Plant Foods Hum Nutr (2017) 72:60–66
DOI 10.1007/s11130-016-0591-0

ORIGINAL PAPER

Use of Banana (*Musa acuminata* Colla AAA) Peel Extract as an Antioxidant Source in Orange Juices

Lucía Ortiz[1] · **Eva Dorta**[2] · **M. Gloria Lobo**[1] · **L. Antonio González-Mendoza**[3] · **Carlos Díaz**[3] · **Mónica González**[1]

Published online: 17 December 2016

Abstract Using banana peel extract as an antioxidant in freshly squeezed orange juices and juices from concentrate was evaluated. Free radical scavenging capacity increased by adding banana peel extracts to both types of orange juice. In addition, remarkable increases in antioxidant capacity using 2,2′-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) radical were observed when equal or greater than 5 mg of banana peel extract per ml of freshly squeezed juice was added. No clear effects were observed in the capacity to inhibit lipid peroxidation. Adding 5 mg banana peel extract per ml of orange juice did not substantially modify the physicochemical and sensory characteristics of either type of juice. However, undesirable changes in the sensory characteristics (in-mouth sensations and colour) were detected when equal or greater than 10 mg banana peel extract per ml of orange juice was added. These results confirm that banana peel is a promising natural additive that increases the capacity to scavenge free radicals of orange juice with acceptable sensory and physicochemical characteristics for the consumer.

Keywords Lipid peroxidation inhibition · Free radical scavenging · Sensory analysis · Bio-wastes · Food antioxidants

Introduction

Antioxidants are health-promoting phytochemicals that provide benefits for human health, in particular by protecting against cellular oxidative stress. Increasing antioxidant concentrations in foods by adding vegetable extracts rich in natural antioxidants has been proposed to improve their antioxidant activity [1]. It also helps to preserve food from oxidation and is an attractive alternative to synthetic antioxidants, whose use is strictly regulated due to their potential health hazards [2]. Due to the low cost and the large quantities of plant bio-wastes produced, it is feasible to extend their use to the food industry, where they can be used as antioxidants to design new functional foods [2–4]. However, adding antioxidants derived from bio-waste to food entails some difficulties. Sensory and physicochemical alterations could appear during industrial production or storage of these functional foods [2]. Pomegranate peel, with high antioxidant activity, has been added to commercial tomato juice and orange juice with strawberries [5]; the samples with higher dried extract concentrations were least acceptable because of the characteristic astringent flavour of pomegranate peel.

From an environmental perspective it is vital to reuse the plant bio-waste produced by the agro-food industry. The main bio-waste of processing bananas is the peel. According to FAO estimates, in 2013 world production of bananas (*Musa acuminata* Colla AAA) was 107,401,205 mT [6]. Many practical uses have been proposed for banana peel, including the production of biomass, protein, ethanol, cellulose, hemicellulose, pectin, minerals and enzymes [7, 8]. But the banana peel

Electronic supplementary material The online version of this article (doi:10.1007/s11130-016-0591-0) contains supplementary material, which is available to authorized users.

✉ Mónica González
glez.glez.monica@gmail.com

[1] Instituto Canario de Investigaciones Agrarias, Apdo, 60, 38200 La Laguna, Spain

[2] Faculty of Chemistry, Pontificia Universidad Católica de Chile, Av. Libertador Bernardo O'Higgins 328, Santiago, Chile

[3] Department of Chemical Engineering and Pharmaceutical Technology, University of La Laguna, Avenida Astrofísico Francisco Sánchez, s/n, 38206 La Laguna, Spain

