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Osmotic Dehydrated Raspberries: Changes in Physical Aspects and Bioactive Compounds

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Raspberries are very labile fruits that have a short postharvest life; therefore, there is a need to develop alternative storage and processing methods for extending their shelf-life. The effect of wet (WI) and dry (DI) sucrose infusions ($a_w = 0.85$) on color and bioactive compounds of raspberries has been studied. Additives were included: citric acid, sodium bisulfite, or both. Moisture content decreased from 85% (w/w) for control fruit to approximately 51% (w/w) for infused samples. The shrinkage of fruits was approximately 27% and 46% after WI and DI, respectively. No major color changes occurred, except for WI-bisulfite treatment. Although the total polyphenols and anthocyanin content were significantly reduced in fruits during osmotic dehydration, mainly due to the dilution effect to the medium, 100 g serving of processed raspberries would supply, in some cases, over 50% of polyphenols provided by a glass of wine. The proposed infusion dehydration methods may be considered an alternative to produce shelf-stable products based on raspberries, with an improved quality in terms of appearance.

Keywords Bioactive compounds; Color; Raspberry; Sugar infusion

INTRODUCTION

The raspberry cultivars grown in Argentine Patagonia are favored by the climatic conditions of the region and constitute about 60% of the national production of this berry.[1] This fruit is one of the most delicate berries, known for being very labile and having a short postharvest life due to its high respiration rate, loss of firmness and freshness, and susceptibility to darkness. For fresh consumption, raspberries tend to be available for only a short season, and although the main destination is the frozen market, the products have limited applications. Industries demand frozen fruits for juices or puree concentrates, jams, preserves, dairy products, or bakery preparations.[2] Therefore, there is a need for alternative processing methods to generate new alternatives for obtaining shelf-stability while minimizing changes in fruit quality attributes. One attractive option is to employ an osmotic dehydration (OD) procedure using the sugar infusion (SI) technique. The use of osmotic dehydration has received increasing attention in the field of fruit preservation processes because browning is reduced, volatile compounds' retention is increased, sweet taste is reinforced, and it produces a partial dehydration of foods with low energy requirements.[3] Together with the water loss, sugars used as osmotic agents are transported inside the fruit. On the other hand, some volatile compounds, flavor precursors, nutrients, and pigments are transferred from the fruit to the osmotic syrups. Thus, it is possible to suggest that osmotic syrups can be successfully used together with dehydrated fruit or applied as natural additives to other products.[3,4] Osmotic dehydration can be carried out to obtain several types of products, such as high-moisture or intermediate-moisture fruits, or it can be used as a pretreatment before drying or freezing.[5–9] As high temperatures are not normally used in OD processes, and no water phase changes occur, changes in sensory attributes, such as color, aroma, flavor, and texture, are minimized.[10,11] Sugar uptake during osmotic dehydration of fruit modifies the composition (sugar to acid ratio) and the taste of the final dehydrated food. This so-called "candying effect" is sometimes desirable, as it improves the taste and acceptability of the final product. In most dehydration cases, however, extensive solute uptake is undesirable, because of its negative impact on the nutritional profile of osmotically dehydrated fruits.[12]

The SI techniques to obtain processed fruit products which are stable at room temperature are very simple and based on combinations of mild heat treatments, reduction of a_w by the addition of humectants (glucose or sucrose), pH control by the addition of acids, and the incorporation of other preservatives in order to have more stable products during storage time. Potassium sorbate is usually used as an antimicrobial agent, and sodium bisulfite is added as an antimicrobial and acts as an inhibitor of

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enzymatic and non-enzymatic browning. Food products formulated under this concept (hurdle effect) are more stable than fresh food without refrigeration while preserving, to a great extent, quality characteristics.[13–15] The blanching step is used for inactivation of enzymes as well as for reduction of some indigenous flora, but in delicate fruits like berries and cherries, heat application might significantly affect sensory properties like color and structure.[16,17] A higher acidity level in the fruit would be an alternative to the use of a thermal treatment in the combined technology for these fruits, maintaining at the same time the characteristic acid taste of the fruit after osmosis. It is known that a pH reduction prevents enzymatic browning, inhibits or reduces bacterial growth, and enhances the action of antimicrobials.[15]

It is important to note that although the combination of factors such as a_w and pH and the incorporation of additives in preserving fruits is important and all play a crucial role in improving the shelf-life of fresh and processed commodities, these additives can also affect the sensory characteristics of some fruits, particularly those with red pigments that can change their structure according to the medium in which they are located. Since color is a critical quality parameter in food due to its influence on consumer acceptance, color measurement has gained the attention of food scientists and industry. To investigate color quality in a systematic way, it is necessary to measure color as well as pigment concentration. Color has always been a great challenge in foods following industrial processing, and many parameters are involved in its stability.[18] In raspberries, the pigments responsible for the attractive red color are the anthocyanins, which are highly unstable and very susceptible to degradation. The color stability of anthocyanins is affected by several factors, such as pH, their own chemical structure and concentration, storage temperature, light, oxygen, and the presence of enzymes, flavonoids, proteins, sugars, and metal ions. In particular, the acid environment provides the highest stability. In fruits, the anthocyanins can exist in four structural forms, depending on pH: the blue quinonoidal base (pH near to 6.0), the red flavylium cation (pH $\approx$ 1.0), and the colorless forms (pseudocarbinol and chalcone; pH $\approx$ 4.5).[17,19,20]

The aim of this work was to study the effect of different sugar infusion treatments on color and bioactive compounds of raspberries (Autumn Bliss var.).

MATERIALS AND METHODS

Fruits

Frozen raspberries (*Rubus idaeus* L.) of the Autumn Bliss cultivar grown at the ''La Piedad'' farm (Plottier, Neuquén province, Argentina) were used in the present study. The climate of the zone, situated in Northwestern Patagonia, can be characterized as temperate or cold-temperate with average temperature of 10°C (autumn-winter) and 18°C (spring-summer). The annual rainfall is between 200 and 400 mm, concentrated in winter and generally in small events. Fruit was totally hand-harvested from the end of November to April from a 3 ha plantation. After harvest, fruits were placed on plastic trays and visually classified according to maturity degree (defined by color) into three groups. Only fruits of intermediate maturity and uniform size ($\approx 2.10^{-6}$ m^3) were selected and then randomly divided into nine lots of 100 g each. Fruits were then individually quick-frozen (IQF) in an air blast tunnel at −48°C and air speed 1.5 ms^{-1}. After freezing, the raspberries were stored at −22°C until use.

Treatments

Osmotic dehydration processes were performed at room temperature in glass vessels ($8 \times 8 \times 16$ cm). The systems were prepared by immersing the frozen fruits into a mixture of the humectant and the preservatives commonly used in the preparation of high- or intermediate-moisture fruits. Potassium sorbate and sodium bisulfite are used as antimicrobial and enzymatic browning preservatives, respectively. Citric acid was added to reduce the pH level of the syrup so that, in the equilibrium, the final pH value of the fruit-syrup system could be in the fresh fruit pH.[14] Reagents were all food grade (Saporiti S.A., Argentina).

