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ELABORATION OF TEA BASED ON GEOPROPOLIS EXTRACT AND MINT (Mentha *piperita L*)

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Beatriz Muniz de Lima Freitas[1], Pablo Ruan Cardoso Forte[2], Antônio José Nogueira Leão[3], Evelyn Carolaine Veloso da Silva[4], Evely Leticia Rodrigues de Lima[5], Josyane Brasil da Silva[6] and Elen Vanessa Costa da Silva[7]

ABSTRACT

The development of new products used in alternative therapies has been gaining prominence due to their properties in biologically active compounds. Geopropolis is widely used because of its rich chemical composition, as it has a wide variety of flavonoids, phenolic compounds, minerals, amino acids, and vitamins. In addition, mint *(Menta piperita L)* has several beneficial properties because it is rich in essential oil in its structure, so the main objective of the present study was the elaboration of a tea based on geopropolis extract and mint. First, the hydroalcoholic extraction of the geopropolis properties was carried out for 60 days. The extract obtained was homogenized with mint and taken to dry in an air circulation oven (4 hours at 50ºC) obtaining the solid material for the preparation of the tea. Physicochemical, microscopic, microbiological and sensory analyses of the product were carried out. The results obtained were similar to those related to tea, with 4.72% acidity, 14.77% ash, 18.68% protein, 6.5% moisture, 1.22% lipids, 22.5mg/100g of

[1] Bachelor of Science in Food Technology
University of the State of Pará
E-mail: beatriz.munizlfreitas@gmail.com
LATTES: http://lattes.cnpq.br/3870453423979729
[2] Bachelor of Science in Food Technology
University of the State of Pará
E-mail: pablo.c.forte@gmail.com
LATTES: http://lattes.cnpq.br/3943605496906873
[3] Bachelor of Science in Biology
University of the State of Pará
Email: antonio.leao@uepa.br
[4] Bachelor of Science in Food Technology
University of the State of Pará
E-mail: evelyn.veloso166@gmail.com
LATTES: http://lattes.cnpq.br/1510339956181082
[5] Bachelor of Science in Food Technology
University of the State of Pará
Email: evelyleticiarl@hmail.com
LATTES: http://lattes.cnpq.br/5918026695909669
[6] Dr. in Animal Health
University of the State of Pará
Email: josyanebr@uepa.br
LATTES: http://lattes.cnpq.br/2789098316302912
[7] Dr. in Food Science and Technology
University of the State of Pará
Email: elen.vanessa@uepa.br
LATTES: http://lattes.cnpq.br/9292369606189635

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vitamin C, 38mg/L of phenolic compounds and 98.59mg/L of Flavonoids. In the microbiological analysis it was obtained absence of *Samolnella sp* and *Escherichia coli,* in the microscopic analysis no dirt was found, sensorially the product had good acceptance (77.77%) and, therefore, being considered nutritious, functional and with commercialization potential.

Keywords: Flavonoids. Meliponas. Drying.

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INTRODUCTION

Geopropolis is a product made by bees of the genus Melipona, consisting of a mixture of earth, clay, propolis, and is used to close cracks, form walls, and protect the internal cavity of the hive from the external environment (Budoia, 2019). The various natural products produced by them have been gaining prominence due to their numerous therapeutic properties, such as anti-inflammatory, healing, hypotensive, anticancer and antimicrobial (Silva, 2019).

For the elaboration of the geopropolis extract, it is necessary to extract the soluble components of the geopropolis and its soluble constituents must be extracted with the use of an appropriate solvent such as neutral alcohol (food grade) or water, by a technological process that is appropriate, which can be applied in medicine or food (Mariano, 2014).

In order to reduce the use of antibiotics, alternative therapies have been gaining strength, with emphasis on natural products and their therapeutic properties, which are characterized by efficacy, accessibility, and low cost, in addition to presenting minimal risks in the face of adverse effects and bacterial resistance (Mello, 2020).

