



## Article

# Designing the Quality Characteristics of Berry Processing Byproducts Using Fermentation

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**Abstract:** In recent years, there has been increasing interest in berry fruit processing byproducts, namely, seeds, pulp, and peel, due to the high content of nutritionally valuable ingredients. The market is seeing an increase in the popularity of fermented products, especially those from vegetables or fruits. Fermented fruit pomace can be used as an ingredient or food additive. Many studies have confirmed that the fermentation process can increase the antioxidant activity of plant extracts due to the decomposition of cell walls. The aim of this study was to evaluate the microbiological quality and antioxidant potential of fermented berry pomace (from chokeberry, blackcurrant, raspberry, and strawberry) in terms of its potential use as an alternative source of valuable ingredients for the design of new food products. The scope of this research included assessing microbiological quality, vitamin C and total phenolic compound (TPC) contents, and antioxidant activity using ABTS, DPPH, and FRAP assays. The polyphenolic compound and vitamin C contents, as well as antioxidant activity, depended on the mixture of microbial strains used for fermentation and the type of fruit pomace. The most favorable parameters for TPC, ABTS, DPPH, and FRAP were obtained for chokeberry pomace samples inoculated with yeast cultures. Chokeberry pomace exhibited the highest vitamin C content when inoculated with a mixture of bacteria.

**Keywords:** byproducts of berries; valorization pomace; management of fruit byproducts; sustainable fermented product; food design



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## 1. Introduction

The valorization of byproducts generated during fruit processing is a consistently growing trend observed in both economic practice and the world of science. These byproducts pose serious problems both economically and ecologically. Currently, these byproducts are often disposed of in landfills, composting plants, or fed into the fermentation process of biogas plants. However, these methods contribute to greenhouse emissions and release waste into the environment. In addition, they result in losses of valuable biomass, nutrients, and economic resources. Therefore, the reuse of these byproducts can lead to both economic and ecological benefits. Activities related to reduction, recycling, and reuse are part of the global trend associated with the concepts of sustainable development and consumption [1–5].

Chokeberry [6,7], blackcurrant [8,9], raspberry [10,11], and strawberry [12,13] represent fruits with a high content of polyphenolic compounds. Their immunity-strengthening,

anti-inflammatory, and antioxidant properties have previously been reported [14–17]. These fruits are an important element of the human diet; most of them, however, are sent for processing in order to obtain juices, nectars, and musts, as well as other products. During fruit juice production, between 20% and 60% of the weight of raw pomace materials is produced [18,19], which can be used for fertilizers, feed, energy, and cosmetics, among other purposes [20–24].

The byproducts of fruit processing, such as chokeberries, blackcurrants, raspberries, and strawberries, similar to the raw materials themselves, are rich in nutritionally valuable ingredients. These include dietary fiber, essential unsaturated fatty acids, exogenous amino acids, dyes, substances with antioxidant properties, and sterols [25–30]. Therefore, they can be a valuable secondary raw material in the food industry due to their use in production, including innovative additives that enrich food products or as ingredients in dietary supplements [31]. Research has shown that raspberry pomace represents a valuable source of fiber and bioactive substances in gluten-free bread [32], waffles [33], and confectionery products [34]. Bobinaite et al. [35] found that adding raspberry pomace extract to fruit purees resulted in an almost threefold increase in the total content of polyphenols, thus improving the functional properties of the products. In another study, the addition of blackcurrant and strawberry pomace to bread increased the antioxidant potential compared to the control bread [36,37]. According to research by Tahvonon et al. [38], supplementation with blackcurrant seed oil lowers LDL cholesterol levels more effectively than supplementation with fish oil. Chokeberry pomace, a source of bioactive compounds, especially anthocyanins, is a valuable secondary raw material used to produce dark anthocyanin dyes [39]. Chitosan films with chokeberry pomace extracts showed higher antioxidant properties than the control sample. Chokeberry pomace extracts strengthened the barrier properties of chitosan films against both UV–Vis light and water vapor, while also reducing their oxygen permeability [40]. Chokeberry pomace has been used as a functional ingredient in preserves [41,42]. According to Goldmeyer, Pena, Melo, and da Rosa [43], blueberry pomace flour can be used to produce fermented drinks. It exhibits microbiological stability during storage and excellent technological properties, which allow it to be used to create new products.

