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Blueberry by-product as a novel food ingredient: physicochemical characterization and study of its application in a bakery product

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jsfa.11812

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Abstract

BACKGROUND

Industrial blueberry juice processing results in a significant amount of by-product, the so called pomace; and could represents a nutritional valuable source of fibre and bioactive compounds to enhance either technological or nutritional characteristics of foods.

The aim of this study was to obtain novel ingredients based on blueberry by-product applying different drying methods: convective (CD), freeze (FD) and vacuum-drying (VD). The powders were physicochemical, functional, and nutritionally characterized. Finally, its application to formulate muffins in replacing 10-20-30% of wheat flour was studied to evaluate the effect on textural, colour and sensorial characteristics.

RESULTS

CD reduced the hydration and functional properties when compared to FD and VD. The powders were characterized by a high content of dietary fibre (273±5 g kg^{-1}) and, a good retention and bioaccessibility of antioxidant compounds (39-85% range).

The powder addition to formulate muffin decreased lightness and chromaticity, without differences due to drying process. The texture parameters were reduced with 10% of ingredient addition; meanwhile 20-30% showed similar values to the muffin control. Sensorial evaluation presented good overall acceptability (>6±2 in a 9-point hedonic scale) and, some specific attributes showed a significant drop in overall acceptability recommending its optimization according to the Penalty Analysis.

CONCLUSION

These results suggest that functional ingredients rich in fibre and bioactive compounds may be obtained from an industrial by-product giving added value and avoiding or reducing their lost; and could also be a promising vehicle to incorporate dietary fibre and bioactive compounds in bakery goods.

Keywords: industrial juice by-products, berries, muffins, antioxidant compounds, *in vitro* bioaccesibility.

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1. Introduction

Fresh blueberries are rich sources of polyphenols such as flavonoids, tannins, and phenolic acids; and specifically in anthocyanins, which are known as potent antioxidants[1]. Several studies have revealed that phytochemical composition and antioxidant capacity of blueberries has an important role in lipid and glucose metabolism, insulin signalling, and inflammation and oxidative stress; with potential health benefits in reducing the development of obesity and its related comorbidities, including type-2 diabetes and chronic inflammation, and as anti-cancer agents [2–4].

Blueberries are highly perishable and a very valuable commodity. The main causes of the loss of blueberry quality are careless picking and handling in the field, delays in cooling, harvesting the fruit in wet conditions, poor field sanitation, white/red drupelets, and mould growth [5]. When the highly quality standards of blueberries are not accomplished for its commercialization in the international markets, it is destined for the elaboration of secondary products such as juices, jams, frozen pulp for production of ice cream, yogurt, and pastries, as well as dehydrated products [6]. Approximately 18% of the total blueberry production is destined to the production of non-alcoholic beverages, mainly as fresh juice or concentrates.

The industrial blueberry juice processing results in a significant amount of by-product, the so-called pomace. This by-product often represents a nutritional valuable source of bioactive compounds and dietary fibre that could enhance either technological or nutritional properties of foods. For instance, authors as Avram *et al.* [7] have studied the recovery of polyphenols from blueberry by-product using nanofiltration, and Tao *et al.* [8] the physical stability of freeze-dried blueberry juice and pomace. However, to our knowledge, the effect of different drying methods on physicochemical, functional and antioxidant properties of blueberry by-product has not been studied so far. It is important to highlight, that a further utilization and/or an added valued of these by-products are desirable from a sustainability point of view. Indeed, there is a global trend in assuring more sustainable application of ingredients in food development, with emphasis in the recovering of nutrients from agro-industrial by-products [9].

Another relevant subject of nutritional matter is the study of the bioaccessibility and bioavailability of antioxidant compounds, which could be affected by the food microstructure [10]. Several studies have demonstrated that polyphenols associated to dietary fibre could be divided in two groups according their bioaccessibility during the pass-through gastrointestinal digestion: 1) polyphenols bioaccessible in the small intestine (e.g. low molecular weight polyphenols), and 2) polyphenols non-bioaccessible that reach the colon because there are linked or trapped into fibre matrix (mainly polymeric and low molecular weight polyphenolic compounds [11,12]. Polyphenols that

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reach the large intestine could be released by fermentation processes performed by the bacterial flora exerting some antioxidant activity [10]. A recent meta-analysis study has demonstrated that polyphenol supplementation from several food sources selectively promoted the abundance of human gut health-promoting microbiota and inhibits the possible pathogenic microbiota. This effect on human gut microbiota was polyphenol intake dose- dependent, concluding that to get the greatest effect for promoting human health, the polyphenol intake dose recommendation would be of 396 mg/day [13]. Therefore, the *in vitro* studies for deeply studying the release behaviour of antioxidant compounds are relevant in food design.

The objective of this work is to develop novel functional ingredients based on blueberry by-product applying different drying methods. After stabilization, physicochemical and functional properties, and the *in vitro* bioaccessibility of bioactive compounds were studied on the powders. Finally, the study of its application as ingredient in a bakery product formulation was also evaluated.

2. Material and methods

2.1. Preparation of Ingredients

The fresh blueberry by-product (FBP) was obtained from an industrial blueberry juice (Starberry S.A., Argentina), and was stored at -20°C until use.

Three drying methods were applied to stabilize the FBP: 1) Convective drying (CD) (FA10-MZ, COBOS, Argentina) at 50°C for 18 h; 2) Vacuum drying (VD) (Freezone 1, Labconco, USA) at room temperature (25°C) with a pressure of 0.040 mbar for 48 h, without freezing the sample previously; 3) Freeze-drying (FD) (Freezone 1, Labconco, USA) in the same condition of 2) but freezing the sample (-20°C) in a previous step. Finally, the powders were milled (Moulinex, Spain), sieved through a mesh with a pore size <840 µm (A.S.T.M. N° 170, ZONYTEST, Argentina) and stored at -20°C until use.

2.2 Physical, functional and hydration properties

The water activity (a_w) was measured on the FBP and on the powders using a hygrometer (HygroLab, Rotronic, USA). The moisture content was determined by drying in an oven at 100 °C until constant weight (AOAC 925.10, 2005).

