



Blueberry by-product used as an ingredient in the development of functional cookies

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Abstract

A by-product of blueberry juice industries was used as an ingredient to develop fiber-enriched cookies. The blueberry pomace, once ground and dried, was used as an ingredient in cookie formulation. A control cookie was elaborated as reference. Cookies were analyzed for composition and functional properties. The fiber content obtained in the fiber-enriched cookie allows it to be labeled as “high fiber” in the European Union and as a “source of fiber” in MERCOSUR. The fiber-enriched cookie presented highly increased values on the antioxidant capacity and the polyphenol content when compared against the control cookie. Sensory evaluation was performed. Acceptability of the fiber-enriched cookie reached a value of 5.3 in a nine-point hedonic scale. Further strategies should be necessary in order to achieve an acceptable product. Cookies were subjected to an *in vitro* digestive process. Results show that the cookies’ phytochemicals are bioaccessible and potentially bioavailable. Therefore, eating this type of food would represent an increase in the amount of antioxidants ingested and redound to a health benefit. In addition to improving both nutritional and functional properties of cookies, the present development represents an innovative strategy for a more sustainable growth of fruit juice industries.

Keywords

Antioxidant fiber, cookies, functional food, consumer acceptability, bioaccessibility

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INTRODUCTION

Over the last few years, consumer demand for nutrition-modified and functional foods has grown rapidly (Bimbo et al., 2017). Consumer’s demand for health-enhancing food has spurred in part because of socio-economic changes, such as longer life expectancy, rise of health care costs, social cost of non-transmittable diseases, and the widespread desire for a better quality of life (Valls et al., 2013). A common approach employed in the food industry to develop added-value foods involves the addition of a healthy component. Benefits could be better if these components are food industry waste and/or by-products with any functional

properties, such as a source of fiber and antioxidants. Ignat et al. (2011) studied how agricultural and industrial residues of fruit and vegetable processing are an important source of natural antioxidants. The use of by-product as an ingredient in the development of new products represents an important approach for a better economic and environmental qualification of these materials (Pasqualone et al., 2014).

The processing of blueberry into juice generates waste that may account for up to 20% of the initial fruit weight (Šarić et al., 2016). This waste, or by-product, usually named “pressed cake” or “pomace,”

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consisting of seeds, stems, and skin (Bobinaitė et al., 2014), is one of the richest sources of phenolic compounds, exhibiting an important antioxidant activity (Ignat et al., 2011; Šarić et al., 2016). It presents good nutritional and functional properties since not only does it contain 70% of polyphenols originally present in berries but also large amounts of cell wall polysaccharides, being considered as a source of dietary fiber (Hilz et al., 2005).

Blueberry by-product can be ground and dried to obtain blueberry pomace powder (BPP) which could be used as an ingredient with a stable shelf life. This product may combine in a single material the physiological effects of both dietary fiber and antioxidants: “antioxidant dietary fiber.” Cookies are the most consumed and popular baked goods worldwide since they are a low-cost, ready-to-eat product, involving a long shelf life and a wide variety (Vitali et al., 2009). Also, consumers are becoming more health conscious. This need for healthy products has led to modifications of the cookies’ nutritional value, which may be improved by adding functional ingredients, such as blueberry pomace.

Nevertheless, these functional characteristics need to be validated on the final product. It is of utmost importance to study the bioaccessibility and bioavailability of polyphenols, since they will only be beneficial if they are available in the target tissue. When the food matrix is rich in dietary fiber, these compounds get trapped inside it. The fiber entrapping the antioxidants makes them less accessible to enzymes, hindering their release; therefore, most of them reach the colon intact. The latter does not make this type of food less beneficial since they contribute to keeping an antioxidant environment in the colonic lumen (Palafox-Carlos et al., 2011). These processes can be studied from an in vitro digestive process approach, which simulates the conditions of gastrointestinal system.

Therefore, the aim of this study was to use blueberry by-product as a functional ingredient in the development of cookies with antioxidants dietary fiber.

MATERIALS AND METHODS

Fiber enriched cookies

To obtain fiber enriched cookies, BPP was used as a source of fiber. BPP was obtained drying blueberry pomace in a convection oven at 40 °C until constant weight and grinding it in a laboratory mill (Retsch ZM 200) using the fraction that passed through the 1 mm sieve. The blueberry pomace was obtained as a by-product from the juice production. The blueberries used were O’Neill variety, from Uruguay.