To date, producers and consumers have not sufficiently utilized the byproducts of berry processing as a source of natural bioactive substances with beneficial effects on the body, primarily due to the low acceptance of their taste. The appropriate processing of berry byproducts can alter their unfavorable organoleptic properties and increase their antioxidant potential. One of the processes that can change the organoleptic characteristics and antioxidant properties of berry pomace products is fermentation [44]. The fermentation process uses microorganisms, such as lactic acid bacteria and yeast, the products of which are characterized by new organoleptic properties and potentially higher nutritional value [45,46]. Fermentation makes it possible to modify the organoleptic characteristics of fermented products, thereby affecting their taste, aroma, consistency, and acidity, as well as their color and antioxidant potential [45,47]. Fermentation represents an opportunity to enhance the economic benefits of berry pomace by enabling the processing of agrifood waste and extending the shelf life of fermented products. Fermented byproducts from berries processing may also encourage producers and consumers to adopt a circular bioeconomy and constitute a potentially new direction for the valorization of berry processing byproducts in the food industry. However, the lack of fermentation standardization is often discussed in the literature due to the use of various raw materials and strains in fermentation, implying different effects on raw materials [46–48]. Therefore, the research gap in relation to this issue should be filled by conducting a comprehensive study.

The novelty of this study lies in its use of three fermentation processes applied to four common byproducts of berry processing. For this purpose, the microbiological quality and antioxidant potential of pomace from fermented berries (i.e., chokeberry, blackcurrant, raspberry, and strawberry) were assessed in terms of their potential use as an alternative source of valuable ingredients for designing new food products.

## 2. Materials and Methods

### 2.1. Materials

The raw materials for the research were randomly selected from commercially available dried chokeberry, blackcurrant, raspberry, and strawberry pomace. The fermentation of pomace was carried out using three processes: (B) pomace inoculated with a mixture of bacteria (*Lactobacillus acidophilus* ATCC 4356, *Lactococcus lactis* subsp. *lactis* ATCC 11454, and *Lactobacillus rhamnosus* ATCC 7496); yeast (D) (*Saccharomyces cerevisiae* ATCC 4098 and *Saccharomyces cerevisiae* ATCC 9763 (WDCM 00058)); and bacteria and yeast (BD) (*Lactobacillus acidophilus* ATCC 4356, *Lactococcus lactis* subsp. *lactis* ATCC 11454, *Lactobacillus rhamnosus* ATCC 7496, *Saccharomyces cerevisiae* ATCC 4098, and *Saccharomyces cerevisiae* ATCC 9763 (WDCM 00058)) (Argenta, Poland). Before inoculation, bacteria were cultured on De Man–Rogosa–Sharpe agar (MRS medium; Argenta, Poland) at 30 °C for 24 h, while yeasts were cultured on Sabouraud dextrose agar (SDA; Argenta, Poland) at 30 °C for 48 h. A total of 30 g of pomace inoculated with a mixture of microorganisms suspended in 300 mL of glucose solution at a concentration of 30 g/L was used for fermentation. The densities of individual strains, determined using a DENSIMAT densitometer (BioMerieux, Poland), were 0.5 McF/mL for bacteria and 1 McF/mL for yeast. After adding the microbial suspension, the flasks were placed in a Biosan ES-20/80C shaking incubator (Biogenet, Poland) at 30 °C for 20 min. Pomace samples were tested before fermentation (control samples) and after two days of fermentation. The fermented pomace was frozen at −18 °C and then subjected to a freeze-drying process. The drying process was carried out in a TG-15 freeze dryer for 16 h. The pomace was stored in barrier packaging, protected from light, at room temperature until the analyses were carried out.

Analyses were carried out on infusions prepared from fermented pomace. The infusions were prepared in accordance with the PN ISO 3103:1996 standard [49]. Each infusion was prepared by combining 2 g of pomace with 200 mL of freshly boiled water and then brewed for 6 min. No sweeteners were added to the prepared infusions. After the appropriate brewing time, the infusions were poured into beakers.

### 2.2. Methods

We assessed the microbiological quality, TPC, and antioxidant activity using the ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), and FRAP (ferric reducing antioxidant power) methods. We also measured the vitamin C content.

