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Physicochemical Stability, Antioxidant Activity, and Acceptance of Beet and Orange Mixed Juice during Refrigerated Storage

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Academic Editor: António Manuel Jordão
Received: 23 May 2017; Accepted: 13 July 2017; Published: 18 July 2017

Abstract: The objective of this study was to mix beet juice and orange juice in two proportions (1:1 and 1:2 v/v), evaluate their physicochemical stability and antioxidant activity during storage (4 °C for 30 days), and evaluate their acceptance by consumers. Beet juice (with or without pasteurization) and pasteurized orange juice were used as controls. The presence of orange juice contributed to the pH, betacyanin, betaxanthin, and antioxidant capacity stabilities during storage, whereas the presence of beet improved the color stability. The mixed juices showed high total phenolic compounds (484–485 µg gallic acid/mL), DPPH scavenging capacity (2083–1930 µg Trolox/mL), and ABTS (1854–1840 µg Trolox/mL), as well as better sensory acceptance than the pasteurized beet juice. However, the mixed juices had a more significant reduction in the ascorbic acid content (completely lost at 15 days of storage) than the pasteurized orange juice (25% reduction at 30 days). The beet and orange mixed juice is an alternative functional beverage that can contribute to an increase in the consumption of beet and orange.

Keywords: phenolics; betacyanin; ascorbic acid; *Beta vulgaris* L.; *Citrus* X *sinensis*

1. Introduction

Beet (*Beta vulgaris* L.) is one of the most produced green vegetables in the world [1]. Beet juice is a convenient alternative for vegetal consumption, and contains beneficial compounds for health, such as potassium, magnesium, folic acid, iron, zinc, calcium, phosphate, sodium, niacin, biotin, the B6 vitamin, and soluble fiber. Furthermore, beet juice is a rich source of polyphenolic compounds, which are biologically available antioxidants [2].

The intense and attractive color of the beet is due to the presence of hydrosoluble pigments called betalains. The betalains are derived from betalamic acid and divided into two sub-classes: betacyanin (purple-red pigments) and betaxanthins (yellow-orange pigments) [3]. These pigments are important antioxidant substances, and present antimicrobial and anti-inflammatory activities, the inhibition of lipid peroxidation, an increased resistance to oxidation of low-density lipoproteins, and chemo-preventive effects [3–5].

Orange juice (*Citrus sinensis*) is responsible for half of the juice produced in the world, and it has great acceptability because of its flavor. Orange juice is a source of ascorbic acid, a nutrient that has a vitaminic action, and is a co-factor in several physiological processes, such as lysine and proline hydroxylation, the synthesis of collagen and other proteins of the conjunctive tissue, the synthesis of the norepinephrine and adrenal hormones, the activation of peptide hormones, and the synthesis of carnitine. Ascorbic acid also acts as an antioxidant, and facilitates iron absorption by the intestines and the maintenance of ferrous ions in blood plasma [6–8].

According to recommendations by the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO), the minimum consumption of fruit and green vegetables for adults must be of 400 g/day (five or more daily portions) [9]. However, according to data from the Chronic Diseases and Risk Factors Surveillance, only 20.2% of Brazilians eat the recommended daily amount [10].

One of the alternatives to redress the balance between the real and the recommended ingestions in a diet is the consumption of functional beverages rich in fruit and/or green vegetables. These products can also be a source of antioxidants, provided that their functional components have been preserved, and their commercialization will be effective if they are widely accepted by consumers [11].

Therefore, the combination of beet juice, rich in antioxidant compounds, with orange juice, highly acceptable sensorially and rich in ascorbic acid and polyphenols, results in an interesting beverage. As far as the authors know, there are no studies concerning the development of beet and orange mixed juices and their stability during refrigerated storage. Therefore, the objective of this study was to evaluate the acceptance as well as the physicochemical stability and antioxidant activity of beet and orange mixed juices (1:1 or 1:2 *v*/*v*) during refrigerated storage for 30 days.

2. Materials and Methods

2.1. Preparation of the Juices

Five formulations of juices were prepared: non-pasteurized beet juice (BJ); pasteurized beet juice (PBJ); pasteurized orange juice (POJ); beet and orange mixed juices, 1:1 (BOMJ 1:1); and beet and orange mixed juices, 1:2 (BOMJ 1:2).

Fresh beets (*Beta vulgaris* L.) cv. Early Wonder were obtained from Central de Abastecimento do Paraná S.A. (CEASA) in the city of Londrina, Brazil. The beets were washed in running water, sanitized (150 mg/L Dinamica® fruit disinfectant with sodium hypochlorite for 30 min), and manually peeled and crushed using a fruit processor (Mondial®, Brazil), originating the beet juice. To obtain the orange juice, concentrated orange juice (66° Brix) was diluted with sterilized water in the proportion of 1:6 (*v*/*v*), according to the manufacturer's recommendation, until the soluble solids value was close to 12° Brix. The concentrated orange juice was sugarless and preservative-free.

The beet and orange mixed juices were prepared from beet juice and the diluted orange juice, mixing them in two proportions, 1:1 (BOMPJ 1:1) or 1:2 (BOMPJ 1:2) (*v*/*v*), respectively. The proportions of the beet and orange in the mixed juices were based on a preliminary analysis in order to obtain a product with suitable phenolic compounds and physicochemical and sensory characteristics. The juices (PBJ, POJ, BOMJ 1:1, and BOMJ 1:2) were placed in transparent glass flasks (Farma®, Brazil), and pasteurized at 70 °C for 30 min in a water bath. The BJ juice was not submitted to the heat treatment.

The juices were stored at 4 °C for 30 days, which is the shelf life of commercial pasteurized Brazilian juices. The products were evaluated on days 0, 5, 10, 15, and 30 of storage.

2.2. Physicochemical Analysis

The pH determination was carried out by a calibrated digital potentiometer (Tecnal, Brazil). The titratable acidity was measured by titration with a 0.01 M NaOH (Anidrol®, Brazil) solution until pH 8.2, and expressed as a citric acid percentage [12].