The physical and functional characterization were determined as detailed in previous works [14,15]. Briefly, the bulk density (ρ) and specific volume (ν) were determined by measuring the volume of a weighed sample in a 15 mL graduated cylinder followed by a vortex for 1 min. For oil holding capacity (OHC), the sample was mixed with sunflower oil and after 18 h at 25°C it was centrifuged at 3.600 rpm for 5 min (5804R, Eppendorf, Germany), the supernatant was discarded, and the weight of the sample was recorded to determine the oil retained.

The hydration properties were determined keeping the sample in contact with distilled water for 18 h at 25°C. Swelling capacity (SC) was determined by measuring the final volume obtained by the hydrated sample. Water holding capacity (WHC) was determined by draining the sample at atmospheric pressure. The weight of the hydrated residue was recorded and then lyophilized for 48 h to finally obtain the weight of the dried residue. Water retention capacity (WRC) was determined recording the weight of water retained by the sample after accelerated decantation (30 min at 4.800 rpm).

2.3 Determination of glass transition temperature (Tg)

Values of Tg for blueberry by-product powders were measured using a differential scanning calorimeter (Q2000, TA Instruments, USA) equipped with a refrigeration system. All samples were equilibrated to 0.22 a_w with $CaCl_2$ salt under vacuum; and then ~5 mg of sample was placed in aluminium hermetic pans of 40µL inner volume. The scans were carried out from −80 to 120°C at a heating rate of 10°C/min, and an empty aluminium pan was used as reference. Thermograms obtained were analysed using Universal Analysis 2000 software (V4.5A, TA Instrument, USA).

2.4 Colour measurement

Instrumental colour was determined using a colorimeter (MiniScan, HunterLab, USA), in the CIE L*a*b* space under illuminant D65 and with an observer angle of 10°. The intensity of colour [Chr = $\sqrt{(a^*)^2 + (b^*)^2}$] was calculated.

2.5 Chemical composition

Protein content was determined by the Kjeldahl method (AOAC 984.13, 2005); lipids remaining by Soxhlet method (AOAC 923.05, 2005); total, soluble and insoluble dietary fibre (DF) by the gravimetric-enzymatic method (AOAC 992.16, 2005); and ash content in a muffle furnace at 550°C (AOAC 936.07, 2005). Total non-DF carbohydrates were determined by difference [15]. The sodium and potassium content were carried out by Atomic Absorption Spectrophotometry (AOAC 985.35, 2005).

2.6 Determination of antioxidant compounds

The antioxidant compounds were determined on the fresh blueberry by-product and on the powders obtained after the dehydration process.

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2.6.1 Total Monomeric Anthocyanins (TMA)

First, ethanolic extract (EE) was prepared by homogenizing ~1.5g of sample with 7.5mL of 95% ethanol acidified with 1.5N HCl, followed by magnetic stirrer for 15 min and then was vacuum-filtrated. The supernatant was raised with acidified ethanol in a 50 mL volumetric flask and stored at -80°C until use (Righi, Argentina).

TMA content was determined using the pH-differential method reported by Archaina *et al.* [16]. An aliquot of sample was diluted (1:4 v/v) in two buffers: pH 1.0 (0.025M potassium chloride) and pH 4.5 (0.4M sodium acetate), and then the absorbance was measured at 510 and 700 nm using a spectrophotometer (Jenway, UK). TMA content was determined using the Eq. 1:

$$\mathrm{TMA} = \frac{\mathrm{A} \times \mathrm{MW} \times \mathrm{DF} \times 1000}{\varepsilon \times \mathrm{OP}} \quad (1)$$

Where, *A* corresponds to the difference of absorbance measures at 510 and 700nm; *MW* is the molecular weight of cyanidin-3-glucoside (449.2 g mol^{-1}); *DF* is referred to the dilution factor, *ε* is the molar absorptivity of cyanidin-3-glucoside (26,900 L cm^{-1} mol^{-1}); and *OP* represents the optical path (1cm).

The results were expressed as g of cyanidin-3-glucoside kg^{-1} powder (d.b).

2.6.2 Extracts for the determination of Total Phenolic Compounds (TPC) and Antioxidant Capacity (AC)

A methanolic extract (ME) was prepared according to Archaina *et al.* [16] with some modifications. Briefly, ~1.0g of sample was mixed with 5 mL of methanol under magnetic stirrer for 5 min and then, it was vacuum-filtrated. The supernatant was recovered, and the sample was washed with methanol up to raised 25mL using a volumetric flask. The ME was stored at -80°C until the colorimetric determination of TPC and AC were performed.

2.6.3 TPC

It was carried out using the Folin-Ciocalteu method described by Archaina *et al.* [16]. Briefly, 100μL of ME was mixed with 900μL of distilled water, 100μL Folin–Ciocalteau reagent (Extrasynthese, China), and 600μL of 20% sodium carbonate. The samples were incubated for 30 min in a thermostatic bath at 40°C, and then the absorbance was measured at 765 nm using a spectrophotometer. The results were expressed as g of gallic acid kg^{-1} powder (d.b.).

2.6.4 AC

The AC was determined by the Trolox Equivalent Antioxidant Capacity (TEAC) method using the 2,2-azinobis-[3-ethylbenzothiazoline-6-sulfonic acid] (A1888-Sigma-Aldrich, USA) to produce the cationic free radical ($ABTS^{•+}$) [16]. $ABTS^{•+}$ was generated by the interaction of $ABTS^{•+}$ reagent and potassium persulfate. The solution was kept for 16 h in dark conditions at room temperature, and then it was diluted with 0.01M phosphate buffer to obtain an absorbance of 1.00±0.01 at 734 nm. An aliquot of 100 μL of ME was mixed with 1.9mL of $ABTS^{•+}$ solution and absorbance was measured at 734 nm. The results were expressed as milliequivalents of Trolox kg^{-1} powder (d.b.).

