applied sciences

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Effect of Fortification of Tahini with Natural Plant Origin Raw Materials on Its Bioactivity

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Abstract: Food fortification is a strategic approach to enhance the nutritional quality of food by incorporating bioactive compounds derived from food by-products. The objective of this study was to assess the impact on bioactivity of tahini enriched with a 4% (*w*/*w*) lyophilized microwave-ultrasound assisted extract of red grape pomace from the Limnio variety and a 6% (*w*/*w*) freeze-dried aquafaba obtained from Lemnian chickpeas of the Panagia variety. The evaluation was conducted through in vitro experiments. The samples under study were extracted by the Bligh and Dyer method and analyzed for their polyphenolic content, as well as for their content in total flavonoids. Assessment of the antioxidant capacity of the extracts was determined in vitro by the ABTS, DPPH, CUPRAC, FRAP and human blood plasma oxidation inhibition (hBPOxi) assays, while the antiplatelet activity of the extracts was determined by the Platelet Activating Factor inhibition assay (PAFi). The bioactivity of fortified tahini (LAC) was increased compared to plain tahini (Control sample). Total phenolics and total flavonoids were increased by 76% and 78%, respectively (10.7 ± 0.2 vs. 6.02 ± 0.3 and 8.5 ± 0.14 vs. 4.76 ± 0.1 μmol Trolox/g, respectively). Antioxidant activities based on ABTS, DPPH, CUPRAC, FRAP and hBPOxi were increased by 101%, 65%, 77%, 73% and 110%, respectively (52.8 ± 0.7 vs. 26.3 ± 0.4; 20.1 ± 1.6 vs. 12.2 ± 0.2; 81.9 ± 0.4 vs. 46.1 ± 2.1; 46.4 ± 4.0 vs. 26.8 ± 0.8; and 12.2 ± 0.9 vs. 5.8 ± 0.2 μmol Trolox/g, respectively), and antiplatelet activity based on PAFi was also increased by 41% (0.19 ± 0.01 vs. 0.32 ± 0.02 mg, respectively). Red grape pomace extract and aquafaba boosted tahini's in vitro antioxidant and antiplatelet capabilities. The results indicate that the valorization of grape and chickpea processing residuals may reduce waste and improve the nutritional value of tahini. Due to its antioxidant and antiplatelet activities, fortified tahini may contribute to the prevention of chronic diseases where oxidation and thrombosis play critical roles. This study exemplifies sustainable resource use and waste reduction, as well as the importance of circular economy strategies in enhancing diets and human welfare.

Keywords: tahini; red grape pomace; aquafaba; bioactivity; food fortification; antioxidant activity; antiplatelet activity

Citation: Achilladelis, P.; Petsas, A.S.; Karantonis, H.C. Effect of Fortification of Tahini with Natural Plant Origin Raw Materials on Its Bioactivity. *Appl. Sci.* **2023**, *13*, 9626. https://doi.org/10.3390/app13179626

Academic Editor: Monica Gallo

Received: 27 July 2023
Revised: 17 August 2023
Accepted: 23 August 2023
Published: 25 August 2023

1. Introduction

Sesame seeds (*Sesamum indicum* L.) contain a multitude of phenolic compounds, which exhibit beneficial effects on human health. Phenolic components, known for their antioxidant, anti-inflammatory and hypolipidemic activities, and, specifically, sesame lignans like sesamin and sesamolin have attracted large interest regarding their nutritional benefits for humans in conditions such as atherosclerosis, hypertension, hyperlipidemia, obesity, diabetes, osteoporosis, arthritis and Alzheimer's disease [1–8]. A number of studies with sesame oil have reported a reduction in oxidative stress and an increase in vitamin E levels [9,10]. However, there is limited data concerning the effect of tahini consumption on human health. A recent study shows that the ingestion of tahini enhances the biomarkers related to metabolic and antioxidant status throughout the postprandial period in healthy

men [11], and a second study presents novel findings indicating that the consumption of tahini may have a beneficial impact on blood pressure, pulse rate and endothelial function in healthy men, implying that including tahini into one's diet as a healthier alternative to snacks with unfavorable lipid profiles could be advantageous [12].

An alternative utilization of food by-products rich in bioactive compounds is their incorporation into other foods, thus making them functional [13–15].

Red grape pomace is a low-cost source of valuable phenolic compounds [16–18]. It has been studied extensively over the past two decades, with clear evidence indicating its effectiveness in reducing the risk of various diseases. Given the plethora of potential health benefits associated with grape phenolic compounds, there has been a growing demand for functional foods fortified with grape extract polyphenols [19,20].

On the other hand, chickpea boiling water called aquafaba produced at home or from canned chickpeas is usually discarded. However, it is valuable not only for its high content of health-promoting compounds, such as polyphenols, but also for its high protein content [21,22].

Red grape pomace and aquafaba are considered by-products of the wine and chickpea industries, respectively. In a circular economy framework, utilizing these by-products as fortifiers for tahini can help minimize waste and create value from materials that would otherwise be discarded. This aligns with the principles of sustainability and resource efficiency. At the same time, fortifying tahini with red grape pomace can enhance its nutritional profile, potential health benefits and appeal to health-conscious consumers.

The purpose of this study was to evaluate in vitro the fortification of tahini with an extract of red grape pomace of the Limnio variety and aquafaba from Lemian chickpeas of the Panagia variety and their effect on its bioactivity. Oxidation, inflammation and thrombosis mechanisms are directly involved in the development of chronic diseases and the study of food substances with possible antioxidant, anti-inflammatory and anti-thrombotic activity can reveal bioactivities that indicate a preventive effect against their development [23–25].

2. Materials and Methods

2.1. Reagents and Chemicals

Tahini was kindly offered by the Achilladelis-Lemnos company (Lemnos, Myrina, Greece). Folin–Ciocalteau reagent, anhydrous Na_2CO_3 and 6-hydroxy-2,5,7,8-tetremethychroman-2-carboxylic acid (Trolox) were purchased from Scharlab (Scharlab, S.L., Barcelona, Spain). 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), 2,20-Azino-bis-(3-ethylbezothiazoline-6-sulphonic acid (ABTS) and Neocuproine were purchased from Glentham Life Sciences (Corsham, UK). Methanol, ammonium acetate, sodium chloride, sodium dihydrogen phosphate dihydrate, copper chloride dihydrate and potassium persulfate were all purchased from Chem-Lab (Zedelgem, Belgium).

