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Research Article

Sea Buckthorn (*Hippophae rhamnoides* L.) and Quince (*Cydonia oblonga* L.) Juices and Their By-Products as Ingredients Showing Antimicrobial and Antioxidant Properties for Chewing Candy: Nutraceutical Formulations

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Sustainable and environmentally friendly approaches to the production of health foods have become very popular. The concept of this study was to develop chewing candy (CC)—nutraceutical formulations based on sea buckthorn (*Hippophae rhamnoides* L.) and quince (*Cydonia oblonga* L.) juice and juice by-products (BuJ, QuJ, BuBP, and QuBP, resp.), as ingredients showing antimicrobial properties against *Klebsiella pneumoniae*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Proteus mirabilis*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis*, *E. faecium*, and *Bacillus cereus*. Two texture-forming agents (agar and gelatin) were tested for CC formulation. BuJ, QuJ, BuBP, and QuBP showed antimicrobial activity against all the pathogens tested, and the largest inhibition zones against *Bacillus* and *Proteus mirabilis* were observed for BuJ and QuJ, respectively. Agar and/or gelatin selection has a significant influence on CC texture ($p = 0.0001$), and interactions of agar and/or gelatin selection × juice or juice by-products and sea buckthorn or quince × juice or juice by-products were also significant ($p = 0.0001$). The best acceptability was shown for CC prepared with agar and BuBP (131.7) and with gelatin and QuJ (132.0). The addition of BuJ, QuJ, BuBP, and QuBP increases the antioxidant activity of CC by five times. Finally, not just juice, but also juice by-products, have great potential as desirable antimicrobial ingredients for the food industry.

1. Introduction

Many efforts have been made to cope with the agroindustrial waste produced by food manufactories. Nowadays, many by-products (BP) are transformed into useful ingredients, and some of these ingredients have been commercialized and widely used in food, nutraceutical, cosmetic, and other industries [1]. However, vegetables and some fruits yield between 25% and 30% of nonedible products, and the BP of fruits and vegetables are made up of skins and seeds of different shapes and sizes that normally have no further usage and are commonly wasted or discarded [2]. Therefore, development of sustainable technologies is still relevant because many BP have until now not been recovered [3]. The very high content of BP after juice production remains. As juice BP have many biologically active compounds which are healthy for consumers, they can be used for preparation of higher value foods and/or supplements. Higher value food should be attractive for consumers, and juice BP can be incorporated into chewing candy (CC) formulations. In some European and Asian countries, sea buckthorn (Bu) and quince (Qu) fruits are very popular as they have a high content of desirable compounds and show good sensory characteristics [4]. Bu is a good source of mineral acids, vitamins, carbohydrates, amino acids, and natural antioxidants, including phenolics, flavonoids, ascorbic acid,

Table 1: Chewing candy formulas.

	GC	Sugar, g	Water, mL	Citric acid, g	A, g	G, g	Bu J, mL	Bu BP, g	Qu J, mL	Qu BP, g
	AC	30	20	0.9	10	—	—	—	—	—
	A + Bu J		—	—			20	—	—	—
A	A + Bu BP		20				—	20	—	—
	A + Qu J		—				—	—	20	—
	A + Qu BP		20				—	—	—	20
	GC		20	0.9	—	10	—	—	—	—
	G + Bu J		—	—			20	—	—	—
G	G + Bu BP		20				—	20	—	—
	G + Qu J		—				—	—	20	—
	G + Qu BP		20				—	—	—	20

GC: gummy candies; A: agar; C: control; G: gelatin; J: juice; BP: juice by-products; Bu: sea buckthorn; Qu: quince.

tocopherols, fatty acids, carotenoids, and organic acids, which possess various biological activities [5]. Qu is described as a fruit with high economic and medicinal potential [6]. Also, Bu and Qu are good candidates as antimicrobial agents. The main components responsible for the antimicrobial properties of plants are polyphenolic compounds, and it has been shown that the oils from different parts of Bu inhibit *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *B. coagulans* [7]. However, literature about the antimicrobial activity of Bu and Qu BP is scarce. Anyway, BP of Bu and Qu juice are not used efficiently enough and taking into account that the botanical dietary supplement segment is anticipated to witness considerable growth over the forecast period on account of the increasing popularity of nutraceuticals with natural ingredients, the development of new products has become very relevant [8]. Nutraceuticals and/or supplements are available in numerous forms including dry, solid or liquid extracts, tablets, capsules, powders, and so on; however, consumers prefer to choose food in traditional form, and incorporation of high value botanical ingredients in CC formulations has become very attractive.

