

Full Length Research Paper

Effect of Brazil nut oil (*Bertholletia excelsa* HBK) on the physical, chemical, sensory and microbiological characteristics of a mayonnaise-type emulsion

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The objective of this study was to evaluate the influence of emulsifiers on the chemico-physical, colorimetric, microscopic and sensorial properties, and the stability of a mayonnaise-type emulsion prepared with Brazil nut oil (*Bertholletia excelsa* H.B.K). For this purpose, two emulsifiers were used: a soya protein isolate and dehydrated and pasteurised egg yolk as a source of protein. Both formulations had high energy and lipid contents but low mineral and carbohydrate contents. The formulation with egg yolk exhibited higher levels of minerals, such as potassium and calcium, than the emulsion with soya. The mean particle diameter of the Brazil nut-soya emulsion ranged from 8.78 to 24.15 µm, and that of the Brazil nut-yolk emulsion ranged from 0.85 to 22.41 µm, indicating that size directly influences the viscosity of the emulsion. Thus, the Brazil nut-soya emulsion can be characterised as a monodisperse emulsion. The Brazil nut-yolk emulsion was darker, or had lower lightness (L^*), compared to the Brazil nut-soya emulsion. The formulation with soya protein was demonstrated to be unsuitable for consumption due to a high microbial load, specifically moulds and yeasts, and was excluded from the sensory evaluation. However, the egg yolk emulsion showed acceptable microbiological parameters according to current legislation. The consumer acceptance means were greater than 6.95 (maximum of 9 on the hedonic scale). Acceptance of the emulsion was also confirmed by purchasing attitude, for which 75% of consumers stated they would purchase the product. Thus, soya protein is not viable for the production of an emulsion with Brazil nut oil, whereas egg yolk is a better emulsifier, which can influence the physicochemical, nutritional and sensory parameters. Additionally, the product can be stored at room temperature, which is an economically feasible feature for the consumer market.

Key words: Brazil nut, egg yolk emulsion, soya emulsion, mayonnaise, nutritional properties.

INTRODUCTION

In the Amazon, a wide variety of foods and medicinal plants exist that have different biological properties, many of which have been rarely or never studied. This is

associated with the cultural diversity of the traditional communities of the region, is a treasure for Brazilian biotechnology. The proper characterisation of this

potential can guide actions for sustainable development in the Amazon (Souza et al., 2008). The region, with its wealth of plant species, is known for producing vegetable oils with unique aromas and tastes. The properties of these vegetable oils have been intensively researched, mainly by international companies, due to their various applications in the food, pharmaceutical and other industries (Pardaul et al., 2007). The Brazil nut (*Bertholletia excelsa* Humb. & Bonpl. Lecythidaceae) is considered one of the most important species with economic exploitation of the Amazon rainforest. Almonds are much appreciated for human consumption due to their high nutritional value and health benefits (Massi et al., 2014).

The Brazil nut (*B. excelsa* H.B.K) is one of the most important extractively exploited non-timber forest products and is native to the Amazon region (Freitas-Silva and Venancio, 2001). The production and extraction of the Brazil nut is a low-environmental-impact activity (Wadt et al., 2005). The nuts are oily, with a high energy value, and are rich in proteins of high biological value. As such, they are considered a good alternative source of nutrients in food fortification and an excellent addition to the diet of children and adults because of the high content of lipids, vitamins, minerals, and proteins (Funasaki et al., 2013). The oil extracted from the nuts has good digestibility, and the extraction residue can be used in foods and animal feed (Pacheco and Scussel, 2006).

The concentration of lipids in the Brazil nut is approximately 66%, and the main fatty acids are linoleic (45%), oleic (29%), palmitic (15%), and stearic (10%) (Kornsteiner et al., 2006; Venkatachalam and Sathe, 2006). Balbi et al. (2014) found that Brazil nuts are an important source of fatty acids, proteins, fibers, minerals and selenium. The results obtained by the physicochemical analyses carried out in *B. excelsa* oil are within the parameters established by the Brazilian legislation; the acidic characteristics, the high degree of unsaturation, indicated that the oil contains polyunsaturated fatty acids to a large extent (Pena Muniz et al., 2015).

Lipids are part of the matrix of many food products, such as emulsions. They modify the physical properties of foods, including the flavour, appearance and structure. It has been reported that lipids influence flavour perception in terms of both aroma release and textural changes (Daget et al., 1987; Malone and Appelqvist, 2003). As a result, the reformulation of flavours consisting of foods with reduced fat requires considerable work so that they can meet the needs and expectations of consumers (Rabe et al., 2003). Considering these aspects, the lipids present in the Brazil nut have

enormous potential for the formulation of mayonnaise-type emulsions, in addition to having good nutritional characteristics. However, the physical properties and chemical stability of the emulsions must be studied.

