Sensory, physico-chemical and microbiological characterization of coffee substitute based on *Brosimum alicastrum*

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Summary

For health or personal reasons, alternatives to the *Coffea arabica* drink are being investigated. In this work, a coffee substitute based on *Brosimum alicastrum* seeds was developed and characterized. Two drying and roasting methods were applied, namely, solar dehydration with solar roasting (M1) and solar dehydration with oven roasting (M2). Three treatments were formulated: T1 (417 g·kg^{-1} M1 and 417 g·kg^{-1} M2), T2 (500 g·kg^{-1} M1 and 334 g·kg^{-1} M2) and T3 (334 g·kg^{-1} M1 and 500 g·kg^{-1} M2). Physico-chemical properties and palatability were evaluated using a randomized design. The microbiological properties met the limits established by the corresponding Mexican standards. T1, T2 and T3 had average pH 5.7 and water activity a_W of 0.5. No differences were in moisture, ash, protein and fibre ($P > 0.05$). T2 and T3 presented higher content of fat (43 g·kg^{-1} and 46 g·kg^{-1}; $P < 0.05$). Absence of coliforms and fungi was observed. The hedonic test indicated that M2 conferred sensory characteristics to T3, registering the same palatability as commercial coffee. It is concluded that with an adequate treatment, the seeds of *Brosimum alicastrum* can be a substitute for coffee.

Keywords

Brosimum alicastrum; breadnut; coffee substitute

Brosimum alicastrum is native to tropical America, predominates in southern Mexico, as well as in Central America and Colombia, Peru, Venezuela, Cuba and Jamaica. It is known by various names, for example, in Honduras it is called “másica”, in Nicaragua “ojoche”, in El Salvador “ojushte”, in Panama “berba”, in Guatemala “ramón” and in Mexico it has more than 50 names, mostly in indigenous languages, among which “Oox” (Yucatán and Quintana Roo), “Moj-cuji” (Veracruz) and “Ash-Ahx” (Chiapas) are mentioned [1]. The seed of *B. alicastrum* was considered a staple food for the Mayan population, who mixed it with maize flour to make tortillas. This seed contains 700 g·kg^{-1} carbohydrates, 15 g·kg^{-1} fat [2] and 110 g·kg^{-1} crude protein. Amino acids lysine, methionine, cystine, tryptophan, threonine, aspartic acid, leucine and valine were detected, as well as calcium, potassium and iron together with vitamins A, E, C and B [2, 3]. In Yucatan, Mexico, *B. alicastrum* forage is used as feed for cattle, goats and pigs. It has a high energy value, which makes it suitable for incorporation into animal feed and food for humans [4].

In Guatemala, they treat kidney infections and the common cold with infusions obtained from the leaves of *B. alicastrum* and the diluted latex is used to assist in the extraction of teeth. In addition, they macerate the bark and with it they make an ointment to treat chest pain and asthma [5]. In that country, there is an association of women who harvest and dehydrate the *B. alicastrum* fruit in order to use it in the preparation of bread, cookies, atole and as a substitute for arti-

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sanal coffee. However, most of the production is exported to the United States of America.

In recent years, bean flour, cereal starch (rice, barley, malt, rye, millet and spelt), roots (ginseng, ginger, beet, chicory) and flowers (rose, jasmine) were studied to formulate coffee substitute beverages. In addition, pistachio (*Terebihiu*), mistol, peanut, orange peel and ginkgo biloba leaves were studied for that purpose [6]. Here, substitutes are defined as products that are not derived from coffee and generally do not contain caffeine [7, 8]. Some people are opposed to drinking coffee due to the high content of caffeine and the effects of this compound on the central nervous and cardiovascular systems, mainly in relation to sleep deprivation and increased blood pressure. Pregnant women are recommended to limit their consumption of coffee to one cup a day, since caffeine in high doses increases the risk of causing spontaneous abortion [9]. For this reason, coffee substitutes are appreciated, in addition to economic reasons, religion reasons or because coffee is not available [10]. The substitutes are also suitable for diabetic and hypertensive people, pregnant women, the elderly and children or adults who simply do not want to consume caffeine. Currently, consumers are looking for beverages that help them improve their health status [11].

