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The use of coffee (*Coffea arabica* L.) pulp in the preparation of a beverage with antioxidant properties

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Abstract

The main by-product of the coffee industry is the fruit pulp. Therefore, finding alternatives for its use is crucial to avoid water and environmental pollution, since the residue contains high amount of organic matter. The aim of the present work was to evaluate the use of coffee pulp in the formulation of a beverage (tisane). Tisanes were formulated by combining coffee pulp, blueberry and strawberry, based on the levels of a rotatable central composite design. Eighteen combinations were evaluated by specialised coffee tasters, and the two best formulations (6 and 14) were selected. These two formulations were then sensory-evaluated by 100 consumers in terms of appearance, colour, aroma, body, sweetness, and flavour. Most consumers enjoyed both formulations, with formulation 14 (3.75 g coffee pulp, 3.75 g blueberry, and 5.75 g strawberry) received higher preference of 92%, 87% would buy it if it became available on the market, and 99% would buy it if it had health benefit. Based on chemical and nutritional analyses, formulation 6 yielded 26.95 mg GAE/g of total phenolic compounds, and 9,110.65 µmol Trolox/g of antioxidant activity. These indicated that the formulated beverage had high antioxidant capacity as well as high consumer acceptance.

Introduction

Reducing pollution from organic residues is one of the biggest challenges faced by the food industry. In coffee-processing industry, pulp is the main by-product; for every two tons of coffee cherries processed, one ton of pulp is obtained. This residue contains a considerable amount of organic matter which pose a problem to the environment (Jaisan and Punbusayakul, 2016; Heeger *et al*., 2017). In order to utilise coffee pulp, several studies have evaluated the production of different materials such as compost, biogas, bioethanol, fuel briquettes, enzymes, and foods such as mushrooms and nutritive flours (Blinová *et al*., 2017). However, there are fewer still studies which focused on food and beverage development from coffee pulp despite the fact that it is rich in polyphenols which provide antioxidant properties that may benefit health, and has a caffeine content similar to that found in coffee beans (Torres-Mancera *et al*., 2011).

Coffee, as a beverage, is widely used as a stimulant, and highly valued for its particular organoleptic properties. Similarly, beverage prepared with dry coffee pulp is increasing in popularity due to the fact that it has protective powers against free radicals, in addition to its pleasant taste (Heeger *et al*., 2017). It has been reported that the preparation of coffee pulp tisanes by hot water infusion allows for the extraction of polyphenols which strengthen the immune system, and reduce the risk of cancers and infections (Cittan *et al*., 2018). Therefore, the present work aimed to look for alternative to utilise the coffee pulp by producing a beverage (tisane). To this end, different formulations based on coffee pulp, blueberry, and strawberry were prepared, and two formulations with the highest sensory scores were selected. The best of the two formulations was then subjected to chemical and nutritional characterisations to determine its antioxidant properties.

Materials and methods

The present work was carried out at the Colegio de Postgraduados (COLPOS), Córdoba

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Campus, located at 18°51’20’’N and 96°51’38’’W in the Municipality of Amatlán de los Reyes, Veracruz, Mexico.

Chemicals and reagents

Chemicals and reagents used in the present work were boric acid (H_3BO_3) (Fagalab, Mocorito, Sinaloa, Mexico), phenol (C_6H_6O) (Sigma-Aldrich, St. Louis, MO, USA), ethanol at 70% (C_2H_5OH) (Meyer, CDMX, Mexico), zinc sulphate ($ZnSO_4$) (Fagalab, Mocorito, Sinaloa, Mexico), potassium ferrocyanide ($C_6FeK_4N_6 \cdot 3H_2O$) (Fermont, Monterrey, Mexico); methanol (CH_3OH) (J.T. Baker, Madrid, Spain), acetone (C_3H_6O) (J.T. Baker, Madrid, Spain), DPPH reagent ($C_{18}H_{12}N_5O_6$) (Sigma-Aldrich, St. Louis, MO, USA), Trolox ($C_{14}H_{18}O_4$) (Sigma-Aldrich, St. Louis, MO, USA), Folin-Ciocalteu reagent ($C_{10}H_5NaO_5S$) (Sigma-Aldrich, St. Louis, MO, USA), sodium bicarbonate ($NaHCO_3$) (J. T. Baker, Madrid, Spain), and gallic acid ($C_7H_6O_5$) (Fagalab, Mocorito, Sinaloa, Mexico).