Pomace samples inoculated with microorganisms were microbiologically tested using the culture method, before and after fermentation. A volume of 1 mL of suspension was taken from the flasks, and a series of 10-fold dilutions were made in a sterile 0.9% sodium chloride physiological solution. The prepared dilutions were inoculated onto plates with the following media: MRS agar (number of lactic acid bacteria and *Saccharomyces cerevisiae*), SDA (number of fungi), and plate count agar (PCA; total number of microorganisms; Argenta, Poland). The cultures were incubated at 30 °C for 24–72 h.

Fermentation was carried out using mixtures of reference strains of bacteria and yeast with a defined composition and count. To standardize the fermentation process, we utilized commercially available berry pomace (chokeberry, blackcurrant, raspberry, and strawberry) and the defined composition of reference strains of lactic acid bacteria and *Saccharomyces cerevisiae* from the ATCC collection. Each strain was added to fermentation at a defined count of  $10^8$  CFU/mL. The number of viable microbial cells was controlled by measuring microbiological cultures for the control sample and the samples after fermentation.

TPCs were measured using the colorimetric method [50], with gallic acid serving as the standard. Volumes of 5 mL of distilled water, 0.5 mL of Folin–Ciocalteu reagent, and 0.1 mL of the sample were added to volumetric flasks (10 mL). After 3 min of incubation at room temperature, 1.5 mL of 20% sodium carbonate solution was added to the flasks. Then, the flasks were filled with distilled water until reaching a designated mark. After incubating the infusions with the reagent for 2 h, the absorbance was measured at a wavelength of

$\lambda = 725$  nm, and the phenolic compound content (in mg GA/L) was determined based on the standard curve.

To assess the free radical scavenging ability, we employed the reaction of the examined samples with ABTS cation radicals. The determination was carried out following the method of Ding et al. [51] with minor modification. Absorbance was measured at a wavelength of  $\lambda = 734$  nm after 6 min of sample incubation at 37 °C. The antioxidant activity of the infusions was determined based on the standard curve and expressed in mmol Trolox/L.

We evaluated the antioxidant properties of the extracts obtained from fruit tea pomaces following the method of Dziecioł et al. [52], modified by using a DPPH radical solution with a concentration of 0.0025 g/100 mL in 96% ethyl alcohol. The DPPH radical (2,2-diphenyl-1-picrylhydrazyl) is stable under normal conditions. This method involves assessing the extent to which antioxidants present in the fruit pomaces reduce DPPH radicals. Absorbance measurements were taken every half minute for 10 min at a wavelength of  $\lambda = 515$  nm. The antioxidant activity of the infusions was determined based on the standard curve and expressed in mmol Trolox/L.

FRAP was measured using a technique previously described by Bratu et al. [53] with minor modification. The FRAP analysis was performed at a wavelength of  $\lambda = 593$  nm. The antioxidant activity of the infusions was determined based on the standard curve and expressed in mmol FeSO<sub>4</sub>/L.

The content of vitamin C, in the form of L-ascorbic acid, was determined using standard methods [54] and expressed in mg/100 g.

The results represent the arithmetic mean of at least three replicates. Analysis of variance (ANOVA) was performed to compare the mean values. Tukey's test was applied to verify the significance of differences between mean values, employing Statistica 13.3 software. A significance level of  $p < 0.05$  was adopted for statistical estimation.