The following ingredients were used for cookie preparation: BPP, wheat flour, vegetable oil, skimmed milk

powder, commercial sucralose (Splenda®, USA), whey protein concentrate 80% from Friesland Campina DMV (the Netherlands), baking powder, flavor vanilla powder, and powdered soy lecithin from Archer Daniels Midland Company ADM, USA.

Cookies’ dough was prepared mixing all ingredients, according to Table 1, with enough water (20% of total dry dough weight) to be kneaded. It was rolled out to disks of 4 cm of diameter and 0.75 cm in height and baked at convection oven for 12 min at 170 °C. In a previous study, results showed that cookies with a 9% dietary fiber (reached with 37.14 g of BPP in 100 g of total dry dough weight), in disks of 0.75 cm of height and baked at 170 °C presented the maximum antioxidant capacity and total polyphenol content according to the experiment design assayed. An image of the fiber enriched cookie is shown in Figure 1.

A control cookie, with no BPP, was assayed as the reference sample.

Proximate compositions

Proximate analyses were performed on the fiber enriched and control cookie. Protein and total dietary fiber were determined using AOAC methods, 984.13, 985.29, respectively (AOAC, 2012). Fat was estimated

Table 1. Cookies formulation

Ingredients	Amount in the dry dough (%)	
	Enriched cookie	Control cookie
Wheat flour	28.52	65.94
Blueberry pomace (BPP)	37.14	–
Sweetener	2.29	2.29
Whey protein concentrate	1.91	1.91
Vegetable oil	17.49	17.49
Skimmed milk powder	10.00	10.00
Vanillin	0.95	0.95
Baking powder	1.43	1.43
Soy lecithin powder	0.28	0.66

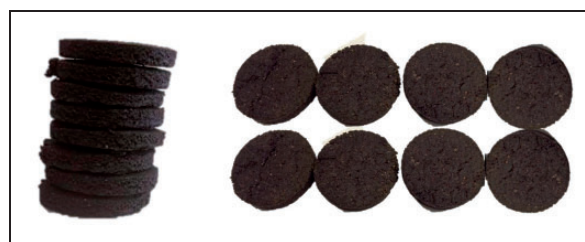


Figure 1. Image of the fiber enriched cookie.

following the ISO-6492-1999 procedure. Moisture content was determined by gravimetric analysis in a convection oven at 105 °C until constant weight. Ash was determined in a muffle furnace following ISO 5984-2002. Total carbohydrates content was obtained by difference between the total weight and the sum of grams of protein, lipids, dietary fiber, moisture, and ash content in 100 g of sample.

Functional properties

Functional properties were performed on both the fiber enriched cookie and the control cookie.

Phenolic compounds

Extraction. Extractable and hydrolysable phenolic compounds were extracted as suggested by Vitali et al. (2009). For the extractable fraction, a mixture of HCl_{conc}/methanol/water (1:80:10 v/v) was used, as proposed by Gao et al. (2002). Briefly, 200 mg of ground sample was extracted with 4 mL of the acidified methanol solution for 2 h at room temperature on a shaker. The mixture was afterward centrifuged at 1358 g for 10 min on a table centrifuge (Thermo Scientific, Sorvall ST-8 R). The supernatant was recovered and used for the determination of total phenolic compounds and antioxidant capacity.

For the extraction of hydrolysable compounds, the sample residues remaining from the first extraction were mixed with 22 mL of acidified methanol (10:1 v/v, methanol/H₂SO_{4conc}), as proposed by Hartzfeld et al. (2002). The mixture was placed in a shaking water bath at 85 °C for 20 h. The supernatants obtained after centrifugation (10 min, 3500 rpm) were used for the determination of antioxidant capacity and total phenolic compounds.

Total polyphenol content. Total phenolic content (TPC) of extracts was determined using Folin–Ciocalteu method (Singleton and Rossi, 1965) modified by Gao et al. (2002). An aliquot of the samples (0.2 mL) was added to 1.5 mL of freshly diluted (1:10) Folin–Ciocalteu reagent. The mixture was allowed to sit for 5 min. After, 1.5 mL of sodium carbonate solution (60 g/L) was added. The mixture was incubated for 90 min and the absorbance read at 725 nm in a Shimadzu 1800 UV-Visible spectrophotometer. Acidified methanol was used as a blanc and gallic acid as standard. The results were expressed in milligrams of gallic acid per gram of sample.