2.7 Bioaccessibility of TPC and AC after in vitro gastrointestinal digestion

The *in vitro* gastrointestinal digestion procedure was conducted according to Genevois *et al.* [14]. Briefly, ~0,6g of powder was mixed with 15mL of the gastric solution [0.3%, w/v pepsin in 0.01M HCl] followed by incubation at 37°C for 2h. Subsequently, the pH was adjusted at 7.5-8.0 with 2M NaOH; and 15mL of intestinal solution [0.6%, w/v bile salts and 0.3%, w/v pancreatin in 0.05 M KH_2PO_4] were added, followed by incubation at 37°C for 2 h. The samples were centrifuged at 3.000xg at 8°C for 15 min and finally, the supernatant (soluble fraction, SF) was stored frozen, and the residue (insoluble fraction, IF) was freeze-dried and stored at -12°C until use.

The SF and IF were extracted with methanol in order to determine TPC as it was detailed previously (2.6.2). The AC was measured in the SF using the TEAC Method as previously described, and in the IF by the QUENCHER Method [16]. Briefly, ~1.0mg of freeze-dried insoluble fraction was mixed with 10mL of $ABTS^{•+}$ and the absorbance was measured on the supernatant at 734 nm. The results were referred to the dried sample before digestions in order to calculate bioaccessibility as a percentage of the total content of the TPC or AC (Eq. 2):

$$\text{Bioaccessibilty (\%)} = \frac{\text{SF}}{\text{SF + IF}} \text{ x } 100 \qquad (2)$$

2.8 Muffins making process

The muffins were made using a recipe reported by Rupasinghe Vasantha *et al.* [17], where wheat flour was replaced by the blueberry by-product powder CD in a range of 10-30%. Solid ingredients: wheat flour (34.1%, Morixe, Argentina), sugar (15.4%; LEDESMA, Argentina), vegetable oil (13.9%, Natura, Argentina), skim milk powder (2.6%; La Serenisima, Argentina), baking powder (1.3%, Royal, Argentina), fresh egg (0.45%), and salt (0.1%; Celusal, Argentina) were mixed with water acidified with juice lemon (32.1%,

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pH 2.7) using a professional stand mixer (Planetaria, Moulinex, Brazil). Dough pieces (~35g) were baked in an electric oven (Marshall, Argentina) for 15min at 165°C. Finally, the muffins were kept at room temperature for 60min and packed in sealed polypropylene bags to avoid dehydration. Analytical measurements were made within 24h.

2.8.1 Physicochemical and textural characterization

Water content and colour measurement were performed as was described in section 2.2 and 2.4.

Texture profile analysis (TPA) of muffins was performed using a texturometer (3345, Instron, USA) provided with a cell charge of 100N using the method described by Genevois *et al.* [15]. Briefly, muffin slices of 10mm thickness and 35mm diameter were compressed using a 36mm diameter cylindrical aluminium probe at a crosshead speed of 1.7mm/sec. The samples were compressed in two sequential cycles up to 40% strain. Texture parameters of hardness [N], cohesiveness [dimensionless], springiness [dimensionless], chewiness [N], and gumminess [N] were calculated from TPA plot Force (N) vs extension (mm) using the Bluehill Lite software (v 2.4.1, USA).

All analytical measurements were performed at least in duplicate from independent samples and the mean values ± standard deviations are reported.

2.8.2 Sensory evaluation

Eighty volunteers (n=80, 68% females and 32% males) within 18- and 72-years old range, frequent or not frequent consumers of muffins were randomly selected at School of Bromatology, UNER, to participate of sensory sessions. The panellists were offered with a half portion of muffins (~15 g) coded with a three-digit random number in an individually partitioned booth. Consumers were first instructed to rinse their mouths with water and eat a cracker between samples to avoid carryover effects. Then they were guided to evaluate the muffins judging the overall acceptability using a 9-point structured hedonic scale and the specific attributes of colour, blueberry smell, hardness, chewiness and gumminess using a 3-point Just-About-Right (JAR) intensity scale [18]. The Penalty Analysis was used to determine if JAR classification for a specific attribute was associated with a drop in overall acceptability >1 in >25% of consumers responses.

2.9 Statistical Analysis

Statistical analysis of results was performed through ANOVA for a level of significance (α) of 0.05 followed by LSD Fisher *post hoc* test. Assumptions of homogeneity of variance was validated by Levene´s test and normality through Shapiro-Wilks´s Test.

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Pearson's product-moment correlations (r, $p<0.05$) were also evaluated. All statistical analysis, regressions, and correlations were performed using the Statgraphics Centurion XV software (V 2.15.06, 2007, USA).

3. Results and discussion

3.1. Physicochemical, functional and hydration properties of the fresh and powders based on blueberry by-product

The results corresponding to the physicochemical, functional, hydration properties and colour parameters of the fresh and dehydrated blueberry by-products are detailed in **Table 1**.

The yield of powders obtained from FBP was the same ($p>0.05$) for the three drying methods proposed, averaging ~23%. The initial water content and a_w of the fresh blueberry by-product were 759±5 g kg^{-1} and 0.921±0.006, respectively. After the different dehydration processes applied, these values were significantly reduced ($p<0.05$), ~72% of the water content and 95% of a_w in the powders obtained. It is important to highlight that a_w between 0.2-0.3 are suitable to provide the most stable conditions for long term storage, where enzymatic, oxidative, and browning reactions as well as microorganisms growth are reduced [19].

The chemical composition of powders based on blueberry by-product did not show differences between dehydration processes applied, and it was characterized by an average content of proteins 53±1 g kg^{-1}, lipids 5.0±0.1 g kg^{-1}, total dietary fibre 273±5 g kg^{-1}, soluble dietary fibre 74±5 g kg^{-1}, insoluble dietary fibre 195±5 g kg^{-1}, carbohydrates 620±10 g kg^{-1}, and ash 9.9±0.1 g kg^{-1}. Authors such as Patel [20] and Michalska *et al.* [21] have reported similar values in the blueberry fruit.

The sodium and potassium content showed an average value of 0.00062±0.00007 g kg^{-1} and 0.55±0.05 g kg^{-1} in the powders, respectively; denoting a decrease in values respect to the fresh fruit [1]. Lower mineral content represents an advantage from a nutritional viewpoint, since some pathologies such as renal insufficiency or hypertension has a restriction of sodium and potassium dietary intake.