Therefore, the objective of this study was to obtain physico-chemical, chemical, and microbiological characteristics of three experimental formulations of coffee substitute based on *B. alicastrum* seed flour obtained through two processes of dehydration and roasting. The sensory preference of substitutes in Tenosique, Tabasco, Mexico, was also studied at a pilot level, in order to propose at least one formulation that meets the quality requirements established by the Mexican standards for commercial coffee and that has the sensory palatability required by the consumers.

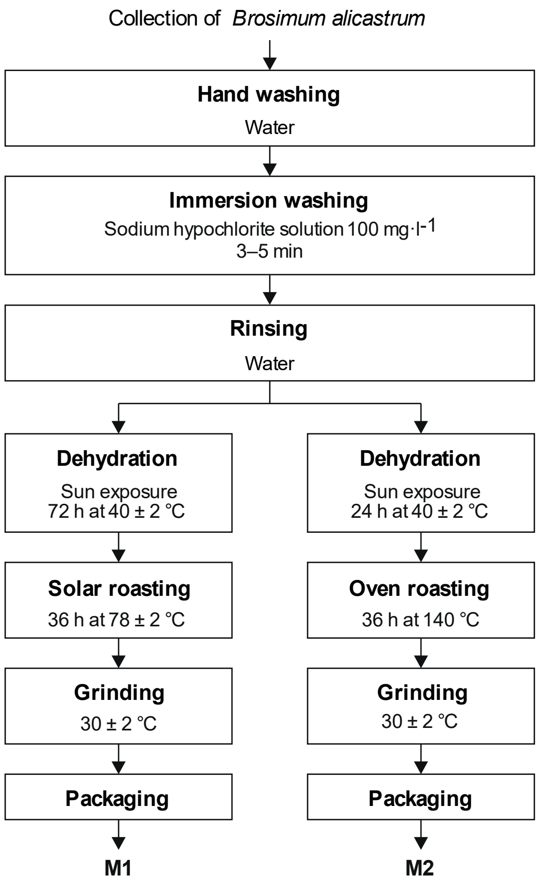

Fig. 1. Process for obtaining the *Brosimum alicastrum* flour.

M1 – solar dehydration followed by solar roasting, M2 – solar dehydration followed by oven roasting.

MATERIALS AND METHODS

Formulation of the coffee substitute

The seeds of *B. alicastrum* were collected in Santa Elena Petén, Guatemala. They were selected on the basis of physiological maturity (yellow-orange colour) and processed following the recommendations of the literature [12]. The collected seed was washed with water to eliminate the residues of soil, leaves or stems. Then it was submerged in a sodium hypochlorite solution of 100 $mg \cdot l^{-1}$ for 3–5 min and finally washed with drinking water. Subsequently, it was dehydrated and roasted using solar dehydration at 40 ± 2 °C for 72 h followed by solar roasting at 78 ± 2 °C for 36 h (M1 method) or using solar dehydration at 40 ± 2 °C for 24 h and controlled roasting in a model B0V900 toaster oven (Breville, Guangzhou, China) at 140 °C for 36 h (M2 method; Fig. 1). The roasted seeds resulting from M1 and M2 methods were ground in a CGF01PBUS model coffee grinder (Smeg, Shenzhen, China). The flour obtained was manually sieved with stainless steel sieves of 20 cm in diameter, mesh No. 20 (0.85 mm; Mont-Inox, Mexico City, Mexico). For the preparation of the coffee substitute, three treatments were formulated with the flour obtained (Tab. 1).

Physico-chemical analysis

The pH reading (n = 3) was recorded with a table potentiometer model PH120 (Conductronic, Puebla, México), previously calibrated with the reference solutions at pH 4 and pH 7. For the analysis, 1 g of the resulting flour was weighed, then it was diluted in 20 ml of distilled water and it was left to settle for 5 min, so that most of the suspended particles settled, finally the pH of each sample was measured.

