoderma viride Spores from Microcapsules Simultaneously Loaded with Chemical and Biological Agents. *Agric. Conspec. Sci.* **2017**, *82*, 395–401.
33. Vinceković, M.; Jurić, S.; Đermić, E.; Topolovec-Pintarić, S. Kinetics and Mechanisms of Chemical and Biological Agents Release from Biopolymeric Microcapsules. *J. Agric. Food Chem.* **2017**, *65*, 9608–9617. [CrossRef]
34. Jurić, S.; Šegota, S.; Vinceković, M. Influence of surface morphology and structure of alginate microparticles on the bioactive agents release behavior. *Carbohydr. Polym.* **2019**, *218*, 234–242. [CrossRef]
35. Jurić, S.; Đermić, E.; Topolovec-Pintarić, S.; Bedek, M.; Vinceković, M. Physicochemical properties and release characteristics of calcium alginate microspheres loaded with *Trichoderma viride* spores. *J. Integr. Agric.* **2019**, *18*, 2534–2548. [CrossRef]
36. Loypimai, P.; Moongngarm, A.; Chottanom, P. Thermal and pH degradation kinetics of anthocyanins in natural food colorant prepared from black rice bran. *J. Food Sci. Technol.* **2016**, *53*, 461–470. [CrossRef]
37. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178. [CrossRef]
38. Ough, C.S.; Amerine, M.A. Nitrogen compounds. In *Methods Analysis of Musts and Wines*; Wiley-Interscience: New York, NY, USA, 1988; pp. 172–195.
39. Ivanova, V.; Stefova, M.; Chinnici, F. Determination of the polyphenol contents in Macedonian grapes and wines by standardized spectrophotometric methods. *J. Serbian Chem. Soc.* **2010**, *75*, 45–59. [CrossRef]
40. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]
41. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
42. Fuleki, T.; Francis, F.J. Quantitative Methods for Anthocyanins: 1. Extraction and Determination of Total Anthocyanins in Cranberries. *J. Food Sci.* **1968**, *33*, 266–274. [CrossRef]
43. IRAC. Susceptibility Test Method 033. Available online: https://irac-online.org/content/uploads/Method_033_Lygus_30March16.pdf (accessed on 15 September 2019).
44. Gylling Data Management Inc. *ARM 2019® GDM Software, Revision 2019.4, 5 August 2019 (B = 25105)*; Gylling Data Management Inc.: Brookings, South Dakota, 2019.
45. Arriola, N.D.A.; Chater, P.I.; Wilcox, M.; Lucini, L.; Rocchetti, G.; Dalmina, M.; Pearson, J.P.; Dias de Mello, R.; Amboni, C. Encapsulation of stevia rebaudiana Bertoni aqueous crude extracts by ionic gelation—Effects of alginate blends and gelling solutions on the polyphenolic profile. *Food Chem.* **2019**, *275*, 123–134. [CrossRef]
46. Dai, Y.; Li, P.; Zhang, J.; Wang, A.; Wei, Q. Swelling characteristics and drug delivery properties of nifedipine-loaded pH sensitive alginate–chitosan hydrogel beads. *J. Biomed. Mater. Res. Part B Appl. Biomater.* **2008**, *86B*, 493–500. [CrossRef]
47. Katsube, T.; Tsurunaga, Y.; Sugiyama, M.; Furuno, T.; Yamasaki, Y. Effect of air-drying temperature on antioxidant capacity and stability of polyphenolic compounds in mulberry (*Morus alba* L.) leaves. *Food Chem.* **2009**, *113*, 964–969. [CrossRef]
48. Sriamornsak, P.; Thirawong, N.; Korkerd, K. Swelling, erosion and release behavior of alginate-based matrix tablets. *Eur. J. Pharm. Biopharm.* **2007**, *66*, 435–450. [CrossRef]
49. Draget, K.I.; Gaserod, O.; Aune, I.; Andersen, P.O.; Storbakken, B.; Stokke, B.T.; Smidsrod, O. Effects of molecular weight and elastic segment flexibility on syneresis in Ca-alginate gels. *Food Hydrocoll.* **2001**, *15*, 485–490. [CrossRef]
50. Roger, S.; Talbot, D.; Bee, A. Preparation and effect of Ca^{2+} on water solubility, particle release and swelling properties of magnetic alginate films. *J. Magn. Magn. Mater.* **2006**, *305*, 221–227. [CrossRef]

51. Korsmeyer, R.W.; Gurny, R.; Doelker, E.; Buri, P.; Peppas, N.A. Mechanisms of solute release from porous hydrophilic polymers. *Int. J. Pharm.* **1983**, *15*, 25–35. [CrossRef]
52. Rousseau, I.; Le Cerf, D.; Picton, L.; Argillier, J.F.; Muller, G. Entrapment and release of sodium polystyrene sulfonate (SPS) from calcium alginate gel beads. *Eur. Polym. J.* **2004**, *40*, 2709–2715. [CrossRef]
53. Martinsen, A.; Skjåk-Braek, G.; Smidsrød, O. Alginate as immobilization material: I. Correlation between chemical and physical properties of alginate gel beads. *Biotechnol. Bioeng.* **1989**, *33*, 79–89. [CrossRef]
54. Stojanovic, R.; Belscak-Cvitanovic, A.; Manojlovic, V.; Komes, D.; Nedovic, V.; Bugarski, B. Encapsulation of thyme (*Thymus serpyllum* L.) aqueous extract in calcium alginate beads. *J. Sci. Food Agric.* **2012**, *92*, 685–696. [CrossRef]
55. Kim, E.S.; Lee, J.-S.; Lee, H.G. Calcium-alginate microparticles for sustained release of catechin prepared via an emulsion gelation technique. *Food Sci. Biotechnol.* **2016**, *25*, 1337–1343. [CrossRef]
56. Ćujić, N.; Trifković, K.; Bugarski, B.; Ibrić, S.; Pljevljakušić, D.; Šavikin, K. Chokeberry (*Aronia melanocarpa* L.) extract loaded in alginate and alginate/inulin system. *Ind. Crops Prod.* **2016**, *86*, 120–131. [CrossRef]
57. Baudier, K.M.; Kaschock-Marenda, S.D.; Patel, N.; Diangelus, K.L.; O'Donnell, S.; Marenda, D.R. Erythritol, a Non-Nutritive Sugar Alcohol Sweetener and the Main Component of Truvia®, Is a Palatable Ingested Insecticide. *PLoS ONE* **2014**, *9*, e98949. [CrossRef]
58. Ahmad, N.; Fazal, H.; Abbasi, B.H.; Iqbal, M. In vitro larvicidal potential against Anopheles stephensi and antioxidative enzyme activities of *Ginkgo biloba*, *Stevia rebaudiana* and *Parthenium hysterophorous*. *Asian Pac. J. Trop. Med.* **2011**, *4*, 169–175. [CrossRef]
59. Castro, A.M.; Tapias, J.; Ortiz, A.; Benavides, P.; Góngora, C.E. Identification of attractant and repellent plants to coffee berry borer, *Hypothenemus hampei*. *Entomol. Exp. Appl.* **2017**, *164*, 120–130. [CrossRef]

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Evaluation of the Characteristics of Native Wild Himalayan Fig (*Ficus palmata* Forsk.) from Pakistan as a Potential Species for Sustainable Fruit Production

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Abstract: Wild Himalayan figs (*Ficus palmata* Forsk.), native to East Asia and the Himalayan region, are closely related to the well-known cultivated fig (*Ficus carica* L.), which is grown mainly in the Mediterranean region. The Pakistani state of Azad Jammu and Kashmir has a rich variety of figs. However, no comprehensive study has been carried out to utilise the diversity of these wild figs for possible use in sustainable fruit production. Therefore, the present study was designed to assess the variability of 35 wild fig accessions using quantitative and qualitative traits. Descriptive statistics were used to measure quantitative characteristics, while the coefficient of variance (CV %) was analysed using SAS® version 9.1. A principal component analysis (PCA) and multivariate analysis were performed using R Studio (v1.1.4). Pearson correlation coefficients between characteristics were obtained using SPSS software. The studied accessions showed high variability and the coefficient of variation (CV) ranged from 4.46–14.81%. Days to maturity varied from 71 to 86, leaf area from 38.55 to 90.06 cm^2. The fruit length, fruit diameter and fruit weight ranged from 11.25 to 29.85 mm, 11.85 to 27.49 mm and 2.65 to 9.66 g, respectively. The photosynthetic activity and total chlorophyll content also varied from 7.94 to 10.22 µmol CO_2 $m^{-2}s^{-1}$ and 37.11 to 46.48 µgml^{-1}. In most of the fig accessions studied, apical dominance was found to be 'absent' while fruit shape was observed to be 'globular'. A strong correlation was observed between all the studied characteristics. In the PCA analysis, all 35 fig accessions were distributed in four quadrants and showed a great diversity. This could be a valuable gene pool for future breeding studies and provide improved quality varieties. Wild Himalayan figs from the wild are well adapted to local pedoclimatic conditions and, combined with easy propagation and production can contribute to the local economy and have a significant impact on the socio-economic and ecological balance. The results of this study show high variability in some of the studied traits of 35 accessions from different parts of Northeast Pakistan, indicating their good potential for further enhancement and utilisation in sustainable agricultural production.

Keywords: native germplasm; temperate fruit; wild fig; biodiversity; conservation

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1. Introduction

Figs are one of the oldest domesticated fruits of the Mediterranean region [1] and are native to western Asia and eastern parts of the Mediterranean countries [2]. They were

domesticated five thousand years earlier than millet and wheat [3]. Based on historical facts, scientists are very interested in exploring the genetic diversity of these species [4]. Figs are gynodioecious species and are pollinated by the wasp (*Blastophaga psenes* L.) [4,5]. The edible part of figs is the fruit, which is fleshy, hollow and receptive [6]. There are three types of figs, depending on how they are grown, e.g., the Common type, the Smyrna type and the San Pedro type. The Common type produces parthenocarpic fruit without pollination for either the breba (first) or the main crop. The Smyrna type, on the other hand, requires caprifig for pollination, while the San Pedro type produces the first fruit without pollination while the second fruit requires caprifig for pollination [7]. Fig types have generally adapted to different soils and climatic conditions and are therefore widely grown in many regions of the world.

Fig fruits are a good source of minerals and bioactive compounds. They are considered healthy fruits because they are a rich source of minerals (iron, calcium, potassium), amino acids (aspartic acid, glutamine) fiber and carotenoids such as lycopene, cryptoxanthin and β-carotene [6,8]. Fresh figs provide a large amount of antioxidants, polyphenols and flavonoids (catechin, epicatechin) [9]. In addition, figs are free of fats and cholesterol and are a good source of sugars (fructose, glucose), organic acids and volatile compounds that enhance the flavour of the fruit [8,9]. In addition, fig fruits are a rich source of phenolic compounds that effectively contribute to colour formation, flavour and aroma [10,11]. Moreover, the colour of the flesh and skin affect the accumulation of phenolic compounds and the antioxidant capacity in fruits [12]. Recently, several studies have shown that fig cultivars and growing locations influence antioxidant potential and other chemical properties such as total soluble solids, sugars, and organic acids [8,12]. It has also been reported that fig fruits, roots and leaves are used in various conventional medicines that are effective against certain ailments such as gastrointestinal, respiratory, cardiovascular and anti-inflammatory problems [13].

The genetic diversity of fruit plant species is under threat by commercialization, which must be preserved using existing genetic resources. This will not only ensure the survival of such species for long term studies but also ensure sufficient variability among species for future breeding programs. It is well known that wild species such as *Ficus palmata* in northeastern Pakistan are well adapted to local pedoclimatic conditions and, combined with easy propagation and production can contribute to the local economy and have a significant impact on socioeconomic and ecological balance. Farmers have good opportunities in this region to use wild figs to increase and secure their income and provide a sustainable food source by using indigenous wild fruit species [14,15].

The unripe fruits and young shoots are cooked and eaten as a vegetables [16]. The leaves of some forage plants are fed to animals in the acute winter season, and *F. palmata* is one of the most important winter forage trees in some areas [17]. Energy plantations have proven to be a viable agroforestry system for the hills and *F. palmata* has proved to be suitable for energy plantations [18]. *F. palmata* biomass is an excellent source of milk-coagulating enzymes [19]. Some studies provide a pharmacological basis for the traditional use of the fruits of *F. palmata* in the treatment of pain and indicate the future prospects of the wild fig in the medicinal industry [20].

In Azad Jammu and Kashmir, most of the edible fruit species are not properly conserved. However, a few species of figs are cultivated by the local population, which reduces the threat to the rare wild edible fruit trees found in the region. The state of Azad Jammu and Kashmir is part of the Lesser Himalayan mountain ranges in north-eastern Pakistan, extending from the low subtropical plains in the south to high alpine slopes with altitudes of 3000 m or more in the north [21]. Topographic variations, altitudinal aspects and vegetation cover have a great influence on the climatic conditions of the mountain ranges of the Lesser Himalaya. Therefore, the inner and outer parts of the ranges differ greatly in terms of rainfall, snowfall and temperature, which in turn affect the production of wild edible fruits [22]. In Azad Jammu and Kashmir, edible figs are generally grown in natural forests and marginal lands, with very low economic returns as most of the fruit is consumed

locally. The total area under fig cultivation is 125 hectares and the production is 500 tonnes in Pakistan, which is very low compared to the rest of the world [22].

Since figs are perishable, they are dried naturally under sunlight in Kashmir [23]. Figs produced in central Kashmir have a very high fiber content. They are also reported to be a very good source of calcium and potassium [23]. Dried Kashmiri figs contain omega-3 and omega-6 fatty acids, which are considered useful for preventing heart disease and can cure sore throats due to their high mucilage content [23]. Due to their delicious taste and freshness, the dried figs enjoy high demand from customers within and outside Kashmir. In Azad Jammu and Kashmir, Pakistani fig species, particularly *F. palmata*, have a large number of accessions, which are under threat of genetic erosion. Furthermore, no comprehensive study has been conducted to exploit the diversity of these wild figs for possible use in sustainable fruit production. Therefore, the present study was undertaken as an initiative measure to assess the morphological and pomological diversity of the existing germplasm of wild edible fig in Azad Jammu and Kashmir and to identify promising trees with high fruit quality.

2. Materials and Methods

Thirty-five wild figs (*Ficus palmate* Forsk.) were collected from 10 districts [Mirpur (MP), Bhimber (BH), Kotli (KT), Sudhnuti (SD), Poonch (PN), Bagh (BG), Haveli (HV), Muzaffarabad (MZ), Jhelum Valley (JV), Neelum (NL)] representing three divisions, i.e., Mirpur, Poonch and Muzaffarabad of Azad Jammu and Kashmir, Pakistan (Table 1, Figure 1).

For data analysis, 10 mature leaves and 10 mature fruits were taken randomly from each tree and 10 trees were used per replicate. A total of 33 traits (13 qualitative and 20 quantitative) were examined. A digital caliper (model: 0–150 mm; MC China) with an accuracy of 0.10 mm was used to measure petiole length, petiole thickness, fruit length, fruit diameter, fruit petiole length, petiole width, and fruit skin thickness. The weight of the fruit was determined with the help of an electric balance. Penetrometer (Willowbank Electronics, Waiohiki, New Zealand) was used to determine fruit firmness, while fruit colour was measured using chromameter (model: CR-400, Konica Minolta, Inc., Tokyo, Japan). Portable photosynthetic apparatus CIRAS-3 (PP Systems, Amesbury, MA, USA) was used to measure photosynthetic activity while total chlorophyll content was measured by Arnon's method [24] using spectrophotometer (Model: SP -3000 Plus Optima, Tokyo, Japan) in postharvest laboratory of Department of Horticulture, PMAS Arid Agriculture, Rawalpindi, Pakistan.

2.1. Evaluation of the Qualitative Characteristics

Thirteen qualitative characteristics, i.e., tree growth habit (TGH), tree vigour (TVg), relative degree of branching (RDB), apical dominance (ADm), leaf colour (LCl), leaf shape (LSh), leaf base shape (LBS), leaf margin (LMr), leaf margin serration (LMrS), leaf veining (LVn), fruit shape (FSh), flesh colour (ClF) and peelability (Pe) were visually recorded to assess diversity using fig descriptors provided by IPGRI and CIHEAM [25].

2.2. Assessment of Quantitative Traits

Twenty quantitative traits, i.e., days to maturity (DtM), shoot length (SLn), number of leaves per shoot (nLS), leaf length (LLn), leaf width (LWd), leaf area (LAr), leaf petiole length (LPLn), leaf petiole thickness (LPTh), number of fruits per shoot (nFS), fruit length (Fln), fruit diameter (FDm), fruit stalk length (FSLn), fruit weight (FWt), ostiole width (OWd), fruit skin thickness (FSTh), fruit firmness (FFn), colour L* [(ClL*), white (100) to black (0)], colour a* [(Cla*), green (−) to red (+)], photosynthetic activity (PAc) and total chlorophyll content (TCC) were observed to analyse diversity among 35 wild fig accessions. DtM means maturity of fruits of the current year calculated from the time of fruit set to the time of fruit maturity.

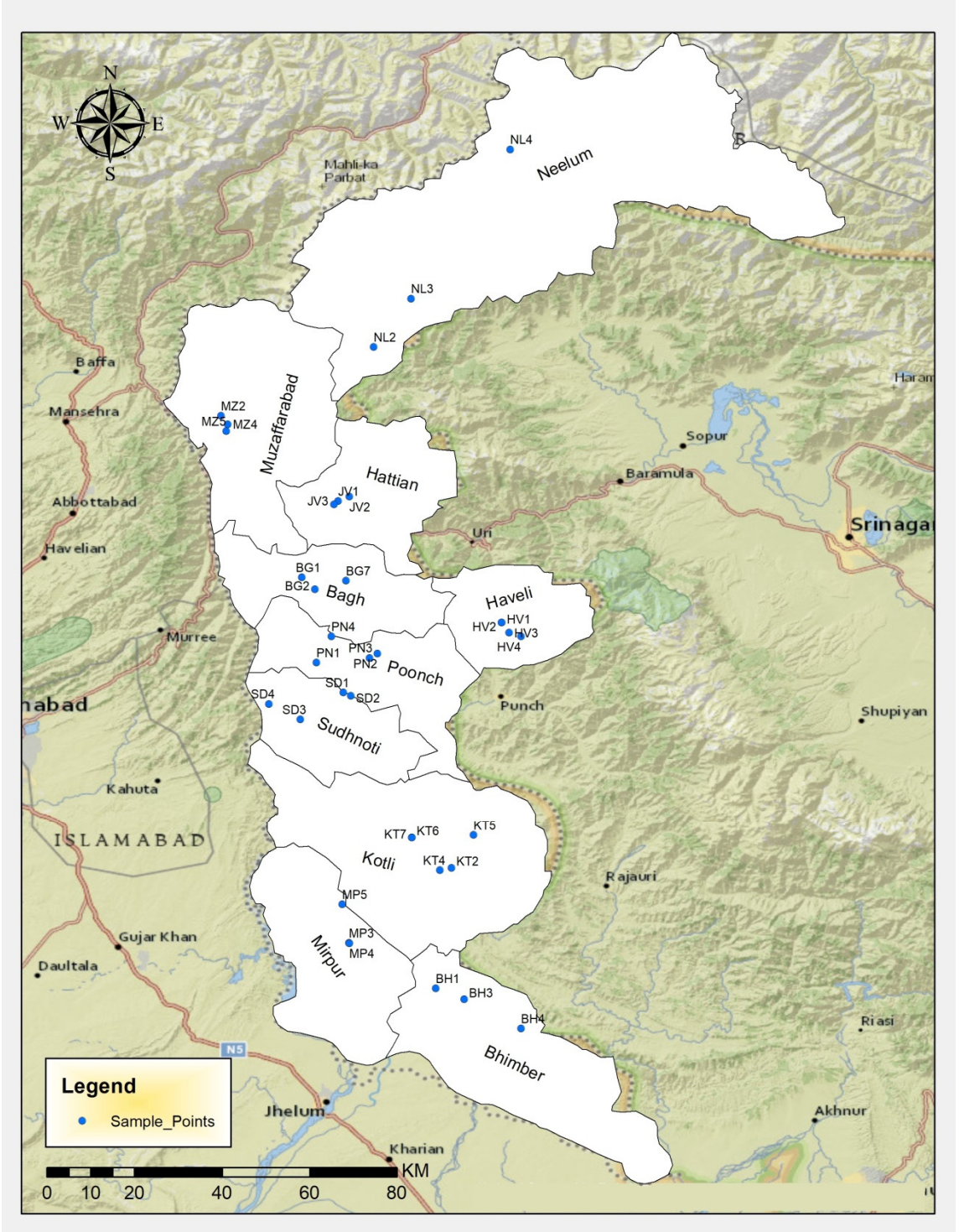

Figure 1. Collection sites of fig accessions in Azad Jammu & Kashmir, Pakistan.

2.3. Statistical Analysis

For the statistical data analysis, average values obtained from 35 wild fig accessions regarding phenotypic measurements linked to 33 characteristics were used. Means, minimum values, maximum values, standard deviation (SD) and coefficient of variance (CV%) were analysed for all the parameters using SAS® version 9.1, while the frequency distribution of qualitative characteristics was analysed using Microsoft Excel 2016. Principal component analysis (PCA) and multivariate analysis were performed using R Studio (v1.1.4) statistics

software. Pearson's correlation coefficients between the characters were determined using SPSS software.

Table 1. Detail of 35 wild fig (*Ficus palmata*) accessions from Azad Jammu and Kashmir, Pakistan.

Accession ID	Collection Site	Latitude (N)	Longitude (E)	Elevation (m.a.s.l)
MP3	Chakshawari	33°15.685	73°46.813	438
MP4	Chakshawari	33°15.651	73°46.880	373
MP5	Dadyal	33°20.689	73°46.739	413
BH1	Dabal Poona	33°10.074	73°57.524	506
BH3	Chadroon	33°08.739	74°01.013	582
BH4	Samahni	33°05.139	74°08.067	595
KT2	Tanyote	33°24.933	73°59.468	621
KT4	Dhongi	33°24.623	73°58.027	766
KT5	Supply Nakyal	33°28.968	74°02.202	595
KT6	Kotli City	33°28.656	73°54.557	642
KT7	Kotli City	33°28.656	73°54.565	629
SD1	Kotara	33°47.118	73°46.635	1843
SD2	Kotara	33°46.219	73°47.045	1726
SD3	Palandri	33°43.334	73°40.836	1361
SD4	Sawa Cross	33°45.244	73°36.919	742
PN1	Kot Maty Khan	33°50.339	73°42.809	1989
PN2	Namnota	33°51.479	73°50.311	1937
PN3	Khaigala	33°50.925	73°49.341	1733
PN4	Lowar Parat	33°53.563	73°44.648	1121
BG1	Mallot	34°00.872	73°41.011	1734
BG2	Jaglari	33°59.402	73°42.637	1561
BG7	Paddar	34°00.459	73°46.456	1232
HV1	Halan North	33°55.278	74°05.663	1743
HV2	Halan North	33°55.271	74°05.669	1742
HV3	Halan South	33°54.026	74°06.574	1555
HV4	Kahuta City	34°53.703	74°06.708	1408
MZ2	Mang Umer Khan	34°20.795	73°31.024	950
MZ4	Langarpura	34°19.771	73°31.843	876
MZ5	Subri	34°18.904	73°31.657	850
JV1	Jigal	34°10.292	73°45.460	972
JV2	Goharabad	34°10.841	73°46.863	1089
JV3	Dhani Shadra	34°09.862	73°44.972	1125
NL2	Barrian	34°26.358	73°48.574	1147
NL3	Jura	34°29.303	73°49.863	1239
NL4	Athmuqam	34°35.287	73°54.483	1460

m.a.s.l: Meter above sea level.

3. Results

3.1. Descriptive Results for Qualitative Characteristics

The results of the qualitative traits including tree, leaf and fruit characteristics are summarised in Table 2. A high variability was observed in all the traits studied except for ease of peeling. Of the two growth forms observed, 'semi-erect' growth form was the predominant (77.14%) while 22.86% of the fig accessions (BH3, SD2, PN2, BG2, MZ2, NL2) had 'spreading' growth form. Most of the fig accessions (74.29%) had 'high' vigour while the remaining 25.71% (MP3, BH4, KT2, KT4, SD4, PN2, BG2, MZ2, NL4) had 'intermediate' tree vigour. Regarding the relative degree of branching, 77.15% of the fig accessions had 'dense' branching, while 22.85% of the fig accessions (MP5, BH4, KT2, PN4, BG7, HV4, MZ5, NL4) had 'intermediate' branching type. No apical dominance was observed in 74.28% of the fig accessions, while apical dominance was present in 25.72% of the fig accessions (BH1, BH4, KT7, SD3, PN4, HV4, MZ5, JV3, NL4). Out of 35 fig accessions, 60% possessed green leaf colour while 40 accessions had dark green leaf colour (SD1, SD3, SD4, PN1, PN2, HV1, MZ2, MZ4, MZ5, JV1, JV2, JV3, NL3, NL4). Of the 35 fig accessions, 91.24% of the accessions had non-lobed leaves, 5.71% of the accessions (KT7, HV2) had calcareous lobed

leaves at the base, while 1 accession (SD2) had cordate trilobed leaves at the base. Most of the fig accessions (71.42%) had round leaf base, while 28.57% of fig accessions (MP3, MP4, MP5, KT7, SD2, BG1, BG2, BG7, HV2) had cordate-shaped leaf base. Regarding leaf margin, 48.57% of the 35 fig accessions had serrated leaf margin, 40% of the fig accessions had serrated leaf margin and the remaining 11.42% of the fig accessions (SD2, SD3, PN4, HV2) had crenate leaf margin. Most of the fig accessions (91.42%) had leaves with fully dented lobe sides, while 8.58% of the fig accessions (SD2, HV2, JV3) had dented leaves at the upper margin. In leaf veining, 88.58% of the fig accessions examined had visible leaf veining while 11.42% of the fig accessions (PN1, PN2, PN3, PN4) had slightly visible leaf veining. A wide variation was observed in the fruit shape of the collected fig germplasm. Out of the 35 fig accessions, 71.42% had globular fruit shape, 25.71% (KT4, KT5, KT6, KT7, SD1, SD2, PN2, HV3, HV4) had an oblate shape and 2.85% (KT2) had an oblong fruit shape. A wide variation in flesh colour was observed in all the fig accessions studied. Of the 35 fig accessions, 40% had amber flesh colour, 37.14% of the accessions were red, while 22.86% of the accessions (SD3, SD4, PN1, PN2, PN3, PN4, BG1, BG2) had pink flesh colour. All 35 fig accessions were difficult to peel.

3.2. Descriptive Results for Quantitative Characteristics

The descriptive statistics of minimum, maximum, means, standard deviations and coefficients of variation (CV) for 20 quantitative traits are presented in Table 3. The results showed a wide range of morphological variability in leaf and fruit traits. Several traits such as leaf width (14.81%), petiole length (14.77%), photosynthetic activity (14.02%), total chlorophyll content (13.43%) showed highest CV while lowest CV was recorded for fruit firmness (4.46%). Days to maturity ranged from 71.00–86.00 for accession KT6 and NL4, respectively. Shoot length varied from 7.69–21.52 cm for NL4 and JV2, respectively. The number of leaves per shoot was different for KT5 and PN3 (7.65–10.30). Leaf length ranged from 7.19–12.89 cm in NL4 and MZ2. Leaf width ranged from 6.47 cm (MP4) to 10.12 cm (BH4). Leaf area varied between 38.55–90.06 cm^2 for accessions MP5 and BG1. Petiole length was different for NL4 and MZ2 (17.49–38.28 mm). Petiole thickness ranged from 1.33–2.99 mm for BG7 and BH3. The number of fruits per shoot varied between 3.60–6.70 in SD4 and BG1. Fruit length was different in HV4 and BG1 (11.25–29.85 mm). Fruit diameter ranged from 11.85–27.49 mm in MP3 and NL4. The fruit stalk length ranged from 5.22–22.38 mm for accession KT6 and BG1, respectively. Fruit weight varied between 2.65 and 9.66 g for BH3 and BG1, respectively. The width of the ostioles was different in NL4 and BH1 (2.17–4.84 mm). The thickness of the fruit skin ranged from 0.10–0.76 mm at KT7 and BG2. Fruit firmness was low in SD1 and high in BH1 (0.46 and 0.69 $kgcm^2$), respectively). The colour value L* ranged between 24.72–59.24 for MP5 and BH3. The colour value a* ranged from 1.14 to 9.51 for NL3 and BH3, respectively. Photosynthetic activity ranged between 7.94–10.22 $\mu mol \cdot CO_2 \cdot m^{-2} s^{-1}$ for BG3 and NL3. Total chlorophyll content ranged from 37.11–46.48 $\mu g\ ml^{-1}$ for BG3 and NL3, respectively. Accessions with high fruit weight, low ostioles width and good fruit firmness with dark colour are preferred for fresh market.

3.3. Correlation for Quantitative Characteristics

A strong correlation was found for all of the quantitative traits studied (Table 4). The highest positive correlation was found for photosynthetic activity and total chlorophyll content (0.85). Additionally, positive correlations were examined between leaf length and leaf width (0.78), fruit length and fruit diameter (0.75), fruit weight and fruit length (0.73), and fruit weight and fruit diameter (0.72). In contrast, negative correlations were observed for quantitative traits such as the leaf petiole thickness and fruit skin thickness (−0.25), leaf area and chlorophyll content (−0.22), ostiole width and total chlorophyll content (−0.20), leaf petiole thickness and photosynthetic activity (−0.17), and ostiole width and fruit skin thickness (−0.15). The study of these traits is helpful in the selection of fig germplasm for documentation.

Table 2. Description of qualitative characteristics of 35 wild fig (*Ficus palmata*) accessions from Azad Jammu and Kashmir, Pakistan.

Accession ID	TGH	TVg	RDB	ADm	LCl	LSh	SLB	LMr	LMrD	LVn	FSh	ClF	EPl
MP3	Semi erect	Intermediate	Dense	Absent	Green	Not lobed	Cordate	Serrate	Lobe sides completely dented	Apparent	Globose	Red	Difficult
MP4	Semi erect	High	Dense	Absent	Green	Not lobed	Cordate	Serrate	Lobe sides completely dented	Apparent	Globose	Red	Difficult
MP5	Semi erect	High	Intermediate	Absent	Green	Not lobed	Cordate	Serrate	Lobe sides completely dented	Apparent	Globose	Red	Difficult
BH1	Semi erect	High	Dense	Present	Green	Not lobed	Round	Serrate	Lobe sides completely dented	Apparent	Globose	Red	Difficult
BH3	Spreading	High	Dense	Absent	Green	Not lobed	Round	Serrate	Lobe sides completely dented	Apparent	Globose	Red	Difficult
BH4	Semi erect	Intermediate	Intermediate	Present	Green	Not lobed	Round	Serrate	Lobe sides completely dented	Apparent	Globose	Red	Difficult
KT2	Semi erect	Intermediate	Intermediate	Absent	Green	Not lobed	Round	Serrate	Lobe sides completely dented	Apparent	Oblong	Red	Difficult
KT4	Semi erect	Intermediate	Dense	Absent	Green	Not lobed	Round	Serrate	Lobe sides completely dented	Apparent	Oblate	Red	Difficult
KT5	Semi erect	High	Dense	Absent	Green	Not lobed	Round	Serrate	Lobe sides completely dented	Apparent	Oblate	Red	Difficult
KT6	Semi erect	High	Dense	Absent	Green	Not lobed	Round	Serrate	Lobe sides completely dented	Apparent	Oblate	Red	Difficult
KT7	Semi erect	High	Dense	Present	Green	Base calcarate lobe lyrate	Cordate	Serrate	Lobe sides completely dented	Apparent	Oblate	Red	Difficult
SD1	Semi erect	High	Dense	Absent	Dark green	Not lobed	Round	Serrulate	Lobe sides completely dented	Apparent	Oblate	Red	Difficult
SD2	Spreading	High	Dense	Absent	Green	Base cordate 3 lobe	Cordate	Crenate	Upper margin dented	Apparent	Oblate	Red	Difficult
SD3	Semi erect	High	Dense	Present	Dark green	Not lobed	Round	Crenate	Lobe sides completely dented	Apparent	Globose	Pink	Difficult
SD4	Spreading	Intermediate	Dense	Absent	Dark green	Not lobed	Round	Serrulate	Lobe sides completely dented	Apparent	Globose	Pink	Difficult
PN1	Semi erect	High	Dense	Absent	Dark green	Not lobed	Round	Serrulate	Lobe sides completely dented	Slightly apparent	Globose	Pink	Difficult
PN2	Semi erect	Intermediate	Dense	Absent	Dark green	Not lobed	Cordate	Serrulate	Lobe sides completely dented	Slightly apparent	Globose	Pink	Difficult
PN3	Spreading	High	Dense	Absent	Green	Not lobed	Round	Serrate	Lobe sides completely dented	Slightly apparent	Oblate	Pink	Difficult
PN4	Semi erect	High	Intermediate	Present	Green	Not lobed	Round	Crenate	Lobe sides completely dented	Slightly apparent	Globose	Pink	Difficult
BG1	Semi erect	High	Dense	Absent	Green	Not lobed	Cordate	Serrulate	Lobe sides completely dented	Apparent	Globose	Pink	Difficult
BG2	Semi erect	Intermediate	Dense	Absent	Green	Not lobed	Cordate	Serrulate	Lobe sides completely dented	Apparent	Globose	Pink	Difficult
BG7	Semi erect	High	Intermediate	Absent	Green	Not lobed	Cordate	Serrate	Lobe sides completely dented	Apparent	Globose	Amber	Difficult
HV1	Semi erect	High	Dense	Absent	Dark green	Not lobed	Round	Serrulate	Lobe sides completely dented	Apparent	Globose	Amber	Difficult
HV2	Semi erect	High	Dense	Absent	Green	Base calcarate lobe lyrate	Cordate	Crenate	Upper margin dented	Apparent	Globose	Amber	Difficult
HV3	Spreading	High	Dense	Absent	Green	Not lobed	Round	Serrulate	Lobe sides completely dented	Apparent	Oblate	Amber	Difficult

Table 2. *Cont.*

HV4	Semi erect	High	Intermediate	Present	Green	Not lobed	Round	Serrulate	Lobe sides completely dented	Apparent	Oblate	Amber	Difficult
MZ2	Spreading	Intermediate	Dense	Absent	Dark green	Not lobed	Round	Serrulate	Lobe sides completely dented	Apparent	Globose	Amber	Difficult
MZ4	Spreading	High	Dense	Absent	Dark green	Not lobed	Round	Serrulate	Lobe sides completely dented	Apparent	Globose	Amber	Difficult
MZ5	Semi erect	High	Intermediate	Present	Dark green	Not lobed	Round	Serrulate	Lobe sides completely dented	Apparent	Globose	Amber	Difficult
JV1	Semi erect	High	Dense	Absent	Dark green	Not lobed	Round	Serrate	Lobe sides completely dented	Apparent	Globose	Amber	Difficult
JV2	Semi erect	High	Dense	Absent	Dark green	Not lobed	Round	Serrulate	Lobe sides completely dented	Apparent	Globose	Amber	Difficult
JV3	Semi erect	High	Dense	Present	Dark green	Not lobed	Round	Serrulate	Upper margin dented	Apparent	Globose	Amber	Difficult
NL2	Spreading	High	Dense	Absent	Green	Not lobed	Round	Serrulate	Lobe sides completely dented	Apparent	Globose	Amber	Difficult
NL3	Semi erect	High	Dense	Absent	Dark green	Not lobed	Round	Serrulate	Lobe sides completely dented	Apparent	Globose	Amber	Difficult
NL4	Semi erect	Intermediate	Intermediate	Present	Dark green	Not lobed	Round	Serrulate	Lobe sides completely dented	Apparent	Globose	Amber	Difficult

Tree growth habit (TGH), tree vigour (TVg), relative degree of branching (RDB), apical dominance (ADm), leaf colour (LCl), leaf shape (LSh), shape of leaf base (SLB), leaf margin (LMr), leaf margin dentation (LMrD), leaf venation (LVn), fruit shape (FSh), colour of flesh (ClF) and ease of peeling (EPl).

Table 3. Descriptive statistics for quantitative characteristics of 35 wild fig (*Ficus palmata*) accessions from Azad Jammu and Kashmir, Pakistan.

Characteristics	Min	Max	Mean	SD	CV (%)
Days to maturity	71.00	86.00	67.70	0.57	11.02
Shoot length (cm)	7.69	21.52	14.60	0.90	9.62
Number of leaf per shoot	7.65	10.30	7.24	0.36	7.83
Leaf length (cm)	7.19	12.89	10.04	0.55	12.44
Leaf width (cm)	6.47	10.12	8.29	0.54	14.81
Leaf area (cm^2)	38.55	90.06	64.31	5.45	10.63
Petiole length (mm)	17.49	38.28	27.88	2.26	14.77
Petiole thickness (mm)	1.33	2.99	2.16	0.10	11.34
Number of fruits per shoot	3.60	6.70	5.15	0.27	7.02
Fruit length (mm)	11.25	29.85	20.55	1.17	12.60
Fruit diameter (mm)	11.85	27.49	19.67	1.07	11.74
Fruit stalk length (mm)	5.22	22.38	13.80	0.92	7.86
Fruit weight (g)	2.65	9.66	6.15	0.57	9.57
Ostiole width (mm)	2.17	4.84	3.50	0.22	7.14
Fruit skin thickness (mm)	0.10	0.76	0.43	0.03	5.62
Fruit firmness ($kgcm^2$)	0.46	0.69	0.71	0.04	4.46
Colour L* value	24.72	59.24	41.98	3.53	8.70
Colour a* value	1.14	9.51	5.32	1.41	6.42
Photosynthetic activity ($\mu mol \cdot CO_2 \cdot m^{-2}s^{-1}$)	7.94	10.22	9.08	0.46	14.02
Total chlorophyll content ($\mu g \cdot ml^{-1}$)	37.11	46.48	41.80	0.67	13.43

Table 4. Correlation coefficients among selected quantitative characters in 35 wild fig (*Ficus palmata*) accessions from Azad Jammu and Kashmir, Pakistan.

	LLn	LWd	LAr	PLn	PTh	FWt	Fln	FDm	FSLn	OWd	FSTh	PAc	TCC
LLn	1												
LWd	0.78 **	1											
LAr	0.67 **	0.70 **	1										
PLn	0.62 **	0.54 **	0.47 **	1									
PTh	0.59 **	0.44 **	0.46 **	0.42 *	1								
FWt	0.43 *	0.46 **	0.54 **	0.30	0.12	1							
Fln	0.56 **	0.39 *	0.33 *	0.39	0.39 *	0.73 **	1						
FDm	0.45 *	0.36 *	0.40 **	0.40 *	0.19	0.72 **	0.75 **	1					
FSLn	0.31	0.25 *	0.20	0.37	0.14	0.44	0.36 *	0.31	1				
OWd	0.29	0.40 *	0.36	0.34	0.42 *	0.12	0.08	0.07	0.17	1			
FSTh	0.11	0.09	0.13	0.02	−0.25	0.58 **	0.45	0.50 **	0.55 **	−0.15	1		
PAc	0.03	0.25	−0.07	0.08	−0.17	0.50	0.31	0.26	0.18	−0.14	0.34	1	
TCC	−0.03	0.09	−0.22	−0.03	−0.14	0.34	0.23	0.14	0.13	−0.20	0.22	0.85 **	1

** is highly significant at $p \leq 0.01$; * is significant at $p \leq 0.05$. Abbreviations: Leaf length (LLn), Leaf width (LWd), Leaf area (LAr), Petiole length (PLn), Petiole thickness (PTh), Fruit weight (FWt), Fruit length (Fln), Fruit diameter (FDm), Fruit stalk length (FSLn), Ostiole width (OWd), Fruit skin thickness (FSTh), Photosynthetic activity (PAc) and Total chlorophyll content (TCC).

3.4. Principal Component Analysis (PCA) for Quantitative Characteristics

A PCA plot was created based on the first two components. In the individual plots, all 35 fig accessions were distributed in four quadrants, showing great diversity (Figure 2). For example, five accessions (BG1, NL4, NL3, NL2, BG2) with the highest leaf length, leaf width, leaf area, leaf petiole length, leaf petiole thickness, fruit length, fruit diameter, fruit weight, ostiole width and fruit firmness were located away from the axis centre on the upper plane. Similarly, fig accessions BH4, BH3 and BH1 had the smallest leaf length, leaf width, leaf area, leaf petiole length, leaf petiole thickness, fruit length, fruit diameter, fruit weight, ostiole width and fruit firmness, and were located at the lower level away from the axis centre.

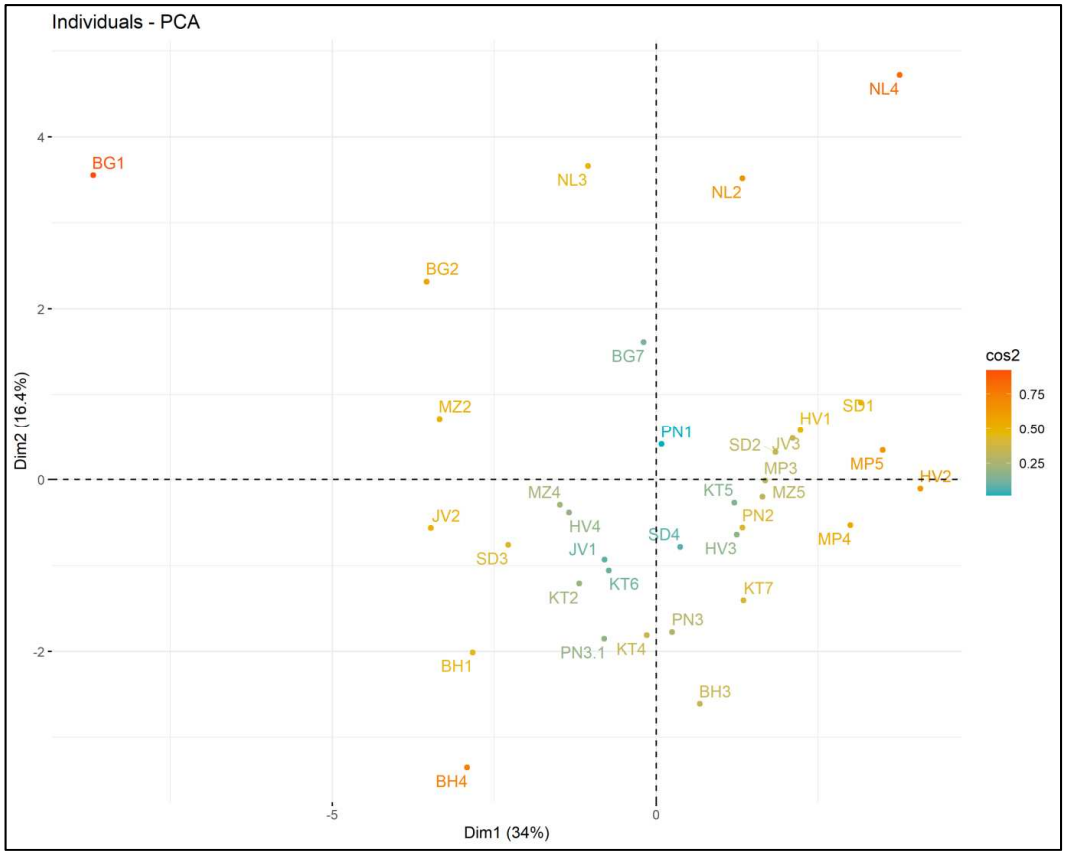

Figure 2. Distribution of 35 wild fig (*Ficus palmata*) accessions in quadrants showing phenotypic variation.

