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ORIGINAL ARTICLE

Colorant and antioxidant properties of freeze-dried extracts from wild berries: use of ultrasound-assisted extraction method and drivers of liking of colored yogurts

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Abstract This work aimed at developing powders rich in antioxidants and pigments from two wild berries: maqui (*Aristotelia chilensis*) and murra (*Rubus ulmifolius*). Fruits were subjected to successive ultrasound-assisted extractions (UAE) and then freeze-dried. Physical properties, anthocyanin stability of powders, and their performance as natural colorants in yogurts were evaluated. The optimum extraction methods were: UAE for 10 min in murra, and without UAE (control) in maqui, with juice extraction yields ranging between 80 and 82%. Maqui powder exhibited ≈ 2.8 times more polyphenol and anthocyanin content than murra. However, murra powder showed better stability characteristics as powder colorant since it exhibited greater protection of anthocyanins by means of copigmentation phenomena. Regarding consumer's perception of colored yogurt, samples with 4% and 8% maqui powder could be considered as future prototypes to be launched into the market. The obtained powders may be used in different industrial food applications.

Keywords Maqui · Murra · Freeze-dried powders · Antioxidants · Natural colorants · Consumer´s perception

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Introduction

Berries have been one of the most dynamic fruit groups in the global food trade for the past decade. Scientific evidence shows that regular consumption of berries would reduce the risk of developing several chronic diseases, including cardiovascular, neurodegenerative, and certain forms of cancer (Nile et al. 2014; Yang and Kortesniemi 2015). Some native or wild berries have received special attention in the last years because of their high antioxidant potential. It has been reported that wild berries growing in cold climates without fertilizers and pesticides contain a higher polyphenol content, compared to those which grow under milder conditions (Jimenez-García et al. 2013). Particularly, certain dark-colored species from highland areas of southern Chile and Argentina, such as maqui (*Aristotelia chilensis)* and wild blackberry *(Rubus ulmifolius)* are not widely known. In the case of maqui, it is used in various dried products produced in Chile and some European countries like Germany, in the form of powders or capsules. However, these products are obtained from direct drying (convective or freeze-drying) of the whole fruit and subsequent grinding, being generally inadequate to be used as ingredients in the food industry.

The application of green-friendly extraction methods and subsequent drying has been used in order to obtain novel antioxidant concentrates or natural colorants from berries, in the form of powders of good physical properties (Archaina et al. 2018; Gagneten et al. 2019). As thermal extraction takes long times and may deteriorate polyphenols, especially anthocyanin compounds (Camel 2000; Lapornik et al. 2005), the colorant power and the antioxidant capacity of products may decrease. Thus, new technologies (i.e. ultrasound, microwaves, and pulsed electric fields) have been explored to reduce the extraction time and

energy consumption, avoiding at the same time the use of high temperatures and unsafe solvents (Chemat et al. 2011). Ultrasound-assisted extraction (UAE) uses acoustic cavitation, and when applied at low frequencies (20–100 kHz), it produces large bubbles that violently implode increasing cellular rupture, improving solvent penetration, and therefore reaching higher extraction yields (Dzah et al. 2020). It has been applied for polyphenol and anthocyanin extraction from several fruits and fruit wastes: *Sambucus nigra* and *Rubus idaeus* (Gagneten et al. 2019), *Rubia sylvatica* (Chen et al. 2020), *Ribes nigrum* (Archaina et al. 2018; Gagneten et al., 2019), *Aronia melanocarpa* (Galvan D'Alessandro et al. 2012), and *Rubus fructicosus* residues (Zafra-Rojas et al. 2016).

Among the drying methods available, freeze-drying appears to be an ideal process for better preservation of the pigments and the bioactive substances due to the low temperature. It has been used for the generation of functional ingredients from juices or extracts of different berries (Pavan et al. 2012; Yamashita et al. 2017; Mar et al. 2020), and to a lesser extent for the development of natural colorants (Duangmal et al. 2008; Estupiñan et al. 2011; Różyło et al. 2019).

In the field of colorants, demand for new sources of natural pigments has increased because of safety issues, together with the worldwide tendency towards the consumption of natural products (Bakowska-Barczak 2005). Anthocyanin pigments, beyond their antioxidant properties, are ideal for coloring acid foodstuff due to their high stability at low pHs (i.e. yogurts or jellies). However, applying fruit pigments as colorants, presents the challenge of color stability, since anthocyanin structure could be modified when the colorant is added at low concentration in foods. Changes in hue and color intensity might be appreciated as a result of pigment transformation or degradation. Anthocyanins tend toward the formation of polymeric compounds, and a potential mechanism for polymerization involves condensation reactions with other phenolic compounds to form colored polymeric pigments, also called anthocyanin-tannin polymers. On the other hand, it must be considered that anthocyanin stability may be improved by intermolecular copigmentation among anthocyanins and other phenolic compounds, and self-association between anthocyanin molecules. These interactions produce a hyperchromic effect and a bathochromic shift in the absorption spectra (UV–vis region). Understanding the process of color stabilization by copigmentation is important when using natural colorants from berries in order to enhance the color in food products, in direct relation with consumers´ perception (Trouillas et al. 2016).

Consumer-based approaches have become very popular in the last decade, in line with the recognition of consumers' ability to reliably characterize products (Ares and Varela 2017). It is necessary to have information about which sensory characteristics are expected to be found in the product, i.e. which sensory attributes drive consumer liking (Ares et al. 2010). In most foods, external quality (appearance and color) is the main determinant of consumers' choices at the point of purchase, whereas their decisions to repeat the purchase are more strongly influenced by the internal product quality (Barman et al. 2015). Therefore, when trying to design novel products that meet consumer sensory expectations, it is advisable to study their drivers of liking.

The aims of this work were: (1) to obtain freeze-dried powders from maqui and murra extracts optimized by ultrasound-assisted extraction process, (2) to study the physicochemical and antioxidant properties of the obtained powders, and (3) to evaluate the powders' performance as natural colorants in yogurt through the consumer´s perception.