The amount of sugars and chemical agents were determined according to the weight of the fruit (100 g) and the final levels required after equilibration of the components of the food system ($a_w = 0.85$). Sucrose concentration in the mixture was calculated using the Ross equation[21] to attain the a_w equilibration value desired between raspberries and the formed syrup. The fruit/sugar ratio was 1.27 for dry infusions and 0.36 for wet infusions.

Final water activity value achieved after infusion process was selected in order to have intermediate-humidity fruits of different acidity levels, as well as to study the impact of sugar concentration, sodium bisulfite, and product pH on the analyzed properties.

Two different infusion treatments were performed: dry infusion (DI) and wet infusion (WI). In DI, fruits were mixed directly with the humectant and the additives. In WI, fruits were immersed in an aqueous solution of the humectant and additives. The systems were prepared as follows:

1. Dry infusion (DI): Fruits and sucrose (the only additive)
2. Dry infusion with citric acid (DI-AC): Fruits and a dry mix of additives (95.8% sugar and 4.2% citric acid)
3. Dry infusion with sodium bisulfite (DI-B): Fruits and a dry mix of additives (sucrose and 250 ppm of sodium bisulfite)
4. Dry infusion with citric acid and sodium bisulfite (DI-BAC): Fruits and a dry mix of additives (95.8% sugar, 4.2% citric acid, and 250 ppm of sodium bisulfite)

5. Wet infusion (WI): Fruits dipped in an aqueous solution of sucrose (61% w/w)
6. Wet infusion with citric acid (WI-AC): Fruits immersed in an aqueous solution (59.4% sugar and 2.3% citric acid)
7. Wet infusion with sodium bisulfite (WI-B): Fruits immersed in an aqueous solution (61% sugar and 250 ppm of sodium bisulfite)
8. Wet infusion with citric acid and sodium bisulfite (WI-BAC): Fruits immersed in an aqueous solution (59.4% sugar, 2.3% citric acid, and 250 ppm of sodium bisulfite)
9. Control samples: Frozen fruits were used as control samples

In all cases, 1000 ppm of potassium sorbate was added. The preparations were gently mixed twice daily and the a_w of the systems was controlled until equilibration was reached (fruit a_w = generated syrup a_w). The time to equilibrate the systems was 10 days. After that, the fruits were taken out of the generated syrup and drained on tissue paper to remove the residual syrup. Both the processed raspberries and the different generated syrups were analyzed and compared with untreated fruits (control).

Samples Analysis

Raspberry Extracts

Fruit extracts were obtained according to Cayupán et al.[22] with some modifications. For extract preparation, 2.5 g of the sample were homogenized in 7.5 mL methanol for 3 min using a blender, and then filtered. The pellet was extracted twice again with 7.5 mL aliquots of methanol. The extracts were combined and methanol was added to constitute a total volume of 25 mL. Extracts were prepared in triplicate. In syrups, analyses were directly assessed. All spectrophotometric measurements from extracts or syrups were carried out using a UV/Vis spectrophotometer model 1700 (Metrolab Instruments, Buenos Aires, Argentina).

Water Content, Water Activity, pH, Total Acidity, Soluble Solids Content, and Ashes

The chemical analysis was carried out by analyzing the following parameters according to AOAC (1990)[23]: moisture (925.09), water activity, pH (945.27), total acidity (945.26), soluble solids content (932–12) and ashes (940–26). Water activity (a_w), pH, total acidity (TA), and soluble solids content (SSC) measurements were carried out from fruit puree. The a_w was measured at 25°C with a psycrometer model Series 3 (Aqua-Lab, Decagon Devices Inc., Pullman, WA), calibrated with saturated salt aqueous solutions. SSC percent in samples liquid phase (Brix) was analyzed by measuring the refraction index in an ABBE refractometer model DR A1 (Atago, Tokyo, Japan) at 25°C. TA is expressed as percent of citric acid in dry matter (%, d.m.). The pH was measured with a pH meter model EA 940 (Orion, Beverly, MA, USA). All measurements were made in triplicate and the average values were informed.

Total Sugar Content (TS) and Reducing Sugars (RS)

The total sugar content was determined by an anthrone/sulfuric acid procedure.[24]

The reducing sugars were spectroscopically determined according to Somogyi and Nelson.[25] Results were expressed as g of glucose in 100 g of wet matter (%, w.b.).

Total Phenolics Content (TPC)

Total phenolics content was determined using the Folin–Ciocalteau reagent according to Singleton and Rossi[26] with some modifications. 150 µL extract was mixed with 950 µL water, 100 µL Folin–Ciocalteau reagent, and 600 µL 20% sodium carbonate in distilled water. After incubation during 30 min at 40°C, the absorbance was measured at 765 nm. A calibration curve was done with gallic acid as standard. The results were expressed as gallic acid equivalents in milligrams per 100 g of wet matter (mg GAE/100 g w.b.).

Total Anthocyanin Content

Extraction method. For total anthocyanin determination, the extracts were obtained according to Nikkhah et al.[27] with some modifications. For extract preparation, 3 g of the sample were homogenized in 35 mL of ethanol acidified with chloridric acid, shaken for 10 min using a blender, and filtered. The pellet was extracted twice again with 10 mL and 5 mL of ethanol acidified with chloridric acid, respectively. The extracts were combined and ethanol was added to constitute a total volume of 50 mL. Extracts were prepared in triplicate. In syrups, analyses were directly assessed.

Monomeric anthocyanin content (ACY). ACY was determined using the pH-differential method.[20] ACY (monomeric anthocyanin content) was expressed as cyanidin-3-glucoside (MW: 445.2; molar extinction coefficient: 29,600 $L\,cm^{-1}\,mol^{-1}$). Calculations were corrected by dry matter content. The results were expressed as mg Cyd-3-glu/100 g of w.b. The absorbance was measured using a UV/Vis spectrophotometer model 1700 (Metrolab Instruments, Buenos Aires, Argentina).

Percent of Polymeric Color (% PC) and Browning Index (BI)

The percent of polymeric color was expressed as a % of total color density (% PC = PC/CD × 100). Color density (CD) and polymeric color (PC) parameters were determined using the bisulfite bleaching method.[20,28] Total color density is a measure of the color strength of the sample solution. Polymeric color (PC) is an indicator of polymerized pigments, including tannins-anthocyanins

complexes and brown compounds. The Browning Index corresponds to the absorbance at 420 nm of the bisulfite bleached samples.

Antioxidant Capacity

The antioxidant activity of raspberries was determined through two different methods: the DPPH• and FRAP assays. An UV/Vis spectrophotometer model 1700 (Metrolab Instruments, Buenos Aires, Argentina) was used for absorbance measurements.