4. Discussion

4.1. Qualitative and Quantitative Characterization

In the present study, the quantitative and qualitative differences among 35 wild fig accessions from Azad Jammu and Kashmir, Pakistan were described to identify unique ecotypes. This study will not only help to understand more about these fig accessions but also help to improve their conservation, management for breeding and organisation of their orchards to achieve high yields. In a recent study by Hssaini et al. [26], it was found that such characterization studies have also proved useful for genetic identification in other fruit crops. Traits related to tree growth habit, leaf length, leaf width, fruit shape, fruit colour, flesh colour, photosynthetic activity and total chlorophyll content were reported to be very important for economic studies. Moreover, these traits can be used by fig breeders and fig farmers. The diversity assessment studies are always a good support for the survival of a population in the face of many natural disturbances and climatic problems [27]. The results of this study will be helpful to understand the morpho-pomological contents of the fruits which could be used to distinguish between cultivars as well as to estimate the genetic affiliation of different fig accessions. Similar studies conducted previously also mentioned the importance of both quantitative and qualitative traits for identification and evaluation of fig germplasm [4,5,26,28]. Based on significant differences between quantitative and qualitative characteristics, many conclusions have been drawn and fruit germplasm has been classified into different groups. In this context, many researchers around the world {Turkey [12,29], Iran [4,30], Malaysia [31], Morocco [2,8], Tunisia [32,33], Spain [34,35], Jordan [36], Algeria [37]} and Kashmir [23,28] have also worked on fig germplasm and reported variabilities in morphological and pomological characteristics. The current results are sufficiently informative and could be used to exploit diversification among the accessions studied. In addition, these results could also lead to the exploration of new potential areas of interest such as breeding and genetics research, domestication

and the commercialization of selected wild fig accessions. In addition, based on the results of this study, various targeted breeding programmes could be initiated to improve fruit quality and fruit size. It is known that fig plants have a wider distribution range, so it is very likely that new ecotypes could be developed [26]. The results also showed that fruit size and flesh colour were highly variable among wild fig accessions. These variations among fig accessions grown in the same geographical regions could be due to differences in genetic makeup or environmental conditions [38,39]. Similar results were reported by Sezen et al. [40], who found that there was a great diversity among fig accessions in terms of fruit size and yield and also for flesh colour. They also found that these traits are usually controlled by the action of additive genes and could be useful for cultivar selection. It is a fact that accessions with larger fruits are suitable for fresh consumption, while accessions with smaller fruits are suitable for processing. Variations in fruit size of figs have also been reported by Simsek et al. [41] and Caliskan et al. [42].

Variations in fruit length and width have also been noted and are considered important traits for breeding programmes. Fruit dimension studies are crucial for the evaluation of packing and shipping [40,41]. Significant variations were observed in leaf length and width among the accessions studied. Leaves are the most important plant parts as they help to absorb sunlight for photosynthesis. Thus, as the leaf size increases, the leaf area also increases, which contributes to an effective supply of primary metabolites for the production of secondary metabolites [43]. Skin colour, flesh colour and fruit firmness are also important traits for commercial purposes. However, among these traits, fruit firmness is most commonly used in research as it helps to assess fruit maturity and quality. Most growers also use fruit firmness as one of the criteria for assessing fruit maturity and quality, as it is directly related to fruit storability. Fruit firmness was determined for the first time in selected fig accessions, and it was found that the values were low in all fig accessions. Skin colour is also an important ripening criterion and a commercially used parameter that could be helpful in the classification and selection of cultivars [40].

A wide phenotypic diversity was also observed in qualitative traits. In the selection of fig cultivars for commercial cultivation, traits such as tree growth habit and type of fruiting are considered very important. Semi-erect growth habit was observed in wild germplasm as it is mostly found in temperate climates with abundant rainfall. Caliskan and Polat [29] and Mir et al. [23] also reported the semi-erect growth habit and fruiting on current year shoot. The most important traits affecting the commercial value of the crop for fresh consumption are fruit shape, fruit weight, length and width [26,28,30]. These traits are polygenic in nature. Fruit shape has been described as oblate, oblong and globose. Most of the wild germplasm studied was accounted with globose fruit shape. Such variations in fruit shape, fruit size and flesh colour were also reported by Mir et al. [23] in central Kashmir and Khadivi et al. [4] in Iran. Fruit ribs varied in morphology from intermediate to none, in addition to prominent ribs [44]. Significant genotypic differences were found by Simsek et al. [41] and Fatahi et al. [30] in the absence of fruit pedicel and ease of peeling. These types of variations may be due to differences in genetic makeup and ecological conditions [40]. All fig accessions studied were sweeter in taste, which can be recommended for fresh fruit consumption. In this study, a wide variation in morphological parameters such as shape, colour and size of fruits was detected. In Azad Jammu and Kashmir, Pakistan, fig growers have given names to different accessions based on their fruit characteristics and their growing locations. Based on these characteristics, local farmers have named fig accessions as Pagwhara (flattened shape, smaller size, dark purple colour).

4.2. Quantitative Correlations

Using a simple correlation coefficient analysis, significant correlations were found between the quantitative characteristics. The strongest positive correlation was found for photosynthetic activity and total chlorophyll content. In addition, positive correlations were examined between leaf length and leaf width, fruit length and fruit diameter, fruit weight and fruit length, and fruit weight and fruit diameter. These results are in agreement

with those of Khadivi-Khub and Anjam [43] and Khadivi et al. [4]. The presence of strong positive correlations between leaf characteristics and photosynthetic activity and total chlorophyll content indicates that larger leaf area leads to higher photosynthetic activity [45]. Negative correlations were also found for quantitative traits such as leaf petiole thickness and fruit skin thickness, leaf area and chlorophyll content, ostiole width and total chlorophyll content, leaf petiole thickness and photosynthetic activity, and ostiole width and fruit skin thickness. The study of these traits is helpful in the selection of fig germplasm for documentation. The presence of negative correlations between leaf area and total chlorophyll content suggests that a greater leaf area should result in lower total chlorophyll content at the fruit maturity stage.

Similar PCA studies have been previously conducted to evaluate the differences among fruit tree species such as apricot [46], cherry [47,48], ber [49] and jaman [50]. Diversity depends on the variations present in the populations under study. Heterosis is generally measured by the degree of genetic diversity between parental combinations. Therefore, traits with high correlations could be useful for future analysis of fig accessions.

4.3. Perspectives of Gene Bank Conservation

Figs are highly nutritious fruits and given the circumstances of malnutrition in the world, they are considered a boon as they can combat the problem of malnutrition to some extent [38]. However, fig borers, mealy bugs and scale insects are important pests while fig mosaic disease and fig rust are important diseases reported to cause heavy losses in fig orchards [51–53]. Modern techniques of selection and breeding of improved cultivars have greatly reduced the diversity of many fruit crops [49,50,54]. This narrowing of plant diversity has resulted in the disappearance of many plant species and has had a negative impact on biodiversity [55]. The accessions collected in this study had a great diversity in quantitative and qualitative traits and a high degree of variation among traits, indicating that these accessions have great strength and potential for further exploitation. Many countries have similar germplasm collections for other minor fruits such as ber, pomegranate, litchi, jaman, etc., consisting of most of the possible varieties of these fruits in these countries. Similarly, fig collections have been reported from different countries such as Tunisia [32,33], Turkey [12,29,56], Morocco [8,57,58], Spain [35,59], Lebanon [60], Iran [4], Kashmir [23,38] and Jordan [36]. These studies have shown that the diversity of morphological and pomological characters could be useful for an efficient marker system to distinguish between different fig genotypes.

The current results support the idea that morphological and pomological traits are reliable parameters to estimate the genetic relationships among fig accessions and can be effectively used to discriminate among different fig accessions. Similarly, many studies [4,26,61,62] have shown that morphological and pomological characters are very helpful in identifying and evaluating fig germplasm. In Azad Jammu and Kashmir, Pakistan, fig is widely distributed in wild and cultivated form and shows very high diversity among different accessions. However, very little attention has been paid to the characterization and conservation of local fig germplasm. This is the first comprehensive study of its kind on the characterization and conservation of 35 wild fig accessions. Thus, the germplasm conserved in this study could be used as source material for future biotic and abiotic studies and also for breeding programs [49].

4.4. Perspectives of Breeding

The basic objective of breeding is to combine the most desired traits in a single variety [63]. In order to exploit the potential of a native crop in a particular area, extensive characterization studies must be conducted [64]. These studies, along with breeding programmes, can result in varieties with better yields, good quality and higher nutritional value of fruits. Pakistan is blessed with rich resources and considerable variability for figs. A little research on characterization, breeding and commercialization can lead to the development of valuable products and in return, rural people can earn a better living.

In our research, correlations were found between the traits studied, which could play an important role in improving fruit yield and quality. Large fruits of wild fig accessions (BG1, NL3, MZ2, BG2) can be crossed to produce high yielding and high quality cultivars. Flesh colour is an important criterion for consumer acceptance, and in our study it ranged from red to pink to amber. Therefore, fig accessions (MP3, MP4, MP5, BH1, BH3, BH4, KT2, KT4, KT5, KT6, KT7, SD1, SD2) with a red flesh colour can be used to produce fruit varieties with red flesh colour. Such types of accessions are good for fresh consumption and can contribute to the improvement of fresh fruit market. Similarly, several promising accessions such as ML-G-17, AH-S-06 and DH-S-04 have been identified by researchers in central Kashmir in the north-western Himalayan region [23] based on flesh colour, external fruit colour and fruit shape.

Diversity between accessions located in the same cluster is minimal and few segregates are possible through hybridization [65]. Therefore, accessions belonging to different clusters should be crossed, as they will produce hybrids with higher heterosis and genetic recombination. Environment can also play an important role in breeding different accessions [66]. So, in order to achieve the best breeding results, the environmental effects must be minimised. In the present study, accessions that have great diversity and are well adapted to the local environment were selected. Thus, in hybridization, the environmental influences could be minimised by selecting those accessions which are considered to be very stable in terms of trait expression [67]. The extent of genetic variation can also be determined using genetic markers for genetic analysis [54]. To utilise the diversity of collected fig germplasm from Azad Jammu and Kashmir, Pakistan, it is proposed to use genetic markers for further confirmation.

5. Conclusions

Significant differences in quantitative and qualitative traits were found in 35 wild fig accessions. The results show that each accession has its own individual characteristics and identity. Studies related to characterization could be useful to identify diversity and highlight the most appropriate variables. Moreover, these studies are of great help in the development of new varieties, either through genetics or breeding programmes. The results of the present study could also be useful in future for conservation of germplasm resources, biochemical studies and other studies for the development of cultivars with large fruits, seedless fruits, fruits with red flesh colour and sweeter taste. The limitation of this study is that it only considers qualitative and quantitative characteristics of plants without genetic studies and collecting chemical data on fruit quality. However, for accurate description of fig germplasm from Azad Jammu and Kashmir, Pakistan, future studies should be carried out for molecular evaluation and biochemical characterization. The traits studied are of importance to further the existing knowledge about native wild figs, which can improve the sustainability of local communities.

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References

1. Khadari, B.; Grout, C.; Santoni, S.; Kjellberg, F. Contrasted genetic diversity and differentiation among Mediterranean populations of *Ficus carica* L.: A study using mtDNA RFLP. *Genet. Resour. Crop Evol.* **2005**, *52*, 97–109. [CrossRef]
2. Hmimsa, Y.; Aumeeruddy-Thomas, Y.; Ater, M. Vernacular taxonomy, classification and varietal diversity of fig (*Ficus carica* L.) among Jbala cultivators in northern Morocco. *Human Ecol.* **2012**, *40*, 301–313. [CrossRef]
3. Hirst, K. Fig trees and archaeology. The history of the domestication of fig trees. *About. Com Archaeol.* 1996. Available online: http://archaeology.about.com/od/domestications/a/fig_trees.htm (accessed on 26 July 2018).
4. Khadivi, A.; Anjam, R.; Anjam, K. Morphological and pomological characterization of edible fig (*Ficus carica* L.) to select the superior trees. *Sci. Hortic.* **2018**, *238*, 66–74. [CrossRef]
5. Kislev, M.E.; Hartmann, A.; Bar-Yosef, O. Early domesticated fig in the Jordan Valley. *Science* **2006**, *312*, 1372–1374. [CrossRef] [PubMed]
6. Duenas, M.; Perez-Alonso, J.J.; Santos-Buelga, C.; Escribano-Bailon, T. Anthocyanin composition in fig (*Ficus carica* L.). *J. Food Compo. Anal.* **2008**, *21*, 107–115. [CrossRef]
7. Flaishman, M.A.; Rodov, V.; Stover, E. The fig: Botany, horticulture and breeding. *Hortic. Rev.* **2008**, *34*, 113–197.
8. Hssaini, L.; Charafi, J.; Hanine, H.; Ennahli, S.; Mekaoui, A.; Mamouni, A.; Razouk, R. Comparative analysis and physio-biochemical screening of an ex-situ fig (*Ficus carica* L.) collection. *Hortic. Environ. Biotechnol.* **2019**, *60*, 671–683. [CrossRef]
9. Slatnar, A.; Klancar, U.; Stampar, F.; Veberic, R. Effect of drying of figs (*Ficus carica* L.) on the contents of sugars, organic acids and phenolic compounds. *J. Agric. Food Chem.* **2011**, *59*, 11696–11702. [CrossRef]
10. Tomas-Barberan, F.A.; Espin, J.C. Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *J. Sci. Food Agric.* **2001**, *81*, 853–876. [CrossRef]
11. Veberic, R.; Colaric, M.; Stampar, F. Phenolic acids and flavonoids of fig fruit (*Ficus carica* L.) in the northern Mediterranean region. *Food Chem.* **2008**, *106*, 153–157. [CrossRef]
12. Ercisli, S.; Tosun, M.; Karlidag, H.; Dzubur, A.; Hadziabulic, S.; Aliman, Y. Color and antioxidant characteristics of some fresh fig (*Ficus carica* L.) genotypes from Northeastern Turkey. *Plant Foods Human Nutr.* **2012**, *67*, 271–276. [CrossRef] [PubMed]
13. Duke, J.A. *Handbook of Medicinal Herbs*; CRC Press: Boca Raton, FL, USA, 2002.
14. Pimbert, M. Sustaining the Multiple Functions of Agricultural Biodiversity. FAO Background Paper Series for the Conference on the Multifunctional Character of Agriculture and Land, The Netherlands, September 1999. Available online: https://pubs.iied.org/sites/default/files/pdfs/migrate/G01250.pdf (accessed on 20 August 2019).
15. Esquinas-Alcázar, J. Protecting crop genetic diversity for food security: Political, ethical and technical challenges. *Nat. Rev. Genet.* **2005**, *6*, 946–953. [CrossRef]
16. Abbasi, A.M.; Shah, M.H.; Khan, M.A. *Wild Edible Vegetables of Lesser Himalayas: Ethnobotanical and Nutraceutical Aspects*; Springer International Publishing: Cham, Switzerland, 2014; Volume 1, p. 360.
17. Ajaib, M.; Khan, Z. Ethnobotanical Studies of Useful Trees of District Kotli, Azad Jammu and Kashmir. *Biologia* **2014**, *60*, 63–71.
18. Bisht, J.K.; Srivastva, A.K.; Gupta, H.S. Sustainable fodder production management in NW Himalaya. *Tech. Bulletin. Almora VPKAS* **2009**, *32*, 1–62.
19. Sbhatu, D.B.; Tekle, H.T.; Tesfamariam, K.H. *Ficus palmata* Forskål (BELES ADGI) as a source of milk clotting agent: A preliminary research. *BMC Res. Notes* **2020**, *13*, 446. [CrossRef]
20. Tewari, D.; Gupta, P.; Bawari, S.; Sah, A.N.; Barreca, D.; Khayatkashani, M.; Khayat Kashani, H.R. Himalayan *Ficus palmata* L. Fruit extract showed in vivo central and peripheral analgesic activity involving COX-2 and Mu opioid receptors. *Plants* **2021**, *10*, 1685. [CrossRef]
21. Mahmood, A.; Qureshi, R.; Mahmood, A.; Sangi, Y.; Shaheen, H.; Ahmad, I.; Nawaz, Z. Ethnobotanical survey of common medicinal plants used by people of district Mirpur, AJK, Pakistan. *J. Med. Plants Res.* **2011**, *5*, 4493–4498.
22. Qureshi, R.A.; Ghufran, M.A.; Gilani, S.A.; Sultana, K.; Ashraf, M. Ethnobotanical studies of selected medicinal plants of Sudhan Gali and Ganga Chotti hills, district Bagh, Azad Kashmir. *Pak. J. Bot.* **2007**, *39*, 2275–2283.
23. Mir, M.M.; Amit, K.; Umar, I.; Mir, S.A.; Rehman, M.U.; Banday, S.A.; Rather, G.H.; Sibat, F. Characterization of fig (*Ficus carica* L.) germplasm in central Kashmir of North Western Himalayan region. *Indian J. Plant Genet. Resour.* **2018**, *31*, 57–63. [CrossRef]
24. Arnon, D.I. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* **1949**, *24*, 1–12. [CrossRef] [PubMed]
25. IPGRI and CIHEAM. Descriptors for Figs. International Plant Genetic Resources Institute (IPGRI), Rome, Italy, and the International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM), Paris, France, 2003. Available online: https://www.bioversityinternational.org/fileadmin/user_upload/online_library/publications/pdfs/907.pdf (accessed on 18 December 2019).

26. Hssaini, L.; Hanine, H.; Razouk, R.; Ennahli, S.; Mekaoui, A.; Ejjilani, A.; Charafi, J. Assessment of genetic diversity in Moroccan fig (*Ficus carica* L.) collection by combining morphological and physicochemical descriptors. *Genet. Resour. Crop Evol.* **2020**, *67*, 457–474. [CrossRef]
27. Awasthi, O.P.; More, T.A. Genetic diversity and status of *Zizyphus* in India. *Acta Hortic.* **2009**, *10*, 33–40. [CrossRef]
28. Podgornik, M.; Vuk, I.; Vrhovnik, I.; Mavsar, D.B. A survey and morphological evaluation of fig (*Ficus carica* L.) genetic resources from Slovenia. *Sci. Hortic.* **2010**, *125*, 380–389. [CrossRef]
29. Caliskan, O.; Polat, A.A. Morphological diversity among fig (*Ficus carica* L.) accessions sampled from the Eastern Mediterranean region of Turkey. *Turkish J. Agric. For.* **2012**, *36*, 179–193.
30. Fatahi, S.; Cheghamirza, K.; Arji, I.; Zarei, L. Evaluation of genetic variation of common fig (*Ficus carica* L.) in West of Iran. *J. Med. Plants By-Prod.* **2017**, *6*, 229–240.
31. Isa, M.M.; Jaafar, M.N.; Kasim, K.F.; Mutalib, M.F.A. Cultivation of fig (*Ficus carica* L.) as an alternative high value crop in Malaysia: A brief review. *IOP Conf. Ser. Mater. Sci. Eng.* **2020**, *864*, 012134. [CrossRef]
32. Saddoud, O.; Baraket, G.; Chatti, K.; Trifi, M.; Marrakchi, M.; Mars, M.; Salhi-Hannachi, A. Using morphological characters and simple sequence repeat (SSR) markers to characterize Tunisian fig (*Ficus carica* L.) cultivars. *Acta Biol. Cracoviensia. Ser. Bot. Wars.* **2011**, *53*, 7. [CrossRef]
33. Aljane, F.; Nahdi, S.; Essid, A. Genetic diversity of some accessions of Tunisian fig tree (*Ficus carica* L.) based in morphological and chemical traits. *J. Nat. Prod. Plant Resour.* **2012**, *2*, 350–359.
34. Pérez-Sánchez, R.; Morales-Corts, M.R.; Gómez-Sánchez, M.A. Agro-morphological diversity of traditional fig cultivars grown in Central-Western Spain. *Genetika* **2016**, *48*, 533–546. [CrossRef]
35. Pereira, C.; Corrales, M.L.; Villalobos, M.C.; Córdoba, M.G.; Serradilla, M.J. Physicochemical and nutritional characterization of brebas for fresh consumption from nine fig varieties (*Ficus carica* L.) grown in Extremadura (Spain). *J. Food Qual.* **2017**, *12*, 6302109.
36. Ateyyeh, A.F.; Sadder, M.T. Growth pattern and fruit characteristics of six common fig (*Ficus carica* L.) cultivars in Jordan. *Jordan J. Agric. Sci.* **2006**, *2*, 105–112.
37. Benettayeb, Z.; Bencheikh, M.; Setti, B.; Chaillou, S. Genetic diversity of Algerian fig (*Ficus carica* L.) cultivars based on morphological and quality traits. *Indian J. Hortic.* **2017**, *74*, 311–316. [CrossRef]
38. Naseer, M.A.; Maqbool, M.; Rafiq, S.; Zahid, N.; Hamid, A.; Shah, S.Z.A. Comparative analysis of physical and biochemical attributes of edible fi (*Ficus carica* L.) collected from three districts of Azad Jammu and Kashmir located at diffrent elevations. *Pak. J. Agric. Res.* **2020**, *33*, 707–713.
39. Saddoud, O.; Baraket, G.; Chatti, K.; Trifi, M.; Marrakchi, M.; Salhi-Hannachi, A.; Mars, M. Morphological variability of fig (*Ficus carica* L.) cultivars. *Int. J. Fruit Sci.* **2008**, *8*, 35–51. [CrossRef]
40. Sezen, I.; Ercisli, S.; Gozlekci, S. Biodiversity of figs (*Ficus carica* L.) in Coruh valley of Turkey. *Erwerbs Obstbau* **2014**, *56*, 139–146. [CrossRef]
41. Simsek, M.; Gulsoy, E.; Kirar, M.Z.; Yucel, Y. Identification and selection of some female fig (*Ficus carica* L.) genotypes from Mardin province of Turkey. *Pak. J. Bot.* **2017**, *49*, 541–546.
42. Caliskan, O.; Bayazit, S.; Iigin, M.; Karatas, N. Morphological diversity of caprifig (*Ficus carica* var. caprificus) accessions in the eastern Mediterranean region of Turkey: Potential utility for caprification. *Sci. Hortic.* **2017**, *222*, 46–56. [CrossRef]
43. Khadivi-Khub, A.; Anjam, K. Morphological characterization of *Prunus scoparia* using multivariate analysis. *Plant Syst. Evol.* **2014**, *300*, 1361–1372. [CrossRef]
44. Polat, A.A.; Caliskan, O. Fruit characteristics of table fig (*Ficus carica*) cultivars in subtropical climate conditions of the Mediterranean region. *N. Zeal. J. Crop. Hortic. Sci.* **2008**, *36*, 107–115. [CrossRef]
45. Zulkarnaini, Z.M.; Sakimin, S.Z.; Mohamed, M.T.M.; Jaafar, H.Z. Changes in leaf area index, leaf mass ratio, net assimilation rate, relative growth rate and specific leaf area two cultivars of fig (*Ficus Carica* L.) treated under different concentrations of brassinolide. *AGRIVITA J. Agric. Sci.* **2019**, *41*, 158–165. [CrossRef]
46. Ruiz, D.; Egea, J. Phenotypic diversity and relationships of fruit quality traits in apricot (*Prunus armeniaca* L.) germplasm. *Euphytica* **2008**, *163*, 143–158. [CrossRef]
47. Khadivi-Khub, A.; Jafari, H.R.; Zamani, Z. Phenotypic and genotypic variation in Iranian sour and duke cherries. *Trees* **2013**, *27*, 1455–1466. [CrossRef]
48. Rakonjac, V.; Mratinić, E.; Jovković, R.; Fotirić Akšić, M. Analysis of morphological variability in wild cherry (*Prunus avium* L.) genetic resources from Central Serbia. *J. Agric. Sci. Technol.* **2014**, *16*, 151–162.
49. Sharif, N.; Jaskani, M.J.; Naqvi, S.A.; Awan, F.S. Exploitation of diversity in domesticated and wild ber (*Ziziphus mauritiana* Lam.) germplasm for conservation and breeding in Pakistan. *Sci. Hortic.* **2019**, *249*, 228–239. [CrossRef]
50. Din, S.; Jaskani, M.J.; Naqvi, S.A.; Awan, F.S. Diversity and divergence in domesticated and wild jamun (*Syzygium cumini*) genotypes of Pakistan. *Sci. Hortic.* **2020**, *273*, 109617. [CrossRef]
51. Himelrick, D.G. Fig production guide. Alabama Cooperative Extension System, ANR-1145, 1999. Available online: https://www.aces.edu/wp-content/uploads/2020/01/ANR-1145_FigProductionGuide_010620-L-G-copy.pdf (accessed on 19 February 2018).
52. Jahén-Rivera, S.N.; Gómez-Rodríguez, O.; Espinosa-Victoria, D. Isolation and identification of pathogens causing stem rot of the fig tree (*Ficus carica*). *Rev. Mex. Fitopatol.* **2020**, *38*, 269–279. [CrossRef]

53. Preising, S.; Borges, D.F.; Ambrósio, M.M.Q.; da Silva, W.L. A fig deal: A global look at fig mosaic disease and its putative associates. *Plant Dis.* **2021**, *105*, 727–738. [CrossRef] [PubMed]
54. Mehmood, A.; Jaskani, M.J.; Khan, I.A.; Ahmad, S.; Ahmad, R.; Luo, S.; Ahmad, N.M. Genetic diversity of Pakistani guava (*Psidium guajava* L.) germplasm and its implications for conservation and breeding. *Sci. Hortic.* **2014**, *172*, 221–232. [CrossRef]
55. Mastretta-Yanes, A.; Gasman, F.A.; Burgeff, C.; Ramírez, M.C.; Piñero, D.; Sarukhán, J. An intitiative for the study and use of genetic diversity of domesticated plants and their wild relatives. *Front. Plant Sci.* **2018**, *9*, 209. [CrossRef] [PubMed]
56. Caliskan, O.; Bayazit, S.; Kilic, D.; Ilgin, M.; Karatas, N. Pollen morphology and variability of caprifig (*Ficus carica* var. caprificus) genetic resources in Turkey using multivariate analysis. *Sci. Hortic.* **2021**, *287*, 110283. [CrossRef]
57. Alter, M.; El Oualkadi, A.; Achtak, H.; Oukabli, A.; Khadari, B. Diversity of the local varieties of the fig tree in the North-Western Morocco. *Acta Hortic.* **2008**, *79*, 69–76. [CrossRef]
58. Achtak, H.; Ater, M.; Oukabli, A.; Santoni, S.; Kjellberg, F.; Khadari, B. Traditional agroecosystems as conservatories and incubators of cultivated plant varietal diversity: The case of fig (*Ficus carica* L.) in Morocco. *BMC Plant Biol.* **2010**, *10*, 28. [CrossRef]
59. Sanchez, M.J.; Melgarejo, P.; Hernandez, F.; Martinez, J.J. Chemical and morphological characterization of four fig tree cultivars (*Ficus carica* L.) grown under similar culture conditions. *Acta Hortic.* **2002**, *605*, 33–36. [CrossRef]
60. Chalack, L.; Chehade, A.; Mattar, E.; Khadari, B. Morphological characterization of fig accessions cultivated in Lebanon. *Acta Hortic.* **2008**, *798*, 54–61. [CrossRef]
61. Condit, I. Fig varieties: A monograph. *Hilgardia* **1955**, *23*, 323–538. [CrossRef]
62. Oukabli, A.; Mamouni, A.; Laghezali, R.; Khadari, B.; Roger, J.P.; Kjellberg, F.; Ater, M. Genetic variability in Morrocan fig cultivars (*Ficus carica*) based on morpholigical and pomological data. *Acta Hortic.* **2002**, *605*, 54–60.
63. Samadia, D.K.; Haldhar, S.M. Breeding strategies and scope of improvement in arid zone fruit crop-plants under abiotic stressed agro-climate: An analysis. *J. Agric. Ecol.* **2017**, *4*, 1–13. [CrossRef]
64. Samadia, D.K.; Pareek, O.P. Fruit quality improvement in pomegranate under hot arid environment. *Indian J. Hortic.* **2006**, *63*, 126–132.
65. Roy, A.; de Melo, J.; Chaurvedi, D.; Thein, T.; Cabrera-Socorro, A.; Houart, C.; Meyer, G.; Blackshaw, S.; Tole, S. LHX2 is necessary for the maintenance of optic idedentity and for the progression of optic morphogenesis. *J. Neurosci.* **2013**, *33*, 6877–6884. [CrossRef]
66. Perfectti, F.; Camacho, J.P.M. Analysis of genotypic differences in developmental stability in *Annona cherimola*. *Evolution* **1999**, *53*, 1396–1405. [CrossRef] [PubMed]
67. Pommer, C.V.; Murakami, K.R.N. Breeding guava (*Psidium guajava* L.). In *Breeding Plantation Tree Crops: Tropical Species*; Jain, S.M., Priyadarshan, P.M., Eds.; Springer Science + Buseniess Media, LLC.: Berlin/Heidelberg, Germany, 2009; pp. 83–120.

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Main Agro-Morphological and Biochemical Berry Characteristics of Wild-Grown Sea Buckthorn (*Hippophae rhamnoides* L. ssp. *caucasica* Rousi) Genotypes in Turkey

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Abstract: Sea buckthorn (*Hippophae rhamnoides* L. ssp. *caucasica* Rousi) is one of the most important wild edible fruits, grown in Turkey for centuries without any chemical treatments. The plant is extremely resistant to adverse environmental conditions. In this study, the main agro-morphological and biochemical berry traits and, to a lesser extent, other plant morphological traits of 10 sea buckthorn genotypes sampled from the eastern Anatolia (Sivas province) region were assessed. Among the 10 genotypes, five of them presented a shrub growth habit, whereas five of them presented tree growth habit, with leaf area ranging from 2.56 to 4.22 cm^2. The majority of genotypes had an oblong berry shape with variable berry skin color ranging from dark orange to orange, light orange, and yellow. The weight of 100 berries varied from 13.85 to 23.87 g, while juice yield and vitamin C content was found to be 44.87–57.15% and 37.45–62.85 mg/100 g fresh berry base, respectively. Soluble solid content (SSC) was in the range of 12.56–14.67%. The genotypes exhibited a great variability in total anthocyanin content (from 9.1 to 38.7 mg/L), with relatively dark-orange sea buckthorn berries containing more anthocyanin than orange, light-orange, and yellow berries. Linoleic acid was the main fatty acid detected in the pulp of sea buckthorn berries, ranging from 24.11% to 36.37%, depending on the genotype. Investigated genotypes proved also to be rich in total phenolic content, showing at the same time great variability in this trait. The results obtained from the relatively limited number of genotypes show promising traits for further valorization of both horticultural and nutritional traits, suggesting potentially even higher variability, if more genotypes are going to be considered in the future.

Keywords: sea buckthorn; biodiversity; biochemical composition; underutilized fruit

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1. Introduction

Wild edible fruits have attracted great attention in recent years. They include a large number of species and genotypes and exhibit a rich biodiversity. The fruits of these species are very rich in terms of vitamins, minerals, dietary fibers, anthocyanins, phenolics, etc. Moreover, for centuries, particularly in rural areas, people used stems, leaves, flowers, and roots of wild edible fruits because of their high potential in traditional medicine [1–3]. More recently, the majority of wild edible fruits, including sea buckthorn, have been labeled as functional foods, which denotes foods used not only for nutrition but also for prevention and cure of various diseases [4–6].

Turkey is accepted as one of the richest countries in wild edible fruits, which played an important role in rural people's life for centuries. Rural communities used wild edible

fruits not only fresh but also processed into several natural products such as molasses, syrups, jams, candied peels, and vinegars [7,8]. In Turkey, it is estimated that there are over 100 wild edible fruit species, and it is well known that they are an integral part of everyday life of Turkish people [9]. In Turkey, nearly one-quarter of the total population lives in rural areas where food shortages are frequent, even in close history, and a large number of these agricultural communities still retain a habit of using wild edible fruits. The rural areas are frequently characterized by harsh climatic conditions coupled with poor crop harvests. Thus, the wild edible fruits are vital for the existence of rural people, and they are frequently educated to rely on wild edible fruits for survival [10–12]. Sea buckthorn shows great diversity in Turkey's flora, and, in general, it occurs in natural stands, mainly in inner, northern, and eastern Anatolia [13].

Sea buckthorn (*Hippophae rhamnoides* L.), which grows in a form of a tree or a shrub, belongs to the Oleaster family (Eleagnaceae) and is mostly found in a broad area of eastern and western Asia, eastern, central, and northern Europe, from the Caucasus to the Carpathian Mountains and northern America including Canada [14–16]. In general, it is found as wild in the fields; however, in some countries such as Sweden, Finland, China, Poland, Romania, Ukraine, Germany, Hungary, Belarus, Russia, Azerbaijan, Czech Republic, and Canada, it was domesticated in the last few centuries due to its valuable horticultural and multiple-use berry characteristics [11,17]. Subspecies, *H. rhamnoides* L. ssp. *caucasica* Rousi is the only *Hippophae* species growing in Turkey and is generally considered as morphologically quite variable [18,19].

All parts of the sea buckthorn plant have broad application. Due to its diverse and attractive shrub and tree characteristics, colorful berries, and narrow silvery leaves, it has considerable ornamental value. Because edible berries are consumed by animals, sea buckthorn is also used for enhancing wildlife habitats. The plant has a great environmental plasticity; thus, it can be used for afforestation and wasteland management, which has led to its large-scale planting. It is resistant to urban conditions. Furthermore, as a xerophyte species, it tolerates drought, cold (up to −40 °C), heat (up to 40 °C), soil salinity, and air pollution [16,20]. Sea buckthorn is a fruit species with a high nitrogen fixation capacity, thus improving soil quality; moreover, because of its extensive root system, it exhibits soil-binding properties [21,22].

Sea buckthorn berries are accepted as a "super food" or "powerful food". The berries have a high amount of proteins, fibers, antioxidants, vitamins (A, C, E), minerals, flavonoids (flavanas), ether oil, organic acids, saponin, and sugar. Berries of sea buckthorn are also used in plant-based medicines because of their valuable contents [23,24]. The leaves, flowers, seeds, and berries of sea buckthorn are used for medicinal purposes [25]. Sea buckthorn boasts all the omega (3, 6, 9 and rare omega-7) essential fatty acids. Furthermore, the pulp of sea buckthorn berries is one of the richest plant sources in the world of omega-7 essential fatty acids [26–28]. The attractive orange color of sea buckthorn berries can be attributed to beta-carotene, a type of carotenoid. Beta-carotene is the most common type of pro-vitamin A found mainly in plant-based foods [15].

Sea buckthorn genotypes have diverse morphological, biochemical, and phenological characteristics, and these characteristics differ from one genotype to another one due to dioicous flowering biology. Each seed-propagated genotype has its own characteristics, and these agro-morphologic and biochemical properties are the basic criteria used to define the genotypes; agro-morphological and biochemical characterizations have been used for a long time, particularly by the breeders [11,15,16,22].

More recently, the use of wild-grown plants as food sources has been a hot topic for researchers globally, and the traditional use of natural plant resources, with minimal ecosystem alteration, is the preferred, emerging approach. Therefore, in this study, we attempted to determine the basic agro-morphological and biochemical traits of 10 seed-propagated sea buckthorn genotypes that grow naturally in the flora of Sivas province, located in eastern Anatolia. To our knowledge, the scientific literature available related to the agro-morphological and biochemical traits of this wild edible fruit grown in Turkey is

scarce. We assume that the obtained results may support breeders' efforts with improved knowledge about the agro-morphological and detailed biochemical properties of diverse sea buckthorn genotypes that could be integrated in future breeding programs of this prominent, but still less utilized species.

2. Materials and Methods

2.1. Plant Materials

Ten promising genotypes of *Hippophae rhamnoides* L. ssp. *caucasica* growing in Sivas province, located at 37°01′ east longitude, 39°75′ north latitude, were chosen according to field observations on the basis of high yield capacity, pest and disease resistance, and attractive berries. In fact, sea buckthorn plants are constantly selected in the fields by farmers who eliminate the bad ones, leaving only the superior plants. Thus, 10 buckthorn genotypes, previously "farmer-selected", were chosen for analysis, each represented with one plant (tree or shrub). All the plants were growing at a similar altitude of 1000 ± 50 m above sea level (a.s.l.).

Sivas province has a continental climate (Köppen climate classification: Dsb) with warm dry summers and cold and snowy relatively long winters. Sea buckthorn plants are abundant in the province and create an important part of the natural landscape of Sivas territory. Local communities have used them for centuries, both as food and in traditional medicine.

In total, 400 g of berries and 100 leaves per genotype were randomly harvested from different parts of the crown and divided into an appropriate number of replicates for different agro-morphological or biochemical analyses.

The berries were harvested in the field when fully ripened, i.e., when they reached their characteristic mature berry skin color and possessed enough sweetness and softness to make them more palatable. The berry and leaf samples taken from each of the sea buckthorn genotypes (trees or shrubs) were labeled, placed into appropriate containers, and immediately transferred to the laboratory. The agro-morphological measurements were done immediately, while the berry samples to be used for biochemical analysis were kept in ultra-low-temperature freezers at −80 °C until analysis.

2.2. Agro-Morphological Measurements

In the field, the growth habit and presence of thorns were determined. In the laboratory, the berry skin color, 100-berry weight, berry shape, and leaf area (cm^2) of 10 genotypes were determined by using 100 leaves and 100 berries per genotype, which were divided into replicates (20 berries per replicate). A trained panel of five experts evaluated visible berry skin color for each genotype. The 1–5 bipolar hedonic scale was used to describe berry skin color [2,3], which was rated as light yellow, yellow, light orange, orange, or dark orange. Berry weight was measured on 100 berries per genotype using a digital balance with a sensitivity of 0.001 g (Scaltec SPB31). Berry length and berry diameter were measured using the digital caliper gauge, once after the harvest, on the longest and the widest point of the berry. The berry shape was determined by using the berry diameter-and-length ratio. Leaf area was measured with a portable laser leaf area meter (Area meter CI 201, Li-Cor Biosciences, Lincoln, NE, USA).

2.3. Juice Yield, SSC (Soluble Solid Content), Vitamin C, Protein, and Lipid

Juice yield, SSC, vitamin C, protein, and lipid content was determined on 100 g of berries per genotype, divided into four replicates (25 g per replicate). Juice yield in the berries of the 10 sea buckthorn genotypes was determined by using pressure extraction, calculated according to Tiitinen et al. [29]. Fruit juice soluble solid content (SSC) was determined using a digital refractometer (Kyoto Electronics Manufacturing Co. Ltd., Kyoto, Japan, Model RA-250 HE). The titratable acidity and vitamin C content of sea buckthorn berries was determined using a RQFlex 10 Reflectometer (Merck, Darmstadt, Germany). The total protein and lipid content of genotypes was determined by AOAC [30]. The

Kjeldahl method was used for total protein content determination of sea buckthorn berries, and Soxhlet extraction was used for lipid determination.

2.4. Extraction and Determination of Specific Sugars

For determination of specific sugars, the procedure of Melgarejo et al. [31] was followed. A total of 10 g of each sea buckthorn berry sample was centrifuged at 12,000 rpm for 2 min at 4 °C. Afterward, the supernatant was filtered with a SEP-PAK C18 cartridge and transferred into a vial for analysis. The standards were used for quantification of the concentrations. Analysis of sugars was performed by HPLC (isocratic program) with a Bondapak-NH_2 column (μg Bondapak/carbohydrate) and a refractive index (RI) detector using 85% acetonitrile as a mobile phase. The calculation of concentrations was based on standards prepared in the laboratory. The mobile phase was acetonitrile/water (85/15) with a flow rate of 1 mL/min, at ambient temperature. Sugars were detected at 210 nm. In calibration curves, standard sugar solutions (glucose, fructose, and sucrose) were prepared to contain between 10 μg/mL and 150 μg/mL. These solutions were injected into the chromatographic system, and the resulting areas of the peaks were plotted against concentration for the calibration curve.

2.5. Total Phenol Determination

The total phenolic content of sea buckthorn berries was determined spectrophotometrically at 765 nm following the Folin–Ciocalteu method as described by Singleton et al. [32]. A standard calibration curve was plotted by using gallic acid (Merck, Germany) in the concentration range 1–500 mg/L. The results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of fresh berries.

2.6. Extraction and Determination of Individual Phenolics

The phenolic acids in sea buckthorn berries were determined following the procedure described by Rodriguez-Delgado et al. [33]. From 200 g of fragmented sample, ca. 50 g of sample (per replicate) was transferred to a centrifuge tube, mixed homogeneously, then diluted 1:1 with distilled water, and centrifuged at 15,000× *g* for 15 min. The supernatant was passed through a 0.45 μm Millex-HV hydrophilic Polyvinylidene fluoride PVDF membrane filter, and then injected into the HPLC system (gradient). The chromatographic separation in the Agilent 1100 series HPLC took place in a DAD detector (Agilent, Santa Clara, CA, USA) with a 250 mm × 4.6 mm, 4 m Octadecylsilyl Groups (ODS) column (HiChrom, Reading, UK). The following solvents in water with a flow rate of 1 mL/min and 20 μL injection volume were used for spectral measurements taken at both 254 nm and 280 nm as the mobile phase: solvent A, methanol–acetic acid–water (10:2:88); solvent B, methanol–acetic acid–water (90:2:8).

2.7. Determination of Trolox Equivalent Antioxidant Capacity (TEAC)

For total antioxidant determination, the Trolox equivalent antioxidant capacity (TEAC) assay was used. TEAC was determined with ABTS (2,2′-azino-bis-3-ethylbenzo-thiazoline-6-sulfonic acid) by dissolving in an acetate buffer using potassium persulfate [34]. For longer stability, the mixture was diluted with 20 mM sodium acetate buffer in an acidic pH of 4.5 and read at 734 nm wavelength, 0.700 ± 0.01. For the spectrometric assay, 3 dm^3 ABTS+ was mixed with a 20 dm^3 fruit extract sample and incubated for 10 min, and then absorbance was detected at 734 nm. The results were expressed as mmol Trolox equivalent/100 g fresh weight (FW).

2.8. FRAP (Ferric Reducing Antioxidant Power) Assay

According to the methods of Benzie and Strain [35], the FRAP assay was used. The assay was conducted using three aqueous stock solutions containing 0.1 mol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ (2,4,6-tris(2-pyridyl)-1,3,5-triazine) acidified with concentrated hydrochloric acid, and 20 mmol/L ferric chloride. These solutions were prepared

and stored in the dark under refrigeration. Stock solutions were combined (10:1:1, $v/v/v$) to form the FRAP reagent just prior to analysis. For each assay, laboratory duplicates from each replicate plus 2.97 mL of FRAP reagent and 30 mL of sample extract were mixed. After 30 min, the absorbance of the reaction mixture at 593 nm was determined on a spectrophotometer. The results were expressed as mmol Trolox/100 g FW.