can also be used as source of natural bioactive compounds, mainly antioxidants such as phenolic compounds, anthocyanins, carotenoids, sterols and triterpenes and catecholamines [9–15]. Flavonols are the most abundant group of phenolic compounds in the peels of plantain cultivars (*Musa balbisiana* Colla). Rutin is a predominant phenolic compound in the flavonol profile of plantain and dessert banana peels, except 'Grande Naine'; their contents are in the range of those reported for buckwheat flour [16], which is one of the most important known food sources of rutin. Moreover, catecholamines [9, 12] are neurotransmitters with important antioxidant activity [17] and their use is being investigated [18] for preventing several illnesses, such as depression and Parkinson's disease. Our research group has confirmed that banana peel extract (BPE) in methanol has a high capacity to scavenge free radicals and inhibit lipid peroxidation [9, 10] due to its high content of phenolic compounds (2.2 ± 0.1 g gallic acid equivalents/100 g banana peel in a dry matter basis, DW) and catecholamines [265 ± 52 and 30 ± 1 mg /100 g banana peel DW, dopamine and L-dopa, respectively] [9, 10]. The antioxidant capacity of BPE is comparable to other plant bio-waste extracts [2, 19]. Therefore, *Musa* sp. peel could be proposed as a novel food ingredient and an important candidate for processing new pharmaceutical and nutraceutical formulations [14, 15, 20].

In this study, the use of BPE as a source of antioxidants in freshly squeezed orange juice (FSJ) and juice from concentrate (JFC) was evaluated. The concentration of BPE in orange juice that produces the maximum antioxidant activity, with acceptable sensory and physicochemical characteristics for the consumer, was determined.

Materials and Methods

Production of Banana Peel Extract (BPE)

Bananas (*Musa acuminata* Colla AAA, cv. 'Grande Naine') bunches ($n = 21$) were obtained from the research fields of the Instituto Canario de Investigaciones Agrarias (Tenerife, Canary Islands, Spain). The second hand from the proximal end of banana bunches were selected, artificially ripened and stored at 18°C and 80–90% relative humidity until full-ripeness was reached (6–7 days) (more details about ripening and maturity stage characterization are included as Electronic Supplementary Material, ESM 1). Peels were then manually separated, cut into small pieces (0.5 × 1 mm), freeze-dried (Christ alpha 1–4 LSC, Osterode, Germany), ground to a fine powder (< 1 mm) and stored at −20°C until the extractions were carried out. Each extraction process was done as many times as required to obtain the necessary amount of BPE to add to the juices. Freeze-dried peel and methanol (Scharlau Chemie, Barcelona, Spain) (peel:solvent ratio, w:v, 1:7) were homogenised with a Politron PT-6000 (Kinematica AG, Lucerne, Switzerland) blender at 12,000 *g* for 1 min and centrifuged at 5000 *g* for 20 min in a Jouan CR-312 centrifuge (Thermo Electron Corporation, Madrid, Spain) [9]. This extraction procedure was repeated three times [10]. The BPE was evaporated to dryness in a Heto VR-1 vacuum evaporator (Allerod, Denmark) at 37°C for 24 h, obtaining a viscous brown residue (dry extract). The extract yield ranged between 35 ± 1 and 50 ± 5 g BPE/100 g banana peel DW. Finally, banana peel dry extract was dissolved in orange juice until the concentration to be assayed was reached. The complete dissolution of the BPE was assured by mechanical agitation within a nitrogen atmosphere in order to avoid antioxidant oxidation.

Preparation of Orange Juices

The FSJ was obtained from oranges (*Citrus sinensis* L. Osbeck, cv. 'Washington Navel'), whose maturity stage was characterised by the colour of the peel and the edible part in eight fruits. The oranges were squeezed (Philips Cucina HR2744/40, Madrid, Spain) without reaching the albedo; the extraction yield, measured in 20 independent extractions, was 0.4 l juice/kg oranges (with 79 ± 5 mg pulp/ml juice). The JFC was prepared from Nova America S.A. orange juice concentrate (Santa Cruz do Rio Pardo, Brazil) (total soluble solids TSS, 66 ± 1 °Brix; citric acid, 4.2 ± 0.2%; pH, 3.8 ± 0.1; TSS/acidity ratio, 16 ± 1; pulp, 10 ± 1%), which was reconstituted with water (diluted five times) and ascorbic acid (Sigma, Madrid, Spain) to a final concentration of 80 mg/100 ml, according to the formula used by EIASA (Tenerife, Canary Islands, Spain). Both FSJ (total 25 L in three batches of 5, 10 and 10 L) and JFC (total 25 L in three batches of 5, 10 and 10 L) were homogenised with the dry extract and packed in sterile (121°C for 30 min) amber glass bottles (250 ml; total 100 bottles in three batches), leaving a minimum headspace volume.