A colorimeter (Minolta®, model CR400, Osaka, Japan) was used for the assessment of the color parameters values (L*, a*, b*, and C (Chroma)). From parameters L*, a*, and b*, the total difference was calculated (ΔE*ab) between each sample at a determined storage time, and the sample at the initial time of storage, as follows: $\Delta E^*ab = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

2.3. Ascorbic Acid Determination by High Performance Liquid Chromatography (HPLC)

The ascorbic acid content was determined according to Souza et al. [13], with modifications. The juice samples that contained orange juice (POJ, BOMJ 1:1, and BOMJ 1:2) were dissolved in 3% metaphosphoric acid and filtered in a PVDF hydrophilic membrane (pores of 0.22 µm) (Millex-GV, Millipore, USA). The chromatograph system (Shimadzu, Japan) consisted of two pumps (model LC-10AD), a Rheodyne injecting valve with a sample handle of 20 µL, a column oven (model CTO-20A), a UV/visible spectrophotometric detector (model SPD-10A), an interface (model CBM-101), the CLASS-CR10 program, version 1.2 (Shimadzu, Japan), and a Spherisorb ODS1 (4.6 × 250 mm, 5 µm) column (Waters®, Ireland). The analysis was conducted at room temperature using an isocratic elution (0.5 mL/min) with a sulfuric acid solution (pH 2.5) as the mobile phase. The ascorbic acid detection was done at 254 nm. The identification was based on retention times and co-elution with standards, and the quantification by external standardization using a calibration curve with six points (measured in duplicate) in the concentration range of 5 to 50 µg/mL.

2.4. Betalains Determination

For the extraction of the betalains, sample aliquots (except POJ) of 1 mL were diluted in 30 mL distilled water by manual agitation for 10 s, and the mix was centrifuged (25 °C) at 6000× *g* for 20 min (Eppendorf centrifuge 5804R, Germany). The betaxanthin and betacyanin content in the supernatants were analyzed through absorbance readings at 476 and 538 nm in a UV/VIS spectrophotometer (Biochrom Libra S22, UK). A reading at 600 nm was used to correct possible impurities (turbidity) according to the methods of Stintzing, Schieber, and Carle [14], with some modifications. The betalain content (BC), expressed in mg/mL of juice, was calculated from the equation = $[(A \times FD \times MM \times 100)/(\varepsilon \times I)]$, where A is the difference between maximum absorption (476 or 538 nm) and the correction absorption at 600 nm, FD is the dilution factor, and I is the length of the cuvette path (1 cm). For the quantification of betacyanin and the betaxanthins, the molecular masses (MM) and molar absorptivity (ε) were: MM = 550 g/mol, ε = 60,000 L/mol cm in H2O and MM = 308 g/mol, ε = 48,000 L/mol cm in H2O, respectively.

2.5. Total Phenolic Compounds Content Determination

The phenolic compounds were determined by the Folin–Ciocalteau method, as described by Singleton, Orthofer, and Lamuela-Raventos [15]. An aliquot (0.2 mL) of sample was added to 1.5 mL of Folin–Ciocalteau aqueous solution (1:10 *v*/*v*). The mix was left to stabilize for 5 min, and then mixed with 1.5 mL of a sodium carbonate solution (60 g/L). Then, it was incubated at room temperature for 90 min and the absorbance of the mix was read at 725 nm in a UV-VIS spectrophotometer, using the respective solvent as the blank. The results were expressed in µg of gallic acid equivalents (µg EAG/100 mL).

2.6. Antioxidant Activity Evaluated by the DPPH Free Radical Scavenging Method

The total antioxidant capacity was evaluated by the DPPH method according to Brand-Williams, Cuvelier, and Berset [16], with some modifications. In a test tube were mixed 1 mL of 100 mM sodium acetate buffer (pH 5.5), 1 mL of absolute ethanol, 0.5 mL of an ethanolic solution of DPPH (250 µmol/L), and 50 µL of each sample. The test tubes were kept in the dark and at room temperature for 30 min, and the absorbance was read at 517 nm in a UV-VIS spectrophotometer. The antioxidant activity quantification was realized by a Trolox standard curve, and the results expressed in µmol of Trolox/mL.

2.7. ABTS+ Cation Radical Scavenging Activity

The antioxidant activity was also analyzed by the sample capacity to eliminate ABTS free radicals, using the modified methodology reported by Ozgen et al. [17]. When combined with an oxidant (potassium persulphate 2.45 mM), the ABTS (7 mM in phosphate tampon 20 mM, pH 7.4) reacts to create a dark greenish-blue stable radical solution after 12–16 h of incubation in the dark. The solution was diluted up to an absorbance of 0.7 ± 0.01 in 730 nm to form the reagent test. Reactional mixes with 10 µL of sample and 4 mL of reagent test were incubated at room temperature for 6 min. The antioxidant activity was calculated in relation to the Trolox standard curve and expressed in µmol of Trolox/mL.

2.8. Sensory Analysis

The sensory analysis was conducted in the juices that had beet in the formulation (PBJ, BOMJ 1:1, and BOMJ 1:2) on the 5th day of storage. The pure orange juice (POJ) was not evaluated for acceptance because it is known that it has great acceptability, and the objective was to produce a juice with the phenolic compounds provided by beet. The acceptance test of attributes (color, aroma, flavor, and overall impression) was conducted with a 9-point hedonic scale (1 = disliked extremely and 9 = liked extremely). The formulations were coded with 3-digit numbers and served at a temperature of 10 °C in acrylic cups, one at a time, in random order. The sensory panel consisted in 72 untrained judges who were habitual consumers of fruit juices. The project was approved by the Ethical Conduct in Research Involving Human Beings Committee from the Londrina State University, through opinion CEP/UEL 342/2011, nº CAAE 0319.0.268.000-11.

2.9. Experimental Design and Statistical Analysis

The physicochemical analysis and antioxidant activity were carried out according to a split plot design, in which the main treatment was the formulation and the secondary treatment was the storage duration. The experiment was replicated three times. In each replication, analyses were conducted in triplicate. The sensory experiment followed a randomized complete blocks design, where the juices were the treatments and the blocks the judges. The data were submitted to an analysis of variance (ANOVA) and the mean comparison Tukey test ($p < 0.05$). The statistical analyses were realized using the Statistical Analysis System version 9.1.3.

