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Research Article

A Square Wave Voltammetry Study on the Antioxidant Interaction and Effect of Extraction Method for Binary Fruit Mixture Extracts

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Square wave voltammetry (SWV) analysis was used to assess the antioxidant interactions (synergism, addition, and antagonism) of fruit mixture extracts from grape (G), lemon (L), and blueberry (B) obtained by conventional extraction, ultrasound-assisted extraction, and high hydrostatic pressure extraction. The experimental results showed antagonistic antioxidant effects in all binary mixture extracts (L-G, L-B, and G-B). In DPPH and FRAP assays, the greatest antioxidant capacity was found in the G-B mixture (108.7 and 108.8 μmol TE g^{-1} dry extract, respectively) obtained by high hydrostatic pressure extraction; however, there were no significant differences when measured by ultrasound-assisted extraction. For TPC and TFC assays, the greatest values were for G-B (6.67 mg GA g^{-1} dry extract) and L-G (1.63 mg QE g^{-1} dry extract), respectively. SWV experiments showed antagonistic behavior in the mixtures. Among the different ratios of the fruit mixture extracts evaluated by SWV, 1 : 1 (w/w) combination showed the greatest antagonistic antioxidant effects. SWV suggests the components of the mixture with the highest antioxidant capacity oxidize after mixing. The results indicate that the presence of natural bioactive antioxidants in fruit mixtures does not guarantee that the interactions are synergistic.

1. Introduction

Natural bioactive antioxidants are nonnutritional constituents present in small quantities in fruits [1]. These compounds are recognized by their organoleptic properties and their beneficial effects on human health [2]. Different mixtures of pure antioxidants or their extracts from fruit sources can enhance the benefits conferred by individual natural bioactive antioxidants [3]. Nevertheless, natural bioactive antioxidants exist in combination in nature, and a combination of different antioxidants might act additively, synergistically, and even antagonistically, resulting in an increased or decreased activity of a mixture when compared with the individual compounds [4]. The interaction between natural bioactive antioxidants can affect their chemical and biophysical properties such as solubility, bioaccessibility, bioavailability, and antioxidant and antimicrobial activities [5].

Electrochemical methods like cyclic voltammetry (CV) or square wave voltammetry (SWV) have become suitable tools for the study of antioxidant capacity in food science [6]. These methods have many advantages such as speed, low cost, and simplicity and depend only on the electrochemical properties of the antioxidants [7]. Although determining the antioxidant capacity of foods is a common procedure in food science, few works have focused on the redox reactions occurring in antioxidant-rich food samples [8–10]. These reactions can be measured by electrochemical methods, such as SWV.

Because fruits are consumed frequently mixed in concentrates, supplement, beverages, and snack, it is important

to determine if the antioxidant effect of a given mixture of fruits is due only to the sum of individual antioxidant capacities or if such effect is decreased or increased with respect to the total effect. However, there is very little scientific evidence to support their use in combination or mixture, and thus we were particularly interested in finding synergistic interactions as a possible way to reduce chronic disease-associated oxidative stress such as cardiovascular disease, diabetes, and cancer. In addition, SWV was used as a good tool to interpret the types of antioxidant interactions (synergism, antagonism, or additive) and the evaluation of the changes in the distribution of molecules which contribute to the antioxidant capacity in a complex matrix as a food. Therefore, the aims of this work were to (a) study the interactions in binary fruit mixture extracts (lemon, grape, and blueberry) obtained by three different extraction methods, (b) evaluate if the interaction promotes a synergistic, additive, or antagonistic effect in the obtained mixtures, and (c) explore the possible mechanism responsible for the observed effect.

2. Materials and Methods

2.1. Reagents. Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; purity ≥98% by titration), analytical grade methanol (Tedia, USA), DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin–Ciocalteau's phenol reagent, and sodium acetate were purchased from Merck (Darmstadt, Germany). Gallic acid standard (purity ≥98%), quercetin standard (purity ≥95%), acetic acid, TPTZ (2,4,6-tripyridyl-*s*-triazine), and ferric chloride hexahydrate ($FeCl_3 \times 6H_2O$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Milli-Q water was obtained through a Millipore filter system (Millipore Co., USA).

2.2. Raw Material, Preparation, and Mixture Formulation. Blueberry (*Vaccinium corymbosum*, O´Neal variety), red grape (*Vitis vinifera*, flame seedless variety), and lemon (*Citrus limon*, Génova variety) were purchased from local markets in La Serena, Chile. The fruits in this study were chosen from Chile's ten most exported fruits [11]. Samples were homogeneously selected based on harvest date (March 2018), color, size, and freshness (without mechanical or microbiological damage) measured by visual analysis. Finally, each fruit was washed, dried with absorbent paper, and stored at −80°C for further analysis. Peel and seeds were discarded from the lemon. The frozen fruit was thawed at 4–6°C, homogenized in a rotor-stator homogenizer (Ultraturrax, T25, IKA, Germany) at full power for 3 × 15 s, and mixed according to Table 1.

2.3. Natural Antioxidant Extraction

2.3.1. Conventional Extraction (CE). Twelve grams of individual fruits (IF) or 24 g (12 g of each fruit) of fruit mixture (FM) were homogenized for 30 s in a 1 : 2 (w/v) ratio (weight/volume) with extraction solvent (80% methanol). The mixtures were extracted by orbital shaking (Boeco, OS20, Germany) at room temperature (RT) for 120 min.

Table 1: Mixture formulation.

Fruit mixture	Percentage (%)		
	Grape (G)	Lemon (L)	Blueberry (B)
L-G	50	50	0
L-B	0	50	50
G-B	50	0	50

After centrifugation (15 min, 20°C, 6000 ×g), the supernatant was recovered and transferred into a 250 ml round-bottom flask. The solvent was evaporated in a rotary evaporator (Büchi RE12, Flawil, Switzerland) under reduced pressure at 40°C, and the dry extract was weighted. The dry extract was dissolved in aqueous methanol (80%) and diluted to a final volume of 25 ml.

2.3.2. Ultrasound-Assisted Extraction (UAE). Twelve grams of IF or 24 g (12 g of each fruit) of FM were homogenized for 30 s in a 1 : 2 ratio (w/v) with extraction solvent (80% methanol). The extraction was carried out in an 2.8 L ultrasound bath at RT (internal dimensions: 240 mm × 140 mm × 100 mm, Branson 2510 E-MT, 42 kHz, 130 W; Danbury, USA) for 15 min. Recycled water was provided to maintain a constant temperature. The samples were then treated as described above (Section 2.3.1).

2.3.3. High Hydrostatic Pressure Extraction (HHPE). Twelve grams of IF or 24 g (12 g of each fruit) of FM were homogenized for 30 s in a 1 : 2 ratio (w/v) with extraction solvent (80% methanol) and hermetically sealed in high-density polyethylene bags. The packaged samples were placed in a 2 L cylindrical loading container at RT and pressurized at 500 MPa for 15 min with pulses of 1 min each in a high hydrostatic pressure equipment (Avure Technologies Incorporated, Kent, WA, USA). Then, the samples were treated as described above (Section 2.3.1).