As regards the ρ significant differences were observed among the blueberry by-products powders. The VD presented the lowest values, followed in decreasing order by the FD and CD. Consequently, contrary order was observed for ν, being VD the powder with highest value.

The OHC also showed significant differences according to the dehydration process applied, higher values were observed in the VD and FD respect to CD powder. Similar values have been reported in dietary fibre concentrates obtained from peach pulp and peel (2.1±0.2 g/g) and quince by-products (1.8±0.1 g/g) obtained by convective drying or freeze-drying [22,23], and in blueberry pomace applying convective drying (~1.9 g/g) [24]. Is noteworthy that OHC depends on the surface properties and the hydrophobic nature of the components of the plant matrix [25].

The WRC and WHC, which represents the proportion of water strongly or weakly associated to the food matrix, respectively, were significantly affected by the dehydration treatments. VD and FD powders presented higher ($p<0.05$) values of WRC and WHC respect to the CD powder. SC presented the same behaviour according to the dehydration processes applied.

These differences observed in the physical, functional and hydration properties could be associated to the physical modifications produced in fibres and lipids when CD is applied, reducing the surface contact of these compounds with the molecules of water and lipids, and producing a more compact matrix as can be inferred through the highest ρ and lowest ν **(Table 1)**, and micrographs showed in **Fig 1** [26]. These physicochemical properties are of interest to the food industry since they provide information of their potential application as food ingredients or additives, or as thickening agents; and also are associated with benefits in health [14].

DSC thermograms of powders obtained by CD, FD and VD are illustrated in **Fig. 2**. The midpoint of Tg was observed at -12±2°C, and no differences ($p>0.05$) were observed due to the drying method applied. According to Tao *et al.* [8] the Tg in blueberry pomace powder was in the range of the values obtained here in, and a dependency between Tg and water activity should be considered. The low Tg value can also be ascribed to the highest non-DF carbohydrates of the sample as was detailed previously [27]. Besides an endothermic peak was observed at 54.4±0.2°C that could be associated to the protein disaggregation [28].

The dehydration methods studied also significantly affected the colour of powders. For instance, CD presented the lowest lightness (L*) value; that means darkest colour being similar to that from the FBP (**Table 1**, **Fig. 1**).

As regards the Chr, it was observed that VD powder presented the highest values, followed in decreasing order by FD and CD; these changes were due to an increase in both, a* (redness) and b* (yellowness) coordinates. As a reference, in **Fig. 1** micrographs images of the powders obtained by different drying methods are shown. The purple and dark red colour indicate the presence of anthocyanin pigments; being a natural option to replace artificial food dyes [29].

3.2. Study of AC and their in vitro bioaccessibility in the blueberry by-product powders

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One of the main purposes of food processing is to preserve the organoleptic and nutritional characteristics of foods; however, some nutrients are labile and could present significant losses during manufacturing processes. Therefore, the bioactive compounds evaluation of nutritional interest during stabilization processes are important when designing novel functional ingredients.

The results corresponding to the content of TMA, TPC and AC of fresh and powders based on blueberry by-products, as well as its *in vitro* bioaccessibility are detailed in **Table 2**. Considering drying processes, the FD samples presented the highest TMA content ($p<0.05$) value among dried powders. While, non-significantly differences ($p>0.05$), were observed among FBP, CD and VD, possibly due to the high variability in TMA value for these samples, showing a lower tendency for the CD sample. The values obtained agreed with data previously reported. For instance, a range of TMA ~1.56-30 g total anthocyanins kg^{-1}, d.b. was reported by Li *et al.* [30] depending on blueberry cultivars; while Rashidinejad (2020) showed average value of 6.83±0.55 g cyn-3-glu kg^{-1}, d.b. Lower values have been reported in pomace blueberry (1.52 mg cyn-3-glu kg^{-1}, d.b.), possibly due to the losses during the juice processing. It is well known that anthocyanins are sensitive to heat treatments, among several factors, and temperature above 70°C have showed significant losses depending on the time of the process [31]. In the present assay, the dehydration applied allowed to retain most of the anthocyanins compounds.

The content of TPC in the FBP presented an average value of 1.85±0.05 g kg^{-1} (d.b.). Authors such as Johnson *et al.* [2] and Li *et al.* [30] have reported that blueberries are characterized by a higher TPC than other berries, presenting a range between 6.4-25 g kg^{-1}, d.b., according to the cultivar. In the case of FBP the TPC content was among 4- and 14-folds lower than values reported in bibliography suggesting the retention of soluble bioactive compounds in the juice during the industrial processing. Nevertheless, it is important to highlight that a considerable amount of TPC remain associated to the blueberry by-product.

On the other hand, the drying methods applied to stabilise the FBP showed that FD and VD processes allowed higher ($p<0.05$) TPC retention compared to the CD process; possibly because high temperature during processing could increase the rate kinetics degradation reactions, which begins with a hydroxylation of monophenols and ends with the formation of *o*-quinones, reducing the TPC in the powder [32].

After *in vitro* gastrointestinal digestion conditions, a higher ($p<0.05$) release of TPC was present in the soluble fractions in all blueberry powders (~72±13%), which means that a greater proportion of these compounds would be bioaccessible to be absorbed through the small intestinal epithelium and, therefore finally be potentially bioavailable. In concordance, lower concentration of non-bioaccessible TPC were presented in the

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colon; where the lowest ($p<0.05$) value was observed in FD powder (**Table 2**). Possibly, some polyphenols are linked to the fibre and are not solubilized by the solvents in the chemical extraction and/or fluids and/or enzymes during the gastrointestinal digestion, and therefore could be metabolized by the gut microbiota, producing metabolites with potential health effects [11]. In addition, after simulated digestion, higher solubilisation of TPC could be registered in the samples summited to drying, evidencing that the drying process induced the bioactive realising during digestion. Similar results have been reported in a cooked enriched with fibre from blueberry pomace, obtaining a good release of hydrolysable TPC (>89%) [33].