2.9. Crude Lipid Extraction

Crude lipid was extracted from 20 g of sea buckthorn berries per genotype using hexane solvent in a Soxhlet apparatus for 6–7 h after solvent was removed by a rotary evaporator (Heidolph, Hei-VAP Value; pressure < 10 mbar, and temperature 40 °C). Berries were placed in a porous thimble of a Soxhlet extractor with a cotton plug at its mouth, and the thimble was placed in an extraction chamber which was suspended in a previously weighed flask containing methanol, methanol–chloroform, or petroleum ether. The whole assembly was adjusted, and the flask was heated using a heating metal for 8–10 h to extract lipid. After the extraction, the thimble was removed from the Soxhlet apparatus and the solvent was removed under reduced pressure to afford lipid. Furthermore, the flask containing lipid was placed in the oven at 100 °C for 30 min to remove residual solvent, cooled in a desiccator, and weighed. The amount of lipid was calculated and expressed as the percentage crude lipid content [36].

2.10. Determination of Fatty Acid Composition by Gas Chromatography

Methanolic HCl was prepared by the gradual addition of 20 mL of acetyl chloride to 100 mL of cold methanol. Fatty acid methyl esters (FAMEs) were prepared from total lipid extracts of sea buckthorn berry tissues by adding 1 mL of methanolic HCl to 1 mg of total lipid and incubating for 1 h at 80 °C. The methylation was quenched by the addition of 1 mL of 0.9% aqueous sodium chloride, and the FAMEs were extracted twice with 2 mL of hexane. The resulting FAME extract was evaporated under nitrogen and resuspended in isooctane for GC/MS analysis. GC/MS analysis of FAMEs was performed on an Agilent 6890 N gas chromatograph with an Agilent 5975 B Inert XL mass selective detector. Chromatographic separation was achieved using a DB-23 capillary column (J&W Scientific, Folsom CA; 30 m × 250 μm × 0.25 μm) with the following temperature program: initial temperature 90 °C, raised at 10 °C/min to 165 °C, held for 5 min, and then raised at 3 °C/min to a final temperature of 230 °C. The inlet was operated in splitless mode at a temperature of 290 °C, with helium as the carrier gas at constant flow of 1.2 mL/min. The transfer line temperature was 250 °C, and the MS ion source and quadrupole temperatures were set to 230 °C and 150 °C, respectively. MS detection was carried out in electron impact (EI) ionization mode, scanning all masses from 30–350 amu. Peaks were identified on the basis of mass spectral comparison with the NIST05 MS library in combination with retention time, matching to external FAME standards [37].

2.11. Statistical Analysis

Differences among genotypes were determined through analysis of variance (ANOVA) using the SPSS 22 software program, and results were evaluated with the least significant difference (LSD) method at $p < 0.05$. In Section 3, mean values and standard deviations are given ($\overline{x} \pm SD$).

3. Results and Discussion

Agro-Morphological Traits

The 100-berry weight, berry shape, berry skin color, ripening time, growth habit, presence of thorns, and leaf area of 10 genotypes are shown in Table 1. Statistically significant differences among genotypes for 100-berry weight ($p < 0.05$) were determined. The genotypes exhibited 100-berry weight in a range between 13.85 g (S-4) and 27.87 g (S-8) indicating a twofold difference among S-4 and S-8 genotypes. Most of the genotypes (six

genotypes) revealed oblong fruit shape, followed by elliptical (two genotypes) and oblate berry shape (two genotypes) (Table 1).

Table 1. Means and SD values of agro-morphological characteristics of the 10 sea buckthorn genotypes.

Genotypes	100-Berry Weight (g)	Berry Shape	Berry Skin Color	Ripening Time	Growth Habit	Thorns	Leaf Area (cm^2)
S-1	17.11 ± 0.8^{d}	Elliptic	Light orange	23 September	Shrub	Few	2.56 ± 0.2^{c}
S-2	21.34 ± 0.9^{bc}	Oblong	Orange	2 October	Tree	Few	3.40 ± 0.2^{ab}
S-3	18.56 ± 0.8^{cd}	Oblong	Light orange	27 September	Tree	Medium	2.89 ± 0.1^{bc}
S-4	13.85 ± 0.6^{e}	Elliptic	Yellow	30 September	Shrub	Few	3.98 ± 0.2^{ab}
S-5	14.47 ± 0.5^{de}	Ovate	Light orange	4 October	Shrub	Few	4.02 ± 0.3^{ab}
S-6	25.11 ± 0.7^{b}	Oblong	Dark orange	7 October	Tree	Medium	4.22 ± 0.3^{a}
S-7	20.38 ± 0.9^{c}	Oblong	Orange	30 September	Tree	Medium	3.70 ± 0.2^{ab}
S-8	27.87 ± 1.1^{a}	Ovate	Yellow	1 October	Shrub	Few	3.23 ± 0.1^{b}
S-9	19.79 ± 0.8^{cd}	Oblong	Light orange	25 September	Shrub	Few	3.04 ± 0.2^{bc}
S-10	16.50 ± 1.0^{de}	Oblong	Yellow	27 September	Tree	Few	2.96 ± 0.1^{bc}

Different letters in same column indicate significant differences at the 0.05 level.

Four genotypes had light-orange berry skin color, three genotypes had yellow berry skin color, two genotypes had orange berry skin color, and only one genotype had dark-orange berry skin color. The ripening time of the 10 genotypes occurred from 23 September (S-1) to 7 October (S-6). Regarding the growth habit, trees and shrubs were represented equally, with five genotypes each. Seven genotypes presented only few thorns per plant, while the remaining genotypes showed medium presence of thorns. Leaf area of the genotypes ranged from 2.56 cm^2 (S-1) to 4.22 cm^2 (S-6) with statistically significant differences ($p < 0.05$) among the analyzed genotypes.

The results indicate that sea buckthorn genotypes were quite variable in most of the agro-morphological characteristics. In fact, previous studies conducted in different countries also showed great diversity in most of the agro-morphological traits in sea buckthorn. For example, Sezen et al. [38] used 30 female seed-propagated sea buckthorn genotypes to determine their landscape and horticulture value and reported quite variable 100-berry weight that ranged between 15 and 26 g. Zheng et al. [39] used a large number of sea buckthorn cultivars belonging to ssp. *mongolica* and found 100-berry weight between 37 and 74 g. Zheng et al. [40] reported 100-berry weight between 9 and 20 g among ssp. *sinensis* genotypes. In India, 100-berry weight of sea buckthorn genotypes was found to be between 11.53 g and 18.87 g [41]. In China, it was reported to vary between 18.5 and 19.5 g among genotypes belonging to *H. rhamnoides* and between 19.0 and 20.5 g among genotypes belonging to *H. salicifolia* [42]. In another study conducted in different valleys in India, diverse 100-berry weight was documented. For example, in Mana valley, they reported average 100-berry weight of 21.25 g, whereas, in Niti valley, the 100-berry weight amounted to 16.73 g [43]. Li et al. [16] used 78 diverse sea buckthorn accessions and reported 100-berry weight between 10.73 g (*H. rhamnoides* ssp. *sinensis*) and 47.69 g (*H. rhamnoides* ssp. *mongolica*).

Yadav et al. [41] used a number of wild-grown sea buckthorn genotypes in India and found that fruit shape varied from round to ovate, while berry skin color ranged from greenish-yellow to yellow-orange in *H. salicifolia* accessions. Sezen et al. [38] found that the majority of *H. rhamnoides* genotypes in Coruh valley in Turkey had oblong berry shape while yellow, light-yellow, dark-yellow, yellow-orange, orange, and dark-orange peel colors were present among wild-grown sea buckthorn genotypes. India is one of the richest countries in terms of variability of sea buckthorn's gene pool, and Dhyani et al. [43] found oval, elliptical, round oval/elliptical, and ovate berry shape with diverse berry skin color including orange yellow, orange, reddish yellow, red orange, and orange. Li et al. [16] used 78 diverse sea buckthorn accessions and reported oblong, ovate, and elliptical berry shape.

Sezen et al. [38] reported early or medium ripening characteristics in sea buckthorn genotypes in Turkey. Singh and Singh [44] also found great variations for this morphological trait in native *H. salicifolia* and *H. rhamnoides* female plants in Himachal Pradesh. Sezen et al. [38] also reported that most of the sea buckthorn genotypes had a bush growth habit, but the tree growth habit was also evident. This was also strongly supported in a study conducted in India, which revealed that most of the sea buckthorn genotypes had a bush growth habit [41].

Previously, Sezen et al. [38] found that most of the sea buckthorn genotypes had few or medium thorns, and the leaf area of these genotypes was between 1.59 and 4.26 cm^2, indicating great variability. In India, leaf area was found to be between 2.28 and 9.35 cm^2 among sea buckthorns belonging to different species [41]. Sabir et al. [20] also reported a quite variable number of thorns and leaf sizes among sea buckthorn genotypes grown in Pakistan. The agro-morphological characteristics varied among studies conducted on different continents. These differences could be connected to diverse origins, species, different parts of the fruit analyzed, climatic and growing conditions, etc.

Table 2 shows the results obtained from the juice analyses, including juice yield, vitamin C content, titratable acidity, SSC, and protein and lipid content of the 10 analyzed genotypes. For all researched parameters, statistically significant differences were evident at the 0.05 level.

Table 2. Means and SD values of juice yield, vitamin C, soluble solid content (SSC), titratable acidity, and protein and lipid content in pulps of the 10 sea buckthorn genotypes.

Genotypes	Juice Yield (%)	Vitamin C (mg/100 g)	Titratable Acidity (%)	SSC (%)	Protein (%)	Lipid (%)
S-1	52.25 ± 2.3^{c}	40.10 ± 2.4^{h}	3.88 ± 0.2^{bc}	12.95 ± 0.6^{cd}	0.74 ± 0.2^{b}	5.70 ± 0.4^{ab}
S-2	57.15 ± 2.7^{a}	57.25 ± 3.9^{c}	3.40 ± 0.2^{bc}	12.56 ± 0.5^{de}	0.66 ± 0.1^{bc}	5.02 ± 0.3^{c}
S-3	54.42 ± 3.9^{b}	44.51 ± 2.0^{g}	4.01 ± 0.3^{b}	12.86 ± 0.7^{cd}	0.72 ± 0.2^{b}	5.49 ± 0.4^{b}
S-4	44.87 ± 3.1^{g}	54.33 ± 3.1^{d}	3.80 ± 0.1^{bc}	14.67 ± 0.7^{ab}	0.60 ± 0.1^{c}	6.03 ± 0.5^{ab}
S-5	50.40 ± 2.9^{d}	37.45 ± 2.7^{i}	3.76 ± 0.2^{bc}	13.17 ± 0.5^{cd}	0.63 ± 0.1^{bc}	5.58 ± 0.3^{ab}
S-6	48.83 ± 2.4^{e}	48.03 ± 3.3^{f}	3.14 ± 0.2^{c}	14.80 ± 0.6^{a}	0.83 ± 0.1^{a}	6.17 ± 0.3^{a}
S-7	47.75 ± 3.2^{ef}	49.28 ± 4.6^{ef}	4.17 ± 0.3^{ab}	14.07 ± 0.5^{b}	0.66 ± 0.2^{bc}	5.40 ± 0.2^{bc}
S-8	46.58 ± 2.7^{f}	62.85 ± 5.4^{a}	3.30 ± 0.2^{bc}	13.90 ± 0.5^{bc}	0.80 ± 0.2^{ab}	5.78 ± 0.4^{ab}
S-9	49.33 ± 2.5^{ef}	60.14 ± 4.2^{b}	4.73 ± 0.3^{a}	13.45 ± 0.6^{c}	0.70 ± 0.1^{b}	6.10 ± 0.3^{ab}
S-10	54.10 ± 4.0^{b}	50.62 ± 2.4^{e}	4.20 ± 0.1^{ab}	12.70 ± 0.4^{d}	0.72 ± 20.1^{b}	5.65 ± 0.3^{ab}

Different letters in same column indicate significant differences at the 0.05 level.

As shown in Table 2, genotypes S-2, S-3, S-10, S-1, and S-5 had higher values of fruit juice yield, i.e., 57.15%, 54.42%, 54.10%, 52.25%, and 50.40%, respectively. The lowest fruit juice yield was obtained from genotype S-4, which amounted to 44.87%. Sezen et al. [38] reported quite variable berry juice yield among 30 seed-propagated female sea buckthorn genotypes grown in Coruh valley in Turkey, ranging between 37.00% and 53.60%, indicating similarity with our study. Zheng et al. [39] used a large number of sea buckthorn cultivars belonging to ssp. *mongolica* and found juice yield between 50.5% and 59.4%. Zheng et al. [40] reported juice yield between 38.9% and 62.7% among ssp. *sinensis* genotypes. Previous researches showed higher fruit juice yield (60–80%) among sea buckthorn genotypes and cultivars [20,41,43,45,46] compared to our study. The observed differences could be the result of plant material used (different species, genotypes, accessions, etc.), growing locality, agronomic practices applied, etc.

The vitamin C content of the 10 sea buckthorn genotypes in this study was in the range of 37.45 mg/100 g (S-5) to 62.85 mg/100 g (S-8), indicating nearly twofold differences between these two genotypes. Sezen et al. [38] found lower vitamin C content (between 19 and 34 mg per 100 g) among sea buckthorn genotypes. Yao et al. [47] studied vitamin C concentrations of 71 *Hippophae rhamnoides* genotypes and found quite variable vitamin C content ranging from 28 to 201 mg/100 g. Jalakas et al. [48] reported vitamin C content to

vary between 49 and 65 mg per 100 g among sea buckthorn cultivars. The great variations in the vitamin C content of sea buckthorn genotypes are characteristic for this unique plant species. This trait could also be affected by the genotype, geographical origin, level of maturity of the berries, growing conditions, etc. [40].

The soluble solid content (SSC) and titratable acidity content of the genotypes in this study varied from 12.56% (S-2) to 14.67% (S-4) and 3.14% (S-6) to 4.73% (S-9), respectively. Genotypes greatly differed from each other for SSC and titratable acidity content at the 0.05 level (Table 2). Sezen et al. [38] reported SSC and titratable acidity among sea buckthorn (*H. rhamnoides*) genotypes to be 10.65–14.60% and 2.75–5.02%, respectively which is in accordance with our results. Sea buckthorn berries are considered highly acidic fruits. In Finland, the SSC content of sea buckthorn was reported to be between 7.4% and 12.6% [29]. Kuhkheil et al. [49] found that the content of vitamin C and SSC were the main variables in chemical constituents for the effective detection of original wild populations of sea buckthorn (*Hippophae rhamnoides* L.) in central Alborz Mountains in Iran. They reported the lowest SSC of 8.60% and average SSC content between 17% and 20% in different years by using a large number of populations. Zheng et al. [39] used a large number of sea buckthorn cultivars belonging to ssp. *mongolica* and found SSC and acidity to be 7.40–9.00% and 2.90–4.99%, respectively. Zheng et al. [40] found SSC to be between 7.3% and 21.8% among ssp. *sinensis* genotypes.

The protein and lipid content of sea buckthorn genotypes is given in Table 2. The genotypes exhibited statistically significant differences for both analyzed parameters at the 0.05 level. Protein and lipid content was 0.60% (S-4) to 0.80% (S-8) and 5.02% (S-2) to 6.17% (S-6), respectively. These results indicate that sea buckthorn berries are a rich source of proteins and lipids (Table 2). Criste et al. [25] analyzed four varieties of sea buckthorn (*H. rhamnoides*) in Romania and found protein content ranging between 0.72% in the Carmen variety and 0.86% in the SF-6 variety. These results agree with our findings on protein content. One of the most important properties of sea buckthorn berries is its lipid content in the mesocarp section, as well as in the seeds [49–51]. The lipid content of whole berries can vary considerably with the variety and other factors. Criste et al. [25] investigated four varieties of sea buckthorn (*H. rhamnoides*) in Romania and reported lipid content of berries (pulp) ranging between 4.61% and 5.71%. The lipid content of the mesocarp (pulp) of sea buckthorn berries is mainly determined by the used genotype, as well as by the environmental conditions. Previous studies also reflected a genotypic effect on the lipid content of fresh berries of *Hippophae* spp.; the reported lipid percentage ranged from 1.4% in ssp. *sinensis* from China up to 13.7% in ssp. *turkestanica* from the Western Pamirs [52]. The specific sugar content of genotypes is given in Table 3. The genotypes contained mainly glucose (0.14–0.71%) and fructose (0.10–0.59%), while a few genotypes contained negligible sucrose content (Table 3).

Table 3. Means and SD values of the contents of specific sugars of the 10 sea buckthorn genotypes.

Genotypes	Glucose (%)	Frucose (%)	Sucrose (%)
S-1	0.39 ± 0.1^{d}	0.25 ± 0.1^{c}	nd
S-2	0.14 ± 0.0^{fg}	0.10 ± 0.0^{d}	nd
S-3	0.30 ± 0.1^{e}	0.19 ± 0.0^{cd}	nd
S-4	0.68 ± 0.2^{ab}	0.52 ± 0.1^{ab}	0.07
S-5	0.50 ± 0.2^{c}	0.30 ± 0.1^{bc}	nd
S-6	0.71 ± 0.2^{a}	0.59 ± 0.1^{a}	0.09
S-7	0.64 ± 0.1^{ab}	0.48 ± 0.1^{ab}	nd
S-8	0.60 ± 0.1^{b}	0.40 ± 0.1^{b}	0.04
S-9	0.55 ± 0.1^{bc}	0.34 ± 0.1^{bc}	nd
S-10	0.22 ± 0.0^{f}	0.15 ± 0.0^{cd}	nd

Different letters in same column indicate significant differences at the 0.05 level; nd, not detected.

We found statistically significant differences in fructose and glucose content among the 10 sea buckthorn genotypes. Previously, Yang [53] studied specific sugars in sea buckthorn berries and reported glucose and fructose as the main sugars in berries for all three major subspecies (*H. rhamnoides* ssp. *sinensis*, ssp. *rhamnoides*, and ssp. *mongolica*). Criste et al. [25] reported glucose and fructose as the main specific sugars in four sea buckthorn cultivars belonging to *H. rhamnoides* in Romania and reported fructose and glucose content of 0.18–1.10% and 0.17–0.46%, respectively. They also found that only one cultivar contained a negligible amount of sucrose, determining at the same time higher glucose than fructose content, similarly to our findings. Glucose content in our research was also similar to the findings of Yang [53] in all samples of *H. rhamnoides*. In another study, Yang et al. [54] found fructose and glucose to range from 0.6% in ssp. *rhamnoides* to 24.2% in berries of ssp. *sinensis*. Zheng et al. [39,40] indicated glucose and fructose as the main sugars in sea buckthorn berries, suggesting that both sugars levels are affected by the cultivars.

In Table 4, the total phenolic content, total anthocyanins, and antioxidant capacity in berries from the 10 sea buckthorn genotypes are shown. As indicated in Table 4, the differences in all analyzed parameters among genotypes were statistically significant ($p < 0.05$).

Table 4. Means and SD values of total phenolic content, total anthocyanins, and antioxidant capacity (ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays) of the 10 sea buckthorn genotypes.

Genotypes	Total Phenolic Content (mg GAE/100 g)	Total Anthocyanin (mg/L)	FRAP (mg Trolox Equivalent/100 g FW)	TEAC (mg Trolox Equivalent/100 g FW)
S-1	486 ± 24^{f}	19.4 ± 0.4^{e}	0.54 ± 0.1^{c}	2.04 ± 0.2^{d}
S-2	522 ± 33^{d}	31.1 ± 0.7^{b}	1.06 ± 0.2^{b}	2.11 ± 0.2^{cd}
S-3	450 ± 18^{hi}	22.5 ± 0.6^{c}	0.77 ± 0.1^{bc}	1.89 ± 0.1^{de}
S-4	412 ± 14^{i}	11.2 ± 0.4^{f}	0.45 ± 0.1^{cd}	1.71 ± 0.1^{e}
S-5	587 ± 20^{c}	20.4 ± 0.5^{d}	1.22 ± 0.2^{ab}	2.34 ± 0.2^{c}
S-6	495 ± 22^{ef}	38.7 ± 0.8^{a}	0.87 ± 0.2^{bc}	1.67 ± 0.1^{e}
S-7	507 ± 19^{e}	29.4 ± 0.3^{c}	1.01 ± 0.2^{bc}	2.28 ± 0.2^{cd}
S-8	622 ± 30^{a}	11.4 ± 0.1^{f}	1.48 ± 0.2^{a}	2.93 ± 0.2^{a}
S-9	604 ± 27^{b}	21.6 ± 0.3^{cd}	1.34 ± 0.2^{ab}	2.78 ± 0.2^{b}
S-10	461 ± 19^{g}	9.1 ± 0.2^{g}	0.37 ± 0.1^{cd}	1.58 ± 0.1^{ef}

Different letters in same column indicate significant differences at the 0.05 level; GAE, gallic acid equivalent; FW, fresh weight.

A high genotypic variation in terms of total phenolic content was observed (412–622 mg GAE/100 g FW). The highest total phenolic content was observed in genotype S-8 (622 mg GAE/100 g FW), followed by the S-5 genotype (587 mg GAE/100 g FW), while the lowest value was recorded in genotype S-4 (412 mg GAE/100 g FW; Table 4).

Total phenolic content was previously reported to be quite variable among sea buckthorn cultivars and genotypes grown in different agro-climatic conditions in the world. Saeidi et al. [55] reported 247 mg GAE/100 g FW of total phenolic content in wild-grown *H. rhamnoides* ssp. *rhamnoides*, indicating lower values than those recorded in our samples. However, Rop et al. [56] and Crieste et al. [25] found total phenolic content in four sea buckthorn cultivars grown in Czech Republic and Romania to range from 862–1417 mg GAE/100 g and 1012–1866 mg GAE/100 g FW, values higher than those recorded in our study. Bittová et al. [57] also reported higher total phenolic content (i.e., between 1070 and 1730 mg GAE/100 g) in berries of sea buckthorn cultivars. All of the abovementioned studies revealed significant differences existing among sea buckthorn cultivars in terms of total phenolic content. Di Mauro et al. [58] reported that the polyphenolic profile in olive is cultivar-dependent. The local sea buckthorn genotypes were characterized by markedly higher contents of total polyphenols compared to blackberry (262 mg/100 g), blueberry (300 mg/100 g), raspberry (322 mg/100 g), strawberry (323 mg/100 g), and blackcurrant

(434 mg/100 g) [59]. The World Health Organization (WHO) recommendation to increase consumption of fruit, vegetables, and fiber is a key lifestyle change that could help to reduce the risk of noncommunicable diseases (NCDs) [60]. Although deficiencies in polyphenol intake do not result in specific deficiency diseases, adequate intake of polyphenols could confer health benefits, especially with regard to chronic diseases. Tea, cocoa, fruits, and berries, as well as vegetables, are rich in polyphenols [61].

Genotype S-6 with dark-orange color had the highest total anthocyanin content in berries (38.7 mg/L), while yellow berry genotypes such as S-4, S-8, and S-10 genotypes had the lowest total anthocyanin content, i.e., 11.2 mg/L, 11.4 mg/L, and 9.3 mg/L, respectively (Table 4). The differences in total anthocyanins among genotypes were found to be statistically significant at $p < 0.05$ (Table 4). Tiitinen et al. [29] previously studied a number of sea buckthorn genotypes in Finland and reported the total anthocyanin content of sea buckthorn berries to range from 7 to 38 mg/L, demonstrating similarities with our study. Sezen et al. [38] also found that relatively dark-orange sea buckthorn berries contain more anthocyanin than yellow and light-yellow sea buckthorn berries. Sabir et al. [20] studied sea buckthorn genotypes in Pakistan and reported anthocyanin content ranging between 0.5 and 25 mg/L, which in accordance with our results.

We found statistically significant differences ($p < 0.05$) among genotypes for antioxidant capacity by using FRAP and TEAC assays. Genotype S-8 showed the highest antioxidant capacity in both methods as 2.93 mmol Trolox equivalent/100 g in the TEAC assay and 1.48 mmol Trolox equivalent/100 g in the FRAP assay (Table 4). In addition, the genotypes that had the highest total phenolic content also showed the highest antioxidant activity in both assays. The FRAP, TEAC, and TPC (total phenolic content) results showed a close relationship, indicating that antioxidant capacity is attributable to the wide range of polyphenols present in sea buckthorn berry skin and flesh. Chen et al. [62] also reported that antioxidant activity in sea buckthorn berries followed the same trend as the concentrations of total phenolics. On the basis of these findings, it can be concluded that differences among the phenolic profiles and antioxidant capacities of sea buckthorn berries significantly depend on the genotype because all plants in our study were growing in similar environmental conditions, receiving similar sun exposure and temperature levels. Makovics-Zsohar et al. [63] investigated six sea buckthorn genotypes in Hungary and revealed a nearly threefold difference between the lowest and highest antioxidant capacities of the tested genotypes. They reported TEAC values that ranged between 1.76 and 3.13 mmol Trolox equivalent/100 g fresh weight and FRAP values that ranged between 0.45 and 1.80 mmol AA equivalent/100 g. They also found that *Hippophae rhamnoides* berries possess in vitro antioxidant activity, strongly determined by the genotype as well as by the harvest time. Criste et al. [25] also reported that the antioxidant capacity of four sea buckthorn genotypes was quite variable among genotypes, and all genotypes had relatively high antioxidant capacity determined by 2.2-diphenyl-1-picrylhdrazyl (DPPH) and TEAC assays.

The fatty acid content of fruits of the 10 sea buckthorn genotypes is given in Table 5. It is obvious that the genotype strongly influenced fatty acid content, and that there were statistically significant differences among genotypes ($p < 0.05$) for individual fatty acids, except stearic acid (Table 5).

Linoleic acid was the main fatty acid in the pulp of sea buckthorn genotypes and varied among genotypes from 24.11% to 36.37%. This fatty acid was followed by palmitoleic acid (18.13–26.44%) and palmitic acid (15.40–21.20%). The content of linolenic and stearic acid was determined to be lower than that of the abovementioned fatty acids and ranged from 3.88–7.02% and 1.80–3.23%, respectively (Table 5). Fatty acids in sea buckthorn berries (pulp) are very important from a nutritive point of view because sea buckthorn berries are edible when ripe, and it is clear that sea buckthorn berries are a valuable source of some biologically active compounds, including antioxidants and fatty acids. Saeidi et al. [55] found that berries of *H. rhamnoides* grown in Iran include linoleic (34.2%), palmitoleic (21.37%), palmitic (17.2%), oleic (12%), linolenic (5.37%), and stearic acid (1.67%) as dominant fatty

acids. It is well documented that, among fruits, macadamia and sea buckthorn are high in concentration of palmitoleic acid [64]. Yang and Kallio [52] also found that sea buckthorn fruit (mesocarp or pulp) exhibits a high content of palmitoleic acid. Regarding fatty acid diversity and content, our results are in accordance with the abovementioned studies. The differences between our results and other studies could be explained by genotype, cultivar used, growing and geographical conditions, and environmental factors [65].

Table 5. Means and SD values of fatty acid content in the pulp of the 10 sea buckthorn genotypes (%).

Genotypes	Linoleic Acid	Palmitoleic Acid	Palmitic Acid	Oleic Acid	Linolenic Acid	Stearic Acid
S-1	36.37 ± 1.2^{a}	18.13 ± 0.7^{e}	17.07 ± 0.5^{c}	9.84 ± 0.2^{c}	3.88 ± 0.2^{b}	1.88 ± 0.1^{NS}
S-2	25.56 ± 0.9^{de}	23.42 ± 0.9^{bc}	19.04 ± 0.7^{b}	10.87 ± 0.3^{bc}	5.15 ± 0.3^{ab}	2.21 ± 0.1
S-3	27.43 ± 1.1^{d}	20.02 ± 0.5^{d}	15.40 ± 0.4^{d}	15.40 ± 0.3^{a}	4.24 ± 0.2^{ab}	1.97 ± 0.1
S-4	30.02 ± 1.3^{c}	19.00 ± 0.5^{de}	21.01 ± 0.3^{ab}	10.36 ± 0.2^{bc}	4.78 ± 0.2^{ab}	2.30 ± 0.2
S-5	24.11 ± 1.1^{e}	24.77 ± 0.8^{b}	20.40 ± 0.5^{ab}	9.23 ± 0.3^{c}	6.44 ± 0.3^{ab}	2.44 ± 0.1
S-6	24.97 ± 0.8^{e}	21.56 ± 1.0^{c}	20.84 ± 0.4^{ab}	13.12 ± 0.2^{b}	6.02 ± 0.4^{ab}	2.27 ± 0.2
S-7	29.27 ± 1.4^{bc}	20.75 ± 0.7^{cd}	18.85 ± 0.3^{bc}	14.56 ± 0.4^{ab}	3.95 ± 0.3^{b}	2.04 ± 0.1
S-8	24.88 ± 1.2^{de}	26.44 ± 1.1^{a}	21.20 ± 0.5^{a}	13.87 ± 0.2^{ab}	5.80 ± 0.3^{ab}	2.15 ± 0.1
S-9	32.11 ± 1.4^{ab}	19.68 ± 0.7^{de}	18.38 ± 0.2^{bc}	11.25 ± 0.4^{bc}	6.67 ± 0.4^{ab}	1.80 ± 0.1
S-10	33.37 ± 1.5^{b}	18.47 ± 0.9^{e}	19.80 ± 0.6^{ab}	9.44 ± 0.3^{c}	7.02 ± 0.4^{a}	3.23 ± 0.2

Different letters in same column indicate significant differences at the 0.05 level; NS, nonsignificant.

Major individual phenolic acids are shown in Table 6. As can be seen, major phenolic acids in pulp of berries belonging to the 10 analyzed sea buckthorn genotypes were gallic acid (5.43–17.12 mg/100 g), followed by quercetin (2.87–11.47 mg/100 g), rutin (2.87–11.47 mg/100 g), quercitrin (2.44–6.57 mg/100 g), luteolin (0.96–5.12 mg/100 g), and kaemferol (0.44–1.29 mg/100 g). Among all sea buckthorn genotypes, significant differences were recorded in phenolic acids at the 0.05 level (Table 6). Criste et al. [25] reported that gallic acid was the main phenolic acid in sea buckthorn pulp belonging to four cultivars, with concentrations varying from 6.51 to 19.37 mg/100 g, thus indicating similarities with our findings. They also reported rutin and quercetin to be the major phenolic acids in sea buckthorn berries. Previously, gallic acid, rutin, and quercetin were reported as main phenolic acids in sea buckthorn berries [64]. Bittova et al. [57] also reported that the main compounds identified in sea buckthorn berries were gallic acid, *p*-coumaric acid, ferulic acid, rutin, and quercitrin

Table 6. Means and SD values of the contents of major individual phenolics in the pulp of the 10 sea buckthorn genotypes (mg/100 g).

Genotypes	Gallic Acid	Quercetin	Rutin	Quercitrin	Luteolin	Kaempferol
S-1	7.44 ± 0.5^{cd}	4.42 ± 0.4^{cd}	3.86 ± 0.5^{d}	2.44 ± 0.3^{c}	2.44 ± 0.2^{b}	0.55 ± 0.1^{bc}
S-2	11.47 ± 0.6^{bc}	8.45 ± 0.7^{b}	8.82 ± 0.8^{ab}	3.66 ± 0.2^{bc}	2.11 ± 0.2^{bc}	1.22 ± 0.2^{a}
S-3	8.23 ± 0.6^{cd}	3.50 ± 0.4^{cd}	9.11 ± 0.6^{ab}	3.11 ± 0.3^{bc}	0.96 ± 0.1^{c}	0.44 ± 0.1^{c}
S-4	6.64 ± 0.5^{cd}	2.87 ± 0.3^{d}	10.24 ± 1.0^{ab}	2.76 ± 0.3^{c}	1.89 ± 0.1^{bc}	1.15 ± 0.2^{ab}
S-5	13.61 ± 1.0^{b}	7.56 ± 0.6^{bc}	8.62 ± 0.9^{b}	3.03 ± 0.2^{b}	2.98 ± 0.2^{ab}	0.82 ± 0.1^{b}
S-6	11.10 ± 1.1^{bc}	9.16 ± 0.8^{ab}	6.45 ± 0.5^{bc}	4.30 ± 0.3^{ab}	5.12 ± 0.3^{a}	1.04 ± 0.1^{ab}
S-7	9.56 ± 0.4^{c}	5.33 ± 0.5^{c}	7.24 ± 0.7^{bc}	4.11 ± 0.2^{ab}	4.04 ± 0.3^{ab}	0.70 ± 0.1^{bc}
S-8	17.12 ± 1.4^{a}	10.64 ± 1.0^{ab}	11.17 ± 0.8^{a}	6.57 ± 0.5^{a}	3.56 ± 0.3^{ab}	0.90 ± 0.1^{ab}
S-9	15.40 ± 1.3^{ab}	11.47 ± 1.3^{a}	9.86 ± 1.0^{ab}	4.77 ± 0.7^{ab}	3.98 ± 0.2^{ab}	1.29 ± 0.2^{ab}
S-10	5.43 ± 0.5^{d}	3.04 ± 0.3^{cd}	6.06 ± 0.6^{c}	2.95 ± 0.3^{bc}	1.44 ± 0.2^{bc}	0.62 ± 0.1^{bc}

Different letters in same column indicate significant differences at the 0.05 level.

4. Conclusions

The main results of this study encompassing 10 sea buckthorn genotypes from eastern Turkey displayed important nutritional and bioactive compounds in the sea buckthorn berries. The attested variability among genotypes in terms of physicochemical profiles and horticultural characteristics also showed their potential value for further breeding programs targeting different breeding purposes. The results also indicated the potential use of berries in bio-industrial applications, which remains unexplored so far. Easy propagation and production of this unique fruit species alongside the sustainable harvest of wild-grown plants can contribute to the local economies and have a significant effect on socioeconomic and environmental balance.

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References

1. Colak, A.M.; Okatan, V.; Polat, M.; Guclu, S.F. Different harvest times affect market quality of *Lycium barbarum* L. berries. *Turk. J. Agric. For.* **2019**, *43*, 326–333. [CrossRef]
2. Ozkan, G.; Ercisli, S.; Zeb, A.; Agar, A.; Sagbas, H.I.; Ilhan, G.; Gundogdu, M. Some Morphological and Biochemical Characteristics of Wild Grown Caucasian Whortleberry (*Vaccinium arctostaphylos* L.) Genotypes from Northeastern Turkey. *Not. Bot. Horti Agrobot.* **2019**, *47*, 378–383. [CrossRef]
3. Gecer, M.K.; Kan, T.; Gundogdu, M.; Ercisli, S.; Ilhan, G.; Sagbas, H.I. Physicochemical characteristics of wild and cultivated apricots (*Prunus armeniaca* L.) from Aras valley in Turkey. *Genet. Resour. Crop Evol.* **2020**, *67*, 935–945. [CrossRef]
4. Sahin, U.; Anapali, O.; Ercisli, S. Physico-chemical and physical properties of some substrates used in horticulture. *Gartenbauwissenschaft.* **2002**, *67*, 55–60.
5. Fu, L.; Xu, B.T.; Xu, X.R.; Qin, X.S.; Gan, R.Y.; Li, H.B. Antioxidant Capacities and Total Phenolic Contents of 56 Wild Fruits from South China. *Molecules* **2010**, *15*, 8602–8617. [CrossRef] [PubMed]
6. Li, Y.; Zhang, J.J.; Xu, D.P.; Zhou, T.; Zhou, Y.; Li, S.; Li, H.B. Bioactivities and Health Benefits of Wild Fruits. *Int. J. Mol. Sci.* **2016**, *17*, 1258. [CrossRef]
7. Ercisli, S.; Orhan, E.; Esitken, A.; Yildirim, N.; Agar, G. Relationships among some cornelian cherry genotypes (*Cornus mas* L.) based on RAPD analysis. *Genet. Resour. Crop Evol.* **2008**, *55*, 613–618. [CrossRef]
8. Yildiz, H.; Ercisli, S.; Hegedus, A.; Akbulut, M.; Topdas, E.F.; Aliman, J. Bioactive content and antioxidant characteristics of wild (*Fragaria vesca* L.) and cultivated strawberry (Fragaria ananassa Duch.) fruits from Turkey. *J. Appl. Bot. Food. Qual.* **2014**, *87*, 274–278.
9. Ercisli, S.; Esitken, A. Fruit characteristics of native rose hip (Rosa spp.) selections from the Erzurum province of Turkey. *N. Z. J. Crop Hort.* **2004**, *32*, 51–53. [CrossRef]
10. Tural, S.; Koca, I. Physico-chemical and antioxidant properties of cornelian cherry fruits (*Cornus mas* L.) grown in Turkey. *Sci. Hortic.* **2008**, *116*, 362–366. [CrossRef]
11. Korekar, G.; Dolkar, P.; Singh, H.; Srivastava, R.B.; Stobdan, T. Variability and the genotypic effect on antioxidant activity, total phenolics, carotenoids and ascorbic acid content in seventeen natural population of Seabuckthorn (*Hippophae rhamnoides* L.) from trans-Himalaya. *LWT Food Sci. Technol.* **2014**, *55*, 157–162. [CrossRef]
12. Hasbal, G.; Yilmaz-Ozden, T.; Can, A. Antioxidant and antiacetylcholinesterase activities of *Sorbus torminalis* (L.) Crantz (wild service tree) fruits. *J. Food Drug Anal.* **2015**, *23*, 57–62. [CrossRef] [PubMed]
13. TUBIVES. Turkish Plants Data Service (TÜBIVES). 2014. Available online: www.tu-bives.com (accessed on 25 June 2020).
14. Wang, Y.; Jiang, H.; Peng, S.; Korpelainen, H. Genetic structure in fragmented populations of Hippophae rhamnoides ssp. sinensis in China investigated by ISSR and cpSSR markers. *Plant Syst. Evol.* **2011**, *295*, 97–107. [CrossRef]
15. Pop, R.M.; Weesepoel, Y.; Socaciu, C.; Pintea, A.; Vincken, J.-P.; Gruppen, H. Carotenoid composition of berries and leaves from six Romanian sea buckthorn (*Hippophae rhamnoides* L.) varieties. *Food Chem.* **2014**, *147*, 1–9. [CrossRef] [PubMed]

16. Li, H.; Ruan, C.; Ding, J.; Li, J.; Wang, L.; Tian, X. Diversity in sea buckthorn (*Hippophae rhamnoides* L.) accessions with different origins based on morphological characteristics, oil traits, and microsatellite markers. *PLoS ONE* **2020**, *15*, e0230356. [CrossRef]
17. Zeb, A. Chemical and nutritional constituents of sea buckthorn juice. *Pak. J. Nutr.* **2004**, *3*, 99–106.
18. Aras, A.; Akkemik, Ü.; Kaya, Z. *Hippophae rhamnoides* L.: Fruit and seed morphology and its taxonomic problems in Turkey. *Pak. J. Bot.* **2007**, *39*, 1907–1916.
19. Rousi, A. The genus *Hippophaë* L. A taxonomic study. *Ann. Bot. Fennici* **1971**, *8*, 177–227.
20. Sabir, S.M.; Maqsood, H.; Hayat, I.; Khan, M.Q.; Khaliq, A. Elemental and nutritional analysis of sea buckthorn (*Hippophae rhamnoides* ssp. *turkestanica) berries of Pakistani origin. J. Med. Food* **2005**, *8*, 518–522.
21. Singh, V. Geographical adaptation and distribution of seabuckthorn. In *Seabuckthorn (Hippophae L.)—A Multipurpose Wonder Plant*; Singh, V., Ed.; Indus Publishing Company: New Delhi, India, 2003; Volume I, pp. 21–34.
22. Ficzek, G.; Mátravölgyi, G.; Furulyás, D.; Rentsendavaa, C.; Jócsák, I.; Papp, D.; Simon, G.; Végvári, G.; Stéger-Máté, M. Analysis of bioactive compounds of three sea buckthorn cultivars (*Hippophaë rhamnoides* L. 'Askola', 'Leikora', and 'Orangeveja') with HPLC and spectrophotometric methods. *Eur. J. Hortic. Sci.* **2019**, *84*, 31–38. [CrossRef]
23. Teleszko, M.; Wojdylo, A.; Rudzinska, M.; Oszmianski, J.; Golis, T. Analysis of Lipophilic and Hydrophilic Bioactive Compounds Content in Sea Buckthorn (*Hippophaë rhamnoides* L.) Berries. *J. Agric. Food Chem.* **2015**, *63*, 4120–4129. [CrossRef] [PubMed]
24. Tiitinen, K.M.; Yang, B.; Haraldsson, G.G.; Jonsdottir, S.; Kallio, H.P. Fast analysis of sugars, fruit acids, and vitamin c in sea buckthorn (*Hippophaë rhamnoides* L.) varieties. *J. Agric. Food Chem.* **2006**, *54*, 2508–2513. [CrossRef] [PubMed]
25. Criste, A.; Urcan, A.C.; Bunea, A.; Furtuna, F.R.P.; Olah, N.K.; Madden, R.H.; Corcionivoschi, N. Phytochemical Composition and Biological Activity of Berries and Leaves from Four Romanian Sea Buckthorn (*Hippophae Rhamnoides* L.) Varieties. *Molecules* **2020**, *25*, 1170. [CrossRef]
26. Cenkowski, S.; Yakimishen, R.; Przybylski, R.; Muir, W.E. Quality of extracted sea buckthorn seed and pulp oil. *Can. Biosyst. Eng.* **2006**, *48*, 309–316.
27. Korekar, G.; Dolkar, P.; Singh, H.; Srivastava, R.B.; Stobdan, T. Genotypic and Morphometric Effect on Fruit Oil Content in Seventeen Natural Population of Seabuckthorn (*Hippophae rhamnoides* L.) from Trans-Himalaya. *Natl. Acad. Sci. Lett.* **2013**, *36*, 603–607. [CrossRef]
28. Zielinska, A.; Nowak, I. Abundance of active ingredients in sea-buckthorn oil. *Lipids Health Dis.* **2017**, *16*, 95. [CrossRef]
29. Tiitinen, K.M.; Hakala, M.A.; Kallio, H.P. Quality Components of Sea Buckthorn (Hippophae rhamnoides) Varieties. *J. Agric. Food Chem.* **2005**, *53*, 1692–1699. [CrossRef]
30. AOAC. Fat (Crude) or Ether Extract in Animal Feed. In *Official Methods of Analysis of AOAC International*; Horwitz, W., Ed.; AOAC International: Gaithersburg, MD, USA, 2000.
31. Melgarejo, P.; Salazar, D.M.; Artes, F. Organic acids and sugars composition of harvested pomegranate fruits. *Eur. Food Res. Technol.* **2000**, *211*, 185–190. [CrossRef]
32. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In *Oxidants and Antioxidants*; Packer, L., Ed.; Elsevier: Amsterdam, The Netherlands, 1999; pp. 152–178.
33. Rodriguez-Delgado, M.Á.; Malovana, S.; Perez, J.; Borges, T.; Garcia-Montelongo, F.J. Separation of phenolic compounds by high-performance liquid chromatography with absorbance and fluorimetric detection. *J. Chromatogr.* **2001**, *912*, 249–257. [CrossRef]
34. Ozgen, M.; Reese, R.N.; Tulio, A.Z.; Scheerens, J.C.; Miller, A.R. Modified 2.2- azino-bis-3- ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and a comparison to ferric reducing antioxidant power (FRAP) and 2.2-diphenyl-1-picrylhdrazyl (DPPH) methods. *J. Agric. Food Chem.* **2006**, *54*, 1151–1157. [CrossRef]
35. Benzie, I.F.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76. [CrossRef] [PubMed]
36. AOAC. *Official Methods of Analysis*; Association of Official Analytical Chemists: Rockville, MD, USA, 1995.
37. Fatima, T.; Snyder, C.L.; Schroeder, W.R.; Cram, D.; Datla, R.; Wishart, D.; Weselake, R.J.; Krishna, P. Fatty Acid Composition of Developing Sea Buckthorn (*Hippophae rhamnoides* L.) Berry and the Transcriptome of the Mature Seed. *PLoS ONE* **2012**, *7*, e34099. [CrossRef] [PubMed]
38. Sezen, I.; Ercisli, S.; Cakir, O.; Koc, A.; Temim, E.; Hadziabulic, A. Biodiversity and Landscape Use of Sea Buckthorn (*Hippophae rhamnoides* L.) in the Coruh Valley of Turkey. *Erwerbs-Obstbau* **2015**, *57*, 23–28. [CrossRef]
39. Zheng, J.; Yang, B.; Trepanier, M.; Kallio, H. Effects of genotype, latitude, and weather conditions on the composition of sugars, sugar alcohols, fruit acids, and ascorbic acid in sea buckthorn (*Hippophaë rhamnoides* ssp. *mongolica*) berry juice. *J. Agric. Food Chem.* **2012**, *60*, 3180–3189. [CrossRef] [PubMed]
40. Zheng, J.; Kallio, H.; Linderborg, K.; Yang, B. Sugars, sugar alcohols, fruit acids, and ascorbic acid in wild Chinese sea buckthorn (*Hippophae rhamnoides* ssp. *sinensis*) with special reference to influence of latitude and altitude. *Food Res. Int.* **2011**, *44*, 2018–2026. [CrossRef]
41. Yadav, V.K.; Sah, V.K.; Singh, A.K.; Sharma, S.K. Variations in morphological and biochemical characters of seabuckthorn (*Hippophae salicifolia* D. Don) populations growing in Harsil area of Garhwal Himalaya in India. *Trop. Agric. Res Ext.* **2006**, *9*, 1–7.
42. Rongsen, L.U. Chemical composition of Hippophae fruits in China. In Proceedings of the International Symposium of Sea-Buckthorn, Barnaul, Russia, 23–25 August 1993; pp. 398–412.