2.4. Antioxidant Capacity Measurements

2.4.1. DPPH Assay. The antioxidant capacity was measured using the 2,2′-diphenyl-1-picrylhydrazyl free-radical scavenging (DPPH) method described by Brand-Williams et al. [12] including a modification to 96-well microplate format. The reaction was read at 517 nm in a spectrophotometric microplate reader (Epoch, BioTek Instruments, Winooski, VT, USA). Antioxidant capacity was calculated from a calibration curve ($y = -0.5389x + 0.5252$; $R^2 = 0.9920$) of the synthetic antioxidant Trolox at concentrations between 80 to 1280 μM. The total antioxidant capacity determined by the DPPH assay was expressed in μmol Trolox equivalents per gram of dry extract (μmol TE g^{-1}). All measurements were carried out in triplicate.

2.4.2. FRAP Assay. A FRAP assay [13] modified for a 96-well microplate was used to determine the reducing power of individual fruits and binary fruit mixtures. FRAP reagent was prepared with 2.5 mL of a 10 mmol L^{-1} TPTZ solution in

40 mmol L^{-1} HCl with 2.5 mL of 20 mm L^{-1} $FeCl_3$ and 25 mL of 0.3 mol L^{-1} acetate buffer at a pH of 3.6. The absorbance of the reaction was read at 593 nm. Antioxidant capacity was calculated from a Trolox calibration curve ($y = 0.0010x + 0.0621$; $R^2 = 0.9986$) at concentrations between 100 and 1500 μM. The values were expressed as micromol Trolox equivalent per gram of dry extract (μmol TE g^{-1}). All measurements were done in triplicate.

2.5. Total Polyphenolic Content (TPC) and Flavonoid Content (TFC) Measurements. Total polyphenolic content (TPC) was determined using the Folin–Ciocalteu (FC) assay [14]. The absorbance was read at 725 nm in the spectrophotometric microplate reader, and the TPC was calculated from a calibration curve ($y = 0.0035x + 0.0369$; $R^2 = 0.9940$), using gallic acid (GA) as standard (50–1000 mg ml^{-1}). The TPC values were expressed as mg GA g^{-1} dry extract. All measurements were done in triplicate.

Total flavonoids content (TFC) was determined according to Dini et al. [15]. The reaction was read at 415 nm in the spectrophotometric microplate reader. The TFC concentration was calculated from a calibration curve ($y = 0.0019x - 0.0142$, $R^2 = 0.9913$) using quercetin as standard (20 to 100 μg ml^{-1}). The TFC values were expressed as mg quercetin equivalents (QE) g^{-1} dry extract. All measurements were done in triplicate.

2.6. Mixture Effect of Antioxidant Capacity and Polyphenolic Compounds

2.6.1. Mixture Index (MI). The mixture index (MI) of binary fruit mixture extract (FME) values was calculated according to the following formula [16]:

$$\mathrm{MI} = \frac{\mathrm{AC_1C_2}}{\mathrm{AC_1} + \mathrm{AC_2}}, \quad (1)$$

where AC_1C_2 is the value of antioxidant capacity for FM extract and ($AC_1 + AC_2$) is the value obtained by the sum of antioxidant capacity for each individual fruit extract (IFE). The following cutoff values were chosen, for the interpretation of obtained results: synergism MI is >1, MI = 1 addition, and MI < 1 would be antagonism.

2.6.2. Regeneration Percentage (X). Regeneration percentage was calculated according to Rúa et al. [17]. The experimental antioxidant capacity (AC_1C_2) of a binary mixture extract can be expressed by the following expression:

$$X(\%) = \frac{\mathrm{AC_1C_2} - \mathrm{AC_1} - \mathrm{AC_2}}{\mathrm{AC_1} - \mathrm{AC_2}}, \quad (2)$$

from which we have deduced the fraction of AC_2 that regenerates AC_1 and AC_1 and AC_2 were the individual antioxidant capacity of extracts.

2.7. Electrochemical Method to Evaluate Mixture Effect. SWV experiments were done according to Uribe et al. [18]. Assays were performed by using a Princeton Applied Research PG 580 potentiostat and a classical three-electrode setup, consisting of a glassy carbon working electrode (3 mm diameter), a platinum (Pt) wire auxiliary electrode, and an silver/silver chloride (Ag/AgCl) reference electrode. Between the above measurements, the working electrode was polished by using a polishing cloth and diamond paste. Lithium perchlorate (1 gram) was added to each sample as an electrolyte to increase the conductivity of the samples. The conditions for SWV were as follows: frequency, 25 Hz; amplitude, 20 mV step size, 5 mV. The potential was scanned between 0.0 and 1.0 V.

2.8. Statistical Analysis. Statgraphics Plus® 5.1 software was used to determine significant differences among samples by using ANOVA (one-way analysis of variance). Fisher's least significant difference test was used as significance testing; differences were taken as statistically significant when $p < 0.05$. Also, the multiple range test (MRT) was used to find homogeneous groups within each of the analyzed parameters. For all samples, three different batches ($n = 3$) were considered to perform the statistical analysis.

3. Results and Discussion

3.1. Antioxidant Capacity

3.1.1. Effect of the Extraction Method on the Antioxidant Capacity. The extractability of natural bioactive antioxidants estimated by DPPH and FRAP assays from individual fruit extract (IFE) and fruit mixture extract (FME) increased with the application of HHPE and UAE as compared to CE (Table 2). Likewise, UAE and HHPE extractions had a significant impact on antioxidant capacity measurements since IFEs obtained by these methods showed higher DPPH and FRAP values than those obtained by CE. The highest antioxidant capacity (218.4 μmol TE g^{-1} dry extract for DPPH and 114.9 μmol TE g^{-1} dry extract for FRAP blueberry dry extract) was obtained by HHPE extracts. The antioxidant capacity of IFEs increased significantly ($p < 0.05$) with extraction methods: from 25.8 to 33.6% for grape, 13.9 to 24.5% for lemon, 39.6 to 45.2% for blueberry by DPPH and from 8.1 to 13.0% for grape, 10.6 to 14.0% for lemon, and 25.0 to 28.2% for blueberry by FRAP with UAE and HHPE, respectively, when compared to CE (Table 2). Antioxidant capacities of IFEs obtained by UAE and HHPE did not show significant differences ($p > 0.05$). The same positive effect by UAE and HHPE extractions over CE is evidenced by the subtle increase in antioxidant capacity in FMEs. However, there was no significant difference ($p > 0.05$) between CE, UAE, and HHPE for L-G (lemon-grape) or G-B (grape-blueberry) (Table 2). Conversely, the L-B (lemon-blueberry) extractability increased significantly ($p < 0.05$) by UAE and HHPE when compared to CE for DPPH and FRAP. Previous studies reported a range between 26.1 and 364.2 μmol TE per gram of grape/grape juice concentrate [19, 20], 14.3 and 122.8 μmol TE per gram of lemon/grape juice concentrate [20–22], and 26.3 and 149.8 μmol TE per gram of blueberry [23, 24] for antioxidant capacity content.