3. Results and Discussion

3.1. Physicochemical Characteristics

The results for pH and titratable acidity of the juices are presented in Table 1. The mixed formulations (BOMJ 1:1 and 1:2) presented titratable acidity (0.41%–0.59% citric acid) with intermediate values between beet juice (BJ or PBJ; 0.08%–0.14% citric acid) and orange juice (POJ; 0.77%–0.79% citric acid) ($p \leq 0.05$), as expected. The same trend was observed for pH values, where the mixed formulations (BOMJ 1:1 and 1:2) presented pH (4–4.21) with intermediate values between beet juice (BJ or PBJ; 4.84–5.71) and orange juice (POJ; 3.83–3.88) ($p \leq 0.05$). The presence of higher quantities of orange juice (BOMJ 1:2) in the mixed juice resulted in more acidic products, with a decrease in the pH values and an increase in the titratable acidity ($p \leq 0.05$) when compared to the product with lower orange content (BOMJ 1:1). The pH and titratable acidities of the beet and orange juices are similar to those reported by other authors [18,19].

Organic acids contribute to the flavor and palatability of fruit juices. The greater acidity of the mixed juices (BOMJ 1:1 or BOMJ 1:2) when compared to the beet juices (BJ or PBJ) could protect them from the development of food spoilage micro-organisms, increasing their shelf life [19]. However, it can decrease the sensory acceptance of the products, because consumers are not attracted to very acidic products [20]. It is noteworthy, however, that the mixed juices (BOMJ 1:1 and BOMJ 1:2) still had lower acidity than the conventional orange juice (POJ).

Table 1. Titratable acidity and pH of juices stored at 4 °C for 30 days.

Titratable Acidity (% Citric Acid)					
Juices	Storage Time (Days)				
	0	5	10	15	30
BJ	0.08 ± 0.00 cD	0.08 ± 0.00 cD	0.09 ± 0.00 cbD	0.09 ± 0.01 bD	0.14 ± 0.06 aA
PBJ	0.08 ± 0.00 aD	0.08 ± 0.00 aD	0.09 ± 0.00 aD	0.08 ± 0.00 aD	0.09 ± 0.01 aB
POJ	0.78 ± 0.00 abA	0.78 ± 0.01 abA	0.78 ± 0.00 abA	0.79 ± 0.00 aA	0.77 ± 0.01 bC
BOMJ 1:1	0.43 ± 0.01 aC	0.42 ± 0.00 bC	0.43 ± 0.01 aC	0.42 ± 0.00 bC	0.41 ± 0.00 bD
BOMJ 1:2	0.59 ± 0.01 aB	0.57 ± 0.01 bB	0.57 ± 0.00 bcB	0.56 ± 0.01 cB	0.54 ± 0.00 dE
pH					
Juices	Storage Time (Days)				
	0	5	10	15	30
BJ	5.71 ± 0.02 aA	5.62 ± 0.01 abA	5.56 ± 0.02 bB	5.36 ± 0.21 cB	4.84 ± 0.26 dB
PBJ	5.70 ± 0.01 aA	5.69 ± 0.01 aA	5.68 ± 0.01 aA	5.68 ± 0.01 aA	5.49 ± 0.40 bA
POJ	3.88 ± 0.00 aD	3.83 ± 0.01 aD	3.87 ± 0.00 aE	3.83 ± 0.00 aE	3.88 ± 0.00 aE
BOMJ 1:1	4.17 ± 0.01 aB	4.18 ± 0.01 aB	4.16 ± 0.00 aC	4.21 ± 0.03 aC	4.16 ± 0.01 aC
BOMJ 1:2	4.03 ± 0.00 aC	4.00 ± 0.01 aC	4.02 ± 0.00 aD	4.03 ± 0.02 aD	4.03 ± 0.01 aD

The values represent the mean ± standard deviation (n = 9). Different superscript low-case letters in the same line indicate significant difference ($p < 0.05$) for each juice in relation to storage time. Different superscript upper-case letters in the same column indicate a significant difference ($p \leq 0.05$) between juices for the same storage day. BJ, natural beet juice; PBJ, pasteurized beet juice; POJ, pasteurized orange juice; BOMJ 1:1, pasteurized beet and orange mix juice 1:1 (v/v); BOMJ 1:2, pasteurized beet and orange mix juice 1:2 (v/v).

During the storage period, the titratable acidity increased ($p < 0.05$) in the BJ juice, decreased in the mixed juices (BOMJ 1:1 and BOMJ 1:2), and was maintained in the PBJ and POJ juices ($p > 0.05$). For the pH values, the products with beet (BJ and PBJ) exhibited a decrease ($p \leq 0.05$) in pH values during storage, and the products with added orange (POJ, BOMJ 1:1, and BOMJ 1:2) maintained ($p > 0.05$) this parameter. Probably, the presence of orange juice (POJ, BOMJ 1:1, and BOMJ 1:2) resulted in an increase in the buffering capacity of the juices, maintaining the pH of the products [20], which did not occur in the juices with beet only.

The color parameters (L*, a*, b* and Croma) are shown in Table 2. The total color difference is presented in Figure 1. The initial values of a* for BJ (0.78) and PBJ (0.95) indicate that the beet-added juices had a slightly red-purple color, probably originating from the presence of betacyanin. The b* values for BJ (1.56) and PBJ (1.67) represent a slight predominance of yellow by the presence of betaxanthins [21]. The POJ juice presented values of L* (36.61), a* (−2.49), and b* (16.34), demonstrating the yellow color of the orange juice. The mixed juices presented color characteristics of both juices (beet and orange) (L* = 23−24; a* = 4.59−8.76; and b* = 2.19−2.75).

During the storage period, all formulations had the same behavior for the L* and a* parameters, as the products stored for 30 days had higher L* and a* values than the newer ones (day 0) ($p \leq 0.05$). These results indicate that the juices became redder and with a lighter color. The juices (BJ, PBJ, POJ, BOMJ 1:1, and BOMJ 1:2) also had a decrease in the parameter b*, demonstrating a discoloration of the yellow color. In the POJ juice, a more intense yellow color was observed (i.e., an increase in b* values), and the total difference in color (ΔE*ab) was the greatest (6.25). Therefore, the beet juice made the color of the juices more stable to the effects of refrigerated storage. The alterations in the color parameters were probably caused by the oxidative and non-oxidative reactions of polyphenols, resulting in colored condensation products. Furthermore, the Maillard Reaction or melanoidins' formation could cause the alteration [20].