2.2. Preparation of Fortification Materials

2.2.1. Preparation of Sesame Cake

The preparation of the sesame cake was done by removing the oil of sesame seeds using n-hexane. After checking for obvious defects or residues, 100.0 g of Lemnian sesame seeds were pulverized for 10 s by a laboratory mill (IKA, A 10 Basic, Staufen, Germany), and then 300.0 mL of n-hexane was added. This was followed by stirring on a magnetic stirrer (Steroglass s.r.l., Steromag, Perugia, Italy) for 30 min, decanting from the solvent and drying in an oven (Binder GmbH, ED 115, Tuttlingen, Germany) at 70 °C for 72 h. The ground-defatted sesame seeds were sieved in a household sieve and stored at room temperature until use (Figure 1A).

Figure 1. Representation of the fortification materials used: (**A**) defatted sesame cake; (**B**) red grape pomace of Limnio variety; (**C**) lyophilized extract of red grape pomace of Limnio variety with defatted sesame cake; and (**D**) freeze-dried aquafaba from Panagia variety Lemnian chickpeas.

2.2.2. Limnio Red Grape Pomace Extract

An amount of 350.0 g of red grape pomace of Limnio variety (Figure 1B) was treated in a microwave extractor for natural products (Milestone Srl, ETHOS X, Milan, Italy) at 350 Watts for 33 min. Then, 25.0 g of the microwave-treated pomace was extracted with ultrasounds in 250 mL of water (10% w/v) in a bath sonicator (Elma Schmidbauer GmbH, Elmasonic P, Singen, Germany) at 37 KHz, with 100% power at 70 °C for 30 min. The extract was filtered and stored at −80 °C until further analysis.

2.2.3. Lyophilization—Drying of Extract

The extracts were lyophilized using a Genevac miVac Duo Concentrator freeze-drying system (Genevac Ltd., Ipswich, UK). The samples were dissolved in dH_2O, and sesame cake was added in an amount so that the ratio was 50:50 (w/v). After concentration, they were pulverized in a laboratory mill (IKA, A 10 Basic, Staufen, Germany) (Figure 1C), while the dry extract weight was calculated, and the final ratio (extract: sesame cake) was (45.35:54.65). The pure proportion of sesame cake used in the fortification of all samples was 5%.

2.2.4. Preparation of Freeze-Dried Aquafaba

The preparation of aquafaba was done according to He et al. [26] with minor modifications. Dry chickpeas of Lemnos variety Panagia were soaked in a ratio of 1:4 with potable soft water for 16 h at 7 °C. The water was then discarded. In a household pressure cooker (Fissler, 8 L, Idar-Oberstein, Germany) 2.0 Kg of soaked chickpeas and 3.0 L of distilled water at a ratio of (2:3) were added and boiled for 30 min.

Aquafaba was separated from cooked chickpeas using a stainless-steel filter with 1 mm holes and stored at −80 °C for 24 h. The aquafaba samples were then freeze-dried (Biobase Biodusrty, Shandong, Co., Ltd., BK-FD10PT, Jinan, China) and stored at room

temperature until use (Figure 1D). The proportion of freeze-dried aquafaba used in the fortification was 6%.

2.3. Tahini Fortification

Tahini was fortified with the red grape pomace of Limnio variety extract at 0.25%, 0.5%, 1.0%, 2.0% and 4.0% (*w*/*w*), leading to increased antioxidant activities based on ABTS assay at 10%, 13%, 20%, 35% and 64%, respectively (Table 1). The 4.0% fortification was acquired for the rest of the study due to its highest effect on the antioxidant activity of the final product. Katarzyna Włodarczyk et al. reported an antioxidant activity for aquafaba based on ABTS assay equal to 20.97 μmol Trolox equivalents per one gram. Based on this study, a 6% fortification was acquired for our study, estimating that this would increase about five times the antioxidant activity of tahini [27]. For better results management, tahini mixtures were prepared with each fortification material separately. Thus, we had the following mixtures with the proportions shown in Table 2: Control: tahini; C: tahini and sesame cake; LC: tahini, sesame cake and lyophilized extract of red grape pomace of Limnio variety; AC: tahini, sesame cake and freeze-dried aquafaba from Panagia variety Lemnian chickpeas; and LAC: tahini, sesame cake, lyophilized extract of red grape pomace of Limnio variety and freeze-dried aquafaba from Panagia variety Lemnian chickpeas. In addition, as a reference, raw materials were also studied: Cake: sesame cake; LMU: lyophilized extract of red grape pomace of Limnio variety; and AF: freeze-dried aquafaba from Panagia variety Lemnian chickpeas.

Table 1. Antioxidant activity of tahini fortified with different amounts of red grape pomace of Limnio variety extract.

% Fortification	μmol Trolox/g
0	26.5 ± 1.3^{a}
0.25	29.2 ± 1.6^{ab}
0.5	30.0 ± 1.2^{bc}
1.0	31.8 ± 1.8^{cd}
2.0	35.8 ± 1.6^{e}
4.0	43.5 ± 2.7^{f}

Results are presented as mean ± standard deviation of μmol Trolox per gram of fortified sample that corresponded to 50% radical scavenging, based on ABTS assay. Samples without a common letter (a–f) are significantly different according to Tukey's test.

2.4. Extraction of the Fortified Tahini

A quantity of the samples under study (2.0 g) was placed in 50.0 mL screw cap polypropylene centrifuge tubes. Methanol in a volume of 4.0 mL was added and stirred in a vortex stirrer (Labbox Labware S.L., LBX instruments V05 series, Barcelona, Spain) for 1.0 min. Then, mixtures were stirred on an orbital shaker (Gesellschaft für Labortechnik mbH, GFL 3017, Burgwedel, Germany) at 250 rpm for 10 min.

Table 2. Proportions of tahini fortification.

Samples	% Tahini	% Sesame Cake	% Pomace Extract	% Aquafaba
Control	100	-	-	-
C	95	5	-	-
LC	91	5	4	-
AC	89	5	-	6
LAC	85	5	4	6

Control: tahini; C: tahini and sesame cake; LC: tahini, sesame cake and lyophilized extract of red grape pomace of Limnio variety; AC: tahini, sesame cake and freeze-dried aquafaba from Panagia variety Lemnian chickpeas; and LAC: tahini, sesame cake, lyophilized extract of red grape pomace of Limnio variety and freeze-dried aquafaba from Panagia variety Lemnian chickpeas.