43. Dhyani, D.; Maikhuri, R.K.; Dhyani, S. Seabuckthorn: An Underutilized Resource for the Nutritional Security and Livelihood Improvement of Rural Communities in Uttarakhand Himalaya. *Ecol. Food Nutr.* **2011**, *50*, 168–180. [CrossRef]
44. Singh, V.; Singh, R.K. Morpho-biochemical variations in seabuckthorn (*Hippophae* spp.) populations growing in Lahaul valley, dry temperate Himalayas. *Indian For.* **2004**, *130*, 663–672.
45. Heilsher, K.; Lorber, S. Processes for Cold Recovery of Clear Juice, Solids and Oil from Seabuckthorn Berries and Use of These Products. German Federal Republic Patent DE 4431394C1, 15 February 1996.
46. Chauhan, A.S.; Rekha, M.N.; Ramteke, R.S.; Eipeson, W.E. Preparation and quality evaluation of processed products from seabuckthorn (*Hippophae rhamnoides* L.) berries. *Beverage Food World* **2001**, *1*, 31–34.
47. Yao, Y.; Tigerstedt, P.M.A.; Joy, P. Variation of Vitamin C Concentration and Character Correlation between and within Natural Sea Buckthorn (*Hippophae rhamnoides* L.) Populations. *Acta Agric. Scand. Sect. B Soil Plant Sci.* **1992**, *42*, 12–17. [CrossRef]
48. Jalakas, M.; Kelt, K.; Kamp, K. The yield and fruit quality of sea buckthorn (*Hippophae rhamnoides* L.) after rejuvenation cutting. *Agron. Res.* **2003**, *1*, 31–36.
49. Kuhkheil, A.; Badi, H.N.; Mehrafarin, A.; Abdossi, V. Chemical constituents of sea buckthorn (*Hippophae rhamnoides* L.) fruit in populations of central Alborz Mountains in Iran. *Res. J. Pharmacog.* **2017**, *4*, 1–12.
50. Yang, B.; Kallio, H. Composition and physiological effects of sea buckthorn (Hippophae) lipids. *Trends Food Sci. Technol.* **2002**, *13*, 160–167. [CrossRef]
51. Ercisli, S.; Orhan, E.; Ozdemir, O.; Sengul, M. The genotypic effects on the chemical composition and antioxidant activity of sea buckthorn (*Hippophae rhamnoides* L.) berries grown in Turkey. *Sci. Hortic.* **2007**, *115*, 27–33. [CrossRef]
52. Yang, B.; Kallio, H.P. Fatty Acid Composition of Lipids in Sea Buckthorn (*Hippophaë rhamnoides* L.) Berries of Different Origins. *J. Agric. Food Chem.* **2001**, *49*, 1939–1947. [CrossRef]
53. Yang, B.; Halttunen, T.; Raimo, O.; Price, K.; Kallio, H. Flavonol glycosides in wild and cultivated berries of three major subspecies of Hippophaë rhamnoides and changes during harvesting period. *Food Chem.* **2009**, *115*, 657–664. [CrossRef]
54. Yang, B.; Zheng, J.; Kallio, H. Influence of origin, harvesting time and weather conditions on content of inositols and methylinositols in sea buckthorn (*H. rhamnoides*) berries. *Food Chem.* **2011**, *125*, 388–396. [CrossRef]
55. Saeidi, K.; Alirezalu, A.; Akbari, Z. Evaluation of chemical constitute, fatty acids and antioxidant activity of the fruit and seed of sea buckthorn (*Hippophae rhamnoides* L.) grown wild in Iran. *Nat. Prod. Res.* **2016**, *30*, 366–368. [CrossRef]
56. Rop, O.; Ercisli, S.; Mlcek, J.; Jurikova, T.; Hoza, I. Antioxidant and radical scavenging activities in fruits of 6 sea buckthorn (*H. rhamnoides* L.) cultivars. *Turk J. Agric. For.* **2014**, *38*, 224–232. [CrossRef]
57. Bittová, M.; Krejzová, E.; Roblová, V.; Kubán, P.; Kubán, V. Monitoring of HPLC profiles of selected polyphenolic compounds in sea buckthorn (*Hippophaë rhamnoides* L.) plant parts during annual growth cycle and estimation of their antioxidant potential. *Cent. Eur. J. Chem.* **2014**, *12*, 1152–1161. [CrossRef]
58. Di Mauro, M.D.; Giardina, R.C.; Fava, G.; Mirabella, E.F.; Acquaviva, R.; Renis, M.; D'Antona, N. Polyphenolic profile and antioxidant activity of olive mill wastewater from two Sicilian olive cultivars: Cerasuola and Nocellara etnea. *Eur. Food Res. Technol.* **2017**, *243*, 1895–1903. [CrossRef]
59. Donno, D.; Cerutti, A.K.; Prgomet, I.; Mellano, M.G.; Beccaro, G.L. Foodomics for mulberry fruit (Morus spp.): Analytical fingerprint as antioxidants' and health properties' determination tool. *Food Res. Int.* **2015**, *69*, 179–188. [CrossRef]
60. World Health Organization. *Diet, Nutrition and the Prevention of Chronic Diseases*, 29th ed.; World Health Organization: Geneva, Switzerland, 2003.
61. Fraga, C.G.; Croft, K.D.; Kennedy, D.O.; Tomás-Barberán, F.A. The effects of polyphenols and other bioactives on human health. *Food Funct.* **2019**, *10*, 514–528. [CrossRef]
62. Chen, C.; Xu, X.M.; Chen, Y.; Yu, M.Y.; Wen, F.Y.; Zhang, H. Identification, quantification and antioxidant activity of acylated flavonol glycosides from sea buckthorn (*Hippophae rhamnoides* ssp. *sinensis*). *Food Chem.* **2013**, *141*, 1573–1579. [CrossRef] [PubMed]
63. Makovics-Zsohár, N.; Hegedûs, A.; Stefanovits-Bányai, É.; Rédei, R.; Papp, N. The antioxidant capacity of sea buckthorn (*Hippophae rhamnoides* L.) berries depends on the genotype and harvest time. *Int. J. Hortic. Sci.* **2014**, *20*, 27–29. [CrossRef]
64. Li, T.S.C.; Beveridge, T.H.J.; Oomah, B.D. Nutritional and medicinal values. In *Sea Buckthorn (Hippophae rhamnoides L.): Production and Utilization*; Li, T.S.C., Beveridge, T., Eds.; NRC Research Press: Ottawa, ON, Canada, 2003; pp. 101–108.
65. Arimboor, R.; Kumar, K.S.; Arumughan, C. Simultaneous estimation of phenolic acids in sea buckthorn (*Hippophaë rhamnoides*) using RP-HPLC with DAD. *J. Pharm. Biomed. Anal.* **2008**, *47*, 31–38. [CrossRef]

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Sustainable Cornelian Cherry Production in Montenegro: Importance of Local Genetic Resources

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Abstract: Cornelian cherries has been grown Balkan peninsula no apparent disease and pest problems for centuries. The most important pomological and technological properties of cornelian cherry genetic resources (eleven local and introduced varieties and selections) in Gornje Polimlje, Montenegro were studied in four-year periods. Fruit weight, stone weight, and mesocarp/stone ratio shows great variation and varied between 1.89 to 6.16 g, 0.32 to 0.64 g and between 76.66 and 90.59%, respectively. Genotypes significantly differed each other in terms of yield determined as per trunk cross section area (TCSA). For better visualization of the agronomical traits important to the yield, i.e., yield per TCSA and fruit weight data is presented in combination as measured in the years of study. The best promising genotypes are Vladimirskiy, Chisinau yellow, and Krupnoplodni NS, which had consistently higher yield and large fruits for sustainable fruit production. Dry matter, total sugars, reducing sugars, sucrose, total acidity, Ca–pectate, vitamin C, total anthocyanins, total polyphenols, and ash content of cornelian cherry cultivars and selections were found between 19.81–24.46%, 10.94–14.47%, 10.44–13.09%, 0.24–1.23%, 1.92–2.51%, 2.76–4.43%, 41.09–101.27 mg/100 g, 0–157.06 mg/100 g, 183.91–307.9 mg/100 g, and 0.89–1.16%, respectively. The amount of potassium, which predominates in percentage of minerals in the ash, ranged from 2888 to 3574 mg/kg. The extensiveness of the study leads, therefore, to several clear conclusions and recommendations. Consistently, the best balance of biochemical elements in combination with good yield and fruit size measurements is found in selection Krupnoplodi NS. If consider fruit size and yield efficiency are priority, Vladimirskiy, Chisinau yellow, Kosten 3, and Krupnoplodni NS have clear advantage over the other genotypes. The study highlights the importance of local cornelian cherry selections for sustainable cornelian cherry production in Montenegro.

Keywords: *Cornus mas*; yield; weight; biochemical characteristics

1. Introduction

Sustainable fruit production is gained much importance more recently and one of the key principles associated with sustainable fruit production includes use and enhance biological diversity and use of genetic resources [1]. Consumers are also tending to buy fruits that can be classed as sustainable, including local provenance of the fruit and how organic or natural it is [2,3].

Plant genetic resources have been indispensable basic raw material due to their importance for food and agriculture since long time. Traditional and local varieties are very valuable resources,

well adopted across a wide range of agroclimatic conditions and remarkable resistance against pests and diseases that indicate higher sustainability. They carry many qualities, such as taste, color, and size [3,4]. Local varieties gain importance in the development of new varieties because they show a high degree of genetic diversity within a particular field [1,5].

Cornelian cherry (*Cornus mas* L.) is spontaneous fruit species, dispersed in East and South regions of Europe and West Asia [6]. It is a slow growing, long living, and very adaptive plant [7]. It grows mostly on the dry, sunny and rocky sides of light deciduous oak forests, together with other shrubs and bushes. Although cornelian cherry has long been known and used as a food and medicine [8], until recently, it belonged to the group of less known fruit species [9].

Lately, there has been an increase in consumer interest in healthy lifestyles, which includes a healthy diet, and producers are returning to this partially forgotten fruit, which is adorned with biologically very valuable fruits for humans with multiple use [10–12]. Ripe cornelian cherry fruits are eaten fresh, frozen, dried or processed into jam, marmalade, slatko (fruit preserve is sugar syrup), composte, fruit juice, syrup, yogurt, wine, liqueur, and brandy, while green fruits are marinated in a saline solution similar to olives [13,14]. Cornelian cherry fruits are rich in essential minerals, vitamins, sugars, acids, tannins, pectins, anthocyanin's, phenolic compounds and other substances, which puts it in line with other more widely used fruit species [9,11,15,16]. Almost all the organs of cornelian cherry are used in traditional medicine to treat diarrhoea, cholera, fever, malaria, kidney stones, urinary tract infections, bleeding, and overheating [17,18]. The healing properties of cornelian cherry are not ignored by medicine of modern age either, and numerous studies indicate its use in the prevention and treatment of arteriosclerosis, diabetes, high cholesterol, hepatic steatosis, and cancer [19–22]. In addition to the before mentioned, cornelian cherry has been found to have antimicrobial, antiparasitic, anti–inflammatory, and antioxidant effects [6,23,24].

Attractive golden–yellow flowers in early spring, as well as decorative leaves with long duration of leaf color change in autumn, allow the use of cornelian cherry for decorative purposes [25]. Thanks to early flowering, cornelian cherry is a valued honey plant because it provides of nectar and pollen in a period of pollen and nectar deficiency [26].

The area of cornelian cherry distribution in Montenegro reaches sea coast in the South and far North of the country, fruiting at 1300 m above sea level [27]. Many products made from cornelian cherry fruits harvested from wild trees from nature are traded in Montenegrin markets. Such fruits are produced in the conditions of undisturbed natural relations, without the application of pesticides and mineral fertilizers, meeting the strictest standards for the production of safe food. Fresh fruits of this fruit species or processed products stored without the use of high temperatures and preservatives can be recommended for the safe diet due to their high nutritional value and beneficial effect on health. However, there is a problem with fruit harvesting, as cornelian cherry, as a forest fruit, often grows in inaccessible terrains. The solution to that problem is to establish orchards of this fruit species.

Therefore, the aim of this paper was to examine the most important pomological and technological characteristics of introduced varieties and selections, as well as selections that were isolated from natural populations in Montenegro, in order to recommend them for further expansion and use in cultivation.

2. Materials and Methods

2.1. Plant Material and Field Evaluation

In the four–year period, four selections of cornelian cherry from Montenegro (Kosten 1, Kosten 2, Kosten 3, and Boro), four selections from Serbia (Apatinski rani, Bačka, Era, and Krupnoplodni NS) and three introduced varieties (Lukyanovskiy, Vladimirskiy, and Chisinau yellow) were studied. The experimental orchard was mainly established in autumn 2008 in the village of Kostenica (43° 02'N, 19° 51'E, 850 m above sea level), near Bijelo Polje in the northern part of Montenegro. Only the Vladimirskiy variety was subsequently planted in the fall of 2011. Plants were produced by grafting

on cornelian cherry seedlings and were planted at a distance of 4 × 4 m. The cultivation form is an improved pyramid. Each variety and selection is represented by five trees. Among the agrotechnical measures in the orchard, fertilization, mulching with green grass under the tree crown, and irrigation were applied. Fertilization was performed with mature bovine manure in the fall of 2011 and 2014 in the amount of 15 kg per tree. Fruit analysis was performed on mature fruits, in an average sample of 100 fruits per variety and selection (20 fruits per tree), and each tree was observed as one replication. The four-year meteorological data (temperature and precipitation) are given in Table 1.

Table 1. The meteorological data of Kostenica between 2012–2015.

Parameters	Years	Jan.	Feb.	Mar	April	May	June	July	August	Sep.	Oct.	Nov.	Dec.	Years
Temperature mean montly (°C)	2012	−1.7	−3.8	6.1	10.9	14.6	20.9	23.6	22.4	18.4	13	8.6	0.1	11.1
	2013	1.9	4.5	7	13.1	16	18.7	21	21.8	16	13.1	7.8	−0.5	11.7
	2014	3.9	7.6	8.6	10.7	14.5	18	20	20	16	11.7	8.6	2.3	11.8
	2015	0.4	2.2	5.1	9.7	16.9	18.9	23.4	22.6	18.7	12.5	6.2	−0.4	11.3
Temperature minimum daily (°C)	2012	−17.0	−20.2	−6.8	−2.4	5.0	8.2	9.2	7.4	2.8	−0.8	0.2	−15.0	−20.2
	2013	−9.0	−4.6	−8.8	1.2	3.8	8.0	7.6	12.4	5.2	−0.4	−4.4	−10.2	−10.2
	2014	−7.0	−6.2	−2.2	−1.0	1.2	9.0	9.0	10.0	4.8	0.6	−2.4	−10.8	−10.8
	2015	−18.8	−10.0	−6.4	−4.8	4.8	7.8	10.0	11.6	4.8	1.4	−3.0	−6.4	−18.8
Temperature maximum daily (°C)	2012	10.0	11.0	23.0	30.2	31.0	35.5	38.0	39.0	34.8	32.0	25.5	14.2	39.0
	2013	17.8	16.0	20.5	30.8	32.0	35.0	36.2	36.8	29.0	27.5	22.0	14.8	36.8
	2014	15.0	22.0	25.5	23.2	30.6	30.6	32.4	33.6	26.0	26.2	21.2	14.2	33.6
	2015	12.0	15.5	21.5	24.5	33.5	32.5	36.5	36.5	36.5	26.5	21.5	10.0	36.5
Precipitation (mm)	2012	44.6	184.5	9.6	70.4	69.6	33.4	13.9	18.3	54.9	129.9	49.2	128.8	807
	2013	124.3	80.4	158.1	35.7	163.5	75.4	73.3	56.9	49.4	96.0	69.3	16.5	999
	2014	69.2	27.9	47.3	145.4	127.5	118.8	78.0	53.9	191.6	69.3	115.0	105.8	1150
	2015	77.0	75.3	130.0	53.5	35.5	90.4	15.5	30.7	59.0	96.3	104.6	0.8	769

2.2. Pomological Analysis

The weight of fruit and stone was determined using analytical scale "Mettler" 1200. The result is expressed in grams with an accuracy of 0.01 g. Utilization of fruit is presented as a content of mesocarp in total fruit weight expressed as a percentage and as the mesocarp stone ratio. Yield is shown using the yield efficiency which represents the ratio between yield in kg and trunk cross sectional area (TCSA) in measured in cm^2 [28].

2.3. Biochemical Analysis

Biochemical analysis of mesocarp included the following tests: Dry Matter (DM), Total Acids (TA), Total Sugar (TS) and Reducing Sugars (RS), sucrose, vitamin C, total anthocyanin, ash, total polyphenols, Ca—pectate, contents of macro and micro elements—sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe), zinc (Zn) copper (Cu), and manganese (Mn). Analyses were performed by conventional methodology [29].

2.4. Statistical Analysis

Data related to fruit of studied indigenous and leading international genotypes of cornelian cherry was presented with standard descriptive measures and differences were tested by general linear models (GLM). In case of the statistically significant difference detected by GLM, the appropriate post–hoc testing was applied in order to test the individual differences and establish similarity groups. We analyzed both pomological and biochemical characteristics separately and in combination. Combination of all studied characteristics and genotypes was analyzed by Principal Components Analysis (PCA) where grouping patterns were established and presented through appropriate biplots. Statistical significance for observed differences was established at the level of $p < 0.05$. Observed statistical differences and groupings were discussed also from perspective of its agronomical significance. Graphical representation and biometrical analysis were conducted with assistance of statistical software packages R 3.1.3 [30] and SPSS 22 [31].

3. Results and Discussion

3.1. Pomological Characteristics

The morphological characteristics of the fruit of the examined varieties and selections are shown in Table 2.

Table 2. Pomological characteristics (mean ± standard error) of studied Cornelian cherry genotypes with statistical analysis of studied factors indicating statistically significant differences and interactions (F and *p* values).

Genotype	Year	Fruit Weight (g)			Pit Weight (g)			Flesh Content (% FW)			Color	Yield Efficiency (kg/cm^2 TCSA]		
		$\overline{X}$	±	$S\overline{x}$	$\overline{X}$	±	$S\overline{x}$	$\overline{X}$	±	$S\overline{x}$		$\overline{X}$	±	$S\overline{x}$
Apatinski rani	2012	2.6	±	0.15	0.49	±	0.06	81.23	±	1.73	-	0.17	±	0.01
	2013	2.91	±	0.23	0.47	±	0.06	84.26	±	1.02	Dark Red			
	2014	2.81	±	0.15	0.37	±	0.02	86.72	±	0.43				
	2015	2.70	±	0.15	0.32	±	0.02	88.23	±	0.3	-			
Bačka	2012	3.27	±	0.15	0.44	±	0.02	86.32	±	0.78	-	0.19	±	0.02
	2013	3.95	±	0.18	0.52	±	0.02	86.65	±	0.39	Dark Red			
	2014	3.72	±	0.09	0.48	±	0.01	87.07	±	0.4				
	2015	4.03	±	0.1	0.51	±	0.02	87.35	±	0.42	-			
Boro	2012	1.89	±	0.04	0.44	±	0.02	76.66	±	0.87	-	0.10	±	0.02
	2013	2.48	±	0.14	0.52	±	0.02	78.73	±	0.89	-			
	2014	2.06	±	0.06	0.41	±	0.01	80.13	±	0.4	Yellow			
	2015	2.17	±	0.05	0.45	±	0.02	79.14	±	0.71	-			
Era	2012	2.43	±	0.12	0.34	±	0.02	85.54	±	1.06	-	0.17	±	0.03
	2013	3.27	±	0.14	0.39	±	0.02	87.90	±	0.61	Dark Red			
	2014	3.08	±	0.12	0.38	±	0.02	87.71	±	0.30				
	2015	3.24	±	0.15	0.38	±	0.01	88.01	±	0.59	-			
Chisinau yellow	2012	3.41	±	0.12	0.46	±	0.02	86.44	±	0.59	-	0.20	±	0.03
	2013	5.09	±	0.17	0.62	±	0.02	87.75	±	0.42	-			
	2014	4.74	±	0.22	0.55	±	0.03	88.11	±	0.74	Yellow			
	2015	4.62	±	0.19	0.53	±	0.03	88.43	±	0.68	-			
Kosten 1	2012	3.15	±	0.08	0.48	±	0.03	84.66	±	0.76	-	0.21	±	0.03
	2013	4.25	±	0.19	0.75	±	0.02	82.29	±	0.59	Dark Red			
	2014	3.31	±	0.1	0.51	±	0.02	84.62	±	0.62				
	2015	3.75	±	0.17	0.54	±	0.03	85.47	±	0.58	-			
Kosten 2	2012	2.82	±	0.1	0.41	±	0.02	85.27	±	0.74	-	0.16	±	0.02
	2013	3.45	±	0.12	0.51	±	0.02	85.16	±	0.41	Dark Red			
	2014	3.14	±	0.15	0.47	±	0.02	84.88	±	0.57				
	2015	2.79	±	0.14	0.41	±	0.02	85.3	±	0.70	-			
Kosten 3	2012	4.09	±	0.18	0.52	±	0.02	87.21	±	0.34	-	0.20	±	0.01
	2013	4.56	±	0.22	0.59	±	0.03	87.07	±	0.1	-			
	2014	4.38	±	0.19	0.53	±	0.02	87.94	±	0.22	Red			
	2015	4.02	±	0.16	0.51	±	0.02	87.15	±	0.67	-			
Krupnoplodni NS	2012	3.38	±	0.11	0.44	±	0.02	86.9	±	0.7	-	0.23	±	0.02
	2013	4.24	±	0.22	0.58	±	0.02	86.09	±	0.37	Dark Red			
	2014	3.72	±	0.09	0.48	±	0.01	87.07	±	0.4				
	2015	4.03	±	0.1	0.51	±	0.02	87.35	±	0.42	-			
Lukyanovskiy	2012	5.01	±	0.23	0.61	±	0.03	87.63	±	0.79	-	0.14	±	0.01
	2013	5.27	±	0.22	0.65	±	0.03	87.72	±	0.55	-			
	2014	4.44	±	0.14	0.56	±	0.03	87.41	±	0.35	Red			
	2015	4.33	±	0.26	0.52	±	0.02	87.79	±	0.52	-			
Vladimirskiy	2014	5.01	±	0.17	0.57	±	0.02	88.58	±	0.37	-	0.21	±	0.03
	2015	6.16	±	0.23	0.57	±	0.02	90.59	±	0.55	Red			
$F_{genotype}$, $p_{genotype}$	-	126.3, $p < 0.001$			29.51, $p < 0.001$			73.53, $p < 0.001$				3.26, $p = 0.006$		
F_{year}, p_{year}	-	44.54, $p < 0.001$			32.76, $p < 0.001$			14.29, $p < 0.001$				-		
$F_{gen.*year}$, $p_{gen*year}$	-	3.18, $p < 0.001$			3.63, $p < 0.001$			2.78, $p < 0.001$				-		

The weight of the fruit varied widely from 1.89 ± 0.04 g in 2012 in the selection Boro to 6.16 ± 0.23 g in 2015 in the variety Vladimirskiy. Apatinski rani had the smallest stone, weighting 0.32 ± 0.02 g (in 2015), while the largest stone was observed in the variety Lukyanovskiy, 0.65 ± 0.03 g (in 2013). The percentage of mesocarp in relation to the total weight of the fruit varied from 76.66 ± 0.87% in the selection Boro in 2012 to 90.59 ± 0.55% in 2015 for the Vladimirskiy variety. Measured fruit morphological characteristics of fruit weight, stone weight and the mesocarp content are significantly interacting with the climatic conditions throughout the years of the research (Table 2).

The data obtained on fruit and stone weight are generally similar to the previously published data [8,10,13,18] which included different varieties and selection of cornelian cherry in different parts of world. Fruit weight is one of the main goals of cornelian cherry selection, because it is strongly positively correlated with the percentage of mesocarp in the total fruit weight [32] (Bijelić et al., 2007). This statement was confirmed in this study, and the fruits of the Vladimirskiy variety had the highest fruit weight and the share of mesocarp in the fruit, while the fruits of the Boro selection had the lowest weight and the lowest yield of mesocarp. Apart from the genetic constitution, the share of mesocarp is also significantly influenced by environmental factors, and there are variations in different years of research, which were also noted by Bjelić et al. [33]. Ninić-Todorović et al. [34] (2005) point out that the share of mesocarp higher than 75% of the total fruit weight is favorable from the point of view of processing in order to obtain confectionery products. Apart from the Boro selection, all other studied varieties and selections have fruits with a high yield of mesocarp, above 80%, which has a positive effect on their economic value. The data for this parameter presented in this paper are in ranges stated by other authors [12,34–36].

In order to observe and present patterns of the most important fruit characteristic, namely fruit weight and flesh content, it is presented (Figure 1) throughout the years of research.

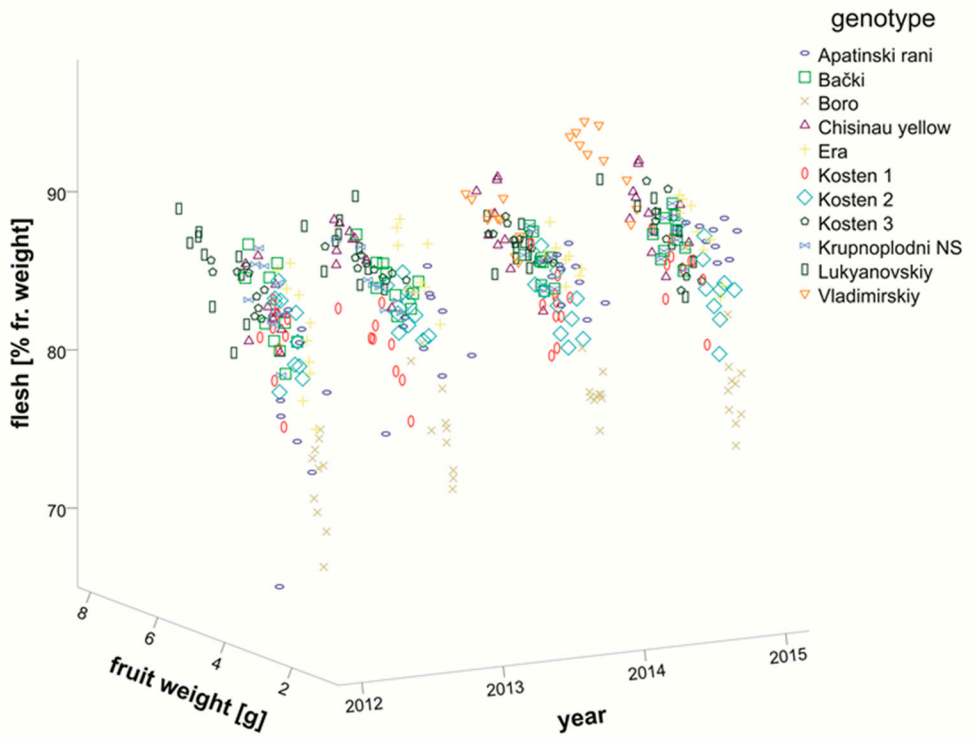

Figure 1. Individual measured fruit characteristics of studied Cornelian cherry genotypes in years of study.

It is clear that genotypes Vladimirskiy, Chisinau yellow, and Lukyanovskiy consistently had larger fruit with more fruit flesh than other studied genotypes. Second best come local varieties Kosten 3, Bačka, and, in some years, Krupnoplodni NS. Smallest fruit with small flesh content is

consistently produced by genotype Boro. In this category with small fruit also belong Kosten 1, Kosten 2, and Apatinski rani. It can be noted that fruit size was generally larger in year 2013 in comparison to other years of study. Measured fruit morphological characteristics of fruit weight, pit weight, and the flesh content are significantly interacting with the climatic conditions throughout the years of the research. The fruits of most examined varieties and selections had a dark red and red color, while yellow color was observed in the fruits of the Chisinau yellow variety and the Boro selection. Color, as an organoleptic property, affects the consumer acceptability of fruits, and red and dark red cherries are in higher demand due to the attractiveness [37]. Dokoupil and Rezníček [38] consider that red color is characteristic of cornelian cherry fruits, although pink and yellow fruits can be found. Jaćimović and Božović [39] state that, when unripe cornelian cherry fruits are marinated like olives in saline solution, the final product is of better quality if yellow fruits are used, while it is better to use red fruits for processing into jam, juice, and liqueur.

3.2. Yield

Productivity is a property that directly affects the economics of cornelian cherry production. The lowest yield efficiency (0.10) was found in the selection Boro, while the highest (0.23) was observed in the selection Krupnoplodni NS. In addition to the selection of Krupnoplodni NS, the Vladimirskiy and Chisinau yellow varieties had a high yield, as did the Kosten 1 and Kosten 3 selections. Yield efficiency above 0.15 indicates good yield [28]. Cultivars, rootstocks, environmental conditions, and applied cultivation techniques significantly affect productivity of fruit trees [40].

Genotypes significantly ($p = 0.006$) differ in yield efficiency (Table 2). For better visualization of the agronomical traits important to the yield, i.e., yield per TCSA and fruit weight data is presented in combination as measured in the years of study (Figure 2). The best genotypes are Vladimirskiy, Chisinau yellow and Krupnoplodni NS characterized with consistently higher yield and large fruit. Good yield and good-sized fruit are measured also in local genotype Kosten 3 and Bačka. Genotype Boro had consistently the lowest yield with the smallest fruit.

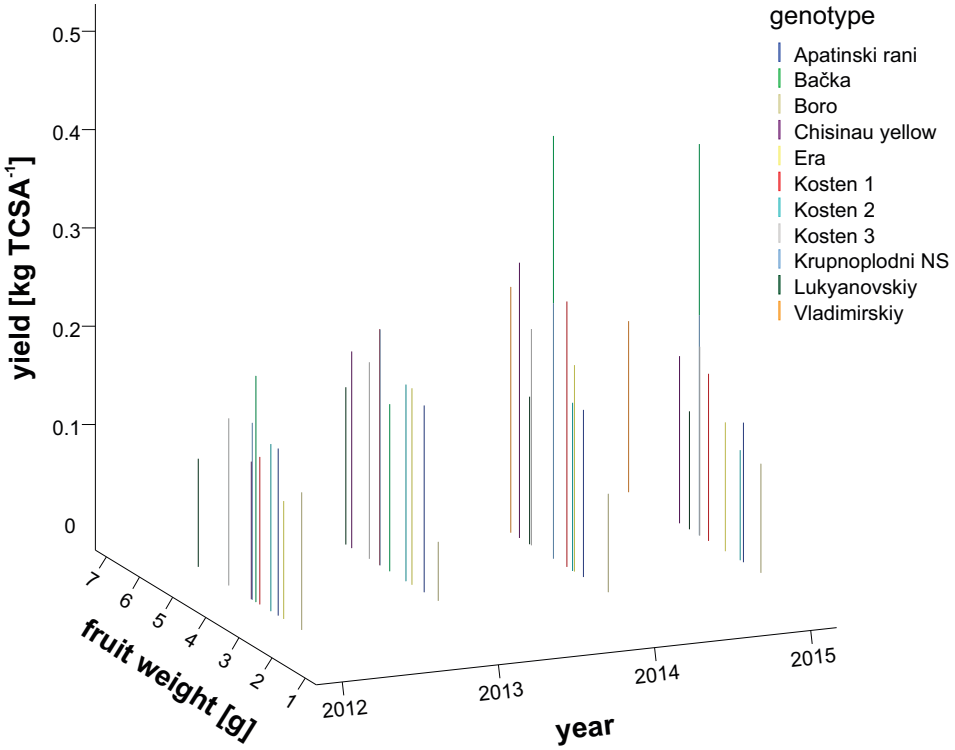

Figure 2. Mean fruit weight and yield of studied Cornelian cherry genotypes in years of study.

3.3. Biochemical Characteristics

Biochemical analyses of varieties and selection of cornelian cherry included the chemical characteristics of the fruit, as well as the content of certain mineral substances, in a four-year period. The chemical characteristics of the fruit are shown in Table 3. The minimal content of dry matter (TDM) was determined in the fruits of the cultivar Chisinau yellow 19.81%, and the maximal 24.46% in the fruits of the selection Krupnoplodni NS presented in Table 2. Similar data for the TDM parameter in fruits of different varieties and genotypes of Cornelian cherry were presented by Tural and Koca [41] as 15.9–28.2% and by Sengul et al. [42] (2014) as 18–23.3% in Turkey, Maghradze et al. [43] reported 17.7–26.1% in Georgia and Jaćimović et al. [14] reported 18.8–27.7% in Montenegro. For the same parameter, slightly higher values were presented by Bijelić et al. [36] as 18.3–33.4% in Serbia. The amount of TDM in Cornelian cherry fruit is significantly influenced by genetic constitution, age, cultivation system, applied agricultural techniques, climatic, and soil factors, as well as the degree of fruit maturity at harvest [18,44].

Cornelian cherry fruit has a significant energy value, mostly due to the abundance of sugar, which dominates the dry matter. Sugar content is the main parameter that affects not only the direct consumption of fruit, but also the fermentation processes during various types of processing. Highest amount of total and reducing sugars had selection Kosten 1 10.94 and 10.44%. Sucrose content ranged from 0.24% (Kosten 2) to 1.23% (Krupnoplodni NS). Similar results on total sugar content in Cornelian cherry fruit were presented by Tural and Koca [41] in Turkey 7.7–15.4% and Brindza et al. [45] in Slovakia as 6.5–15.1%. Compared to the presented data for total sugars, Bijelić et al. [36] found a higher amount of total sugars, 13.49–25.23% in cornelian cherry fruits from Serbia, while Maghradze et al. [43] and Drkenda et al. [46] observed lower values in fruits from Georgia and Bosnia and Herzegovina, respectively, which ranged from 6.2 to 9.2%. The determined differences in the content of total sugars can be interpreted as a result of different climatic and soil conditions.

The reducing sugars contained in the cornelian cherry fruits from Poland examined by Kucharska et al. [47] were 9.9–14.7%, and in the cornelian cherry fruits from Turkey measured by Sengul et al. [42] as 8.6–15.2%, which is in accordance with our research. The low amount of sucrose in the studied fruits confirmed the conclusion of Kucharska et al. [47] that reducing sugars make the main part of total sugars in cornelian cherry fruits (about 90%). Akagić et al. [48] believe that the lower sucrose content can be explained by the high activity of invertase in the final stage of fruit ripening. The acidity of cornelian cherry fruit occurs due to the presence of organic acids, of which malic acid predominates [46]. The total acidity in these studies varied from 1.92% in the Boro selection to 2.51% in the Vladimirskiy variety. Similar data for total acidity in cornelian cherry fruit, which ranged from 1.1 to 3.7, were presented by Klimenko et al. [49]; Tural and Koca [41]; Maghradze et al. [43]; Ercisli et al. [37]; and Bijelić et al. [36]. The processes involved in the metabolism and accumulation of malic and citric acid in mesocarp cells are under genetic and environmental control [50]. Pectic substances are dietary fibres of high nutritional value. The lowest amount of pectin was in the fruits of the Era selection 2.76%, and the highest in the fruits of the Bačka selection 4.43%. Lower results than ours on the content of pectic substances in Cornelian cherry fruit, from 0.37 to 2.47%, were presented by Maghradze et al. [43]; Bijelić et al. [36]; Jaćimović et al. [9], which can be explained by the high dependence of this parameter on the environmental factors. In years when the air temperature is high during ripening, and the amount of precipitation is low, an increased content of pectic substances in the fruit can be expected [9]. Pectins have the property that in the presence of sugars and acids are transformed into a gelatinous mass called jelly, which is especially used in the fruit processing industry [51].

Vitamins are essential nutrients that the human body cannot synthesize, but consumes through food. Vitamin C strengthens the immune system and as an antioxidant plays a protective role against cardiovascular diseases [6]. Cornelian cherry fruits contain more vitamin C than fruits of many other fruit species that are considered to be rich sources of this vitamin, e.g., strawberries, kiwis, lemons, oranges, etc. [15,26,52]. Fruits of the varieties and selection of cornelian cherry presented in this paper

had vitamin C from 41.09 mg/100 g (Lukyanovskiy) to 101.27 mg/100 g (Kosten 3). In accordance with our data, the results on the content of this vitamin presented by Yilmaz et al. [35] (2009) as 29–112 mg/100 g and Maghradze et al. [43] as 50.5–128 mg/100 g. Cornelian cherry fruits are often used in the processed state without the application of heat treatment so that vitamin C, as well as other thermo labile substances, remain preserved in the final product [39].

Anthocyanins are natural nutritive bioactive components that are found in fruits and have a beneficial effect on human health. They are among the most important bioactive compounds that prevent cardiovascular disease and cancer, which are considered the most common diseases today [53–55]. The fruits of selection Kosten 1 with a content of 157.06 mg/100 g were the most abundant in total anthocyanins of all studied varieties and selections. In the fruits of the Chisinau yellow variety and the Boro selection, anthocyanins were not determined, which confirms the statement made by Ochmian et al. [56] that genotypes that have a yellow skin color do not contain anthocyanin compounds in the fruit. The concentration of anthocyanins in fruit varieties tested by Kucharska et al. [47] ranged from 33.7 to 149.6 mg/100 g and Bijelić et al. [37] from 37.6 to 116.4 mg/100 g, which is consistent with the data presented in this paper. Kazimierski et al. [26] indicate that according to the literature, the total content of anthocyanins in cornelian cherry fruits is between 35 and 300 mg/100 g, depending on the color of the epidermis, which varies from pink to dark red.

Phenolic compounds are very widespread products of secondary metabolism of plants, which are recently receiving great attention. They are found in significant quantities in food products of plant origin, and numerous studies indicate that their regular consumption reduces the risk of many serious diseases [57,58]. Cornelian cherry fruits contain significant amounts of phenolic bioactive substances, which contribute to their antioxidant value. In our study, the lowest total polyphenols were found in the fruits of the Chisinau yellow variety 183.91 mg/100 g, and the highest in the fruits of the selection Krupnoplodni NS 307.9 mg/100 g. The content of total polyphenols in Cornelian cherry fruits from Bosnia and Herzegovina studied by Drkenda et al. [46] ranged from 119.1 to 230.6 mg GAE/100 g FW, and in fruits from Romania shown by Cosmulenca et al. [59] from 163.7 to 359.28 mg GAE/100 g FW, which is close to results presented in this paper. Much wider variation in the content of total polyphenols in Cornelian cherry fruits was presented by Stanković et al. [60] from 12.8 to 341.1 mg GAE/100 g FW. In addition to the genetic constitution, the amount of phenolic compounds in fruits is influenced by climatic and geographical factors, applied agro technics, and fruit ripeness.

The most important source of minerals in the diet is food of plant origin, which is also biologically more valuable. According to the presented research, the total amount of ash ranged from 0.89% (Lukyanovskiy) to 1.16% (Bačka). A smaller amount of mineral substances ranging from 0.61 to 0.81% had genotypes of the Cornelian cherry fruits from Turkey studied by Sengul et al. [42] and in accordance with our results, Cornelian cherry genotypes from Montenegro had mineral substances content from 0.65 to 1.59% [9] and from Serbia 0.53–1.23% [36].

3.4. Content of Certain Mineral Substances in the Fruit

The content of the most important mineral substances in the fruits of the studied varieties and selections is presented in Table 4. Potassium is the dominant mineral element in the fruit of cornelian cherry [16,61]. From the presented data, it can be noted that the variety Chisinau yellow has the lowest content of potassium in the fruit 2888 mg/kg, while the fruits of the selection Kosten 1 demonstrate good supply of this important element 3574 mg/kg. Dokoupil and Rezniček [38] reported that Cornelian cherry fruit content of potassium is in the range from 3441 to 3798 mg/kg, and Gozlekci et al. [16] report potassium content ranges from 2780 to 3340 mg/kg, which is close to data presented in this paper. Potassium regulates the normal function of the nervous system, heart and other muscles. An important function of potassium as an electrolyte is to maintain the acid–base balance in human body, and an adequate supply of this macroelement prevents acidification of the body. Eating fruits that are rich in potassium can reduce blood pressure, and it is recommended for people suffering from hypertension [62].

Table 3. Chemical characteristics in the fruit of studied Cornelian cherry genotypes fruit (mean ± standard error) with statistical analysis indicating statistically significant differences between the genotypes (*F* and *p* values).