Table 2: DPPH and FRAP assays for different extraction methods from fruit and fruit mixture.

Assay		Sample	Extraction methods		
			CE (μmol TE g^{-1} dry extract)	UAE (μmol TE g^{-1} dry extract)	HHPE (μmol TE g^{-1} dry extract)
DPPH	IFE	Grape (G)	81.5 (10.4)^{a}	102.5 (5.2)^{b}	108.9 (0.7)^{b}
		Lemon (L)	116.9 (1.2)^{a}	133.1 (7.0)^{b}	145.5 (4.1)^{b}
		Blueberry (B)	150.4 (4.0)^{a}	209.9 (7.8)^{b}	218.4 (2.3)^{b}
	FME	L-G	92.9 (7.3)^{a}	98.1 (5.7)^{a}	101.7 (4.3)^{a}
		L-B	93.1 (0.9)^{a}	104.5 (0.2)^{b}	105.9 (1.1)^{b}
		G-B	103.6 (1.7)^{a}	103.6 (3.2)^{a}	108.7 (3.6)^{a}
FRAP	IFE	Grape (G)	75.5 (2.1)^{a}	81.6 (2.5)^{ab}	85.3 (1.7)^{b}
		Lemon (L)	80.0 (1.6)^{a}	88.5 (1.8)^{b}	91.2 (2.6)^{b}
		Blueberry (B)	89.6 (4.3)^{a}	112.0 (3.5)^{b}	114.9 (0.5)^{b}
	FME	L-G	96.1 (2.5)^{a}	96.9 (3.8)^{a}	97.1 (1.5)^{a}
		L-B	97.5 (0.3)^{a}	104.9 (2.0)^{b}	105.2 (1.4)^{b}
		G-B	104.1 (0.5)^{a}	104.9 (1.2)^{a}	108.8 (4.6)^{a}

Mean values of extraction methods with different superscript letters (a-b) in rows were significantly different ($p < 0.05$) by Fisher's test. Mean and standard deviation are presented in brackets.

The increased antioxidant capacity associated with UAE can be explained by several mechanisms involved in the extraction including fragmentation, erosion, sonocapillary effect, sonoporation, local shear stress, and destruction-detexturation of plant structures. In addition, the intense mixing effect generated by the propagation of the ultrasound wave in the liquid medium enhances the mass transfer, greatly improving the solute transfer rate and thus the extraction [25]. The high efficiency of natural bioactive antioxidant (polyphenolic and flavonoid compounds) extraction by HHPE relates to the short time necessary to reach the equilibrium pressure between the inside and outside of the cells. Consequently, the diffusion speed of the solvent is high, allowing natural bioactive antioxidants to be quickly released obtaining a greater antioxidant capacity [26].

3.1.2. Effect of Mixture Index and Regeneration Percentage on Antioxidant Capacity. Evaluating the antioxidant capacity of natural bioactive antioxidants in foods is a topic that has attracted the attention of many researchers. Thus far, many studies have concluded that it is impossible to predict the antioxidant potential of a given food by studying a single type of bioactive antioxidant. In some cases, there are possible synergistic or antagonistic effects between the various natural bioactive antioxidants present in the different food matrices, as discussed by Aoun and Makris [27], Gironés-Vilaplana et al. [28], Jain at al. [29], and Jiang et al. [3].

Table 3 shows the mixture effect on antioxidant capacity of FMEs as the mixture index (MI) and regeneration percentage (X) obtained by CE, HHPE, and UAE. The MI for antioxidant capacity by DPPH and FRAP exhibited values lower than one in all FMEs and for all extraction methods. Thus, the binary mixtures of lemon, grape, and blueberry (L-G, L-B, and G-B) showed antagonistic interactions due to the fact that experimental antioxidant capacity values differed significantly from the summed antioxidant capacity of each IFE. These interactions could be explained by the formation of hydrogen bonds in the available active hydroxyl groups among the natural bioactive antioxidants present in IFEs, reducing the free-radical scavenging capacity. Another possible phenomenon was that one or more antioxidants in the mixture are oxidized and become a free radical which can receive electrons or hydrogen atoms donated by other antioxidants of the mixture to regenerate itself. Several authors have reported that different mixture interactions can be explained by the regeneration of one antioxidant by another [17, 27, 30]. A synergistic effect occurs when one (or more) less efficient molecule regenerates the more efficient one [16]. On the contrary, an antagonistic effect occurs when a more efficient molecule regenerates the less efficient one [16], as observed in this investigation. We calculated this regeneration as the percentage of antioxidant capacity in FMEs obtained using DPPH and FRAP for three extraction methods that showed an antagonistic effect. The FRAP assay for L-G resulted in a higher percentage of regeneration than the DPPH assay, where percentages ranged from 10.64% to 13.68% (Table 3). The regeneration percentage of L-B showed no significant differences between the extraction methods by DPPH. On the contrary, there were significant differences ($p < 0.05$) in the regeneration percentage of G-B between the extraction methods by DPPH and FRAP, where percentages ranged from 3.11% to 7.53%. This is supported by the findings of Pinelo et al. [31] and Velderrain-Rodríguez et al. [32] who reported an antagonistic behavior in antioxidant capacity of polyphenol mixtures by DPPH. Other authors reported that they have not found a synergistic effect between flavonoids by the ABTS assay [33]. Thus, the consumption of IFE may provide the higher antioxidant balance needed to quench the ROS (reactive oxygen species), which are implicated in almost all cancers and are known to promote tumorigenesis [2].

On the contrary, our results were opposed to studies for some binary food mixtures with synergistic interactions: tomato-onion, tomato-garlic, tomato-lettuce for ABTS, and xanthine oxidase inhibitory assays [34]; eggplant-tomato, purple potato-tomato, carrot-eggplant, carrot-purple potato, and eggplant-purple potato for DPPH and ABTS assays [3]; tomato-purple cauliflower, soybean-adzuki bean, raspberry-mushroom, apple-tomato, and raspberry-soybean for ORAC assay [35]; apple-purple cauliflower for DPPH assay

Table 3: Mixture index (MI) and regeneration percentage of DPPH and FRAP assays from fruit mixtures.