DPPH• free radical–scavenging capacity. The bleaching method of the radical 1,1-diphenyl-2-picrylhydrazyl (DPPH•)[29] with some modifications was used. 3 mL of DPPH• solution in methanol was placed into a cuvette and mixed with aliquots of raspberry extract. The absorbance decrease at 515 nm was monitored in 30 cycles in periods of 1 min. The initial radical absorbance was 1.00 ± 0.01 AU at 515 nm when a solvent aliquot was added instead of the extract. The absorbance of the system at the steady state was estimated by mathematical fitting of kinetic curves performed with Origin 8.0 software. Percentages of radical consumption for different aliquots of raspberry extract were measured in order to find EC_{50} value that corresponds to the concentration that scavenged 50% of the radicals. The antiradical power (ARP), defined as the inverse of EC_{50} expressed in wet basis, was used for comparison of different raspberry extracts.

FRAP ferric-ion-reducing ability. The antioxidant capacity was determined using the FRAP method (the ferric reducing/antioxidant power) described by Benzie and Strain.[30] The change in absorbance was expressed in Fe^{2+} μmoles/100 g sample in wet basis.

Color Characteristics of Infused Fruits

The superficial color of raspberries and syrups was determined by measuring tristimulus parameters (CIELAB color space) with a Minolta photocolorimeter (model CR 400) using illuminant C and 2° observer angle. The instrument was standardized each time with a white ceramic plate. The parameters L^*, a^*, b^* of CIELAB color space were recorded. L^* represents color lightness (0 = black and 100 = white). a^* scale indicates the chromaticity axis from green (−) to red (+) while the b^* axis ranged from blue (−) to yellow (+). These numerical values were converted into "total color difference" (ΔE^*_{ab}), "chroma" (C^*_{ab}), and "hue angle" (h_{ab}).[31] For ΔE^*_{ab}, differences were calculated taking into account L^*, a^* and b^* values of control raspberries before treatments. Color determinations were made in 10 raspberries for each infusion with two readings in the equatorial zone.

Volumetric Shrinkage (Sh)

The shrinkage caused by the osmotic dehydration was evaluated through measurements of sample volume change. Samples volume was estimated gravimetrically by displacement of water in a volumetric flask.[32] Shrinkage was expressed in terms of percent of volume change.

$$S_h = \left(\frac{V_0 - V}{V_0}\right) 100 \quad (1)$$

where V_0 = initial average volume (taken from 10 control raspberries) and V = volume of each raspberry after treatment. Volume displacement was determined in quintuple.

Mass Transfer Parameters

The weight loss (ΔM), the water loss (ΔM_W), and the solids gain (ΔM_{SS}) occurred in samples after osmosis were determined by considering water content, soluble solids content, and weight of raspberries before and after they were treated under different experimental conditions of infusions. Each experiment was performed in triplicate. Calculations were then made using the following relationships:[33,34]

$$\Delta M = \frac{w_0 - w}{w_0} \quad (2)$$

$$\Delta M_W = \frac{w_{w0} - w_w}{w_0} \quad (3)$$

$$\Delta M_{SS} = \frac{w_S - w_{S0}}{w_0} \quad (4)$$

Where w_0 = initial weight of the sample (g), w = final weight of the sample (g), w_{w0} = initial weight of water in the sample (g), w_w = final weight of water in the sample (g), w_{S0} = initial weight of soluble solids in the sample (g), w_S final weight of soluble solids (g).

Statistical Analysis

The experimental design was a completely randomized design. For all determinations, except for superficial color, three replicates were measured. The results were expressed by mean and standard deviation of the mean (SD). All of the measured variables used to characterize the raspberries under the different infusions were descriptively compared with an analysis of principal components (PCA). Two-way analysis of variance (ANOVA) was carried out to establish the presence or absence of significant differences in parameters according to the factors "additive" and "type of infusion." Multiple comparisons were performed using the Tukey test and significance level was set at $p < 0.05$. In the case of significant interactions between factors, the Tukey test was run for the interaction. For insignificant interaction between factors, a Tukey test of main effects

was performed; uppercase letters were used for expressing significant difference between means of "type of infusion" factor, and lowercase letters were used for expressing significant difference between means of "additive" factor. All statistical analyses were carried out using the data analysis software system STATISTICA version 8.0. (StatSoft, Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

Mass Transport and Compositional Changes

When a cellular tissue is immersed in a hypertonic solution to reduce its water activity, mass transfer phenomena and tissue shrinkage spread simultaneously from the surface to the center of the tissue throughout process time and the different cell layers will experience different conditions of water loss, solid gain, and tissue shrinkage.[35] Figure 1 shows that raspberries suffered ≈34% weight loss under wet infusion (WI) and ≈51% under dry infusion (DI), and these values were not affected by the type of preservatives used in the formulation of osmotic medium (sucrose combined with acid, bisulfite or with both). Raspberries subjected to dry infusion treatments exhibited greater water loss (WL) and lower solid gain (SG) values than samples treated with wet infusions. Moisture content decreased from 85% (w/w) for fresh fruit (Table 1) to ≈51% (w/w) for infused samples in all cases, with a final a_w of 0.85 (Table 2). Thus, the products obtained belong to the foods known as "intermediate moisture fruits."

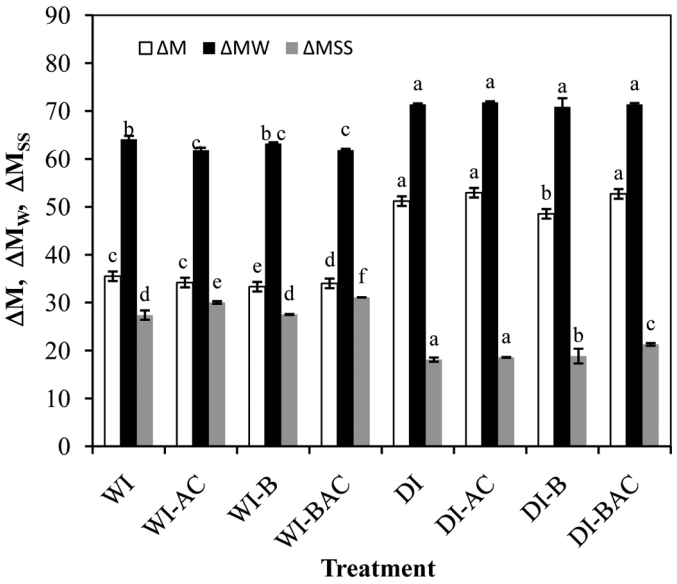

FIG. 1. Weight loss (ΔM), water loss (ΔM_W), and sugar gain (ΔM_{SS}) of raspberries after infusion treatments (wet infusions: WI, WI-B, WI-AC, and WI-BAC; dry infusions: DI, DI-B, DI-AC, and DI-BAC). Vertical bars represent standard errors of the means. Means with a different letter are significantly different ($p < 0.05$).