The mixtures were subjected to further separation into their hydrophilic and lipophilic components through a biphasic solvent system formation according to the method of Bligh and Dyer [28] with slight modifications. Volumes of 1.6 mL dH_2O and 2.0 mL chloroform were added, resulting in a ratio of chloroform:methanol:water equal to 1:2:0.8 ($v/v/v$), and stirred on the orbital shaker at 250 rpm for another 5 min. The solutions were centrifuged (Orto Alresa—Alva-rez Redondo SA, Digicen 21R, Madrid, Spain and HERMLE Labortechnik GmbH, Z383, Wehingen, Germany) at 1500× g for 10 min and the supernatant was poured into 15 mL polypropylene screw tubes. Then, 2.0 mL chloroform and 2.0 mL dH_2O were added to obtain a final ratio of chloroform:methanol:water equal to 1:1:0.9 ($v/v/v$) to create the biphasic system. The phases were separated by centrifugation at 1500× g for 5 min (HERMLE Labortechnik GmbH, Z383, Wehingen, Germany); the lower phase (chloroformic) was discarded and the upper (water-methanolic) was stored at −40 °C for 24 h. Then, 1.0 mL extract was transferred to 1.5 mL capped polypropylene Eppendorf vials and centrifuged (Hermle Labortechnik GmbH, Z216 MK, Wehingen, Germany) at 20,000× g for 5 min. After that, 0.800 mL without sediment were transferred to new capped polypropylene Eppendorf vials and stored at −40 °C until further analysis.

2.5. Determination of Total Phenolic Content

Total phenolic content in the extracts was determined according to the method of Singleton and Rossi [29] with minor modifications. Total phenolics assay was conducted by mixing aliquots of fortified tahini extracts with distilled water up to the volume of 1.8 mL and 0.1 mL Folin–Ciocalteu reagent. After that, the samples were vigorously stirred and incubated in the dark for 2 min. Then, 0.3 mL of aqueous Na_2CO_3 20% (w/v) was added and the samples were vigorously stirred and incubated in a water bath at 40 °C for 30 min. Absorbance was measured at 765 nm using a photometer (Rayleigh, VIS-7220G, Shenzhen, China). A standard curve was prepared with gallic acid. Final results were given as mg of gallic acid equivalents per one g of final product. Every sample was tested in triplicate.

2.6. Determination of Total Flavonoid Content

Total flavonoid assay was conducted by mixing aliquots of fortified tahini extracts with distilled water up to the volume of 0.740 mL and 0.030 mL 5% (w/v) $NaNO_2$. After vigorously vortexing at 2500 rpm for 5 s and leaving samples for 5 min, 0.03 mL of 10% (w/v) $AlCl_3$ was added, followed by stirring. After 6 min, 0.2 mL NaOH 1 M was added, and the samples were vortexed again. Absorbance was measured at 510 nm using a photometer (Rayleigh, VIS-7220G, Shenzhen, China). A standard curve was prepared with rutin. Results were given as mg of rutin equivalents per one g of final product. Every sample was tested in triplicate.

2.7. Evaluation of the Antioxidant Capacity

The antioxidant capacity of the extracts was assessed by the ABTS, DPPH, CUPRAC and FRAP assays.

2.7.1. ABTS Assay

Determination of ABTS radical scavenging activity of extracts was performed as described elsewhere [30] with minor modifications. ABTS radical cation ($ABTS^{\bullet+}$) was generated by the oxidation of ABTS with potassium persulphate ($K_2S_2O_8$). The $ABTS^{\bullet+}$ was produced by reacting to a 7.0 mmol/L stock solution of ABTS with 2.45 mmol/L potassium persulphate (final concentration). The $ABTS^{\bullet+}$ solution was diluted (with distilled water) to an absorbance of 0.700 at 734 nm. A volume of 1.0 mL of $ABTS^{\bullet+}$ was added to aliquots of fortified tahini extracts, or appropriate standard solution of Trolox, and were vigorously stirred. Samples were incubated in the dark for 15.0 min at ambient temperature, and the absorbance was measured at 734 nm using a photometer (Rayleigh, VIS-7220G, Shenzhen, China). Results were expressed as µmol Trolox/g final product using the standard Trolox

solutions assayed at conditions similar to the sample extracts. Every sample was tested in triplicate.

2.7.2. DPPH Assay

The capacity of the fortified tahini extracts to scavenge the free radical of DPPH was assessed by a modified method as previously described [30]. DPPH working solution was 0.6 mM in methanol with an absorbance of 0.600 at 515 nm. A volume of 1.0 mL of the working solution of DPPH was added to aliquots of fortified tahini extracts or the appropriate standard solution of Trolox, followed by vigorous stirring. Samples were incubated in the dark for 15 min at ambient temperature, and the absorbance was measured at 515 nm against a reference sample containing methanol instead of a sample aliquot using a photometer (Rayleigh, VIS-7220G, Shenzhen, China). Results were expressed as μmol Trolox/g final product using the standard Trolox solutions assayed at conditions similar to the sample extracts. Every sample was tested in triplicate.

2.7.3. CUPRAC Assay

The reducing potential of the sample was determined using the CUPRAC assay as previously described [31]. Different volumes of the extracts were transferred in test tubes and solutions of 0.3 mL $CuCl_2 \cdot 2H_2O$ 10 mM, 0.3 mL neocuproine 7.5 mM and 0.3 mL ammonium acetate buffer 1M, pH = 7.0, were added. A final volume of 1.2 mL was reached with dH_2O. The samples were well stirred and incubated at room temperature for 30 min. After incubation, the absorbance of the samples was measured at 450 nm (Rayleigh, VIS-7220G, Shenzhen, China). A standard curve was prepared using different concentrations of Trolox. The antioxidant activity was expressed as μmol Trolox/g final product. Every sample was tested in triplicate.

2.7.4. FRAP Assay

The reducing potential of the samples was determined using the FRAP assay as described elsewhere [32]. The method is based on the reduction of the Fe^{3+}-tripyridyltriazine complex to its ferrous-colored form at low pH in the presence of antioxidants. The FRAP reagent was freshly prepared daily and contained a ratio of 1:1:1 ($v/v/v$) of TPTZ (2,4,6-tripyridyl-s-triazine) 10 mM solution in HCl 40 mM, $FeCl_3 \cdot 6H_2O$ 20 mM and acetate buffer 3.0 mM, pH = 3.6. The aliquots of the extracts were transferred in glass test tubes and dissolved in distilled water to a final volume of 0.9 mL, followed by an addition of 0.3 mL of FRAP solution. The samples were stirred and incubated for 10 min in a 40 °C water bath, and the absorbance was measured at 593 nm using a photometer (Rayleigh, VIS-7220G, Shenzhen, China). A standard curve was prepared using different concentrations of Trolox. The antioxidant activity was expressed as μmol Trolox/g final product. Every sample was tested in triplicate.