Table 2. Color parameters for juices stored at 4 °C for 30 days

	L*				
Juices	**Storage Time (Days)**				
	0	**5**	**10**	**15**	**30**
BJ	22.45 ± 0.16 cD	22.87 ± 0.04 bD	22.31 ± 0.07 Cd	22.37 ± 0.02 cD	24.16 ± 0.47 aD
PBJ	22.37 ± 0.02 cD	22.71 ± 0.10 bD	22.34 ± 0.14 cD	22.23 ± 0.06 cD	23.72 ± 0.20 aE
POJ	36.61 ± 0.14 dA	38.18 ± 0.04 bA	36.73 ± 0.03 dA	37.04 ± 0.17 cA	42.42 ± 0.73 aA
BOMJ 1:1	23.21 ± 0.04 cC	23.76 ± 0.06 bC	23.15 ± 0.02 cC	23.14 ± 0.05 cC	24.78 ± 0.17 aC
BOMJ 1:2	23.77 ± 0.04 cB	24.33 ± 0.07 bB	23.81 ± 0.06 cB	24.15 ± 0.04 bB	26.18 ± 0.27 aB
	a*				
Juices	**Storage Time (Days)**				
	0	**5**	**10**	**15**	**30**
BJ	0.78 ± 0.06 bD	0.68 ± 0.03 cD	0.74 ± 0.03 bcD	0.81 ± 0.03 bC	1.07 ± 0.16 aD
PBJ	0.95 ± 0.03 bC	0.82 ± 0.04 cC	0.87 ± 0.06 bcC	0.91 ± 0.03 bcC	1.19 ± 0.13 aC
POJ	−2.49 ± 0.02 dE	−2.87 ± 0.02 bE	−2.51 ± 0.04 dE	−2.63 ± 0.05 cD	−3.64 ± 0.14 aE
BOMJ 1:1	4.80 ± 0.05 cB	5.18 ± 0.03 bB	4.78 ± 0.07 cB	4.59 ± 0.13 dB	6.13 ± 0.18 aB
BOMJ 1:2	6.65 ± 0.04 cA	7.07 ± 0.05 cA	6.65 ± 0.03 cA	6.68 ± 0.10 cA	8.76 ± 0.32 aA
	b*				
Juices	**Storage Time (Days)**				
	0	**5**	**10**	**15**	**30**
BJ	1.56 ± 0.04 aE	1.52 ± 0.03 aD	1.56 ± 0.03 aD	1.57 ± 0.03 aC	0.97 ± 0.16 bE
PBJ	1.67 ± 0.02 aD	1.61 ± 0.03 aD	1.63 ± 0.05 aD	1.64 ± 0.03 aC	1.21 ± 0.11 bD
POJ	16.34 ± 0.31 dA	17.03 ± 0.02 bA	16.46 ± 0.08 cA	15.95 ± 0.16 eA	18.34 ± 0.17 aA
BOMJ 1:1	2.39 ± 0.03 aC	2.38 ± 0.03 aC	2.35 ± 0.04 aC	2.19 ± 0.14 bB	2.23 ± 0.07 bC
BOMJ 1:2	2.75 ± 0.03 abB	2.82 ± 0.03 aB	2.68 ± 0.02 bB	2.26 ± 0.06 dB	2.47 ± 0.02 cB
	Chroma				
Juices	**Storage Time (Days)**				
	0	**5**	**10**	**15**	**30**
BJ	1.74 ± 0.03 aE	1.66 ± 0.02 aE	1.73 ± 0.03 aA	1.77 ± 0.02 aA	1.45 ± 0.12 bA
PBJ	1.92 ± 0.02 aD	1.80 ± 0.03 abD	1.85 ± 0.03 aB	1.88 ± 0.03 aA	1.69 ± 0.16 bB
POJ	16.53 ± 0.31 dA	17.27 ± 0.02 bA	16.65 ± 0.08 cC	16.16 ± 0.16 eB	18.70 ± 0.19 aC
BOMJ 1:1	5.36 ± 0.05 cC	5.70 ± 0.03 bC	5.33 ± 0.05 cD	5.08 ± 0.17 dC	6.52 ± 0.17 aD
BOMJ 1:2	7.19 ± 0.04 cB	7.61 ± 0.04 bB	7.17 ± 0.02 cdE	7.05 ± 0.11 dD	9.10 ± 0.31 aE

The values represent the mean ± standard deviation (n = 9). Different superscript low-case letters in the same line indicate significant difference ($p < 0.05$) for each juice in relation to storage time. Different superscript upper-case letters in the same column indicate significant difference ($p \leq 0.05$) between juices for the same storage day. BJ, raw beet juice; PBJ, pasteurized beet juice; POJ, pasteurized orange juice; BOMJ 1:1, pasteurized beet and orange mix juice 1:1 (*v*/*v*); BOMJ 1:2, pasteurized beet and orange mix juice 1:2 (*v*/*v*).

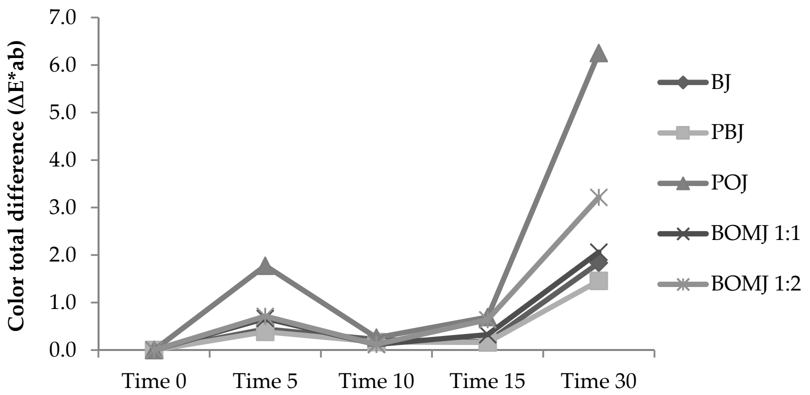

Figure 1. Total color difference (ΔE *ab) in juices stored at 4 °C for 30 days. BJ, raw beet juice; PBJ, pasteurized beet juice; POJ, pasteurized orange juice; BOMJ 1:1, pasteurized beet and orange mix juice 1:1 (*v*/*v*); BOMJ 1:2, pasteurized beet and orange mix juice 1:2 (*v*/*v*).