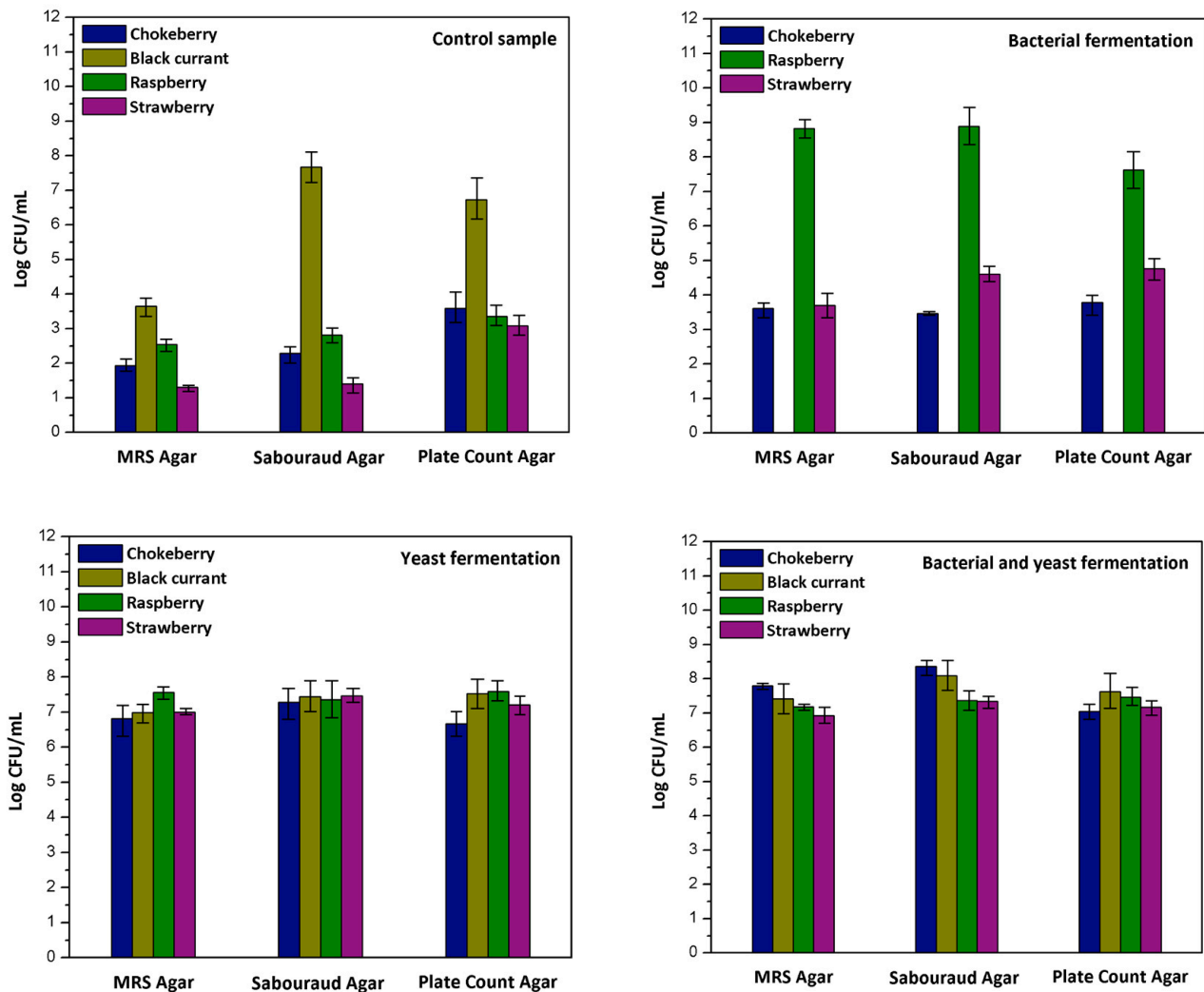
### 3. Results

#### 3.1. Fermentation of Pomace

Three fermentation variants were carried out using various strains of microorganisms. In the first variant (B), lactic acid bacteria strains were used, namely, *Lactobacillus acidophilus* ATCC 4356, *Lactococcus lactis* subsp. *lactis* ATCC 11454, and *Lactobacillus rhamnosus* ATCC 7496. In the second variant (D), yeast strains were utilized, namely, *Saccharomyces cerevisiae* ATCC 4098 and *Saccharomyces cerevisiae* ATCC 9763 (WDCM 00058). In the third variant, all the abovementioned strains were employed (BD). Microorganisms were added at an amount of  $1 \times 10^8$  of each strain per mL of fermentation solution.

#### 3.2. Microbiological Analysis of Fermented Pomace

Microbiological tests were performed on pomace samples before and after fermentation to determine the number of microorganisms grown on MRS, Sabouraud agar, and plate count agar. The results are summarized in Figure 1. In the control, the highest number of colonies was observed in the blackcurrant pomace sample ( $4.5 \times 10^3$  cfu/mL on MRS,  $4.7 \times 10^7$  on Sabouraud agar, and  $5.3 \times 10^6$  on PCA), while the fewest colonies grew from the inoculation of strawberry pomace ( $2.0 \times 10^1$  cfu/mL on MRS,  $2.5 \times 10^1$  on Sabouraud agar, and  $1.2 \times 10^3$  on PCA). After 48 h of fermentation using lactic acid bacteria, the highest number of colonies per mL of the sample was observed on all media with inoculations from raspberry pomace ( $6.8 \times 10^8$  on MRS,  $7.5 \times 10^8$  on Sabouraud agar, and  $4.0 \times 10^7$  on PCA). Due to the intense growth of mold on the plates with inoculation of blackcurrant pomace fermented using lactic acid bacteria, it was not possible to determine the number of microorganisms for this sample. Samples fermented with yeast demonstrated fewer visible differences in the number of living microorganism cells across all pomace samples. The fewest colonies grew on the PCA medium for the chokeberry pomace ( $4.7 \times 10^6$  cfu/mL), while the highest number of colonies was determined on this medium for raspberry pomace ( $3.8 \times 10^7$  cfu/mL).