2.8. In Vitro Inhibition of Plasma Oxidation

In vitro inhibition of plasma oxidation was evaluated as previously reported [33] with minor modifications. Aliquots of the extracts were transferred in glass test tubes and dried under a stream of nitrogen. Then, they were dissolved in phosphate buffer solution, pH = 7.4, 146 mM in NaCl to a final volume of 0.88 mL. A volume of 0.02 mL of human plasma was added, followed by gentle shaking. In the next step, the oxidation reaction was started by the addition of 0.1 mL of $CuSO_4 \cdot 5H_2O$ 1 mM aqueous solution and the samples were placed immediately into the chamber of the spectrophotometer (PerkinElmer Inc., Lambda 25, Waltham, MA, USA) equipped with an 8 position thermostated sample changer. The absorbance was continuously recorded for 3.0 h at 245 nm at 37 °C. Lag times from the resultant sigmoid curves were calculated as the time needed for the antioxidants to be oxidized, corresponding to the period of time that plasma resists oxidation. The percentage of the inhibition of in vitro plasma oxidation induced by the extracts was evaluated by comparing the lag time of each tested sample containing extract to a reference sample that

did not contain extract. Also, a standard curve was prepared using different concentrations of Trolox. Results were expressed as μmol Trolox/g final product using the standard Trolox solutions assayed at conditions similar to the sample extracts.

2.9. In Vitro Antiplatelet Activity

The in vitro antiplatelet activity of extracts was evaluated on a Born-type aggregometer by their ability to inhibit the thrombotic and inflammatory lipid mediator of Platelet Activating Factor (PAF) towards human platelet rich plasma (PRP) [33]. Aliquots of the extracts and PAF solution were evaporated under a stream of nitrogen and reconstituted in bovine serum albumin (BSA) 2.5 mg/mL saline. After that, aliquots of 0.25 mL of PRP along with stir bars were placed in siliconized glass cuvettes and were incubated for 15 min in the incubation wells of the aggregometer at 37 °C (Chrono-log Corporation, 500CA, Havertown, PA, USA). The platelet response induced by PAF 2.6×10^{-7} M, final concentration, in human PRP before (considered as 0% inhibition) and after the addition of various amounts of the examined samples was recorded by the aggregometer upon stirring at 1200 rpm. The amounts of extracts required for 50% inhibition (IC_{50}) of PAF activity were calculated using the linear plot of the percentage inhibition, ranging from 20 to 80%, versus different amounts of the samples. The in vitro antiplatelet activity was expressed as mg final product required for 50% inhibition of PAF activity towards human PRP.

2.10. Statistical Analysis

Case samples tested in triplicate data are presented as mean ± standard deviation. One-way ANOVA with post-hoc Tukey test was performed to assess statistical significance ($p < 0.05$). Statistical analysis was performed with the statistical package IBM SPSS Statistics for Windows (IBM, version 28.0, Armonk, NY, USA).

3. Results and Discussion

3.1. Total Phenolics

The mixture of tahini, sesame cake, lyophilized pomace extract and freeze-dried aquafaba (LAC) showed the highest concentration of total phenolics (10.71 ± 0.18 mg GAE/g final product), followed by the mixture of tahini, sesame cake and lyophilized pomace extract (LC) (9.01 ± 0.12 mg GAE/g final product), the mixture of tahini, sesame cake and freeze-dried aquafaba (AC) (7.34 ± 0.34 mg GAE/g final product), the plain tahini (Control) (6.02 ± 0.03 mg GAE/g final product) and, finally, the mixture of tahini and sesame cake (C) (5.82 ± 0.16 mg GAE/g final product) (Table 3). Both the lyophilized pomace extract (LMU) (44.86 ± 3.32 mg GAE/g final product) and the freeze-dried aquafaba (AF) (18.87 ± 0.66 mg GAE/g final product) had a higher concentration of total phenolics than the tahini sample, so the order of its fortified samples in the content of phenolic components was as expected: LAC > LC > AC > C ≈ Control, since LMU > AF > Cake > Control. Among the fortified samples, all three (LC, AC, LAC) had significantly higher amounts of total phenolics than plain tahini (Control) ($p < 0.001$, $p = 0.002$ and $p < 0.001$, respectively). Sesame cake (Cake) had 6.89 ± 0.19 mg GAE/g final product (Table 3).

3.2. Total Flavonoids

The mixture of tahini, sesame cake and lyophilized pomace extract (LC) together with the mixture of tahini, sesame cake, lyophilized pomace extract and freeze-dried aquafaba (LAC) presented the highest concentration of total flavonoids (9.00 ± 0.36 mg RE/g final product and 8.45 ± 0.14 mg RE/g final product, respectively), followed by the plain tahini (Control) (4.76 ± 0.10 mg RE/g final product), the mixture of tahini and sesame cake (C) (4.49 ± 0.01 mg RE/g final product) and, finally, the mixture of tahini, sesame cake and freeze-dried aquafaba (AC) (4.07 ± 0.04 mg RE/g final product) (Table 3).

Freeze-dried aquafaba (AF) had the lowest content of total flavonoids of the fortification materials, but also in relation to the plain tahini (Control), with only 1.10 ± 0.03 mg RE/g final product.

Therefore, the reduction of total flavonoids of AC relative to Control ($p = 0.001$) and the approximately equal concentrations of LC and LAC samples were expected. However, there is a significant difference between these samples ($p = 0.009$). The lyophilized pomace extract (LMU) had a total flavonoid content of 61.76 ± 3.74 mg RE/g final product, resulting in an almost twofold increase of the flavonoid content in the fortified samples LC and LAC compared to the plain tahini (Control) ($p < 0.001$ and $p < 0.001$, respectively). Sesame cake (Cake) had 5.01 ± 0.02 mg RE/g final product (Table 3).

Table 3. Results of total phenolics and total flavonoids.