Water activity (a_W) was analyzed according by Water Activity Meter Pawkit equipment (Decagon Devices, Pullman, Washington, USA), calibrated with reference standards: NaCl standard solution at 6 mol·kg^{-1} for 0.760 a_W, LiCl solution at 13.41 mol·kg^{-1} for 0.250 a_W and NaCl standard solution at 2.33 mol·kg^{-1} for 0.920 a_W. The sample was placed in the analyzer of the equipment and a reading was taken at 26 °C (n = 3).

The proximate chemical analysis was carried out with the methods described by AOAC [13]: moisture No. 930.15 (41.1.00), ash No. 942.05 (4.1.10), ether extract No. 920.39 (4.5.01), protein No. 2001.11 (4.2.11) and crude fibre No. 962.09 (4.6.01). Nitrogen-free extract was calculated by subtracting the moisture, ash, protein, fat and fibre content. The determinations were done in triplicate.

Microbiological analysis

The products resulting from the formulated treatments (T1, T2 and T3) were subjected to the analysis of moulds and yeasts [14], as well as quantification of total coliforms (*TC*), using the most probable number (MPN) technique [15].

Sensory analysis

Tasting was carried out according to the official Mexican standard NOM-169-SCFI-2007 [16], which establishes that each cup of coffee to be evaluated must have a ratio of 7 g of ground coffee for every 100 ml of hot water at boiling point (100 °C). It is also established that the sample must first be placed in the cup, then hot water poured in and allowed to settle for 5 min, so that most of the suspended particles settle. In this study, the sample for tasting was prepared on the basis of 1 200 ml. The volume of the evaluation sample for each consumer judge was 20 ml, placed in number 0 disposable pre-coded cups. To preserve the consumption temperature of the samples (45 °C), the infusions were kept in insulating containers for a period of less than 30 min.

For the selection of consumer judges, an intentional non-probabilistic sampling was carried out (for convenience), the main characteristic being that the candidate judge had to meet was to be a frequent coffee consumer. To locate them, home-to-home interviews were conducted in the city of Tenosique, Tabasco, Mexico. In these interviews, each candidate was explained the purpose of the research and was asked if they met the main characteristic of interest. In total, the inhabitants of 300 homes were interviewed and 60 consumer judges were selected. The evaluations were carried out in the home of each consumer judge, who was served the samples T1, T2, T3 and T4 (registered brand of genuine coffee) in coded glasses.

Tab. 1. Formulations of the coffee substitute.

Treatment	Flour [g·kg^{-1}]		Sugar [g·kg^{-1}]
	M1	M2	
T1	417	417	166
T2	500	334	166
T3	334	500	166

M1 – solar dehydration followed by solar roasting, M2 – solar dehydration followed by oven roasting.

Each consumer judge indicated in a record sheet the level of liking or disliking of each of the samples. The record sheet indicated the following instruction to the consumer judge: “Taste the samples provided and indicate your perception on the following scale: I dislike it very much, I dislike it, I neither like it nor dislike it, I like it, I like it very much” [17].

To analyse the results of the sensory evaluation, the hedonic scale was transformed into a numerical scale, where “I dislike very much” corresponded to 1, “I dislike” corresponded to 2, “I neither like nor dislike” corresponded to 3, “I like” corresponded to 4 and “I like it a lot” corresponded to 5.

Statistical analysis

For the statistical analysis of the physicochemical and sensory results, a completely randomized design was used. In order to establish significance of the differences between treatments, Tukey’s mean comparison technique was applied at $P \leq 0.05$. For this, JMP 6.0 software (SAS Institute, Cary, North Carolina, USA) was used.

RESULTS AND DISCUSSION

To establish the degree of roasting, the chromatic gradient of the coffee bean presented by Portillo [18] was used. The M1 method, allowed to obtain a product in the grade corresponding to light roasting, while the M2 method, in the medium roasting grade. This is important given that the Mexican standard NMX-F-013-SCFI-2010 [19] indicates that roasting is essential to develop coffee aroma and colour. Regarding particle size, the resulting flour had a diameter of 0.72–0.85 mm, greater than the 0.5 mm found in a roasted soya flour used as a coffee substitute [20]. According to the Mexican standard [19], an average grinding corresponds to a particle size of 0.72–1.70 mm in coffee beans, so it was considered that the particle size obtained in the flour of toasted seeds of *B. alicastrum* was suitable to prepare coffee in a percolator.