Plant materials

The coffee pulp was obtained from the wet process facility at the Colegio de Postgraduados (COLPOS), Córdoba Campus, from healthy and mature fruits of the *Coffea arabica* L. (Costa Rica variety). Pulping was done using a Penagos model 2500 ecological module (Santander, Colombia). The strawberries (2 kg, frozen, Great Value® brand) and blueberries (120 g, dehydrated, Verde Valle® brand) used in the formulations of tisane were purchased from a local market.

Preparation of ingredients and formulations

Strawberries and coffee pulps were dehydrated in a Terlab oven (Guadalajara, Jalisco, Mexico) for 24 - 48 h; 35°C for strawberries, and 40 ± 5°C for coffee pulps, until they reached 12% moisture content. Ingredients were stored in hermetic containers at room temperature. The coffee pulp-blueberry-strawberry infusions were prepared in accordance with the formulations established in a rotatable central composite design (RCCD) with 18 formulations each with 1 L of water, as shown in Table 1.

Table 1. Tisane formulations.

Formulation	Coffee pulp (g)	Blueberry (g)	Strawberry (g)	CCRD
1	2.5	2.5	2.5	Factorial points
2	5.0	2.5	2.5	
3	2.5	5.0	2.5	
4	5.0	5.0	2.5	
5	2.5	2.5	5.0	
6	5.0	2.5	5.0	
7	2.5	5.0	5.0	
8	5.0	5.0	5.0	
9	1.75	3.75	3.75	Axial points
10	5.75	3.75	3.75	
11	3.75	1.75	3.75	
12	3.75	5.75	3.75	
13	3.75	3.75	1.75	
14	3.75	3.75	5.75	
15	3.75	3.75	3.75	Central points
16	3.75	3.75	3.75	
17	3.75	3.75	3.75	
18	3.75	3.75	3.75	

For the RCCD, three factors were considered namely coffee pulp, blueberry, and strawberry; each at two levels: low (2.5 g) and high (5.0 g). The centre point was 3.75 g, while the axial points were 1.75 and 5.75 g.

Sensory evaluation

To select the two most preferred tisanes out of the 18 formulations (Table 1), an order test was applied according to the degree of preference, with eight trained tasters specialised in the sensory evaluation of coffee brews who have several years of experience in the coffee industry. The evaluation was carried out in two steps at the Coffee Quality Laboratory of the Colegio de Postgraduados. Formulations were served in 200 mL tasting cups, and coded in random order. In the first step, each taster was given all 18 formulations in randomly selected batches of six treatments at a time by adapting the Specialty Coffee Association of America protocol for coffee cupping (SCAA, 2015), and using water to rinse between each formulation. The six samples of any batch were ordered according to the degree of preference, and scored from 1 (least preferred) to 6 (most preferred). The scores from the eight tasters for a given sample were summarised, and samples with more than 30 points were selected for a second-step evaluation, in a similar manner.

From the sensory evaluation by the specialised tasters, the two formulations (6 and 14) with the highest preference scores were selected and evaluated by 100 consumers in the city of Tehuacán, Veracruz, Mexico. A nine-point hedonic test was applied; 1 = dislike very much; 2 = dislike a lot; 3 = dislike enough; 4 = dislike a bit; 5 = neither like nor dislike; 6 = like a bit; 7 = like enough; 8 = like a lot; and 9 = like very much. This scale was used to evaluate the appearance, colour, aroma, body, sweetness, and flavour of the two selected formulations. Samples were presented in identical containers, and coded with random three-digit numbers.

The chemical and nutritional characterisations were performed for the formulation (6) with the highest preference of the two, which had 5.0 g of coffee pulp, 2.5 g of blueberry, and 5.0 g of strawberry.

Proximate analysis

The moisture content was determined by method NMX-F-428-1982 (Norma Mexicana, 1982) using a Mettler Toledo HG63 thermobalance (Columbus, OH, USA). The crude fat (ethereal extract) was determined by the Soxhlet method NMX-F-089-S-1978 (Norma Mexicana, 1978b). The fibre content was determined by method NMX-F-090-S-1978 (Norma Mexicana, 1978c). The ash content was determined by the dry incineration method NMX-F-066-S-1978 (Norma Mexicana, 1978a) using a Scorpion Scientific muffle model A51120 (Mexico City, Mexico). The crude protein content was determined by method NMX-F-068-S-1980 (Norma Mexicana, 1980).