Samples	Total Phenolics (mg GAE/g)	Total Flavonoids (mg RE/g)
Control	6.02 ± 0.03 ^{a}	4.76 ± 0.1 ^{ae}
C	5.82 ± 0.16 ^{a}	4.49 ± 0.01 ^{ac}
LC	9.01 ± 0.12 ^{b}	9 ± 0.36 ^{b}
AC	7.34 ± 0.34 ^{c}	4.07 ± 0.04 ^{c}
LAC	10.71 ± 0.18 ^{d}	8.45 ± 0.14 ^{d}
Cake	6.89 ± 0.19 ^{c}	5.01 ± 0.02 ^{e}
LMU	44.86 ± 3.32 ^{e}	61.76 ± 3.74 ^{f}
AF	18.87 ± 0.66 ^{f}	1.1 ± 0.03 ^{g}

Results for fortified tahini samples and raw materials are expressed as mean ± standard deviation (n = 3) in mg GAE/g final product for total phenolics and in mg RE/g final product for total flavonoids. Control: tahini; C: mixture of tahini and sesame cake; LC: mixture of tahini, sesame cake and lyophilized pomace extract; AC: mixture of tahini, sesame cake and freeze-dried aquafaba; LAC: mixture of tahini, sesame cake, lyophilized pomace extract and freeze-dried aquafaba; Cake: sesame cake; LMU: lyophilized pomace extract; AF: freeze-dried aquafaba; GAE: gallic acid equivalents; and RE: rutin equivalents. In each column, values without a common letter are significantly different according to Tukey's test.

3.3. Antioxidant Capacity

3.3.1. ABTS Assay

All samples showed antioxidant activity through scavenging radical cation of ABTS. The best results were shown by the mixture of tahini, sesame cake, lyophilized pomace extract and freeze-dried aquafaba (LAC), which exerted an activity equivalent to 52.8 ± 0.66 µmol Trolox per one gram of final product. LAC was followed by the mixture of tahini, sesame cake and lyophilized pomace extract (LC), with 47.8 ± 2.50 µmol Trolox/g, the mixture of tahini, sesame cake and freeze-dried aquafaba (AC), with 31.6 ± 0.86 µmol Trolox/g, and, finally, the plain tahini (Control) and the mixture of tahini and sesame cake (C), with 26.3 ± 0.36 µmol Trolox/g and 25.3 ± 0.72 µmol Trolox/g, respectively (Table 4).

Table 4. Results of antioxidant capacity.

Samples	ABTS	DPPH	CUPRAC	FRAP
Control	26.3 ± 0.36 ^{a}	12.2 ± 0.18 ^{a}	46.1 ± 2.14 ^{a}	26.8 ± 0.75 ^{a}
C	25.3 ± 0.72 ^{a}	11.6 ± 0.18 ^{a}	46.1 ± 1.44 ^{a}	26.9 ± 0.50 ^{a}
LC	47.8 ± 2.50 ^{b}	20.9 ± 0.46 ^{b}	75.0 ± 2.22 ^{b}	43.7 ± 1.60 ^{b}
AC	31.6 ± 0.86 ^{c}	12.0 ± 0.19 ^{a}	49.6 ± 2.60 ^{a}	26.9 ± 0.62 ^{a}
LAC	52.8 ± 0.66 ^{b}	20.1 ± 1.65 ^{b}	81.8 ± 0.44 ^{c}	46.4 ± 3.99 ^{b}
Cake	31.1 ± 2.71 ^{c}	12.2 ± 0.32 ^{a}	51.2 ± 1.03 ^{a}	29.9 ± 0.08 ^{a}
LMU	338.3 ± 2.54 ^{d}	181.7 ± 10.1 ^{c}	488.0 ± 5.04 ^{d}	263.3 ± 7.98 ^{c}
AF	38.8 ± 3.00 ^{e}	10.6 ± 1.10 ^{a}	62.7 ± 4.46 ^{e}	26.4 ± 2.34 ^{a}

Results concerning ABTS, DPPH, CUPRAC and FRAP are expressed as mean ± standard deviation (n = 3) µmol trolox/g final product. Control: tahini; C: mixture of tahini and sesame cake; LC: mixture of tahini, sesame cake and lyophilized pomace extract; AC: mixture of tahini, sesame cake and freeze-dried aquafaba; LAC: mixture of tahini, sesame cake, lyophilized pomace extract and freeze-dried aquafaba; Cake: sesame cake; LMU: lyophilized pomace extract; and AF: freeze-dried aquafaba. Values in each column without a common letter are significantly different according to Tukey's test.

LAC and LC had similar results ($p > 0.05$) although freeze-dried aquafaba (AF) with activity equivalent to 38.8 ± 3 µmol trolox/g was lower compared to 26.3 ± 0 µmol

trolox/g required from the plain tahini (Control) ($p < 0.001$); this was possibly due to the very potent lyophilized pomace extract (LMU) with activity equal to 338.1 ± 2.54 μmol Trolox/g. However, among the fortified samples, all three (LC, AC and LAC) had a significant difference compared to plain tahini (Control) ($p < 0.001$, $p = 0.002$ and $p < 0.001$, respectively). As for the sesame cake (Cake), the activity was 31.1 ± 2.71 μmol trolox/g (Table 4).

3.3.2. DPPH Assay

All samples showed antioxidant activity using the DPPH free radical scavenging assay. Specifically, as in the ABTS assay, the best results were presented by the mixture of tahini, sesame cake, lyophilized pomace extract and freeze-dried aquafaba (LAC) and the mixture of tahini, sesame cake and lyophilized pomace extract (LC) with 20.1 ± 1.65 μmol Trolox and 20.9 ± 0.46 μmol Trolox per g final product, respectively. They were followed by plain tahini (Control) (12.2 ± 0.18 μmol Trolox/g final product), the mixture of tahini, sesame cake and freeze-dried aquafaba (AC) (12.0 ± 0.19 μmol Trolox/g final product) and the mixture of tahini and sesame cake (C) (11.7 ± 0.18 μmol Trolox/g final product) (Table 4).

According to the DPPH assay, the antioxidant capacity of the freeze-dried aquafaba (AF) with a value of 10.6 ± 1.10 μmol Trolox/g final product was not statistically different compared to plain tahini (Control) ($p > 0.05$). In addition, sesame cake (Cake) was also at the same level of antioxidant capacity with a value equal to 12.2 ± 0.32 μmol Trolox/g final product ($p > 0.05$). Therefore, similar results of Control, AC and C samples were expected. The lyophilized pomace extract (LMU) again showed very high activity with 181.7 ± 10.1 μmol Trolox/g final product, creating a significant difference between both LC and LAC samples compared with plain tahini (Control) ($p < 0.001$ and $p < 0.001$, respectively), while there was no significant difference between them ($p > 0.05$) (Table 4).