**Figure 1.** The viable cell count of microorganisms determined in both the fermented pomace and control samples ( $n = 3$ ).

For all samples fermented with a mixture of bacteria and yeast, a lower number of microorganisms was observed on PCA than on MRS and Sabouraud media. This difference was most visible for the chokeberry pomace sample, the inoculation of which showed a colony number of  $6.2 \times 10^7$  per mL on the MRS plate,  $2.4 \times 10^8$  on Sabouraud agar, and  $1.1 \times 10^7$  on the PCA medium.

### 3.3. Total Polyphenol Content, Antioxidant Activity, and Vitamin C Content of Fermented Pomaces

Table 1 shows the total phenolic compound content, antioxidant activity, and vitamin C content in chokeberry, blackcurrant, raspberry, and strawberry pomace samples before and after fermentation. The tested samples differed significantly in their TPC content, antioxidant properties, and vitamin C content.

The polyphenol content, antioxidant activity, and vitamin C content depended on the microbial culture and fruit pomace used. Among the tested pomace samples, those made from fermented chokeberry, raspberry, and strawberry, inoculated with a yeast mixture and incubated for 48 h, exhibited the highest content of TPCs compared to the control samples, at 158.21 mg GA/L, 82.06 mg GA/L, and 78.72 mg GA/L, respectively. In the case of blackcurrant pomace, the highest content of phenolic compounds was recorded for the sample fermented with a mixture of bacteria (78.72 mg GA/L). A similar relationship was

observed when evaluating antioxidant activity via the ABTS, DPPH, and FRAP methods, the highest concentration of which was recorded for fermented chokeberry, raspberry, and strawberry pomace inoculated with a yeast mixture and incubated for two days. However, for blackcurrant, the highest concentration was recorded for the fermented sample with a mixture of bacteria.

**Table 1.** Total polyphenol content, antioxidant activity, and vitamin C content in the fermented pomace and control sample (n = 3).