Determination of the Quality Parameters

All the juices were characterised by their antioxidant activity, sensory attributes, analysed by a sensory analysis panel, and physicochemical parameters. All analyses were done in triplicate in independent replicates.

Antioxidant Activity

The capacity to scavenge free radicals was determined by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) [10, 19] and 2,2′-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS) radicals [10, 21]. The capacity to inhibit lipid peroxidation was evaluated using the β-carotene bleaching method, which is based on the capacity of antioxidants to decrease the loss of β-carotene (Aldrich) in a β-carotene/linoleic acid

(Sigma) emulsion [10, 22]. The antioxidant activity was expressed as g trolox (Aldrich, Madrid, Spain) equivalents (TE)/100 ml juice [10]. The antioxidant activity was also measured [10, 21–23] by dissolving the BPE in water at different concentrations between 1.3 and 7.5 mg/ml, in order to evaluate the BPE concentration that achieves maximum antioxidant activity.

Sensory Analysis

Sensory analyses of the orange juice were carried out in a standardised test room (ISO 8589:2007 [24]). The sensory panel was made up of 12 panellists specialised in sensory analysis (wine and honey). Each juice sample (40 ml) was served to panellists in wine-testing glasses, at a serving temperature of 13 ± 2°C and coded with a random number. Prior to carrying out the sensory evaluation, the panellists were trained on the sensory characteristics of the conventional (with no BPE added) juice and on the methodology of the tests. In addition, the criteria used to evaluate the quality of the juices and the recognition of perceived sensations were discussed.

Difference, triangle (ISO 4120:2004 [25]) and A-not A (ISO 8588:1987 [26]) tests were used to select the concentration range of the BPE in the orange juices. In the triangle test, three juice samples were presented simultaneously to the panellists, two of which were identical and the other different, in order to identify which of the samples was different. In the A-not A test, the panellists were presented with seven juice samples arranged in a circle: six contained different concentrations of BPE and one was a sample of conventional juice. The only information that the panellists were given was that the samples may or may not contain BPE. They were asked to evaluate each against a reference sample of conventional juice and indicate which were different from the reference. To mask colour differences between samples, the test was performed under red light. The percentage of panellists that found sensory differences between the samples with BPE and conventional juice was evaluated. In addition, when identifying the different samples the panellists indicated whether they were 'barely', 'somewhat' or 'very' different from the reference.

In descriptive and preference tests, the panellists provided a description of the samples that were detected as 'different', focusing on what they thought were the sensory differences between conventional juice and juice with banana peel added. The sensory characteristics described were visual appearance (colour, turbidity and browning), odour or in-nose sensation (characteristic aroma and off-odour) and overall flavour or in-mouth sensation (characteristic flavour and off-flavour) of the juices. Rather than using scores, the panellists wrote down what they understood as the specific differences in the samples and how extreme those differences were; they also discussed how easy/difficult was to detect the differences. They also indicated their opinion of each sensory characteristic, defining each as positive, negative or unimportant.

Physicochemical Analysis

Colour (lightness L*, hue angle h° and chromaticity C*) was measured with a Minolta Chroma meter CR-300 (Ramsey, NJ, USA) colour difference meter. TSS was determined using an Atago ATC-1 (Tokyo, Japan) refractometer and pH was measured by a WTW (St. Woburn, MA, USA) pH-meter. After determining pH, titratable acidity (g citric acid/ml juice) was measured with a 0.1 N sodium hydroxide standard solution (Merck, Darmstadt, Germany) up to pH 8.1.