3.3.3. CUPRAC Assay

Based on the results, it appears that tahini and its fortification materials exhibit antioxidant activity through the reduction of copper ions by antioxidants, with the mixture of tahini, sesame cake, lyophilized pomace extract and freeze-dried aquafaba (LAC) showing greater antioxidant capacity (81.9 ± 0.44 μmol Trolox/g final product). The order of the samples with the highest antioxidant capacity after LAC was as follows: mixture of tahini, sesame cake and lyophilized pomace extract (LC) (75.0 ± 2.22 μmol Trolox/g final product) > mixture of tahini, sesame cake and freeze-dried aquafaba (AC) (49.7 ± 2.60 μmol Trolox/g final product) > mixture of tahini and sesame cake (C) (46.1 ± 1.44 μmol Trolox/g final product) > tahini (Control) (46.1 ± 2.14 μmol Trolox/g final product) (Table 4).

Both lyophilized pomace extract (LMU) (488.0 ± 5.04 μmol Trolox/g final product) and freeze-dried aquafaba (AF) (62.7 ± 4.46 μmol Trolox/g final product) had a higher antioxidant capacity than the plain tahini, so the order of its fortified samples in antioxidant activity through reduction of copper ion capacity by antioxidants was as expected; LAC > LC > AC ≈ C ≈ Control, since LMU > AF > Cake ≈ Control. Among the fortified samples, only LC and LAC had a significant difference compared to plain tahini (Control) ($p < 0.001$ and $p < 0.001$, respectively), while they also had a significant difference between themselves ($p = 0.041$). Sesame cake (Cake) had 51.2 ± 1.03 μmol Trolox/g final product (Table 4).

3.3.4. FRAP Assay

Based on the results, it appears that tahini and its fortification materials exhibit antioxidant activity through the reduction of ferric to ferrous form of TPTZ, with the mixture of tahini, sesame cake, lyophilized pomace extract and freeze-dried aquafaba (LAC) exhibiting greater antioxidant capacity (46.4 ± 3.99 μmol Trolox/g final product). The order of the samples with the highest antioxidant capacity after LAC was as follows: mixture of tahini, sesame cake and lyophilized pomace extract (LC) (43.7 ± 1.60 μmol Trolox/g final product) > mixture of tahini, sesame cake and freeze-dried aquafaba (AC) (26.9 ± 0.62 μmol Trolox/g

final product) = mixture of tahini and sesame cake (C) (26.9 ± 0.50 μmol Trolox/g final product) > tahini (Control) (26.8 ± 0.75 μmol Trolox/g final product) (Table 4).

According to the FRAP assay, freeze-dried aquafaba (AF) and sesame cake (Cake) had approximately the same antioxidant capacity (26.4 ± 2.34 and 29.9 ± 0.08 μmol Trolox/g final product, respectively) compared to the plain tahini (Control) ($p > 0.05$), thus the similar results of Control, AC and C samples were expected ($p > 0.05$). The lyophilized pomace extract (LMU) showed a very high activity again with 263.3 ± 7.98 μmol Trolox/g final product, so the LC and LAC samples did not have a significant difference between them ($p > 0.05$), while they did have it with plain tahini (Control) ($p < 0.001$ and $p < 0.001$, respectively) (Table 4).

3.4. In Vitro Inhibition of Plasma Oxidation

Oxidation of plasma lipoproteins plays an important role in atherogenesis, since, at the molecular level, oxidatively modified molecules on oxidized LDLs initiate the events of atherosclerosis development [24]. The in vitro inhibition of plasma oxidation from extracts was expressed as μmol Trolox/g final product. The more Trolox equivalents per g of final product correspond to a sample, the greater the protective effect against the oxidation of plasma lipoproteins.

The mixture of tahini, sesame cake and lyophilized pomace extract (LC) and the mixture of tahini, sesame cake, lyophilized pomace extract and freeze-dried aquafaba (LAC) had the highest activities equal to 13.9 ± 1.1 and 12.2 ± 0.9 μmol Trolox/g final product, respectively ($p < 0.05$). Lower activities were recorded in tahini (Control) with 5.8 ± 0.2 μmol Trolox/g final product, mixture of tahini and sesame cake (C) with 6.0 ± 0.4 μmol Trolox/g final product and mixture of tahini, sesame cake and freeze-dried aquafaba (AC) with 5.2 ± 0.2 μmol Trolox/g final product (Figure 2).

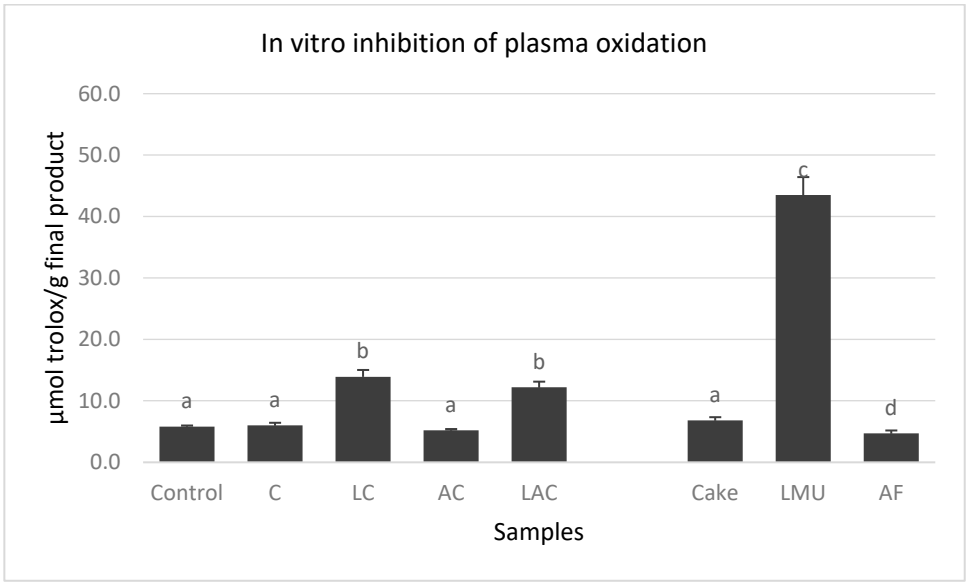

Figure 2. Human plasma oxidation assay results, expressed in μmol Trolox/g final product. Control: tahini; C: mixture of tahini and sesame cake; LC: mixture of tahini, sesame cake and lyophilized pomace extract; AC: mixture of tahini, sesame cake and freeze-dried aquafaba; LAC: mixture of tahini, sesame cake, lyophilized pomace extract and freeze-dried aquafaba; Cake: sesame cake; LMU: lyophilized pomace extract; and AF: freeze-dried aquafaba. Results are expressed as mean ± standard deviation (n = 3). Samples without a common letter (a–d) are significantly different according to Tukey's test.