Sample		Fermentation Time (day)	TPC (mg GA/L)	ABTS (mmol Trolox/L)	DPPH (mmol Trolox/L)	FRAP (mmol FeSO <sub>4</sub> /L)	Vitamin C (mg/100 g)
Chokeberry pomace	Control sample	0	122.51 ± 0.84 <sup>b</sup>	1.30 ± 0.09 <sup>b</sup>	0.44 ± 0.02 <sup>b</sup>	0.68 ± 0.09 <sup>b</sup>	0.62 ± 0.08 <sup>a</sup>
	B	2	90.43 ± 1.28 <sup>a</sup>	1.08 ± 0.19 <sup>a</sup>	0.35 ± 0.04 <sup>a</sup>	0.50 ± 0.03 <sup>a</sup>	1.19 ± 0.04 <sup>c</sup>
	D	2	158.21 ± 3.17 <sup>d</sup>	1.67 ± 0.07 <sup>c</sup>	0.67 ± 0.04 <sup>d</sup>	0.77 ± 0.05 <sup>d</sup>	0.75 ± 0.04 <sup>a,b</sup>
	BD	2	140.36 ± 2.69 <sup>c</sup>	1.29 ± 0.03 <sup>b</sup>	0.59 ± 0.05 <sup>c</sup>	0.75 ± 0.04 <sup>c</sup>	0.77 ± 0.02 <sup>a,b</sup>
Blackcurrant pomace	Control sample	0	56.96 ± 2.11 <sup>a</sup>	0.57 ± 0.09 <sup>b</sup>	0.14 ± 0.04 <sup>a</sup>	0.39 ± 0.05 <sup>b</sup>	0.42 ± 0.02 <sup>a</sup>
	B	2	78.72 ± 1.28 <sup>c</sup>	0.63 ± 0.10 <sup>c</sup>	0.36 ± 0.03 <sup>d</sup>	0.43 ± 0.06 <sup>c</sup>	0.73 ± 0.04 <sup>b</sup>
	D	2	72.58 ± 1.28 <sup>b</sup>	0.58 ± 0.08 <sup>b</sup>	0.25 ± 0.05 <sup>c</sup>	0.40 ± 0.04 <sup>b</sup>	0.66 ± 0.07 <sup>b</sup>
	BD	2	70.91 ± 1.93 <sup>b</sup>	0.55 ± 0.04 <sup>a</sup>	0.21 ± 0.04 <sup>b</sup>	0.35 ± 0.03 <sup>a</sup>	0.42 ± 0.04 <sup>a</sup>
Raspberry pomace	Control sample	0	80.39 ± 1.74 <sup>b</sup>	0.42 ± 0.05 <sup>b</sup>	0.20 ± 0.05 <sup>b</sup>	0.27 ± 0.05 <sup>b</sup>	0.77 ± 0.05 <sup>a</sup>
	B	2	69.79 ± 3.02 <sup>a</sup>	0.33 ± 0.06 <sup>a</sup>	0.21 ± 0.04 <sup>b</sup>	0.29 ± 0.07 <sup>c</sup>	1.14 ± 0.04 <sup>b</sup>
	D	2	82.06 ± 2.94 <sup>b</sup>	0.70 ± 0.16 <sup>c</sup>	0.36 ± 0.13 <sup>c</sup>	0.47 ± 0.02 <sup>d</sup>	0.81 ± 0.04 <sup>a</sup>
	BD	2	77.32 ± 1.67 <sup>b</sup>	0.41 ± 0.17 <sup>b</sup>	0.17 ± 0.02 <sup>a</sup>	0.25 ± 0.03 <sup>a</sup>	0.83 ± 0.04 <sup>a</sup>
Strawberry pomace	Control sample	0	67.56 ± 1.28 <sup>a</sup>	0.59 ± 0.09 <sup>a</sup>	0.14 ± 0.09 <sup>a</sup>	0.38 ± 0.04 <sup>b</sup>	0.49 ± 0.05 <sup>a</sup>
	B	2	71.46 ± 1.67 <sup>a,b</sup>	0.71 ± 0.07 <sup>b</sup>	0.15 ± 0.07 <sup>b</sup>	0.35 ± 0.04 <sup>a</sup>	0.81 ± 0.04 <sup>b</sup>
	D	2	78.72 ± 2.94 <sup>c</sup>	0.80 ± 0.03 <sup>d</sup>	0.21 ± 0.04 <sup>d</sup>	0.49 ± 0.07 <sup>c</sup>	0.53 ± 0.04 <sup>a</sup>
	BD	2	77.04 ± 3.77 <sup>b,c</sup>	0.76 ± 0.06 <sup>c</sup>	0.20 ± 0.02 <sup>c</sup>	0.38 ± 0.02 <sup>b</sup>	0.58 ± 0.01 <sup>a</sup>

Data are expressed as mean ± standard deviation. Microorganisms used for fermentation: B—bacteria; D—yeast; BD—bacteria and yeast. Means that do not share the same letter (a, b, c, and d) are significantly different at  $p < 0.05$  relative to the mixture of microbial strains within a given type of pomace.

When analyzing the influence of microorganisms on the content of vitamin C, the highest concentration was observed for all pomace samples when inoculated with a mixture of bacteria and incubated for 48 h. Chokeberry pomace had the highest vitamin C content (1.19 mg/100 g) when inoculated with a mixture of bacteria.

#### 4. Discussion

In order to determine the number of selected groups of microorganisms in pomace samples, they were inoculated onto three different microbiological media. The growth of lactic acid bacteria and yeast strains was observed on the MRS medium, including those used for fermentation. These microorganisms grew as white, creamy colonies, with the bacteria forming small colonies, while *Saccharomyces* sp. were much larger. Molds and yeast grew on Sabouraud agar. Colonies of various groups of aerobic microorganisms grew on plate count agar, including the yeast used for fermentation. Lactic acid bacteria, however, did not grow on it. By comparing the viable cell counts, the optimal fermentation process was identified, inhibiting mold growth in contaminated fruit pomace, thus restricting its