Assay	FME	Extraction method	MI	Interaction	X (%)
DPPH	L-G	CE	0.47 (0.06)^{a}	An	3.10 (0.14)^{a}
		UAE	0.42 (0.03)^{a}	An	4.58 (0.61)^{b}
		HHPE	0.40 (0.01)^{a}	An	4.20 (0.37)^{ab}
	L-B	CE	0.30 (0.01)^{a}	An	1.92 (0.16)^{a}
		UAE	0.33 (0.00)^{b}	An	1.95 (0.14)^{a}
		HHPE	0.32 (0.00)^{ab}	An	2.02 (0.01)^{a}
	G-B	CE	0.49 (0.04)^{a}	An	5.20 (0.01)^{a}
		UAE	0.35 (0.01)^{b}	An	3.11 (0.12)^{b}
		HHPE	0.34 (0.00)^{b}	An	3.50 (0.12)^{c}
FRAP	L-G	CE	0.57 (0.04)^{a}	An	13.28 (1.06)^{a}
		UAE	0.57 (0.04)^{a}	An	10.64 (0.77)^{a}
		HHPE	0.55 (0.02)^{a}	An	13.68 (1.16)^{a}
	L-B	CE	0.59 (0.01)^{a}	An	4.28 (0.42)^{a}
		UAE	0.53 (0.00)^{b}	An	3.03 (0.32)^{b}
		HHPE	0.53 (0.01)^{b}	An	3.22 (0.32)^{ab}
	G-B	CE	0.59 (0.01)^{a}	An	7.53 (0.90)^{a}
		UAE	0.57 (0.02)^{a}	An	4.15 (0.36)^{b}
		HHPE	0.58 (0.03)^{a}	An	4.15 (0.39)^{b}

Sy, synergistic interaction; Ad, additive interaction; An, antagonistic interaction. Mean values of each FME by CE, UAE, and HHPE with different superscript letters (a-b) in rows were significantly different ($p < 0.05$) by Fisher's test.

[35]; raspberry-adzuki bean for FRAP, DPPH, and ORAC assays [35]; and lettuce-green tea and lettuce-grape seed extracts for the liposome oxidation assay [36].

3.2. TPC and TFC

3.2.1. Extraction Method Effect on TPC and TFC. Total polyphenolic and total flavonoid contents of IFE and FME obtained by CE, UAE, and HHPE are determined, as shown in Table 4. The TPC values for all extracts (IFE and FME) obtained by UAE and HHPE were higher than for extracts obtained by CE. HHPE had a significant effect on the extractability of TPC, giving the higher return for IFE for blueberry (13.75 mg GA g^{-1} dry extract) when extraction was carried out at 500 MPa over 15 min (Table 4). Of the FMEs analyzed, G-B displayed higher TPC values for UAE and HHPE (6.65 and 6.67 mg GA g^{-1} dry extract, respectively). TPC values for all extracts increased significantly ($p < 0.05$) when extracted via UAE and HHPE as compared to CE: between 14.9–19.8% and 28.5–36.3% for IFEs, respectively; and between 20.8–46.6% and 21.0–48.2% for FMEs, respectively. These results agree with other investigations such as in *Moringa oleifera* [37] and dried fruit of *Azadirachta indica A. Juss (Meliaceae)* [38] for UAE and apple [39] and soy smoothie [40] for HHPE. To compare our TPC results with past evaluations, Wang et al. [24] measured 2.1–4.6 mg AG g^{-1} for blueberries, García-Salas et al. [21] reported lemon values of 10.1 and 10.4 mg AG g^{-1}, and Sun et al. [41] found a value of 2.5 mg AG g^{-1} for red grape.

Likewise, TFC extractability increased with UAE and HHPE for all extract measurements as compared to CE. The highest TFC was obtained by extracting IFE for lemon (2.92 mg QE g^{-1} dry extract) using HHPE (Table 4). The UAE and HHPE over CE that increased in TFC of all extracts were between 2.4–6.6% and 5.4–11.0% for IFEs, respectively, and between 3.9–6.4% and 7.2–15.7% for FMEs, respectively. This behavior has also been observed using UAE and HHPE in orange peel (*Citrus sinensis* L.) [42] for UAE and propolis [43] for HHPE. Similar results regarding extractability of TFC from IFE demonstrate TFC values ranging from 0.3 to 17.1 QE g^{-1} in 13 citrus species [44], from 0.8 to 1.0 QE g^{-1} for blueberry [45], and from 3.4 to 14.2 for grape [46]. TPC and TFC likely increased because of the different effects produced by UAE and HHPE as discussed above (Section 3.1.1).

3.2.2. Mixture Index and Regeneration Percentage on TPC and TFC. Table 5 indicates the effect of the mixture on the TPC and TFC of FMEs as the mixture index (MI) and regeneration percentage (X) obtained by CE, HHPE, and UAE. It was observed that MI values for TPC and TFC of L-G, L-B, and G-B mixtures ranged from 0.2 to 0.3 for all extraction methods. Thus, it can be indicated that the interactions in all mixtures studied resulted in antagonism. This antagonistic response in FME showed that polyphenolic compound content is less than would be expected if the polyphenolic compound content of each IFE was added. It has been shown that the same ratio in the mixture with these combinations of fruit was less effective than with the corresponding individual fruits in TPC and TFC assays. Hidalgo et al. [47] reported that phenolic compounds such as flavonoids tended to have antagonistic effect because by the formation of hydrogen bonds between two different flavonoids that reduced the availability of the active hydroxyl groups for radical scavenging activities. Therefore, the data indicate that when these fruits are mixed, an interaction takes place affecting their TPC and TFC. Likewise, the results of the binary mixtures from TPC and TFC may be due to coupled reactions of regeneration, as proposed by Peyrat-Maillard et al. [16] and discussed above (Section 3.1.2). In summary, the results of this work support that TPC and TFC could reduce in part the antioxidant capacity in FME.

Table 4: Total polyphenolic content (TPC) and total flavonoid content (TFC) for different extraction methods from fruit and fruit mixture.

Assay	Sample		Extraction methods		
			CE	UAE	HHPE
TPC (mg GA g^{-1} dry extract)	IFE	Grape (G)	7.64 (0.08)^{a}	8.77 (0.54)^{ab}	9.81 (0.50)^{b}
		Lemon (L)	9.56 (0.39)^{a}	11.17 (0.40)^{b}	12.35 (0.02)^{c}
		Blueberry (B)	10.09 (0.15)^{a}	12.10 (0.10)^{b}	13.75 (0.31)^{c}
	FME	L-G	4.16 (0.01)^{a}	6.09 (0.00)^{b}	6.16 (0.10)^{b}
		L-B	5.10 (0.28)^{a}	6.30 (0.05)^{b}	6.38 (0.10)^{b}
		G-B	5.50 (0.05)^{a}	6.65 (0.25)^{b}	6.67 (0.11)^{b}
TFC (mg QE g^{-1} dry extract)	IFE	Grape (G)	2.16 (0.09)^{a}	2.30 (0.01)^{ab}	2.38 (0.03)^{b}
		Lemon (L)	2.77 (0.02)^{a}	2.84 (0.01)^{b}	2.92 (0.00)^{c}
		Blueberry (B)	2.06 (0.08)^{a}	2.19 (0.01)^{ab}	2.28 (0.05)^{b}
	FME	L-G	1.48 (0.12)^{a}	1.57 (0.02)^{a}	1.63 (0.02)^{a}
		L-B	1.46 (0.06)^{a}	1.51 (0.06)^{a}	1.56 (0.04)^{a}
		G-B	1.34 (0.04)^{a}	1.42 (0.02)^{a}	1.52 (0.02)^{b}

Mean values of extraction methods with different superscript letters (a–c) in rows were significantly different ($p < 0.05$) by Fisher's test. Mean and standard deviation are presented in brackets.