Materials and methods

Fruits

Fruits were manually harvested from argentine Patagonia forests: wild blackberry "murra" (*Rubus ulmifolius*) and "maqui" (*Aristotelia chilensis*). Fully mature fruits were selected, fractionated in bags, and frozen applying individual quick freezing (IQF) in an air blast tunnel at −48 °C and air speed 1.5 ms^{-1}. Then, samples were stored at −18 °C until use.

Preparation of fruit extracts by ultrasound-assisted extraction method

Fruits (50 g) were thawed at 40 °C for 10 min, blanched with steam at T > 98 °C for 1 min, and blended for 1 min with water 2:1 ratio (water: fruit). After 10 min cooling over an ice bath, the product was divided into three samples: (1) control, (2) and (3) were subjected to UAE for 5 min (U5) and 10 min (U10), respectively, using a UP100H ultrasonic processor (Teltow, Germany) at a frequency of 30 kHz and 100% amplitude. Samples were weighed and centrifuged (9000 rpm, 4 °C) for 20 min and the supernatants (extracts) were separated. The procedure was repeated twice more over the precipitates, completing three successive cycles. The selection of the best extraction process was statistically determined considering the extract yield (YE), the polyphenols yield (YP), and the bioactive compounds content considering in all cases the total extract volume (supernatants of the consecutive cycles):

$$YE = \frac{\text{g extract}}{FF + gH_2O} \times 100 \quad (1)$$

$$YP = \frac{\text{mg gallic acid in extract}}{\text{mg gallic acid in FF}} \times 100 \quad (2)$$

where FF is the mass of fresh fruit = 50 g.

Freeze-drying process

Optimum extracts were freeze-dried using maltodextrin DE-12 (Givaudan, Buenos Aires, Argentina) as carrier matrix (20%, w/w). Extracts were frozen at −80 °C for 24 h in an ultrafreezer Presvac FH-80 (Presvac SRL, Buenos Aires, Argentina) and dehydrated in an Alpha 1-4LD/2-4 LD-2 freeze-drier (Martin Christ, Gefriertrocknungsanlagen GmbH, Osterode, Germany) at the following conditions: 48 h, chamber temperature −84 °C, chamber pressure 0.04 mbar. Dried samples were then grinded and the powders were hermetically stored.

Functional and physicochemical properties

Determinations were done on the following samples: fruits, aqueous extracts, freeze-drier feed solution, and the reconstituted powders considering a ratio 1:6 (powder:water, w/w).

Functional properties

For functional properties, a spectrophotometer T60 UV–visible (PG-instruments, Leicestershire, United Kingdom) was used according to guidelines given by Gagneten et al. (2019).

Alcoholic extracts preparation Two different extracts were obtained: 1) ethanolic extracts using a solution of 95% ethanol:1.5 N HCl (85:15 v/v) for monomeric anthocyanin content and polymeric color determinations, and 2) methanolic extracts using absolute methanol as a solvent for antioxidant capacity and total phenolic content determinations. For both extraction types, 1 g of sample was homogenized using a magnetic stirrer in 5 ml of extraction solvent for 5 min and then filtered under vacuum. The same procedure was applied to the pellets to reach a final volume of 10 ml extract. In the case of the fruits, the same procedure was followed, with a final ratio of 2 g sample (maqui) in 100 ml of solvent or 4 g sample (murra) in 100 ml.

Monomeric anthocyanin content (ACY), polymeric color (PC), and Vis-Absorption spectra The pH differential method for ACY was used. Ethanolic extracts were diluted (1:4 v/v) in two buffer solutions, one of pH 1.0 and the other one of pH 4.5. The absorbance at 510 and 700 nm was then measured and results were expressed as cyanidin-3-glucoside (molar absorptivity ε = 26,900 L mol^{-1} cm^{-1}, molecular weight MW = 449.2 g/mol) per 100 g of sample. ACY content was calculated using Eq. (3):

$$ACY = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1} \quad (3)$$

where,

$$A = (A_{510} - A_{700})_{pH_{1,0}} - (A_{510} - A_{700})_{pH_{4,5}}$$

DF = dilution factor,

l = optical path length,

Polymeric color determination is based on the treatment of an anthocyanin colored sample with sodium bisulfite (bleaching agent). For each fruit, two samples were prepared: a bleached sample that consisted in 2.8 ml of ethanolic extract with the addition of 0.2 ml of a 20% metabisulfite solution, and a control sample adding 0.2 ml of distilled water to 2.8 ml of ethanolic extract. Both samples were kept in darkness for 15 min and absorbance determinations at 420, 510, and 700 nm were taken. Polymeric color and color density were calculated using Eq. (4) with bleached samples and Eq. (5) with control samples, respectively. In order to comply with the linear range of absorbance in the spectrophotometer, samples were diluted with distilled water when necessary. Results were informed as PC percentage (%), according to Eq. (6).

$$PC = [(A_{420nm} - A_{700nm}) + (A_{510nm} - A_{700nm})] \times DF \quad (4)$$

$$CD = [(A_{420nm} - A_{700nm}) + (A_{510nm} - A_{700nm})] \times DF \quad (5)$$

$$PC\ (\%) = \left(\frac{PC}{CD}\right) \times 100 \quad (6)$$

Absorption spectra of the reconstituted powders at pH 1, 4.5, and 10, were obtained in the visible region in the range of 400–800 nm.

Total phenolic content (TPC) and Antioxidant capacity (AC) Total phenolic content was determined using the Folin–Ciocalteu reagent. 100 µl of methanolic extract was added to 900 µl of distilled water, 100 µl of reagent, and 600 µl of a 20% sodium carbonate in 0.1 N NaOH solution. Then, it was incubated at 40 °C for 25 min, centrifuged at 10,000 rpm for 5 min, and finally, the supernatant absorbance was measured at 765 nm. Antioxidant capacity was measured by using the bleaching method of 2, 2-azinobis-[3-ethylbenzothiazoline-6-sulfonic acid] radical cation ($ABTS^{+\cdot}$). For the activation of the

radical cation, a 7mN ABTS solution was prepared in distilled water and incubated for 16 h in the dark at room temperature with a 2.5 mM potassium persulfate solution. To achieve a final absorbance of 1.00 ± 0.01 at 734 nm, phosphate buffer was used as diluent. Then, 0.1 ml of methanolic extract with 1.9 ml $ABTS^{+}$ solution was incubated at 25 °C for 30 min in dark, and finally, the absorbance was measured. For both determinations, gallic acid was used as standard for the calibration curve, and results were expressed as mg gallic acid equivalent (GAE) per 100 g of sample.

Attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR) Infrared spectra of freeze-dried fruits and freeze-dried powders were recorded according to the procedure of Roa et al. (2014), using an FT-IR (Spectrum 400, Perkin Elmer Inc., Shelton CT, USA) with a deuterated triglycine sulfate (DTGS) detector and an attenuated total reflectance accessory (ATR, PIKE Technologies, Inc., Madison WI, USA). Spectra were obtained in the range of 4000–400 cm^{-1}. Each sample was scanned 32 times and the reported spectrum corresponded to the scan average. Shifts in functional groups were calculated according to the equation Δ_{υ}^{-}=(X–Y), where Δ_{υ}^{-} is the difference of each wavenumber (cm^{-1}), X is the maximum carbonyl wavenumber corresponding to the fruit (cm^{-1}), and Y is the maximum carbonyl wavenumber in the freeze-dried powder (cm^{-1}).

Physicochemical properties

For fresh fruit analysis, AOAC methods (AOAC 2006) were used for the measurement of soluble solids (932.12), acidity (942.15b), pH (981.12), and water content (934.06).

For freeze-dried powders, water content, water activity, glass transition temperature (T_g), and superficial color properties, as well as sample hygroscopicity and water sorption isotherms, were assessed according to procedures described by Franceschinis et al. (2014).

Fresh fruits Water content of fruits was determined gravimetrically in a vacuum oven at 60 ± 1 °C and 0.05 mbar with desiccant, while soluble solids were measured at 25 °C with an ABBE digital refractometer DR A1 model (Atago, Tokyo, Japan) calibrated with distilled water. pH was determined by potentiometry with an EA 940 (ORION, Beverly, USA) pH-meter. Acidity was evaluated by titration with NaOH 0.1 N; as fruits are strongly colored, the final point was determined using the glass electrode method at pH = 8.1.

Freeze-dried powders Water content was determined volumetrically performing a titration at 20 ± 1 °C with a Karl Fisher titrator DL 31 from Mettler-Toledo, using Hydranal Titrant Composite 5 (Riedel-de Haën, Germany) as reagent. Pure methanol was used as solvent, and approximately 20 mg of samples were analyzed.

Water activity was measured using an Aqualab Series 3TE (Decagon Devices, Pullman, Washington, USA) electronic dew-point water activity meter.

For water sorption isotherms and hygroscopicity values, 0.5 g of powder were placed in glass vials and immediately put under incubation at 20 °C, in desiccators with constant relative humidity (RH) provided by saturated salt solutions of LiCl (11%), CH_3COONa (22%), $MgCl_2$ (33%), K_2CO_3 (43%), $Mg(NO_3)_2$ (52%) and NaCl (75%). Water content was determined with Karl Fisher titration after equilibrium, which was reached when the difference in weight was less than 0.0005 g. Hygroscopicity (Hi) value corresponded to the water content at the equilibrium condition under 75% RH.

T_g (onset values) of fruit powders were analyzed by differential scanning calorimetry (DSC), using a DSC 822e Mettler Toledo calorimeter (Schwerezenbach, Switzerland). Instrument calibration was carried out with indium (156.6 °C), lead (327.5 °C), and zinc (419.6 °C). Measurements were performed with a heating rate of 10 °C/min, between -50 and 100 °C. Hermetically sealed medium pressure pans (40 µl capacity) were used, and an empty pan was used as reference. Thermograms analyses were performed with Mettler Star^{e} program.

Finally, superficial color was measured using a handheld colorimeter (Minolta Co, model CR-400, Japan). Color functions were calculated in the CIELAB uniform color space, for illuminant C at 2° standard observer. Values of L* (luminosity/darkness), a* (redness/greenness), and b* (yellowness/blueness) were obtained. Powders were analyzed using glass vials containing 1 cm height of powder, and initial fruits were directly assessed. Measurements were standardized using a white cylindrical cup to cover the vial.

Sensory study

Product preparation

In order to evaluate the performance of berry powders as colorants, color acceptability and consumer perception of colored yogurts were evaluated. Six samples were prepared with commercial plain yogurt and the addition of murra and maqui powders in different concentrations: 4, 8 and 12% (w/w). High quality color images of each yogurt sample were taken and identified by 3-digit random codes.

Consumers' perception analysis

Participants were given a poll through social networks containing pictures and a short description (see Online Resource 1, Supplementary Fig. 1), and they were asked to score their color liking using a 9-point hedonic scale. Additionally, they were requested to complete open-ended questions (Ares et al. 2010).

The purchase intention (PI) was inquired using a 'yes/no' scale and justified through an open-ended question. The poll was responded by 169 consumers from Argentina, ages ranging between 15 and 79, who reported having consumed yogurt at least once a week.

All the words, descriptions, and associations provided for the "Open-ended question analysis" were considered and grouped in categories. The final categories were those mentioned by more than 10% of the participants (for at least one of the samples) and were established by consensus between three researchers (Jaeger et al. 2018) (see Online Resource 1, Supplementary Tables 1 and 2).

Statistical analysis

STATISTICA version 8.0 and R software version 3.4.1 (R Core Team 2018) were used. ANOVA analysis and multiple comparisons using Tukey's test ($p < 0.05$) were applied to detect differences between treatments. Determinations involved three replicates ($n = 3$) and the results were expressed in terms of mean and standard deviation. For color analysis, 10 replicates were used. A correspondence analysis (CA) was applied in order to visualize the relationship between yogurt samples and categories.