usability. On all media in the control sample, the highest number of microbial colonies was determined in the case of blackcurrant pomace. These pomace samples were characterized by the highest initial microbiological contamination, exceeding 3 log cfu/mL compared to the other samples. All plates containing cultures of these samples, after fermentation with only lactic acid bacteria, were overgrown with mold. When yeast and a mixture of bacterial and yeast cultures were used for fermentation, mold growth on the seeded plates was not observed, indicating the inhibition of mold growth, likely due to metabolites produced by the yeast strains. As proven in numerous studies, bioactive compounds (phenolics, flavonoids, organic and fatty acids, peptides, volatiles, and enzymes) resulting from fermentation can inhibit the growth of commonly occurring food spoilage mold and be used as natural preservatives [55–59]. This effect was also observed in the experiments that were performed. Although molds grew on both PCA and Sabouraud agar in the control, their growth was not observed on the fermented sample cultures (except for blackcurrant samples fermented with lactic acid bacteria). Notably, Hathout and Abdel-Nasser [60] demonstrated the ability of *Saccharomyces cerevisiae* strains to inhibit the production of mycotoxins. The inhibition of microorganism growth in pomace was also determined by changing the quantitative ratio of individual microorganism groups in the control and fermented pomace samples. For all control samples, except for blackcurrant, the most numerous colonies observed grew on the medium used to determine the total number of microorganisms (PCA). Following 48 h of fermentation using lactic acid bacteria, notably fewer colonies were observed on the PCA medium for the raspberry pomace sample. This was also observed for the chokeberry pomace sample fermented with yeast and a mixture of lactic acid bacteria and yeast. The obtained results confirm the beneficial effect of fermentation on the microbiological quality of the tested pomace.

Fermentation with microorganisms presents a potential approach to modifying the composition of berry pomace by increasing the content of key compounds that contribute to its biological activity. Additional benefits of fermentation include the low temperature required, which helps preserve heat-sensitive compounds, such as vitamin C and polyphenols.

Improving the biological activity of fruit pomace is one of the potential and emerging applications of fermentation. In this work, this strategy was applied to obtain berry pomace with higher TPC and ascorbic acid (vitamin C) contents, as well as antioxidant activity (assessed via ABTS, DPPH, and FRAP assays). Three different mixtures of microorganisms were applied to improve the biological activity of chokeberry, blackcurrant, raspberry, and strawberry pomace samples (Table 1).

Vitamin C is a water-soluble vitamin that is present in fruits and has many nutritional and clinical benefits for human health. In particular, it acts as a strong antioxidant through its electron donation potential [61]. Fermentation with a mixture of lactic acid bacteria (*Lactobacillus acidophilus*, *Lactococcus lactis* subsp. *lactis*, and *Lactobacillus rhamnosus*) led to a significant increase in vitamin C content in all berry pomace samples compared with the control samples. These results are in agreement with previous studies [62,63] that reported an increase in ascorbic acid content in orange juice following fermentation with lactic acid bacteria. The greatest increase in vitamin C content occurred when chokeberry pomace was fermented with lactic acid bacteria. The growth was over 90% compared to the control sample. All fermented berry pomace samples had a higher level of vitamin C than the control sample, suggesting that microorganisms may play a role in synthesizing vitamin C during fermentation [64]. However, fermentation with *Saccharomyces cerevisiae* led to moderate growth of vitamin C for chokeberry and blackcurrant samples, exhibiting increases of over 20% and 55%, respectively. In raspberry and strawberry samples fermented with yeast, vitamin C was not statistically significant compared to the control sample. In terms of the fermentation process, a mixture of lactic acid bacteria and yeast led to an increase in vitamin C content only for chokeberry pomace.

Phenolic acids are associated with the biological activity and sensory qualities of foods [65]. Therefore, we investigated the evolution of TPC in berry pomace samples during fermentation with *Saccharomyces cerevisiae*. The results showed that TPC increased

by over 38%, 29%, and 16% for blackcurrant, chokeberry, and strawberry pomace samples, respectively. In the case of raspberry pomace, the increase in TPC was not statistically significant compared to the control sample. The increase in phenolic compounds in fermented pomace is due to the action of cellulolytic, lignolytic, and pectinolytic enzymes, which are produced mainly during the growth of fungi [66,67]. These enzymes effectively break down the cell wall components and hydrolyze the ester bonds that connect individual phenolic compounds with the cell wall. Consequently, this process increases valuable secondary metabolites in berry pomace.