In general, mayonnaise is a semi-solid emulsion of oil in water containing 70 to 80% fat. It is traditionally prepared by carefully mixing egg yolk, vinegar, oil and spices (especially mustard). Largely influenced by concerns about health, there has been pressure on the food industry to reduce the amount of fat, sugar, cholesterol, salt and certain additives in the diet (Liu et al., 2007).

Therefore, the objective of the present study was to investigate the influence of emulsifiers on the chemico-physical, colorimetric, microscopic and sensorial properties, and the stability of mayonnaise-type emulsions made with Brazil nut oil.

MATERIALS AND METHODS

Emulsion preparation

Two emulsifiers were used with different protein sources: soya isolate and dehydrated and pasteurised egg yolk. For the preparation of emulsions, the ingredients were pre-weighed in a Filizola analytical balance. The dry ingredients (soya protein isolate (Levlife) or pasteurised and dehydrated egg yolk (Salto's)), salt, and guar gum (Maxfoods) were mixed, and then 1/3 of the Brazil nut oil was added, and the ingredients were homogenised for 5 min in a mixer. Next, vinegar and the remaining oil were added, followed by homogenisation for 2 min (Nikzade et al., 2012).

Physicochemical characterisation

For proximate composition, moisture was determined by gravimetry in a conventional oven at 105°C to a constant weight. Protein content was determined by the Kjeldahl method, and lipid content was estimated by direct extraction in a Soxhlet apparatus, using petroleum ether as the solvent. The ashes were quantified using gravimetry by incinerating the sample in a muffle furnace at 550°C. Carbohydrate content was calculated by the difference method, by subtracting from 100 the sum of the moisture, protein, lipid and ash contents, following the methodology of the Adolfo Lutz Institute (Instituto Adolfo Lutz, 2008).

Minerals

Mineral content was determined in triplicate by atomic absorption spectrometry as recommended by the Adolfo Lutz Institute (IAL, 2008) and according to the Varian manual (2000). Samples were digested in a MARS Xpress microwave digester (CEM Corporation, Model – 2591) in organic matter with the use of concentrated nitric acid. The reading was performed directly in dilute solutions in an atomic absorption spectrophotometer (Spectra AA, model 220 FS, Varian, 2000). The following mineral elements were quantified: Ca, K, Na, Mg, Fe, Zn, Mn and Cu. Controls for the analysis followed the recommendations of Cornelis (1992), with the certified reference

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material Peach leaves (NIST-SRM 1547).

Antioxidant activity of the emulsion

Antioxidant activity was evaluated using the free radical-scavenging assay of the reaction of DPPH (2,2-diphenyl-1-picrylhydrazyl) in absolute ethanol (2 mg DPPH in 12 mL of ethanol). Emulsion test solutions were prepared in various concentrations: 0, 7.8, 15.6, 31.25, 62.5, 125, 250, 500 and 1000 µg/mL. Thereafter, 30 µL of the samples was added to the microplate wells. Next, 270 µL of DPPH solution or ethanol was added to the test samples or blanks, respectively. The plates were incubated in the dark for 30 min. Readings were taken at 492 nm in a microplate reader (Multimode Detector DTX 800, Beckman Coulter). The ability to eliminate the DPPH radical (% antioxidant activity) was assessed following the method of Molyneux, 2004.

Microbiological analysis

The microbiological analysis methods were adopted from the Compendium of Methods for the Microbiological Examination of Foods by the American Public Health Association (APHA, 2001). Total coliforms, *Salmonella* spp, moulds and yeasts were analysed. The analyses were performed on a qualitative basis; the results are expressed as the presence or absence of these microorganisms in 25 g of food.

Microscopic analysis

Emulsion patterns were analysed using a Nikon E-800 microscope (Kawasaki, Japan) with bright-field illumination at 40× magnification. The emulsions were observed after 24 h of cooling at 4°C. For this purpose, 4.0 mL of the emulsion was trickled with a microsyringe over a drop of water (3 mL), previously deposited onto a slide (76 × 26 mm), and covered with a coverslip (24 × 32 mm). Digital images were acquired with a Nikon DXM-1200 camera. Particle sizes were measured from the previously calibrated images (Poyato et al., 2013).