The concept of this study was to develop chewing candy (CC)— nutraceutical formulations based on sea buckthorn (*Hippophae rhamnoides* L.) and quince (*Cydonia oblonga* L.) juice and juice by-products (BuJ, QuJ, BuBP, and QuBP, resp.), as ingredients showing antimicrobial properties against *Klebsiella pneumoniae*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Proteus mirabilis*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis*, *E. faecium*, and *Bacillus cereus*. Two texture-forming agents (agar and gelatin) were tested for CC formulation.

2. Materials and Methods

2.1. Materials Used for Chewing Candy Preparation. Bu (*Hippophae rhamnoides* L., variety "Щербинка") and Qu (*Cydonia oblonga* L., variety "Rondo") were purchased from local farms (from Klaipeda and Alytus districts, resp., Lithuania). Bu and Qu juice (BuJ and QuJ, resp.) prepared from fresh fruits and juice by-products (BuBP and QuBP, resp.), the dry part after juice preparation, were prepared at laboratory scale by using a "KENWOOD JE850" juicer at the Lithuanian University of Health Sciences (Kaunas, Lithuania). Juice and by-products were used fresh without pasteurisation and drying, as well as by-products were used with seeds. Agar powder (*Gelidium sesquipedale* algae, Rapunzel, Germany) was used as a polymer with mucoadhesive properties for CC formation. Gelatin (Klingai, Kaunas, Lithuania) was also tested. Sugar was obtained from Nordic Sugar (Kedainiai, Lithuania), xylitol from Natur Hurtig (Nuremberg, Germany), and citric acid from Sanitex (Kaunas, Lithuania).

2.2. Evaluation of the Acidity Parameters of Sea Buckthorn and Quince Products. The pH values of BuJ, QuJ, BuBP, and QuBP were measured and recorded using a pH electrode (PP-15; Sartorius, Goettingen, Germany). The total titratable acidity (TTA) was determined for a 10 g sample of BP and/or juice homogenized with 90 mL of distilled water and expressed as millilitres of 0.1 mol·L^{-1}·NaOH required to achieve a pH of 8.2.

2.3. Chewing Candy Formulas. The control CC formula consisted of sugar (30 g), water (20 g), citric acid (0.90 g), agar (10.0 g), and/or gelatin (10.0 g) (Table 1). Chewing candies were prepared by addition to the main formula of Bu (*Rhamnus*, family *Rhamnaceae*) and/or Qu (*C. oblonga*, family *Rosaceae*) juice or BP. It should be mentioned that candies with juice and/or BP were prepared without the addition of citric acid. For preparation of candies with agar, firstly, agar powder was soaked in water for 30 min and then melted by heating for 5 min; then, sugar and/or xylitol was added and dissolved under boiling conditions. The mixture obtained was further heated to 103 ± 2°C under stirring. Citric acid was incorporated into the control CC mass at the end of the process. For preparation of CC with gelatin, firstly, gelatin powder was soaked in water for 30 min, and then melted at 80 ± 2°C; then, sugar and/or xylitol and citric acid were added and dissolved. After mixing, the mass obtained (both that prepared with agar and prepared with gelatin) was poured into a mould, and CC were dried at 22–24°C for 24 h to get a gel-hard form.

2.4. Evaluation of Antimicrobial Activity of Sea Buckthorn and Quince Juices and Their By-Products. An agar well diffusion assay was used for testing the antimicrobial activity of BuJ,

QuJ, BuBP, and QuBP. For this purpose, a 0.5 McFarland unit density suspension of each pathogenic bacteria strain was inoculated onto the surface of cooled Mueller–Hinton agar (Oxoid, UK) using sterile cotton swabs. Wells 6 mm in diameter were punched in the agar and filled separately with BuJ, QuJ, BuBP, and/or QuBP. Juice and juice production by-products were used fresh without pasteurisation and drying. The antimicrobial activity against tested bacteria was determined by measuring the diameter of inhibition zones (mm). The experiments were repeated three times, and the average of inhibition zones was calculated.

2.5. Determination of Total Phenolic Compound (TPC) Content, Antioxidant Activity, and Colour Characteristics of Chewing Candies. The TPC in juice and BP samples was determined by a spectrophotometric method, as reported elsewhere [9]. The absorbance of samples was measured at 765 nm using a J.P. Selecta S.A. V-1100D spectrophotometer (Barcelona, Spain). Antioxidant activity of the samples was evaluated according to the method reported by Zhu et al. [10]. Colour characteristics were evaluated using a CIE $L^*a^*b^*$ system (Croma Meter CR-400, Konica Minolta, Japan) [11].