However, fermentation with a mixture of lactic acid bacteria (namely, *Lactobacillus acidophilus*, *Lactococcus lactis* subsp. *lactis*, and *Lactobacillus rhamnosus*) led to a reduction in TPC of about 35% and 25% for chokeberry and raspberry pomace samples, respectively. In the case of strawberry pomace, the increase in TPC was not statistically significant compared to the control sample. Phenolics can bind with sugar or amino acids [65], thus lowering soluble phenolic contents [68]. During fermentation, the total soluble phenolics that increase in the fermented fruit substrate might also be mobilized and degraded, resulting in lower phenolic contents [69].

Fermentation with a mixture of lactic acid bacteria and yeast led to the growth of TPC by over 25%, 15%, and 14% in blackcurrant, chokeberry, and strawberry pomace samples, respectively. In the case of fermented raspberry pomace, a reduction in TPC was observed.

Different factors influence antioxidant activity in complex heterogeneous biological systems; thus, an evaluation cannot rely only on a one-assay protocol. For this reason, three assays (namely, ABTS, DPPH, and FRAP) were chosen to evaluate the antioxidant capacity of berry pomace during fermentation. While ABTS is based on the hydrogen atom transfer mechanism, DPPH and FRAP are based on the electron transfer mechanism [70]. After fermentation with lactic acid bacteria, the free radical scavenging capacity showed a slight reduction in the case of chokeberry and raspberry pomace samples, which may be attributed to the decrease in the polyphenolic content. These results are in agreement with an earlier study [64] that reported a reduction in antioxidant activity in cashew apple juice after fermentation with lactic acid bacteria. However, in the case of blackcurrant and strawberry pomace samples, no statistical difference in comparison with the control sample was observed.

Fermentation of berry pomace with *Saccharomyces cerevisiae* led to increased antioxidant activity, as evidenced by the enhanced DPPH, FRAP, and ABTS radical scavenging activities. A significant increase in antioxidant activity was detected for chokeberry, raspberry, and strawberry pomace samples. For example, the antioxidant activity of raspberry pomace in the DPPH, FRAP, and ABTS assays increased by 67%, 74%, and 80%, respectively, compared to the control sample. In the case of blackcurrant pomace, the increase in radical scavenging activities was not statistically significant, with the exception of the DPPH assay.

The results also indicated that the antioxidant activity of fermented chokeberry pomace is positively correlated with TPC and vitamin C contents. In the case of blackcurrant pomace, DPPH radical scavenging activity is also positively correlated with TPC and vitamin C contents.

Fermentation with a mixture of lactic acid bacteria and yeast led to an increase in antioxidant activity, as assessed via DPPH and ABTS assays, specifically for strawberry pomace. Moreover, this was positively correlated with TPC and vitamin C contents. In the case of chokeberry pomace, this fermentation process caused an increase in radical scavenging activities, as evidenced by the DPPH and FRAP methods. This was also positively correlated with TPC and vitamin C contents. Fermentation of raspberry pomace with a mixture of lactic acid bacteria and yeast reduced antioxidant activity, which was positively correlated with TPC content.

In summary, the highest antioxidant activity for all berry pomace samples was achieved via fermentation with yeast. Vitamin C content also increased under these fermentation conditions in the case of chokeberry and blackcurrant pomace samples. All pomace samples fermented with lactic acid bacteria achieved the highest vitamin C content.



## 5. Conclusions

Fermentation with microorganisms presents a potential approach to modifying berry pomace composition by increasing the content of key compounds that contribute to its biological activity. In the present work, this strategy was applied to improve the quality of berry pomace in terms of polyphenol and ascorbic acid contents, as well as antioxidant activity. The results show that the fermentation process has an impact on the microbiological quality, polyphenol content, and antioxidant activity of berry pomace. The appropriate selection of microbial strains for fermentation enabled the inhibition of mold growth, even in pomace with significant initial microbiological contamination. Notably, yeast showed a stronger preservative effect than bacteria. When analyzing the impact of microorganisms on vitamin C content, the highest concentration was recorded in samples inoculated with a mixture of bacteria and incubated for 48 h across all pomace types. Fermented chokeberry, raspberry, and strawberry pomace inoculated with a yeast mixture exhibited the highest concentration of polyphenols and antioxidant activity. In future research, we intend to use fermented pomace to design new food products, such as fruit teas. For this purpose, it will be necessary to perform sensory tests on fermented pomace to determine their degree of acceptability. These results will aid in selecting fermentation process parameters that yield products with optimal functionality and sensory appeal.

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