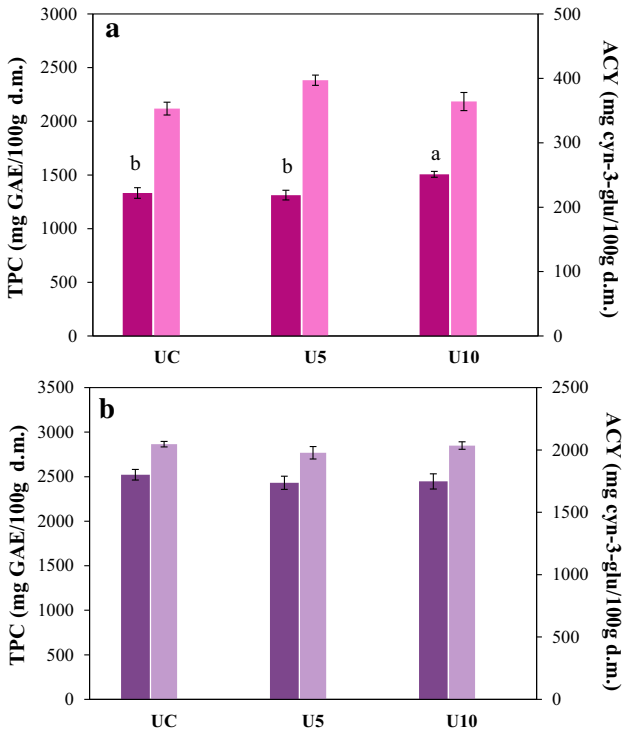


Fig. 1 Total polyphenol content (dark colored bars) and monomeric anthocyanin content (light colored bars) in the total extract (dry matter) of murra (a; pink) and maqui (b; purple). Different letters indicate significant differences between treatments; the absence of letters indicates not significant differences

Results and discussion

Wild berries characteristics

Table 1 shows the characterization of fresh fruits. Water content, ACY, and TPC values were within the range reported by Tardío et al. (2016) for *Rubus ulmifolius* Schott when studying Mediterranean wild edible plants: 57.8–83.8 g H_2O/100 g fresh fruit, 89–123 mg Cyd-3-glu/100 g fresh fruit, and 247–951 mg GAE/100 g fresh fruit, respectively. On the other hand, the wild murra was richer in polyphenols and anthocyanins, compared with blackberry (*Rubus fruticosus)* cultivars of similar zones of Argentina. For example, Franceschinis et al. (2014) and Van de Velde et al. (2016) informed TPC values ranging between 139.8 and 161.8 mg GAE/100 g fresh fruit, respectively and ACY values between 91.4 and 107.3 mg Cyd-3-glucoside/100 g fresh fruit.

The wild maqui exhibited a much lower humidity than the maqui from southern Chile reported by Rodríguez et al. (2016) (68.2 g H_2O/100 g fresh fruit). Regarding bioactive compounds, the concentration obtained was extremely high and was in agreement with the range of TPC (1070–2050 mg GAE/100 g fresh fruit) and ACY (660–1500 mg cyd-3-glu/100 g fresh fruit) of wild maqui from different geographical regions in Chile, provided by Fredes et al. (2014). These results confirm the high potential of maqui as raw material to create functional products and ingredients. Substantial differences (at least 4 times higher in bioactive compounds) were observed when compared with other cultivated berries widely known for their antioxidant properties, such as blueberries (You et al. 2011) or blackcurrants (Djordjević et al. 2013; Archaina et al. 2018). Furthermore, the bioactive concentration was also superior to those obtained for other twelve native Canadian fruits reported by Dudonné et al. (2015), with phenolic values ranging between 70–760 mg GAE/100 g of fresh frozen weight, and anthocyanin content ranging from 2–503 mg/100 g of fresh frozen weight.

Selection of the extraction process

The extraction efficiency is controlled by the morphology and the composition of the fruits, as well as the distribution

Table 1 Physicochemical properties of wild berries

Physicochemical properties	Murra	Maqui
Water content (g H_2O/100 g fresh fruit)	72.8 ± 0.7	45.40 ± 0.03
pH	3.9 ± 0.2	3.90 ± 0.01
Total acidity (mg citric acid/100 g fresh fruit)	0.73 ± 0.08	1.9 ± 0.7
Total soluble solids (°Brix)	16.8 ± 0.2	38.2 ± 0.5
Monomeric Anthocyanin (mg Cyd-3-glu/100 g fresh fruit)	118 ± 31	626 ± 129
Total phenolic (mg GAE/100 g fresh fruit)	358 ± 18	1202 ± 162
Antioxidant capacity (mg GAE/100 g fresh fruit)	209 ± 20	533 ± 74
% Polymeric color (%PC)	15.6 ± 2.5	7.1 ± 0.8
Color variables		
L*	18.6 ± 0.2	20 ± 2
a*	1.7 ± 0.3	0.6 ± 0.2
b*	0.8 ± 0.2	0.05 ± 0.03

Values expressed as means ± standard deviation

of bioactive compounds in the tissue. Maqui is a very poor juicy fruit (fresh juice yield ≈ 0.5%), with low water content (Table 1), implying that some of the water and most of the polyphenols are probably attached to the solid phase (Struck et al., 2016). This makes it necessary to apply an adequate extraction process to increase YP and antioxidant potential, as well as the total volume of obtained extract. After applying three extraction cycles, YE of maqui increased from 61.5% (first cycle) to ≈80% (all the unified extracts), without significant effects of UAE treatment. On the other hand, murra is a juicer fruit (fresh juice yield ≈ 40%), and YE after the first cycle (72–77%) was slightly improved after three cycles (81–82%), getting with only one cycle high liquid recovery from the fruit tissue. In maqui, it was necessary to perform three extraction cycles to improve liquid recovery from tissue structure.