TABLE 1
Physicochemical properties of the frozen raspberries studied

Physicochemical properties of raspberries	Mean ± SD
Water content (g H_2O/100 g w.b.)	85 ± 3
Water activity (a_w measured at 25°C)	0.97 ± 0.02
pH	3.13 ± 0.02
Total acidity (TA, g citric acid/100 g w.b.)	0.267 ± 0.004
Total soluble solids (Brix)	8.8 ± 0.8
Ash (%)	0.363 ± 0.012
Total sugar (TS, g glucose/100 g w.b.)	14.5 ± 0.4
Reducing sugars (RS, g glucose/100 g w.b.)	1.4 ± 0.2
Anthocyanins (ACY, mg Cyd-3-glu/100 g w.b.)	81.3 ± 5.1
Total phenolics content (TPC, mg gallic acid/100 g w.b.)	236 ± 2
ARP ($1/EC_{50}$) (100 g^{-1} w.b.)	63 ± 3
FRAP (μmol Fe^{2+}/100 g w.b.)	3152 ± 17

Both osmotic treatments resulted in a decrease in sample volume. The shrinkage of fruits with wet infusion was small (≈27%), while treatments with dry infusion resulted in a more severe volume change (≈46%). The higher water loss of DI raspberries could be ascribed to the greater driving force that occurred in the early stages of the process. This led to a rapid volume reduction, thus reducing the transfer of solutes inside the fruit, mainly into the intercellular spaces of the tissue, where diffusion mechanism mostly occurs. Also, the development of a concentrated solids' surface layer during osmosis could have reduced sugar penetration during the final stages of osmosis. Thus, trans-membrane water flux is probably prevailing in these samples instead of diffusion of external solutes, which reduced SG/WL ratio, indicating a better efficiency of water removal with lower sugar uptake. During the DI process, more than 70% of water was removed from fruits and, at the same time, the solid gain reached to ≈20% of the initial mass. The corresponding values for fruits treated by WI were 63% WL and 30% SG. In spite of the differences observed in water loss and solute penetration, as expected, the final water and sugar content of infused fruits was independent of type of treatment (Table 2), the values being defined by the final equilibrium condition ($a_w = 0.85$) described in the methodology.

Although the total sugar content (TS) of raspberries significantly increased after infusions, there was a reduction in reducing sugars content (RS). The statistical study of the equilibrium RS content showed slight but significant differences between dry and wet infused samples. On the other hand, samples exhibited a significant decrease in TA when compared to the control, mainly after wet

TABLE 2
Water content, total sugars (TS), reducing sugars (RS), pH, acidity (TA), and volumetric shrinkage (Sh) of raspberries subjected to different infusion treatments

Sample	Water content (%, w.b.)	TS (%, w.b.)	RS (%, w.b.)	pH	TA (%, w.b.)	Sh (%)
WI	49.6 ± 1.2^{a}	55.6 ± 1.5^{b}	1.72 ± 0.07^{Aa}	3.513 ± 0.012^{d}	0.32 ± 0.03^{a}	27 ± 3^{Aa}
WI-AC	50.7 ± 0.8^{ab}	58.5 ± 0.4^{cd}	1.74 ± 0.02^{Aa}	2.36 ± 0.03^{a}	2.16 ± 0.04^{d}	30.94 ± 4.04^{Aa}
WI-B	48.4 ± 0.4^{a}	53.9 ± 0.1^{a}	1.75 ± 0.12^{Ab}	3.58 ± 0.03^{d}	0.309 ± 0.009^{a}	23 ± 3^{Ab}
WI-BAC	51.1 ± 0.4^{ab}	59.9 ± 0.1^{ef}	1.69 ± 0.02^{Aa}	2.36 ± 0.05^{a}	2.01 ± 0.58^{d}	$27 \pm 3^{Aa.b}$
DI	51.4 ± 0.5^{ab}	54.4 ± 0.9^{b}	1.76 ± 0.02^{Ba}	3.19 ± 0.03^{c}	0.76 ± 0.02^{b}	50.75 ± 2.08^{Ba}
DI-AC	52.5 ± 0.5^{b}	57.5 ± 0.1^{de}	1.77 ± 0.06^{Ba}	2.53 ± 0.04^{b}	2.45 ± 0.03^{e}	47 ± 4^{Ba}
DI-B	51.6 ± 3.4^{a}	53 ± 3^{c}	1.66 ± 0.06^{Bb}	3.16 ± 0.03^{c}	0.73 ± 0.04^{b}	40 ± 2^{Bb}
DI-BAC	53.7 ± 0.6^{b}	62.9 ± 0.6^{f}	1.72 ± 0.09^{Ba}	2.50 ± 0.02^{b}	2.21 ± 0.05^{e}	47 ± 7^{Bab}

Wet infusions: WI, WI-B, WI-AC, and WI-BAC; dry infusions: DI, DI-B, DI-AC, and DI-BAC. Means within columns with a different lowercase superscript letter are significantly different ($p < 0.05$). For RS and Sh variables, uppercase and lowercase superscript letters were used for main effect of factors: "type of infusion" and "additive," respectively.

infusions. Samples subjected to a more acid medium (AC or BAC) experimented a significant acidity increase for both dry and wet infusions. The stabilization of TA values in samples (Table 2) and also in the corresponding syrup occurred due to the formation of an acid equilibrium between the fruit and the osmotic medium and to the water and solute transport in the fruit tissue. Although the corresponding lowering in pH of these samples might have accelerated acid hydrolysis of sucrose along the process time (10 days), the leakage rate of natural reducing sugars appeared to be the main factor, since similar RS concentration was observed in both acidified and non-acidified fruits. This result is in agreement with those reported by other authors[4,36] in their studies concerning reuse of syrups in osmotic dehydration of apples and peaches, respectively, in which they concluded that the increase in RS of syrups was due to extraction from the fruit and not to enzymatic reactions or acid hydrolysis of non-reducing sugars, even when heating was applied during treatments.

The obtained syrups presented TS values ranging between 51 ± 0.1 and 54 ± 0.1 g sugar/100 g syrup, which means that, in spite of the dilution related to fruit dehydration, these syrups have a considerable osmotic potential to be reused as osmotic solution instead of discarding them. Furthermore, the reduction in syrup pH (≈2.4 units) due to acidification of the initial formulation in some conditions could be beneficial with respect to its conservation, making microbial growth more difficult during reuse.