Total monomeric anthocyanins. Quantification of anthocyanins was performed by the pH differential method of Wrolstad (2001). Samples were diluted 1:50

(blueberry pomace) or 1:10 (blueberry cookies) in pH 1.0 and pH 4.5 buffers and measured at 510 and 700 nm in a Shimadzu 1800 UV-Visible spectrophotometer. The absorbance values of the diluted samples (A) were calculated as follows:

$$A = \left[(A_{s10} - A_{700})_{pH1.0} - (A_{s10} - A_{700})_{pH4.5} \right]$$

Total monomeric anthocyanins method was based on a cyanidin 3-glucoside molar extinction coefficient of 26,900 (ε) and a molecular weight of 449.2 g/mol and was calculated as follows:

$$\text{TMA} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l}$$

Results were expressed in mg of anthocyanins per 100 g of dry sample.

Antioxidant capacity. Antioxidant capacity was measured using the ABTS radical cation decolouration assay, as proposed by Re et al. (1999) with modifications.

ABTS stock solution was prepared by diluting the reagent with distilled water to a concentration of 2.5 mM to generate the ABTS radical, 2.5 mL of this solution was mixed 44 μL of potassium persulfate (140 mM) and left standing in the dark for 16 h for the complete formation of ABTS radical cation. Trolox standard curve was prepared diluting Trolox in ethanol:water (50:50 v/v), from 0 to 2.0 mM final concentration.

For the study, the radical solution was diluted with a mixture of ethanol:water (50:50) to an absorbance of 0.70 (±0.02) at 734 nm. Three millilitres of this solution were added to 30 μL of extract or Trolox standard solution and left standing for 30 min in the dark. Absorbance was read at 734 nm in a Shimadzu 1800 UV-Visible spectrophotometer.

Consumer acceptability

The fiber enriched and the control cookies were evaluated by 96 consumers. They were students and workers of the Universidad Católica del Uruguay and regular cookies consumers. They were 33% men and 67% women, and their ages ranged between 18 and 65 years. One cookie of each sample was served to consumers on plastic plates coded using three-digit random numbers. They were asked to try each of the samples and to indicate their overall liking using a nine-point hedonic scale (1: “I dislike extremely”; 9: “I like extremely”) and to answer a check-all-that-apply (CATA) questions. CATA questions were composed of 19 terms (Table 4).

In vitro digestion. To study potential bioaccessibility of the phenolic compounds, an in vitro digestion was carried out on the fiber enriched cookie and on the control cookie, following the method proposed by Hollebeeck et al. (2013). This method consists of three stages: salivary, gastric, and intestinal.

All incubations were performed in closed Erlenmeyer flasks of 50 mL, in a shaking water bath at 37 °C and 200 rpm. All volumes were adjusted with phosphate buffer saline (PBS) 10 mM, pH 6.9.

For the first step, incubation volume was 10.43 mL, with α -amylase (90 units/mL, 0.43 mL). The incubation time was 5 min. For the gastric step, pepsin was added and pH adjusted to 2.0 with HCl 1 M. Volume was adjusted to 22.73 mL with PBS; reaction time was 90 min.

In the last step, pancreatin and bile were added, and pH was adjusted to 7.0 with NaHCO₃ 0.1 M. Volume was completed to 30.09 mL, and the sample was incubated for 150 min.

When all the steps were finished, enzymatic reactions were stopped in a water bath at 90 °C for 10 min. Samples were centrifuged at 10,000 rpm for 10 min. After separating the supernatant from solid residue, samples were frozen at -80 °C and lyophilized.

Bioaccessibility was calculated as the ratio between TPC and sumatory of extractable and hydrolysable phenolic compounds.

Data analysis

Analyses were performed in triplicate, and data reported as mean \pm SD. Student's *t* test was performed on the acceptability data. The frequency of mention of each of the CATA question terms was determined. Cochran's Q test was used to evaluate significant differences between samples. Analyses were performed using XLSTAT Version 2011 (Addinsoft 1995–2010, France).

RESULTS AND DISCUSSION

Proximate compositions and functional properties

Proximate compositions of the fiber enriched cookie included the estimation of proteins, fat, ash, dietary fiber, and carbohydrates (Table 2).