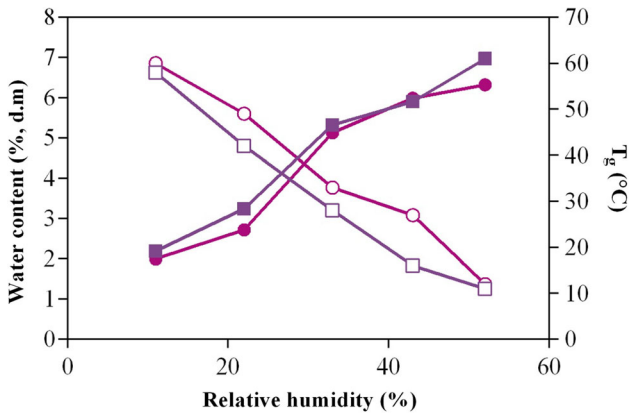


Fig. 2 Glass transition temperature (empty symbols) as a function of RH (%), and water sorption isotherms at 20 °C (filled symbols) of murra powders (pink lines) and maqui powders (purple lines)

Figure 1 shows the total bioactive compounds content achieved in the extracts obtained after the three extraction cycles. U10 treatment applied on murra increased TPC, while no significant effect ($p < 0.05$) of UAE treatment was detected in maqui. No evidence of improvement of

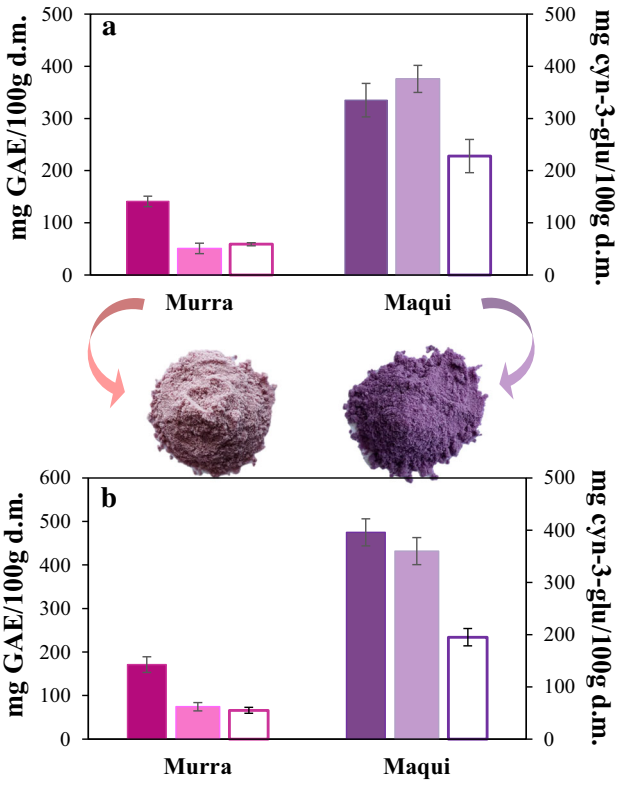


Fig. 3 Bioactive compounds content for murra (pink tone bars) and maqui (purple tone bars) formulated extracts (**a**) and freeze-dried powders (**b**). TPC (dark colored bars) and AC (white bars) can be read on left y-axis and ACY content (light colored bars) on right y-axis

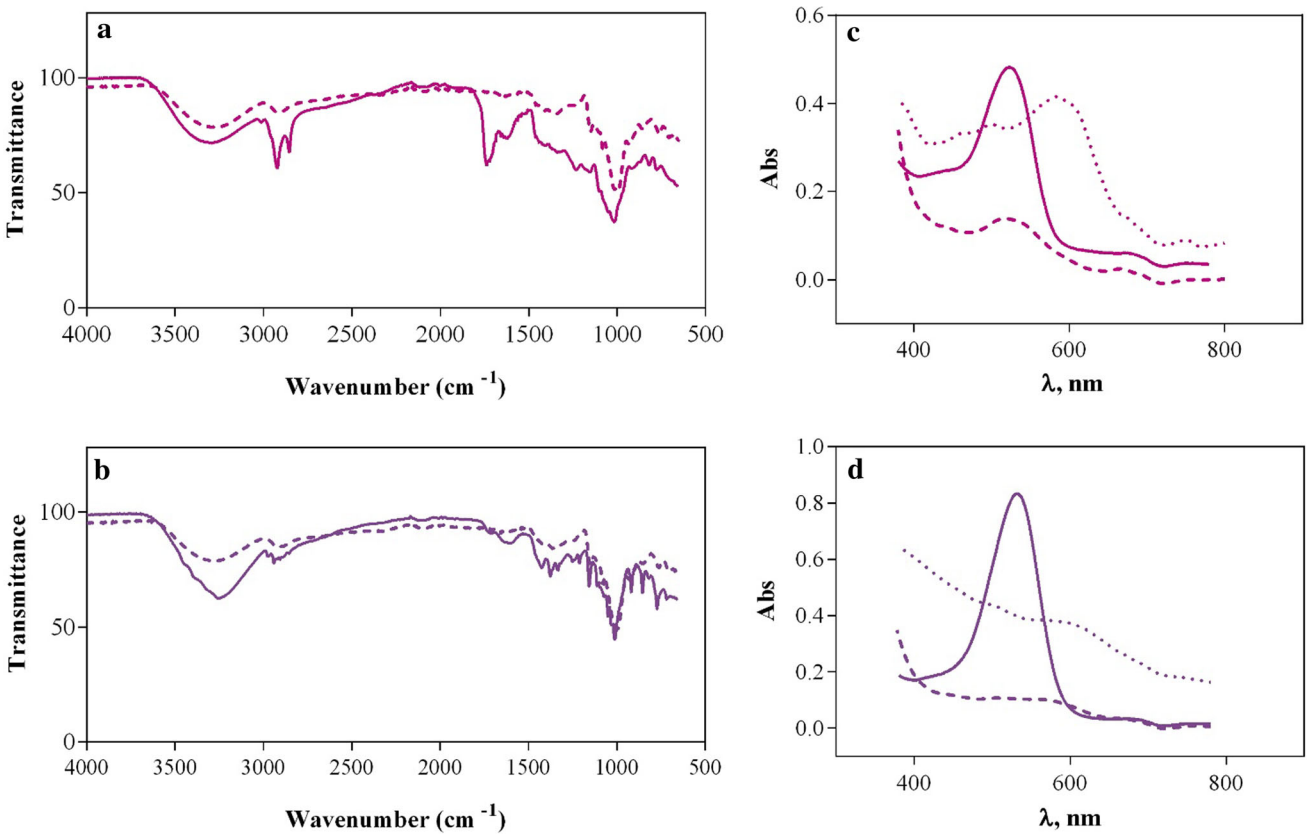


Fig. 4 ATR-FTIR (**a**, **b**) and UV–vis (**c, d**) spectra profiles for murra (pink lines) and maqui (purple lines) samples. Continuous and dashed lines correspond to initial fruit and powder samples, respectively, for **a** and **b** spectra; for **c** and **d** spectra continuous, dashed, and pointed lines correspond to pH values of 1, 4.5, and 10, respectively

monomeric anthocyanin content was observed. Other authors, when using UAE method, reported different results regarding polyphenol and juice extraction. For instance, Galvan d'Alessandro et al. (2012) reported an 85% increase in phenolic yield in black chokeberry with ultrasound extraction (30.8 kHz, power of 100 W), while Radziejewska-Kubzdela et al. (2020) observed an increase of 20% of anthocyanin content in barberry fruits after ultrasound treatment (20 kHz, power of 140 W), but non significant effect of ultrasound was detected in juice yield.