Bioactive Compounds and Antioxidant Capacity

Total phenolics and anthocyanins content were significantly reduced ($p \leq 0.05$) during osmotic dehydration, mainly due to the dilution effect to the medium, compared to the control fruit (Table 3). Higher retention of phenolics was obtained after dry infusion (DI) compared to wet infusion (WI), and the addition of bisulfite caused the highest losses (Table 3). In the case of anthocyanin content, in comparison with the TPC, the decrease was much more evident after wet infusion treatments (Table 3). Several studies have also demonstrated that the osmotic dehydration leads to bioactive compounds' loss by diffusion to the osmotic solution. Osorio et al.[3] observed an anthocyanin reduction of 86% in Andes berries after osmotic dehydration with sucrose solutions at 30°C and Chottamom et al.[10] reported 52–61% reduction in anthocyanins and 51–68% reduction in phenolics in mulberries after 6 h soaking in 60% sucrose solutions at 35°C. Kucner et al.[11] studied the influence of temperature (30–70°C), osmotic dehydration duration (5–240 min), and some pretreatment methods on phenolics' content in highbush blueberry fruits. They concluded that 13.8, 17.5, and 54.7% of total phenolics content present in fresh blueberries migrated to syrups after 4 h of processing at 50, 60, and 70°C, respectively. Araya-Farias et al.[37] observed a 12% decrease in phenolics content of seabuckthorn berries osmotically dehydrated for six hours at 40°C.

The obtained syrups contained considerable amounts of bioactive compounds, especially those obtained with dried infusion treatments, where a minor syrup to fruit ratio is generated throughout the whole process. The final syrup/fruit ratio was ≈7 in wet infusions and ≈2 in dry infusions. Not only the enrichment of all syrups in polyphenols and anthocyanins could be verified, but also, in some cases (DI-B e DI-BAC), the consumption of 100 g syrup would be more beneficial in terms of bioactive compounds' intake than the consumption of 100 g processed raspberries. In the United States, average daily intake of anthocyanins has been estimated at 215 mg during the summer and 180 g during the winter,[38] these values being higher for regular red wine consumers. If we consider the consumption of a glass of red wine, which provides around 115 mg of

TABLE 3
Total phenolics content (TPC), monomeric anthocyanin content (ACY), antiradical activity (ARP), and antioxidant capacity (FRAP) of raspberries and syrups obtained after the application of wet infusions (WI, WI-B, WI-AC, and WI-BAC) and dry infusions (DI, DI-B, DI-AC, and DI-BAC) (wet basis)

Sample	TPC (mg gallic acid/100 g w.b.)	ACY (mg Cyd-3-glu/100 g w.b.)	ARP ($1/EC_{50}$) ($100\,g^{-1}$ w.b.)	FRAP (μmol Fe^{2+}/100 g w.b.)
		Fruits		
WI	140 ± 3^{b}	13.5 ± 1.2^{a}	38 ± 2^{b}	1773 ± 62^{a}
WI-AC	134.9 ± 0.9^{b}	10.2 ± 0.3^{a}	41 ± 3^{bc}	2146 ± 37^{a}
WI-B	119 ± 3^{a}	14 ± 2^{a}	24 ± 3^{a}	2067 ± 20^{a}
WI-BAC	136.2 ± 0.4^{b}	15.1 ± 1.5^{a}	38 ± 2^{b}	2113 ± 26^{a}
DI	160 ± 2^{c}	34 ± 2^{b}	37.5 ± 0.3^{b}	2099 ± 28^{a}
DI-AC	181 ± 4^{d}	29.3 ± 1.2^{b}	43.4 ± 0.9^{c}	2008 ± 70^{a}
DI-B	154 ± 4^{c}	30.4 ± 0.3^{b}	40.0 ± 1.2^{bc}	2097 ± 138^{a}
DI-BAC	179 ± 6^{d}	32.1 ± 1.2^{b}	44.6 ± 0.4^{c}	1880 ± 12^{a}
		Syrups		
WI	45 ± 2^{a}	4.7 ± 0.2^{a}	0.898 ± 0.007^{b}	178.2 ± 1.2^{bc}
WI-AC	46.8 ± 1.4^{a}	5.48 ± 0.07^{ab}	0.828 ± 0.006^{a}	178.19 ± 0.15^{bc}
WI-B	54 ± 3^{a}	13.4 ± 0.8^{c}	1.08 ± 0.02^{c}	177.02 ± 0.15^{ab}
WI-BAC	51.6 ± 1.3^{a}	6.20 ± 0.11^{b}	0.872 ± 0.012^{ab}	175.9 ± 0.4^{ab}
DI	173 ± 2^{d}	26.97 ± 0.12^{d}	1.154 ± 0.008^{d}	180.5 ± 0.6^{cd}
DI-AC	198 ± 4^{b}	28.9 ± 0.4^{e}	1.336 ± 0.008^{e}	182.6 ± 1.2^{de}
DI-B	205 ± 9^{bc}	37.23 ± 0.14^{g}	1.297 ± 0.003^{e}	184 ± 2^{e}
DI-BAC	211 ± 4^{c}	33.1 ± 0.2^{f}	1.189 ± 0.008^{d}	174.5 ± 1.2^{a}

For fruits and syrups, means within columns with a different lowercase superscript letter are significantly different ($p < 0.05$).

polyphenols,[39] a 100 g serving of the infused raspberries obtained in the present study would supply, in some cases (DI-AC and DI-BAC), over 50% of polyphenols provided by a glass of wine. Moreover, if this serving is composed of fruit immersed in syrup (fruit/syrup ratio = 5), as in canned fruit, the contribution of 100 g intake would be ≈60% higher.

It was observed that, when the balance of total compounds in fruits and syrups was carried out before and after treatments (data not shown), the total quantity of polyphenols in the whole system (fruits +syrup) after infusions increased around 70% when compared to control fruit, being more evident in samples with the combination of sodium bisulfite and citric acid. This could be ascribed to the way in which phenolic compounds were analyzed, which may require soluble molecules to be detected. It is noteworthy that all of the soluble phenolic compounds are accumulated in the cell vacuole. However, phenolic acids may be present in free and bound forms. Free compounds are extractable by aqueous organic solvents, but when phenolic acids exist as insoluble bound complexes, which are coupled to cell wall polymers through ester and glycosidic links, they are not extractable by solvents and they are typically liberated by hydrolysis with acids or bases or both.[40] Häkkinen et al.[41] indicated that ellagic acid released after acid hydrolysis was the main phenolic compound in the berries belonging to the genus *Rubus* sp, constituting 77–88% of the total phenolics. Therefore, considering the procedures used in this work, the total polyphenol content of control samples would not account for the phenolic acids bound to the cell wall of the fruit tissue. However, when a dehydration process is applied to a plant tissue, the integrity of the main structural elements at cellular level (cell wall, middle lamella, and plasma membrane) are modified.[13] Prinzivalli et al.,[42] when studying the effect of osmosis time on strawberry tissue subjected to osmotic treatments at 25°C, found a clear relationship between time of osmosis and solubilization of cell walls' polymers. Therefore, the changes in cell wall integrity and membranes due to osmosis would render the phenolics compounds of treated raspberries more accessible to extraction, which would explain the higher polyphenols amount observed in the global system (syrup + processed fruit) when compared to control fruit.