In this study, the blueberry juice production by-product was used as a functional ingredient to substitute part of the wheat flour. In accordance with a previous study, BPP presents dietary fiber (25.15 \pm 0.23 g/100 g dwb), with antioxidant capacity (total polyphenol content 232.32 \pm 1.28 mg gallic acid/g ww; antioxidant capacity 117.22 \pm 1.78 μ M TE/g ww).

Table 2. Proximate composition of the fiber enriched and the control cookie in g/100 g dwb

	Fiber enriched cookie	Control cookie
Proteins	10.02 \pm 0.62	12.55 \pm 0.37
Lipids	18.39 \pm 0.01	15.73 \pm 0.18
Ash	2.43 \pm 0.01	1.86 \pm 0.03
Dietary fiber	14.64 \pm 4.40	9.39 \pm 1.40
Carbohydrates	54.50 ^a	55.10 ^a

^aCarbohydrate content was obtained by difference.

Table 3. Functional parameters of the fiber enriched and the control cookie

	Fiber enriched cookie	Control cookie
ABTS extractable fraction ^a	27.69 \pm 0.06	0.97 \pm 0.06
ABTS hydrolysable fraction ^a	159.77 \pm 4.29	46.26 \pm 2.67
Total antioxidant capacity ^{a,b}	187.46 \pm 4.29	47.23 \pm 2.67
Extractable phenols ^c	90.01 \pm 3.20	17.19 \pm 0.59
Hydrolysable phenols ^c	287.90 \pm 12.77	93.90 \pm 0.81
Total phenols ^{b,c}	321.30 \pm 13.16	111.09 \pm 1.00
Anthocyanins ^d	35.76 \pm 3.83	n.d. ^e

^a μ M TE/g cookies dry weight basis (dwb).

^bTotal fraction refers to the sum of extractable and hydrolysable fraction.

^cmg gallic acid equivalents/g cookies dwb.

^dmg cyanidin-3-glucoside/100 g cookie dwb.

^eNot detected.

Cookies were obtained by substituting 56% of wheat flour with blueberry pomace, obtaining a fiber content of 14.64 \pm 4.40 g/100 g of cookies dwb. Vitali et al. (2009) studied the effect of the addition of integral raw materials (white soy, amaranth, carob, oat fiber, and apple fiber) on the fiber content of cookies, only obtaining a similar value with oat fiber (14.48 g/100 g dwb). Aksoylu and Çag (2015) got lower fiber levels, obtaining 1.61 \pm 0.01 g/100 g dwb of dietary fiber with the incorporation of defatted poppy seed powder. Mildner-Szkudlarz et al. (2012), who enriched cookies with 30% of white grape pomace, reported 11.03 g/100 g dwb of dietary fiber, finding blueberry pomace as a higher source of dietary fiber.

The fiber content obtained in this work allows the cookie to be labeled as “high fiber” in the European Union (at least 6 g of dietary fiber per 100 g) and as a “source of fiber” in MERCOSUR (at least 2.5 g of dietary fiber per 30 g).

To evaluate functional properties, both the fiber enriched cookie and the control cookie were analyzed. Table 3 summarizes the functional characterization of

cookies. There is a significant difference ($p > 0.05$) between the ABTS scavenging activity result of the control cookie and the one supplemented with blueberry pomace. The fiber enriched cookie exhibits higher antioxidant activity than commercial cookies, as demonstrated by comparing it with the control cookie. The ABTS antioxidant activity obtained from the soluble fraction was higher ($27.69 \pm 0.06 \mu\text{mol TE/g dwb}$) than values reported by Vitali et al. (2009), on all the matrices tested (highest value is $20.10 \pm 0.36 \mu\text{mol TE/g dwb}$).

The relation between the extractable and hydrolysable fraction is consistent with the aforementioned fact of the bounded antioxidants to dietary fiber (Pérez-Jiménez and Saura-Calixto, 2005). This fraction of the polyphenols can be found in the residues of the first extraction.

The results of the Folin–Ciocalteu assay showed that only 28.01% of total polyphenols is extractable, which is consistent with the findings reported by Saura-Calixto (2011), showing that most of the polyphenols are bound to dietary fiber and are not soluble in common solvents. On ABTS assay, the fraction is lower (14.77%), probably due to overestimation of the Folin–Ciocalteu assay, that estimates all the reducing substances of the sample.