The freeze-dried aquafaba (AF) with a value of 4.7 ± 0.5 exerted the lowest activity ($p < 0.05$) followed by sesame cake (Cake) with an activity of 6.8 ± 0.5, which was comparable to Control, C, AC and Cake ($p > 0.05$) with values equal to 5.8 ± 0.2, 6.0 ± 0.4,

5.2 ± 0.2 and 6.8 ± 0.5 µmol Trolox/g final product, respectively. Lyophilized pomace extract (LMU) had the highest activity equal to 43.5 ± 2.9 µmol Trolox/g final product, which was about three times more than those of the LC and LAC ($p < 0.05$) (Figure 2).

3.5. In Vitro Antiplatelet Activity

The in vitro antiplatelet activity of each extract was expressed as the equivalent amount, in mg final product, required for 50% inhibition of PAF activity (IC_{50}) toward human platelet rich plasma (PRP). The smallest required amount of mg final product to achieve the IC_{50}, thus the greater potential antiplatelet capacity, was presented by the mixture of tahini, sesame cake, lyophilized pomace extract and freeze-dried aquafaba (LAC) with a requirement of 0.19 ± 0.01 mg final product. It is followed by plain tahini (Control) with 0.32 ± 0.02 mg final product, the mixture of tahini, sesame cake and freeze-dried aquafaba (AC) with 0.37 ± 0.01 mg final product, the mixture of tahini and sesame cake (C) with 0.44 ± 0.03 mg final product and the mixture of tahini, sesame cake and lyophilized pomace extract (LC) with 0.45 ± 0.04 mg final product (Figure 3).

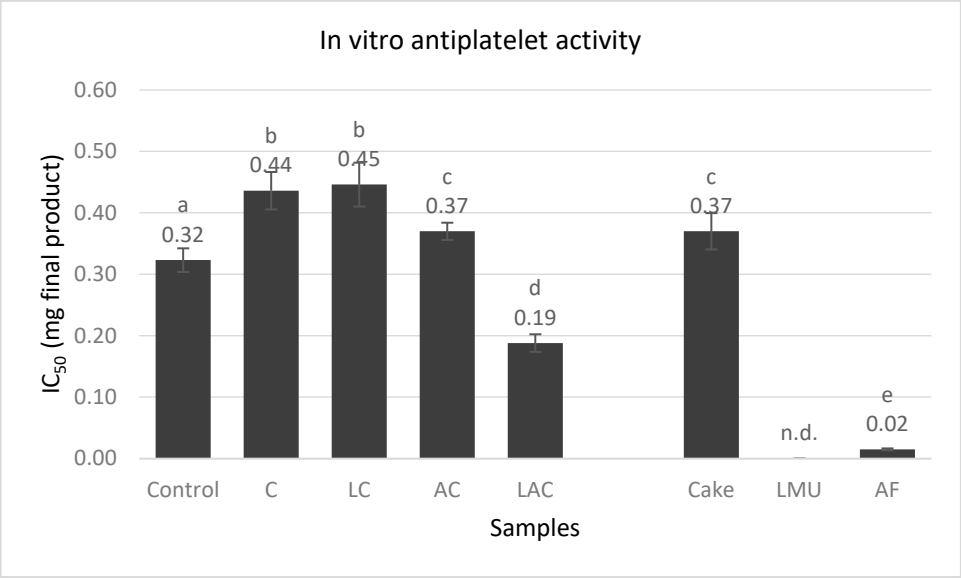

Figure 3. PAF inhibition toward PRP assay results expressed as an equivalent amount of mg final product required for 50% inhibition (IC_{50}). Control: tahini; C: mixture of tahini and sesame cake; LC: mixture of tahini, sesame cake and lyophilized pomace extract; AC: mixture of tahini, sesame cake and freeze-dried aquafaba; LAC: mixture of tahini, sesame cake, lyophilized pomace extract and freeze-dried aquafaba; Cake: sesame cake; LMU: lyophilized pomace extract; and AF: freeze-dried aquafaba; n.d. stands for not detected. Results are expressed as mean ± standard deviation (n = 3). Samples without a common letter (a–e) are significantly different according to Tukey's test.

No inhibitory activity was detected in lyophilized pomace extract (LMU). Freeze-dried aquafaba (AF) had the highest activity of the fortification materials with 0.02 ± 0.00 mg final product required for 50% inhibition of PAF activity toward PRP. LC is in agreement with the not detectable activity for LMU, while LAC seems to be in line with the results of AF, while sesame cake (Cake) required 0.37 ± 0.03 mg final product (Figure 3). The results show lower antiplatelet activities for all extracts related to sesame and grape pomace (Control, C, LC, LAC, Cake and LMU) compared to aquafaba (AF) and indicate that antiplatelet activity in the fortified tahini (LAC) comes from the addition of AF and its positive synergistic activity with the extract of grape pomace (LMU). This is in line with the synergistic effect that has been recently reported for grape seed and grape skin extracts, where grape seed and grape skin extracts did not exert antiplatelet activity when tested individually, while the combination of them significantly inhibited platelet activation [34].

Sesame cake lacks the majority of the lipophilic molecules that are contained in sesame seeds and tahini. Since PAF is a lipid mediator, it is possible that lipophilic molecules in tahini interact with its receptor in order to inhibit its activity more effectively. Therefore, the incorporation of sesame cake and red grape pomace extract in C and LC lowers the concentration of lipophilic molecules with antiplatelet activity against PAF, leading to increased IC_{50} values. Moreover, the significant difference noticed in IC_{50} values for LAC compared to AC seems to be the result of a positive synergistic activity that leads to a higher antiplatelet activity when AF and LMU coexist.

It is worth noting that there is a difference between the fortification materials about their antioxidant and antiplatelet activities. Antiplatelet activity has been referred to as independent of antioxidants due to the different mechanisms and receptors that participate in each case [35]. Moreover, Platelet Activating Factor, due to its biochemical actions, has attracted the attention of the scientific community as a molecule involved in all the fundamental states of the development of atherosclerosis, the inhibition of which could be a way to counteract the development of atherosclerosis and, by extension, the prevention of cardiovascular diseases [24].