The antioxidant activity of raspberries produced by different infusion treatments is shown in Table 3. The inclusion of different assays (FRAP and DPPH•) was considered important so as to provide comprehensive information on the total antioxidant capacity of processed raspberries and to make precise estimates and comparisons

from the different samples.[43] In agreement with the observed loss of polyphenols and anthocyanins, the infusion treatments also led to a large reduction of antioxidant activity of raspberries. Moreover, some differences between one method and the other were detected when the different infusion treatments were compared. All samples exhibited the same antioxidant activity values against FRAP assay (Table 3) with ≈20% retention with respect to control fruit, which indicates that the decrease in antioxidant activity does not follow the same pattern as the decrease in the analyzed bioactive compounds.

In fact, ACY and TPC showed no correlation to changes in antioxidant capacity, indicating that factors other than anthocyanins and polyphenols are involved in the antioxidant potency in terms of the ability to reduce metals. Some authors have stated that the ascorbic acid is another compound present in raspberry fruits that contributes to the antioxidant capacity.[44] Sun-Waterhouse et al.,[45] when studying the effect of apple cell walls and pectin extracts on natural antioxidants by FRAP assay, found that water-soluble components leached from cell walls, including pectic polysaccharides, affected the antioxidant activity of ascorbic acid and quercetin. They showed that apple cell-wall components largely stabilized ascorbic acid antioxidant activity but offered little protection against quercetin degradation. On the other hand, several workers have demonstrated that Maillard reaction products can also increase antioxidant activities.[46]

When the effect of processing on DPPH$^{\bullet}$ radical scavenging ability of raspberries was analyzed (Table 3), the antioxidant capacity appeared to be more related to total phenolic content than to anthocyanin content, showing a relatively high positive correlation with TPC values ($p < 0.05$, $r = 0.78$) and a lower correlation with ACY ($p < 0.05$, $r = 0.57$). In spite of the fact that anthocyanin retention in dried infused samples was twice the values observed in samples subjected to wet infusions, this difference between the types of infusion was not observed in the phenol amounts obtained. Thus, its contribution to total antioxidant capacity was not significant.

Among the different treatments, the samples with bisulfite had the lowest antiradical capacity and samples with acid or with a combination acid/bisulfite presented the highest antiradical capacity.

In the case of syrups, as occurred in fruits, differences between TPC values of wet and dry infusions were not evidenced in the antioxidant activity by FRAP assay, while ARP of syrups obtained from dry infusions were higher than those from wet infusions. However, it is remarkable that the values of antioxidant capacity were very low despite the considerable high phenols content in all syrups. This could be explained because of the mobility that all molecules gain in solution, so that the interactions of phenolic compounds with other macromolecules released from cell wall cannot be avoided. These interactions are frequent in drinks such as wine and berry juice. For instance, during the aging of wine in the presence of air, the most significant modification consists of the appearance of complexes between tannins and macromolecules, such as proteins and polysaccharides, the progressive disappearance of free anthocyanins, and the appearance of complexes between tannins and anthocyanins.[47] This binding of tannin by macromolecules would limit the capacity of tannin to react with other compounds, which would explain the low antioxidant capacity obtained in syrups after 10 days of processing.

Since the antioxidant capacity determined by both assays is based on different reaction mechanisms, there may be certain differences when comparing particular samples. For instance, when antioxidant capacity is determined by FRAP assay, other compounds may absorb at 595 nm, and also any compound with a redox potential lower than 0.77 V, although it does behave as an antioxidant, may reduce iron.[43]

Superficial Color

Anthocyanins are the main pigments responsible for the color of raspberries; therefore, it would be expected that the superficial color of the processed raspberries would change due to the decrease of these compounds. Figure 2 shows the global color changes of the different treated fruits compared to the control sample, and Fig. 3 shows the visual aspect of the different samples. The highest

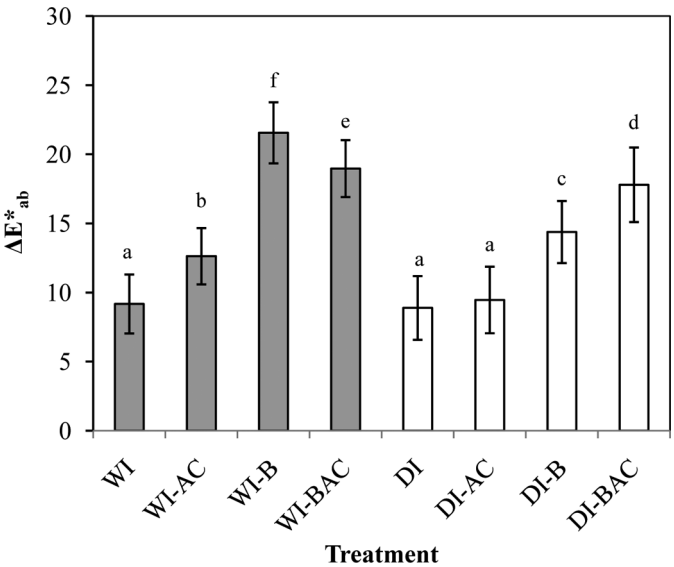

FIG. 2. Global color change (ΔE^*_{ab}) of raspberries after the application of treatments. Wet infusions: WI, WI-B, WI-AC, and WI-BAC. Dry infusions: DI, DI-B, DI-AC, and DI-BAC. Vertical bars represent standard deviation of the mean. Means with a different lowercase letter are significantly different ($p < 0.05$).

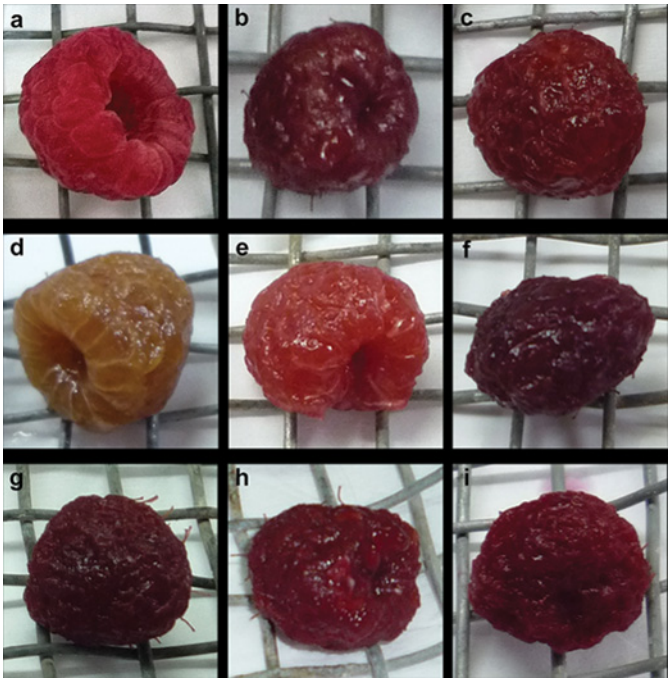

FIG. 3. Images obtained after the application of sugar infusions treatments. Control (a). Wet infusions: WI (b), WI-AC (c), WI-B (d), and WI-BAC (e). Dry infusions: DI (f), DI-AC (g), DI-B (h), and DI-BAC (i).