Genotype	DM (%)			Total Sugars (TS) (%)			Reducing Sugars (RS) (%)			Sucrose (%)			Total Acids (TA) (%)			Ca–Pectate (%)			Vitamin C (mg/100 g)			Anthocyanins (mg/100 g)			Polyphenols (mg/100 g)			Ash (%)		
	$\bar{X}$	±	$S_{\bar{x}}$	$\bar{X}$	±	$S_{\bar{x}}$	$\bar{X}$	±	$S_{\bar{x}}$	$\bar{X}$	±	$S_{\bar{x}}$	$\bar{X}$	±	$S_{\bar{x}}$	$\bar{X}$	±	$S_{\bar{x}}$	$\bar{X}$	±	$S_{\bar{x}}$	$\bar{X}$	±	$S_{\bar{x}}$	$\bar{X}$	±	$S_{\bar{x}}$	$\bar{X}$	±	$S_{\bar{x}}$
Apatinski rani	24.36	±	1.37	14.47	±	0.45	12.64	±	0.33	0.49	±	0.10	2.31	±	0.34	4.20	±	0.31	61.10	±	6.89	100.44	±	4.42	208.55	±	20.88	1.15	±	0.16
Bačka	21.68	±	1.48	12.94	±	0.78	12.10	±	1.02	0.60	±	0.29	2.12	±	0.31	4.43	±	0.90	57.78	±	5.77	32.54	±	5.86	216.25	±	20.40	1.16	±	0.20
Boro	21.17	±	2.05	12.42	±	1.58	11.90	±	1.57	0.37	±	0.09	2.51	±	0.02	3.13	±	0.46	89.64	±	5.79		–		265.95	±	7.79	1.07	±	0.16
Era	21.58	±	1.53	11.79	±	1.32	11.26	±	1.16	0.35	±	0.05	2.29	±	0.24	2.76	±	0.49	79.83	±	2.42	65.23	±	8.25	233.09	±	19.99	1.02	±	0.18
Chisinau yellow	19.81	±	1.28	12.83	±	1.02	12.38	±	0.91	0.45	±	0.11	2.06	±	0.03	2.98	±	0.56	58.14	±	6.33		–		183.91	±	5.47	1.08	±	0.25
Kosten 1	22.36	±	1.22	10.94	±	0.52	10.44	±	0.54	0.45	±	0.04	2.37	±	0.19	3.55	±	0.45	101.27	±	3.39	157.06	±	32.87	255.80	±	44.27	1.14	±	0.07
Kosten 2	24.45	±	2.30	13.26	±	0.54	12.62	±	0.53	0.24	±	0.03	2.08	±	0.16	4.13	±	0.28	86.41	±	4.73	60.05	±	2.06	258.21	±	38.95	1.09	±	0.09
Kosten 3	23.83	±	1.65	14.07	±	1.59	13.09	±	1.26	0.92	±	0.33	2.02	±	0.21	4.18	±	1.00	64.52	±	2.72	104.09	±	38.52	219.15	±	12.57	0.94	±	0.29
Krupnoplodni NS	25.46	±	1.76	12.47	±	0.33	11.17	±	0.60	1.23	±	0.27	2.15	±	0.25	4.18	±	0.70	65.11	±	2.88	80.07	±	17.50	307.90	±	23.99	1.14	±	0.07
Lukyanovskiy	20.58	±	0.40	13.53	±	0.28	12.87	±	0.29	0.63	±	0.19	2.28	±	0.11	3.45	±	1.02	41.09	±	12.79	47.88	±	12.09	217.44	±	9.02	0.89	±	0.13
Vladimirskiy	21.45	±	1.12	12.90	±	0.20	11.80	±	0.25	0.90	±	0.01	1.92	±	0.13	3.43	±	1.12	72.40	±	5.10	59.26	±	2.14	243.75	±	13.55	1.08	±	0.09
$F_{genotype}$, $p_{genotype}$	1.1, $p = 0.459$			0.8, $p = 0.605$			0.6, $p = 0.780$			2.1, $p = 0.073$			0.5, $p = 0.868$			0.5, $p = 0.840$			6.2, $p < 0.001$			5.3, $p = 0.001$			1.6, $p = 0.177$			0.2, $p = 0.994$		

In the fruits of the tested varieties and selection of Cornelian cherry, other macroelements were present in the following amounts: Na from 4.99 mg/kg (Kosten 3) to 32.3 mg/kg (Boro), Ca from 239.6 mg/kg (Lukyanovskiy) to 444.6 mg/kg (Boro), Mg from 70.7 mg/kg (Chisinau yellow) to 165 mg/kg (Krupnoplodni NS) and P from 179 mg/kg (Kosten 2) to 221.6 mg/kg (Bačka). Sodium has an important role in maintaining fluid balance in the body. Sodium and potassium are closely related, because the first element is an antagonist of the second, which means that it has the opposite action. Balance between these two electrolytes is necessary to maintain homeostasis throughout the body [16]. Compared to our data, cornelian cherry genotypes examined by Dokoupil and Rezníček [38] (2012) and Gozlekci et al. [16] had a higher amount of sodium in the fruit 51–82 mg/kg. In cornelian cherry fruits, Sotiropoulos et al. [8] showed lower concentrations of biogenic elements compared to those presented in this paper Ca 20–30 mg/kg, Mg 40–50 mg/kg and P 80–90 mg/kg, and Bjelić et al. [36] found higher variation in Ca content from 24.7 to 526 mg/kg and Mg from 10.12 to 160 mg/kg. Sufficient amount of phosphorus and calcium in the diet enables proper functioning of bones and teeth, muscle and heart. Magnesium is essential for the proper metabolic processes, as well as the normal functioning of the neuromuscular and cardiovascular systems [16].

Tested varieties and selections of cornelian cherry contained in fruits important microelements in following concentrations: Fe from 3.85 mg/kg (Kosten 1), to 14.21 mg/kg (Vladimirskiy), Zn from 1.17 mg/kg (Kosten 1) to 3.05 mg/kg (Era), Cu from 1 mg/kg (Kosten 2) to 1.27 mg/kg (Krupnoplodni NS), and Mn from 2.27 mg/kg (Chisinau yellow) to 5.40 mg/kg (Kosten 1). Slightly lower amounts of these trace elements were found in Cornelian cherry genotypes presented by Bijelić et al. [36], much lower in cultivars studied by Sotiropoulos et al. [8], and higher in genotypes studied by Gozlekci et al. [16]. This variability is conditioned by genotype, climate, soil and geographical factors, substrate used, etc.

Analysis of biochemical characteristics indicated statistically highly significant differences among studied genotypes in Na, Mg, P, Fe, Zn, Mn, vitamin C, and anthocyanins ($p < 0.001$) content, while there was no observed statistically significant difference ($p > 0.07$) in K, Ca, Cu, TDM, ash, TA, total sugars (TS), reducing sugars (RS), sucrose, Ca—pectate, and polyphenols (Tables 3 and 4).

Table 4. Mineral contents (mg/kg) in the fruit of studied Cornelian cherry genotypes fruit (mean ± standard error) with statistical analysis indicating statistically significant differences between the genotypes (*F* and *p* values).

Genotype	K			Na			Ca			Mg			P			Fe			Zn			Cu			Mn		
	$\overline{X}$	±	$S\overline{x}$	$\overline{X}$	±	$S\overline{x}$	$\overline{X}$	±	$S\overline{x}$	$\overline{X}$	±	$S\overline{x}$	$\overline{X}$	±	$S\overline{x}$	$\overline{X}$	±	$S\overline{x}$	$\overline{X}$	±	$S\overline{x}$	$\overline{X}$	±	$S\overline{x}$	$\overline{X}$	±	$S\overline{x}$
Apatinski rani	3136	±	206	16	±	1.23	320	±	14.1	113.3	±	4.35	200	±	2	4.5	±	0.31	1.79	±	0.09	1.01	±	0.14	5.13	±	0.53
Bačka	3380	±	106	18.5	±	3.17	310.9	±	29.5	108.5	±	8.77	221.6	±	2.5	5.17	±	0.11	1.84	±	0.23	1.04	±	0.25	4.2	±	0.18
Boro	3136	±	128	32.3	±	1.55	444.6	±	5.52	130.2	±	6.74	202.5	±	5.82	4.1	±	0.09	1.52	±	0.04	1.15	±	0.07	4.2	±	0.49
Era	3009	±	83	20.1	±	0.51	402.1	±	37.1	105.6	±	7.74	206.1	±	3.77	7.87	±	2.97	3.05	±	0.12	1.26	±	0.12	3.67	±	0.08
Chisinau yellow	2885	±	34.4	20.4	±	0.33	315.9	±	50.7	70.07	±	6.58	192.5	±	1.53	4.98	±	0.04	1.38	±	0.22	1.07	±	0.08	2.27	±	0.08
Kosten 1	3574	±	46	15.9	±	2.1	312.8	±	3.96	122.1	±	8.09	192.4	±	3.2	4.37	±	0.12	1.41	±	0.13	1.16	±	0.04	5.4	±	0.57
Kosten 2	3452	±	257	16.5	±	1.31	293.7	±	12.3	84.82	±	2.34	179	±	3.9	5.1	±	0.44	1.7	±	0.27	1	±	0.04	4.63	±	0.32
Kosten 3	3259	±	118	4.99	±	0.25	326.3	±	63.8	99.77	±	12.6	203.2	±	5.25	3.85	±	0.55	1.17	±	0.19	1.09	±	0.08	2.37	±	0.08
Krupnoplodni NS	3461	±	101	25.9	±	1.49	301.4	±	20.9	165	±	6.03	182.7	±	9.39	4.2	±	0.22	1.86	±	0.09	1.27	±	0.21	3.53	±	0.19
Lukyanovskiy	3193	±	98.5	11.6	±	0.86	239.6	±	33.3	77.49	±	7.4	206.6	±	4.21	4.61	±	0.71	1.88	±	0.17	1.04	±	0.11	2.9	±	0.22
Vladimirskiy	3282	±	155	11.7	±	1.42	317	±	25.5	99.84	±	2.26	203.6	±	3.25	14.21	±	0.31	1.42	±	0.11	1.02	±	0.08	2.95	±	0.25
$F_{genotype}$, $p_{genotype}$	1.7, $p = 0.134$			17.2; $p < 0.001$			2.1, $p = 0.071$			9.8, $p < 0.001$			5.1, $p = 0.001$			5.2, $p = 0.001$			6.9, $p < 0.001$			0.4, $p = 0.923$			8.1, $p < 0.001$		

By analyzing the all measured biochemical characteristics of studied Cornelian cherry genotypes through principal components analysis we have observed general influence of the year of study (Figure 3). Namely, in 2013, all genotypes grouped together along the negative part of the first principal component. This component is positively correlated with dry matter, anthocyanins, ash, and sugar content. Bearing in mind the larger fruit recorded for all genotypes in 2013, it can be linked to lower measures of those biochemical characteristics.

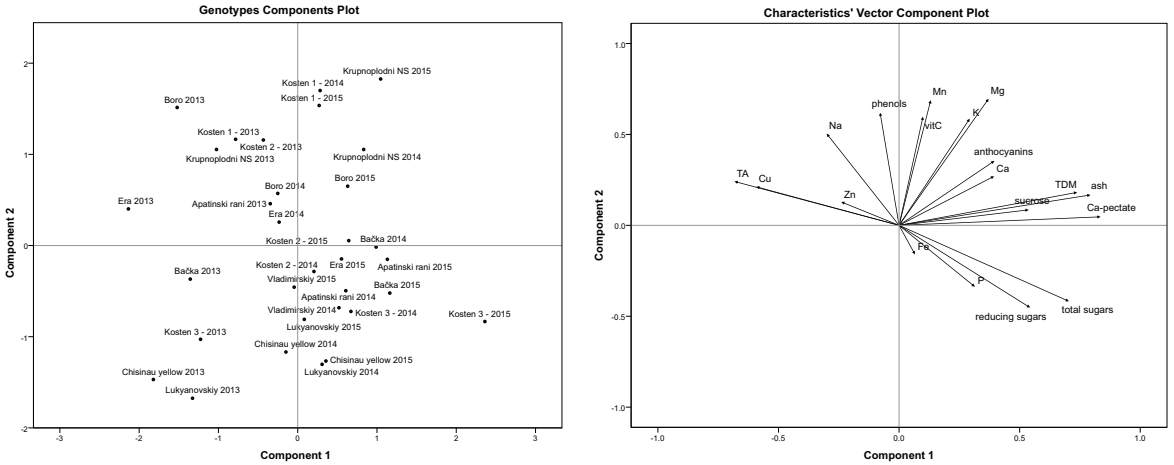

Figure 3. Principal components analysis of fruits biochemical characteristics of studied genotypes in years of study with both genotypes components plots (left) and the vectors of individual biochemical characteristics (right).

Another important grouping factor is C vitamin and anthocyanin content. Generally larger fruiting varieties Vladimirskiy, Lukyanovskiy, Chisinau yellow, and selection Bačka have consistently measured lower content in those and also most other measured biochemical characteristics. Selection Kosten 3 has larger fruit but also has higher anthocyanins content. Highest anthocyianins and C vitamin content was recorded in selection Kosten 2, coupled with lowest sugar content in this genotype. Those genotypes are grouped along the negative part of the second principal component.

Krupnoplodni NS, Kosten 1, and Boro represent group of genotypes with consistently higher to medium biochemical content. Those genotypes are grouped along the positive part of the second principal component for all years of study.

The change in biochemical and other measured characteristics has to be observed through weather conditions during the growing seasons [63–65]. In 2013, the growing season provided optimum precipitation and temperatures [63]. Contrary to that season in 2014 and 2015, there were different climate extremes. Namely, in 2014, during the growing season, precipitations were at the decades' maximum with almost 50% more precipitations in comparison to the long-term average [64]. Contrary to 2014, in 2015, growing season was dry, with almost 50% less precipitations than the long-term average [65]. Climate change as peaks in precipitation and temperature leading to floods and droughts in 2014 and 2015, respectively, have, to a large extent, similarly influenced the fruit size and quality of studied cornelian cherry genotypes. Namely, most genotypes grouped close for its biochemical characteristics in 2014 and 2015.

4. Conclusions

Studied cornelian cherry international and local varieties and selections have intrinsically mixed combination of fruit size, yield, and biochemical content characteristics. Those are further significantly influenced by climate and especially climate change extremes. In all studied varieties and selections, we found that climate conditions which favored larger fruit and yield generally led to lower content of important biochemicals. The extensiveness of the study leads, therefore, to several clear conclusions

and recommendations. Consistently, the best balance of biochemical elements in combination with good yield and fruit size measurements is found in selection Krupnoplodi NS. If fruit size and yield are priority, then varieties and selections Vladimirskiy, Chisinau yellow, Kosten 3, and Krupnoplodni NS have a clear advantage over other genotypes. In environments and years with unfavorable conditions, it is expected to have smaller fruit with more concentrated biochemical content.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Ercisli, S.; Akbulut, M.; Ozdemir, O.; Sengul, M.; Orhan, E. Phenolic and antioxidant diversity among persimmon (*Diospyrus kaki* L.) genotypes in Turkey. *Int. J. Food Sci. Nutr.* **2008**, *59*, 477–482. [CrossRef]
2. Ozkan, G.; Ercisli, S.; Zeb, A.; Agar, G.; Sagbas, H.I.; Ilhan, G.; Gundogdu, M. Some morphological and biochemical characteristics of wild grown Caucasian Whortleberry (*Vaccinium arctostaphylos* L.) genotypes from Northeastern Turkey. *Not. Bot. Horti. Agrobot.* **2019**, *47*, 378–383. [CrossRef]
3. Gecer, M.K.; Kan, T.; Gundogdu, M.; Ercisli, S.; Ilhan, G.; Sagbas, H.I. Physicochemical characteristics of wild and cultivated apricots (*Prunus armeniaca* L.) from Aras valley in Turkey. *Genet. Resour. Crop Evol.* **2020**, *67*, 935–945. [CrossRef]
4. Eyduran, S.P.; Ercisli, S.; Akin, M.; Beyhan, Ö.; Geçer, M.K. Organic acids, sugars, vitamin c, antioxidant capacity, and phenolic compounds in fruits of white (*Morus alba* L.) and black (*Morus nigra* L.) mulberry genotypes. *J. Appl. Bot. Food Qual.* **2015**, *88*, 134–138.
5. Ercisli, S.; Ipek, A.; Barut, E. SSR marker–based DNA fingerprinting and cultivar identification of olives (*Olea europaea*). *Biochem. Genet.* **2011**, *49*, 555–561. [CrossRef]
6. Szczepaniak, O.M.; Kobus–Cisowska, J.; Kusek, W.; Przeor, M. Functional properties of cornelian cherry (*Cornus mas* L.): A comprehensive review. *Eur. Food Res. Technol.* **2019**, *245*, 2071–2087. [CrossRef]
7. Vilotić, D.; Tucović, A.; Radošević, G. Comparative analysis of anatomical–technological properties of cornel tree (*Cornus mas* L.) and red dogwood (*Cornus sanguinea* L.). In *Proceedings Books of 7th Symposium on Flora of Southeastern Serbia and Neighbouring Regions*; Bulgarian Botanical Society: Dimitrovgrad, Bulgaria, 2002; pp. 117–120.
8. Sotiropoulos, T.; Petridis, A.; Koutinas, N.; Therios, I. 'Ntoulia 1' and 'Ntoulia 2' cornelian cherries (*Cornus mas*). *Hortscience* **2011**, *46*, 955–957. [CrossRef]
9. Jaćimović, V.; Božović, Đ.; Ercisli, S.; Ognjanov, V.; Bosančić, B. Some fruit characteristics of selected cornelian cherries (*Cornus mas* L.) from Montenegro. *Erwerbs Obstbau.* **2015**, *57*, 119–124. [CrossRef]
10. Ercisli, S. A short review of the fruit germplasm resources of Turkey. *Genet. Resour. Crop Evol.* **2004**, *51*, 419–435. [CrossRef]
11. Sochor, J.; Jurikova, T.; Ercisli, S.; Mlcek, J.; Baron, M.; Balla, S.; Yilmaz, S.O.; Necas, T. Characterization of cornelian cherry (*Cornus mas* L.) genotypes–genetic resources for food production in Czech Republic. *Genetika* **2014**, *46*, 915–924. [CrossRef]
12. Hassanpour, H.; Hamiddoghli, Y.; Samizadeh, H. Some fruit characteristics of Iranian cornelian cherries (*Cornus mas* L.). *Not. Bot. Horti. Agrobo.* **2012**, *40*, 247–252. [CrossRef]
13. Bajic–Ljubicic, J.; Popovic, Z.; Matic, R.; Bojovic, S. Selected phenolic compounds in fruits of wild growing *Cornus mas* L. *Indian J. Tradit. Know.* **2018**, *17*, 91–96.

14. Jaćimović, V.; Božović, Đ. Biological traits of cornelian cherry genotypes (*Cornus mas* L.) from teritory of Montenegro. *Genetika* **2014**, *46*, 427–436.
15. Seeram, N.; Schuzki, R.; Chandra, A.; Nair, M.G. Characterization, quantification and bioactivities of anthocyanins in *Cornus* species. *J. Agric. Food Chem.* **2002**, *50*, 2519–2523. [CrossRef]
16. Gozlekci, S.; Esringu, A.; Ercisli, S.; Eyduran, S.P.; Akin, M.; Božović, Đ.; Nehgreanu–Pirjol, T.; Sagbas, H.I. Mineral content of cornelian cherry (*Cornus mas* L.) fruits. *Oxid. Commun.* **2018**, *40*, 301–308.
17. Memedov, N.; Craker, L.E. Cornelian cherry: A prospective source for phytomedicine. *Acta Hortic.* **2002**, *629*, 83–86. [CrossRef]
18. Demir, F.; Kalyoncu, I.H. Some nutritional, pomological, and physical properties of cornelian cherry (*Cornus mas* L.). *J. Food Eng.* **2003**, *60*, 335–341. [CrossRef]
19. Yousefi, B.; Abbasi, M.; Abbasi, M.M.; Jahanban–Esfahlan, R. Anti–proliferative properties of *Cornus mas* fruit in different human cancer cells. *Asian Pac. J. Cancer Prev.* **2015**, *16*, 5727–5731. [CrossRef]
20. Hosseinpour–Jaghdani, F.; Shomali, T.; Gholipour–Shahraki, S.; Rahimi–Madiseh, M.; Rafieian–Kopaei, M. *Cornus mas*: A review on traditional uses and pharmacological properties. *J. Complement. Integr. Med.* **2017**, *11*, 63–69. [CrossRef]
21. Czerwinska, M.E.; Melzig, M.F. *Cornus mas* and *Cornus officinalis*– Analogies and differences of two medicinal plants traditionally used. *Front. Pharmacol.* **2018**, *9*, 894. [CrossRef]
22. Gholamrezayi, A.; Yaghubi, E.; Ghafouri, A. A review of probable effects of cornelian cherry. *Fruit J. Biochem. Tech.* **2019**, *2*, 71–74.
23. Milenković–Andjelković, A.; Andjelković, M.; Radovanović, A.; Radovanović, B.; Nikolić, V. Phenol composition, DPPH radical scavenging and antimicrobial activity of cornelian cherry (*Cornus mas*) fruit and leaf extracts. *Hem. Ind.* **2015**, *69*, 331–337. [CrossRef]
24. Moldovan, B.; Filip, A.; Clichici, S.; Suharoschi, R.; Bolfa, P.; David, L. Antioxidant activity of cornelian cherry (*Cornus mas* L.) fruits extract and the in vivo evaluation of its anti inflammatory effects. *J. Funct. Foods* **2016**, *26*, 77–87. [CrossRef]
25. Rop, O.; Mlcek, J.; Kramarova, D.; Jurikova, T. Selected cultivars of cornelian cherry (*Cornus mas* L.) as a new food source for human nutrition. *Afr. J. Biotehnol.* **2010**, *9*, 1205–1210.
26. Kazimierski, M.; Regula, J.; Molska, M. Cornelian cherry (*Cornus mas* L.)–characteristics, nutritional and pro–health properties. *Acta Sci. Pol. Technol. Aliment.* **2019**, *18*, 5–12.
27. Jaćimović, V.; Božović, Đ.; Jovančević, M. Selection of cornelian cherry (*Cornus mas* L.) in Upper Polimlje area. *Agro Knowledge J.* **2003**, *4*, 32–39.
28. Karadeniz, T. Promising cornelian cherries (*Cornus mas* L.) from the Northeastern Anatolia region of Turkey. *J. Am. Pom. Soc.* **2002**, *56*, 164–167.
29. AOAC. *Official Methods of Analysis*, 17th ed.; Association of Official Analytical Chemists: Washington, DC, USA, 2002.
30. Available online: https://CRAN.R--project.org/doc/FAQ/ (accessed on 22 June 2020).
31. IBM Corp. *IBM SPSS Statistics for Windows*; Armonk, SPSS Inc.: North Castle, NY, USA, 2019.
32. Bijelić, S.; Ninić–Todorović, J.; Jaćimović, G.; Gološin, B.; Cerović, S.; Vidicki, B. Morphometric characteristics of selected cornelian cherry genotypes. *Contemp. Agric.* **2007**, *56*, 130–137.
33. Bijelić, S.; Ninić-Todorović, J.; Gološin, B.; Cerović, S.; Ognjanov, V. Cornelian cherry (*Cornus mas* L.) selection at the Faculty of Agriculture in Novi Sad. In Proceedings of the 43th Croatian and 3th International Symposium on Agriculture, Opatija, Croatia, 18–21 February 2008; pp. 901–904.
34. Ninić–Todorović, J.; Cerović, S.; Gološin, B.; Bijelić, S.; Miletić, R. Pomological–biochemical characteristics of selected Cornelian cherry genotypes. *Pomology* **2005**, *39*, 395–399.
35. Yilmaz, K.U.; Ercisli, S.; Zengin, Y.; Sengul, M.; Kafkas, E.Y. Preliminary characterisation of cornelian cherry (*Cornus mas* L.) genotypes for their physico–chemical properties. *Food Chem.* **2009**, *114*, 408–412. [CrossRef]
36. Bijelić, S.; Gološin, B.; Ninić–Todorović, J.; Cerović, S.; Popović, B. Physicochemical fruit characteristics of cornelian cherry (*Cornus mas* L.) genotypes from Serbia. *Hortscience* **2011**, *46*, 849–853. [CrossRef]
37. Ercisli, S.; Yilmaz, S.O.; Gadze, J.; Džubur, A.; Hadžiabulić, S.; Aliman, J. Some fruit characteristics of cornelian cherries (*Cornus mas* L.). *Not. Bot. Horti. Agrobot.* **2011**, *39*, 255–259. [CrossRef]
38. Dokoupil, L.; Rezníček, V. Production end use of the cornelian cherry–*Cornus mas* L. *Acta Univ. Agric. at Silvic. Mendel Bruno.* **2012**, *LX*, 49–58. [CrossRef]

39. Jaćimović, V.; Božović, Đ. Chemical composition of cornelian cherry (*Cornus mas* L.) products and their relevance in human consumption. *Contemp. Agric.* **2007**, *56*, 150–157.
40. Božović, Đ.; Bosančić, B.; Velimirović, A.; Ercisli, S.; Jaćimović, V.; Keles, H. Biological characteristics of some plum cultivars grown in Montenegro. *Acta Sci. Pol. Hortoru.* **2017**, *16*, 35–45.
41. Tural, S.; Koca, I. Physico–chemical and antioxidant properties of cornelian cherry fruits (*Cornus mas* L.) grown in Turkey. *Sci. Hortic.* **2008**, *116*, 362–366. [CrossRef]
42. Sengul, M.; Eser, Z.; Ercisli, S. Chemical properties and antioxidant capacity of cornelian cherry genotypes grown in Coruh valley of Turkey. *Acta Sci. Pol. Hortoru.* **2014**, *13*, 73–82.
43. Maghradze, D.; Abashidze, E.; Bobokashvili, Z.; Tchipashvili, R.; Maghlakelidze, E. Cornelian cherry in Georgia. *Acta Hortic.* **2009**, *818*, 65–72. [CrossRef]
44. Ognjanov, V.; Cerović, S.; Ninić–Todorović, J.; Jaćimović, V.; Gološin, B.; Bijelić, S.; Vračević, B. Selection and utilization of table cornelian cherry (*Cornus mas* L.). *Acta Hortic.* **2009**, *814*, 121–124. [CrossRef]
45. Brindza, P.; Brindza, J.; Toth, D.; Klimenko, S.; Grigorieva, O. Slovakian cornelian cherry (*Cornus mas* L.) potential for cultivation. *Acta Hortic.* **2007**, *760*, 433–437. [CrossRef]
46. Drkenda, P.; Spahic, A.; Begić–Akagić, A.; Gaši, F.; Vranac, A.; Hudina, M.; Blanke, M. Pomological characteristics of some autochthonous genotypes of cornelian cherry (*Cornus mas* L.) in Bosnia and Herzegovina. *Erwerbs–Obstbau* **2014**, *56*, 59–66. [CrossRef]
47. Kucharska, A.; Sokol–Letowska, A.; Piorecki, N. Morphological, physical & chemical, and antioxidant profiles of Polish varieties of cornelian cherry fruit (*Cornus mas* l.). *Food Sci. Technol. Qual.* **2011**, *3*, 78–89.
48. Akagić, A.; Vranac–Oras, A.; Oručević–Žuljević, S.; Spaho, N.; Drkenda, P.; Bijedić, A.; Memić, S.; Hudina, M. Geographic variability of sugars and organic acids in selected wild fruit species. *Foods* **2020**, *9*, 462.
49. Klimenko, S. The cornelian cherry (*Cornus mas* L.) collection, preservation and utilization of genetic resources. *J. Fruit Ornam. Plant Res.* **2004**, *12*, 93–98.
50. Etienne, A.; Genard, M.; Lobit, P.; Mbeguie, A.; Mbeguie, D.; Bugaud, C. What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. *J. Exp. Bot.* **2013**, *64*, 1451–1469. [CrossRef] [PubMed]
51. Milatović, D. *Apricot*; Scientific Pomological Society of Serbia: Čačak, Serbia, 2013.
52. Pantelidis, G.E.; Vasilakakis, M.; Manganaris, G.A.; Diamantidis, G. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and cornelian cherries. *Food Chem.* **2007**, *102*, 777–783. [CrossRef]
53. De Pascual, T.B.; Sanchez, S. Anthocyanins, from plant to health. *Phytochem. Rev.* **2008**, *7*, 281–299. [CrossRef]
54. Ćujić, N.; Kundaković, K.; Šavikin, K. Anthocyanins–chemical analysis and biological activity. *Lek. Sirov.* **2013**, *32*, 19–37.
55. Dinda, B.; Kyriakopoulos, A.M.; Dinda, S.; Zoumpourlis, V.; Thomaidis, N.S.; Velegraki, A.; Markopoulos, C.; Dinda, M. *Cornus mas* L. (cornelian cherry), an important European and Asian traditional food and medicine: Etnomedicine, phytochemistry and pharmacology for its commercial utilization in drug industry. *J. Ethopharmacol.* **2016**, *193*, 670–690. [CrossRef]
56. Ochmian, I.; Oszmiański, J.; Lachowicz, S.; Krupa–Małkiewicz, M. Rootstock effect on physico–chemical properties and content of bioactive compounds of four cultivars cornelian cherry fruits. *Sci. Hortic.* **2019**, *256*, 108588. [CrossRef]
57. Ramos, S. Cancer chemoprevention and chemotherapy: Dietary polyphenols and signalling pathways. *Mol. Nutr. Food Res.* **2008**, *52*, 507–526. [CrossRef] [PubMed]
58. Mehta, R.G.; Murillo, G.; Naithani, R.; Peng, X. Cancer chemoprevention by natural products: How far have we come? *Pharm. Res.* **2010**, *27*, 950–961. [CrossRef] [PubMed]
59. Cosmulescu, S.; Trandafir, I.; Cornescu, F. Antioxidant capacity, total phenols, total flavonoids and color component of cornelian cherry (*Cornus mas* L.) wild genotypes. *Not. Bot. Horti. Agrobo.* **2019**, *47*, 390–394. [CrossRef]
60. Stanković, M.S.; Zia–Ul–Haq, M.; Bojović, B.M.; Topuzović, M.D. Total phenolics, flavonoid content and antioxidant power of leaf, flower and fruits from cornelian cherry (*Cornus mas* L.). *Bulg. J. Agric. Sci.* **2014**, *20*, 358–363.
61. Cetkovská, J.; Diviš, P.; Vespalcová, M.; Pořízka, J.; Řezníček, V. Basic nutritional properties of cornelian cherry (*Cornus mas* L.) cultivars grown in the Czech Republic. *Acta Aliment. Hung.* **2015**, *44*, 357–364. [CrossRef]

62. Zlatković, B. *The Technology of Processing and Storage of Fruit*; Agricultural University: Belgrade, Serbia, 2003.
63. Kandic, R. *Meteorological and Hydrological Yearbook 2013*; IHMS, MNE: Podgorica, Montenegro, 2016. Available online: http://www.meteo.co.me/godisnjaci/GODISNJAK%202013.pdf (accessed on 19 October 2020).
64. Kandic, R. *Meteorological and Hydrological Yearbook 2014*; IHMS, MNE: Podgorica, Montenegro, 2016. Available online: http://www.meteo.co.me/godisnjaci/GODISNJAK%202014.pdf (accessed on 19 October 2020).
65. Kandic, R. *Meteorological and Hydrological Yearbook 2015*; IHMS, MNE: Podgorica, Montenegro, 2018. Available online: http://www.meteo.co.me/godisnjaci/GODISNJAK%202015.pdf (accessed on 19 October 2020).

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Assessment of Morphological Traits, Nutritional and Nutraceutical Composition in Fruits of 18 Apricot cv. Sekerpare Clones

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Abstract: Apricot (*Prunus armeniaca* L.) is one of the most important members of *Prunus* and its trees bears delicious and nutritious fruits during summer months in the temperate zones in the world. Apricot cultivars are propagated asexually which consists of clones. Information on inter-clonal variations in apricot cultivars can assist us in the selection of better clones from commercial cultivars. We aimed to determine morphological traits (fruit weight, seed weight, kernel weight, flesh/seed ratio, shape index, fruit firmness, color index), nutritional (sugars and organic acids) and nutraceutical (total phenolic, total flavonoids, total carotenoid and antioxidant activity) composition of 18 clones of Sekerpare apricot cultivar grown together in Kagizman district in eastern Turkey. Results showed significant differences among clones concerning most of the morphological traits, nutritional and nutraceutical compositions. Fruit weight, flesh/seed ratio and fruit firmness of clones were in range of 23.14–27.11 g, 11.21–13.14 and 3.88–5.11 kg/cm^2, respectively. Fruit shape index was slightly similar among all clones which was between 0.95 and 1.03. Citric acid and sucrose were found to be the predominant organic acid and sugar among clones which varied from 728 to 915 mg/100 g and 7.11 to 9.94 g/100 g, respectively. The clone 'KS2' exhibited the highest level of total phenol (67.1 mg gallic acid equivalent per 100 g) and antioxidant activity (2.16 µmol trolox equivalent per g). The study confirmed the diversity among Sekerpare clones and effectiveness of combining morphological, nutritional and nutraceutical analyses in assessment of Şekerpare clones and its use for future pre-breeding programs.

Keywords: apricot; Sekerpare; nutraceuticals

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1. Introduction

Due to suitable climate and soil conditions, Turkey is among the most important fruit producer countries in the world in terms of both the number of fruit species and the amount of production. Turkey ranks first in the world's apricot, fig, hazelnut, sweet cherry and quince production [1]. Nine apricot species and subspecies are known in the world. Among these species, *Prunus armeniaca* L. is cultivated in main apricot growing countries and spreads over the widest geographical area in the world [2,3]. The origin of apricot, which has been cultivated since ancient times, covers a wide area from Turkistan to Western China. Although apricots are grown geographically almost everywhere in the world, commercial production mostly occurs in countries of southern Europe, north America and north Africa [4].

Apricot can be consumed fresh, dried and canned throughout the year. The fruits of apricots are important for human nutrition, being rich in sugars, organic acids, fiber, vitamin A, vitamin E and potassium [5–9].

According to the data of the Food and Agriculture Organization (FAO) based on the year 2018, the amount of apricot production increased 19.5% compared to the previous year, while the area shows a decrease of 2% in apricot growing countries. When the data for the last five years (2014–2018) are examined, apricot production increased from 3.3 million tons to 3.8 million tons worldwide, but the production area stayed stable [1]. Turkey is leading world apricot production with a yearly average 750 thousand tons production. The country shares 20% of the world apricot production and is followed by Uzbekistan (13%), Iran (9%), Algeria (6%) and Italy (6%) [1].

In Turkey, apricot trees are grown mainly in the Aegean region, the Mediterranean region, and in particular the Central and Eastern Anatolia regions. Within the regions, Malatya, Elazig, Erzincan, Kahramanmaras, Kars, Mersin and Iğdır provinces are well known for commercial apricot cultivation and significant portions of the apricots are dried traditionally in these areas. Except for drying, apricots are generally used in the fruit juice industry in Turkey as well [10,11]. In recent years, depending on the technological developments, apricot fruits are frozen and become widespread in the market outside of the production period [12].

Each apricot growing region in Turkey has their own apricot cultivars and inter-regional cultivar transfer generally results in negative results. This is because apricots show low environmental adaptability, and the introduction of foreign germplasm may also result in fluctuating or limited yield. This is associated with differences in fertilization, chilling requirements, late-frost resistance, cold-hardiness, etc. [13]. In Turkey, the cultivar–region relationship is very strong as well in apricots. However, the Şekerpare cultivar can be grown in every region and shows great environmental plasticity. The cultivars are mostly grown in Malatya, Erzincan provinces and Aras valley in Turkey and show variable fruit weight ranging from 20 to 40 g [2,4,10,14]. The cultivar called Shakarpara in Pakistan and India and Shekarpareh in Iran shows great phenotypic variability as well. Phenotypic variation within Sekerpare grown in similar ecological conditions arises from an accumulation of somatic mutations due to vegetative propagation during centuries in different Sekerpare growing countries [2,15,16]. The concept of sustainable apricot production can be described as a "three-legged stool", with legs of economic viability, environmental soundness, and social acceptability. Communicating the health benefits of apricot fruit to consumers is an essential ingredient in sustaining apricot product demand, which is a prerequisite for sustainable apricot production. Thus, the cultivar Sekerpare grown in different parts of the world could be adding value for economic viability, environmental soundness, and social acceptability.

Sekerpare is found in most of the apricot growing regions in Turkey and still retains importance and provides interesting economic results in local markets, remaining a popular option for most of the apricot growing regions. This locally adapted cultivar is appreciated for its superior flavor and suitability for both fresh consumption and as a dried product [2].

The Aras valley (Kars-Igdir region) is one of the important apricot growing areas of Turkey. Kagizman district provides almost all of the apricot production in Kars province. In the district, Aprikoz, Sekerpare and wild apricots are grown [17–19]. There are numerous clones of Sekerpare available in the Kagizman district that exhibit differences in key horticultural traits.

Identifying plant varieties is an age-old human endeavor. Historically, morphological traits and later nutritional and nutraceutical characteristics were used to categorize specimens into families, genera, species, cultivars, genotypes, landraces (for perennials: a plant selected from seedlings and asexually re-propagated for its desired characteristics). Thus, varietal characterization based on morphological, nutritional and nutraceutical traits is an important component of fruit tree improvement and breeding [20,21].

Apricot has gained great value in human consumption and commercial importance in recent years, attracting researchers to study its morphological, nutritional and in particular nutraceutical traits.

Advances in fruit species improvement programmes is only possible when intense and more defined genetic variability exists. The phonological expression of any fruit tree species is mostly governed by two factors viz. heredity and environment. Given the fact that environmental variations can be reduced by growing the identical genotypes under uniform site and climatic conditions, studying genetic parameters is of immense use to obtain superior genotypes of any species.

The present study intended to capture variability across morphological, nutritional and nutraceutical parameters of 18 clones of Sekerpare apricot from a particular similar environmental condition.

2. Materials and Methods

2.1. Plant Samples

Twenty fruits were harvested from different parts of trees of 18 Sekerpare clones grown together in Kagizman district during July in 2018. Kagizman is located at 40. 1406° N and 43.1191° E and 1406 m above mean sea-level. All trees of the 18 Sekerpare clones were found at nearly the same altitude in Kagizman district. All examined trees were pre-selected clones according to higher yield, pest and disease free status and more attractive bigger fruit characteristics. Special attention was given on harvest and fruits were harvested in the same period with the same degree of maturity. A total of 80 fruits per clone were collected and then sorted and cleaned. Mature and healthy fruits were transported to the laboratory and divided into two equal parts for morphological measurements and nutritional and nutraceutical analysis.

2.2. Morphological Parameters

A total 40 fruits per clone were used for morphological measurements which included fruit weight, seed weight, kernel weight, flesh/seed ratio, shape index and fruit color coordinates (L, a and b values). Fruit weight (g) was measured with a digital scale sensitive to 0.01 g (Scaltec SPB31). Fruit firmness was determined with a non-destructive Acoustic Firmness Sensor (Aweta B.V., Pijnacker, The Netherlands) expressed as kg/cm^2. Fruit shape index (SI) was calculated with the following equation [22].

$$\mathrm{SI}\frac{\mathrm{W}+\mathrm{T}}{2\mathrm{L}} \quad \text{where W : Width, T : Thickness and L : Length} \tag{1}$$

Color coordinates (*L**, *a**, and *b**) of fruit skin were determined by a Konica Minolta, CR-400 Plus fruit colorimeter (Konica Minolta, Inc., Chiyoda City, Tokyo, Japan) at four different positions around the equator of the fruits [23].

2.3. Nutritional and Nutraceutical Composition

2.3.1. Sample Preparation and Extraction

A representative of 40 fruits/clone were randomly selected from the harvested lot at commercial maturity stage. The fruits were then introduced to a High-Speed Pulp Ejection Juicer (Omega Products International, Corona, CA, USA), allowing the separation of pomace and juice. The juice was stored at −80 °C until use for nutritional and nutraceutical content. During the analysis, the frozen fruits were taken and thawed to 24–25 °C. A laboratory blender was used to homogenize the fruit samples (100 g lots of fruits per clone) and a single extraction procedure (taking 3 g aliquots transferred inside tubes and extracted for 1 h with 20 mL buffer including acetone, water (deionized), and acetic acid (70:29.5:0.5 *v*/*v*)) was used [24].

2.3.2. Organic Acids

Organic acid composition in fruits of Sekerpare apricot clones was determined by Bevilacqua and Califano [25]. Fruit extracts were obtained by crushing the fruits in cheesecloth. Then, 0.009 N H_2SO_4 was added and shaken for 1 h. The mixture was then centrifuged at 15,000 rpm for 15 min and the supernatants were filtered twice through a

0.45 µm membrane filter with a coarse filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, MA, USA) and passed through a SEP-PAK C18 cartridge. Organic acid readings were performed by HPLC using the Aminex column (HPX-87 H, 300 × 7.8 mm, Bio-Rad Laboratories, Richmond, CA, USA) at 214 and 280 nm wavelengths in the Agilent package program (Agilent Technologies, Santa Clara, CA, USA). Results are expressed as mg/100 g.

2.3.3. Determination of Individual Sugars

For individual sugar (fructose, glucose, and saccharose) analyses, the method of Melgarejo et al. [26] was used. First, homogenized fruits (5 g) were diluted with purified water and homogenate was centrifuged at 6000 rpm for 5 min. Supernatants were filtered through a 0.45 µm membrane filter (Iwaki Glass, Jawa Barat, Indonesia) before the analysis. The HPLC analysis was conducted using a PerkinElmer HPLC system with Amino NH_2 column, and 85% acetonitrile/15% H_2O (v/v) as a mobile phase. Refractive index detector (RID) was used. Samples were identified and quantified by standards. Results were expressed as g/100 g fw. To specify the sweetness perception of 40 fruit per clone, their sweetness indices (SI) were calculated according to Roussos et al. [27]. The SI index considers the relative sweetness as a factor of each of the three sugars measured. It is described in the following equation (1): where *Glu* stands for glucose concentration, *Fru* for fructose concentration, and *Sacch* stands for saccharose concentration.

$$\mathrm{SI} = 1.00 \cdot Glu + 2.3 \cdot Fru + 1.35 \cdot Sacch \quad (2)$$

2.3.4. Total Phenol Content

The total phenolic content (TPC) of the samples was evaluated using the Folin–Ciocalteu method according to Singleton and Rossi [24]. In this procedure, each extract (1 mL) was mixed with Folin–Ciocalteu's reagent and water 1:1:20 (v/v). The samples were incubated for 8 min. Then, sodium carbonate (10 mL) with a concentration of 7% (w/v) was added. After incubation for 2 h, the absorbance at 750 nm was measured. The total phenolic content was calculated against the reference standard calibration curve of gallic acid. The TPC was expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh sample.

2.3.5. Total Carotenoid Content

The total carotenoid content was determined by Lichtenthaler [28]. For total carotenoid content, 1 g of fruit sample was homogenized with 5 mL of acetone in a cold porcelain mortar in an ice bath. Then, 1 g of anhydrous sodium sulfate ($Na_2 \cdot SO_4$) was added to the homogenate, which was elutriated using a paper filter. The filtered solution was made up to 10 mL with acetone and centrifuged at 2600× g for 10 min. The upper phase was collected and the absorbance of the solution at 662, 645 and 470 nm was measured. Acetone was used as control. Total carotenoid content is expressed as mg per 100 g fresh fruit sample.