Anthocyanins content remains high compared to published data. Pasqualone et al. (2014) report $14.0 \pm 0.9 \text{ mg malvidin } 3\text{-O-}\beta\text{-D-glucoside}/100 \text{ g}$ cookies in cookies enriched with grape marc. The theoretical amount added, corresponding to the added powder, is $40.36 \text{ mg anthocyanins in } 100 \text{ g}$ of BPP cookies. A loss of 11.27% of anthocyanins content was identified, imputable to the baking process. Nevertheless, there is no consensus regarding the effect of baking conditions on the anthocyanin content.

Consumer acceptability

Acceptability score for the fiber enriched and the control cookie presented no significant difference ($p = 0.582$) with values of 5.3 and 5.5, respectively. Cookies did not reach an acceptable value on a nine-point hedonic scale according to Muñoz et al. (1992). These authors considered an acceptability score of 6.0 in a nine-point hedonic scale (the first score in the liking category) as a commercial or quality limit. This is in agreement with Carrillo et al. (2012) which studied acceptability of 10 enriched sweet cookies, remarking that participants were not able to sacrifice taste for health in that kind of product. Without information they obtained scores between 3.7 and 6.9 in a nine-point scale.

The percentages of terms that consumers found for each cookie sample are shown in Table 4. Frequencies varied significantly among samples for 13 out of the 19

Table 4. Frequency of use (%) of the sensory terms included in the CATA question for the evaluation of the fiber enriched and the control cookie

Term	Fiber enriched cookie	Control cookie
Crunchy	0	3
Off-flavour	9	0
Tasty	33	35
Barely sweet	30	50
Sweet	24	16
Healthy	26	15
Intense flavour	48	2
Fibrous	32	3
Bitter	9	0
Fruity	67	1
Non-characteristic flavour	22	5
Crambly	22	10
Aftertaste	17	10
Poor tasty	4	46
Soft	71	73
Hard	0	1
Dry	6	31
Rough	8	1
Floury	8	26

CATA: check-all-that-apply.

Terms highlighted in bold mean significant difference between cookies ($p < 0.05$).

attributes. Within the most relevant, the fiber enriched cookie was considered fruity, tasty, fibrous, and with an intense flavor. This characteristic made that consumer reject or accept the product but not feel indifferent. This behavior was observed analyzing acceptability data not as an average value but divide the nine-point scale in ranges. Considering this, 31% of the consumer ranked the overall acceptability between one and four in the nine-point hedonic scale resulting in an unacceptable judge, while 52% of consumer evaluated the fiber enriched cookie between six and nine points of the range meaning they liked it. So, despite the overall acceptability resulted in a media value under the commercial quality limit, there is an important group of consumer that really like the product. It is interesting to mention that 26% of consumer perceived the fiber enriched cookie as healthy. In the other side, considering the negative terms that consumer mentioned, off flavor and soft were the most relevant. Kim and Kim (2017) compared the acceptance of madeleines made with commercial wheat flours versus antioxidant wheat flours. The overall acceptability for baked products made with antioxidant wheat flours was lower than those made with commercial wheat flour.

Nevertheless the willingness to pay for madeleines made with antioxidant wheat flours tended to increase when information about the antioxidant properties was provided to consumers. Carrillo et al. (2012) observed that in the expected scenario, brand, familiarity with the product and familiarity with the claim were found to play an important role in enhancing the overall acceptance of some biscuits. Kim and Kim (2017) identified different trends appeared when consumers were stratified, resulting that 25% of consumers were health benefit conscious and preferred madeleine made with antioxidant wheat flours.

For that reason, a factor to be considered that could improve overall acceptability could be the inclusion of “healthy benefits” information. In order to improve the likelihood of product acceptance, an adequate diffusion of functional properties exposed in label information may be necessary. Claims, illustrations, and symbols convey important information on what one can expect on the product by looking at the package. Previous studies have shown that package characteristics, and especially those related to the label, can influence, either positively or negatively the overall image of the product and likewise product expectative, acceptability and consumer choice (Deliza and Mac Fie, 1996; Ares and Deliza, 2010; Torres et al., 2012; Reis et al., 2017).