Total sugars

The total sugars were determined by the Dubois method (phenol-sulfuric method) (Ávila *et al*., 2012) using a Genesys 10S UV/vis spectrophotometer (Thermo Fisher, Madison, WI, USA). Glucose was used as the standard. Measurements were done in triplicate at 490 nm.

Reducing sugars

The reducing sugars were determined by the Miller method (DNS) (Cortes Ortiz *et al*., 2015) also using a Genesys 10S UV/vis spectrophotometer. Glucose was used as the standard. Measurements were done in triplicate at 490 nm.

Antioxidant activity

The extraction of antioxidant compounds was performed following the method of Pérez-Jiménez *et al*. (2008) with slight modifications. Samples were dehydrated to up to 12% moisture content. Then, 10 mL of methanol/water acidified with 2 N HCl (50:50 v/v, pH 2) was added to 0.5 g of the dehydrated sample. The mixture was subjected to a water bath with agitation at 50°C for 30 min. The mixture was then centrifuged at 4,000 rpm for 15 min at 4°C, the supernatant was then recovered and stored in the dark in 50 mL volumetric flask at 4°C. The residue was subjected to a second extraction with 10 mL of acetone/water (70:30 v/v), and agitated at 50°C for 30 min. Subsequently, the mixture from the second extraction was centrifuged at 4,000 rpm for 15 min at 4°C, and the supernatant was recovered. A total of four acetone/water (70:30 v/v) extractions were performed. Finally, the five supernatants were pooled and brought to 50 mL with double-distilled water, then the extract obtained was stored in tubes at -20°C in the dark until further analysis.

The antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazil) technique. Firstly, 0.1 mM DPPH solution was prepared in methanol, and the absorbance was adjusted to 0.7 ± 0.1 at 515 nm with methanol. Into 3.9 mL of this solution, 0.1 mL of extract was added. It was then vortexed for 30 s. After 30 min, absorbance was measured in triplicate at 515 nm using a spectrometer (Genesys 10S UV-visible Thermo Scientific, Thermo Fisher, Madison, WI, USA). For the calibration curve, Trolox (6-hydroxy-2,5,7,8-tetramethylcroman-2-carboxylic acid) was used. The results were expressed as percentage of inhibition of the DPPH radical and mM Trolox/g of dried pulp, and were computed using Eq. 1.

$$\% \text{ inhibition of DPPH} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \quad \text{(Eq. 1)}$$

Phenolic compounds

The concentration of total phenolic compounds was determined using the Folin-Ciocalteu reagent following the modified method of Rojas-Barquera and Narváez-Cuenca (2009). To 20 µL of extract, 1.58 mL of distilled water and 100 µL of a 10% solution of Folin-Ciocalteu reagent were added. The mixture was vortexed and left to stand in the dark for 5 min. Subsequently, 300 µL of a 20% solution of sodium bicarbonate was added to this mixture, shaken, and left to react for 2 h at room temperature. The absorbance was measured in triplicate at 765 nm using a spectrometer (Genesys 10S UV-visible Thermo Scientific, Thermo Fisher, Madison, WI, USA). A calibration curve was prepared using gallic acid. Results were expressed as mg gallic acid equivalent (GAE) per g of sample.

Statistical analysis

Wilcoxon rank-sum tests for independent samples were performed to determine if the distribution of scores in the two formulations was the same for the different sensory attributes. The analysis was complemented with one-way non-parametric analysis of variance (Kruskal-Wallis rank-sum test), to determine if, for any sensory attribute, the two formulations had an identical score distribution. The relationship between the different sensory attributes was evaluated by computing Spearman's correlation coefficient (rho). Analyses were performed using the statistical software R (http://www.rproject.org) version 3.6.2.

Results and discussion

Sensory evaluation

Following the sensory evaluation of the 18 formulations, the two most preferred formulations listed by the tasting panel were selected (Table 2).

Table 2. Sensory score of tisane formulations.