2.6. Evaluation of Chewing Candy Texture. The hardness of CC was evaluated by using a TA.XT2 texture analyser (Stable Micro Systems Ltd., Godalming, UK) (compression force 0.5 N, test speed 0.5 mm·s^{-1}, posttest speed 2 mm·s^{-1}, distance 6 mm).

2.7. Evaluation of Overall Acceptability of Chewing Candies. Overall acceptability of CC was evaluated according to ISO method 8586-1 [12] for preliminary sensory acceptability, using a 150 mm hedonic line scale ranging from 150 (extremely like) to 0 (extremely dislike).

2.8. Statistical Analysis. The results were expressed as the mean value of at least three measurements ± standard deviation. In order to evaluate the effects of the different formula components on CC quality parameters, data were analysed by analysis of variance (IBM SPSS Statistics, ver. 22). Results were recognized as statistically significant at $p \leq 0.05$.

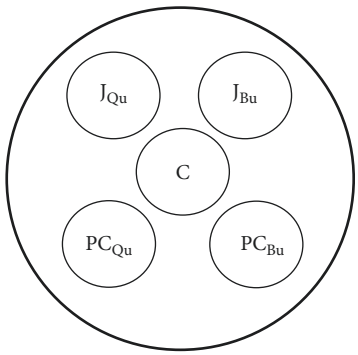
3. Results and Discussion

3.1. Antimicrobial Activity of Sea Buckthorn and Quince Juices and Their By-Products. The antimicrobial activity of BuJ, QuJ, BuBP, and QuBP is shown in Table 2. BuJ, QuJ, BuBP, and QuBP showed antimicrobial activity against all the pathogenic bacteria tested; comparing the antimicrobial activity of juice, the largest inhibition zones for BuJ and QuJ were observed against *B. cereus* (29.0 ± 0.5 and 30.0 ± 0.7 mm, resp.) and *Proteus mirabilis* (25.0 ± 0.6 and 26.0 ± 0.4 mm, resp.). In most cases, BuBP and QuBP showed weaker antimicrobial activity than the juice (on average, BuBP inhibition zones were 0 to 7 mm smaller, and QuBP zones were 0 to 5 mm smaller). The antimicrobial activity of BuBP ranged from 8.0 ± 0.4 to 25.0 ± 0.5 mm (for *K. pneumoniae* and *B. cereus* inhibition, resp.) and of QuBP from 8.0 ± 0.4 to 26.0 ± 0.5 mm (for *S. enterica* and *B. cereus* inhibition, resp.). The inhibition zones for juice and BP were found to be the same for *S. enterica* (11.0 and 8.0 mm for Bu and Qu products, resp.). Antibiotic resistance is one of the biggest threats to global health, food security, and development today [13], and natural botanical sources showing antimicrobial activity against antibiotic-resistant pathogens can be very important. It was published that n-hexane and chloroform extracts of Bu berries and n-hexane extract of Bu leaves show significant ($p < 0.05$) antibacterial activity comparable with vancomycin, and it was concluded that extracts of Bu berries and leaves have antibacterial activity against MRSA [14]. According to Fattouch et al. [15], polyphenolic extract of Qu peel shows antimicrobial activity against Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and yeast (*Candida albicans*). The MIC of ethanolic extract of Qu peel ranges between 0.1 and 5.0 mg·polyphenol·mL^{-1} [16]. Both Bu and Qu have a high phenolic compound content, and recent studies have shown that phenolic compounds mostly modulate the composition of gut microbial communities through the inhibition of pathogenic bacteria and stimulation of beneficial bacteria; in the latter, phenolic compounds may exert a prebiotic function and increase the population of beneficial bacteria, including probiotics, suggesting a mutual relationship between phenolic compounds and probiotics [17–19]. The main components responsible for the antimicrobial properties of plants are polyphenolic compounds; they exhibit anti-inflammatory activity *in vitro* and *in vivo*, and their mechanism of action is the inhibition of enzymes (phospholipase oxygenase), that is, by binding with hydrosulphide groups and inactivating bacterial proteins [20]. According to our results, not just the juice, but also BuBP and QuBP have great potential as antimicrobial ingredients for the food industry.