The physicochemical results demonstrated that the presence of orange juice in the mixed juices improved the pH stability of the products to the effects of refrigerated storage, as demonstrated by the maintenance of the pH of the orange and mixed juices. The alterations in the titratable acidity were slight (0.2% citric acid for BOMJ 1:1 and 0.05% citric acid for BOMJ 1:2). On the other hand, the presence of beet juice improved the color stability of the products, as the mixed juices showed a reduction of only 7%–10% in yellow color intensity and an increase in color purity (Chroma) by 22%–27%, probably due to the betalains' stability. These results reinforced the viability of the production of mixed juices with orange and beet. The physicochemical stability is desirable, because it confirms that the products remain similar to those that are newly manufactured, even after several weeks of storage [19].

3.2. Ascorbic Acid Content

The ascorbic acid content is shown in Figure 2. The initial values were 37.19, 25.93, and 39.84 mg/100 mL for POJ, BOMJ 1:1, and BOMJ 1:2, respectively, with the mixed juice with a higher quantity of orange juice (BOMJ 1:2) presenting higher ascorbic acid content than the other mixed juice (BOMJ 1:1), as expected. The pasteurized orange juice (POJ) showed an ascorbic acid loss of 25% after 30 days of storage ($p \leq 0.05$). The ascorbic acid stability is influenced by many factors, such as storage temperature, concentration of salt, sugars, and minerals, pH, oxygen levels, and the presence of enzymes or light [22]. However, the final values were in compliance with the legislation that established a minimum limit of ascorbic acid of 25 mg/100 mL for juice [23].

In the mixed juices (BOMJ 1:1 and BOMJ 1:2), a rapid loss of the ascorbic acid content occurred, with reduction right after 5 days of storage ($p \leq 0.05$). In BOMJ 1:1, the ascorbic acid content was completely lost at 15 days of storage, whereas in the BOMJ 1:2 juice a very low content was observed at day 30. The ascorbic acid can act as an antioxidant, removing the oxygen and controlling the activity of polyphenol oxidases enzymes, increasing the stability of the pigments [24]. In this study, it is possible that the decrease in the ascorbic acid content in the mixed juices is associated with a greater stability of the betalains pigments.

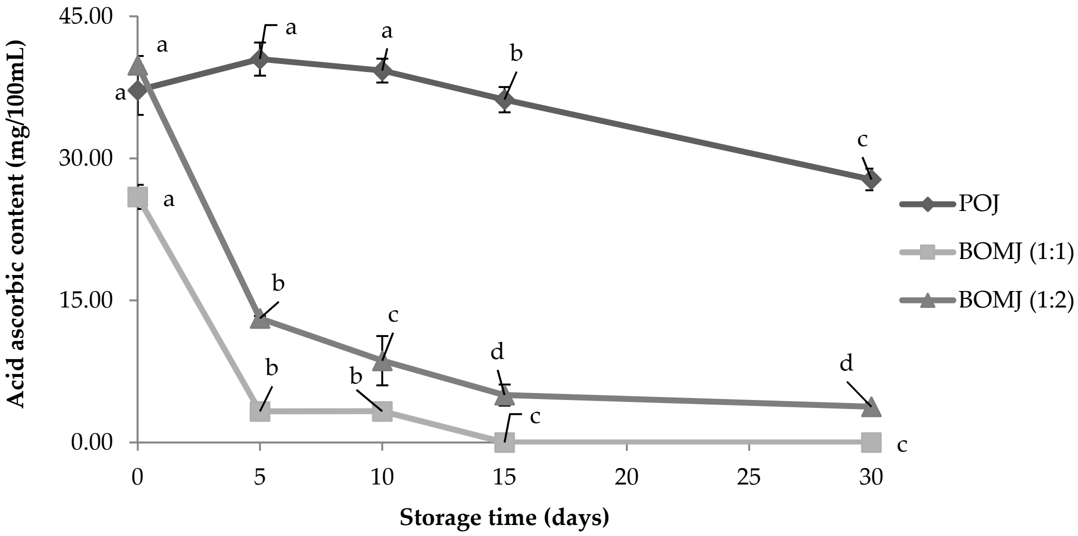

Figure 2. Acid Ascorbic Content (mg/100mL) in juices stored at 4 °C for 30 days. The values represent the mean ± standard deviation (n = 9). Different superscript lower case letters indicate significant difference ($p < 0.05$) for each juice in relation to storage time. POJ, pasteurized orange juice; BOMJ 1:1, pasteurized beet and orange mix juice 1:1 (v/v); BOMJ 1:2, pasteurized beet and orange mix juice 1:2 (v/v).

3.3. Betalain Content

Table 3 shows the results for the betacyanin and betaxanthin content during the refrigerated storage of the juices. The initial betacyanin content (Day 0) in pasteurized pure beet juice (PBJ) (53.39 mg/100 mL) was lower than the content in the raw beet juice (BJ) (65.32 mg/100 mg), showing that the pasteurization caused a reduction in betacyanins by 18% ($p \leq 0.05$). For the betaxanthins, the thermal treatment had no significant effect ($p > 0.05$).

Table 3. Betacyanin and betaxanthins content (mg/100 mL) in juices stored at 4 °C for 30 days.

Juices	Storage Time (Days)				
	0	**5**	**10**	**15**	**30**
Betacyanin					
BJ	65.32 ± 1.58 aA	61.18 ± 1.72 bA	55.34 ± 2.09 cA	51.76 ± 3.51 cA	40.64 ± 7.79 dA
PBJ	53.39 ± 3.31 aB	50.52 ± 5.89 bcB	49.48 ± 2.61 cB	42.31 ± 3.68 dB	31.55 ± 5.64 eB
BOMJ 1:1	31.12 ± 0.89 aC	31.99 ± 0.59 aC	31.63 ± 0.76 aC	28.27 ± 5.19 bC	30.29 ± 1.94 abC
BOMJ 1:2	22.31 ± 0.98 abD	23.17 ± 0.77 aD	22.85 ± 0.78 abD	20.37 ± 3.77 bD	22.32 ± 1.40 abD
Betaxanthins					
BJ	29.99 ± 1.86 aA	28.84 ± 0.79 abB	28.42 ± 2.34 bB	27.82 ± 2.77 bA	22.79 ± 5.61 cB
PBJ	31.76 ± 0.51 aA	30.43 ± 0.08 bA	30.40 ± 2.29 bA	27.80 ± 1.44 cA	25.69 ± 0.22 dA
BOMJ 1:1	11.96 ± 0.30 abB	12.08 ± 0.16 aC	11.76 ± 0.25 abC	11.44 ± 0.29 abB	10.74 ± 0.93 bC
BOMJ 1:2	8.80 ± 0.29 aC	8.84 ± 0.16 aD	8.52 ± 0.16 aD	8.21 ± 0.18 aC	7.82 ± 0.60 aD

The values represent the mean ± standard deviation ($n = 9$). Different superscript low-case letters in the same line indicate significant difference ($p < 0.05$) for each juice in relation to storage time. Different superscript upper-case letters in the same column indicate significant difference ($p \leq 0.05$) between juices for the same storage day, BJ (raw beet juice), PBJ (pasteurized beet juice), BOMJ 1:1 (pasteurized beet and orange mix juice 1:1 (*v*/*v*) and BOMJ 1:2 (pasteurized beet and orange mix juice 1:2 (*v*/*v*)).