Around the world, there are approximately 300 genera and 7,500 species of the *Lamiaceae* family, among these genera, *Mentha* or *Mentha piperita L can be highlighted.*, popularly known in Brazil as mint. Originally in Europe, this plant has an intense odor due to the aromatic compounds present, commonly used in teas and use in cooking (Malaquias et al., 2014).

Mint is very popularly known, it has always been used the dried leaf, in natura, in the form of tea, it is so common within homes, that each family has its own way of using it, there are people who use the dried leaf bought in health food stores, others buy industrialized tea in bags, others prefer the plant in natura in cultivation in their own backyard (BRASIL, 2015).

Teas have been known since ancient times and widely consumed around the world. The infusions are prepared by adding heated or boiling water over parts of the plant, a method of preparing herbal infusions (known as teas in Brazil) favors the extraction of phenolic compounds from plants, making the drinks excellent sources of these natural antioxidants. The consumption of herbal infusions is, in addition to a cultural aspect, an efficient and low-cost alternative for various therapeutic purposes (Morais, 2009; Santos, 2020).

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Tea is one of the most consumed beverages in the world, characterized by having a pleasant aroma and flavor that contributed to the popularization of this drink, but it is due to its medicinal properties that it has spread to different cultures. These properties are due to the presence, in its chemical composition, of biologically active compounds such as: flavonoids, polyphenols, alkaloids, vitamins and mineral salts (Schmitz, 2005).

Based on the above, the work aimed to develop a tea based on geopropolis and mint extract and evaluate its proximate composition, microbiology, microscopy and sensory acceptability.

MATERIAL AND METHODS

The raw material was acquired in a Meliponary located in the municipality of Castanhal-PA. For the collection, the lids of the boxes were opened and the geopropolis was removed through its detachment, later they were deposited in plastic bags and taken to the food laboratory of the State University of Pará – Campus XX.

To obtain the hydroalcoholic extract of geopropolis, 300g of geopropolis sample was weighed and mixed with 700ml of 70% hydroalcoholic cereal alcohol solution, homogenized and left to extract the compounds for 60 days away from light and constant agitation. After the period, the sample was filtered twice on filter paper, and the supernatant was reserved according to the methodology described by Silva et al. (2023). The hydroalcoholic extract of geopropolis was stored in amber glass bottles in order not to lose its properties due to the photosensitivity of the bioactive compounds (Frazão, 2017)

To obtain the dry extract of geopropolis and mint, 4.5 ml of hydroalcoholic extract of geopropolis and 4.5 g of dry mint were added in a becker, and then the mixture was homogenized. It was then taken for drying in an air circulation oven for approximately 4 hours at a temperature of 50°C until the sample was completely dried.

PHYSICOCHEMICAL ANALYSIS

All analyses and equipment were made available by the food laboratory at the State University of Pará, Campus xx – Castanhal. The physicochemical analyses were performed on the dry extract in triplicate according to the Adolfo Lutz Institute (2008), and the analyses of flavonoids and phenolic compounds were made according to the method described by Zhishen, Mengcheng, Jianming (1999) and by the Folin-Ciocalteau method carried out by Chandra and Mejia (2004), respectively.

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Determination of Acidity

For acidity, the methodology of the IAL (2008) was followed, the sample was weighed 2g, transferred to a 125 mL Erlenmeyer flask together with 50mL of distilled water and homogenization was performed. 4 drops of phenolphthalein solution were added and titration was performed with 0.1M sodium hydroxide solution until pink color.

Determination of Ashes

The 3 crucibles were taken with the previously dried sample and placed in the muffle furnace at an initial temperature of 150°C, then increased by 100°C every hour until it reached a temperature of 550°C, when it reached 550°C it remained at this temperature for 4 hours. Finally, with the help of metal tweezers, the crucibles were left in the desiccator for 30 minutes and then the final weight was checked.