Table 5: Mixture index (MI) and regeneration percentage of TPC and TFC assays from fruit mixtures.

Assay	FME	Extraction method	MI	Interaction	X (%)
TPC	L-G	CE	0.24 (0.00)^{a}	An	6.82 (0.36)^{a}
		UAE	0.31 (0.01)^{b}	An	5.81 (0.74)^{a}
		HHPE	0.28 (0.01)^{b}	An	6.27 (0.44)^{a}
	L-B	CE	0.29 (0.02)^{a}	An	5.18 (0.55)^{a}
		UAE	0.30 (0.01)^{a}	An	4.25 (0.38)^{a}
		HHPE	0.27 (0.00)^{a}	An	4.33 (0.46)^{a}
	G-B	CE	0.28 (0.01)^{a}	An	15.20 (0.05)^{a}
		UAE	0.29 (0.02)^{a}	An	18.19 (0.99)^{b}
		HHPE	0.26 (0.01)^{a}	An	14.03 (0.35)^{a}
TFC	L-G	CE	0.30 (0.02)^{a}	An	5.75 (0.68)^{a}
		UAE	0.30 (0.02)^{a}	An	6.65 (0.18)^{a}
		HHPE	0.31 (0.00)^{a}	An	6.91 (0.51)^{a}
	L-B	CE	0.35 (0.03)^{a}	An	4.74 (0.59)^{a}
		UAE	0.34 (0.01)^{a}	An	5.53(0.24)^{a}
		HHPE	0.33 (0.01)^{a}	An	5.57 (0.53)^{a}
	G-B	CE	0.28 (0.00)^{a}	An	4.39 (0.17)^{a}
		UAE	0.28 (0.00)^{a}	An	4.42 (0.22)^{a}
		HHPE	0.29 (0.01)^{b}	An	4.97 (0.37)^{a}

Sy, synergistic interaction; Ad, additive interaction; An, antagonistic interaction. Mean values of each FME by CE, UAE, and HHPE with different superscript letters (a-b) in rows were significantly different ($p < 0.05$) by Fisher's test.

Leopoldini et al. [48] reported that phenolic compounds are capable of transferring electrons to other antioxidant compounds, promoting their chemical regeneration. This possible regeneration was calculated indicating that the highest regeneration percentage of TPC and TFC for FME was obtained for L-B and G-B, respectively, for all extraction methods. The regeneration percentage of TPC was found to be between 4.3 and 27.3% for all extraction methods (Table 5). For TFC, regeneration percentage ranged from 4.7 to 31.3% for all extraction methods.

Another possible phenomenon was that some compounds in the mixture could promote antagonistic effects by decreasing their stability and thus inhibiting their biological activity [49]. It was also postulated that the measured antioxidant interactions include the polarity of the interacting compounds, reaction rates of antioxidants, the efficient concentration of the antioxidants at the oxidation site, and the possible formation of antioxidant complexes [17]. According to Luís et al. [50], berry mixtures containing carbonyl and hydroxyl groups have exhibited antagonistic interactions by favoring the presence of hydrogen bonds, thereby preventing hydrogen atoms from leaving and reacting with free radicals.

3.3. Electrochemical Analysis of the Antagonist Effect. In general, in electrochemical methods, a current flux between a working electrode and an analyte solution is monitored when the electrode is polarized at different potentials.

The voltammograms of pure compounds usually show one or more maximum values that can be associated with the corresponding electrochemical processes the analyte is experiencing (oxidation or reduction). The potentials where these maxima appear give information about the thermodynamic tendency of the analytes to participate in electron transfer reactions. A substance will act as an antioxidant in an electron transfer mechanism, when it displays a thermodynamic tendency to be oxidized, as is generally associated with a low positive oxidation potential.

Electrochemical analysis of the different mixtures (1 : 1 w/w) was assessed to find the antagonistic effect between

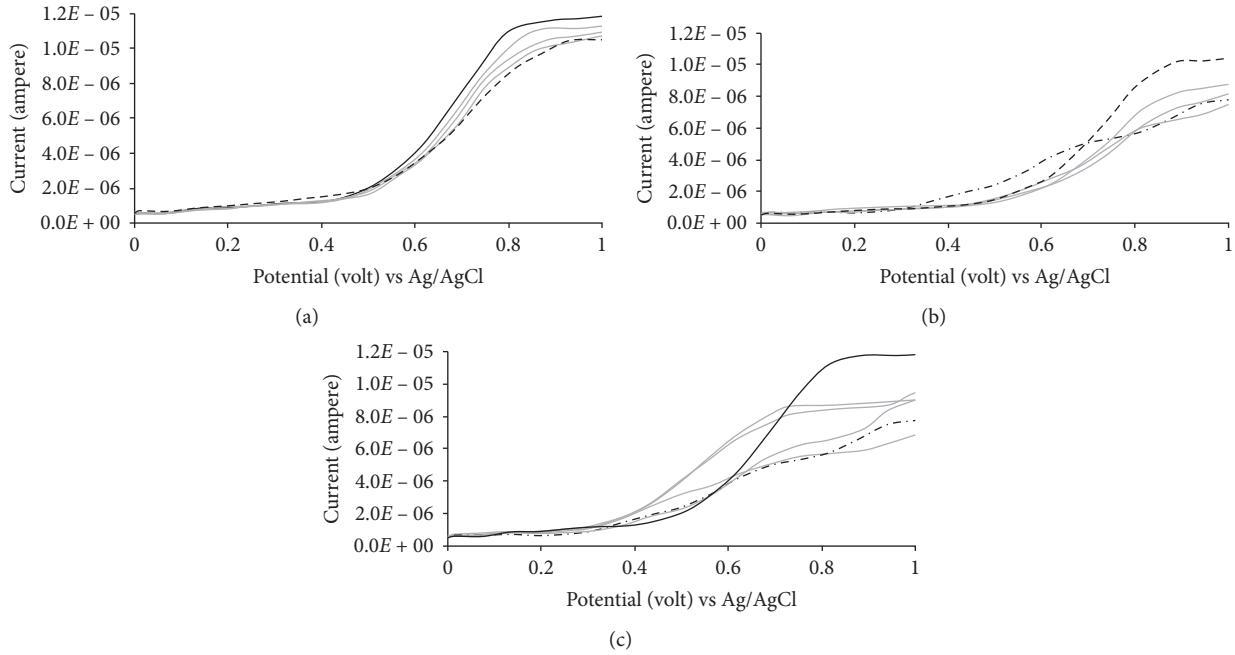

Figure 1: Square wave voltammograms of extracts for CE: (a) lemon and blueberry; (b) blueberry and grape; (c) lemon and grape. Lemon - - -, blueberry ——, and grape - · - ·. The gray lines represent the binary mixtures in different ratios of fruit (25/75, 50/50, and 75/25 w/w).