The dehydration processes applied to stabilise the FBP did not affect ($p>0.05$) the content of AC in the FD and VD powders. Nevertheless, following the same tendency as in TPC, a significant reduction (~28%) was observed in the powder obtained by CD respect to FBP. The FD and VD powders did not show a significant ($p>0.05$) loss of AC respect to the FBP, indicating a good retention of bioactive compounds after the stabilization processes.
Following with the *in vitro* gastrointestinal digestion, a higher release of AC compounds in the soluble fraction was observed in all powders (39-45% range) respect to the FBP. Even though, higher proportion of these compounds was presented in the insoluble fraction (48-57% range) respect to the soluble fraction (**Table 2**). There are several studies that supports the presence of AC in large intestine might play a role in maintaining the redox balance against different oxidants, preventing diseases of the intestinal tract [34]. Indeed, recent studied reveal that AC of blueberry compounds could exert a prebiotic action associated to health benefits [35].

3.3. Effect of novel ingredients based on blueberry by-products on muffin making process: physical, colour, textural and sensory evaluation

The water content, colour and texture characteristics of muffins containing the different blueberry by-product powders, and the control system are detailed in **Table 3**.
The water content of muffins with the addition of different levels (10-20-30%) of blueberry powders showed significant differences among the systems. In general, the muffins with VD and FD ingredients had a lower water content vs CD and control system; possibly due to the good hydration properties of VD and FD powders (**Table 1**). In concordance, a negative correlation between water content and WHC was observed (r:-0.81; p-value 0.0087).

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As regards the muffins colour, the L* was significantly reduced with the addition of the powders in all formulations; and in general, no differences ($p>0.05$) due to drying process applied were observed.

The same tendency was observed in Chr, showing a reduction of colour intensity with the increase of the powders addition; these changes were ascribed to variations in both chromatic coordinates, a* and b*. The colour of the muffins changed from a yellowness (control system) to a dark violet colour when the CD, FD and VD powders were added (**Table 3**). These powders could present itself as a natural ingredient to replace the artificial colours used in the food industry for good bakery, and as option for consumers with preferences towards clean labels and health foods [29].

The texture profile, in general, did not present differences among systems containing the same level addition of CD, FD, and VD blueberry powder. Nevertheless, the level of powder addition affected the muffins texture. For instance, hardness of muffins was reduced ~29% at the lowest levels (10-20%) compared to the control system. Meanwhile, the hardness of systems with 30% of powders presented similar values ($p>0.05$) to the traditional muffin.

The chewiness and the gumminess presented a similar trend. Up to 10% of powder addition, a reduction of ~26% was registered; and in general, 20-30% did not show significant differences ($p>0.05$) compared to the control system.

In concordance to the above results, a significant and positive correlation was observed between chewiness and hardness (r:0.98; p-value 0.01), and chewiness and gumminess (r:1.00; p-value 0.01).

Springiness and cohesiveness did not present changes among muffins added with powders at different levels respect to the control system, presenting an average values of 0.84±0.01 and 0.63±0.03, respectively.

The main differences registered between muffins formulations, were mainly due to the percentage of the wheat flour replacement, but not because due to the drying method applied. Therefore, batches of muffins with CD addition at the three levels were made for the subsequent sensory analysis.

The JAR scales are commonly used in new product development as a consumer research technique; and besides are useful to identify whether attributes present in the food are well optimized or if need to increase or decrease the intensity level according to consumers opinions [18]. JAR scales and Penalty Analysis of muffins formulated with different levels of the blueberry by-product CD powder are shown in **Fig. 3**.

The intensity scale of colour showed that muffins were considered as “JAR” by ~34% of consumers in all systems; and the “Too High” intensity responses increased from ~48%

in F1 to ~62% in F2 and F3, denoting that the changes in muffins colour were perceived by consumers due to the different levels of powder addition.

The blueberry smell was perceived as “Too Low” by ~68% of consumers responses in all formulations studied. In concordance, a significant drop in overall acceptability was observed in F1 and F2, showing that this attribute should be improved in these formulations. The low perception of blueberry smell could be possibly explain because aromatic compounds are lost during industrial juice processing and subsequent stabilization, and also during baking process. Besides, the consumption in Argentina is estimated in approximately 45-50 g/year *per capita*, being lower compared to other countries, having an impact in the familiarity with this kind of berries [6]. Authors as Perez et al.[33] have reported that familiarity with the product could have an effect in the overall acceptability of baked goods.

Chewiness was perceived as “JAR” by most of the consumers (43%) for F1; however as long as the ingredient was increased in the formulation the intensity perception moved towards “Too High” (~41%, F2 and F3). This attribute received a punctuation of 7±2 being classified as “like moderately”. Nevertheless, a significant drop in overall acceptability was observed and should be considered for its modification. These results agreed with the textural measurements discussed previously (**Table 3**).

Sensorial intensity of gumminess received a classification of “JAR” by most of the consumers in F1 and F2 (~41%); meanwhile, F3 was perceived mainly as “Too Low” with a significant drop in overall acceptability.

Hardness of muffins was classified as “JAR” by 55% of consumers in all systems, and a drop in overall acceptability was observed in F3 denoting that a decrease in this attribute should be considered.

The overall acceptability of muffins with different levels of blueberry by-products powders obtained by convective drying did not show significant differences, receiving an average punctuation of 6±2 in the 9-point hedonic scale with a classification of “like slightly”. It is important to highlight that novels products that obtain high scores in Affective Test are more likely to be successful in the market [18].

The present study showed that replacement of wheat flour by the lowest levels (10-20%) of the blueberry powder in a muffin formulation significantly improved the colour, texture, and sensorial characteristics, resulting in a colourfully and softer product with good overall acceptability in consumers. Meanwhile, higher levels of the ingredient presented a similar profile texture to the traditional muffin. The replacement of 10, 20 and 30% by the novel ingredients in a muffin recipe would provide 1.7, 3.4 and 5.1 g of fibre, and 10, 20 and 30 mg of gallic acid per serving size (~40g). Therefore, its consumption would

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contribute with 6.8-20.3% and 4.5-13.4% of the daily dietary fibre intake in women and men of 18- to 50- years-old, respectively [36].