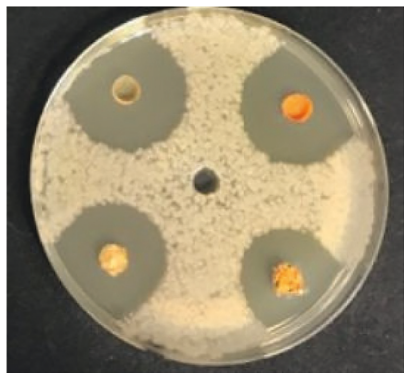
3.2. Acidity Parameters and Colour Coordinates of Sea Buckthorn and Quince Juices and Their By-Products. Acidity parameters and colour coordinates of BuJ, QuJ, BuBP, and QuBP are shown in Figures 1(a) and 1(b). Comparing juice and BP, lower pH values were obtained for the juice (compared to BP, BuJ pH was lower by 24.0% and QuJ pH was lower by 10.4%) (Figure 1(a)). Opposite to pH values, BuJ TTA was two times lower than that of BP; however, QuJ TTA was 1.9 times higher than that of BP. BuBP and QuBP showed higher lightness (L^*) values than juice; L^* coordinates ranged from 29.82 ± 0.96 to 35.84 ± 1.27 for BuJ and BuBP, respectively, and from 36.23 ± 1.63 to 48.53 ± 1.08 for QuJ and QuBP, respectively (Figure 1(b)). Different tendencies of Bu and Qu redness (a^*) were observed when comparing juice and BP. Coordinates of a^* for BuJ were 49.9% lower than those for BP; however, a^* coordinates for QuJ were 30.7% higher than those for BP. In all cases, higher yellowness (b^*) coordinates were found for BP than for juice (BuBP b^* coordinates 155.2% higher, QuBP 56.2% higher).

Table 2: Antimicrobial activity of buckthorn and quince juices and their 363 by-products.

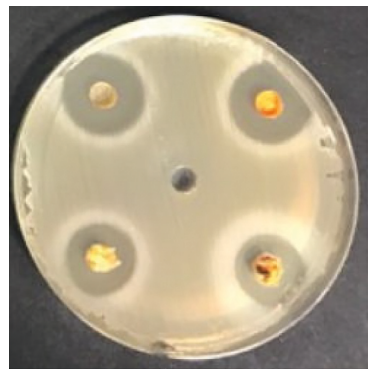
Microorganisms	J		BP	
	Bu	Qu	Bu	Qu
	Inhibition zones, mm			
Klebsiella pneumoniae	14.0 ± 0.3^{d}	13.0 ± 0.3^{c}	8.0 ± 0.4^{a}	10.0 ± 0.3^{b}
Salmonella enterica	11.0 ± 0.2^{b}	8.0 ± 0.2^{a}	11.0 ± 0.3^{b}	8.0 ± 0.4^{a}
Pseudomonas aeruginosa	23.0 ± 0.4^{b}	23.0 ± 0.3^{b}	20.0 ± 0.2^{a}	20.0 ± 0.2^{a}
Acinetobacter baumannii	20.0 ± 0.5^{c}	22.0 ± 0.3^{d}	16.0 ± 0.1^{a}	19.0 ± 0.3^{b}
Proteus mirabilis	25.0 ± 0.6^{c}	$26.0 \pm 0.4^{c,d}$	20.0 ± 0.2^{a}	23.0 ± 0.4^{b}
MRSA	22.0 ± 0.3^{c}	22.0 ± 0.2^{c}	17.0 ± 0.2^{a}	18.0 ± 0.2^{b}
Enterococcus faecalis	25.0 ± 0.4^{c}	23.0 ± 0.3^{b}	18.0 ± 0.3^{a}	18.0 ± 0.2^{a}
Enterococcus faecium	20.0 ± 0.3^{b}	22.0 ± 0.3^{c}	18.0 ± 0.2^{a}	22.0 ± 0.4^{c}
Bacillus cereus	29.0 ± 0.5^{c}	30.0 ± 0.7^{d}	25.0 ± 0.5^{a}	$26.0 \pm 0.5^{a,b}$