Colour analysis

The colour of the emulsion was measured in the L*, a*, b* system using a spectrophotometer (Mini Hunter Lab Scan XE Plus, Model 45/0-G), which was calibrated using a black and white porcelain plate. For direct reading, the samples were added in a sufficient amount to cuvettes. In this colour system, L* represents the changes in lightness, ranging from 0 (black) to 100 (white), and a* and b* are the colour coordinates responsible for the chromaticity, where -a*=green and +a*=red and -b*=blue and +b*=yellow (HunterLab, 2001).

Shelf life

The shelf life of the emulsion was established using packaging that prevents contact between food and oxygen for seven different time periods (0, 30, 60, 90, 120, 150 and 180 days), during which the samples were stored at room temperature.

Sensory analysis

Sensory tests were performed in individual booths, illuminated with white light. Sensory analysis of the emulsions was performed using

the global acceptance and purchasing attitude tests. Two samples of approximately 10 g each were served refrigerated (10°C ± 1°C), individually, in disposable cups (50 mL) with a plastic spatula, and coded with three random digits. To cleanse the palate, water and salt biscuits were served.

The acceptance test used a hedonic scale applied to 68 untrained judges, aged 22–45 years. A structured nine-point scale was used, where 1 corresponded to "disliked extremely", and 9 to "liked extremely". Purchasing attitude was assessed with the sample that obtained better acceptance in the hedonic scale, using a five-point scale, where 1 corresponded to "I would never purchase this product" and 5 to "I would certainly purchase this product".

Data analysis

Data are presented as the mean ± standard deviation. Data were submitted to a test of normality. To compare each characteristic of the Brazil nut emulsion with egg yolk or soya protein, Student's t test or Mann-Whitney U test was used, depending on the normality test. In all cases, the significance level was 5% (P < 0.05).

RESULTS AND DISCUSSION

Physical, chemical and mineral characterisation of emulsions

The results of the physicochemical parameters of moisture, protein, lipid, ash, carbohydrate and energy are shown in Table 1. It is observed that the values of the above parameters are similar in the Brazil nut–soya (BS) and Brazil nut–dehydrated egg yolk (BY) emulsions, with the exception of the energy value and calcium and potassium contents, which are higher in the BY emulsion. Additionally, it was found that the BS and BY emulsions have more proteins, lipids, energy and minerals than a traditional mayonnaise emulsion (TACO, 2006).

The energy values of the two Brazil nut emulsions (492–597 kcal 100 g⁻¹) were low compared to the values estimated for commercial mayonnaise. Traditional mayonnaise has, on average, 680 kcal 100 g⁻¹, whereas the low-fat versions (light) have 340 kcal 100 g⁻¹ (USDA, 2002). According to TACO (2006), traditional industrialised mayonnaise with eggs has, on average, 302 kcal 100 g⁻¹.

The emulsions studied are an important source of minerals. These minerals perform essential functions in the body as components of prosthetic groups in proteins or ions dissolved in body fluids that regulate the activities of many enzymes and maintain the acid–base balance and osmotic pressure necessary for physiological homeostasis (Andrade et al., 2003). The minerals found at higher concentrations were sodium, calcium and potassium. Calcium and potassium are important for neuromuscular and muscle activity, bone tissue, cell growth, intracellular homeostasis and hormonal function regulation. Deficiency in these minerals may result in osteoporosis in adults and even rickets in children (Shils et al., 2003).

Table 1. Proximate and mineral composition of Brazil nut–soya (BS) and Brazil nut–egg yolk (BY) emulsions.

Mineral composition	BS emulsion	BY emulsion	Traditional*
Moisture (% wb#)	40.41	31.99	58
Protein (%)	6.12	9.6	1
Lipids (%)	51.22	55.84	30
Ash (%)	0.69	1.01	2.6
Carbohydrates (%)	1.55	1.55	8
Total energy (kcal ⁻¹ g)	491.98	547.16	302
Calcium (mg)	15.30	38.71	3.0
Magnesium (mg)	4.30	4.41	1
Potassium (mg)	11.4	62.05	16
Sodium (mg)	870.48	710.48	787
Manganese (mg)	0.30	0.30	<LQ
Copper (mg)	Nd	Nd	Na
Zinc (mg)	0.11	Tr	Na
Iron (mg)	2.62	0.94	0.1

wb = wet basis. * Traditional mayonnaise, as in TACO (2006). Tr , Traces; Na, not analysed; Nd, not detecte; <, LQ below the limit of quantification.

Table 2. Antioxidant activity of Brazil nut–soya protein (BS) and Brazil nut–egg yolk protein (BY) emulsions. The BS and BY values are negative. Gallic acid was used as a standard.