4. Conclusion

Pomace, blueberry by-product from juice extraction process, was dried by different methods obtaining powders with technological and functional characteristics. In general, AC remaining in the pomace could be stabilized without losses by the drying processes proposed. Convective-drying slightly reduced the hydration and functional properties when compared to dehydration processes with vacuum and freeze-dried. The powders presented higher fibre content and, good retention and bioaccessibility of antioxidant compounds.

The addition of blueberry powders to formulate muffins, in general, improved the physical, textural and colour characteristics, with good sensorial acceptability by the consumers. This could represent an effective strategy to increase the intake of fibre and phytochemicals in consumer´s diet.

Blueberry by-products can be transformed in functional ingredients giving added value to an industrial waste recirculating nutrients and bioactive compounds to the food industry avoiding or reducing their lost.

Acknowledgements

This study was funded by the Ministry of Education "Universities adding value 2017", UNER (PID-9112), National Agency of Scientific and Technical Research (PICT-2018-01019), CONICET and Starberry S.A.

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FIGURES AND TABLES CAPTIONS

Figure 1. Images and micrographs of the (A) fresh blueberry by-product, and the powders obtained by (B) convective-drying, (C) vacuum-drying, and (D) freeze-drying methods.

Figure 2. Thermograms of blueberry by-products powders obtained by convective-drying (CD), freeze-drying (FD), and vacuum-drying (VD) methods.

Figure 3. Consumer responses obtained in the Just about Right scale and Penalty Analysis of muffins formulated with different levels of the blueberry powder convective dried.

Table 1. Physicochemical, functional, hydration and colour properties of fresh and blueberry by-products powders.

Table 2. Content of total monomeric anthocyanins, total phenolic compounds, and antioxidant capacity in fresh and dried blueberry by-products powders. Percentage of the total antioxidant compounds and capacity solubilised or not during *in vitro* bioaccessibility assay.

Table 3. Physicochemical, colour and textural characteristics of muffins containing blueberry powders dried by different methods.

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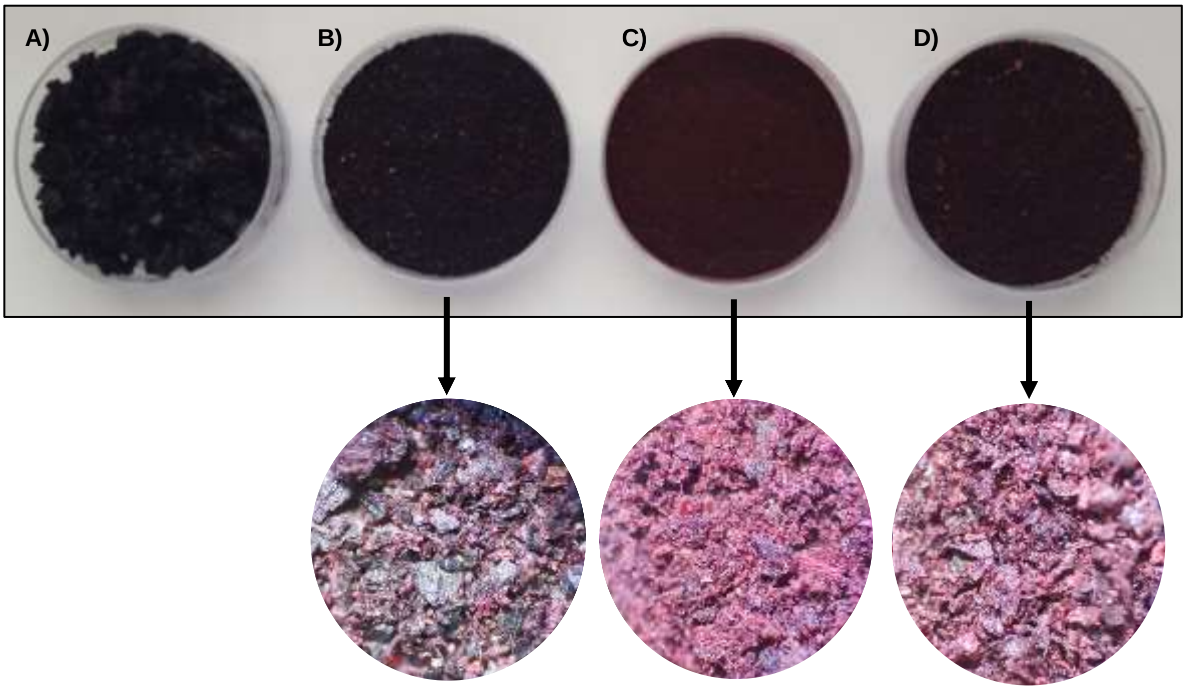

Figure 1. Images and micrographs of the (A) fresh blueberry by-product, and the powders obtained by (B) convective-drying, (C) vacuum-drying, and (D) freeze-drying methods.

Micrographs of powders with magnifications up to 5000× were obtained using a stereo microscope (SMZ 745T, Nikon, USA).

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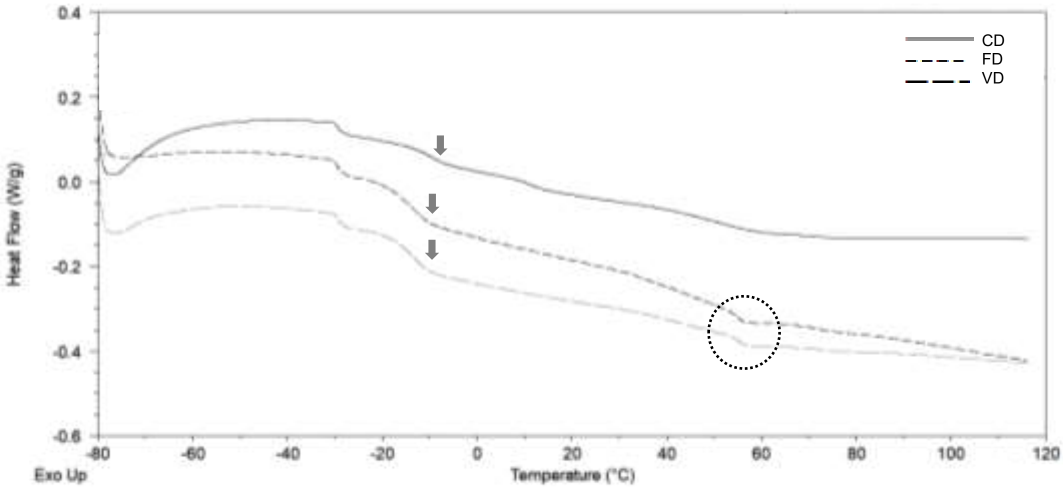

Figure 2. Thermograms of blueberry by-products powders obtained by convective-drying (CD), freeze-drying (FD), and vacuum-drying (VD) methods.