Results concerning total phenolic and flavonoid content in Limnio grape pomace are in line with recent studies that showed total phenolics in the range of 33.39 ± 0.59 to 92.15 ± 1.75 mg GAE/g [36–38] and total flavonoids in the range of 12.15 ± 1.20 to 51.36 mg quercetin equivalent/g [36], depending on the extraction conditions. On the other hand, other investigators have determined lower total phenolics in aquafaba (5.50 mgGAE/g) compared to our study (18.87 mgGAE/g) [39]. This difference may exist due to the different varieties and storage conditions of chickpeas [40].

The Limnio grape pomace extract and freeze-dried aquafaba of Lemnian chickpeas from Panagia exhibit a notably greater concentration of total phenolics in comparison to tahini (44.86 ± 3.32 and 18.87 ± 0.66 > 6.02 ± 0.03, respectively; $p < 0.05$). The Limnio grape pomace extract exhibits a notably greater concentration of total flavonoids in comparison to tahini (61.76 ± 3.74 > 4.76 ± 0.1; $p < 0.05$). The findings of this study suggest that the utilization of Limnio grape pomace and aquafaba derived from Lemnian chickpeas sourced from Panagia can be considered as suitable options for fortifying tahini. Incorporating these raw materials in small quantities can effectively enhance the overall content of total phenolics and flavonoids in tahini. The fortification of tahini with the aforementioned materials at percentages of 4% and 6% (w/w) resulted in a significant increase in the levels of total phenolics and flavonoids, with an average increase of 77.9 ± 2.1% and 77.5 ± 0.8%, respectively.

Fortification of tahini with Limnio grape pomace extract and Lemnian chickpeas aquafaba from Panagia increased the antioxidant capacity of the fortified tahini by 100.8 ± 0.2%, 64.6 ± 11.1%, 77.7 ± 7.3% and 72.9 ± 10.1%, as was assessed by ABTS, DPPH, CUPRAC and FRAP assays, respectively. In vitro plasma oxidation was also significantly increased in fortified tahini compared to the Control by 110.2 ± 8.3% ($p < 0.05$).

Polyphenols are the predominant class of antioxidants found in the human diet through plant foodstuffs. At the same time, it has become widely accepted that oxidative stress is an important contributor to the onset and progression of a number of different diseases. These chemicals have distinct ways by which they exert their antioxidant capacity. The constant link between the consumption of polyphenol-rich foods and the prevention of certain chronic and degenerative diseases has led to an increase in interest in phenolic compounds. This interest has been growing steadily over the past many years. It has been demonstrated that these molecules are capable of performing a diverse range of biological tasks, such as the neutralization of free radicals, amongst others [41–44].

Grape pomace is a significant reservoir of antioxidant chemicals such as phenolics that have been demonstrated to have free radical inhibitory properties in many in vitro experiments [45]. The antioxidant capacity of aquafaba has been previously discussed. There exists documented evidence suggesting that aquafaba, when discarded, contains bioactive polyphenols. Furthermore, previous studies have provided evidence that chick-

peas' polyphenols have the potential to leach out into the water during the boiling process, resulting in their degradation into smaller, more bioactive molecules. In the case of chickpeas' aquafaba, the process involves at least the degradation of lignin into syringic acid and the conversion of anthocyanins into 4-hydroxy benzoic acid [46,47].

In addition, in the present study, in vitro antiplatelet activity toward PAF was significantly increased by 31.6 ± 1.7% in fortified tahini compared to the Control ($p < 0.05$).

According to our knowledge, there are no studies concerning anti-PAF activity from red grape pomace extracts. However, studies in wine samples report the existence of lipid molecules that may come from grape skin with antiplatelet activities toward PAF [48]. Moreover, the acetylation of phenolic compounds that render them more hydrophobic may increase their antiplatelet activity toward PAF [49]. The results of this study are in line with the above findings concerning the high antioxidant capacity of LMU and the not detectable antiplatelet activity.

According to our knowledge, there are no studies concerning the antiplatelet activity of aquafaba from chickpeas. However, phenolic compounds from legumes have been referred to not only for their antioxidant activities but also for their antithrombotic ones [50]. Antiplatelet activity toward TRAP-6 and ADP has been recently reported for aqueous extracts of common beans (*Phaseolus vulgaris* L.) [51]. The results of this study are in accordance with the above findings concerning the detected antioxidant and antiplatelet capacities of AF.

Overall, the results indicated the effectiveness of grape pomace extracts from Limnio and aquafaba from Lemnian chickpeas of Panagia in increasing antioxidant and antiplatelet bioactivities of tahini, thus giving it an added value due to a higher nutritional value.

4. Conclusions

Both the extract of red grape pomace of the Limnio variety and the aquafaba from Lemnian chickpeas of the Panagia variety increased the bioactivity of tahini. The percentages of fortification and the parallel study of the lipophilic compounds, as they may act synergistically, presenting completely different results is an area for further research.

When these foods are part of a balanced diet, enhancing the antioxidant and antiplatelet activities the foods by creating highly active natural additives, especially when derived from food by-products, can be beneficial to human health and help prevent the development of chronic diseases, which have as their main causal mechanisms oxidative stress, inflammation and thrombosis as occurs in cardiovascular diseases, diabetes mellitus type II, obesity and cancer.

The combination of red grape pomace extract and aquafaba presents a substantial supply of antioxidants and antiplatelet agents, resulting in a notable enhancement of the in vitro antioxidant and antiplatelet properties of tahini. Through the utilization of the residual materials generated by the grape and chickpea processing sectors, this intervention effectively mitigates wastage while concurrently converting these remnants into valuable resources that augment the health-promoting attributes of the end product. This practice not only adheres to the principles of the circular economy, which emphasize the efficient use of resources throughout the production process, but also serves as a prime example of value creation within the food industry.

Author Contributions: Conceptualization, H.C.K.; methodology, H.C.K.; software, P.A.; formal analysis, P.A.; investigation, P.A., H.C.K. and A.S.P.; resources, H.C.K.; data curation, P.A. and H.C.K.; writing—original draft preparation, P.A.; writing—review and editing, H.C.K. and A.S.P.; supervision, H.C.K.; project administration, H.C.K.; funding acquisition, H.C.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by EPAnEk -NRSF 2014–2020; Operational Program "Competitiveness, Entrepreneurship and Innovation", Call 111 "Support of Regional Excellence" in the context of the implementation of the program AGRICA II: AGrifood Research and Innovation Network of Excellence of the Aegean, which is co-financed by the European Regional Development Fund (ERDF), MIS code: 5046750.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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