Physico-chemical and proximate chemical analysis

Tab. 2 shows the results of the physico-chemical parameters and proximate chemical analysis of the coffee substitute based on *B. alicastrum* seeds.

pH

Coffee substitute formulations had pH values of 5.7 ($P > 0.05$). According to the Mexican standard NOM-169-SCFI-2007 [16], in *Coffea arabica*, it is desirable to perceive acidity. An acid coffee has a pH value below 4.9 and pH 5.2 is considered to result in a bitter coffee [21]. Therefore, in relation to pH, in the treatments T1, T2 and T3, the characterized values conferred sensory notes of bitter taste to the formulations. On the other hand, on the pH scale the value of 5.7 corresponds to a slightly acidic substance. Food products gain stability with respect to bacterial growth due to acidic pH values, since the growth of most bacteria is suppressed at such pH values [22].

Water activity

The formulated treatments registered an a_w value between 0.53 and 0.55. This is also an indicator of microbiological stability, since it was found that a minimum a_w of 0.9 is required for bacterial growth. For fungi, this value is 0.80 [23].

Moisture

In relation to humidity, no statistical differences were found between the treatments ($P > 0.05$). A moisture content of 88 g·kg^{-1} was recorded in the samples. In previous studies [6] they reported 83 g·kg^{-1} of moisture in toasted seeds of *B. alicastrum*. The Mexican standard NMX-F-013-SCFI-2010 [19] establishes a maximum value of 60 g·kg^{-1} of moisture for pure roasted ground coffee with or without caffeine, while the Mexican standard NMX-F-173-SCFI-2011 [24] establishes 80 g·kg^{-1} of moisture for roasted coffee mixed with sugar. It is important to control the humidity in this type of products, since high humidity values support the growth of bacteria that could deteriorate the sensory characteristics of the product and also its shelf life.

Ash

Regarding the ash content, there were no significant differences between the treatments ($P > 0.05$). The experimental treatments yielded from 26 g·kg^{-1} to 27 g·kg^{-1} of ash. These levels are within the provisions of the Mexican standard

Tab. 2. Physico-chemical, proximate chemical and microbiological parameters of the coffee substitute.

	Experimental coffee substitute formulations		
	T1	T2	T3
Physico-chemical parameters			
pH	5.73 ± 0.01^{a}	5.73 ± 0.01^{a}	5.72 ± 0.00^{a}
a_w	0.53 ± 0.01^{b}	0.54 ± 0.01^{ab}	0.55 ± 0.01^{a}
Proximate chemical parameters			
Moisture [g·kg^{-1}]	88 ± 2^{a}	88 ± 7^{a}	88 ± 1^{a}
Ash [g·kg^{-1}]	26 ± 0^{a}	27 ± 1^{a}	27 ± 0^{a}
Protein [g·kg^{-1}]	76 ± 2^{a}	74 ± 4^{a}	73 ± 1^{a}
Fat [g·kg^{-1}]	29 ± 2^{b}	43 ± 4^{a}	46 ± 0^{a}
Fibre [g·kg^{-1}]	47 ± 5^{a}	43 ± 2^{a}	52 ± 7^{a}
Nitrogen-free extract [g·kg^{-1}]	734 ± 2^{a}	726 ± 7^{a}	715 ± 1^{a}
Microbiological parameters			
Moulds and yeasts	Absent	Absent	Absent
Total coliforms	Absent	Absent	Absent

Mean ± standard deviation ($n = 3$) values are presented. Different letters in superscript in the same row mean statistically significant differences according to Tukey's test ($P < 0.05$).
T1, T2, T3 – composition of coffee substitute formulations is given in Tab. 1.