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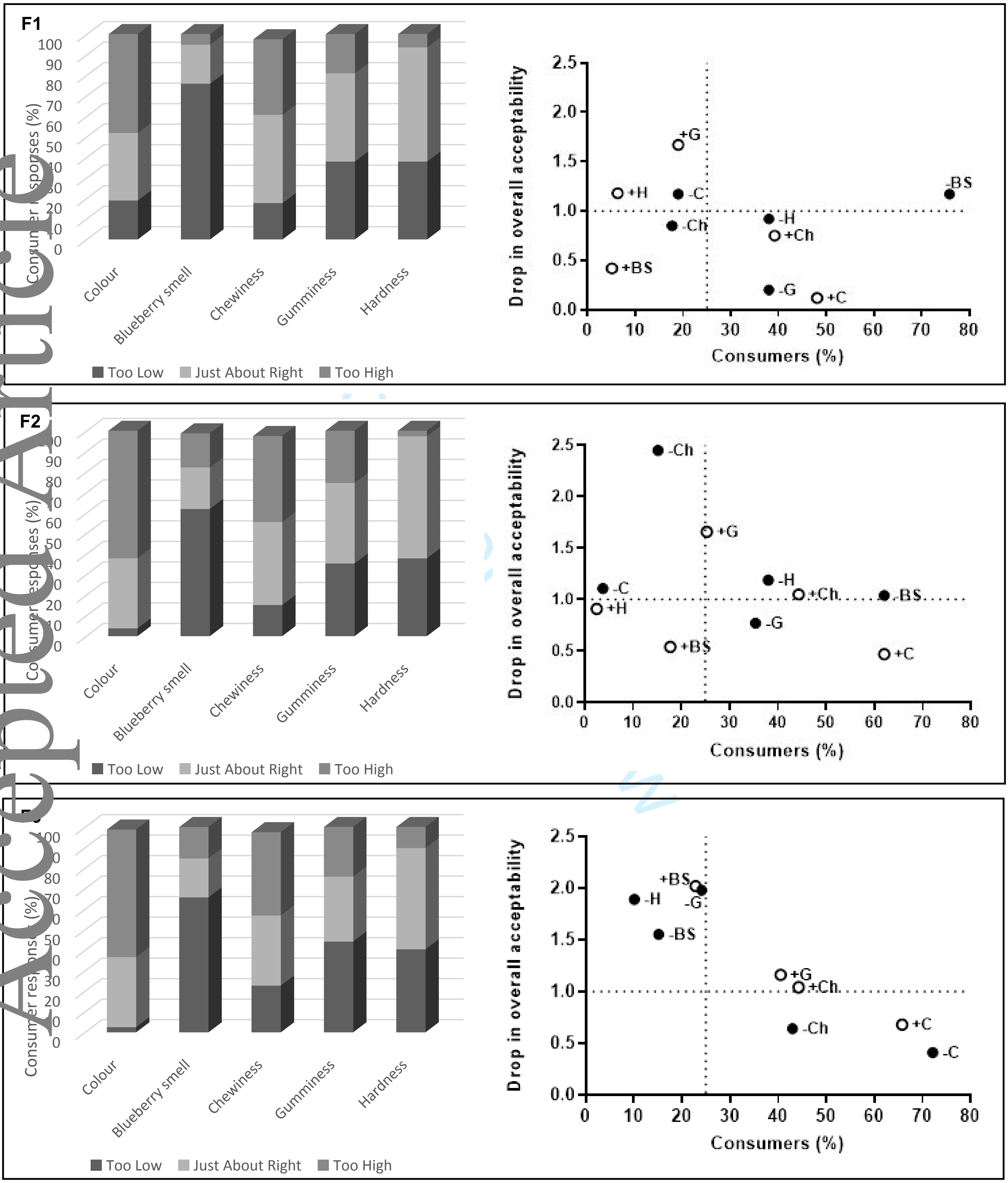
Figure 3. Consumer responses obtained in the Just about Right scale and Penalty Analysis of muffins formulated with different levels of the blueberry powder convective dried.



Muffins added with 10% (F1), 20% (F2) and 30% (F3) of the blueberry powder convective dried.
C: colour; BS: blueberry smell; H: hardness; Ch: chewiness; G: gumminess.
(+) Too High; (-) Too Low.

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Table 1. Physicochemical, functional, hydration and colour properties of fresh and blueberry by-products powder.

	Systems			
	FBP	CD	VD	FD
Physicochemical and Functional Properties				
Water content (g kg^{-1})	759±5 ^{a}	39±} ^{b}	31.2±0.9 ^{c}	39±1 ^{b}
a_w	0.921±0.006 ^{a}	0.28±0.03 ^{b}	0.22±0.02 ^{b}	0.24±0.01 ^{b}
ρ (g/cm^3)	NA	0.58±0.02 ^{a}	0.35±0.01 ^{b}	0.43±0.01 ^{c}
ν (cm^3/g)	NA	1.73±0.05 ^{a}	2.83±0.01 ^{b}	2.34±0.07 ^{c}
OHC (g/g)	NA	1.93±0.03 ^{a}	2.88±0.08 ^{b}	2.31±0.02 ^{c}
Hydration Properties				
SC (mL/g)	NA	3.3±0.1 ^{a}	4.2±0.2 ^{b}	3.7±0.1 ^{c}
WHC (g/g)	NA	8.4±0.6 ^{a}	9.6±0.4 ^{b}	9.4±0.5 ^{b}
WRC (g/g)	NA	9.7±0.2 ^{a}	11.4±0.4 ^{b}	10.4±0.3 ^{c}
Colour parameters				
L*	5.1±0.5 ^{a}	5.4±0.1 ^{a}	7.0±0.05 ^{b}	6.9±0.05 ^{b}
a*	2.2±0.1 ^{a}	4.6±0.1 ^{b}	9.1±0.3 ^{c}	7.1±0.1 ^{d}
b*	0.49±0.04 ^{a}	0.96±0.02 ^{b}	2.8±0.2 ^{c}	2.5±0.1 ^{d}
Chr	2.28±0.09 ^{a}	4.7±0.1 ^{b}	9.6±0.3 ^{c}	7.5±0.2 ^{d}