Statistical Analysis

Data analysis was carried out with Statgraphics-Plus 5.1 software (Statistical Graphics, Rockville, MD, USA). Grubbs' test was applied to detect outliers in the data set and analysis of variance was used to evaluate how BPE concentration affected the quality of the juice. Fisher's least-significant-difference test, at 5% significance level, was applied to experimental results to assess intra-pair significant differences. Simple linear correlation analysis was used to measure the correlation between BPE concentration and the antioxidant activity of BPE (dose-response curves). The interpretation of the results obtained by difference tests was done according to ISO 8588:1987 [26] and ISO 4120:2004 [25].

Results and Discussion

Antioxidant Activity

Evaluating the relationship between BPE concentration and in-vitro antioxidant activity of banana peel allowed determining the BPE concentration with maximum antioxidant activity. This was previously carried out in triplicate using dose-response curves of the BPE in water (see Figure included as Electronic Supplementary Material, ESM 2). The capacity to scavenge DPPH and ABTS radicals and inhibit lipid peroxidation was detected at concentrations greater than 1.3 mg BPE/ml. Above this concentration, antioxidant activity increased linearly. However, linear increases in the capacity to scavenge radicals and inhibit lipid oxidation only occurred up to a concentration of around 7.5 mg BPE/ml water (0.78 ± 0.01, 0.85 ± 0.06 and 0.28 ± 0.01 g TE/100 g banana peel DW, for $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging and lipid peroxidation inhibition, respectively); higher concentrations did not increase antioxidant capacity. Some differences can be observed in the antioxidant capacity based on the different study systems that were used. At the same concentration, the antioxidant effect of BPE is much higher in homogeneous

systems such as those based on the formation of stable free radicals like DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ with a single phase, than in heterogeneous or compartmentalised systems such as the β-carotene bleaching system. Antioxidant capacity against DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ increased around eight times when the BPE concentration increased from 1.3 to 7.5 mg BPE/ml; on the other hand, the inhibition of the β-carotene oxidation (mediated by lipid peroxidation) only increased around three times. These results can be explained by considering that the distribution of the antioxidants from BPE in the micelles of the β-carotene system is low. The main water-soluble antioxidant compounds present in BPE (phenolic compounds and catecholamines) have a low lipophilicity, thus, it is difficult for them to be distributed between the lipophilic zone of the micelle (where the β-carotene is found) and the hydrophilic aqueous phase [27, 28].

On the other hand, different preliminary sensory difference tests (triangle tests) with banana peel added to orange juice at concentrations between 3.5 and 85 mg BPE/ml revealed that BPE in concentrations higher than 15 mg BPE/ml had unacceptable sensory attributes; therefore, the range of BPE concentrations selected to develop subsequent studies is between 1.3 and 15 mg BPE/ml orange juice.

Effect on the Capacity to Scavenge Free Radicals

There was an increase ($P < 0.05$), approximately proportional to concentration, in the capacity of FSJ to scavenge DPPH radicals when BPE was added at a concentration ≥ 2.5 mg BPE/ml juice (Fig. 1). The increase compared to conventional juice (without BPE) was about 21–28% for concentrations between 5 and 15 mg/ml. Similarly, adding concentrations between 2.5 and 15 mg/ml to JFC increased its capacity to scavenge DPPH radicals by around 25%, when compared to the control (Fig. 1).

The capacity of FSJ to scavenge ABTS radicals did not increase significantly with the addition of 1.3–3.8 mg BPE/ml juice. However, when the BPE was added in concentrations ≥5 mg/ml, a remarkable increase was detected (185% for a concentration of 15 mg BPE/ml juice) (Fig. 1). JFC displayed a different behaviour when BPE was added: adding different concentrations of BPE (1.3 and 5 mg BPE/ml juice) tended to slightly decrease its capacity to scavenge ABTS radicals (142 ± 13 mg TE/100 ml) (Fig. 1). These differences in the capacity to scavenge free radicals shoed by the different types of juices could be a consequence of the modification of chemical components, particularly phenolic compounds, of JFC caused by the processes of concentration and pasteurization carried out on the concentrate. Moreover, a high amount of ascorbic acid is added in the reconstitution of JFC to balance the losses produced during these processes. The different chemical composition of both types of juices can generate synergistic and antagonistic interactions with the antioxidant components of the banana peel, which implies changes in the antioxidant capacity (more details are included as Electronic Supplementary Material, ESM 3).