The betacyanin and betaxanthins content of the mixed juices BOMJ 1:1 (31.12 mg/100 mL and 11.96 mg/100 mL) and BOMJ 1:2 (22.31 mg/100 mL e 8.80 mg/100 mL) were lower ($p \leq 0.05$) than those of the juices only with beet (BJ with 65.32 mg/100 mL and 29.99 mg/100 mL and PBJ with 53.39 mg/100 mL e 31.76 mg/100 mL). These results were expected, due to the dilution of the beet juice with the orange juice, proportional to each mixed juice formulation.

At the end of the storage time, the beet juices (BJ and PBJ) showed a significant reduction of 37–41% in betacyanin content, while for the betaxanthins, a reduction of 19–24% was observed. In the mixed juices (BOMJ 1:1 and BOMJ 1:2), the betacyanin (22.31–31.63 mg/100 mL) and betaxanthin (7.82–12.08 mg/100 mL) content was maintained, when comparing the products at 0 and 30 days of storage ($p > 0.05$). As discussed before, the results demonstrate that the orange juice contributed to the stability of both classes of betalains in the mixed juices, probably due to the action of the citric acid and the ascorbic acid. The citric acid is an antioxidant and chelating agent, and acts synergistically with the ascorbic acid. The maintenance of these beneficial compounds in the mixed juices is of primary importance in the development of functional foods.

3.4. Total Phenolic Compounds Content and Antioxidant Activity

Total phenolic content and the antioxidant activity (DPPH Trolox or ABTS trolox) are presented in Table 4. All juices were significant sources of polyphenols. The raw beet juice (BJ) and the pasteurized beet juice (PBJ) presented 521 and 497 µg EAG/mL, respectively; the mixed juices had 484–485 µg EAG/mL; and the orange juice (POJ) had 448 µg EAG/mL at day 0. In fact, orange juices are good sources of polyphenolic compounds, including hydroxycinnamic acids and flavonoids (mainly flavonas) [25], while beet juice has a high antioxidant action and is a rich source of polyphenols. Among the beet extract phenolic acids, the 4-hydroxybenzoic acid is the main constituent, followed by the cynamic, vanillic, and chlorogenic acids, the trans ferulic acid, and caffeic acid [2].

During storage time, the beet juices (BJ and PBJ) showed a significant loss ($p \leq 0.05$) of phenolic compounds by 14% and 13%, respectively, while the orange and mixed juices did not show any significant variation ($p > 0.05$) when comparing the products at day 0 and 30 of storage. This was probably due to the joint action of the citric and ascorbic acids, which inhibited the oxidative reactions of the phenolic compounds presented in the juices, demonstrating the beneficial effect of the addition of orange juice to the beet juice.

In both methodologies of antioxidant activity evaluation (DPPH and ABTS methods), the samples with greater antioxidant activity were the raw (2733 and 2179 µg Trolox/mL) and pasteurized beet (2813 and 2081 µg Trolox/mL) juices, and the sample with the lowest antioxidant activity was the orange juice (1408 and 1872 µg Trolox/mL). In fact, betalains' antioxidant activity is greater than that of ascorbic acid, and beet is one of the most powerful green vegetables with regard to antioxidant activity [26], when beet juice is compared to other fruits that are well-accepted as having high antioxidant contents, such as pomegranate and cranberry [27]. The mixed juices had intermediated antioxidant activity, with 1930–2083 µg Trolox/mL in the DPPH method and 1840–1854 µg Trolox/mL in the ABTS method.

During storage, the antioxidant activity decreased in all of the juices evaluated in one or both methodologies (DPPH or ABTS) ($p \leq 0.05$). Pasteurized beet juice (PBJ) showed the greatest loss (37%) in the DPPH method, followed by POJ (32%), BJ (21%), BOMJ 1:2 (19%), and BOMJ 1:1 (no significant loss). In the ABTS methodology, the decrease was: POJ (21%), PBJ (14%), BOMJ 1:1 (13%), and BOMJ 1:2 (12%). The decrease in ascorbic acid and total phenolic compounds is the probable cause for the reduction in antioxidant capacity in the juice samples analyzed in this study. Therefore, this study demonstrated that the orange juice maintained the stability of the phenolic compounds, and reduced the loss of the antioxidant activity of the juices.

Table 4. Total Phenolic compounds (µg EAG/mL), DPPH Trolox (µg/mL), and ABTS Trolox (µg/mL) in juices stored at 4 °C/30 days.

Juices	Total Phenolic Compounds				
	Storage Time (Days)				
	0	5	10	15	30
BJ	521 ± 19 bA	518 ± 9 bA	571 ± 86 aA	515 ± 62 bA	448 ± 48 cB
PBJ	497 ± 26 bABD	494 ± 20 bAB	555 ± 76 aA	517 ± 54 bA	431 ± 19 cB
POJ	448 ± 19 abC	473 ± 10 aB	477 ± 18 aBD	458 ± 15 abB	433 ± 41 bB
BOMJ 1:1	484 ± 33 cDB	499 ± 18 abcAB	519 ± 50 aC	514 ± 48 abA	485 ± 65 bcA
BOMJ 1:2	485 ± 50 abB	506 ± 29 aA	505 ± 42 aCD	490 ± 42 aA	459 ± 59 bAB
DPPH					
BJ	2733 ± 188 aA	2527 ± 255 abA	2307 ± 325 bcA	2323 ± 419 bA	2157 ± 243 cA
PBJ	2813 ± 264 aA	2210 ± 285 bB	2040 ± 296 bcB	1870 ± 236 cdB	1760 ± 261 dB
POJ	1408 ± 245 aC	773 ± 96 bcD	1037 ± 153 bD	797 ± 167 bcD	957 ± 135 bcC
BOMJ 1:1	2083 ± 89 aB	2027 ± 187 abBC	1317 ± 318 cC	1670 ± 369 bBC	1853 ± 231 abB
BOMJ 1:2	1930 ± 246 aB	1803 ± 146 abC	1452 ± 120 cC	1596 ± 200 bcC	1554 ± 119 cB
ABTS					
BJ	2179 ± 126 aA	1972 ± 209 bA	1950 ± 173 bA	2059 ± 145 bA	1720 ± 352 cAD
PBJ	2081 ± 142 aA	1741 ± 155 cB	1957 ± 206 bA	1999 ± 63 abA	1798 ± 62 cA
POJ	1872 ± 241 aB	1515 ± 93 bC	1507 ± 103 bcB	1575 ± 148 bB	1400 ± 113 cB
BOMJ 1:1	1854 ± 75 aB	1746 ± 136 abB	1642 ± 61 bcC	1711 ± 92 bcC	1604 ± 54 cC
BOMJ 1:2	1840 ± 131 aB	1667 ± 68 bB	1552 ± 51 cBC	1605 ± 84 bcBC	1614 ± 146 bcCD