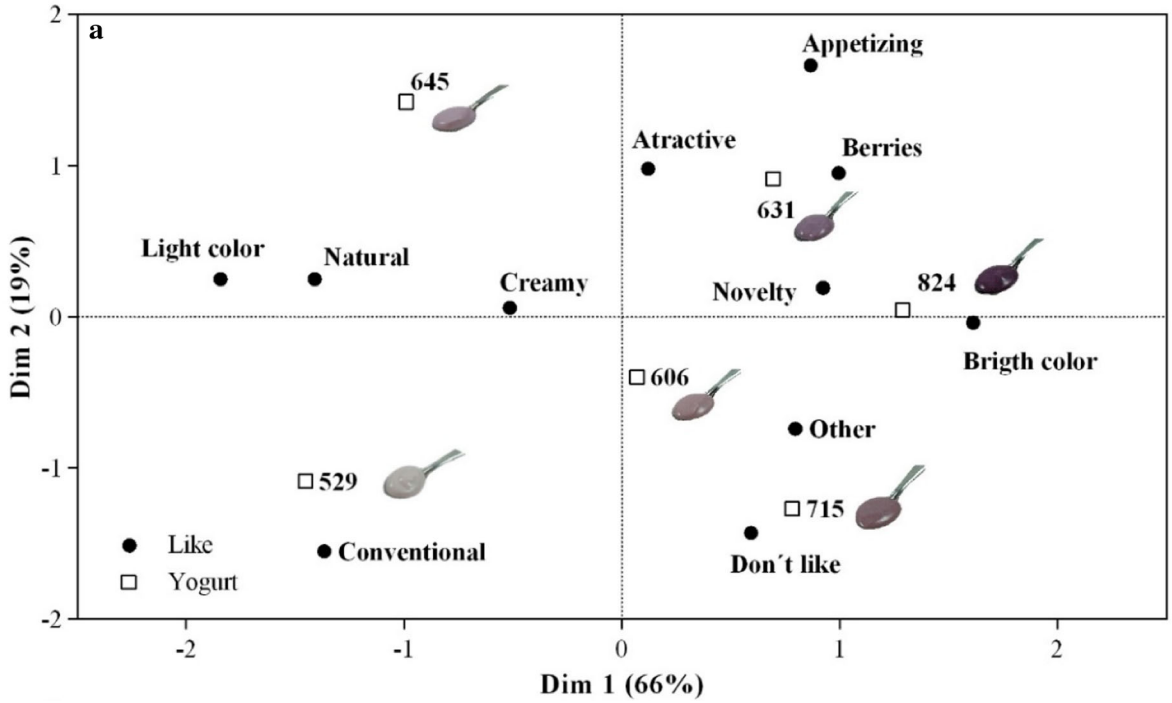
Regarding polyphenol recovery from fruit matrix (YP), it was around 54% in maqui after the third cycle, suggesting that a substantial amount of polyphenols remained retained in the waste. In contrast, the application of U10 in murra improved bioactive compounds extraction not only from the fruits (4.3%) but also from the waste produced upon the first cycle (9%), reaching 65% after the third cycle.

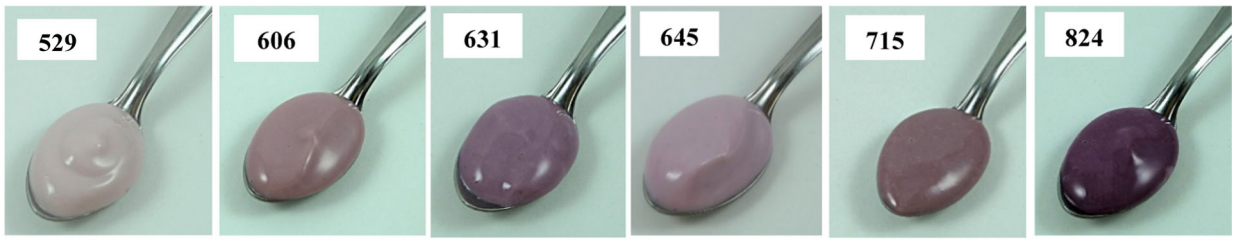
According to these results, U10 treatment was selected as optimum for murra and U0 for maqui, and the extract obtained by the three extraction cycles was considered for powder production.

Freeze-dried powders

The selected extracts were formulated with maltodextrin (20% w/w) to produce the fluid feeds for the freeze-drying process. Both berry-powders presented very low values of water content ($\approx$ 1.2%, w/w) and water activity (< 0.06), together with high T_g values (119 °C for murra and 95 °C for maqui), indicating that the powders exhibited good physical characteristics to be stored at room temperature, preserving their glassy state, without risk of physical damage. Water activity and water content were much lower than those obtained by Franceschinis et al. (2014) for blackberry juice freeze-dried with the same carrier type and concentration. The better drying efficiency obtained when using berry extracts could be ascribed to the reduced content of low-molecular-weight sugars and organic acids in comparison with the juices (obtained by milling, centrifugation, and filtration), thus contributing to stickiness decrease during freeze-drying process.