Selection step	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Step 1	10	25	**36**	25	30	**38**	**37**	**39**	20	25	24	**36**	9	**36**	**35**	30	25	24
Step 2			17			**46**	18	29				24		**35**	15			

These were formulation 6 (5.0 g of coffee pulp, 2.5 g of blueberry, and 5.0 g of strawberry) and formulation 14 (3.75 g of coffee pulp, 3.75 g of blueberry, and 5.75 g of strawberry). These formulations were sensory-evaluated by 100 consumers, 60% women and 40% men aged from 18 to 24 years (36%), 25 to 34 years (18%), 35 to 44 years (14%), 45 to 54 years (20%), and 55 years and older (12%). Among these, 84% said they consumed tea, herbal tea, or infusions. The frequency of consumption was eventually (43%), once a week (29%), once a month (14%), and once a day (14%), with 57% preferring herbal teas, 18% preferring fruit teas, 3% preferring energizers, 2% preferring detoxifiers, and 4% preferring other types of commercial teas. With regard to the form of consumption, 56% of the surveyed population consumed hot tisanes where 29% preferred it warm, 14% preferred it cold, and 4% preferred iced tisanes.

The results of the consumers' acceptability evaluation for the two formulations based on appearance, colour, aroma, body, sweetness, and flavour are shown in Figure 1. It can be seen that most of the consumers evaluated the two formulations with scores of between 7 and 8 in all the evaluated attributes. This is consistent with Jiménez *et al*. (2017) who mentioned that the sensory quality of foods of plant origin is mainly influenced by the phenolic compounds present which determine the appearance, flavour, and aroma.

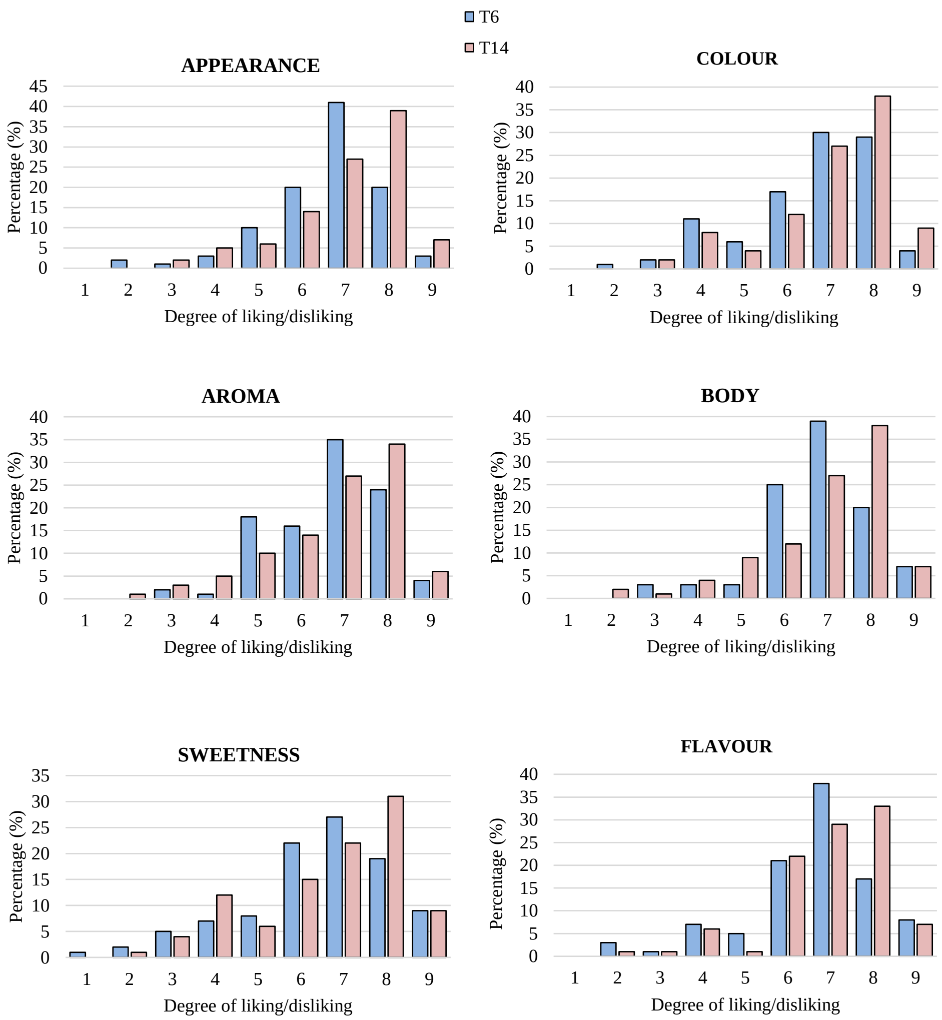

Figure 1. Enjoyment level of sensory attributes for formulation 6 (5.0 g coffee pulp, 2.5 g blueberry, and 5.0 g strawberry) and formulation 14 (3.75 g coffee pulp, 3.75 g blueberry, and 5.75 g of strawberry), represented as percentage (%) in each of nine-point hedonic scale, where 1 = dislike very much; 2 = dislike a lot; 3 = dislike enough; 4 = dislike a bit; 5 = neither like nor dislike; 6 = like a bit; 7 = like enough; 8 = like a lot; and 9 = like very much.