NMX-F-173-SCFI-2011 [24] for roasted coffee mixed with sugar, which indicates a maximum ash of 60 $g\cdot kg^{-1}$. Ash at 53.9 $g\cdot kg^{-1}$ was reported in roasted Cuban arabica coffee and at 52.9 $g\cdot kg^{-1}$ in Brazilian robusta coffee. In other types of roasted grains such as Canadian peas and Cuban chickpeas, values of 34 $g\cdot kg^{-1}$ and 39 $g\cdot kg^{-1}$ of ash were reported, respectively [25]. In a previous study [26], 34 $g\cdot kg^{-1}$ of ash was recorded in *B. alicastrum* seed meal, while in a drink made from the same source, the ash content decreased to 6 $g\cdot kg^{-1}$. From the nutritional point of view, the ash found in the *B. alicastrum* seed meal substitutes is important, since it is an indicator of the mineral composition of the product (calcium, iron, zinc and sodium) [2].

Protein

Concerning protein, no significant statistical differences were recorded between the treatments ($P > 0.05$). The protein determined in the treatments T1, T2 and T3, was from 73 $g\cdot kg^{-1}$ to 76 $g\cdot kg^{-1}$, being lower than previously recorded for toasted *B. alicastrum* flour (from 106 $g\cdot kg^{-1}$ to 121 $g\cdot kg^{-1}$ protein) [6, 27]. However, 73 $g\cdot kg^{-1}$ protein was found in a roasted *Myrciaria dubia* seed coffee substitute [28], similar to what was obtained in this investigation. The decrease in protein content can be attributed to the fact that sugar was used as an ingredient in the coffee substitute formulations based on *B. alicastrum* seeds, which could have caused a replacement in the protein content. In addition, it is important to indicate that the age of the plant, the physiological maturity of the seed, the type of soil, the climate and the availability of water are decisive factors in the chemical composition of the coffee substitute obtained.

Fat

Significant differences were found in fat content ($P < 0.05$), with treatments T2 and T3 having the highest content (43 $g\cdot kg^{-1}$ and 46 $g\cdot kg^{-1}$, respectively) compared to T1 (29 $g\cdot kg^{-1}$). In a study of *B. alicastrum* seeds toasted at 160 °C, a fat content of 14 $g\cdot kg^{-1}$ [27] was shown, while in another study 7 $g\cdot kg^{-1}$ were recorded in seeds toasted at 220–230 °C [6]. In the substitutes formulated in this work, a higher fat content was obtained in comparison to those mentioned. This was probably due to the fact that the roasting temperature influenced the fat content, since increasing the temperature above 150 °C begins the processes of lipid loss by oxidation and hydrolysis [29]. It should be noted that the fat limit established by the Mexican standard NMX-F-013-SCFI-2010 [19] for pure roasted coffee beans without decaffeination is from 80 $g\cdot kg^{-1}$ to 180 $g\cdot kg^{-1}$, and for roasted coffee mixed with sugar according to the Mexican standard NMX-F-173-SCFI-2011 [24] is from 75 $g\cdot kg^{-1}$ to 120 $g\cdot kg^{-1}$. Although the values for fat found in the substitute formulations of this work were not within the ranges established by the Mexican standards for coffee, an important content was found that could favour the palatability of the product, since fats also modify the flavour of many foods through the mouthfeel they cause [29].

Fibre

The three analysed treatments T1, T2 and T3 had a fibre content of 47 $g\cdot kg^{-1}$, 43 $g\cdot kg^{-1}$ and 52 $g\cdot kg^{-1}$, respectively, without statistical differences ($P > 0.05$). In a preliminary study [6], it was found that the cooked flour of *B. alicastrum* seeds contained 37 $g\cdot kg^{-1}$ of fibre, while the toasted flour 39 $g\cdot kg^{-1}$. In a substitute coffee based on *Myrciaria dubia* seeds, 36 $g\cdot kg^{-1}$ of fibre were reported [28]. Another study [30] reported 60 $g\cdot kg^{-1}$ of raw fibre in a bean-based coffee substitute. The raw fibre present in the coffee grounds formulated in this work originated in the fibre contained naturally in the cell walls of plants and varied depending on the type of plant product.