On the other hand, water sorption may affect physical properties and compromise the stability of powders during storage. The analysis of water sorption isotherms along with T_g evaluation can contribute to the selection of


a
Appetizing
645
Atractive
Berries
631
Light color
Natural
Creamy
Novelty
824
Brigth color
606
Other
529
715
Like
Yogurt
Conventional
Don´t like
Dim 2 (19%)
Dim 1 (66%)


529
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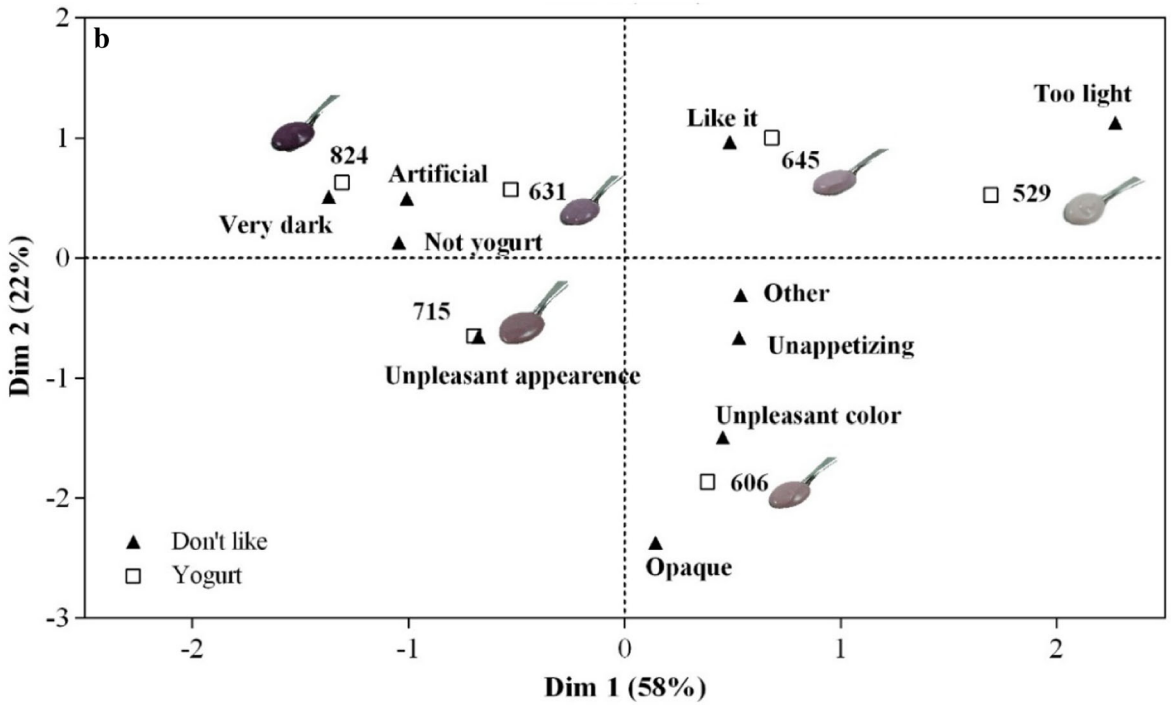
b
Too light
Like it
824
Artificial
631
645
529
Very dark
Not yogurt
Other
715
Unappetizing
Unpleasant appearence
Unpleasant color
606
Don't like
Yogurt
Opaque
Dim 2 (22%)
Dim 1 (58%)

◀**Fig. 5** Correspondence analysis of terms used to describe the color perception of yogurts colored with wild berries powders through the open-ended questions: "I like the color because…" (**a**), and "I don't like the color because…" (**b**). Murra yogurt samples: 529, 606, and 715 (4, 8, and 12% powder, respectively). Maqui yogurt samples: 645, 631, and 824 (4, 8, and 12% powder, respectively)

adequate storage conditions. Water sorption at 20 °C of both powders exhibited similar behavior, while glass transition temperatures for murra were slightly higher than those observed for maqui (Fig. 2). Powders kept the glassy state at room temperature (20 °C) up to 43% RH for murra and up to 33% for maqui. As expected, both powders were highly hygroscopic reaching equilibrium values within 7–8 days ($H_i \approx$ 17 g H_20/100 g d.m.). Relatively close values were informed by Pereira Souza et al. (2017), when studying freeze-dried jaboticaba powders, using the same type and concentration of carrier, at 25 °C after 8 days. These results indicate that it would be advisable to store the powders protected from the environmental conditions using a hermetic packaging.

Bioactive compounds of maqui powders were considerably higher than those of murra, which combined with its intense purple color, makes it interesting for its use as both functional ingredient and natural colorant (Fig. 3). In murra, the much lower ACY and the higher polymeric color (PC = 18) could indicate a higher polymerization degree of the anthocyanins, with the consequent higher stability of the powder color when exposed at different conditions. In maqui powders, the lower polymeric color (PC = 6) suggested a higher contribution of monomeric anthocyanins, which are less stable than the polymerized compounds. The particularly high ACY content of maqui powders provided a purple color ($L^* = 45 \pm 5$; $a^* = 18.6 \pm 1.3$; $b^* = -7.9 \pm 0.6$), while murra powders presented a light pink hue ($L^* = 60 \pm 2$; $a^* = 12.7 \pm 0.7$; $b^* = 1.0 \pm 0.5$). Both powders presented attractive colors for their use as natural food colorants. Polyphenols content in maqui powders was $\approx$ 2.8 times higher than those obtained in murra powders.