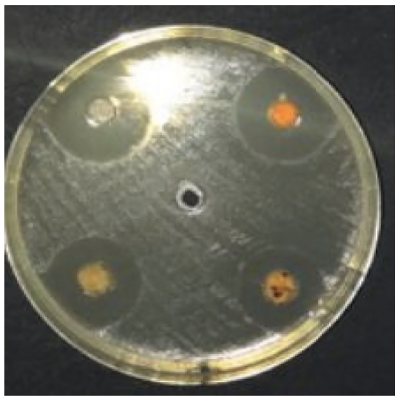
Experimental design



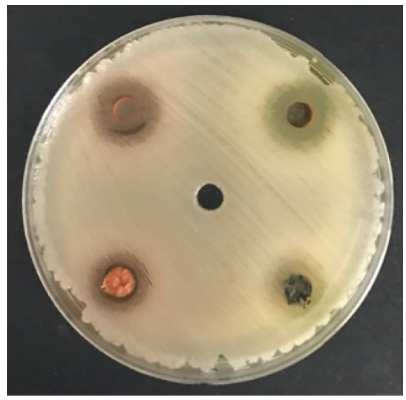
Bacillus cereus



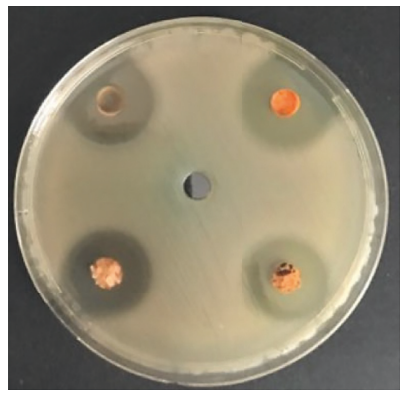
MRSA



Proteus mirabilis



Klebsiella pneumoniae



Pseudomonas aeruginosa

J: juice; BP: juice by-products; Bu: sea buckthorn; Qu: quince; C: control; MRSA: methicillin-resistant *Staphylococcus aureus*. Data expressed as means ($n = 3$) ± standard deviation (SD). $^{a-d}$Mean values with different letters are significantly different when $p \leq 0.05$.

The main components of Bu identified are ascorbic acid, carotenoids, and various phenolics, including proanthocyanidins, gallic acid, ursolic acid, caffeic acid, coumaric acid, ferulic acid, catechin and epicatechin derivatives, quercetin, kaempferol, and isorhamnetin glycoside derivatives [21]. However, colour is influenced by carotenoids, and the main carotenoids of Bu are α-, β-, and δ-carotene and lycopene [22]. It was published that the edible parts of Qu can have 16.8 mg·100·g^{-1} ascorbic acid; this is the main compound which influences the sour taste of these fruits [6].

3.3. Texture, Colour Characteristics, and Overall Acceptability of Chewing Candies. CC texture, colour characteristics, and overall acceptability are shown in Table 3. Comparing the CC group prepared with agar (A), the hardest texture was obtained for the CC prepared with BuBP (hardness of A + BuBP samples was 1.00 ± 0.01 mJ). Compared with control CC (AC), CC prepared with the addition of juice and/or BP were harder in all cases (from 35% to 400% for CC prepared with BuJ and BuBP, resp.). Comparing the CC group prepared with gelatin (G), the hardest texture was obtained for the CC with BuJ (hardness of G + BuJ samples was 3.13 ± 0.12 mJ); in contrast, CC prepared with the addition of BuBP had the softest texture (37.1% softer). In all cases, CC prepared with juice were harder than the candies prepared with the addition of BP. Selection of A and/or G

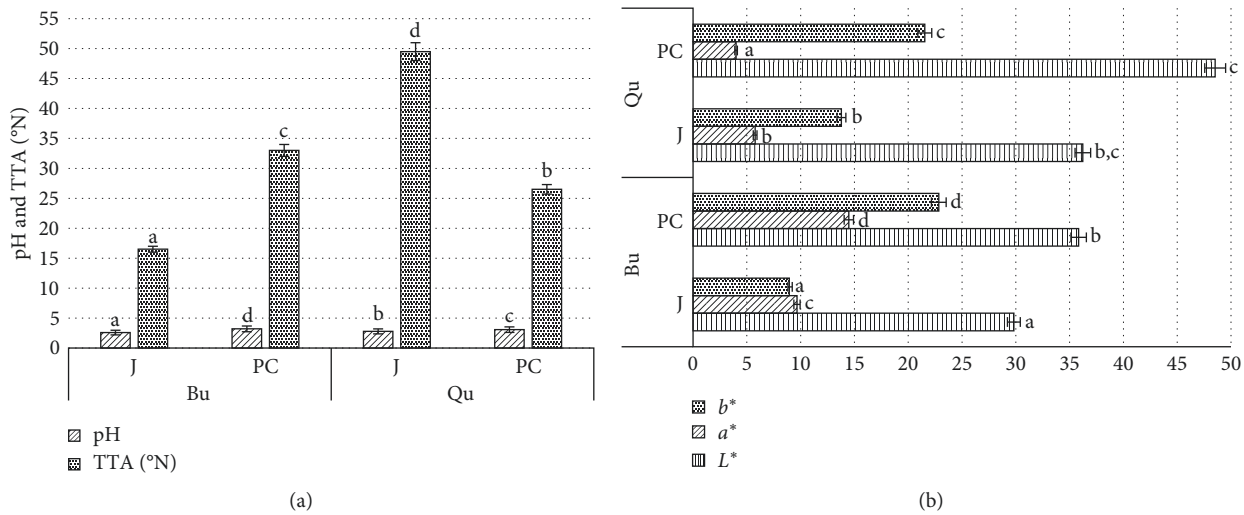


Figure 1: Acidity parameters (a) and colour coordinates (b) of sea buckthorn (Bu) and quince (Qu) juice (J) and juice by-products (PC) (BuJ, QuJ, BuBP, and QuBP, resp.).

had a significant influence on CC texture ($p = 0.0001$), and interactions of A and/or G selection × juice or BP and Bu or Qu × juice or BP were also significant ($p = 0.0001$).