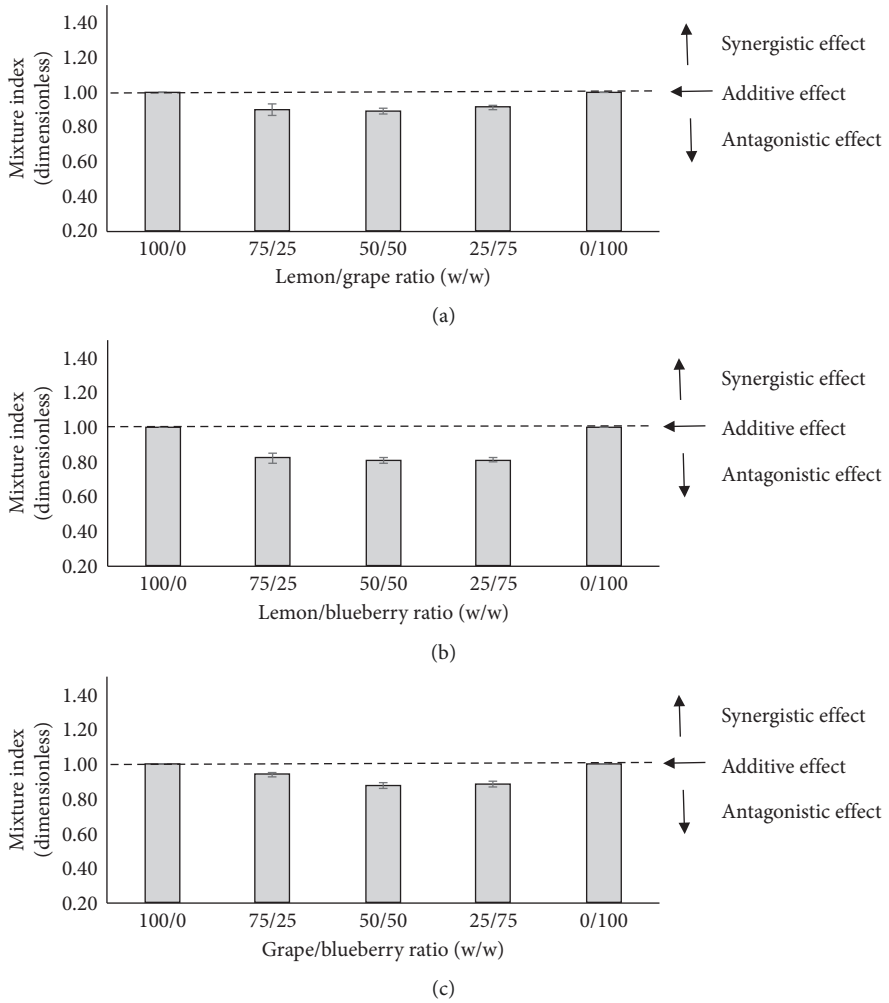

Figure 2: Different ratios (w/w) evaluated when combining fruits by SWV for CE.

natural antioxidants of extract fruits (lemon, blueberry, and grape). For IFE or FME, there was a distribution of the compounds which varied in nature and ratio; a superposition of the anodic waves of the electroactive compounds is observed instead of a few well-defined anodic waves. Although, as previously mentioned, no clear anodic waves were observed; however, the area under the curve (AUC) can be employed to calculate the antioxidant capacity of an extract.

Figure 1 shows the square wave voltammograms of the extracts used in this study for CE (UAE and HHPE exhibited the same behavior and are not shown). In Figure 1(a), the AUC of pure blueberry extract appears greater than the AUC of the lemon extract. The SWV voltammogram of the blueberry extract starts to increase at +0.5 V reaching a maximum at +0.8 V. This corresponds to the main electrochemically active components in the extract (showing anodic waves in this region). Lemon extracts showed a similar behavior at a lower current as compared to blueberry, at +0.4 V, indicating that, in this extract, some of the components are more prone to oxidation. The current at this potential decreased when the extracts were obtained from fruit mixtures. This explains the antagonist behavior of the samples since the components of the lemon extract, which contributes to the current at +0.4 V, will have the thermodynamic tendency to transfer electrons to molecules of the blueberry extract. A similar behavior was shown in Figures 1(b) and 1(c) for the other mixtures.

However, some authors have reported that antioxidant mixtures of two compounds at various ratios could show different interactions such as synergistic, additive, or antagonistic [3, 44]. Therefore, the binary mixture effect of combination from lemon, blueberry, and grape at different ratios was examined using SWV for CE (Figure 2), to evaluate their potential synergistic, additive, or antagonistic effects (UAE and HHPE exhibited the same behavior and therefore are not shown). Figure 2 shows that an increase or decrease in ratio of one fruit in the binary mixture in all cases presented antagonistic interactions. According to Jiang et al. [3], the differences in antioxidant interaction might be caused by the different ratios of bioactive compounds in the mixture as well as the antioxidant mechanism of phytochemicals. Different combination ratios have been reported by García et al. [51] for 44 binary mixtures with the isolated compounds from *Citrus sinensis*; 32 additive interactions, 7 synergistic interactions, and 5 antagonistic interactions were detected. The antagonistic interactions can occur as discussed above (Sections 3.1.2 and 3.2.2).

4. Conclusion

Our results indicate that measurements of antioxidant capacity and polyphenolic and flavonoid content recovery from IFE and FME can be maximized using UAE and HHPE. The extraction procedure was found to influence significantly the total polyphenolic content and antioxidant activity, as differences were observed among UAE, HHPE, and CE. Thus, UAE and HHPE enhance the extraction yield by reducing processing time. Antagonistic interactions were found in all FMEs for all extraction methods. SWV experiments show that compounds with a higher antioxidant capacity on IFE are consumed upon mixing. The results have revealed the importance of choosing the best combination of antioxidants for the design of functional foods. Thus, more investigations are necessary to explore the types of interactions for the different food categories as well as to establish mixtures that contain synergistic interactions that lead to the development of new functional foods.

Abbreviations

B: Blueberry
G: Grape
L: Lemon
IF: Individual fruit
IFE: Individual fruit extract
FM: Fruit mixture
FME: Fruit mixture extract
RT: Room temperature
CE: Conventional extraction
UAE: Ultrasound-assisted extraction
HHPE: High hydrostatic pressure extraction
TE: Trolox equivalent
TPC: Total polyphenolic content (mg GA g^{-1} dry extract)
GA: Gallic acid
TFC: Total flavonoid content (mg QE g^{-1} dry extract)
QE: Quercetin equivalent
MI: Mixture index (dimensionless)
AC_1: Antioxidant capacity of IFE "1"
AC_2: Antioxidant capacity of IFE "2"
AC_1C_2: Antioxidant capacity of FME
X: Regeneration percentage (%)
SWV: Square wave voltammetry.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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References

[1] P. Kris-Etherton, K. D. Hecker, A. Bonanome et al., "Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer," *The American Journal of Medicine*, vol. 113, no. 9, pp. 71–88, 2002.