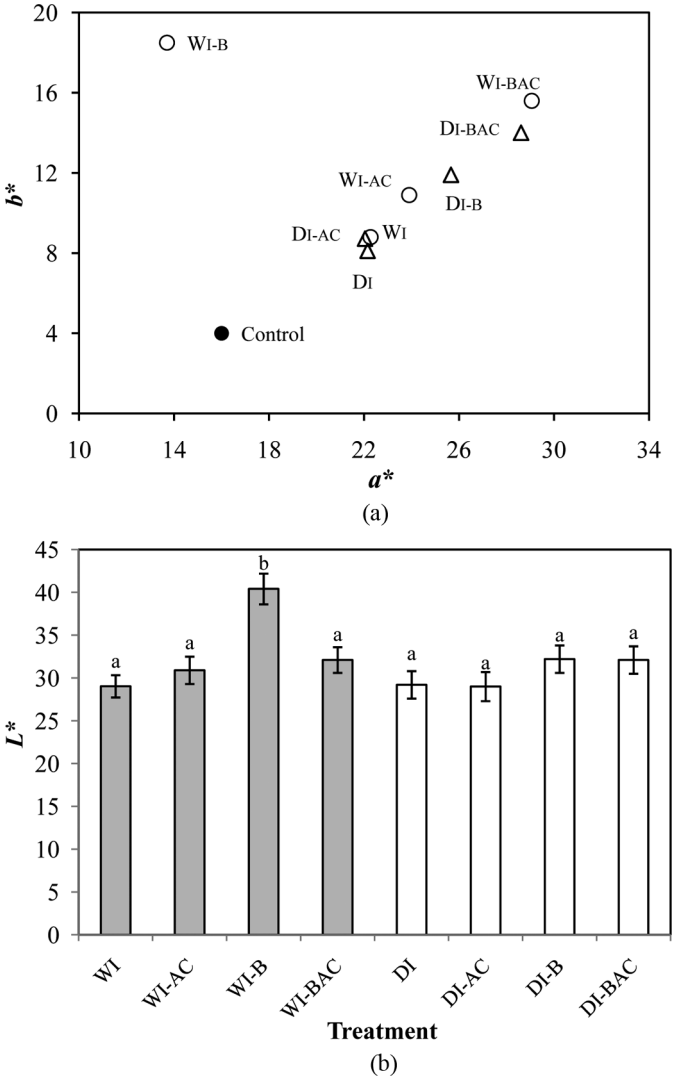

FIG. 4. Colorimetric parameters obtained in raspberries after the application of treatments. (a) b^* versus a^*; (○) wet infusions (WI, WI-B, WI-AC, and WI-BAC); (△) dry infusions (DI, DI-B, DI-AC, and DI-BAC). (b) Lightness (L^*). Vertical bars represent standard deviation of the mean. Means with a different letter are significantly different ($p < 0.05$).

ΔE^*_{ab} values were observed for the samples containing sodium bisulfite, particularly WI-B.

To better understand the causes of the global color changes, the variables L^*, a^* and b^* were analyzed (Fig. 4). In general, a displacement to higher a^* and b^* values can be seen with respect to the control sample (Fig. 4a). The addition of sodium bisulfite alone or a combination of sodium bisulfite and acid to the infusions caused the highest increases in both color coordinates, except for WI-B, which showed a large increase in b^* and a decrease in a^*, turning the sample to a yellowish color. L^* variable (Fig. 4b) presented an increase for all of the treated fruits when compared to the control sample ($L^* = 24 \pm 2$). The WI-B samples showed the largest L^* increase. The images presented in Fig. 3 showed that the color changes detected by measuring tristimulus parameters were subtle to the eye, indicating no major changes in the appearance of osmotically concentrated raspberries, except for fruits treated by WI-B treatment. The particular behavior observed for the WI-B samples could be attributed to the combination of the anthocyanin pigments with sodium bisulfite to form colorless compounds (sulfonic adducts).[20] This effect was not observed in the superficial color of DI-B samples. In this case, the ACY content was twice that observed for wet infusion samples, because the dilution effect to the medium was less relevant and therefore the added bisulfite was not enough to react with the entire sample. This reaction can be reversed in an acid medium, and this fact could explain the higher a^* values observed in samples treated with bisulfite and acid (WI-BAC) compared to those that only had bisulfite (WI-B). For this reason, even though the WI-B samples look colorless, they have the same ACY content as the WI samples. According to these results, it can be concluded that color deterioration cannot be characterized by changes in ACY alone, which was also stated by other authors for some berry products.[48]

Also, the pH differential method is a measure of the monomeric anthocyanins. The polymeric anthocyanins formed by condensing monomeric anthocyanins with other

phenolic compounds form colored polymeric compounds. These polymerized anthocyanin pigments, brown pigments that originated in enzymatic browning, Maillard reactions, or degradation of anthocyanins, do not exhibit a reversible behavior with pH. Therefore, they are excluded from the calculation of the absorbance, but contribute to the color intensity.[27] For this reason, we determined other parameters which may allow us to better explain the color changes observed, such as % polymeric color (%PC) and Browning Index (BI) (Fig. 5). In general, samples with dry infusion showed higher BI values than those with wet infusions, except for DI-BAC. This behavior can be ascribed to the fact that fruits were initially placed in contact with a dry mixture of solutes, so that some zones of the fruit surface were exposed to oxygen, at least during the early stages of the process, when syrups were still not generated. As a result, a partial decompartmentalization of cells during thawing of control raspberries upon osmosis could cause a certain level of browning. In contrast, raspberries under wet infusions were surrounded by syrup throughout the complete course of the process. This way, enzymatic browning was retarded by the elimination of oxygen from the surface, allowing the preservatives to act.

The infusion treatments caused a higher % of compounds with polymeric structure in comparison with the control. PC values include the presence of brown polymers' pigments and monomeric anthocyanins that, during the infusion process, may be polymerized. Percentage of polymeric color (%PC) is a measure of the pigment resistance to bleaching and indicates, to some degree, the anthocyanins' polymerization. The DI-BAC samples had the lowest percentage of polymeric color, exhibited a low browning index, and were among the samples with higher anthocyanin content. The acidity achieved in these fruits increased over 100% with a corresponding decrease of 0.63 units of pH, which was beneficial for color stabilization. On the one hand, the acid environment allows the anthocyanin resonance structures to move to the formation of flavylium cation, a molecular structure with greater stability than the other resonance forms. In addition, not only the absorption of this cation on a suitable substrate (copigment) could stabilize anthocyanins by intermolecular copigmentation, but also anthocyanins could form strong bonds with groups of organic acids (in this case citric) favoring intramolecular copigmentation.[17,19] On the other hand, the higher acidity could inhibit enzymatic browning of fruits, either by acting on polyphenol oxidase (PPO) directly or by reducing the o-quinones produced by PPO catalysis to the less reactive diphenols, preventing the development of later condensation of complex brown melanins.