In all cases, higher lightness (L^*) coordinates were obtained for CC prepared with A (on average 48.3% higher compared to CC prepared with G). Most of the factors analysed had a significant influence on CC L^* coordinates (except A and/or G selection). Comparing control CC prepared with A and G, no significant changes were found for redness (a^*) coordinates; however, comparing a^* coordinates of CC prepared with the addition of Bu and Qu products (juice and/or BP), a different influence of A and/or G selection on a^* coordinates was established. Samples prepared with BuBP and A had 60.5% higher redness than samples prepared with G; in contrast, CC prepared with G and QuJ and BP had 143.6% and 35.4% higher redness, respectively, than samples prepared with agar. Comparing yellowness (b^*), CC prepared with G had b^* coordinates 44.6% lower than those of the control CC. However, b^* coordinates of CC were more influenced by the addition of Bu and Qu products (juice and/or BP), and the highest b^* was obtained for the CC prepared with G and BuJ (27.44 ± 2.15). Bu or Qu selection ($p = 0.0001$) and juice or BP selection ($p = 0.0001$) had a significant influence on CC a^* and b^* coordinates, and interactions of A and/or G × Bu or Qu, A and/or G × juice or BP, and Bu or Qu × juice or BP were also significant.

No significant differences were obtained for overall acceptability between the control CC samples prepared with A and G; however, most of the CC prepared with the addition of Bu and Qu products (juice and/or BP) were more acceptable than controls. The best acceptability was obtained for CC prepared with A and BuBP (131.7 ± 10.7) and CC prepared with G and QuJ (132.0 ± 7.8). CC prepared with A and BuBP was also hardest; however, no correlation was found between hardness and overall acceptability of CC. A significant influence of the interaction of analysed factors A and/or G selection × juice or BP on overall acceptability was established ($p = 0.001$).

Pressing of juice produces large amounts of pomace, which are currently discarded as waste or utilized rather inefficiently; therefore, considerable amounts of nutrients are lost annually [23]. Finally, development of new products in which the whole BP can be used is very important; it leads to reduced production costs because extracts should not be prepared. Further, in our experiment, the best acceptability was found for CC formulas improved by changing saccharose to xylitol (1 : 1). In comparing the parameters of CC samples with saccharose and xylitol, no significant changes were observed, except in overall acceptability which was significantly higher for the CC with xylitol.

3.4. Total Phenolic Compound Content and Antioxidant Activity of Chewing Candies. The TPC content and antioxidant activity of the CC are presented in Table 4. Comparing CC groups prepared with A and G, in all cases, control CC samples had a lower TPC content (in the CC group prepared with A, from 132.7% to 312.5% lower, for CC with BuBP and QuJ, resp.; in the group prepared with G, from 44.5% to 160.9% lower, for CC with QuJ and BuBP, resp.) than CC prepared with the addition of Bu and Qu products (juice and/or BP). Also, comparing CC control groups, a 45.0% higher TPC content was obtained for the CC group prepared with G; however, no significant differences in antioxidant activity of control CC groups were established. In all cases (in both CC groups prepared with A and with G), stronger antioxidant activity was established for the CC prepared with Qu and Bu products (juice and/or BP): in the CC group prepared with A, from 130.7% to 563.7% higher (in CC prepared with the addition of BuJ and QuBP, resp.), and in the group prepared with G, from 95.2% to 325.8% higher (in CC prepared with the addition of BuJ and QuBP, resp.). A significant influence of the parameters analysed (A or G selection; Bu or Qu; juice or BP) on TPC content in CC ($p = 0.0001$) and their antioxidant activity ($p = 0.0004$, $p = 0.0001$ and $p = 0.0001$, resp.) was observed. Interactions of Bu or Qu × juice or BP were also significant for the antioxidant properties of CC

Table 3: Hardness, colour coordinates, and overall acceptability of gummy candies.