[2] W. Maldonado-Rojas and J. Olivero-Verbel, "Potential interaction of natural dietary bioactive compounds with COX-2," *Journal of Molecular Graphics and Modelling*, vol. 30, pp. 157–166, 2011.

[3] H.-W. Jiang, H.-Y. Li, C.-W. Yu et al., "The evaluation of antioxidant interactions among 4 common vegetables using isobolographic analysis," *Journal of Food Science*, vol. 80, no. 6, pp. C1162–C1169, 2015.

[4] D. Liu, J. Shi, A. Colina Ibarra, Y. Kakuda, and S. Jun Xue, "The scavenging capacity and synergistic effects of lycopene, vitamin E, vitamin C, and β-carotene mixtures on the DPPH free radical," *LWT—Food Science and Technology*, vol. 41, no. 7, pp. 1344–1349, 2008.

[5] L. M. Ciesla, K. A. Wojtunik-Kulesza, A. Oniszczuk, and M. Waksmundzka-Hajnos, "Antioxidant synergism and antagonism between selected monoterpenes using the 2,2-diphenyl-1-picrylhydrazyl method," *Flavour and Fragrance Journal*, vol. 31, no. 6, pp. 412–419, 2016.

[6] J. Sochor, J. Dobes, O. Krystofova et al., "Electrochemistry as a tool for studying antioxidant properties," *International Journal of Electrochemical Science*, vol. 8, pp. 8464–8489, 2013.

[7] F. M. A. Lino, L. Z. de Sá, I. M. S. Torres et al., "Voltammetric and spectrometric determination of antioxidant capacity of selected wines," *Electrochimica Acta*, vol. 128, pp. 25–31, 2014.

[8] N. N. Sazhina, "Determination of antioxidant activity of various bioantioxidants and their mixtures by the amperometric method," *Russian Journal of Bioorganic Chemistry*, vol. 43, no. 7, pp. 771–775, 2017.

[9] N. Sazhina, E. Plotnikov, E. Korotkova, E. Dorozhko, and O. Voronova, "Electrochemical oxidability of antioxidants: synergism and antagonism in mixes," *Journal of Pharmacy And Bioallied Sciences*, vol. 10, pp. 60–65, 2018.

[10] S. Buratti, S. Benedetti, and M. S. Cosio, "Evaluation of the antioxidant power of honey, propolis and royal jelly by amperometric flow injection analysis," *Talanta*, vol. 71, no. 3, pp. 1387–1392, 2007.

[11] Odepa (Oficina de Estudios y Políticas Agrarias), *Panorama Agricultura Chilena/Chilean Agriculture Overview*, Odepa (Oficina de Estudios y Políticas Agrarias), Santiago, Chile, 2017.

[12] W. Brand-Williams, M. E. Cuvelier, and C. Berset, "Use of a free radical method to evaluate antioxidant activity," *LWT—Food Science and Technology*, vol. 28, no. 1, pp. 25–30, 1995.

[13] I. F. F. Benzie and J. J. Strain, "The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay," *Analytical Biochemistry*, vol. 239, no. 1, pp. 70–76, 1996.

[14] F. Que, L. Mao, X. Fang, and T. Wu, "Comparison of hot air-drying and freeze-drying on the physicochemical properties and antioxidant activities of pumpkin (*Cucurbita moschata* Duch.) flours," *International Journal of Food Science & Technology*, vol. 43, no. 7, pp. 1195–1201, 2008.

[15] I. Dini, G. C. Tenore, and A. Dini, "Antioxidant compound contents and antioxidant activity before and after cooking in sweet and bitter Chenopodium quinoa seeds," *LWT—Food Science and Technology*, vol. 43, no. 3, pp. 447–451, 2010.

[16] M. N. Peyrat-Maillard, M. E. Cuvelier, and C. Berset, "Antioxidant activity of phenolic compounds in 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced oxidation: synergistic and antagonistic effects," *Journal of the American Oil Chemists' Society*, vol. 80, no. 10, pp. 1007–1012, 2003.

[17] J. Rúa, D. de Arriaga, M. R. García-Armesto, F. Busto, and P. del Valle, "Binary combinations of natural phenolic compounds with gallic acid or with its alkyl esters: an approach to understand the antioxidant interactions," *European Food Research and Technology*, vol. 243, no. 7, pp. 1211–1217, 2017.

[18] E. Uribe, A. Delgadillo, C. Giovagnoli-Vicuña, I. Quispe-Fuentes, and L. Zura-Bravo, "Extraction techniques for bioactive compounds and antioxidant capacity determination of chilean papaya (*Vasconcellea pubescens*) fruit," *Journal of Chemistry*, vol. 2015, Article ID 347532, 8 pages, 2015.

[19] K. L. Wolfe, X. Kang, X. He, M. Dong, Q. ZHang, and R. H. Liu, "Cellular antioxidant activity of common fruits," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 18, pp. 8418–8426, 2008.

[20] E. I. Oikeh, E. S. Omoregie, F. E. Oviasogie, and K. Oriakhi, "Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates," *Food Science & Nutrition*, vol. 4, no. 1, pp. 103–109, 2016.

[21] P. García-Salas, A. M. Gómez-Caravaca, D. Arráez-Román et al., "Influence of technological processes on phenolic compounds, organic acids, furanic derivatives, and antioxidant activity of whole-lemon powder," *Food Chemistry*, vol. 141, no. 2, pp. 869–878, 2013.

[22] C. Guo, J. Yang, J. Wei, Y. Li, J. Xu, and Y. Jiang, "Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay," *Nutrition Research*, vol. 23, no. 12, pp. 1719–1726, 2003.

[23] W.-Y. Huang, H.-C. Zhang, W.-X. Liu, and C.-Y. Li, "Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing," *Journal of Zhejiang University SCIENCE B*, vol. 13, no. 2, pp. 94–102, 2012.

[24] H. Wang, X. Guo, X. Hu, T. Li, X. Fu, and R. H. Liu, "Comparison of phytochemical profiles, antioxidant and cellular antioxidant activities of different varieties of blueberry (*Vaccinium* spp.)," *Food Chemistry*, vol. 217, pp. 773–781, 2017.

[25] F. Chemat, N. Rombaut, A.-G. Sicaire, A. Meullemiestre, A.-S. Fabiano-Tixier, and M. Abert-Vian, "Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications: a review," *Ultrasonics Sonochemistry*, vol. 34, pp. 540–560, 2017.

[26] V. Briones-Labarca, M. Plaza-Morales, C. Giovagnoli-Vicuña, and F. Jamett, "High hydrostatic pressure and ultrasound extractions of antioxidant compounds, sulforaphane and fatty acids from Chilean papaya (Vasconcellea pubescens) seeds: effects of extraction conditions and methods," *LWT—Food Science and Technology*, vol. 60, no. 1, pp. 525–534, 2015.