2.3.6. Antioxidant Capacity

The TEAC value of each sample was detected according to the method described by Rice-Evans et al. [29]. The 7 mM ABTS reagent solutions were prepared and diluted with sodium acetate ($C_2H_3NaO_2$) until 0.700 ± 0.01 spectrophotometrical absorbance level at 734 nm. Following this, 2.97 mL buffered solution was mixed with 30 µL fruit extract and kept in dark at room temperature for 10 min and their absorbance levels were measured at 734 nm using a spectrophotometer. Obtained results were calculated according to TEAC standard calibration curve and expressed as µmol of trolox equivalent/g fresh fruit weight (µmol TE/g FW).

2.4. Statistical Analysis

All data were analyzed using SPSS software and procedures. Analysis of variance tables were constructed using the least significant difference (LSD) method at $p < 0.05$.

3. Results and Discussion

3.1. Morphological Traits

As presented in Table 1, statistically significant differences ($p < 0.05$) were recorded in fruits of among 18 clones of cv. Sekerpare for most of the morphological traits. Fruit weight and skin color are the most important and distinct external fruit traits in apricots for consumer acceptance. Skin color is a practical and simple indicator with which to instruct harvesters on what to harvest. In addition, consumers in general prefer attractive medium-sized apricot fruits. The flesh/seed ratio is also an important fruit characteristic for apricots [2].

Table 1. Fruit weight, seed weight, kernel weight, flesh/seed ratio, shape index and fruit firmness of 18 Sekerpare clones.

Clones	Fruit Weight (g)	Seed Weight (g)	Kernel Weight (g)	Flesh/Seed Ratio	Shape Index	Fruit Firmness (kg/cm^2)
KS1	23.02c	1.85bc	0.78bc	11.44bc	0.98b	4.96ab
KS2	24.56bc	1.86bc	0.68de	12.20ab	0.99ab	4.55ab
KS3	27.11a	2.09a	0.84a	11.97bc	1.01ab	3.98c
KS4	24.03bc	1.93bc	0.74cd	11.45bc	1.00ab	4.32b
KS5	26.33ab	2.01ab	0.79b	12.09abc	0.95c	3.95c
KS6	25.18abc	1.90bc	0.75c	12.25ab	1.03a	4.20bc
KS7	26.55ab	2.03ab	0.82ab	12.08b	1.00ab	4.32b
KS8	25.78ab	1.89bc	0.72cd	12.64ab	0.98b	4.56ab
KS9	25.30b	1.85bc	0.72cd	13.14a	1.00ab	4.30b
KS10	23.14c	2.02ab	0.74c	10.46cd	0.98b	5.11a
KS11	24.98bc	1.85c	0.70d	12.50ab	1.03a	4.74ab
KS12	23.66bc	2.04ab	0.73cde	10.60c	0.97bc	4.70ab
KS13	24.58bc	1.94abc	0.81ab	11.67bc	0.99ab	4.55ab
KS14	23.86bc	1.83bc	0.70d	12.04b	0.96bc	4.85ab
KS15	23.44bc	1.92bc	0.76c	11.21bc	0.98b	5.02ab
KS16	23.40bc	1.80cd	0.70d	12.00b	0.96bc	4.80ab
KS17	26.13ab	1.96b	0.84a	12.33ab	0.97abc	3.88c
KS18	25.41ab	1.92bc	0.75c	12.24ab	1.00ab	4.33b

Different letters in the same column indicate significant differences ($p < 0.05$) among clones.

Fruit weight, seed weight and kernel weight of Sekerpare clones differed from each other statistically ($p < 0.05$) which ranged from 23.02 to 27.11, 1.80 to 2.09 and 0.68 to 0.84 g, respectively (Table 1). The variation in fruit and seed weight of the clones was also reflected in the fruit flesh/seed ratio which were in the range of 10.46–13.14.

Shape index and fruit firmness of clones were found between 0.96–1.03 and 3.88–5.11 kg/cm^2 (Table 1). KS3 and KS9 were notable among clones due to relatively high fruit weight and flesh/seed ratio.

Studies on apricots in different parts of Turkey identified variations in apricot fruit weight [18,30–32]. Turkish national apricot cultivars have relatively small fruit size and previous studies indicated this fact. For example, Akin et al. [33] studied main apricot cultivars grown in Malatya and determined fruit weight between 21.16 and 38.24 g. Asma and Ozturk [31] reported that 128 Turkish apricot cultivars that belong to the Iran-Caucasian ecogeographical group generally had low fruit weight (lower than 50 g). The authors reported that the fruit weight of only seven apricot cultivars was over 50 g, and the rest of cultivars had lower fruit weight. Karaat and Serce [10] reported fruit weight as 25.12 g in Cagataybey cultivar and 25.65 g in Sekerpare cultivar in Malatya which supports our findings. They also found seed weight to be 1.97 g, flesh/seed ratio as 12.02 and fruit firmness as 2.58 kg/cm^2 in cv. Sekerpare. Akca and Asma [34] conducted a clonal selection study on cv. Kabasi that aimed to find better Kabaasi clones with promising horticultural characteristics. They determined 13 promising clones among 450 Kabaasi trees. Fruit weight, seed weight, kernel weight and flesh/seed ratio were found between 31.81 and

60.91 g, 2.35–3.01 g, 0.52–0.98 g and 12.38–16.64, respectively, indicating similarities with our study. Previously, the flesh/seed ratios of the foreign apricot cultivars grown in Turkey varied between 8.9 and 21.8 [35,36]. In the literature, the shape index of apricots was reported between 0.91 and 1.09 [37,38]. In apricots, if fruit shape index values are found around 1, fruit tend to have a round shape. The Sekerpare cultivar in general gave round shaped fruits while if these values are higher than 1, fruits correspond to ovoid shape.

L^*, a^* and b^* color coordinates of clones are given in Table 2 and it was found that the L^*, a^* and b^* values of the Sekerpare clones significantly differed from each other at $p < 0.05$ (Table 2).

Table 2. Fruit skin color parameters of 18 Sekerpare clones.

Clones	L^*	a^*	b^*	Ground Color	Red Blushed Skin
KS1	61.21cd	11.38d	42.56ab	Yellow	Absent
KS2	63.45bc	14.29bc	40.14c	Yellow	Present
KS3	59.89de	14.90bc	44.13a	Dark yellow	Present
KS4	63.11bc	15.57ab	39.88cd	Yellow	Present
KS5	60.18d	15.39b	40.55bc	Light orange	Present
KS6	64.10b	12.55cd	38.15d	Light yellow	Absent
KS7	63.23bc	10.67de	41.23bc	Yellow	Absent
KS8	56.15f	14.44bc	43.32ab	Dark yellow	Present
KS9	58.33de	10.14def	42.56ab	Dark yellow	Absent
KS10	65.41ab	12.80cd	37.45de	Yellow	Absent
KS11	58.45de	13.38c	37.89de	Light orange	Present
KS12	57.10ef	17.12a	39.11cd	Light orange	Present
KS13	64.96ab	10.77de	40.15c	Yellow	Present
KS14	66.32a	14.20bc	38.68cd	Light yellow	Present
KS15	63.50bc	16.41ab	43.33ab	Dark yellow	Absent
KS16	60.25d	11.00de	41.22bc	Yellow	Absent
KS17	62.27c	10.70de	43.09ab	Dark yellow	Present
KS18	58.00e	12.07cd	42.25b	Light orange	Absent

Different letters in the same column indicate significant differences ($p < 0.05$) among clones.

Color is of primary importance for consumers in the judgment of different fruit groups and accepted as one of the important quality attributes. Lightness (L^*), red/greenness (a^*), and yellow/blueness (b^*) values of the 18 clones of cv. Sekerpare are shown in Table 2. The highest L^* values were observed in clones KS14 as 66.32 and followed by KS10 (65.41) while KS18 had the lowest L^* values (58.00) compared to the other clones. The lightness (L^*) was dependent on exposure to sun [39]. Higher a^* and b^* values were observed in KS12 (17.12) and 44.13 (KS3). The chromaticity coordinate a^* is the most important factor of maturity appearance describing color of the fruit. The intensity of red color normally indicates full maturity and ripeness [40]. Karaat [41] indicated L^*, a^* and b^* values in Sekerpare fruit as 64.17, 14.07 and 42.27, respectively, which is in agreement with our results. Karatas and Sengul [4] reported L^*, a^* and b^* values as 48.66, 19.41 and 19.72 in Sekerpare fruits which indicated differences with our study. In India, L^*, a^* and b^* values of cv. Shakarpara (Sekerpare) were 71.51, 1.03 and 40.56, respectively [16]. These results also reveal that Sekerpare is probably the name of a group of apricots because quite diverse results were obtained from different countries even in the same countries and also strong clonal variation is evident because different clones of the cultivar show significant variation in color values as well.

Among 18 clones, seven had yellow ground color, five clones had dark yellow ground color, four clones had light orange ground color and two clones had light yellow ground color (Table 2).

The majority of clones had red blushed skin and eight clones lacked red blushed skin development (Table 2). The majority of apricot (*Prunus armeniaca* L.) cultivars display orange or yellow background skin, whereas some cultivars are particularly preferred by consumers because of their red blushed skin on the background. Anthocyanins are

responsible for the blushed skin of apricots and the PaMYB10 gene was found as a positive regulator of anthocyanin biosynthesis in apricots and demonstrates that blush formation depends on light [42]. Apricots with a blush on orange or yellow skin are becoming more and more popular in the market due to their colorful appearance and excellent nutritional value [43].

3.2. Nutritional Traits

3.2.1. Organic Acids

The results on organic acid content in fruits of 18 clones of cv. Sekerpare apricots are reported in Table 3. The order of organic acid depending on their content was in descending order citric > malic > ascorbic > tartaric for all clones. Citric acid was the predominant organic acid for all studied clones that ranged from 728 to 915 mg/100 g. Malic acid and tartaric acid content of 18 Sekerpare clones were in range of 261–452 and 2.8–5.7 mg/100 g, respectively (Table 3). Ascorbic acid was identified in all clones from 13.9 mg to 18.6 mg/100 g and this indicates that apricot fruits contain a moderate level of ascorbic acid among fruit species. Organic acid results also indicated that citric, malic, tartaric and ascorbic acid concentrations are greatly varied among clones ($p < 0.05$). Organic acids are of increasing interest because of their role in plant physiology as cofactors, buffering agents, etc. [44]. The organic acid content and profile of fruit species differs depending on species, cultivars, accessions, etc. Alajil et al. [16] showed that citric acid comprised approximately 55% of the organic acids in apricot fruits and ranged from 550 to 1170 mg/100 g, followed by malic acid, which comprised approximately 25% of the organic acids and ranged from 400 to 1430 mg/100 g. Some organic acids have an antioxidant role (tartaric, malic and citric acids). Fruit acids that allow nutrient digestion and stimulate blood circulation are considered among the quality parameters of apricot fruits. Numerous studies have quantified and detailed the organic acid content of apricot fruits and there have been differences between them due to the species, location, used methods, sampling periods, etc. [16,44–52]. Fan et al. [49] showed that malic acid was mainly responsible for sourness of apricots, although malic acid was not the prominent organic acid in all apricot cultivars. It has also been reported that malic acid has an apparent acidic taste compared to citric acid or other organic acids in fruit [53].

Table 3. Organic acids in fruits of 18 Sekerpare clones (mg/100 g).

Clones	Citric Acid	Malic Acid	Ascorbic Acid	Tartaric Acid
KS1	880ab	275de	17.2ab	5.0ab
KS2	915a	261e	18.0ab	3.2cd
KS3	885ab	296de	18.6a	3.6c
KS4	904ab	405bc	17.6ab	4.4bc
KS5	822bc	350cd	15.0bc	4.0bc
KS6	816bc	427ab	15.8bc	5.7a
KS7	734cd	304d	14.0c	5.0ab
KS8	829bc	380bc	15.1bc	2.8cd
KS9	728d	369c	14.7bc	4.0bc
KS10	763cd	387bc	16.0b	5.0ab
KS11	838b	344cd	13.9c	3.6c
KS12	874ab	395bc	18.4ab	4.7b
KS13	769c	360cd	17.9ab	5.5ab
KS14	745cd	452a	15.7bc	4.2bc
KS15	796bc	412b	18.0ab	4.0bc
KS16	778c	435ab	18.2ab	3.4c
KS17	810bc	365cd	14.0c	3.2cd
KS18	785bc	422ab	14.4bc	3.6c

Different letters in the same column indicate significant differences ($p < 0.05$) among clones.

3.2.2. Individual Sugars and Sweetness Indices

Sugar content in different Sekerpare clones is shown in Table 4. The dominant sugar was sucrose in 18 clones that ranged from 7.11 to 9.94 mg/100 g, followed by glucose in the range of 2.03–3.31 g/100 g, respectively. Fructose content of fruits was relatively lower and found between 0.78 and 1.05 g/100 g (Table 4). Overall, the highest sucrose, glucose and fructose contents were found in KS17, KS1, KS3 and KS4 clones, respectively. Sweetness index (SI) was obtained between 13.35 (KS13) and 18.46 (KS17). Alajil et al. [16] used a number of apricots including Sekerpare in nutritional analysis and reported that sucrose was the dominant sugar, accounting for more than 63% of total sugars and ranged from 4.15 to 10.13 g/100 g, glucose contributed about 22% of total sugars and ranged from 2.28 to 4.31 g/100 g, and fructose contributed about 15% of total sugars and ranged from 1.22 to 4.19 g/100 g which shows parallel values with our study. Saridas and Agcam [44] examined individual sugars and organic acids in Agerik and Teberze apricot cultivars and reported that both contents change greatly according to cultivars. They reported sucrose, glucose and fructose content between 5.33 and 8.57, 1.90 and 2.95 and 0.60 and 0.88 g/100 g, respectively. Furthermore, the composition of individual sugars in the current study agrees with that documented by Akin et al. [33] for different Turkish apricot cultivars. İmrak et al. [45] found that the dominant sugar in apricot fruits was sucrose. Karataş and Sengul [4] reported sucrose content between 1.83 and 3.97 g/100 g in main apricot cultivars sampled in Malatya province in Turkey. Su et al. [46] used local apricots in sugar analysis and found that sucrose was the main sugar. Individual sugars differ in sweetness, with fructose perceived as sweeter than sucrose and sucrose perceived as sweeter than glucose [27]. The sweetness is important to apricot consumers and breeders, and it also leads to market acceptance of the fruit [47].

Table 4. Individual sugars (g/100 g) and sweetness indices (SI) in fruits of 18 Sekerpare clones.

Clones	Sucrose	Glucose	Fructose	Sweetness Index (SI)
KS1	9.06ab	3.24a	0.95ab	17.66ab
KS2	7.67bc	2.70bc	0.89b	15.10bc
KS3	8.23bc	3.02ab	1.05ab	16.55b
KS4	7.77bc	2.90abc	1.09a	15.90bc
KS5	8.11bc	3.10ab	0.86bc	16.03bc
KS6	7.90bc	2.22cd	0.67c	14.43cd
KS7	7.61bc	2.03d	0.78bc	14.09cd
KS8	8.10bc	2.78bc	0.99ab	16.00bc
KS9	8.40b	2.55bc	0.80bc	15.73bc
KS10	7.86bc	3.20a	1.02ab	16.16bc
KS11	8.33b	3.31a	0.90b	16.63b
KS12	7.85bc	2.39bcd	1.00ab	15.29bc
KS13	7.11c	2.14cd	0.70c	13.35d
KS14	7.56bc	2.29cd	0.82bc	14.39cd
KS15	8.06bc	2.63bc	1.02ab	15.78bc
KS16	7.37bc	2.55bc	1.05ab	14.92c
KS17	9.94a	2.85b	0.95ab	18.46a
KS18	9.12ab	2.47c	0.75bc	16.33bc

Different letters in the same column indicate significant differences ($p < 0.05$) among clones.

The sweetness index (SI) in fruits of Sekerpare clones ranged from 13.35 to 18.46 (Table 3). Previously, the sweetness index (SI) ranged from 13.58 to 22.30 in apricot fruits grown in India and Shakarpara reported a SI of about 13.58 [16], indicating lower values than our study. In Greece, SIs were found between 8.16 and 11.25 among apricot cultivars [27]. Our findings are consistent with those published SIs for Spanish apricot genotypes ranging from 8.5 to 15.9 [48]. Despite the fact that SI determines taste, the final perception of fruit sweetness is influenced by the presence of other compounds such as phenolics and other aroma compounds [49].

3.3. Nutraceutical Traits

Total Phenolic Content, Total Flavonoids, Total Carotenoids and Antioxidant Activity

Table 5 shows total phenolic, total flavonoid, total carotenoid content and antioxidant activity in fruits of 18 clones of cv. Sekerpare. We found statistically significant differences among clones in terms all nutraceutical traits at 0.05 level (Table 5).

Table 5. Nutraceuticals in fruits of 18 Sekerpare clones (fresh weight basis).

Clones	Total Phenolic Content (mg GAE/100 g)	TEAC (μmol TE/g)	Total Flavonoids (mg CE/100 g)	Total Carotenoid (mg/100 g)
KS1	58.4c	1.96bc	11.9c	7.80e
KS2	67.1a	2.16a	13.8ab	7.72e
KS3	56.0cd	1.92bc	12.7abc	9.30bc
KS4	61.3bc	2.06ab	10.5de	8.84c
KS5	59.3bc	1.96bc	9.3ef	10.05ab
KS6	64.4ab	2.12ab	12.9b	8.28cd
KS7	62.0b	2.09ab	12.1bc	8.65bc
KS8	58.0c	1.94bc	10.5de	9.25abc
KS9	49.9e	1.88bc	9.8e	9.10bc
KS10	55.0d	1.90bc	9.2ef	8.58cd
KS11	59.0bc	1.98bc	10.9d	8.51cd
KS12	63.0ab	2.09ab	13.3ab	10.13a
KS13	61.0bc	2.04ab	11.7abc	9.41b
KS14	58.9bc	1.99bc	13.6ab	8.44cd
KS15	49.5e	1.86c	11.4bc	8.16d
KS16	57.2cd	1.92bc	10.5de	8.95bc
KS17	60.4bc	2.01b	11.4cd	8.40cd
KS18	60.7bc	2.04ab	14.1a	8.88c

Different letters in the same column indicate significant differences ($p < 0.05$) among clones.

As mentioned in Table 5, total phenolic content was found between 49.5 and 67.1 mg GAE/100 g fresh weight basis. Saeed et al. [54] used fruits of eight apricot cultivars sampled from Pakistan and reported total phenolic content between 50 and 220 mg GAE/100 g FW indicating higher values than our results. Gecer et al. [18] used a number of wild apricots and cv. Aprikoz and found total phenolic content between 34.2 and 52.8 mg GAE/100 g which is in accordance with our study. In Hungary, a large number of apricot cultivars were used in nutraceutical analysis and total phenolic content greatly varied among cultivars from 12.0 to 89.0 mg GAE/100 g [7]. In Turkey, Karaat and Serce [10] used main apricot cultivars in Malatya and reported total phenolic content between 35.1 and 90.7 mg GAE/100 g. In the Mediterranean region in Turkey, apricots show total phenolic content between 14.4 and 177.1 mg GAE/100 g, with a mean value of 64.4 mg GAE/100 g indicating similarities with our samples [55]. Alajil et al. [16] reported total phenolic content among apricots, ranging from 25.31 (Shakarpara) to 89.95 mg GAE/100 g (Roxana). Wani et al. [56] and Leccese et al. [57] previously reported similar findings in apricots grown in India and Italy, respectively.

Total flavonoids were in range of 9.2–14.1 mg CE/100 g (Table 5). Saeed et al. [54] reported total flavonoids between 48 and 382 mg QE/100 g on fresh weight basis in apricots indicating higher values than our results. Alajil et al. [16] found that total flavonoid amounts in apricot genotypes ranged from 5.00 to 15.46 mg CE/100 g which indicated good agreement with our study. Our results are also consistent with those reported by Carbone et al. [58], who reported total flavonoid content (TFC) ranging from 1.9 to 12.0 mg CE/100 g for different apricot genotypes. Kafkaletou et al. [59] and Wani et al. [56] found TFC values ranging from 16.87 to 41.42 and 12.2 to 36.2 mg/100 g in apricot genotypes grown in Greece and India, respectively. Phenolics and flavonoids are essential measures of nutraceutical quality and have been linked to the treatment of a variety of chronic diseases, including cancer, cardiovascular disease, and neurodegeneration [60–64].

Total carotenoid content of 18 apricot clones of cv. Sekerpare were found between 7.72 and 10.13 mg/100. Gecer et al. [18] reported total carotenoid content ranged from 1.1 to 12.5 mg/100 g of edible portion in wild apricots and cv. Aprikoz. Ruiz et al. [65] found total carotenoid content between 1.5 and 16.5 mg/100 g among a large number of apricot cultivars in Spain. Shemesh et al. [66] found total carotenoid content between 0.5 and 9.5 mg/100 g among 113 apricot cultivars in Israel. These studies are in harmony with our results. The content and composition of carotenoids in apricots determine their fruit color. Apricots are high in carotenoids, which influence the color and visual appearance of the fruit; the color of the fruit can vary from yellow to orange depending on the carotenoids content [67]. Carotenoids are also essential dietary sources of vitamin A.

The antioxidant activity determined by TEAC assay in fruits of the 18 Sekerpare clones were evaluated and the results are presented in Table 5. Amongst the 18 clones of Sekerpare cultivar, the KS15 clone showed the lowest antioxidant activity (1.88 μmol TE/g), whereas the KS2 clone showed the highest antioxidant activity (2.16 μmol TE/g) (Table 5). The KS6 clone showed the second highest antioxidant activity (2.12 μmol TE/g) and the clones KS7 and KS12 showed the third highest antioxidant activity (2.09 μmol TE/g). In Italy, among the apricot cultivars analyzed, the variability of the antioxidant capacity was obtained showing a range from 1.36 to 4.55 μmol TE/g. They found that the latest cultivars had two-fold higher TEAC values with respect to the earliest ones [68].

Previous studies conducted in different horticultural plants indicated that horticultural crops rich for antioxidant components and antioxidant activity were found to be cultivar/genotype/clone dependent [69–80].

4. Conclusions

A detailed morphological, nutritional and nutraceutical traits analysis was reported here for the first time in a large number of clones of cv. Sekerpare. The results indicated that even in a small single growing location, Sekerpare clones showed rich diversity on most of the morphological traits and nutritional and nutraceutical compositions. The KS3 clone showed the highest fruit weight as 27.11 g. KS2, KS7 and KS12 had the highest antioxidant activity. The promising clones could be used as breeding material. The results could have practical implications for orchard management to select better Sekerpare clones and bring them into production.

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References

1. FAO. Food and Agriculture Organization of the United Nations. 2020. Available online: http://www.fao.org/faostat/en/#data/QC (accessed on 18 April 2021).
2. Ercisli, S. Apricot culture in Turkey. *Sci. Res. Essays* **2009**, *4*, 715–719.
3. Ruiz, D.; Egea, J. Phenotypic diversity and relationships of fruit quality traits in apricot (*Prunus armeniaca* L.) germplasm. *Euphytica* **2008**, *163*, 143–158. [CrossRef]
4. Karatas, N.; Sengul, M. Some important physicochemical and bioactive characteristics of the main apricot cultivars from Turkey. *Turk. J. Agric. For.* **2020**, *44*, 651–661. [CrossRef]
5. Gurrieri, F.; Audergon, J.M.; Albagnac, G.; Reich, M. Soluble sugars and carboxylic acids in ripe apricot fruit as parameters for distinguishing different cultivars. *Euphytica* **2001**, *117*, 183–189. [CrossRef]

6. Munzuroglu, O.; Karatas, F.; Geckil, H. The vitamin and selenium contents of apricot fruit of different varieties cultivated in different geographical regions. *Food Chem.* **2003**, *83*, 205–212. [CrossRef]
7. Hegedus, A.; Engel, R.; Abranko, L.; Balogh, E.; Blazovics, A.; Herman, R.; Halasz, J.; Ercisli, S.; Pedryc, A.; Stefanovits-Banyai, E. Antioxidant and antiradical capacities in apricot (*Prunus armeniaca* L.) fruits: Variation from genotypes, years, and analytical methods. *J. Food Sci.* **2010**, *75*, C722–C730. [CrossRef]
8. Sochor, J.; Zitka, O.; Skutkova, H.; Pavlik, D.; Babula, P.; Krska, B.; Horna, A.; Adam, V.; Kizek, R.; Provaznik, I. Content of phenolic compounds and antioxidant capacity in fruits of apricot genotypes. *Molecules* **2010**, *15*, 6285–6305. [CrossRef]
9. Ali, S.; Masud, T.; Abbasi, K.S. Physico-chemical characteristics of apricot (*Prunus armeniaca* L.) grown in Northern Areas of Pakistan. *Sci. Hortic.* **2011**, *130*, 386–392. [CrossRef]
10. Karaat, F.E.; Serce, S. Total phenolics, antioxidant capacities and pomological characteristics of 12 apricot cultivars grown in Turkey. *Adyütayam* **2019**, *7*, 46–60.
11. Kayran, S.; Doymaz, I. Drying of Cataloglu apricots: The effect of sodium metabisulfite solution on drying kinetics, diffusion coefficient, and color parameters. *Int. J. Fruit Sci.* **2021**, *21*, 270–283. [CrossRef]
12. Gundogdu, M. Effect of rootstocks on phytochemical properties of apricot fruit. *Turk. J. Agric. For.* **2019**, *43*, 1–10. [CrossRef]
13. Pennone, F.; Abbate, V. Prospettive per la valorizzazione della coltura dell'albicocco nel Mezzogiorno attraverso il miglioramento genetico. *Riv. Fruttic. Ortofloric.* **2004**, *66*, 36–39.
14. Asma, B.; Kan, T.; Birhanli, O. Characterization of promising apricot (*Prunus armeniaca* L) genetic resources in Malatya, Turkey. *Genet. Resour. Crop Evol.* **2007**, *54*, 205–212. [CrossRef]
15. Molaie, S.; Soleimani, A.; Zeinolabedini, M. Evaluation of quantitative and qualitative traits of some apricot cultivars grown in Zanjan region. *J. Hortic. Sci.* **2016**, *30*, 35–48.
16. Alajil, O.; Sagar, V.R.; Kaur, C.; Rudra, S.G.; Sharma, R.R.; Kaushik, R.; Verma, M.K.; Tomar, M.; Kumar, M.; Mekhemar, M. Nutritional and phytochemical traits of apricots (*Prunus armeniaca* L.) for application in nutraceutical and health industry. *Foods* **2021**, *10*, 1344. [CrossRef]
17. Atis, E.; Celikoglu, S. Apricot producing in Kagizman district and its contribution to the economy of territory. *Marmara Geogr. J.* **2017**, *36*, 191–205.
18. Gecer, M.K.; Kan, T.; Gundogdu, M.; Ercisli, S.; Ilhan, G.; Sagbas, H.I. Physicochemical characteristics of wild and cultivated apricots (*Prunus armeniaca* L.) from Aras valley in Turkey. *Genet. Resour. Crop Evol.* **2020**, *67*, 935–945. [CrossRef]
19. Karatas, N. Some important fruit characteristics of apricot (cv. Aprikoz) accessions grown in Kagizman district in Turkey. *J. Inst. Sci. Technol.* **2021**, *11*, 1176–1182. [CrossRef]
20. Engin, S.P.; Mert, C. The effects of harvesting time on the physicochemical components of aronia berry. *Turk. J. Agric. For.* **2020**, *44*, 361–370. [CrossRef]
21. Skender, A.; Kurtovic, M.; Drkenda, P.; Becirspahic, D.; Ebrahimi, A. Phenotypic variability of autochthonous walnut (*Juglans regia* L) genotypes in northwestern Bosnia and Herzegovina. *Turk. J. Agric. For.* **2020**, *44*, 517–525. [CrossRef]
22. Mohsenin, N.N. *Physical Properties of Plant and Animal Materials*; Gordon and Breach Science Publisher: New York, NY, USA, 1986.
23. Ozturk, I.; Ercisli, S.; Kalkan, F.; Demir, B. Some chemical and physico-mechanical properties of pear cultivars. *Afr. J. Biotechnol.* **2009**, *8*, 687–693.
24. Singleton, V.L.; Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.
25. Bevilacqua, A.E.; Califano, A.N. Determination of organic acids in dairy products by high performance liquid chromatography. *J. Food Sci.* **1989**, *54*, 1076–1079. [CrossRef]
26. Melgarejo, P.; Salazar, D.M.; Artes, F. Organic acids and sugars composition of harvested pomegranate fruits. *Eur. Food Res. Technol.* **2000**, *211*, 185–190. [CrossRef]
27. Roussos, P.A.; Sefferou, V.; Denaxa, N.K.; Tsantili, E.; Stathis, V. Apricot (*Prunus armeniaca* L.) fruit quality attributes and phytochemicals under different crop load. *Sci. Hortic.* **2011**, *129*, 472–478. [CrossRef]
28. Lichtenthaler, H.K. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol.* **1987**, *148*, 350–382.
29. Evans, R.H.; Van Soestbergen, A.W.; Ristow, K.A. Evaluation of apple juice authenticity of organic acid analysis. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 1517–1520. [CrossRef]
30. Akca, Y.; Askin, A. Clonal selection in apricot cultivar Hacıhaliloglu. *Acta Hortic.* **1995**, *384*, 169–171. [CrossRef]
31. Asma, B.M.; Ozturk, K. Analysis of morphological, pomological and yield characteristics of some apricot germplasm in Turkey. *Genet. Resour. Crop Evol.* **2005**, *52*, 305–313. [CrossRef]
32. Yilmaz, K.U.; Paydas Kargi, S.; Kafkas, S. Morphological diversity of the Turkish apricot (*Prunus armeniaca* L.) germplasm in the Irano-Caucasian ecogeographical group. *Turk. J. Agric. For.* **2010**, *36*, 688–694.
33. Akin, E.B.; Karabulut, I.; Topcu, A. Some compositional properties of main Malatya apricot (*Prunus armeniaca* L.) varieties. *Food Chem.* **2008**, *107*, 939–948. [CrossRef]
34. Akca, Y.; Asma, B.M. Clonal selection from cv. Kabasi apricot. *Turk. J. Agric. For.* **1997**, *21*, 519–521.
35. Polat, A.A.; Yilmaz, M. Investigations on the adaptations of some native and foreign apricot cultivars to Adana ecological conditions. *J. Sci. Eng.* **1988**, *2*, 127–146.

36. Polat, A.A.; Durgac, C.; Kamiloglu, O.; Caliskan, O. Investigation on the adaptation of some low-chill apricot cultivars to Kirikhan (Turkey) ecological conditions. *Acta Hortic.* **2004**, *636*, 395–400. [CrossRef]
37. Miloševic, T.; Miloševic, N.; Glišic, I.; Glišic, I.S. Determination of size and shape properties of apricots using multivariate analysis. *Acta Sci. Pol. Hortorum Cultus* **2014**, *13*, 77–90.
38. Abd El Abd El-Rzek, E.; Abd El-Migeed, M.M.M.; Abdel-Hamid, N. Effect of spraying garlic extract and olive oil on flowering behavior, yield and fruit quality of 'Canino' apricot trees. *Am.-Eur. J. Agric. Environ. Sci.* **2011**, *11*, 776–781.
39. Peavey, M.; Goodwin, I.; McClymont, L.; Chandra, S. Effect of shading on red colour and fruit quality in blush pears "ANP-0118" and "ANP-0131". *Plants* **2020**, *9*, 206. [CrossRef]
40. Dobrzański, B.; Rybczyński, R. Colour change of apple as a result of storage, shelf-life, and bruising. *Int. Agrophys.* **2002**, *16*, 261–268.
41. Karaat, F.E. *Variation and Heritability of Phytochemical Characters in Some Apricot Cultivars and Hybrids*; Graduate School of Natural and Applied Sciences, Department of Agricultural Genetic Engineering, Nigde Omer Halisdemir University: Niğde, Turkey, 2018; p. 88.
42. Xi, W.; Feng, J.; Liu, Y.; Zhang, S.; Zhao, G. The R2R3-MYB transcription factor PaMYB10 is involved in anthocyanin biosynthesis in apricots and determines red blushed skin. *BMC Plant Biol.* **2019**, *19*, 287. [CrossRef]
43. Yuan, H.; Zhang, J.X.; Nageswaran, D.; Li, L. Carotenoid metabolism and regulation in horticultural crops. *Hortic Res.* **2015**, *2*, 15036. [CrossRef]
44. Saridas, M.A.; Agcam, E. Detailed fruit quality contents of 'Teberze' and 'Ağerik' apricot (*Prunus armeniaca* L.) cultivars grown in Iğdır province. *Harran J. Agric. Food Sci.* **2021**, *25*, 214–224.
45. Imrak, B.A.; Küden, V.; Yurtkulu, E.; Kafkas, S.; Ercişli, S.; Kafkas, E. Evaluation of some phenological and biochemical characteristics of selected new late flowering dried apricot cultivars. *Biochem. Genet.* **2017**, *55*, 234–243. [CrossRef]
46. Su, C.; Zheng, X.; Zhang, D.; Chen, Y.; Xiao, J.; He, Y.; Shi, X. Investigation of sugars, organic acids, phenolic compounds, antioxidant activity and the aroma fingerprint of small white apricots grown in Xinjiang. *J. Food Sci.* **2020**, *85*, 4300–4311. [CrossRef]
47. Schmitzer, V.; Slatnar, A.; Mikulic-Petkovsek, M.; Veberic, R.; Krska, B.; Stampar, F. Comparative study of primary and secondary metabolites in apricot (*Prunus armeniaca* L.) cultivars. *J. Sci. Food Agric.* **2011**, *91*, 860–866. [CrossRef]
48. Melgarejo, P.; Calín-Sánchez, Á.; Carbonell-Barrachina, Á.A.; Martínez-Nicolás, J.J.; Legua, P.; Martínez, R.; Hernández, F. Antioxidant activity, volatile composition and sensory profile of four new very early apricots (*Prunus armeniaca* L.). *J. Sci. Food Agric.* **2014**, *94*, 85–94. [CrossRef]
49. Fan, X.; Zhao, H.; Wang, X.; Cao, J.; Jiang, W. Sugar and organic acid composition of apricot and their contribution to sensory quality and consumer satisfaction. *Sci. Hortic.* **2017**, *225*, 553–560. [CrossRef]
50. Pereira, C.; Barros, L.; Carvalho, A.M.; Ferreira, I.C. Use of UFLC-PDA for the analysis of organic acids in thirty-five species of food and medicinal plants. *Food Anal. Methods* **2013**, *6*, 1337–1344. [CrossRef]
51. Bae, H.; Yun, S.K.; Yoon, I.K.; Nam, E.Y.; Kwon, J.H. Assessment of organic acid and sugar composition in apricot, plumcot, plum, and peach during fruit development. *J. Food Qual. Appl. Bot.* **2014**, *87*, 24–29.
52. Cirilli, M.; Baccichet, I.; Chiozzotto, R.; Spinardi, A.; Bassi, D. Organic acids content in fruit flesh and skin from a large apricot collection. *Acta Hortic.* **2020**, *1290*, 197–200. [CrossRef]
53. Colaric, M.; Veberic, R.; Stampar, F.; Hudina, M. Evaluation of peach and nectarine fruit quality and correlations between sensory and chemical attributes. *J. Sci. Food Agric.* **2005**, *85*, 2611–2616. [CrossRef]
54. Saeed, I.; Guo, X.; Azeem, M.; Elshikh, M.S.; Zainab, B.; Ayaz, Z.; You, L.; Alwahibi, M.S.; Abbasi, A.M. Comparative assessment of polyphenolics' content, free radicals' scavenging and cellular antioxidant potential in apricot fruit. *J. King Saud Univ.-Sci.* **2021**, *33*, 101459. [CrossRef]
55. Çaliskan, O.; Bayazit, S.; Sumbul, A. Fruit quality and phytochemical attributes of some apricot (*Prunus armeniaca* L.) cultivars as affected by genotypes and seasons. *Not. Bot. Hortic. Agrobot. Cluj-Napoca* **2012**, *40*, 284–294. [CrossRef]
56. Wani, A.A.; Zargar, S.A.; Malik, A.H.; Kashtwari, M.; Nazir, M.; Khuroo, A.A.; Ahmad, F.; Dar, T.A. Assessment of variability in morphological characters of apricot germplasm of Kashmir, India. *Sci. Hortic.* **2017**, *225*, 630–637. [CrossRef]
57. Leccese, A.; Bartolini, S.; Viti, R. From cultivar to apricot fruit quality: The antioxidant properties contribution. *Plant Food Hum. Nutr.* **2012**, *67*, 317–325. [CrossRef]
58. Carbone, K.; Ciccoritti, R.; Paliotta, M.; Rosato, T.; Terlizzi, M.; Cipriani, G. Chemometric classification of early-ripening apricot (*Prunus armeniaca* L.) germplasm based on quality traits, biochemical profiling and in vitro biological activity. *Sci. Hortic.* **2018**, *227*, 187–195. [CrossRef]
59. Kafkaletou, M.; Kalantzis, I.; Karantzi, A.; Christopoulos, M.V.; Tsantili, E. Phytochemical characterization in traditional and modern apricot (*Prunus armeniaca* L.) cultivars–Nutritional value and its relation to origin. *Sci. Hortic.* **2019**, *253*, 195–202. [CrossRef]
60. Yamada, T.; Hayasaka, S.; Shibata, Y.; Ojima, T.; Saegusa, T.; Gotoh, T.; Ishikawa, S.; Nakamura, Y.; Kayaba, K. Frequency of citrus fruit intake is associated with the incidence of cardiovascular disease: The Jichi Medical School cohort study. *J. Epidemiol.* **2011**, *21*, 169–175. [CrossRef]
61. Giampieri, F.; Tulipani, S.; Alvarez-Suarez, J.M.; Quiles, J.L.; Mezzetti, B.; Battino, M. The strawberry: Composition, nutritional quality, and impact on human health. *Nutrition* **2012**, *28*, 9–19. [CrossRef]

62. McCullough, M.L.; Peterson, J.J.; Patel, R.; Jacques, P.F.; Shah, R.; Dwyer, J.T. Flavonoid intake and cardiovascular disease mortality in a prospective cohort of US adults. *Am. J. Clin. Nutr.* **2012**, *95*, 454–464. [CrossRef]
63. Singh, M.; Suman, S.; Shukla, Y. New enlightenment of skin cancer chemoprevention through phytochemicals: In vitro and in vivo studies and the underlying mechanisms. *Biomed. Res. Int.* **2014**, 243452.
64. Rodríguez-Morató, J.; Xicota, L.; Fitó, M.; Farré, M.; Dierssen, M.; De la Torre, R. Potential role of olive oil phenolic compounds in the prevention of neurodegenerative diseases. *Molecules* **2015**, *20*, 4655–4680. [CrossRef] [PubMed]
65. Ruiz, D.; Egea, J.; Tomás-Barberán, F.A.; Gil, M.I. Carotenoids from new apricot (*Prunus armeniaca* L.) varieties and their relationship with flesh and skin colour. *J. Agric. Food Chem.* **2005**, *53*, 6368–6374. [CrossRef]
66. Shemesh, K.; Zohar, M.; Bar-Yaakov, I.; Hatib, K.; Holland, D.; Isaacson, T. Analysis of carotenoids in fruit of different apricot accessions reveals large variability and highlights apricot as a rich source of phytoene and phytofluene. *Fruits* **2017**, *72*, 185–202. [CrossRef]
67. Fratianni, F.; Ombra, M.N.; d'Acierno, A.; Cipriano, L.; Nazzaro, F. Apricots: Biochemistry and functional properties. *Curr. Opin. Food Sci.* **2018**, *19*, 23–29. [CrossRef]
68. Leccese, A.; Bartolini, S.; Viti, R. Total antioxidant capacity and phenolics content in fresh apricots. *Acta Aliment.* **2008**, *37*, 65–76. [CrossRef]
69. Zia-Ul-Haq, M.; Ahmad, S.; Qayum, M.; Ercisli, S. Compositional studies and antioxidant potential of *Albizia lebbeck* (L.) Benth. Pods and seeds. *Turk. J. Biol.* **2013**, *37*, 25–32.
70. Bolat, I.; Dikilitas, M.; Ercisli, S.; Ikinci, A.; Tonkaz, T. The effect of water stress on some morphological, physiological, and biochemical characteristics and bud success on apple and quince rootstocks. *Sci. World J.* **2014**, *2014*, 769732. [CrossRef]
71. Dogan, H.; Ercisli, S.; Jurikova, T.; Temim, E.; Leto, A.; Hadziabulic, A.; Tosun, M.; Narmanlioglu, H.K.; Zia-Ul-Haq, M. Physicochemical and antioxidant characteristics of fruits of cape gooseberry (*Physalis peruviana* L.) from Turkey. *Oxid. Commun.* **2014**, *37*, 1005–1014.
72. Dogan, H.; Ercisli, S.; Temim, E.; Hadziabulic, A.; Tosun, M.; Yilmaz, S.O.; Zia-Ul-Haq, M. Diversity of chemical content and biological activity in flower buds of a wide number of wild grown caper (*Capparis ovate* Desf.) genotypes from Turkey. *C. R. Acad. Bulg. Sci.* **2014**, *67*, 1593–1600.
73. Gundogdu, M.; Ozrenk, K.; Ercisli, S.; Kan, T.; Kodad, O.; Hegedus, A. Organic acids, sugars, vitamin C content and some pomological characteristics of eleven hawthorn species (*Crataegus* spp.) from Turkey. *Biol. Res.* **2014**, *47*, 21. [CrossRef]
74. Ersoy, N.; Kupe, M.; Sagbas, H.I.; Ercisli, S. Phytochemical diversity among barberry (*Berberis vulgaris* L.). *Not. Bot. Horti Agrobo.* **2018**, *46*, 198–204. [CrossRef]
75. Ersoy, N.; Kupe, M.; Gundogdu, M.; Gulce, I.; Ercisli, S. Phytochemical and antioxidant diversity in fruits of currant (Ribes spp.) cultivars. *Not. Bot. Horti Agrobo.* **2018**, *46*, 381–387. [CrossRef]
76. Ozkan, G. Phenolic compounds, organic acids, vitamin C and antioxidant capacity in Prunus spinose. *C. R. Acad. Bulg. Sci.* **2019**, *72*, 267–273.
77. Göttingerová, M.; Kumšta, M.; Nečas, T. Health-benefitting biologically active substances in edible apricot flowers. *HortScience* **2020**, *55*, 1372–1377. [CrossRef]
78. Ozkan, G.; Ercisli, S.; Sagbas, H.I.; Ilhan, G. Diversity on fruits of wild grown European cranberrybush from Coruh valley in Turkey. *Erwerbs-Obstbau* **2020**, *62*, 275–279. [CrossRef]
79. Bujdosó, G.; Cseke, K. The Persian (English) walnut (*Juglans regia* L.) assortment of Hungary: Nut characteristics and origin. *Sci. Hortic.* **2021**, *283*, 110035. [CrossRef]
80. Rampáčková, E.; Göttingerová, M.; Gála, P.; Kiss, T.; Ercişli, S.; Nečas, T. Evaluation of protein and antioxidant content in apricot kernels as a sustainable additional source of nutrition. *Sustainability* **2021**, *13*, 4742. [CrossRef]

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Determination of Selected Beneficial Substances in Peach Fruits

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Abstract: Peaches (*Prunus persica* L.) are a popular and sought-after dessert fruit. This is mainly due to their flavour, aroma, attractive appearance, and high content of substances that play an important role in human nutrition. The present study was carried out to determine some important analytical properties (sugars/sucrose, glucose, fructose and sorbitol), total acid, total phenolics, flavonoids, antioxidant capacity, carotenoids and anthocyanins of 34 selected peach varieties. The analyses are also complemented by colorimetric measurements of peach skin colour using CIELAB and other chromatic parameters. The results show, for example, that all peach varieties are good sources of phenolic compounds (9.43–577 mg gallic acid equivalent (GAE).100 g^{-1}), flavonoids (1.12–95.1 mg catechin equivalent (CAE).100 g^{-1}), and antioxidant capacity (136–462 mg Trolox equivalent (TE).100 g^{-1}).