Protein Determination

For the determination of proteins, 0.1g of anhydrous sodium carbonate PA was weighed (dried at 105°C for 4 hours). The weight was noted, up to the 4th decimal place. Transfer the sodium carbonate to a 250ml Erlenmeyer and add 100ml of distilled water. It was agitated until the complete dissolution of the solute. The solution was heated until the beginning of boiling. 8 drops of 0.1% methyl red indicator solution were added. It was titrated with 0.1N hydrochloric acid solution, until it turned from yellow to red. The CO2 was eliminated by heating the solution again. It was waited for it to cool down and titration was performed again, this operation was repeated until the red stain was permanent.

We then weighed 0.1 of the sample into a watch glass and transferred to the Kjeldahl tube, then added to the tube 2 g of catalytic mixture. Subsequently, 5 ml of concentrated sulfuric acid was pipetted to the tube, the tube was stirred for better sample dissolution and catalytic mixing. The tubes were placed in the digester block and the device was set to 450ºC. The equipment will gradually increase the temperature. Finally, after digestion was finished, 20 ml of boric acid solution and five drops of mixed indicator were added to a 250 ml Erlenmeyer Erlenmeyer test. In the distillation set, the Kjeldahl tube and the Erlenmeyer tube are coupled with boric acid solution and mixed indicator. After these steps, 25 ml of 50% sodium hydroxide was added to the measuring cup of the equipment. At the end of the process, the volume of the distillate was 100 ml and the color of the

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solution changed from pink to green. At the end, Titrated with hydrochloric acid, the distilled solution to its turning point.

Moisture Determination

To determine the humidity, the crucibles were first separated. Then he took them to an air circulation oven with a temperature of 105° for an hour of time and then weighed each one. Then, approximately 5g of the sample was weighed in each crucible and taken back to the oven under a temperature of 105°C for 3 hours, after which time the crucibles were transferred to a desiccator for 30 minutes and then their first weighing was carried out.

Subsequently, taken back to the oven at 105°C for 1 hour and 30 minutes in the desiccator to make the next weighing, this procedure was carried out until the weight of the crucible was constant.

Lipid Determination

The reboiller cups were dried in the oven at 105°C for 2 hours, and after the cooling time, the value obtained was weighed and noted. A paper cartridge was made using a filter paper and 2g of the sample that was previously dried was added to it. In the cellulose cartridge of the equipment, the paper cartridge was added and coupled to the stainless steel cradle of the equipment. In the reboiller cup, previously dry and heavy, 50ml of petroleum ether was added where the cup was placed in the vat, with the equipment ready, the adjustment of the stop flow turner and extraction was made for 4 hours, after the necessary time, the dripping process was finished and the reboiller cup was taken to the oven to remove residues of the petroleum ether, After 20 minutes the reboiller cup was weighed again, the result was noted and later the calculation was made.

Determination of Vitamin C Content

For the determination of vitamin C, oxalic acid 2%, ascorbic acid and 2,6-dichlorophenoldophenol solution (DCFI) were used, for oxalic acid 2g of oxalic acid monohydrate was weighed, transferred to a 100ml volumetric flask and completed the volume with distilled water. In ascorbic acid, 0.05g was weighed for 50ml of oxalic acid. He diluted 5ml of this solution to 50ml with 2% oxalic acid (intended for the standardization of the 2,6-dichlorophenoldophenol solution. In the 2,6-dichlorophenoldophenol (DCFI)

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solution, 0.05g of DCFI sodium salt was dissolved in 250ml of hot distilled water, containing 0.042g of sodium bicarbonate, the 250ml volumetric flask wrapped in aluminum foil was measured.

In the standardization, to perform a white test on a 250 ml Erlenmeyer test, 10 ml of oxalic acid and 50 ml of distilled water were added. It was titrated with the 2,6-dichlorophenoldophenol (DCFI) solution until persistent pinkish color for 15 seconds. A second duplicate titration was performed by adding 5 ml of ascorbic acid, 5 ml of oxalic acid and 50 ml of distilled water to a 250 ml erlenmeyer sequence.

For the method, a 15ml capacity falcon tube was required, the sample was added and then centrifuged for 5 minutes in rotation 8. At the end of this process, 5ml of the formed supernatant, 5ml of oxalic acid and 50ml of distilled water were added to a 250ml erlenmeyer machine.