Analysis of Principal Component (PCA)

PCA analysis was applied to detect patterns between the variables and samples analyzed. In this study, PCA resumed the information of 17 variables measured on eight different infusions in two new, uncorrelated variables termed "principal components" (PC1 and PC2). PC1 explained 42.4% of the total variance of the data set while PC2 explained 24.6%. Several observations may be made from the sample score plot for PC1 vs. PC2 (Fig. 6). On the one hand, samples subjected to dry infusions are located on the right side of the graph, while samples with wet infusions are located on the left side. Additionally,

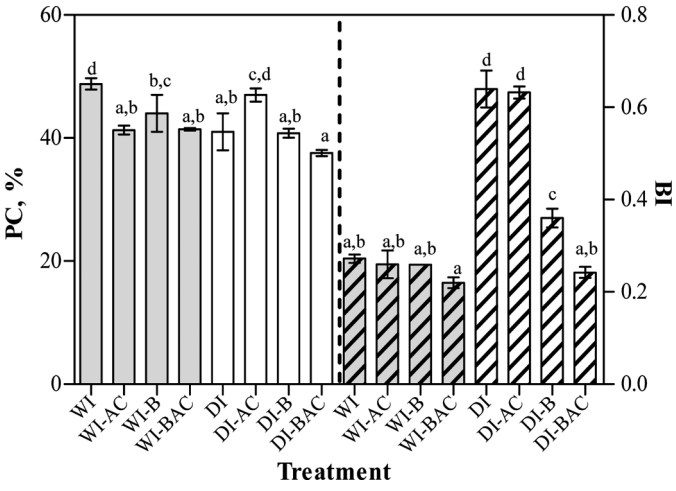

FIG. 5. Percent of polymeric color (% PC) (empty bars) and browning index (BI) (striped bars) of raspberries after the application of treatments. Wet infusions: WI, WI-B, WI-AC, and WI-BAC. Dry infusions: DI, DI-B, DI-AC, and DI-BAC. Vertical bars represent standard deviation of the mean. Means with a different letter are significantly different ($p < 0.05$).

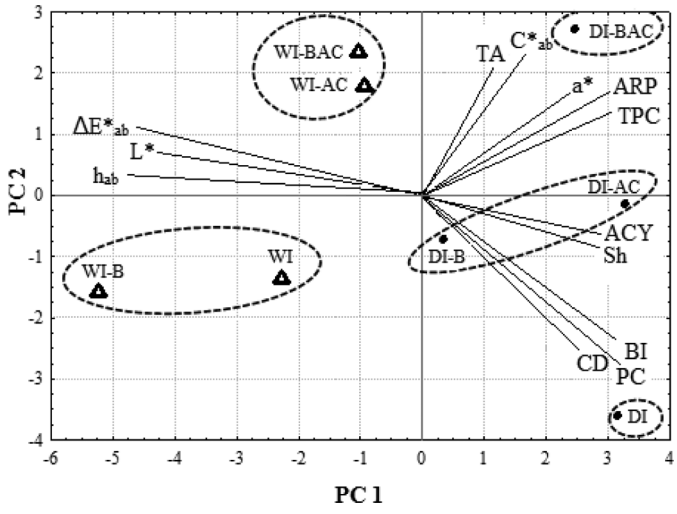

FIG. 6. PCA two-dimensional scatter plot based on the first two principal components (PC1 and PC2) generated for the studied infusions and based on data of the analyzed variables. (△) Wet infusions: WI, WI-B, WI-AC, and WI-BAC. (●) Dry infusions: DI, DI-B, DI-AC, and DI-BAC).

samples without additives (WI and DI) or with only bisulfite (WI-B and DI-B) are in the lower half, and samples with the addition of both additives (WI-BAC and DI-BAC) were grouped into the top half of the graph. The figure also shows that shrinkage and anthocyanin content are in opposite directions to the variables h_{ab}, L^* and ΔE^*_{ab}, thus suggesting that samples with lower concentration of pigments, despite exhibiting lower shrinkage, also presented greater overall change in color and yellow hues. The colorimetric variables, C^*_{ab} and a^*, are clustered together on the right quadrant along with the variables acidity (TA), total phenolics content (TPC), and antiradical activity (ARP), as a result of the correlation between the capacity of scavenger free radicals (ARP) with conservation of redness (C^*_{ab} and a^*) and phenolic compounds in samples with higher acidity, especially in dry infusion ones (DI-AC and DI-BAC). Samples subjected to DI presented higher values of BI, PC, and CD parameters. Principal component analysis is a useful tool to evaluate globally the samples' behavior, so that a new variable called "quality" can be drawn with similar direction to that of ARP, TPC, and a^* variables, considering the best attributes of quality related to color conservation ($>a^*$, $>C^*_{ab}$, $<h_{ab}$, and >ACY retention), a higher content of polyphenols, and therefore higher antiradical activity. According to this, raspberries treated with DI had better attributes than those treated with WI; DI-BAC samples appeared to be a product with higher quality, while WI-B samples could be considered minor-quality products. Also, the diagram shows that, in both infusions without additives, or with sodium bisulfite and citric acid added separately the final quality of the product was damaged, while the combination of both additives improved the color and the retention of bioactive compounds.

CONCLUSIONS

The methodological approach carried out in the present work allowed us to conclude that the evaluation of color in processed raspberries must be done not only through measurements of colorimetric parameters and monomeric anthocyanin content, as frequently performed in most studies concerning berries and cherries. Studies must be accompanied by the determination of other parameters, such as browning index and polymeric color percentage, in order to analyze more deeply brown pigments' development and red pigments' degradation, both aspects with a big impact on color quality.

In this work, we applied infusion technologies that may represent efficient and reliable alternatives to generate shelf-stable products of intermediate humidity based on raspberries, very similar to some commercial products like canned fruits or preserves, but with improved quality in terms of appearance, particularly in color retention. In this sense, the best osmotic treatment for raspberries was DI-BAC, which included a dry infusion with sucrose and the addition of citric acid and sodium bisulfite. The incorporation of acid as an ingredient may provide an advantage, as the increase in acidity of fruits and syrups would compensate for the sweetness increase due to the added sugars.

The processed raspberries obtained can be consumed as is or can be used as an ingredient in foods such as desserts and dairy and confectionary products. If fruits are presented with surrounding syrup, the total quantity of polyphenols incorporated in a serving would be higher than that provided by infused fruits.

However, it is necessary to streamline processes in terms of bioactive component retention. In particular, the numerous and complex mechanisms by which bioactive compounds degrade during sugar infusion treatments demand more research focus on finding new combinations of preservation factors that increase bioactive retention.

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