GC		Texture, mJ	Colour coordinates			Overall acceptability
			L^*	a^*	b^*	
A	AC	0.20 ± 0.01^{a}	46.05 ± 4.32^{d}	1.57 ± 0.28^{c}	13.63 ± 1.44^{e}	85.3 ± 4.2^{a}
	A + Bu J	0.27 ± 0.05^{a}	28.42 ± 4.55^{a}	3.72 ± 0.21^{e}	13.30 ± 2.18^{e}	114.7 ± 9.5^{d}
	A + Bu BP	1.00 ± 0.01^{d}	27.27 ± 1.49^{a}	2.38 ± 0.23^{d}	13.20 ± 1.35^{e}	131.7 ± 10.7^{f}
	A + Qu J	0.43 ± 0.06^{b}	31.39 ± 1.98^{b}	0.94 ± 0.16^{a}	10.88 ± 1.05^{c}	107.0 ± 12.1^{d}
	A + Qu BP	0.80 ± 0.01^{c}	27.40 ± 0.81^{a}	0.96 ± 0.06^{a}	10.08 ± 0.39^{b}	124.0 ± 10.4^{e}
	A + Bu BP + Xy	1.00 ± 0.01^{d}	28.30 ± 1.23^{a}	2.34 ± 0.17^{d}	13.04 ± 1.14^{e}	147.7 ± 2.3^{g}
G	GC	2.07 ± 0.15^{e}	48.45 ± 1.46^{e}	1.56 ± 0.32^{c}	7.55 ± 1.52^{a}	90.4 ± 5.1^{b}
	G + Bu J	3.13 ± 0.12^{h}	49.44 ± 0.16^{e}	3.93 ± 0.39^{e}	27.44 ± 2.15^{f}	108.7 ± 22.9^{d}
	G + Bu BP	1.97 ± 0.25^{e}	34.60 ± 0.88^{c}	0.94 ± 0.11^{a}	12.49 ± 0.21^{d}	$99.3 \pm 17.6^{b,c}$
	G + Qu J	2.70 ± 0.11^{g}	58.81 ± 4.53^{f}	2.29 ± 0.17^{d}	12.74 ± 1.04^{d}	132.0 ± 7.8^{f}
	G + Qu BP	2.53 ± 0.01^{f}	46.60 ± 0.96^{d}	1.30 ± 0.14^{b}	7.54 ± 0.32^{a}	96.3 ± 6.7^{b}
	G + Qu J + Xy	2.67 ± 0.09^{f}	58.62 ± 3.21^{f}	2.26 ± 0.12^{d}	12.68 ± 0.93^{d}	142.0 ± 3.6^{g}

Influence of different texture-forming agents (A or G), different plants (Bu or Qu), and J or BP C of plants on gummy candy parameters

Source	*Dependent variable*		*Mean square*	*F*	*p*
A or G	Texture		26.520	2501.853	0.0001
	Colour coordinates	L^*	1433.441	252.122	0.0001
		a^*	0.043	0.837	0.371
		b^*	1.222	0.708	0.410
	Overall acceptability		199.180	1.217	0.283
Bu or Qu	Texture		0.003	0.287	0.598
	Colour coordinates	L^*	253.110	44.519	0.0001
		a^*	11.261	216.690	0.0001
		b^*	216.300	125.212	0.0001
	Overall acceptability		21.094	0.129	0.723
J or C	Texture		0.020	1.871	0.186
	Colour coordinates	L^*	425.884	74.884	0.0001
		a^*	10.534	202.689	0.0001
		b^*	158.672	91.852	0.0001
	Overall acceptability		70.384	0.430	0.519
A or G × Bu or Qu	Texture		0.011	1.022	0.324
	Colour coordinates	L^*	146.718	25.806	0.0001
		a^*	3.197	61.524	0.0001
		b^*	77.508	44.868	0.0001
	Overall acceptability		550.084	3.362	0.082
A or G × J or C	Texture		2.214	208.900	0.0001
	Colour coordinates	L^*	205.686	36.177	0.0001
		a^*	2.653	51.055	0.0001
		b^*	153.167	88.665	0.0001
	Overall acceptability		2503.084	15.297	0.001
Bu or Qu × J or C	Texture		0.149	14.041	0.001
	Colour coordinates	L^*	1.109	0.195	0.663
		a^*	4.234	81.462	0.0001
		b^*	34.058	19.715	0.0001
	Overall acceptability		313.204	1.914	0.182
A or G × Bu or Qu × J or C	Texture		0.683	64.475	0.0001
	Colour coordinates	L^*	5.881	1.034	0.321
		a^*	0.154	2.956	0.101
		b^*	33.773	19.550	0.0001
	Overall acceptability		313.204	1.914	0.182

GC: gummy candies; A: agar; C: control; G: gelatin; J: juice; BP: juice by-products; Bu: sea buckthorn; Qu: quince; Xy: xylitol. L^*: lightness; a^*: redness ($-a^*$: greenness); b^*: yellowness ($-b^*$: blueness). Data are represented as means ($n = 3$) ± SD. $^{a-g}$Mean values with different letters are significantly different when $p \leq 0.05$.