Keywords: *Prunus persica* L.; colour; chemical contents; antioxidant capacity; sugar

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1. Introduction

There are a great variety of peach trees (*Prunus persica* L.), not only in terms of the length of ripening period, but also in terms of the pomological characteristics of the fruit, where we can distinguish yellow-fleshed, white-fleshed, red-fleshed, fully separable from the stone or clings, flat-shaped varieties, referred to as Peento, that are very popular in southern Italy and Asia. There are also well-known selections of varieties without any anthocyanin content, originating in Italy (the 'ice peach'), and the Californian 'Royal' series of varieties, which are characterised by their very hard flesh and very low acid content, giving the fruit a sweet taste.

From a nutritional point of view, peaches contain a number of beneficial substances, making them an interesting addition to the human diet. Peaches are a rich source of dietary fibre (1.5 g.100 g^{-1}) and provitamin A [1]. This fruit is considerably rich in antioxidants and is an important source of vitamins A, B, and C, carotenoids and phenolic compounds. Among the most important phenolic acids are chlorogenic and neochlorogenic acids, catechin, epicatechin, 3-glucoside of cyanidin (chrysanthemin), and quercetin derivatives [2–5]. Polyphenols represent the majority of antioxidants present in the diet and their daily intake should exceed 1 g/day, which is much higher than that of all other classes of phytochemicals and known dietary antioxidants [6]. They are low in fat and contain a lot of water, approximately 89 g per 100 g of fruit [7,8]. Peaches are very low in sugars (9–20 °Rf), with the main sugars present being sucrose, fructose, sorbitol, and glucose. The proportions of these sugars undergo changes during fruit ripening, with glucose and fructose being present in greater amounts in immature fruit and increasing as ripening progresses. At full maturity, sucrose content dominates [9–11]. Carbohydrates are an important source

of energy in the human diet and also play an important role in the regulation of the gut microbiota [12]. They also have low levels of organic acids (0.13–1.16%) such as malic, citric, and folic acids. The content of L-ascorbic acid (vitamin C) in peaches is relatively low compared to other fruits such as kiwifruit or oranges, in which it is the most important antioxidant. Quinic, fumaric, and shikimic acids are present in smaller concentrations [13,14]. Amino acids (arginine, asparagine, isoleucine, lysine, serine, threonine, valine, leucine, phenylalanine, tryptophan, tyrosine, proline, and alanine) also contribute to the flavour of fruit and are found in peaches in different concentrations depending on the cultivar [15,16]. Among the mineral elements, they contain nitrogen, phosphorus, potassium, calcium, magnesium, iron, manganese, zinc, cooper, chromium, nickel, cobalt, lead, selenium, and fluoride [17,18]. Similar to apricots, the glycoside amygdalin (26%), protein amandine (3.8%), enzymes, lactase, and oleic acids are present in peach kernels. The leaves contain about 1% prunasin and are used against rheumatism, gastritis, headaches, and as a diuretic; when used externally, they are effective against eczema, ulcers, and other dermatoses [19].

The potential of peaches, especially those rich in phenolics, lies in delaying or even preventing the onset of neurogenerative diseases such as Alzheimer's and Parkinson's. They also help in the prevention of inflammation, atherosclerosis, diabetes, obesity, and cardiovascular disease. Due to their low sugar content, they can easily be included in nutritional therapy. They are easily digestible, have a strong alkaline effect on the body, and stimulate the secretion of digestive juices. They have both a laxative and a diuretic effect. Peach phenolics have been shown to display several biological activities such as antioxidant activity [20,21], anti-allergic and anti-inflammatory activities [22], antibacterial activity [23], hepatoprotective activity [24], nephroprotective activity [25], antiproliferative [26], chemopreventive, and anticancer activities [27,28].

The aim of this study was to compare varieties from different pomological groups as well as different geographical origins and thus get an overview of the differences in content composition from the point of view of titratable acidity, soluble solid content, sugars, phenolic compounds, flavonoids, antioxidant activity, carotenoids, and total anthocyanin content.

2. Materials and Methods

2.1. Site of Planting and Plant Material

In total, 34 peach cultivars of different origin were analysed in this study (Table 1). 20 cultivars from USA, 6 from Yalta, 5 from Italy, 1 from Czech Republic, 1 from France, and 1 from Slovakia. Trees of these cultivars were grown in the experimental orchard at the Faculty of Horticulture in Lednice, Mendel University in Brno (localisation 48.80°N/16.80°E, at an altitude of 172 m), with an average annual temperature of 9.7 °C.

Table 1. The cultivars obtained in this study and their flesh colour, fruit type, and origin.

Cultivars	Flesh Colour	Fruit Type	Origin	Cultivars	Flesh Colour	Fruit Type	Origin
Admiral de Wey	Yellow	Peach	USA	Iris Rosso	White	peach	Italy
Alexandra	White	Peach	USA	Krasava	Creamy	peach	Czech Republic
Anita	White	Peach	USA	Lakomyj	Yellow	peach	Yalta, Crimea
Aurelia	Yellow	Peach	Italy	Narjadnyj Nikitskij	Yellow	peach	Yalta, Crimea
Avalon Pride	Yellow	Peach	USA	Nerine	Yellow	peach	USA
Benedicte	Creamy	Peach	France	Otličnik	Yellow	peach	Yalta, Crimea
Candor	Yellow	Peach	USA	Queen Lady	Yellow	peach	USA
Carolina Belle	White	Peach	USA	Red Robin	White	peach	USA
Dixigem	Yellow	Peach	USA	Redhaven	Yellow	peach	USA
Dostojnyj	Yellow	Peach	Yalta, Crimea	Romea	Yellow	cling	Italy
Early Glo	Yellow	Peach	USA	Royal Glory	Yellow	peach	USA
Early Redhaven	Yellow	Peach	USA	Royal Majestic	Yellow	peach	USA
Favorita Morettini	Yellow	Peach	Italy	Sonet	Yellow	peach	Yalta, Crimea
Fénix	Yellow	Peach	Slovakia	Strelec	Yellow	peach	Yalta, Crimea
Fidelia	White	Peach	USA	Suncrest	Yellow	peach	USA
Harvester	Yellow	Peach	USA	Sunshine	Yellow	peach	USA
Helene	White	Peach	USA	UFO 3	White	peento	Italy

Five fruits from each variety were harvested at their harvest maturity and transported to the laboratory for chemical analyses.

2.2. Determination of Titratable Acidity

The determination of titratable acidity was performed by potentiometric titration, with a solution of 0.1 mol.L^{-1} NaOH of a known factor up to pH 8.1 measured by a combined SenTix™ 81 pH electrode (WTW™, Prague, Czech Republic) coupled with inoLab 7110 pH meter (WTW™, Prague, Czech Republic). Titratable acidity was expressed as % malic acid equivalent [29]. Mixed fruits were used as a sample for titration.

2.3. Preparation of the Plant Samples for Analysis of Total Phenolic Content, Total Flavonoids, and Total Antioxidant Capacity

Prior to determination of content of secondary metabolites (phenolic compounds, flavonoids, and antioxidant capacity), methanol extract from fresh fruit material was performed. Five grams of the sample was homogenized with a hand blender in 25 mL 75% methanol. The extract was left to stand for 24 h and then filtered through a filter paper into a 50 mL measuring flask. The filtrate was then adjusted to the line with 75% methanol. Samples were transferred into a 20 mL plastic bottles and kept at −20 °C until the analysis [30].

2.4. Determination of Total Phenolic Content, Total Flavonoids, and Total Antioxidant Capacity

Analyses of all parameters were carried out according to the protocols of Zloch et al. (2004) [31] by using a SPECORD® 50 PLUS spectrophotometer (Analytik, Jena, DE). Total phenolic content was measured after reaction of sample extracts with Folin–Ciocalteu reagent at a wavelength of 765 nm and expressed in milligrams GAE per 100 g FW. Total flavonoid content was determined by using chloride and sodium nitrite and the results were expressed in milligrams CAE per 100 g FW. For determination of total antioxidant activity, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was used. This method is based on the decolorizing property of the hydrogen radical of DPPH with hydrogen donors, which are included in phenolic compounds as well. Trolox (6-hydroxy-2.5.7.8-tetramethylchroman-2-carboxylic acid) was used as a standard, and the measurement was performed at 515 nm and then expressed in milligrams TE per 100 g FW.

2.5. Determination of Total Carotenoids

Prior to determination of the carotenoid content, sliced thin fruit sections were dried in a heat chamber FED 400 (Binder, Tuttlingen DE) at 50 °C for 24 h and pulverised in a mill Pulverisette 11 (Fritsch, Weimar, DE). Next, acetone was used to extract the pigments from the samples. Determination of photosynthetically active pigments (carotenoids) was performed with a SPECORD® 50 PLUS spectrophotometer (Analytic Jena AG, Germany) at 440 nm according to Holm (1954) [32]. Total carotenoids were expressed in milligrams per 100 g dry weight (DW).

2.6. Determination of Total Anthocyanin Content (TAC)

The determination of TAC was based on a pH differential method using changes in the colour of samples containing anthocyanins in various pH value environments. Five grams of homogenized whole fruit of peach was mixed with 25 mL of 0.1 M HCl. After 1 h of extraction, the solution was filtered and 0.5 mL of the filtrate was pipetted into 6 test tubes. A 2.5 mL (0.025 mol.L^{-1}) of KCl solution of pH 1 was added into the first 3 test tubes and 2.5 mL (0.4 mol.L^{-1}) solution of $C_2H_3NaO_2$ of pH 4.5 was added into the remaining 3 test tubes. Prepared rest tubes were measured at wavelengths of 510 nm and 700 nm with a spectrophotometer SPECORD® 50 PLUS (Analytic Jena AG, Germany). The results were expressed in mg.100 g^{-1} fresh weight (FW).

2.7. Determination of Sugar Content

The soluble solids content was determined using the Abbé refractometer and expressed in weight percentage.

The determination of sugar content was performed by high performance liquid chromatography (HPLC). Juice was squeezed from the fruit and diluted with distilled water at a 1:4 ratio (2 mL juice + 8 mL H_2O). The diluted sample was filtered through a microfilter and analysed. A Clarity chromatography station (Watrex, Prague, Czech Republic) with a Polymer IEX Ca_SN8422 column (250 × 8 mm; Watrex, Prague, Czech Republic) was used for making the analysis. The flow rate of the mobile phase (deionized water) was 0.5 mL.min^{-1}, pressure 1.9 MPa, temperature 80 °C. A refractometric detector was used for making the evaluation. Fructose, glucose, sucrose, and sorbitol contents were converted into the fresh weight of plant material and expressed as g sugar per 100 g fruit.

2.8. Colour Analysis

Colour of cleaned skin of 5 fruits was analysed using colorimeter CR-400 (Konica Minolta®, Tokyo, Japan), equipped with D65 illuminant. The over colour and ground colour were distinguished where possible within the analysis. The data were processed by software SpectraMagic NX Lite (Konica Minolta®, Tokyo, Japan). The analysis is based on CIELAB scale. The colour parameters L^*, a^*, b^* are directly measured in terms of standard observed and standard illuminant [33], where parameter L^* represents the lightness of the fruit, parameter a^* represents the axis in the direction from green to red and parameter b^* represents the axis in the direction from blue to yellow. Values were displayed with the mean ± standard deviation. Cylindrical coordinates C^*_{ab} and h°_{ab} were calculated from coordinates a^* and b^* by Equations (1) and (2) [34]:

$$C^*_{ab} = (a^{*2} + b^{*2})^{1/2} \quad (1)$$

$$h^\circ_{ab} = \tan^{-1}(b^*/a^*) \quad (2)$$

C^*_{ab} denotes the purity of saturation of the colour [35], which means the higher is the chroma (C^*_{ab}) the colour is more intense. Hue angle (h°_{ab}) refers to the colour wheel and is measured in angles [36]. The colour difference ΔE^*_{ab} was accomplished for cultivars with measurable ground and over colour. Values were displayed with the mean ± standard deviation of ten replications. Given two colours in the CIELAB colour space, (L^*_1, a^*_1, b^*_1) and (L^*_2, a^*_2, b^*_2), the CIE76 colour difference formula is defined as (3):

$$\Delta E^*_{ab} = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2} \quad (3)$$

$\Delta E^*_{ab} \approx 2.3$ corresponds to a JND (just noticeable difference) [37].

2.9. Statistical Analysis

Statistical analysis was performed in Statistica 12 (TIBCO, USA) and Microsoft Excel software. Single-factor ANOVA analysis (level of significance $\alpha = 0.05$) was used for statistical processing and the Tukey HSD test was subsequently used to evaluate the statistical significance of differences between the individually measured values (TAC and chromatic parameters L^*, a^*, b^*). Between colour parameters and TAC, the Spearman's correlation coefficient ρ was determined using Statistica 12 (TIBCO, USA) and regression function with coefficient of determination R^2 were determined using Microsoft Excel.

3. Results

The highest acid content was recorded in the fruit of the varieties 'Benedicte' (1.32% malic acid), 'Helene' (0.91% malic acid), and 'Royal Majestic' (0.85% malic acid). The varieties with the lowest acid content were 'UFO 3' (0.25% malic acid), 'Fidelia' (0.26% malic acid) and 'Royal Glory' (0.26% malic acid, Figure 1). The average value of the test set

was 0.59% malic acid. The differences between the varieties were confirmed as statistically highly significant (Table 2).

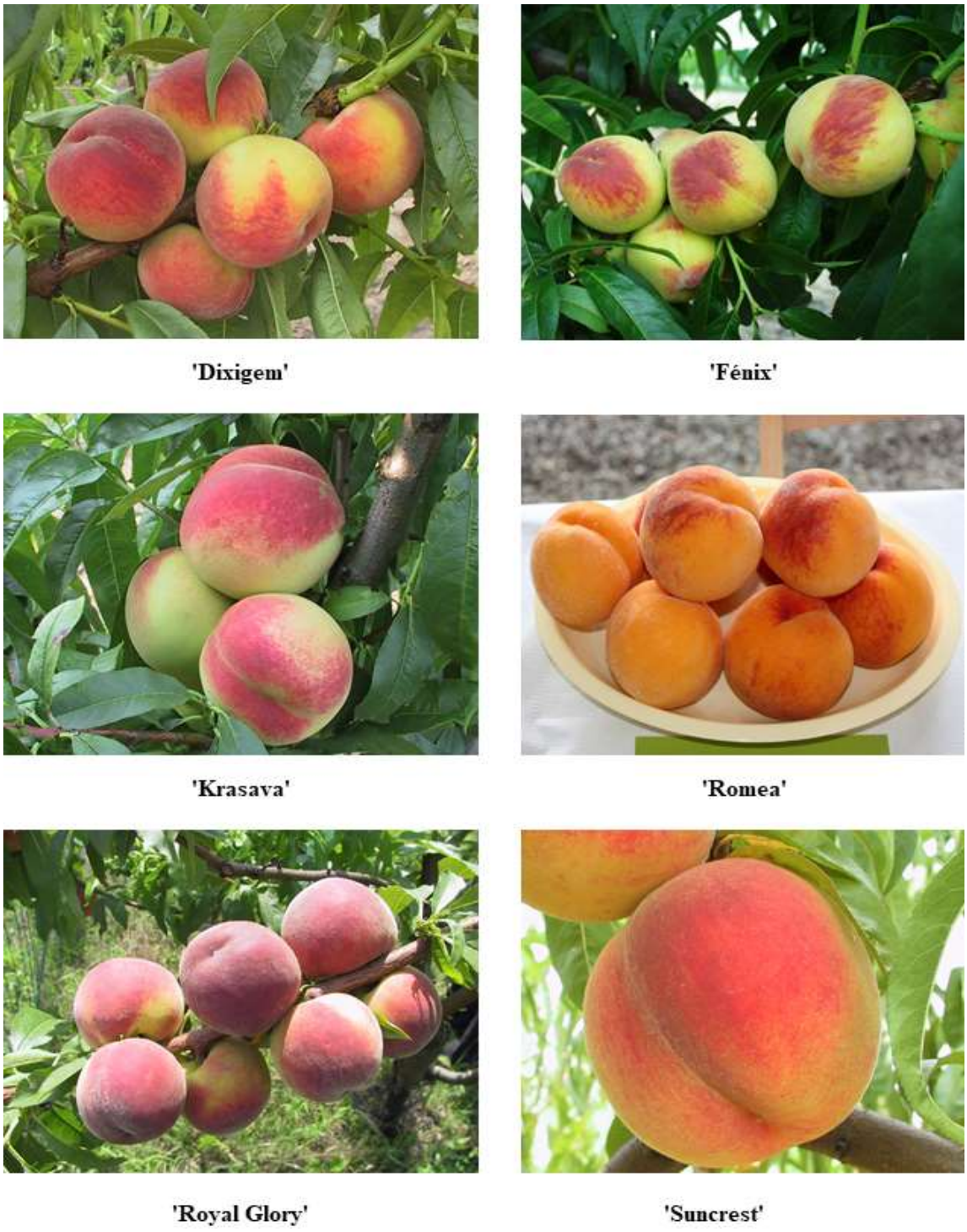

Figure 1. The photos of some cultivars obtained in this study.

Table 2. The total content of titratable acids in peach cultivars. The data are displayed as the mean ± standard deviation of three replications; a–p refer to the grouping based on the Tukey HSD test.

Cultivars	Titratable Acidity [%]	Cultivars	Titratable Acidity [%]
Admiral de Wey	$0.44 \pm 0.01^{b,c}$	Iris Rosso	$0.676 \pm 0.009^{j,k,l,m}$
Alexandra	$0.452 \pm 0.004^{b,c}$	Krasava	0.84 ± 0.02^{n}
Anita	$0.44 \pm 0.01^{b,c}$	Lakomyj	$0.591 \pm 0.007^{f,g,h}$
Aurelia	$0.643 \pm 0.004^{i,j}$	Narjadnyj Nikitskij	0.513 ± 0.002^{d}
Avalon Pride	$0.61 \pm 0.01^{g,h,i}$	Nerine	$0.450 \pm 0.002^{b,c}$
Benedicte	1.32 ± 0.04^{p}	Otličnik	$0.533 \pm 0.002^{d,e,f}$
Candor	$0.58 \pm 0.01^{f,g}$	Queen Lady	$0.636 \pm 0.004^{h,i,j}$
Carolina Belle	$0.691 \pm 0.006^{k,l,m}$	Red Robin	0.72 ± 0.01^{m}
Dixigem	$0.573 \pm 0.005^{e,f,g}$	Redhaven	$0.53 \pm 0.05^{d,e}$
Dostojnyj	0.415 ± 0.005^{b}	Romea	$0.562 \pm 0.009^{e,f,g}$
Early Glo	0.463 ± 0.006^{c}	Royal Glory	0.264 ± 0.006^{a}
Early Redhaven	$0.428 \pm 0.006^{b,c}$	Royal Majestic	0.850 ± 0.003^{n}
Favorita Morettini	$0.645 \pm 0.006^{i,j,k}$	Sonet	$0.564 \pm 0.005^{e,f,g}$
Fénix	$0.655 \pm 0.001^{j,k}$	Strelec	$0.712 \pm 0.006^{l,m}$
Fidelia	0.26 ± 0.01^{a}	Suncrest	$0.55 \pm 0.03^{d,e,f}$
Harvester	$0.712 \pm 0.001^{l,m}$	Sunshine	$0.668 \pm 0.007^{j,k,l}$
Helene	0.910 ± 0.008^{o}	UFO 3	0.25 ± 0.02^{a}

Significantly, the highest representation of total phenolic compounds was found in fruits of the variety 'Carolina Belle' (577.72 mg GAE.100 g^{-1} FW), then in the variety 'Krasava' (334.02 mg GAE.100 g^{-1} FW, Figure 1), 'Dixigem' (285.24 mg GAE.100 g^{-1} FW, Figure 1), and in the variety 'Benedicte' (238.09 mg GAE.100 g^{-1} FW). On the other hand, the lowest values of phenolic compounds content were observed in fruits of 'Favorita Morettini' (9.43 mg GAE.100 g^{-1} FW), 'Early Redhaven' (12.90 mg GAE.100 g^{-1} FW), and 'Strelec' (17.39 mg GAE.100 g^{-1} FW). In the studied set of cultivars, the total phenolic content in fruits ranged from 9.43 to 577 mg GAE.100 g^{-1} FW. The differences between the values were highly statistically significant (Table 3).

Table 3. Total phenolic content in peach cultivars. The data are displayed as the mean ± standard deviation of three replications; a–w refer to the grouping based on the Tukey HSD test.

Cultivars	Total Phenolic Content [mg GAE.100 g^{-1}]	Cultivars	Total Phenolic Content [mg GAE.100 g^{-1}]
Admiral de Wey	104.4 ± 0.4^{m}	Iris Rosso	103.4 ± 0.9^{m}
Alexandra	$19 \pm 1^{c,d}$	Krasava	334 ± 2^{v}
Anita	44.6 ± 0.4^{f}	Lakomyj	$18.4 \pm 0.4^{b,c}$
Aurelia	139.3 ± 0.7^{o}	Narjadnyj Nikitskij	66.7 ± 0.6^{h}
Avalon Pride	162.2 ± 0.8^{q}	Nerine	115 ± 2^{n}
Benedicte	238 ± 1^{t}	Otličnik	73 ± 2^{i}
Candor	54.8 ± 0.3^{g}	Queen Lady	151.2 ± 0.7^{p}
Carolina Belle	577 ± 2^{w}	Red Robin	65.6 ± 0.4^{h}
Dixigem	285.2 ± 0.4^{u}	Redhaven	193 ± 7^{r}
Dostojnyj	110.5 ± 0.3^{n}	Romea	72.8 ± 0.3^{i}
Early Glo	47.0 ± 0.2^{f}	Royal Glory	$78 \pm 4^{i,j}$
Early Redhaven	$12.9 \pm 0.3^{a,b}$	Royal Majestic	95 ± 3^{l}
Favorita Morettini	9.4 ± 0.3^{a}	Sonet	34.0 ± 0.4^{e}
Fénix	80 ± 1^{j}	Strelec	$17.39 \pm 0.04^{b,c}$
Fidelia	86.3 ± 0.9^{k}	Suncrest	197 ± 2^{r}
Harvester	203.0 ± 0.6^{s}	Sunshine	195.0 ± 0.6^{r}
Helene	152.0 ± 0.5^{p}	UFO 3	25 ± 2^{d}

The highest concentration of flavonoids was measured in the fruits of 'Carolina Belle' (95.1 mg CAE.100 g^{-1} FW), 'Benedicte' (53.2 mg CAE.100 g^{-1} FW), and 'Admiral de Wey' (50.8 mg CAE.100 g^{-1} FW). The lowest values were observed in 'UFO 3', 'Favorita Morettini', 'Alexandra' and 'Candor' (1.12; 3.37; 4.09 and 5.16 mg CAE.100 g^{-1} FW). The average flavonoid value in the test set was 22.3 mg CAE.100 g^{-1} FW. The differences between the varieties were confirmed as statistically highly significant (Table 4).

Table 4. Total flavonoid content in peach cultivars. The data are displayed as the mean ± standard deviation of three replications; a–v refer to the grouping based on the Tukey HSD test.

Cultivars	Flavonoids [mg CAE.100 g^{-1}]	Cultivars	Flavonoids [mg CAE.100 g^{-1}]
Admiral de Wey	50.8 ± 0.3^{t}	Iris Rosso	$12 \pm 1^{h,i}$
Alexandra	$4.1 \pm 0.5^{b,c}$	Krasava	45.1 ± 0.5^{s}
Anita	27.7 ± 0.2^{n}	Lakomyj	$10.6 \pm 0.1^{g,h}$
Aurelia	18.0 ± 0.2^{k}	Narjadnyj Nikitskij	31.8 ± 0.1^{p}
Avalon Pride	24.2 ± 0.4^{m}	Nerine	15.0 ± 0.1^{j}
Benedicte	53.2 ± 0.5^{u}	Otličnik	$9.60 \pm 0.09^{f,g}$
Candor	5.16 ± 0.06^{c}	Queen Lady	20.1 ± 0.1^{l}
Carolina Belle	95.1 ± 0.8^{v}	Red Robin	34.1 ± 0.2^{q}
Dixigem	35.6 ± 0.2^{r}	Redhaven	24.5 ± 0.2^{m}
Dostojnyj	12.7 ± 0.1^{i}	Romea	$8.2 \pm 0.1^{d,e}$
Early Glo	27.4 ± 0.8^{n}	Royal Glory	$9.5 \pm 0.7^{e,f,g}$
Early Redhaven	$8.68 \pm 0.09^{d,e,f}$	Royal Majestic	10.0 ± 0.7^{g}
Favorita Morettini	3.4 ± 0.3^{b}	Sonet	14.8 ± 0.2^{j}
Fénix	7.55 ± 0.08^{d}	Strelec	$11.8 \pm 0.2^{h,i}$
Fidelia	10.0 ± 0.3^{g}	Suncrest	46.1 ± 0.3^{s}
Harvester	23.3 ± 0.1^{m}	Sunshine	30.20 ± 0.09^{o}
Helene	18.4 ± 0.4^{k}	UFO 3	1.12 ± 0.02^{a}

Using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, values of antioxidant activity in peach fruits ranging from 136 to 462 mg TE.100 g^{-1} FW were determined. Specifically, the cultivar 'Carolina Belle' (249.08 mg TE.100 g^{-1} FW) had the highest value. All other varieties analysed showed relatively high values. The results varied within a few units. High values were also found in the fruit of the variety 'Admiral de Wey' (280.46 mg TE.100 g^{-1} FW) and in the variety 'Dixigem' (255.61 mg TE.100 g^{-1} FW). The Czech variety 'Krasava' also had high antioxidant capacity (250.07 mg TE.100 g^{-1} FW). The lowest total antioxidant capacity was measured in the fruits of 'Favorita Morettini' (136.15 mg TE.100 g^{-1} FW) and 'Candor' (150.72 mg TE.100 g^{-1} FW). The differences in the values were highly statistically significant (Table 5).

Table 5. Antioxidant activity in peach cultivars. The data are displayed as the mean ± standard deviation of three replications; a–z refer to the grouping based on Tukey HSD test.

Cultivars	Antioxidant Activity [mg.100 g^{-1}]	Cultivars	Antioxidant Activity [mg.100 g^{-1}]
Admiral de Wey	280.5 ± 0.2^{y}	Iris Rosso	184.59 ± 0.09^{k}
Alexandra	184.09 ± 0.02^{k}	Krasava	250.1 ± 0.3^{w}
Anita	230.9 ± 0.2^{u}	Lakomyj	178.20 ± 0.07^{j}
Aurelia	203.44 ± 0.09^{p}	Narjadnyj Nikitskij	211.48 ± 0.09^{s}
Avalon Pride	184.54 ± 0.08^{k}	Nerine	204.4 ± 0.1^{q}
Benedicte	200.52 ± 0.08^{o}	Otličnik	188.07 ± 0.04^{l}
Candor	150.72 ± 0.06^{b}	Queen Lady	210.7 ± 0.1^{s}

Table 5. *Cont.*

Cultivars	Antioxidant Activity [mg.100 g^{-1}]	Cultivars	Antioxidant Activity [mg.100 g^{-1}]
Carolina Belle	462.41 ± 0.84 ^{z}	Red Robin	206.9 ± 0.1 ^{r}
Dixigem	256 ± 1 ^{x}	Redhaven	233.1 ± 0.2 ^{v}
Dostojnyj	175.11 ± 0.05 ^{h}	Romea	169.16 ± 0.06 ^{e}
Early Glo	220.1 ± 0.1 ^{t}	Royal Glory	165.0 ± 0.3 ^{c}
Early Redhaven	164.53 ± 0.06 ^{c}	Royal Majestic	199.84 ± 0.05 ^{o}
Favorita Morettini	136 ± 0 ^{a}	Sonet	176.8 ± 0.1 ^{i}
Fénix	166.76 ± 0.03 ^{d}	Strelec	195.18 ± 0.05 ^{n}
Fidelia	191.05 ± 0.07 ^{m}	Suncrest	231.07 ± 0.06 ^{u}
Harvester	203.9 ± 0.1 ^{p,q}	Sunshine	233.9 ± 0.2 ^{v}
Helene	173.5 ± 0.1 ^{g}	UFO 3	172.01 ± 0.05 ^{f}

The average carotenoids content in the fruits of the studied varieties reached 1.67g.100 g^{-1} DW. The varieties with the highest carotenoids (4.77 mg.100 g^{-1} DW) include fruits of the variety 'Romea' (3.50 mg.100 g^{-1} DW, Figure 1), followed by fruits of the variety 'Royal Majestic' (3.14 mg.100 g^{-1} DW), 'Favorita Morettini' (3.12 mg.100 g^{-1} DW), and 'Early Redhaven' (3.12 mg.100 g^{-1} DW). On the other hand, the lowest total carotenoids content was determined in the fruits of 'Krasava', 'Fidelia', and 'Anita' (0.05; 0.24 and 0.24 mg.100 g^{-1} DW). Total carotenoids content was not detected in the cultivars 'Benedicte' and 'Royal Glory'. The differences between the varieties were confirmed as statistically highly significant (Table 6).

Table 6. Total carotenoids in peach cultivars. The data are displayed as the mean ± standard deviation of three replications; a–j refer to the grouping based on the Tukey HSD test.

Cultivars	Carotenoids [mg.100 g^{-1}]	Cultivars	Carotenoids [mg.100 g^{-1}]
Admiral de Wey	2.20 ± 0.04 ^{d,e,f,g}	Iris Rosso	0.40 ± 0.01 ^{a,b}
Alexandra	0.45 ± 0.04 ^{a,b}	Krasava	0.05 ± 0.59 ^{a}
Anita	0.24 ± 0.02 ^{a,b}	Lakomyj	2.13 ± 0.02 ^{d,e,f}
Aurelia	1.1 ± 0.2 ^{c}	Narjadnyj Nikitskij	1.8 ± 0.6 ^{d}
Avalon Pride	2.0 ± 0.1 ^{d,e}	Nerine	1.88 ± 0.06 ^{d}
Benedicte	*	Otličnik	0.56 ± 0.01 ^{b}
Candor	2.46 ± 0.03 ^{e,f,g}	Queen Lady	2.24 ± 0.03 ^{d,e,f,g}
Carolina Belle	0.36 ± 0.02 ^{a,b}	Red Robin	0.67 ± 0.03 ^{b,c}
Dixigem	2.47 ± 0.04 ^{f,g}	Redhaven	2.27 ± 0.05 ^{d,e,f,g}
Dostojnyj	1.88 ± 0.04 ^{d}	Romea	4.8 ± 0.2 ^{j}
Early Glo	2.0 ± 0.01 ^{d,e,f}	Royal Glory	*
Early Redhaven	3.12 ± 0.01 ^{h,i}	Royal Majestic	3.51 ± 0.05 ^{i}
Favorita Morettini	3.14 ± 0.06 ^{i}	Sonet	2.26 ± 0.05 ^{d,e,f,g}
Fénix	3.03 ± 0.04 ^{h,i}	Strelec	1.904 ± 0.009 ^{d}
Fidelia	0.24 ± 0.06 ^{a,b}	Suncrest	2.300 ± 0.003 ^{a,b}
Harvester	2.64 ± 0.04 ^{g,h}	Sunshine	2.12 ± 0.02 ^{d,e,f}
Helene	0.31 ± 0.03 ^{a,b}	UFO 3	0.30 ± 0.03 ^{a,b}

* Not measured.

High levels of anthocyanins were measured in the fruits of 'Helene' (3.74 mg.100 g^{-1} FW), 'Royal Majestic' (2.64 mg.100 g^{-1} FW), and 'Favorita Morettini' (2.13 mg.100 g^{-1} FW). On the other hand, low values were recorded in fruits of 'Early Redhaven', 'UFO 3', 'Dostojnyj', 'Strelec' and 'Admiral de Wey' (0.05; 0.05 0.14; 0.18 mg.100 g^{-1} FW). The average value of total anthocyanins of the tested set of varieties reached 0.70 mg.100 g^{-1} FW. The differences between the varieties were confirmed as statistically highly significant (Table 7).

Table 7. Total anthocyanin content (TAC) in peach cultivars. The data are displayed as the mean ± standard deviation of three replications; a–p refer to the grouping based on the Tukey HSD test.

Cultivars	Total Anthocyanin Content [mg.100 g^{-1}]	Cultivars	Total Anthocyanin Content [mg.100 g^{-1}]
Admiral de Wey	$0.2 \pm 0.4^{a,b,c,d,e}$	Iris Rosso	$1.13 \pm 0.08^{j,k,l}$
Alexandra	$1.4 \pm 0.3^{l,m}$	Krasava	*
Anita	1.65 ± 0.06^{m}	Lakomyj	*
Aurelia	$0.63 \pm 0.06^{f,g,h,i}$	Narjadnyj Nikitskij	$0.5 \pm 0.1^{c,d,e,f,g,h}$
Avalon Pride	$1.2 \pm 0.2^{k,l,m}$	Nerine	$0.47 \pm 0.06^{c,d,e,f,g,h}$
Benedicte	*	Otličnik	*
Candor	$0.26 \pm 0.07^{a,b,c,d,e,f}$	Queen Lady	$0.58 \pm 0.06^{e,f,g,h,i}$
Carolina Belle	$0.95 \pm 0.03^{i,j,k}$	Red Robin	$0.56 \pm 0.04^{d,e,f,g,h,i}$
Dixigem	$0.5 \pm 0.1^{c,d,e,f,g,h}$	Redhaven	*
Dostojnyj	$0.14 \pm 0.04^{a,b,c}$	Romea	*
Early Glo	$0.9 \pm 0.3^{h,i,j,k}$	Royal Glory	$1.3 \pm 0.2^{k,l,m}$
Early Redhaven	$0.05 \pm 0.03^{a,b}$	Royal Majestic	2.6 ± 0.1^{o}
Favorita Morettini	2.13 ± 0.08^{n}	Sonet	$0.815 \pm 0.003^{g,h,i,j}$
Fénix	$0.37 \pm 0.03^{a,b,c,d,e,f}$	Strelec	$0.17 \pm 0.03^{a,b,c,d}$
Fidelia	$0.55 \pm 0.07^{d,e,f,g,h,i}$	Suncrest	$0.4 \pm 0.1^{b,c,d,e,f,g}$
Harvester	$0.2 \pm 0.1^{a,b,c,d,e,f}$	Sunshine	$0.38 \pm 0.05^{a,b,c,d,e,f}$
Helene	3.7 ± 0.2^{p}	UFO 3	$0.05 \pm 0.06^{a,b}$

* Not measured.

In the set of varieties studied, the total soluble solids content of the fruit ranged from 8.3 to 14.7 °Rf. The varieties with the highest content were 'Royal Majestic' (14.7 °Rf), followed by 'Helene' (13.8 °Rf) and 'Nerine' (13.7 °Rf). The lowest values of the evaluated set of varieties were measured for the fruits of the 'Fénix' variety (8.3 °Rf, Figure 1), 'Krasava' and 'Romea', which had the same soluble solids value for both varieties (9.2 °Rf). The differences in the values found were highly statistically significant (Table 8).

Table 8. Soluble solid content (SSC) in peach cultivars. The data are displayed as the mean ± standard deviation of three replications; a–k refer to the grouping based on Tukey HSD test.

Cultivars	Soluble Solid Content [°Rf]	Cultivars	Soluble Solid Content [°Rf]
Admiral de Wey	$10.7 \pm 0.6^{b,c,d}$	Iris Rosso	$12.5 \pm 0.5^{d,e,f,g,h,i}$
Alexandra	$10.2 \pm 0.3^{a,b}$	Krasava	$12.2 \pm 0.3^{c,d,e,f,g,h,i}$
Anita	$11.8 \pm 0.8^{b,c,d,e,f,g,h}$	Lakomyj	$12.3 \pm 0.3^{d,e,f,g,h,i}$
Aurelia	$12.2 \pm 0.3^{c,d,e,f,g,h,i}$	Narjadnyj Nikitskij	$13.5 \pm 0.5^{g,h,i,j}$
Avalon Pride	$11.30 \pm 1.04^{b,c,d,e,f}$	Nerine	$13.7 \pm 0.8^{h,i,j}$
Benedicte	15.7 ± 0.3^{k}	Otličnik	$12.0 \pm 0.5^{b,c,d,e,f,g,h,i}$
Candor	$12.3 \pm 0.3^{d,e,f,g,h,i}$	Queen Lady	$11.0 \pm 0.9^{b,c,d,e}$
Carolina Belle	$11.8 \pm 0.3^{b,c,d,e,f,g,h}$	Red Robin	$10.8 \pm 0.8^{b,c,d}$
Dixigem	$11.8 \pm 0.3^{b,c,d,e,f,g,h}$	Redhaven	$13 \pm 1^{e,f,g,h,i,j}$
Dostojnyj	$13.2 \pm 0.3^{f,g,h,i,j}$	Romea	$11.8 \pm 0.8^{b,c,d,e,f,g,h}$
Early Glo	$10.3 \pm 0.3^{b,c}$	Royal Glory	$13.3 \pm 0.6^{g,h,i,j}$
Early Redhaven	$11.7 \pm 0.3^{b,c,d,e,f,g}$	Royal Majestic	$14.7 \pm 0.3^{j,k}$
Favorita Morettini	$11.8 \pm 0.8^{b,c,d,e,f,g,h}$	Sonet	$13.5 \pm 0.5^{g,h,i,j}$
Fénix	8.3 ± 0.5^{a}	Strelec	$12.8 \pm 0.3^{e,f,g,h,i,j}$
Fidelia	$12.1 \pm 0.5^{c,d,e,f,g,h,i}$	Suncrest	$13.8 \pm 0.8^{i,j,k}$
Harvester	$12.2 \pm 0.3^{c,d,e,f,g,h,i}$	Sunshine	$11.2 \pm 0.3^{b,c,d,e}$
Helene	$13.8 \pm 0.3^{i,j,k}$	UFO 3	$10.8 \pm 0.3^{b,c,d}$

The average sucrose, glucose, fructose, and sorbitol contents of the fruit were determined for each variety. The average sucrose content was 9.62 g.100 g^{-1} FW. The highest sucrose content was measured in the varieties 'Narjadnyj Nikitskiy' (16.57 g.100 g^{-1}) and

'Sonet' (16.44 g.100 g^{-1}). The lowest contents were observed in the cultivars 'Alexandra', 'Suncrest'(Figure 1), and 'Iris Rosso' (4.89, 4.69 and 4.66 g.100 g^{-1}, respectively). The glucose content ranged from 0.74 to 3.67 g.100 g^{-1}. The highest contents were determined in the varieties 'Sunshine' (3.67 g.100 g^{-1}) and 'Admiral de Wey' (3.50 g.100 g^{-1}). The lowest content was measured in the varieties 'UFO 3' (0.82 g.100 g^{-1}) and 'Nerine' (0.74 g.100 g^{-1}). The average value of glucose content was 1.94 g.100 g^{-1}. In the studied set of varieties, the total fructose content ranged from 0.48 to 2.39 g.100 g^{-1}, with an average value of 1.37 g.100 g^{-1}. The highest content was measured in the cultivars 'Sunshine' and 'Dixigem' (2.39 and 2.36 g.100 g^{-1}). The lowest fructose content was observed in the variety 'UFO 3' (0.48 g.100 g^{-1}). The average value of alcoholic sugar sorbitol in our study was 0.23 g.100 g^{-1}. The variety 'Benedicte' greatly exceeded all other varieties in sorbitol content, with its content being determined at 1.57 g.100 g^{-1}. Very low amounts were measured in the cultivars 'Lakomyj', 'Nerine', 'Iris Rosso', and 'Alexandra' (0.09; 0.09; 0.08 and 0.06 g.100 g^{-1}). The differences in the values found were highly statistically significant (Table 9).

Table 9. Sugars in peach cultivars. The data are displayed as the mean ± standard deviation of three replications; a–p refer to the grouping based on the Tukey HSD test.