Table 3. Sensory characteristics of selected tisane formulations.

Formulation	Appearance	Colour	Aroma	Body
6	Herbal tea	Yellow	Chamomile, orange, and tamarind	Velvety
14	Medicine	Orange-reddish	Chamomile, orange, and tamarind	Slightly astringent

Descriptors of these properties for the two selected formulations are presented in Table 3.

According to consumers, the appearance of formulation 6 seemed more familiar to them as herbal tea, while formulation 14 resembled a medicine. Despite this, the appearance of formulation 14 was more liked than the appearance of formulation 6, with significant differences ($p < 0.05$) in the distribution of scores in favour of formulation 14. Formulation 14 was liked a lot by 39%, and very much liked by 7% of consumers, whereas formulation 6 was liked a lot by 20%, and very much liked by only 3% of consumers. Similar to appearance, the colour of formulation 14 (orange-reddish) was more liked than the colour of formulation 6 (yellow), with significant differences ($p < 0.05$) in the distribution of scores in favour of formulation 14. Jin *et al*. (2016) stated that colour is one of the most important characteristics of teas and infusions, and particularly relevant for market acceptance since colour is the first quality attribute evaluated in foods by consumers (Di and Da-Wen, 2013). It is interesting to note that there was a high correlation (Spearman's rho = 0.84) between the perception of colour and the evaluation of appearance in both formulations.

The two evaluated formulations contained three ingredients that provided particular colour, aroma, and flavour characteristics. Coffee pulp provided caffeine together with blueberries, chlorogenic, and caffeic acids (Diaconeasa *et al*., 2014; Blinová *et al.*, 2017). Most of these compounds are yellow to dark brown in colour, and confer bitterness and astringency. In addition, the oxidation of phenolic compounds to quinones produces enzymatic browning that impacts the colour of foods (Taranto *et al*., 2017).

Consumers detected aromatic notes similar to chamomile, orange, and tamarind, among other fruits for both formulations. Although more people (34%) liked a lot the aroma of formulation 14, as compared to 24% of those who liked a lot the aroma of formulation 6, the distribution of scores for this attribute in both formulations did not present significant differences ($p > 0.05$).

For body, formulation 14 was perceived as slightly astringent, while formulation 6 was perceived as velvety. However, the distribution of scores in both formulations did not present significant differences ($p > 0.05$). Nonetheless, 38% of the respondents liked a lot the body of formulation 14, as opposed to 20% who liked a lot the body of formulation 6. In addition, 7% of the respondents liked the body of both formulations very much.

The sweetness of both formulations did not present a significant difference ($p < 0.05$). Most of the consumers liked a lot or very much the sweetness of both formulations. It is interesting to note that the sweetness of the formulations was highly correlated (Spearman's rho = 0.85) with the perception of acidity in these beverages.

Beverage flavour is one of the most important parameters for evaluating beverage acceptability. In the present work, the two selected formulations did not show significant difference ($p > 0.05$) in the distribution of scores for this attribute, although 33% of consumers reported that they liked a lot formulation 14, as compared to 17% who reported that they liked a lot formulation 6.

The results of the sensory evaluation showed that formulation 14 was slightly superior, at least in appearance and colour, to formulation 6, possibly due to a higher proportion of strawberry. Finally, 92% of consumers considered formulation 14 acceptable, 87% would buy it if it was available on the market, and 99% would buy it if it had health benefits. In the case of formulation 6, it had an acceptance rate of 93%; where 84% would buy it if it was available on the market, and 97% would also buy it if it had health benefits.

Chemical and nutritional characterisations

Chemical and nutritional characterisations were performed for formulation 6 which scored the highest preference (Table 4).

Table 4. Chemical, nutritional, and antioxidant properties of the most preferred tisane sample (formulation 6).