FBP: Fresh blueberry by-products. CD: Blueberry by-products powders obtained by convective drying. VD: Blueberry by-products powders obtained by vacuum drying. FD: Blueberry by-products powders obtained by freeze drying

a_w: water activity. ρ: bulk density. ν: specific volume. OHC: oil holding capacity. SC: swelling capacity. WHC: water holding capacity. WHR: water holding retention. L*: lightness. Chr: intensity of colour. NA: not applied.

Different letters denote significant difference between systems at p-value < 0.05.

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Table 2. Content of total monomeric anthocyanins, total phenolic compounds, and antioxidant capacity in fresh and dried blueberry by-products powders. Percentage of the total antioxidant compounds and capacity solubilised or not during *in vitro* bioaccessibility assay.

Systems	TMA [1]	TPC [2]	AC [3]	*In Vitro* Bioaccessibility			
				TPC		AC	
				SF (%)	IF %	SF (%)	IF (%)
FBP	7.3 ± 0.8 [a]	1.85 ± 0.05 [a]	29 ± 4 [a]	60 ± 4 [a]	38 ± 5 [a]	19 ± 3 [a]	90 ± 10 [a]
CD	6.82 ± 0.02 [a]	1.62 ± 0.01 [b]	21 ± 3 [b]	78 ± 2 [b]	24 ± 3 [b]	42 ± 5 [b]	48 ± 6 [b]
VD	7.2 ± 0.2 [a]	1.8 ± 0.01 [a]	28 ± 2 [a]	78 ± 10 [b]	31 ± 4 [c]	39 ± 1 [b]	55 ± 8 [b]
FD	8.12 ± 0.06 [b]	2.01 ± 0.06 [c]	28 ± 2 [a]	85 ± 9 [b]	16 ± 5 [d]	45 ± 5 [b]	57 ± 5 [b]

[1] TMA: total monomeric anthocyanins expressed as g cyn-3-glu kg^{-1} (d.b). [2] TPC: total phenolic compounds expressed as g of gallic acid kg^{-1} (d.b.). [3] AC: antioxidant capacity expressed as meq Trolox kg^{-1} (d.b).
SF: Soluble Fraction (%). IF: Insoluble Fraction (%). Based on original sample before digestion.
* Different letters denote significant difference between systems at p-value < 0.05.
FBP: Fresh blueberry by-products
CD: Blueberry by-products powders obtained by convective drying
VD: Blueberry by-products powders obtained by vacuum drying
FD: Blueberry by-products powders obtained by freeze drying

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Table 3. Physicochemical, colour and textural characteristics of muffins containing blueberry powders dried by different methods.

Systems		Water content (%)	Colour parameters		Textural parameters		
			Lightness	Chroma	Hardness (N)	Chewiness (N)	Gumminess (N)
Control		36.0±0.4 ^{a}	78±1 ^{a}	24.1±0.4 ^{a}	7.8±0.5 ^{a}	3.8±0.3 ^{bcd}	4.5±0.4 ^{abc}
M-CD	10%	37.8±0.1 ^{d}	18.5±0.3 ^{b}	3.6±0.1 ^{b}	5.1±0.9 ^{b}	2.9±0.4 ^{a}	3.3±0.3 ^{e}
	20%	37.5±0.1 ^{d}	12.7±0.3 ^{d}	3.1±0.2 ^{cd}	5.7±0.5 ^{b}	3.3±0.5 ^{ab}	4.2±0.4 ^{abc}
	30%	37.1±0.2 ^{d}	10.7±0.6 ^{e}	2.8±0.1 ^{de}	7.9±0.5 ^{a}	4.2±0.4 ^{cd}	4.9±0.5 ^{c}
M-VD	10%	34.9±0.9 ^{b}	16.1±0.9 ^{c}	3.4±0.2 ^{bc}	5.5±0.4 ^{b}	2.8±0.3 ^{a}	3.4±0.4 ^{ed}
	20%	34.9±0.9 ^{b}	12.7±0.5 ^{d}	2.5±0.5 ^{ef}	5.8±0.8 ^{b}	3.3±0.6 ^{ab}	4.0±0.4 ^{ad}
	30%	35.4±0.6 ^{ab}	11.7±0.5 ^{de}	1.8±0.1 ^{g}	7.5±0.8 ^{a}	4.4±0.2 ^{d}	4.7±0.3 ^{bc}
M-FD	10%	34.9±0.7 ^{b}	17.5±0.2 ^{bc}	3.7±0.4 ^{b}	5.2±0.4 ^{b}	2.7±0.3 ^{a}	3.3±0.3 ^{e}
	20%	35.7±0.1 ^{ab}	12.7±0.6 ^{d}	2.6±0.1 ^{de}	5.9±0.6 ^{b}	3.6±0.3 ^{bc}	4.2±0.4 ^{ab}
	30%	35.5±0.4 ^{ab}	10.3±0.8 ^{e}	2.0±0.1 ^{fg}	8.0±0.8 ^{a}	4.2±0.4 ^{cd}	4.8±0.5 ^{bc}

M-CD: muffin containing blueberry powder convective dried. M-VD: muffin containing blueberry powder vacuum dried. M-FD: muffin containing blueberry powder freeze dried.
Different letters denote significant difference between systems at p-value < 0.05.

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