Cultivars	Sucrose (g.100 g^{-1})	Glucose (mg.100 g^{-1})	Fructose (mg.100 g^{-1})	Sorbitol (g.100 g^{-1})
Admiral de Wey	$14.6 \pm 0.2^{m,n}$	$3.5 \pm 0.2^{j,k}$	$2.22 \pm 0.08^{j,k}$	$0.18 \pm 0.05^{d,e,f,g,h,i,j}$
Alexandra	$4.9 \pm 0.1^{a,b}$	$1.24 \pm 0.06^{a,b,c,d}$	$0.92 \pm 0.04^{b,c,d,e}$	0.055 ± 0.002^{a}
Anita	12.2 ± 0.6^{k}	$2.8 \pm 0.4^{g,h}$	$2.24 \pm 0.05^{j,k}$	$0.17 \pm 0.02^{c,d,e,f,g,h,i,j}$
Aurelia	$6.6 \pm 0.1^{c,d,e}$	$1.3 \pm 0.1^{a,b,c,d}$	$0.99 \pm 0.02^{b,c,d,e}$	$0.151 \pm 0.007^{b,c,d,e,f,g,h}$
Avalon Pride	$7.22 \pm 0.07^{c,d,e,f,g,h}$	$1.71 \pm 0.06^{d,e}$	$1.12 \pm 0.05^{b,c,d,e,f}$	$0.16 \pm 0.02^{b,c,d,e,f,g,h,i}$
Benedicte	$8.06 \pm 0.04^{f,g,h,i}$	$3.37 \pm 0.03^{i,j,k}$	$1.9 \pm 0.1^{i,j}$	1.57 ± 0.03^{o}
Candor	$7.0 \pm 0.3^{c,d,e,f,g}$	$1.22 \pm 0.03^{a,b,c,d}$	$1.0 \pm 0.1^{b,c,d,e}$	$0.12 \pm 0.02^{a,b,c,d,e}$
Carolina Belle	$8.09 \pm 0.06^{g,h,i}$	$2.288 \pm 0.007^{f,g}$	$1.4 \pm 0.2^{f,g,h}$	$0.28 \pm 0.02^{k,l,m}$
Dixigem	$15.3 \pm 0.3^{m,n,o,p}$	3.4 ± 0.1^{k}	2.36 ± 0.05^{k}	$0.29 \pm 0.02^{k,l,m}$
Dostojnyj	$16 \pm 1^{n,o,p}$	$2.7 \pm 0.7^{f,g,h}$	$1.85 \pm 0.09^{h,i,j}$	$0.17 \pm 0.07^{c,d,e,f,g,h,i,j}$
Early Glo	$6.80 \pm 0.08^{c,d,e,f}$	$1.31 \pm 0.07^{b,c,d}$	$1.03 \pm 0.04^{b,c,d,e}$	$0.114 \pm 0.002^{a,b,c,d,e}$
Early Redhaven	$13.09 \pm 0.05^{k,l}$	$2.393 \pm 0.006^{f,g}$	$1.7 \pm 0.1^{g,h,i}$	$0.13 \pm 0.01^{a,b,c,d,e,f}$
Favorita Morettini	$15.57 \pm 0.03^{n,o,p}$	$2.72 \pm 0.02^{f,g,h}$	$1.45 \pm 0.01^{f,g,h}$	$0.21 \pm 0.01^{f,g,h,i,j,k}$
Fénix	$6.11 \pm 0.09^{b,c}$	$1.20 \pm 0.02^{a,b,c,d}$	$0.80 \pm 0.01^{a,b}$	$0.128 \pm 0.002^{a,b,c,d,e,f}$
Fidelia	$7.86 \pm 0.05^{e,f,g,h,i}$	$1.38 \pm 0.03^{b,c,d}$	$1.32 \pm 0.05^{e,f,g}$	$0.28 \pm 0.01^{k,l,m}$
Harvester	$8.8 \pm 0.2^{i,j}$	$1.71 \pm 0.05^{d,e}$	$1.1 \pm 0.1^{b,c,d,e,f}$	$0.23 \pm 0.03^{h,i,j,k}$
Helene	$7.1 \pm 0.5^{c,d,e,f,g,h}$	$1.12 \pm 0.02^{a,b,c}$	$0.90 \pm 0.03^{b,c,d}$	$0.24 \pm 0.01^{i,j,k,l}$
Iris Rosso	4.7 ± 0.3^{a}	$1.4 \pm 0.4^{c,d}$	$1.0 \pm 0.1^{b,c,d,e}$	$0.077 \pm 0.003^{a,b}$
Krasava	$6.6 \pm 0.3^{c,d,e}$	$1.4 \pm 0.2^{c,d}$	$1.25 \pm 0.07^{c,d,e,f}$	$0.22 \pm 0.01^{g,h,i,j,k}$
Lakomyj	$7.03 \pm 0.03^{c,d,e,f,g,h}$	$1.26 \pm 0.04^{a,b,c,d}$	$0.9 \pm 0.2^{a,b,c}$	$0.092 \pm 0.004^{a,b,c,d}$
Narjadnyj Nikitskij	16.6 ± 0.3^{p}	$2.9 \pm 0.2^{g,h,i}$	$2.0 \pm 0.4^{i,j,k}$	$0.16 \pm 0.02^{b,c,d,e,f,g,h,i}$
Nerine	$7.3 \pm 0.3^{c,d,e,f,g,h}$	0.740 ± 0.005^{a}	$0.90 \pm 0.06^{b,c,d}$	$0.087 \pm 0.005^{a,b,c}$
Otličnik	$12.83 \pm 0.05^{k,l}$	$2.16 \pm 0.08^{e,f}$	$1.69 \pm 0.01^{g,h,i}$	$0.181 \pm 0.002^{e,f,g,h,i,j}$
Queen Lady	$14.1 \pm 0.5^{l,m}$	$3.1 \pm 0.3^{h,i,j,k}$	$2.0 \pm 0.2^{i,j,k}$	$0.33 \pm 0.09^{l,m}$
Red Robin	$7.621 \pm 0.004^{d,e,f,g,h,i}$	$1.47 \pm 0.03^{c,d}$	$0.9 \pm 0.1^{b,c,d,e}$	$0.2 \pm 0.0^{b,c,d,e,f,g,h}$
Redhaven	$8.73 \pm 0.03^{i,j}$	$1.56 \pm 0.04^{c,d}$	$1.11 \pm 0.04^{b,c,d,e,f}$	$0.138 \pm 0.001^{a,b,c,d,e,f,g}$
Romea	9.7 ± 0.2^{j}	$1.57 \pm 0.03^{c,d}$	$1.14 \pm 0.09^{b,c,d,e,f}$	$0.144 \pm 0.009^{b,c,d,e,f,g,h}$
Royal Glory	$7.39 \pm 0.08^{c,d,e,f,g,h}$	$1.462 \pm 0.003^{c,d}$	$1.2 \pm 0.1^{c,d,e,f}$	$0.21 \pm 0.03^{f,g,h,i,j,k}$
Royal Majestic	$8.3 \pm 0.7^{h,i}$	$1.3 \pm 0.1^{b,c,d}$	$1.1 \pm 0.3^{b,c,d,e,f}$	0.44 ± 0.05^{n}
Sonet	$16 \pm 1^{o,p}$	$3.1 \pm 0.4^{h,i,j}$	$2.0 \pm 0.2^{i,j,k}$	$0.25 \pm 0.02^{j,k,l}$
Strelec	$6.5 \pm 0.3^{c,d}$	$1.14 \pm 0.04^{a,b,c,d}$	$0.86 \pm 0.08^{a,b,c}$	$0.144 \pm 0.007^{b,c,d,e,f,g,h}$
Suncrest	4.7 ± 0.1^{a}	$1.50 \pm 0.05^{c,d}$	$1.30 \pm 0.03^{d,e,f,g}$	$0.124 \pm 0.002^{a,b,c,d,e,f}$
Sunshine	$15.3 \pm 0.1^{m,n,o}$	3.67 ± 0.03^{k}	2.4 ± 0.1^{k}	0.34 ± 0.02^{m}
UFO 3	$8.19 \pm 0.03^{g,h,i}$	$0.819 \pm 0.006^{a,b}$	0.475 ± 0.009^{a}	$0.21 \pm 0.03^{f,g,h,i,j,k}$

Colorimetric parameters *L**, *a**, *b** for the basic skin colour of the fruit were measured for all varieties. In the varieties 'Alexandra', 'Anita', 'Helene', 'Iris Rosso', 'Royal Glory'

and 'Royal Majestic', the skin was completely covered by the blush. The average values of L^*, a^*, b^* are summarised in Table 10. The highest values of L^* were found for the basic colour in the varieties 'Krasava', 'Aurelia', 'Sunshine' and for the cheek in the varieties 'Romea', 'Dostojnyj', 'Carolina Belle'. In our study the highest value of a^* were found for 'Nerine', 'Admiral de Wey', 'Avalon Pride', the lowest value were found for 'Krasava', 'Otličnik', 'Carolina Belle' and 'Queen Lady'. For chromatic parameter b^* the highest values were measured for 'Romea', 'Otličnik', 'Lakomyj' and the lowest values were found for 'Fidelia', 'UFO 3' and 'Red Robin'. Colour intensity is represented by the chromatic parameter C^*_{ab}, which was determined using the chromatic parameters a^* and b^*, and its highest values were found for the basic colour of 'Romea', 'Otličnik', 'Lakomyj' and for the cheek colour of 'Romea', 'Sunshine', 'Admiral de Wey'. From the measured values for base colour and cheek colour, the greatest colour difference ΔE^*_{ab} (Table 11) was found for the cultivars 'Otličnik', 'Lakomyj', 'Queen Lady'. These varieties had the richest cheeks when compared to the base colour. On the other hand, the lowest ΔE^*_{ab} were found for the varieties 'Red Robin', 'Romea', 'UFO 3', where the cheek almost merged with the base colour. Figure 2 captures the exact colour found in the L^*, a^*, b^* coordinates.

Table 10. The average values of individual chromatic parameters for peach skin ground colour and over colour of peach.

Ground Colour	***L****	***a****	***b****	***C****	***h****
Average	69.3	7.17	43.8	45.0	0.96
Deviation	3.83	7.12	8.04	7.67	1.01
Over colour	*L**	*a**	*b**	*C**	*h**
Average	36.5	29.8	17.0	34.5	0.51
Deviation	4.83	4.67	5.44	6.06	0.11

Table 11. Values of chromatic parameters for ground colour skin and over colour skin of the peach cultivars.

	Ground Colour					**Over Colour**					ΔE^*_{ab}
Cultivar	***L****	***a****	***b****	***C****	***h***	***L****	***a****	***b****	***C****	***h***	
Admiral de Wey	65.5	19.3	39.1	43.6	1.1	35.8	37.6	18.0	41.7	0.45	40.8
Alexandra	-	-	-	-	-	29.1	21.9	10.2	24.2	0.43	-
Anita	-	-	-	-	-	36.0	33.5	15.5	37.0	0.43	-
Aurelia	73.6	1.96	50.8	50.8	1.53	39.6	30.0	19.1	35.6	0.57	54.3
Avalon Pride	67.5	16.8	40.8	44.1	1.18	37.8	33.6	18.2	38.2	0.50	41.0
Benedicte	72.5	0.36	29.8	29.8	1.56	33.1	32.1	16.6	36.1	0.48	52.3
Candor	68.7	10.8	44.3	45.7	1.33	35.3	33.7	17.3	37.9	0.48	48.6
Carolina Belle	71.9	−2.44	32.3	32.4	−1.50	42.5	29.6	18.8	35.1	0.57	45.5
Dixigem	73.2	9.21	47.6	48.5	1.38	37.4	29.3	14.5	32.7	0.46	52.7
Dostojnyj	69.9	0.78	52.4	52.4	1.56	48.4	25.7	32.5	41.4	0.90	38.4
Early Glo	72.6	9.38	45.9	46.9	1.37	39.4	31.8	18.8	37.0	0.53	48.4
Early Redhaven	72.4	8.69	48.9	49.7	1.40	36.8	33.1	19.0	38.2	0.52	52.5
Favorita Morettini	64.1	10.3	43.6	44.8	1.34	39.0	25.3	13.4	28.6	0.49	42.1
Fénix	65.9	9.11	43.2	44.1	1.36	35.5	27.9	17.1	32.7	0.55	44.2
Fidelia	66.7	16.2	28.5	32.8	1.05	33.0	33.0	14.9	36.2	0.42	40.0
Harvester	69.2	7.98	48.0	48.6	1.41	36.0	30.0	15.7	33.9	0.48	51.3
Helene	-	-	-	-	-	30.8	28.5	12.3	31.0	0.41	-
Iris Rosso	-	-	-	-	-	33.2	16.0	7.01	17.5	0.41	-
Krasava	74.5	−6.95	33.8	34.5	−1.37	40.1	34.1	19.9	39.5	0.53	55.4
Lakomyj	73.1	0.71	52.7	52.7	1.56	37.7	35.3	20.6	40.9	0.53	58.9
Narjadnyj Nikitskij	62.3	13.1	43.1	45.0	1.28	35.0	29.7	16.9	34.2	0.51	41.4
Nerine	64.9	20.3	45.8	50.1	1.15	37.8	34.8	20.3	40.3	0.53	39.9
Otličnik	71.6	−6.38	53.5	53.9	−1.45	37.5	26.8	16.5	31.4	0.55	60.2
Queen Lady	69.9	−2.00	47.2	47.2	−1.53	34.3	29.3	16.0	33.4	0.50	56.8
Red Robin	59.8	11.4	29.2	31.3	1.20	36.2	30.1	16.2	34.2	0.49	32.7

Table 11. *Cont.*

Cultivar	Ground Colour					Over Colour					ΔE^*_{ab}
	*L**	*a**	*b**	*C**	*h*	*L**	*a**	*b**	*C**	*h*	
Redhaven	71.3	9.62	49.3	50.3	1.38	37.1	30.6	18.8	35.9	0.55	50.4
Romea	71.9	13.7	54.5	56.2	1.32	50.4	32.1	33.1	46.1	0.80	35.5
Royal Glory	-	-	-	-	-	25.6	30.0	10.0	31.6	0.32	-
Royal Majestic	-	-	-	-	-	27.0	20.3	7.17	21.6	0.34	-
Sonet	66.5	6.81	48.3	48.8	1.43	37.4	32.5	20.9	38.6	0.57	47.5
Strelec	69.5	4.39	47.8	48.0	1.48	34.1	28.3	14.3	31.7	0.47	54.3
Suncrest	70.9	5.23	50.2	50.5	1.47	35.2	25.3	13.6	28.7	0.49	54.0
Sunshine	73.7	4.27	48.4	48.6	1.48	39.0	36.4	21.8	42.4	0.54	54.3
UFO 3	65.7	8.29	28.8	29.9	1.29	36.3	23.8	12.1	26.7	0.47	37.2

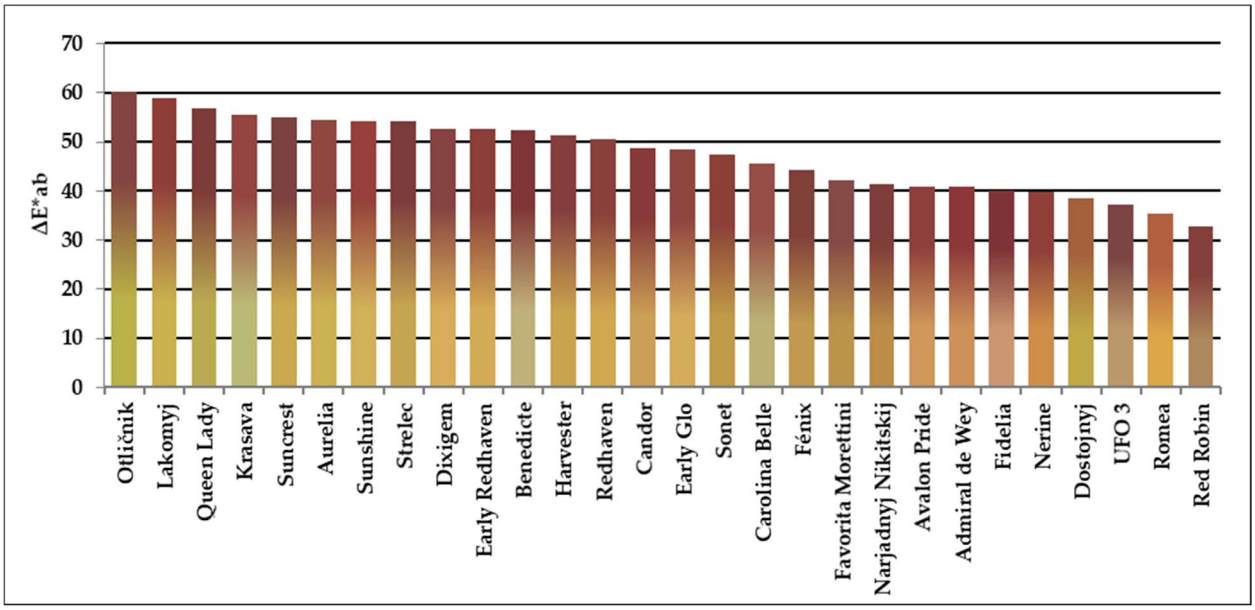

Figure 2. Values of ΔE^*_{ab} of individual peach cultivars. The ground colour is shown in the lower part of the column, and over colour is shown in the upper part according to the measured coordinates *L**, *a**, *b**.

4. Discussion

The acid content of fruit is a key quality parameter and is an important factor in determining the taste of the fruit. Titratable acidity indicates the concentration of organic acids present in the fruit. Peaches have a very low level of organic acids. The total titratable acid content found in our set of varieties ranged from 0.26 to 1.32% malic acid on fresh weight. These values are similar to the results found in many other publications. Scordino et al. (2012) [38] reported TA contents ranging from 0.52–0.86% malic acid in Sicilian yellow flesh peaches on fresh weight. Similar values were also found in the work by Tomás-Barberán et al. (2010) [39], where the contents ranged from 0.53–0.97% malic acid in yellow flesh peaches on fresh weight, and 0.15–0.34% malic acid in white flesh peaches on fresh weight. Gil et al. (2002) [40] investigated the differences between white- and yellow-fleshed peach cultivars grown in California. The average TAC content found in the white-fleshed varieties was 0.22%, and in the yellow-fleshed varieties it was 0.69%.

In a publication by Cantin et al. (2009) [41], the total phenolic content ranged from 12.7 to 71.3 mg GAE.100 g^{-1} FW, with an average of 36.4 mg GAE.100 g^{-1} FW. In our selected set of cultivars, the average content reached 122.4 mg GAE.100 g^{-1} FW. Marinova et al. (2005) [42] investigated the determination of all phenolic compounds in fruit grown in Bulgaria. The total phenolic content in peach fruits was 50.9 mg GAE.100 g^{-1} FW, and similar values were reached by figs—*Ficus carica* (59.0 mg GAE.100 g^{-1} FW). Another

publication by Saidani et al. (2017) [43] dealt with the determination of phenolic compounds separately in the peel and in the pulp. In the peel, contents ranging from 88.9 to 277 mg GAE.100 g^{-1} FW were determined, while in the pulp, contents ranging from 25.1 to 139 mg GAE.100 g^{-1} FW were determined. Previously, Zhao et al. (2015) [44] monitored the content of total phenolics in selected Chinese peach cultivars, ranging from 4.58 to 12.68 mg gallic acid equivalent (GAE).100 g^{-1} DW in the peel and from 0.82 to 6.52 mg GAE.100 g^{-1} DW in the pulp.

The obtained results of total flavonoids content in the tested set of varieties ranged from 1.1 to 95.1 mg CAE.100 g^{-1} FW. Di Vaio et al. (2015) [45] determined the total flavonoid content, and it ranged from 35.05-58.85 g CAE.kg^{-1} FW within the test set. In another publication by Cantin et al. (2009) [40], total flavonoid content ranged from 1.8 to 30.9 mg CAE.100 g^{-1} FW, with an average of 8.8 mg CAE.100 g^{-1} FW. Marinova et al. (2005) [42] investigated the determination of all phenolic compounds, as well as flavonoids in crops grown in Bulgaria. The total flavonoid content in peach fruits was 15.0 mg CAE.100 g^{-1} FW; similar values are seen in figs—*Ficus carica* (20.2 mg CAE.100 g^{-1} FW) and sweet cherries (19.6 mg CAE.100 g^{-1} FW). The highest representation of flavonoids was found in this work in blueberries (190.3 mg CAE.100 g^{-1} FW). Saidani et al. (2017) [43] determined the flavonoid content in the skin of peach fruits to be between 39 and 245 mg CAE.100 g^{-1} FW, and in the flesh between 8.18 and 112 mg CAE.100 g^{-1} FW.

Analyses of antioxidant components in products are fast becoming a recognized profile, primarily emphasizing antioxidant capacity as a quality index for many fruits and vegetables. The high phenolic content showed an increased antioxidant capacity in the studied varieties. The average value of antioxidant capacity determined by the DPPH (1-diphenyl-2,2-picrylhydrazyl) method showed values of 205.7 mg TE.100 g^{-1} FW. The authors of Di Vaio et al. (2015) [45] determined average antioxidant capacity values of 111.1 mg TE.100 g^{-1} FW in four peach cultivars. Saidani et al. (2017) [43], in a tested set of peach cultivars, determined the antioxidant capacity value in the skin of the fruit ranging from 133 to 401 mg TE.100 g^{-1} FW, and in the flesh ranging from 22.7 to 194 mg TE.100 g^{-1} FW. Zhao et al. (2015) [44] found antioxidant capacity contents in Chinese peach cultivars from 6.35 to 19.84 mg trolox equivalent antioxidant capacity (TE).100 g^{-1} DW in the peel and from 1.05 to 15.01 mg TE.100 g^{-1} DW in the pulp.

The content of carotenoids (especially β-carotene, zeaxanthin, lutein, neoxanthin) and anthocyanins increases with fruit maturity, largely due to the colouring of the fruit (formation of the cheek). The results obtained for total anthocyanin content in the tested set of cultivars ranged from 0.04 to 3.74 mg.100 g^{-1} FW. Cantín et al. (2009) [41] monitored the content of total anthocyanins in selected cultivars and found contents ranging between 0.1 and 26.7 mg of C3GE.kg^{-1} FW (0.1-26.7 mg of cyanidin-3-glucoside equiv. (C3GE) per kg of FW). In another publication by Saidani et al. (2017) [43], they discussed the determination of total anthocyanins separately in the peel and in the pulp. The average anthocyanin content in the peel was 5.53 mg C3GE.100 g^{-1} FW, while in the pulp the average content was 0.37 mg C3GE.100 g^{-1} FW. In other research on total anthocyanin content in apricot fruits, Rababah et al. (2011) [46] reported an average anthocyanin content of 2.54 mg.100 g^{-1} FW, whereas Contessa et al. (2013) [47] reported an anthocyanin content of 0.99 mg C3GE.100 g^{-1} FW.

The carotenoids content found in the set of cultivars ranged from 0.00 to 4.77 mg.100 g^{-1} DW. Gil et al. (2002) [40] observed differences in carotenoids content between white- and yellow-skinned peach cultivars. The average carotenoids content found in white-fleshed cultivars was 11.6 μg.100 g^{-1}, while in yellow-fleshed cultivars it was 131.6 μg.100 g^{-1}. Vizzotto et al. (2007) [48] also found higher carotenoids content in genotypes with yellow flesh (0.8 to 3.7 milligrams β-carotene per 100 g tissue) than in peaches with white flesh (0.0 to 0.1 milligrams β-carotene per 100 g tissue).

Soluble solid content (SSC) is an important characteristic of fruit, as it is closely related to consumer satisfaction and how well the fruit is liked. Zhao et al. (2015) [44] evaluated the soluble solid content of different Chinese peach cultivars; their findings ranged from 8.34

to 15.48 °Rf. These results are similar to ours, with values ranging from 8.30 to 14.70 °Rf in our set of cultivars. In another work, Gil et al. (2002) [40] investigated the differences between white- and yellow-fleshed peach cultivars grown in California. The average SSC content found in the white-fleshed varieties was 11.22 °Rf, while in the yellow-fleshed varieties it was 11.90 °Rf. For the Spanish varieties, Legua et al. (2011) [49] found SSC contents between 9.98 and 18.36 °Rf. Tavarini et al. (2008) [50] determined an average SSC value of 12.42 °Rf for Italian varieties.

In this study, sucrose, glucose, fructose, and sorbitol were determined as the basic sugars of peaches and there were differences found among the cultivars (Table 10). The mean values of sucrose, glucose, fructose, and sorbitol were 9.62 g.100 g^{-1}, 1.94 g.100 g^{-1}, 1.37 g.100 g^{-1} and 0.23 g.100 g^{-1}, respectively. These values are very similar to those determined by Forcada et al. (2014) [9]. The values found ranged from 3.5–9.8 g.100 g^{-1} sucrose, 0.4–1.5 g.100 g^{-1} glucose, 0.2–1.4 g.100 g^{-1} fructose, and 0.2–3.5 g.100 g^{-1} sorbitol. Nowicka et al. (2019) [51] investigated the sugar content of 20 peach cultivars. They determined sucrose content ranging from 3.4–5.4 g.100 g^{-1}, glucose 0.27–0.84 g.100 g^{-1}, fructose 0.41–1.03 g.100 g^{-1}, and sorbitol content ranging from 0.15–0.74 g.100 g^{-1}. Colaric et al. (2005) [13] determined sucrose levels between 46.14-66.92 g.kg^{-1} in some nectarine and peach cultivars. Cantin et al. (2009) [41] determined a similar sucrose content (47.10–64.00 g.kg^{-1}), and further investigated the determination of glucose (5.60–8.00 g.kg^{-1}) and fructose (6.9–10.3 g.kg^{-1}) in peach and nectarine fruits. Gecer (2020) [52] measured sucrose (5216.3–9122.4 mg.100 g^{-1}), glucose (721.7–1902.1 mg.100 g^{-1}), and fructose (325.7–1048.1 mg.100 g^{-1}) in some peach and nectarine cultivars. Robertson et al. (1990) [53] determined the average sorbitol content in yellow-fleshed cultivars, 0.46% and in white-fleshed cultivars, 0.37%. The colour of the fruit is an important parameter that influences the attractiveness of the fruit to consumers. A colorimetric analysis can also provide information on the degree of ripeness of the fruit. The colour of peaches using CIELAB was measured in studies before [54–57]. The value of a^* has been suggested as colour index maturity [54]. The study was associated with changes of a^* with chlorophyl degradation and an increase of anthocyanin content. Because of low values of anthocyanin in most cultivars of peaches, Ferrer et al. (2010) [55] found that changes of chromatic parameter b^* can be a good indicator of ripeness of peach fruit. These changes correlated to an increase of carotenoids pigments. In our study the correlation relationship between carotenoids and chromatic parameter b^* with a correlation coefficient R = 0.7951 and C^* with R = 0.8051 were found (Figure 3). The cultivars 'Otličnik' and 'Aurelia' were accomplished as outliers and they are not included in the correlations; this can be attributed to insufficient maturity of these two cultivars.

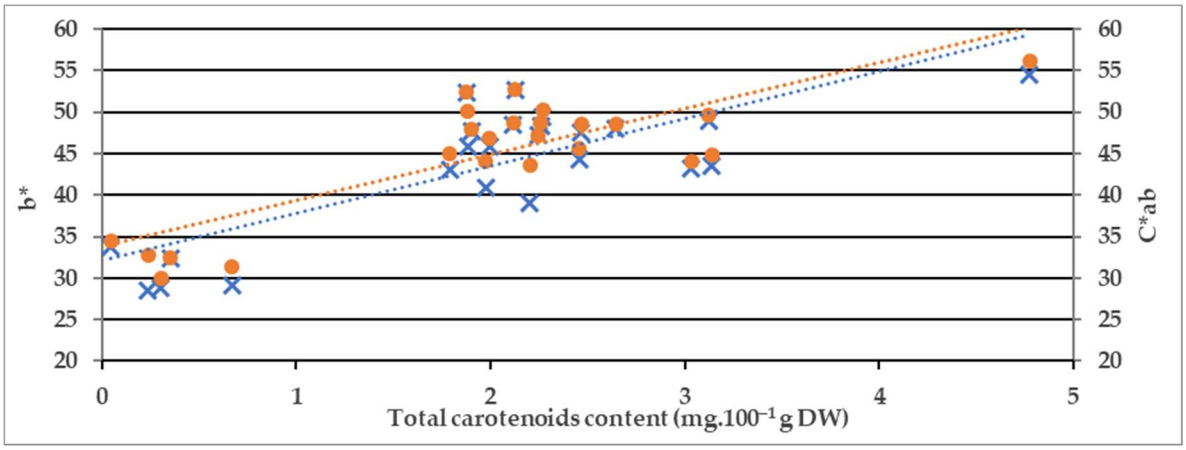

Figure 3. Relationship between b^*, C^*_{ab}, and total carotenoids content (TCC) in peach cultivars. Blue points represent correlation between b^* and TCC, orange points represent correlation between C^*_{ab} and TCC.

5. Conclusions

Peach fruits have an important specific nutritional status among stone fruits. This means that peaches can serve as a source of sugars, mainly sucrose, as well as phenolics, carotenoids, and anthocyanins, and can also provide valuable antioxidants. The Czech 'Krasava' variety was found to be a variety that has a very high content of titratable acids, phenolics, flavonoids, and antioxidant capacity. It can be said that this variety is very interesting from a biochemical point of view and offers a certain potential. Peach consumption represents one of the main fruit incomes during the summer months and is subject to seasonal demand, i.e., the short period of availability in the year. While pome fruits may form the bulk of typical dietary intake during longer periods of the year, peaches are only a seasonal concern.

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References

1. Janick, J.; Paull, R.E. *The Encyclopedia of Fruit & Nuts*; CABI: Wallingford, UK, 2008; p. 954.
2. Davidović, S.M.; Veljović, M.S.; Pantelić, M.M.; Baošić, R.M.; Natić, M.M.; Dabić, D.Č.; Pecić, S.P.; Vukosavljević, P.V. Physicochemical, Antioxidant and Sensory Properties of Peach Wine Made from Redhaven Cultivar. *J. Agric. Food Chem.* **2013**, *61*, 1357–1363. [CrossRef] [PubMed]
3. Campbell, O.E.; Padilla-Zakour, O.I. Phenolic and Carotenoid Composition of Canned Peaches (*Prunus persica*) and Apricots (*Prunus armeniaca*) as Affected by Variety and Peeling. *Food Res. Int.* **2013**, *54*, 448–455. [CrossRef]
4. Cantin, C.M.; Moreno, M.A.; Gogorcena, Y. Evaluation of the antioxidant capacity, phenolic compounds, and vitamin C content of different peach and nectarine [*Prunus persica* (L.) Batsch] breeding progenies. *J. Agric. Food Chem.* **2009**, *57*, 4586–4592. [CrossRef] [PubMed]
5. Liu, H.; Cao, J.; Jiang, W. Evaluation and Comparison of Vitamin C, Phenolic Compounds, Antioxidant Properties and Metal Chelating Activity of Pulp and Peel from Selected Peach Cultivars. *LWT Food Sci. Technol.* **2015**, *63*, 1042–1048. [CrossRef]
6. Scalbert, A.; Johnson, I.T.; Saltmarsh, M. Polyphenols: Antioxidants and beyond. *Am. J. Clin. Nutr.* **2005**, *81*, 215S–217S. [CrossRef] [PubMed]
7. Bento, C.; Gonçalves, A.C.; Silva, B.; Silva, L.R. Peach (*Prunus persica*): Phytochemicals and Health Benefits. *Food Rev. Int.* **2020**, *36*, 1–32. [CrossRef]
8. Remorini, D.; Tavarini, S.; Degl'Innocenti, E.; Loreti, F.; Massai, R.; Guidi, L. Effect of Rootstocks and Harvesting Time on the Nutritional Quality of Peel and Flesh of Peach Fruits. *Food Chem.* **2008**, *110*, 361–367. [CrossRef] [PubMed]
9. Forcada, C.F.I.; Gradziel, T.M.; Gogorcena, Y.; Moreno, M.Á. Phenotypic diversity among local Spanish and foreign peach and nectarine [*Prunus persica* (L.) Batsch] accessions. *Euphytica* **2014**, *197*, 261–277. [CrossRef]
10. Caniato, F.F.; Galvao, J.C.C.; Finger, F.L.; Ribeiro, R.A.; Miranda, G.V.; Puiatti, M. Study of total soluble sugars, reducing sugars and starch composition in immature kernels of corn cultivars. *Rev. Bras. Milho Sorgo.* **2004**, *3*, 38–44. [CrossRef]
11. Cirilli, M.; Bassi, D.; Ciacciulli, A. Sugars in peach fruit: A breeding perspective. *Hortic. Res.* **2016**, *3*, 1–12. [CrossRef]
12. Sánchez-Moreno, C.; Pascual-Teresa, S.D.; Ancos, B.D.; Cano, M.P. Nutritional Values of Fruits. In *Handbook of Fruits and Fruit Processing*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2006; p. 29.
13. Colaric, M.; Veberic, R.; Stampar, F.; Hudina, M. Evaluation of peach and nectarine fruit quality and correlations between sensory and chemical attributes. *J. Sci. Food Agric.* **2005**, *85*, 2611–2616. [CrossRef]
14. Versari, A.; Castellari, M.; Parpinello, G.P.; Riponi, C.; Galassi, S. Characterisation of peach juices obtained from cultivars Redhaven, Suncrest and Maria Marta grown in Italy. *Food Chem.* **2002**, *76*, 181–185. [CrossRef]

15. Iordănescu, O.A.; Alexa, E.; Radulov, I.; Costea, A.; Dobrei, A.; Dobrei, A. Minerals and amino acids in peach (*Prunus persica* L.) cultivars and hybrids belonging to world germoplasm collection in the conditions of West Romania. *Agric. Agric. Sci. Procedia* **2015**, *6*, 145–150. [CrossRef]
16. Kim, H.R.; Kim, I.D.; Dhungana, S.K.; Kim, M.O.; Shin, D.H. Comparative assessment of physicochemical properties of unripe peach (*Prunus persica*) and Japanese apricot (*Prunus mume*). *Asian Pac. J. Trop. Biomed.* **2014**, *4*, 97–103. [CrossRef]
17. Başar, H. Elemental Composition of Various Peach Cultivars. *Sci. Hortic.* **2006**, *107*, 259–263. [CrossRef]
18. Melo, G.W.B.; Sete, P.B.; Ambrosini, V.G.; Freitas, R.F.; Basso, A.; Brunetto, G. Nutritional Status, Yield and Composition of Peach Fruit Subjected to the Application of Organic Compost. *Acta Sci. Agron.* **2016**, *38*, 103. [CrossRef]
19. Valíček, P. *Léčivé Rostliny Číny a Vietnamu: (j-z) 2. díl. 1. Vyd*; Start: Benešov, Czech Republic, 2010; p. 351. (In Czech)
20. Li, C.; Wang, M.H. Antioxidant activity of peach blossom extracts. *J. Korean Soc. Appl. Biol. Chem.* **2011**, *54*, 46–53. [CrossRef]
21. Rossato, S.B.; Haas, C.; Raseira, M.D.C.B.; Moreira, J.C.F.; Zuanazzi, J.Â.S. Antioxidant potential of peels and fleshes of peaches from different cultivars. *J. Med. Food.* **2009**, *12*, 1119–1126. [CrossRef] [PubMed]
22. Shin, T.Y.; Park, S.B.; Yoo, J.S.; Kim, I.K.; Lee, H.S.; Kwon, T.K.; Kim, M.K.; Kim, J.C.; Kim, S.H. Anti-allergic inflammatory activity of the fruit of *Prunus persica*: Role of calcium and NF-κB. *Food Chem. Toxicol.* **2010**, *48*, 2797–2802. [CrossRef]
23. Cevallos-Casals, B.A.; Byrne, D.; Okie, W.R.; Cisneros-Zevallos, L. Selecting new peach and plum genotypes rich in phenolic compounds and enhanced functional properties. *Food Chem.* **2006**, *96*, 273–280. [CrossRef]
24. Lee, C.K.; Park, K.K.; Hwang, J.K.; Lee, S.K.; Chung, W.Y. The extract of *Prunus persica* flesh (PPFE) attenuates chemotherapy-induced hepatotoxicity in mice. *Phytother. Res.* **2008**, *22*, 223–227. [CrossRef]
25. Lee, C.K.; Park, K.K.; Hwang, J.K.; Lee, S.K.; Chung, W.Y. Extract of *Prunus persica* flesh (PPFE) improves chemotherapeutic efficacy and protects against nephrotoxicity in cisplatin-treated mice. *Phytother. Res.* **2009**, *23*, 999–1005. [CrossRef]
26. Geng, M.; Li, W.; Yang, R.; Cui, C.; An, H.; Liu, H.; Wang, X. Antioxidant and antiproliferative aktivity (in vitro) of polyphenols from peach blossom. *J. Food Agric. Environ.* **2013**, *11*, 429–433.
27. Noratto, G.; Porter, W.; Byrne, D.; Cisneros-Zevallos, L. Identifying peach and plum polyphenols with chemopreventive potential against estrogen-independent breast cancer cells. *J. Agric. Food Chem.* **2009**, *57*, 5219–5226. [CrossRef] [PubMed]
28. Noratto, G.; Porter, W.; Byrne, D.; Cisneros-Zevallos, L. Polyphenolics from peach (*Prunus persica* var. Rich Lady) inhibit tumor growth and metastasis of MDA-MB-435 breast cancer cells *in vivo*. *J. Nutri. Biochem.* **2014**, *25*, 796–800. [CrossRef]
29. Goliáš, J.; Němcová, A. *Skladování a zpracování ovoce a zeleniny: (návody do cvičení). 1. vyd.*; Mendelova zemědělská a lesnická univerzita v Brně: Brno, Czech Republic, 2009.
30. Zloch, Z.; Čelakovský, J.; Aujezdská, A. *Stanovení Obsahu Polyfenolů a Celkové Antioxidační Kapacity v Potravinách Rostlinného Původu. Závěrečná zpráva o plnění výzkumného projektu podpořeného finančně Nadačním fondem Institutu Danone*; (In Czech). Ústav Hygieny Lékařské Fakulty UK Plzeň: Plzeň, Czech Republic, 2004.
31. Holm, G. Chlorophyll mutations in barley. *Acta Agr. Scand.* **1954**, *4*, 457–471. [CrossRef]
32. Zbíral, J. *Analýza Rostlinného Materiálu: Jednotné Pracovní Postupy; Vyd. 2, rozš. A přeprac*; (In Czech). Ústřední Kontrolní a Zkušební Ústav Zemědělský: Brno, Czech Republic, 2005.
33. Lancaster, J.E.; Lister, C.E.; Reay, P.F.; Triggs, C.M. Influence of Pigment Composition on Skin Colour in a Wide Range of Fruit and Vegetables. *J. Amer. Soc. Hort. Sci.* **1997**, *122*, 594–598. [CrossRef]
34. Clydesdale, F.M.; Ahmed, E.M. Colorimetry—Methodology and Applications. *CRC Crit. Rev. Food Sci. Nutri.* **1978**, *10*, 243–301. [CrossRef] [PubMed]
35. Voss, D.H. Relating colourimeter measurement of plant colour to the royal horticultural society colour chart. *HortScience* **1992**, *27*, 1256–1260. [CrossRef]
36. McGuire, R.G. Reporting of objective colour measurements. *HortScience* **1992**, *27*, 1254–1255. [CrossRef]
37. Sharma, G. *Digital Color Imaging Handbook*; CRC Press: Boca Raton, FL, USA, 2003.
38. Scordino, M.; Sabatino, L.; Muratore, A.; Belligno, A.; Gagliano, G. Phenolic Characterization of Sicilian Yellow Flesh Peach (*Prunus persica* L.) Cultivars at Different Ripening Stages. *J. Food Qual.* **2012**, *35*, 255–262. [CrossRef]
39. Tomás-Barberán, F.A.; Gil, M.I.; Cremin, P.; Waterhouse, A.L.; Hess-Pierce, B.; Kader, A.A. HPLC—DAD − ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. *J. Agric. Food Chem.* **2001**, *49*, 4748–4760. [CrossRef] [PubMed]
40. Gil, M.I.; Tomás-Barberán, F.A.; Hess-Pierce, B.; Kader, A.A. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *J. Agric. Food Chem.* **2002**, *50*, 4976–4982. [CrossRef]
41. Cantin, C.M.; Gogorcena, Y.; Moreno, M.A. Analysis of phenotypic variation of sugar profile in different peach and nectarine [*Prunus persica* (L.) Batsch] breeding progenies. *J. Sci. Food Agric.* **2009**, *89*, 1909–1917. [CrossRef]
42. Marinova, D.; Ribarova, F.; Atanassova, M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. *J. Univ. Chem. Technol. Metall.* **2005**, *40*, 255–260.
43. Saidani, F.; Giménez, R.; Aubert, C.; Chalot, G.; Betrán, J.A.; Gogorcena, Y. Phenolic, sugar and acid profiles and the antioxidant composition in the peel and pulp of peach fruits. *J. Food Composit. Anal.* **2017**, *62*, 126–133. [CrossRef]
44. Zhao, X.; Zhang, W.; Yin, X.; Su, M.; Sun, C.; Li, X.; Chen, K. Phenolic composition and antioxidant properties of different peach [*Prunus persica* (L.) Batsch] cultivars in China. *Int. J. Mol. Sci.* **2015**, *16*, 5762–5778. [CrossRef]
45. Di Vaio, C.; Marallo, N.; Graziani, G.; Ritieni, A.; Di Matteo, A. Evaluation of fruit quality, bioactive compounds and total antioxidant activity of flat peach cultivars. *J. Sci. Food Agric.* **2015**, *95*, 2124–2131. [CrossRef]

46. Rababah, T.M.; Al-Mahasneh, M.A.; Kilani, I.; Yang, W.; Alhamad, M.N.; Ereifej, K.; Al-u'datt, M. Effect of jam processing and storage on total phenolics, antioxidant activity, and anthocyanins of different fruits. *J. Sci. Food Agric.* **2011**, *91*, 1096–1102. [CrossRef]
47. Contessa, C.; Mellano, M.G.; Beccaro, G.L.; Giusiano, A.; Botta, R. Total antioxidant capacity and total phenolic and anthocyanin contents in fruit species grown in Northwest Italy. *Sci. Hort.* **2013**, *160*, 351–357. [CrossRef]
48. Vizzotto, M.; Cisneros-Zevallos, L.; Byrne, D.H.; Ramming, D.W.; Okie, W.R. Large variation found in the phytochemical and antioxidant activity of peach and plum germplasm. *J. Amer. Soc. Hort. Sci.* **2007**, *132*, 334–340. [CrossRef]
49. Legua, P.; Hernández, F.; Díaz-Mula, H.M.; Valero, D.; Serrano, M. Quality, bioactive compounds, and antioxidant activity of new flat-type peach and nectarine cultivars: A comparative study. *J. Food Sci.* **2011**, *76*, C729–C735. [CrossRef] [PubMed]
50. Tavarini, S.; Degl'Innocenti, E.; Remorini, D.; Massai, R.; Guidi, L. Preliminary characterisation of peach cultivars for their antioxidant capacity. *Int. J. Food Sci. Technol.* **2008**, *43*, 810–815. [CrossRef]
51. Nowicka, P.; Wojdylo, A.; Laskowski, P. Principal component analysis (PCA) of physicochemical compounds' content in different cultivars of peach fruits, including qualification and quantification of sugars and organic acids by HPLC. *Eur. Food Res. Technol.* **2019**, *245*, 929–938. [CrossRef]
52. Gecer, M.K. Biochemical content in fruits of peach and nectarine cultivars. *Turk. J. Agric. For.* **2020**, *44*, 500–505. [CrossRef]
53. Robertson, J.A.; Horvat, R.J.; Lyon, B.G.; Meredith, F.I.; Senter, S.D.; Okie, W.R. Comparison of quality characteristics of selected yellow-and white-fleshed peach cultivars. *J. Food Sci.* **1990**, *55*, 1308–1311. [CrossRef]
54. Delwiche, M.J.; Baumgardner, R.A. Ground colour as a peach maturity index. *J. Am. Soc. Hort. Sci.* **1985**, *110*, 53–57.
55. Ferrer, A.; Remón, S.; Negueruela, A.I.; Oria, R. Changes during the ripening of the very late season Spanish peach cultivar Calanda Feasibility of using CIELAB coordinates as maturity indices. *Sci. Hortic.* **2005**, *105*, 435–446. [CrossRef]
56. Pinto, C.; Reginato, G.; Shinya, P.; Mesa, K.; Díaz, M.; Atenas, C.; Infante, R. Skin color and chlorophyll absorbance: Indices for establishing a harvest date on non-melting peach. *Sci. Hortic.* **2015**, *195*, 231–236. [CrossRef]
57. Cáceres, D.; Díaz, M.; Shinya, P.; Infante, R. Assessment of peach internal flesh browning through colorimetric measures. *Postharvest Biol. Technol.* **2016**, *111*, 48–52. [CrossRef]

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