($p = 0.0001$), and interactions of A or G selection × juice or BP and Bu or Qu × juice BP selection were significant for TPC content in CC. The main phytochemicals present in Qu (quercetin, 5-O-caffeoylquinic acid, rutin, β-carotene, lycopene, β-cryptoxanthin, lutein, plus zeaxanthin) have received attention due to their high antioxidant potential and capability of preventing and treating pathologies [24]. Total antioxidant activity in Bu is significantly associated with total phenolics, isorhamnetin-3-rutinoside, and isorhamnetin-3-glucoside [25]. Therefore, strong antioxidant activity of Bu has been reported by several authors [26]. Finally, BuJ, QuJ, BuBP, and QuBP increased the TPC content and antioxidant activity of the CC, and

Table 4: Total phenolic compounds (TPC) content and antioxidant activity of gummy candies.

GC		TPC content, RE mg·100·g^{-1}	Antioxidant activity, %
A	AC	47.1 ± 3.1^{a}	12.4 ± 1.9^{a}
	A + Bu J	153.5 ± 6.4^{f}	28.6 ± 1.7^{c}
	A + Bu BP	194.3 ± 7.2^{i}	35.2 ± 2.8^{f}
	A + Qu J	109.6 ± 4.3^{d}	41.8 ± 3.4^{g}
	A + Qu BP	162.1 ± 4.7^{g}	57.5 ± 3.1^{i}
	A + Bu BP + Xy	186.1 ± 4.5^{i}	36.4 ± 1.8^{f}
G	GC	68.3 ± 3.4^{b}	12.4 ± 0.7^{a}
	G + Bu J	149.4 ± 5.6^{e}	24.2 ± 1.6^{b}
	G + Bu BP	178.2 ± 7.0^{h}	31.6 ± 2.3^{d}
	G + Qu J	98.7 ± 4.3^{c}	37.3 ± 2.9^{f}
	G + Qu BP	143.5 ± 6.2^{e}	52.8 ± 3.4^{h}
	G + Qu J + Xy	100.3 ± 3.9^{c}	34.1 ± 1.6^{e}

Influence of different texture-forming agents (A or G), different plants (Bu or Qu), and J or BP of plants on gummy candy parameters

Source	*Dependent variable*	*Mean square*	*F*	*p*
A or G	TPC content	45.679	1.565	0.225
	Antioxidant activity	65.473	10.259	0.004
Bu or Qu	TPC content	9780.844	335.144	0.0001
	Antioxidant activity	1827.015	286.276	0.0001
J or C	TPC content	10445.854	357.931	0.0001
	Antioxidant activity	766.140	120.047	0.0001
A or G × Bu or Qu	TPC content	32.434	1.111	0.304
	Antioxidant activity	0.540	0.085	0.774
A or G × J or C	TPC content	145.534	4.987	0.037
	Antioxidant activity	0.135	0.021	0.886
Bu or Qu × J or C	TPC content	287.734	9.859	0.005
	Antioxidant activity	110.940	17.383	0.0001
A or G × Bu or Qu × J or C	TPC content	6.934	0.238	0.631
	Antioxidant activity	0.375	0.059	0.811

GC: gummy candies; A: agar; C: control; G: gelatin; J: juice; BP: juice by-products; Bu: sea buckthorn; Qu: quince; TPC: total phenolic compounds; RE: rutin equivalents. L^*: lightness; a^*: redness ($-a^*$: greenness); b^*: yellowness ($-b^*$: blueness). Data are represented as means ($n = 3$) ± SD. $^{a-i}$Mean values with different letters are significantly different when $p \leq 0.05$.

incorporation of these ingredients into the main formula leads to the preparation of products with better functionality.

4. Conclusions

BuJ, QuJ, BuBP, and QuBP showed antimicrobial activity against all the pathogenic bacteria tested; according to the results obtained, not just the juice, but also BP have great potential as desirable antimicrobial ingredients for the food industry. In all cases, BuJ, QuJ, BuBP, and QuBP increase the TPC content and antioxidant activity of the CC. However, A and/or G selection ($p = 0.0001$) and interactions of A and/or G selection × juice or BP and Bu or Qu × juice or BP ($p = 0.0001$) have a significant influence on CC texture. Combination of A and BuBP and of G and QuJ leads to the preparation of products with a high degree of overall acceptability containing desirable antimicrobials.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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