The values represent the mean ± standard deviation ($n = 9$). Different superscript low-case letters in the same line indicate significant difference ($p < 0.05$) for each juice in relation to storage time. Different superscript upper-case letters in the same column indicate significant difference ($p \leq 0.05$) between juices for the same storage day. BJ, raw beet juice; PBJ, pasteurized beet juice; POJ, pasteurized orange juice; BOMJ 1:1, pasteurized beet and orange mix juice 1:1 (*v*/*v*); BOMJ 1:2, pasteurized beet and orange mix juice 1:2 (*v*/*v*).

3.5. Sensory Acceptance

Consumer acceptance is presented in Table 5. Pasteurized beet juice (PBJ) acceptance was lower ($p \leq 0.05$) than that of the mixed juices (BOMJ 1:1 and BOMJ 1:2) with regard to the attributes aroma, flavor, and overall acceptance. For the color attribute, it was not different from the BOMJ 1:2 ($p > 0.05$). The PBJ juice had satisfactory acceptance in the color parameter (a score of 7.5 in a 9-point hedonic scale), but, in aroma, flavor, and overall acceptance, the scores were low (<5.9).

Table 5. Acceptance of the juices.

Juices	Color	Aroma	Flavor	Overall Acceptance
PBJ	7.5 ± 1.8 b	5.9 ± 1.7 b	4.9 ± 2.2 b	5.6 ± 1.8 b
BOMJ (1:1)	8.0 ± 0.9 a	6.6 ± 1.7 a	6.3 ± 1.7 a	6.8 ± 1.5 a
BOMJ (1:2)	7.8 ± 1.1 ab	6.9 ± 1.9 a	6.6 ± 2.0 a	6.9 ± 1.8 a

The values represent the mean ± standard deviation, where different lower case superscript letters in the same column indicate significant differences ($p < 0.05$) between the juices for the same attribute or overall acceptance. PBJ, pasteurized beet juice; BOMJ 1:1, pasteurized beet and orange mix juice 1:1 (v/v); BOMJ 1:2, pasteurized beet and orange mix juice 1:2 (v/v). On the scale of acceptance, 1 = extremely dislike and 9 = like extremely.

The addition of orange juice to the beet juice increased the acceptance of the products in all evaluated parameters (color, aroma, flavor, and overall acceptance). The mixed juices received scores between 6.3 and 8 in a 9-point hedonic scale, indicating that the consumers liked the products from slightly to much. The color was the attribute with the highest score (7.8–8), probably because of the intensity and attractiveness that the beet gives to the product due to the presence of betalains.

The results of the present study demonstrate the applicability of the mixed juices, as the products were well accepted. The pasteurized beet juice (PBJ) had a higher content of betacyanin, betaxanthin, total phenolic content, and antioxidant activity; however, it was not well accepted by consumers, and the functional components were less stable to the effects of refrigerated storage in this juice.

Currently, several studies have focused on the antioxidant capacity and consumption benefits of the fruit and green vegetables juices, including beet juice [2,28,29]; however, there are no data related to the sensory acceptance of these types of beverages by population.

4. Conclusions

The beet and orange mixed juices developed in this study could provide consumers with strong natural antioxidant compounds and bioactive phytochemicals, and they had good acceptance. The presence of orange juice contributed to the pH, betacyanin, betaxanthin, and antioxidant capacity stabilities during storage, whereas the presence of beet improved the color stability. The mixed juices showed high total phenolic compounds (484–485 μg gallic acid/mL), DPPH scavenging capacity (2083–1930 μg Trolox/mL), and ABTS (1854–1840 μg Trolox/mL). The beet and orange mixed juice is an alternative functional beverage that can contribute to an increase in the consumption of beet and orange.

Acknowledgments: The authors gratefully acknowledge the financial support of Conselho Nacional de Pesquisa (CNPq) and Fundação Araucária.

Author Contributions: All authors contributed equally to this work.

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

References

1. FAO. Crop Water Information: Sugarbeet. 2015. Available online: http://www.fao.org/nr/water/cropinfo_sugarbeet.html (accessed on 22 February 2017).
2. Wootton-Beard, P.C.; Moran, A.; Ryan, L. Stability of the antioxidant capacity and total polyphenol content of 23 commercially available vegetable jucies before and after in vitro digestion as measured by FRAP, DPPH, ABTS and Folin Ciocalteau methods. *Food Res. Int.* **2011**, *44*, 217–224. [CrossRef]
3. Wruss, J.; Waldenberger, G.; Huemer, S.; Uygun, P.; Lazerstorfer, P.; Müller, U.; Höglinger, O.; Weghuber, J. Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Upper Austria. *J. Food Comps. Anal.* **2015**, *42*, 46–55. [CrossRef]
4. Gengatharan, A.; Dykes, G.A.; Choo, W.S. Betalains: Natural plant pigments with potential application in functional foods. *LWT Food Sci. Technol.* **2015**, *64*, 645–649. [CrossRef]