Effect on the Capacity to Inhibit Lipid Peroxidation

FSJ (with or without added BPE) did not present capacity to inhibit lipid peroxidation. In addition, JFC without BPE added displayed a very limited capacity to inhibit lipid peroxidation (6 ± 1 mg TE/100 ml juice); and a small increase was detected when BPE was added. Capacity to inhibit lipid peroxidation was 8 ± 1 mg TE/100 ml juice, at a concentration of 1.3 mg BPE/ml juice, and around 12 ± 1 mg TE/100 ml, at concentrations between 2.5 and 15 mg BPE/ml, respectively.

Sensory Evaluation

In the difference tests (Table 1), the panellists noted differences in the colour of FSJ or JFC after adding BPE at

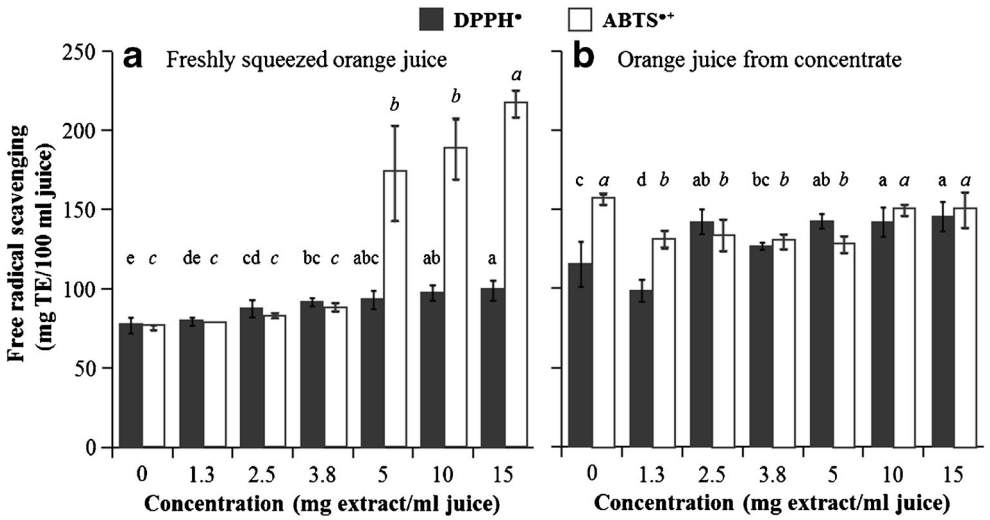

Fig. 1 Antioxidant capacity to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH; dark bars) and 2,2′-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS; light bars) free radicals, of freshly squeezed and concentrate orange juices added with banana peel extracts (BPE). Different letters (a-e for DPPH$^{\bullet}$, and *a-c* for ABTS$^{\bullet+}$) denote significant differences ($P < 0.05$) between extract concentrations. TE, Trolox equivalents

Table 1 Panellists (%) who found sensory differences between orange juice with and without banana peel extract (BPE) added at different concentrations in the A-not A test

Concentration (mg BPE/ml juice)	Freshly squeezed juice				Juice from concentrate			
	In-nose**		In-mouth**		In-nose**		In-mouth**	
0 (without BPE)	29	*(50)*	29	*(50)*	0	*(0)*	29	*(50)*
1.3	57	*(50)*	29	*(50)*	43	*(33)*	57	*(0)*
2.5	57	*(50)*	57	*(50)*	57	*(0)*	43	*(0)*
3.8	57	*(50)*	29	*(0)*	57	*(0)*	57	*(25)*
5	57	*(25)*	57	*(75)*	71*	*(0)*	57	*(50)*
10	86*	*(14)*	86*	*(17)*	86*	*(0)*	86*	*(0)*
15	100*	*(0)*	100*	*(0)*	100*	*(0)*	100*	*(0)*