Parameter	Content
Moisture	15.4%
Fat	0.7%
Fibre	4.8%
Ash	2.5%
Protein	0.4%
Reducing sugars	520.82 ± 84.20 µg/mL
Total sugars	1,867.09 ± 91.28 µg/mL
Antioxidant activity	9,110.65 µmol Trolox/g
Total phenols	26.95 mg GAE/g

Tisanes are fruit infusions or herbal-fruit mixtures, prepared with very hot or boiling water to dissolve the soluble fraction of their components. Given the composition of its ingredients, the protein and fat contents in formulation 6 was low, since strawberry and blueberry have less than 1% of each macronutrient, while coffee pulp has a protein content of 10.85% and 1.21% fat (Munguía *et al*., 2018).

Polyphenols have a biological activity that can prevent chronic diseases related to inflammation such as diabetes, obesity, neurodegeneration, cancers, and cardiovascular diseases (Goszcz *et al*., 2017; Shalaby, 2018; Yahfoufi *et al*., 2018). Synthetic antioxidants are in disuse based on studies that showed their carcinogenic effects; so, interest has turned towards natural antioxidants (Shalaby, 2018).

Agro-industrial residues, such as coffee pulp, are inexpensive and they have proven to contain significant levels of phenolic compounds such as caffeic and chlorogenic acids, which have antioxidant properties as well as hypoglycaemic and antiviral activities (Arellano-González *et al*., 2011; Vijayalaxmi *et al*., 2015; Olmos-Padilla *et al*., 2019). In the case of blueberries, large concentrations of caffeic and chlorogenic acid have been found (Diaconeasa *et al*., 2014), thus enhancing the beneficial effects of the formulated tisane. The main flavonoids present in the fruits are quercetin and kaempferol, especially in strawberries (Panche *et al*., 2016).

Since fruit and herbal infusions are major sources of polyphenolic antioxidants, related studies have focused on evaluating their antioxidant capacity, as well as their total phenolic content (Belščak *et al*., 2011). Fu *et al*. (2011) reported a range from 32 to 1,395 mg GAE/L with a high correlation between phenols and antioxidant activity in 51 types of commercial teas made in China, thus showing that phenolic compounds are the major components responsible for the antioxidant activity in this type of beverage.

Jungmin *et al.* (2013) reported the extractions of phenolic compounds in different tisanes using water or ethanol, and presented their results in mg GAE/g. The amount of extracted phenols depended on the tisane, *e.g.*, peppermint (water, 75.31; ethanol, 33.68), blackberry leaves (water, 11.64; ethanol, 10.98), bamboo leaves (water, 11.50; ethanol, 14.93), and mate (water, 27.93; ethanol, 66.86). The tisane with coffee pulp formulated in the present work showed a phenolic content similar to that of mate extracted in water.

Şahin (2013) showed that phenol, flavonoid, and anthocyanin contents in tisanes from 16 different fruits were dominant, and increased with infusion temperature. For pomegranate, blueberry, and strawberry at 100°C, the phenolic contents were 6.9, 5.6, and 4.6 mg GAE/g, respectively, and the antioxidant activities were 67, 50, and 43 mg Trolox/g, respectively. In the case of coffee pulp, different values of total phenols on a dry basis have been found, *e.g.*, 6.08, 9.17, and 4.09 mg GAE/g (Heeger *et al*., 2017; Fierro-Cabrales *et al*., 2018). In comparison to those reports, the formulation with coffee pulp, blueberry, and strawberry prepared in the present work showed a higher amount of phenolic compounds and antioxidant activity.

Conclusion

In the present work, two formulations of tisane based on coffee pulp, blueberry, and strawberry were selected out of 18 different treatment combinations. Formulation 6 (5.0 g coffee pulp, 2.5 g blueberry, and 5.0 g strawberry) and formulation 14 (3.75 g pulp, 3.75 g blueberry, and 5.75 g strawberry) were chosen by a group of specialised coffee tasters, based on their degree of preference. These two formulations were sensory evaluated, through a hedonic test, by 100 consumers. Both formulations scored higher on the positive part of the scale for all attributes (appearance, colour, aroma, body, sweetness, and flavour), showing significant differences ($p < 0.05$) only in appearance and colour. Chemical and nutritional analyses on formulation 6 were performed, showing a large quantity of total phenols as well as a high antioxidant capacity, which indicated that phenolic compounds were mainly responsible for the antioxidant activity. As a result, tisanes based on coffee pulp could be a promising alternative for the use of this by-product to obtain a natural beverage with antioxidant properties and excellent acceptance by consumers.

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