5. Farabegoli, F.; Scarpa, E.S.; Frati, A.; Serafini, G.; Papi, A.; Spisni, E.; Antonini, E.; Benedetti, S.; Ninfali, P. Betalains increase vitexin-2-O-xyloside cytotoxicity in CaCo-2 cancer cells. *Food Chem.* **2017**, *218*, 356–364. [CrossRef] [PubMed]
6. Tarrago-Trani, M.T.; Phillips, K.M.; Cotty, M. Matrix-specific method validation for quantitative analysis of vitamin c in diverse foods. *J. Food Comps. Anal.* **2012**, *26*, 12–25. [CrossRef]
7. Oikeh, E.I.; Omoregie, E.S.; Oviasogie, F.E.; Oriakhi, K. Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates. *Food Sci. Nutr.* **2016**, *4*, 103–109. [CrossRef] [PubMed]
8. Fusco, R.; Cirmi, S.; Gugliandolo, E.; di Paola, R.; Cuzzocrea, S.; Navarra, M. A flavonoid-rich extract of orange juice reduced oxidative stress in an experimental model of inflammatory bowel disease. *J. Funct. Foods* **2017**, *30*, 168–178. [CrossRef]
9. FAO. Food Based Dietary Guidelines for Sri Lankans. 2011. Available online: http://www.fao.org/nutrition/education/food-based-dietary-guidelines/regions/countries/sri-lanka/en/ (accessed on 22 February 2017).
10. Vigitel Brasil 2011: Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico. Available online: http://bvsms.saude.gov.br/bvs/publicacoes/vigitel_brasil_2011_fatores_risco_doencas_cronicas.pdf (accessed on 18 July 2017).
11. Wootton-Beard, P.C.; Ryan, L. Improving public health?: The role of antioxidant-rich fruit and vegetable beverages. *Food Res. Int.* **2011**, *44*, 3135–3148. [CrossRef]
12. Association of Official Analytical Chemistry (AOAC). *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Washington, DC, USA, 2004.
13. Souza, M.C.C.; Benassi, M.T.; Meneghel, R.F.A.; Silva, R.S.S.F. Stability of unpasteurized and refrigerated orange juice. *Braz. Arch. Biol. Technol.* **2004**, *47*, 391–397. [CrossRef]
14. Stintzing, F.C.; Schieber, A.; Carle, R. Evaluation of colour properties and chemical quality parameters of cactus juices. *Eur. Food Res. Technol.* **2003**, *216*, 303–311. [CrossRef]
15. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteau reagent. *Methods Enzymol.* **1999**, *299*, 152–178.
16. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free-radical method to evaluate antioxidant activity. *LWT Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]
17. Ozgen, M.; Reese, R.N.; Tulio, A.Z.; Scheerens, J.C.; Miller, A.R. Modified 2,2- azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods. *J. Agric. Food Chem.* **2006**, *54*, 1151–1157. [CrossRef] [PubMed]
18. Yoon, K.Y.; Woodams, E.E.; Hang, Y.D. Fermentation of beet juice by beneficial lactic acid bacteria. *LWT Food Sci. Technol.* **2005**, *38*, 73–75. [CrossRef]
19. Pimentel, T.C.; Madrona, G.S.; Garcia, S.; Prudencio, S.H. Probiotic viability, physicochemical characteristics and acceptability during refrigerated storage of clarified apple juice supplemented with *Lactobacillus paracasei* ssp. *paracasei* and oligofructose in different package type. *LWT Food Sci. Technol.* **2015**, *63*, 415–422. [CrossRef]
20. Costa, G.M.; Silva, J.V.C.; Mingotti, J.D.; Barão, C.E.; Klososki, S.J.; Pimentel, T.C. Effect of ascorbic acid or oligofructose supplementation on L. paracasei viability, physicochemical characteristics and acceptance of probiotic orange juice. *LWT Food Sci. Technol.* **2017**, *75*, 195–201. [CrossRef]
21. Delgado-Vargas, F.; Jiménez, A.R.; Paredes-López, O. Natural pigments: Carotenoids, anthocyanins, and betalains—Characteristics, biosynthesis, processing, and stability. *Crit. Rev. Food Sci. Nutr.* **2000**, *40*, 173–289. [CrossRef] [PubMed]
22. Tiwari, B.K.; Donnell, C.P.O.; Muthukumarappan, K.; Cullen, P.J. Ascorbic acid degradation kinetics of sonicated orange juice during storage and comparison with thermally pasteurised juice. *LWT Food Sci. Technol.* **2009**, *42*, 700–704. [CrossRef]
23. *Ministério da Agricultura e do Abastecimento Instrução Normativa n° 1, de 7 de Janeiro de 2000. Complementa Padrões de Identidade e Qualidade Para Suco de Laranja*; Diário Oficial da União da República Federativa do Brasil: Brasília (DF), Brasil, 2000; Seção 1; p. 54.
24. Cardoso-Ugarte, G.A.; Sosa-Morales, M.E.; Ballard, T.; Liceaga, A.; San Martín-González, M.F. Microwave-assisted extraction of betalains from red beet (*Beta vulgaris*). *LWT Food Sci. Technol.* **2014**, *59*, 276–282. [CrossRef]

25. Gliszczynska-Swiglo, A.; Wroblewska, J.; Lemanska, K.; Klimczak, I.; Tyrakowska, B. The contribution of polyphenols and vitamin C to the antioxidant activity of commercial orange juices and drinks. In Proceedings of the 14th IGWT Symposium Focussing New Century, Commodity-trade Environment, Beijng, China, August 2004; pp. 121–126.
26. Zitnanova, I.; Ranostajova, S.; Sobotova, H.; Demelova, D.; Pechan, I.; Durackova, Z. Antioxidative activity of selected fruits and vegetables. *Biologia* **2006**, *61*, 279–284. [CrossRef]
27. Ryan, L.; Prescott, S.L. Stability of the antioxidant capacity of twenty-five commercially available fruit juices subjected to an in vitro digestion. *Int. J. Food Sci. Technol.* **2010**, *45*, 1191–1197. [CrossRef]
28. Ravichandran, K.; Ahmed, A.R.; Knorr, D.; Smetanska, I. The effect of different processing methods on phenolic acid content and antioxidant activity of red beet. *Food Res. Int.* **2012**, *48*, 16–20. [CrossRef]
29. Ravichandran, K.; Saw, N.M.M.T.; Mohdaly, A.A.A.; Gabr, A.M.M.; Kastell, A.; Riedel, H.; Cai, Z.; Knorr, D.; Smetanska, I. Impact of processing of red beet on betalain content and antioxidant activity. *Food Res. Int.* **2013**, *50*, 670–675. [CrossRef]