*Significant differences ($P < 0.05$) according to ISO 8588:1987 (ISO, 1987) norm

**The percentage of panellists who detected very small differences between orange juice samples with BPE and control are indicated between brackets and italics

concentrations >5 mg/ml juice, which match with a higher content of antioxidant compounds capable to scavenge free radicals (Fig. 1). However, these differences were evaluated as positive or unimportant, especially at concentrations above 10 mg/ml. The FSJ with BPE was described as having a darker and more intense colour than conventional juice; while JFC with BPE was described as more vivid and brighter than conventional JFC.

When evaluating odour and flavour, the panellists found statistically significant differences in both types of juice containing BPE in concentrations of 10 and 15 mg/ml; the addition of 5 mg BPE/ml to JFC was also detected when evaluated by odour (Table 1). At average concentrations (1.3–3.8 mg BPE/ml), the panellists stressed that it was extremely difficult to detect differences between samples with and without banana peel and that the identification proved more difficult in-mouth than in-nose; for concentrations between 1.3 and 5 mg/ml, a large number of panellists (up to 57%) detected differences in the odour and flavour of both types of juices, but a high percentage of them noted that it was only a small difference (Table 1). Moreover, at average concentrations, when BPE was added to both types of juices the flavour was described as less acidic and sweeter than the corresponding conventional juices, which was evaluated as positive by the panellists. High concentrations (≥ 15 mg BPE/ml) of BPE, in FSJ, produced negative in-nose sensations due to loss of fruitiness and freshness, and the appearance of aromas that were described as dry leaves, wet grass and grapefruit, and the odour and flavour of the JFC was described as very unpleasant (damp, earthy, ripe banana). Therefore, adding concentrations between 1.3 and 5 mg/ml of BPE did not affect the sensory characteristics of the juice, while adding concentrations of 10 and 15 mg BPE/ml juice was detected through sensory evaluation. Further research is needed to confirm these promising results, as it is crucial to develop consumer acceptance tests that evaluate the market acceptability of the juices.

Table 2 Physicochemical parameters in freshly squeezed orange juice and orange juice from concentrate without and with banana peel extract (BPE) added at different concentrations

Physicochemical parameters	Freshly squeezed juice mg (BPE /ml juice)				Juice from concentrate (mg BPE /ml juice)			
	0	5	10	15	0	5	10	15
Colour								
L*	38.9 ± 0.1	38.9 ± 0.1	39.0 ± 0.1	39.2 ± 0.2	45.3 ± 0.8 a	45.0 ± 0.5 a	46.1 ± 0.8 a	43.8 ± 0.6 b
h°	108.9 ± 0.4 a	108.1 ± 0.2 b	107.1 ± 1.0 c	106.2 ± 1.0 c	106.9 ± 0.3 a	107.0 ± 0.5 a	106.5 ± 0.1 a	105.5 ± 0.1 b
C*	16.5 ± 0.2 c	16.8 ± 0.3 c	21.0 ± 0.1 b	23.3 ± 0.1 a	22.1 ± 0.8 b	21.6 ± 0.6 b	23.1 ± 0.9 ab	24.4 ± 0.6 a
TSS (°Brix)	9.1 ± 0.1 c	9.3 ± 0.2 bc	9.6 ± 0.1 ab	9.7 ± 0.1 a	10.4 ± 0.1 b	10.3 ± 0.1 b	10.5 ± 0.1 ab	10.7 ± 0.1 a
pH	3.48 ± 0.01	3.50 ± 0.01	3.50 ± 0.01	3.51 ± 0.02	3.64 ± 0.01	3.66 ± 0.01	3.66 ± 0.01	3.66 ± 0.01
Titratable acidity (g citric acid/100 ml)	1.08 ± 0.07	1.10 ± 0.11	1.13 ± 0.08	1.10 ± 0.15	1.08 ± 0.09	0.92 ± 0.09	0.93 ± 0.07	1.06 ± 0.15