Nitrogen-free extract

The nitrogen-free extract obtained in T1, T2 and T3 was 734 $g\cdot kg^{-1}$, 726 $g\cdot kg^{-1}$ and 715 $g\cdot kg^{-1}$, respectively. These values were similar to the 712 $g\cdot kg^{-1}$ of the total carbohydrates present in the *B. alicastrum* seed flour including the sugar added to the formulations, reported by another study [31]. Therefore, the final product can be called a coffee substitute mixed with sugar.

Microbiological status

The experimental formulations did not show the presence of coliforms or fungus, this absence of microbial contamination can be attributed to the a_w of 0.5. The Mexican standard NMX-F-173-SCFI-2011 [24] establishes that food products such as roasted coffee mixed with sugar must not contain intentionally added pathogenic microorganisms and microbial inhibitors. However, compounds with antimicrobial activity may be present naturally, that is, as part of the chemical composition of the grains or seeds. Such is the case of *B. alicastrum*, where different substances with antimicrobial activity have been identified, such as *p*-hydroxybenzoic acid, vanillic acid, epicatechin, gallic acid, caffeic acid or *p*-coumaric acid [32]. On the other hand, the Mexican standard NOM-218-SSA1-2011 [33] indicates that for non-alcoholic, flavoured and heat-treated powdered beverages,

< 10 CFU·g^{-1} of coliforms are tolerated. In addition, according to the Mexican standard NOM-247-SSA1-2008 [34], in foods based on edible seeds < 30 CFU·g^{-1} of coliforms and 300 CFU·g^{-1} of fungi are tolerated. Considering the above, the treatments formulated for the seed coffee substitute *B. alicastrum* complied with Mexican standards.

Sensory analysis

Fig. 2 shows the results of palatability of the coffee substitutes formulated based on *B. alicastrum* seed flour (T1, T2 and T3), compared with a commercial brand of genuine coffee (T4). Significant differences were observed in the sensory test ($P < 0.05$). T1 and T2 were different from T3 and T4. Statistical equality was observed in the palatability of treatment T3 and T4, both had averages of 4, which indicated that the evaluators "liked it". This suggested that the T3 coffee substitute had the same sensory acceptance as commercial genuine coffee. The acceptance registered for the substitute formulated with the T3 treatment may be due to the fact that it had a higher proportion of *B. alicastrum* flour obtained by the M2 method, which, when subjected to a temperature of 140 °C, transformed sugars and proteins into other low molecular weight compounds, conferring colour, flavour and aroma to the product [19]. Variations in time and temperature profiles within the roasting process are known to directly impact the rate of moisture loss, internal bean temperature, microchemistry, as well as colour, flavour and aroma development in the final roasted coffee bean [35, 36]. These could positively influence the level of pleasure perceived by consumer judges.

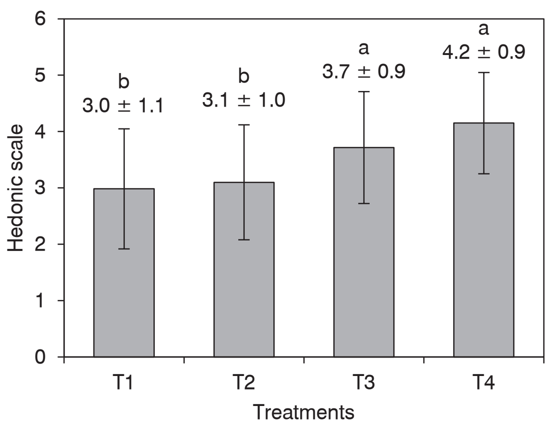

Fig. 2. Palatability of the coffee substitute treatments.

Mean ± standard deviation ($n = 60$) values are presented. Different letters mean statistically significant differences according to Tukey's test ($P < 0.05$).

CONCLUSIONS

Seeds of *B. alicastrum* dehydrated and roasted by the M1 and M2 method allowed to obtain a flour with convenient contents of protein, ash, fat and nitrogen-free extract, together with low a_w that inhibited microbial growth. The T3 treatment led to the same palatability as commercial coffee. Therefore, the T3 treatment is recommended for the formulation of the coffee substitute based on roasted *B. alicastrum* seeds, which can be an alternative to coffee for people with diabetes, hypertension, pregnant women, the elderly and children.

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