ATR-FTIR spectra of freeze-dried powders and fruits are shown in Fig. 4a, b. Maqui fruit spectrum exhibited typical absorption bands of anthocyanin and polyphenol-rich fruits, such as hydroxyls groups (O–H) stretching vibration (maximum at 3251 cm^{-1}), anti-symmetric stretching vibrations –CH and $–CH_2$ for methyl groups (at 2941 cm^{-1}), stretching vibration of carbonyls groups (–C=O) at 1705 cm^{-1} and –C=C stretching vibration of the aromatic rings (at 1606 cm^{-1}). Maqui powder spectrum was very similar to the fruit spectrum. Only the characteristic peak corresponding to –OH vibration exhibited a lower intensity and a left shift ($\Delta\nu = -43$ cm^{-1}). The band shift to higher wavenumbers indicates a weakening of the hydrogen bonds, which can be ascribed to the occurrence of intermolecular copigmentation phenomena of anthocyanins. Mioč et al. (2000) reported a shift of 34 cm^{-1} and a fall of intensity of peaks when copigments were formed in model systems with malvin chloride and three flavones. Murra spectra presented important differences between fruit and powder. The main noticeable difference was the decreased intensity of the band group around 2800 cm^{-1} and a shift to lower wavenumbers ($\Delta\nu = 15$ cm^{-1}), which indicated the presence of strong hydrogen bonds formed via the oxonium groups of the copigments (Mioč et al. 2000). The existence of oxonium ions can be found in the fruit in the region of bending vibrations, bands around 1738 cm^{-1}, which can be attributed to the v_4 vibration of the H_3O^+ ion. Its intensity fell to 1688 cm^{-1} in the powder sample when the copigments were formed.

The occurrence of copigmentation phenomena was also appreciated in the uv–visible spectra of both powders at different pH values (Fig. 4c, d). In the case of maqui, the far less colored system (significant absorbance decrease) observed at pH 4.5 was indicative of higher monomeric anthocyanin content. On the contrary, murra powder showed colored structures in all the pH range, suggesting higher protection of the chromophores due to the stronger bonds verified by FTIR analysis and also through the higher PC values.

Application of powders as natural colorants in yogurts: consumer perception

The color acceptability scores of yogurt samples colored with wild berry powders are shown in Online Resource 1 (Supplementary Table 3). Samples with murra powder had acceptability mean values lower than 6 (between 4.4 and 5.3), suggesting that this powder would not be suitable as yogurt colorant. Samples with 8 and 12% murra powder presented more than 50% of the scores in "I dislike" and "I dislike very much". Maqui samples had the highest acceptability means, except for that with 12% powder (mean = 4.4) since 64% of the values were observed in the reject zone. Yogurt with 8% maqui powder exhibited an acceptability value of 5.5, and that with 4% (mean = 6.8) obtained 74% of hedonic scores in "I like it" (30%) and "I like it very much" (44%).

The relations between colored yogurts and the categories for the open-ended question are presented in the correspondence analysis (CA) shown in Fig. 5. In Fig. 5a, drivers of liking of color could be appreciated since 85% inertia was explained by CA. Yogurts colored with murra powder were located in the lower quadrant while those with maqui powder were in the upper one, which is related to positive attributes. Sample 631 (maqui 8%) was related

to categories "Berries", "Appetizing", "Attractive" and "Novelty". Yogurts with both 4% powders (529 and 645) were linked to "Light color", "Natural" and "Creamy", which are very important sensory attributes for acceptability. However, only sample 645 was linked with an attractive tone, while 529 color was categorized as "Conventional", that is, similar to the yogurts available in the Argentinean market. Sample 715 (murra 12%) was associated with the least preferred color. These results suggest that the "attractive tone" acted as a driver of liking of color in yogurts colored with wild berry powders, particularly samples with 4 and 8% maqui powder.

The CA (80% inertia) carried out on the open question "I don't like the color because…" (Fig. 5b) showed that the low acceptability exhibited by sample 715 was related to an unpleasant aspect of the yogurt. Sample 606 (murra 8%) was related to "Unpleasant color" category represented by terms such as "Brown", "Dull", "Ugly color" and "Unattractive"; other categories like "Opaque tone" and "Unappetizing" were also used to describe the color of this yogurt. Sample 529 (murra 4%) was associated with lack of color. Sample 645 (maqui 4%) presented an attractive color ("there is nothing to dislike") and samples 631 and 824 were related to "Artificial", and "Not yogurt", being 824 (12% maqui) defined as "too dark". Different drivers of disliking of color were found according to the berry powder added since the "unpleasant color" was pointed for yogurts colored with murra powder (4, 8, and 12%), and "too dark" was indicated for yogurts with maqui powder at high concentrations (8 and 12%).

Purchase decisions of consumers are often based on a prompt emotional response towards a product (Tepsongkroh et al. 2020). In this case, consumers manifested a very high PI (93.5%) for yogurt colored with wild berry powders, being higher the preference for maqui powder colors (78%) compared to murra powder colors (22%). Additionally, light tones were preferred. Prototype 645 presented the highest PI (46%) followed by 631 (18%) and 529 (17%), in accordance with the average hedonic scores. Among the justifications for the purchase choice, 27% were related to appearance (pleasant color, appearance, and texture), 26% were motivated by good flavor expectation (I expect a good taste, I would like to try it), 22% considered the product natural and healthy, 14% mentioned terms such as red fruits, fruity, Patagonian, regional product, and only 9% qualified it as novel product.

Results from CA, PI, and color acceptability analysis suggest that samples 645 and 631 could be selected for future sensory studies of consumer perception in order to evaluate the global acceptability.

Conclusion

It was possible to achieve high bioactive compounds recovery from wild underutilized berries through UAE and successive extraction cycles. Freeze-dried berry-powders with excellent physical properties were achieved. Maqui powders exhibited extraordinarily high bioactive compounds content and very attractive color, being a good option as functional ingredient or food colorant. Murra powders showed greater stability of anthocyanin pigments which could be interesting for certain food applications. The potential of powders as natural colorants in yogurts was evaluated. The use of open-ended questions provided interesting information about the most relevant attributes for consumers and the possible improvements for the development and marketing of new yogurts formulated with natural colorants. Yogurts colored with 4 and 8% maqui powder should be further tested in future sensory characterization by consumers to find the launch prototype.

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Author contributions MGM and AS carried out the experiments, analyzed data and wrote the MS; RC and MG performed specific experiments and analyzed data; LF performed specific experiments, analyzed data and wrote the MS; CS and DS conceived and supervised the work, analyzed data, wrote and revised the manuscript and were responsible for funding and resources acquisition.

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Data availability The datasets used and/or analyzed during the current study are available from the.corresponding author on reasonable request.

Declaration

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The research being reported has been conducted in an ethical and responsible manner.

Informed consent All authors gave their consent to participate in the publication, as well as the responsible authorities at the institute where the work has been carried out. All authors gave their consent for publication of the work.

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