Samples	Mean
BS (10 mg mL ⁻¹)	-2.12396±1.42
BY (10 mg mL ⁻¹)	-2.12366±1.42
Gallic acid (10 mg mL ⁻¹)	86.22544

Antioxidant activity

The antioxidant activity assays show that none of the emulsions played an antioxidant role detectable by the DPPH assay (Table 2). These data were compared to gallic acid, which was used as a standard. All values were negative, indicating no antioxidant activity. However, lipid peroxidation is the main cause of the deterioration of fatty bodies and is responsible for modifying the odour and flavour of food, as well as the loss of nutritional quality, resulting in depreciation and/or rejection by the consumer (Silva et al., 1999).

Microbiological analysis

A microbiological evaluation of the emulsions was performed over six months. Microbes were detected only in the Brazil nut–soya protein (BS) emulsion, where high contamination by yeasts and moulds was observed at the beginning of the experiment. The Brazil nut oil–dehydrated and pasteurised egg yolk (BY) emulsion did

not present a microbiological risk because the limits conformed to those specified in current legislation (Table 3). Reis et al. (2014) the storage conditions of nuts seem to have an important influence on the population of fungi *Aspergillus section Flavi*.

Size of emulsion particles

The Brazil nut–soya protein (BS) emulsion produced larger and more uniform droplets than the Brazil nut–egg yolk protein (BY) emulsion. Thus, according to Worrasinchai et al. (2006), the BS emulsion can be classified as a monodisperse emulsion (uniform droplet size), in contrast to the BY emulsion. The mean particle diameter of the BS emulsion ranged from 8.78 to 24.15 µm, whereas that of the BY emulsion ranged from 0.85 to 22.41 µm, indicating that size directly influences the emulsion. The microscopic images (Figure 1) of the two emulsions confirm the suitable structure for an oil-in-water emulsion.

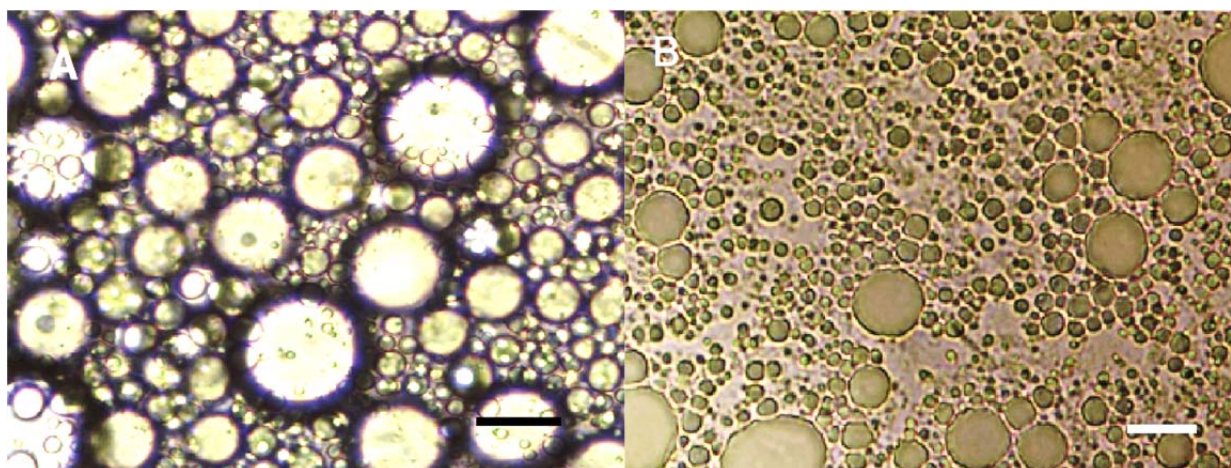
Colour analysis

The L parameter represents how light or dark the sample is, and it ranges from 0 (very dark) to 100 (very light) (Bonagurio et al., 2003). The results obtained for colour (Table 4) show that the BY emulsion had a lower lightness values (L*) than the BS emulsion, indicating that the BY emulsion is darker due to the egg yolk formulation, in contrast with the BS emulsion with soya protein, which had a lighter colour. All of the mayonnaise samples tended more towards white than black because

Table 3. Microbiological analyses of Brazil nut oil–soya protein (BS) and Brazil nut oil–dehydrated egg yolk (BY) emulsion samples.