[27] M. Aoun and D. P. Makris, "Binary mixtures of natural polyphenolic antioxidants with ascorbic acid: impact of interactions on the antiradical activity," *International Food Research Journal*, vol. 19, pp. 603–606, 2012.

[28] A. Gironés-Vilaplana, P. Valentão, D. A. Moreno, F. Ferreres, C. García-Viguera, and P. B. Andrade, "New beverages of lemon juice enriched with the exotic berries maqui, Açaí, and blackthorn: bioactive components and in vitro biological properties," *Journal of Agricultural and Food Chemistry*, vol. 60, no. 26, pp. 6571–6580, 2012.

[29] D. Jain, S. Pancholi, and R. Patel, "Synergistic antioxidant activity of green tea with some herbs," *Journal of Advanced Pharmaceutical Technology & Research*, vol. 2, no. 3, p. 177, 2011.

[30] M. Colon and C. Nerín, "Synergistic, antagonistic and additive interactions of green tea polyphenols," *European Food Research and Technology*, vol. 242, no. 2, pp. 211–220, 2016.

[31] M. Pinelo, L. Manzocco, M. J. Nuñez, and M. C. Nicoli, "Interaction among phenols in food fortification: negative synergism on antioxidant capacity," *Journal of Agricultural and Food Chemistry*, vol. 52, no. 5, pp. 1177–1180, 2004.

[32] G. R. Velderrain-Rodríguez, M. Ovando-Martínez, M. Villegas-Ochoa et al., "Antioxidant capacity and bioaccessibility of synergic mango (cv. Ataulfo) peel phenolic compounds in edible coatings applied to fresh-cut papaya," *Food and Nutrition Sciences*, vol. 6, no. 3, pp. 365–373, 2015.

[33] H. J. Heo, Y. J. Kim, D. Chung, and D.-O. Kim, "Antioxidant capacities of individual and combined phenolics in a model system," *Food Chemistry*, vol. 104, no. 1, pp. 87–92, 2007.

[34] U. Gawlik-Dziki, "Changes in the antioxidant activities of vegetables as a consequence of interactions between active compounds," *Journal of Functional Foods*, vol. 4, no. 4, pp. 872–882, 2012.

[35] S. Wang, K. A. Meckling, M. F. Marcone, Y. Kakuda, and R. Tsao, "Synergistic, additive, and antagonistic effects of food mixtures on total antioxidant capacities," *Journal of Agricultural and Food Chemistry*, vol. 59, no. 3, pp. 960–968, 2011.

[36] A. Altunkaya, V. Gökmen, and L. H. Skibsted, "pH dependent antioxidant activity of lettuce (L. *sativa*) and synergism with added phenolic antioxidants," *Food Chemistry*, vol. 190, pp. 25–32, 2016.

[37] C. Rodríguez-Pérez, R. Quirantes-Piné, A. Fernández-Gutiérrez, and A. Segura-Carretero, "Optimization of extraction method to obtain a phenolic compounds-rich extract from Moringa oleifera Lam leaves," *Industrial Crops and Products*, vol. 66, pp. 246–254, 2015.

[38] J. A. M. De Paula, L. F. Brito, K. L. F. N. Caetano, M. C. De Morais Rodrigues, L. L. Borges, and E. C. da Conceição, "Ultrasound-assisted extraction of azadirachtin from dried entire fruits of Azadirachta indica A. Juss. (Meliaceae) and its determination by a validated HPLC-PDA method," *Talanta*, vol. 149, pp. 77–84, 2016.

[39] J. M. George, T. Senthamizh Selvan, and N. K. Rastogi, "High-pressure-assisted infusion of bioactive compounds in apple slices," *Innovative Food Science & Emerging Technologies*, vol. 33, pp. 100–107, 2016.

[40] V. Andrés, L. Mateo-Vivaracho, E. Guillamón, M. J. Villanueva, and M. D. Tenorio, "High hydrostatic pressure treatment and storage of soy-smoothies: colour, bioactive compounds and antioxidant capacity," *LWT—Food Science and Technology*, vol. 69, pp. 123–130, 2016.

[41] J. Sun, Y.-F. Chu, X. Wu, and R. H. Liu, "Antioxidant and antiproliferative activities of common fruits," *Journal of Agricultural and Food Chemistry*, vol. 50, no. 25, pp. 7449–7454, 2002.

[42] M. K. Khan, M. Abert-Vian, A.-S. Fabiano-Tixier, O. Dangles, and F. Chemat, "Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (*Citrus sinensis* L.) peel," *Food Chemistry*, vol. 119, no. 2, pp. 851–858, 2010.

[43] Z. Shouqin, X. Jun, and W. Changzheng, "High hydrostatic pressure extraction of flavonoids from propolis," *Journal of Chemical Technology & Biotechnology*, vol. 80, no. 1, pp. 50–54, 2005.

[44] K. Ghasemi, Y. Ghasemi, and M. A. Ebrahimzadeh, "Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues," *Pakistan Journal of Pharmaceutical Sciences*, vol. 22, no. 3, pp. 277–281, 2009.

[45] A. Bunea, O. D. Rugina, A. M. Pintea, Z. Sconţa, C. I. Bunea, and C. Socaciu, "Comparative polyphenolic content and antioxidant activities of some wild and cultivated blueberries from Romania," *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, vol. 39, no. 2, pp. 70–76, 2011.

[46] P. Doshi, P. Adsule, and K. Banerjee, "Phenolic composition and antioxidant activity in grapevine parts and berries (*Vitis vinifera* L.) cv. Kishmish Chornyi (Sharad Seedless) during maturation," *International Journal of Food Science and Technology*, vol. 41, no. s1, pp. 1–9, 2006.

[47] M. Hidalgo, C. Sánchez-Moreno, and S. de Pascual-Teresa, "Flavonoid-flavonoid interaction and its effect on their antioxidant activity," *Food Chemistry*, vol. 121, no. 3, pp. 691–696, 2010.

[48] M. Leopoldini, T. Marino, N. Russo, and M. Toscano, "Antioxidant properties of phenolic compounds: H-atom versus electron transfer mechanism," *The Journal of Physical Chemistry A*, vol. 108, no. 22, pp. 4916–4922, 2004.

[49] A. Kirakosyan, E. Mitchell Seymour, K. R. Noon et al., "Interactions of antioxidants isolated from tart cherry (*Prunus cerasus*) fruits," *Food Chemistry*, vol. 122, no. 1, pp. 78–83, 2010.

[50] Â. Luís, A. P. Duarte, L. Pereira, and F. Domingues, "Interactions between the major bioactive polyphenols of berries: effects on antioxidant properties," *European Food Research and Technology*, vol. 244, no. 1, pp. 175–185, 2018.

[51] B. García, A. Torres, and F. Macías, "Synergy and other interactions between polymethoxyflavones from citrus byproducts," *Molecules*, vol. 20, no. 11, pp. 20079–20106, 2015.