L*, Lightness; h°, Hue angle; C*, Chromaticity; TSS, Total soluble solids

Values are the mean ± standard deviation of three determinations. Within each row (a-c), different letters denote significant differences ($P < 0.05$) between extract concentrations. No letters denote no significant differences between extract concentrations

Physicochemical Characterisation

Results of the physicochemical parameters determined in FSJ and JFC with different concentrations of BPE added are shown in Table 2. L* of the FSJ remained unchanged by adding the BPE and only modifications in its h° and C* were found. Therefore, at concentrations up to 1.3 mg/ml there was a progressive decrease in h°. The addition of 10 and 15 mg BPE /ml caused an increase in the C*, compared to conventional FSJ. The addition of the BPE to the JFC, at concentrations equal or lower 10 mg/ml, did not generate substantial modification in L*, h° or C*. However, these colour parameters significantly decreased (L* and h°) or increased (C*) for the addition of 15 mg/ml compared to the colour of the conventional JFC. This is consistent with the colour perception highlighted by the panellists in the sensory analysis which described orange juices with BPE as having a darker and more intense colour than conventional juices. The TSS amount in the FSJ was not modified significantly when 1.3 mg and 5 BPE/ml were added; when the concentration added was ≥10 mg/ml, a significant increase of TSS (0.5–0.6 °Brix) was detected. This TSS increase is related to the presence of soluble components in BPE. There was a slight but significant increase in the JFC when 15 mg BPE/ml was added; this increase in TSS content is consistent with the flavour modifications described in sensory analysis. These juices were perceived as less acidic and sweeter than the corresponding conventional juices. Adding BPE did not cause significant variations in the pH or titratable acidity of either FSJ or JFC.

Conclusions

BPE is a promising natural additive that increases the capacity to scavenge free radicals of FSJ and JFC; however, the capacity to inhibit lipid peroxidation was low for both juices, and no increases were detected in orange juices with BPE. Only slight modifications were detected in the sensory analysis of FSJ and JFC when BPE was added at concentrations less or equal than 5 mg/ml juice. At higher concentrations (10–15 mg BPE/ml), some positive in-mouth sensations and colour were detected, which is consistent with the results obtained by colour and TSS analysis; however, the odour was unpleasant in both types of juice with these BPE concentrations. Therefore, a concentration of 5 mg BPE/ml orange juice could be proposed as optimal because it significantly increased the antioxidant capacity, allowing acceptable juices with adequate characteristics for the consumer to be obtained, from a sensory and physicochemical point of view. Further research is needed to confirm these promising results, as it is crucial to determine the individual phenolic compounds and to establish the mechanisms involved in the antioxidant capacity of these biowastes. Moreover, the performance of these juices with BPE added during processing, conservation and storage should be studied in a greater depth.

ABTS, 2,2′-Azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid; BPE, Banana peel extract; C*, Chromaticity; DPPH, 2,2-Diphenyl-1-picrylhydrazyl; DW, Dry matter basis; FSJ, Freshly squeezed juice; h°, Hue angle; JFC, Juice from concentrate; L*, Lightness; TE, Trolox equivalents; TSS, Total soluble solids.

Acknowledgements L. Ortiz wishes to thank the Spanish Ministry of Education for the collaboration grant. The authors are grateful to the Spain's INIA (RTA2006-00187) and Canary Islands Government (ULL APD-08/01) for financial support. The collaboration of all panellists is especially acknowledged. EIASA is also acknowledged for the provision of the orange juice concentrate.

Compliance with Ethical Standards

Conflict of Interest The authors declare that there are no conflicts of interests.

Human and Animal Rights This article does not contain any studies with human or animal subjects.

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