In vitro digestion. An in vitro digestion was carried out on the fiber enriched cookie and on the control cookie to simulate the digestive process and study their bioaccessibility, as the antioxidants need to be released to exert their beneficial effects on the gastrointestinal tract. From a nutritional and functional point of view, this fraction is much more important than the concentration of the bioactive compounds in the cookie (Rodríguez-Roque et al., 2015). The content of bioaccessible phenols and the relation with phenol content is shown in Table 5.

The in vitro digestive process showed that 35% of the extractable and hydrolysable phenols are bioaccessible. Saura-Calixto et al. (2007) estimated that about 48% of total polyphenols from solid vegetable foods in the diet is bioaccessible. The obtained fraction is lower,

probably due to the large amount of dietary fiber in the cookie. Dietary fiber hinders the bioaccessibility of phenols by not allowing them to be released from the food matrix and entrapping the compounds on the first steps of the digestive process (Palafox-Carlos et al., 2011). This also explains the higher percentage of phenolic bioaccessibility of the control cookie, as it has a lower content of dietary fiber and therefore phenolics are more bioaccessible. Vitali et al. (2009) studied potential phenols bioaccessibility on their cookies but obtained lower values (30.5% for the cookie enriched with amaranth).

The antioxidant capacity of the physiological extract was lower than the one of the chemical extract (14%). Nevertheless, when comparing the antioxidant capacities of both cookies, a 2.5 times fold increase was observed, ensuring the bioactivity of the blueberry pomace. The lower extraction is due to the entrapment of the antioxidant compounds within the food matrix, as happens with phenol content. The chemical extraction procedures are much more aggressive, allowing more compounds to be released from the food matrix. The difference in the increase of phenol content and antioxidant capacity between control cookie and the enriched one is notorious, where wheat flour was replaced by blueberry flour.

Although the bioaccessibility of these phytochemicals is limited, they are still beneficial. When non-extractable polyphenols reach the colon intact, some will be released and new compounds will be formed through the action of colonic microbiota or enzymes able to break covalent bonds. Some of them may be absorbed and new metabolites may be formed (Pérez-Jiménez et al., 2013). Even those that are not absorbable are valuable, as they maintain an antioxidant environment on the colonic lumen against prooxidant conditions (Palafox-Carlos et al., 2011). Although carrying an in vitro fermentation to study the bioaccessibility of these compounds would be useful, it would only represent an estimate rather than an accurate determination, as the fermentation degree depends on each person’s microflora (Saura-Calixto et al., 2007).

It has been shown that these non-extractable polyphenols exert many benefits on the gastrointestinal

Table 5. Antioxidant capacity and phenol content on the physiological extract of cookies samples

	Antioxidant capacity ^a	Bioaccessible phenols ^b	Phenolic bioaccessibility ^c (%)
Fiber enriched cookie	22.21 ± 0.92	113.50 ± 10.75	35.32
Control cookie	8.37 ± 0.26	71.48 ± 1.50	68.15

^aµM TE/g sample wwb; on the physiological extract.

^bmg gallic acid/g sample wwb.

^cBioaccessibility was calculated as proposed by Vitali et al. (2009), as percentage of total phenolic content.

health. The fecal content shows higher antioxidant capacity, preventing against reactive oxygen species of the colon. These compounds also show prebiotic effect, stimulating *Lactobacillus* growth, reducing the colonization of non-beneficial bacteria species (Pérez-Jiménez et al., 2013).

CONCLUSIONS

Adding blueberry pomace as an ingredient for cookies led to better nutritional and functional properties. One of the goals of the conducted recipe modifications was the increase of dietary fiber of supplemented cookies, which was achieved. The fiber content obtained allows the cookie to be labeled as “high fiber” in the European Union, and as a “source of fiber” in MERCOSUR.

Another goal was improving functional properties which were achieved by the incorporation of the BPP. The supplemented cookie presented highly increased values on the antioxidant capacity and the polyphenol content when compared against the control cookie.

Regarding sensory analysis, further strategies should be necessary to achieve an acceptable product in the Uruguayan market. In order to improve the likelihood of product acceptance, an adequate diffusion of functional properties may be necessary.

The in vitro digestive process showed that the cookies' phytochemicals are bioaccessible and potentially bioavailable. Therefore, eating this type of food would represent an increase in the amount of antioxidants ingested and redound to a health benefit.

The present development represents an innovative strategy for a more sustainable growth of the juice fruit industry, improving cookies' nutritional and functional properties.

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