Microbiological parameter	BS emulsion	BY emulsion
Total coliforms	0.0 NMP/g	0.0 NPM/g
Coliforms at 45°C*	0.0 NMP/g	0.0 NMP/g
<i>Staphylococcus aureus</i> *	<10 UFC/g	<10 UFC/g
<i>Salmonella</i> spp.*	Absent	Absent
Yeasts and moulds	11x10 ⁴ UFC/g	<10 UFC/g

*Following the Board of Directors Resolution nº 12 of January 2001/Brazilian National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária – ANVISA)/Ministry of Health (Brasil, 2001).

**Figure 1.** (A) Emulsion with Brazil nut oil and soya protein (BS). (B) Emulsion with Brazil nut oil and dehydrated egg yolk (BY). The scales on the images correspond to 20 µm.**Table 4.** Colour parameters L*, a*, b* in Brazil nut oil–soya protein (BS) and Brazil nut oil–dehydrated egg yolk (BY) emulsions.

Samples	L*	a*	b*
BS Emulsion	71.68	2.53	24.52
BY Emulsion	70.48	0.33 [§]	13.59 [§]

[§]Indicates significant difference (Student's t test, P <0.05) between the two types of emulsions.

all produced values above 60, which differed significantly between the two different emulsions. Similar values were found by Pereira et al. (2013), who studied the ideal profile of commercial mayonnaise. Differences existed for the parameters a* and b* among the samples. The a* parameter corresponds to the scale from green to red, where a is negative for green, and a is positive for red; the b* values correspond to the scale from blue to yellow, where b is negative for blue and b is positive for yellow, according to Bonagurio et al. (2003). Thus, all of the emulsion samples tended to red because all values found

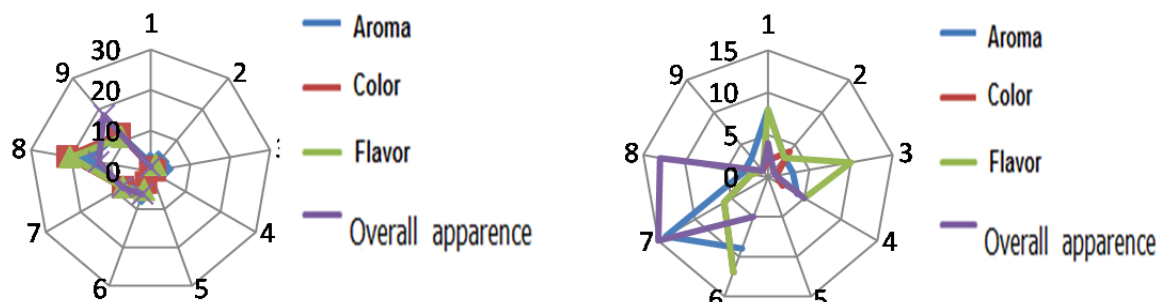
for a* were positive.

In general, the emulsions exhibited yellow pigmentation because all values were positive, which is a characteristic inherent to this type of product.

The main factors that cause industrial mayonnaise to be yellow are egg yolk, mustard and dyes (Dickinson and Miller, 2001). The colour of egg yolks is attributed to the carotenoids xanthophylls, lutein, zeaxanthin, β-cryptoxanthin and β-carotene, which are solubilised in the yolk (by Li-Chan and Kim 2008).

Sensory analysis

The results of the sensory analysis acceptance test for aroma, flavour, colour and overall appearance of the emulsion samples are presented in Figures 2. The mean scores obtained for the overall appearance lie in the acceptance zone of the graph (scores of 7 and 8) and, on the hedonic scale, correspond to "liked slightly" and "liked moderately". The colour attribute obtained a score of 8 on the hedonic scale, corresponding to "liked moderately".



Commercial Emulsion

BY Emulsion

Figure 2. Representation of the acceptance of the commercial emulsion and Brazil nut oil–dehydrated egg yolk (BY) emulsions scored on a hedonic scale. The formulation with soya protein (BS) was determined to be unsuitable for consumption due to the high load of *Bacillus cereus* and was excluded from the sensory analysis.

This may be associated with the egg yolk, which provides a favourable colour to the emulsion. A score of 7 was obtained for aroma, corresponding to "liked slightly", with no reports of distinct odour in the product due to the addition of Brazil nut oil. The flavour attribute was the parameter with the lowest score on the hedonic scale (6), corresponding to "neither liked nor disliked", and no attribute evaluated was rejected.

Conclusions

Soya protein is not viable for the production of an emulsion with Brazil nut oil. In contrast, egg yolk was a suitable emulsifier. The physicochemical, nutritional and sensory parameters were influenced by the emulsion and also because the product was stored at room temperature, storage makes it economically feasible for the consumer market.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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