Determination of Phenolic Compounds

The determination of total polyphenols was performed by the Folin-Ciocalteau method, described by Chandra and Mejia (2004), with modifications. For this, the sample was diluted to a concentration of 0.150 mg/mL in water and added to 2 mL of 20% sodium carbonate solution. After 5 minutes, 0.5 mL of the Folin-Ciocalteau 2N reagent was added. The solution was incubated for 10 minutes and the absorbances were measured in a spectrophotometer at a wavelength of 730nm, in triplicate. The analysis was tailored for the best results with the liquid sample. The total polyphenol content was expressed in milligrams of gallic acid equivalent per gram of dry plant, based on the gallic acid calibration curve.

Determination of Flavonoids

First, the standard curve was made using known concentrations with the quercetin solution. For the determination, 2g was weighed and the mixture of ethanol and water (1:1 v/v) was added until 20 g was completed. Finally, the stirring was done for 10 min. It was centrifuged for 5 min at 3000 rpm and 1 mL of the supernatant was collected for analysis.

MICROBIOLOGICAL ANALYSIS

The tea samples were subjected to microbiological analyses of *Salmonella sp. and Escherichia Coli,* according to the methodology described in ISO 6579-1:2002 and the

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EMBRAPA method (2011) respectively, described in Normative Instruction No. 161, of July 1, 2022 (BRASIL, 2022).

SENSORY ANALYSIS

For sensory analysis, the project was submitted to the UEPA Ethics Committee and approved with CAAE 75669823.9.0000.5174.

In the sensory analysis, a sample of geopropolis and mint tea was served. The drink was prepared and served moments before the sensory analysis. About 50 untrained tasters received a form with a 9-point hedonic scale ranging from extremely liked to extremely disliked.

2.4 MICROSCOPIC ANALYSIS

The microscopic analyses were carried out evaluating the requirements cited by Brasil (1998), 10g of the sample was used and it was evaluated under a microscope according to an adaptation of the methodology proposed by Firmino (2011).

RESULTS AND DISCUSSIONS

RESULTS OF PHYSICOCHEMICAL ANALYSES

Table 2 shows the results of the physicochemical analyses of tea based on geopropolis and mint extract.

Table 2: Results of the physicochemical analysis of tea based on the extract of geopropolis and mint (*Menta piperita L).*

Analysis	Average ± SD
Acidity	4.72% ± 0.02
Ashes	14.77% ± 0.1
Protein	18.68% ± 0.0
Moisture	6.50% ± 0.13
Lipids	1.22% ± 0.2
Vitamin C	22.5mg/100g ± 0.57
Phenolic compounds	38 mg/L ± 0.38
Flavonoids	98.59 mg/L ± 0.27

In the acidity evaluation, the result obtained was 4.72%, a value that is directly reflected by the compounds found in the geopropolis such as gallic acids, caffeic acid (CAPE) and caffeic acids (Silva *et al*., 2013), and also by mint, since according to Félix et al., (2012), there is the presence of some acids such as rosmarinic acid in mint. Vicentin et al, (2023) in their work on kombuchas based on mint tea and green tea infusions, when evaluating the acidity, obtained a value of 17.49%.

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In the evaluation of the ash content, a value of 14.77% was obtained in relation to the product on a dry basis. According to Brasil (1998), in relation to the regulation of mixed tea without fruits, up to 14% ashes are allowed. Ash, when present, according to Krumreich et al, (2013), refers to the amount of inorganic materials such as chloride, potassium, and other mineral compounds. It is suggested that the value found in the present study is related to the sample of propolis, since it is a complex mixture, containing resins, balsams and wax, among others (Funaro, 2006).

In the work of Carneiro and Valentini (2018), when evaluating the quality parameters of samples of commercial teas from the region of Campo Mourão – Paraná, ash values for mint tea ranged between 2%, 4% and 10%. Diniz (2013), when evaluating new strategies for simultaneous classification of the type and geographic origin of teas, obtained ash results that did not exceed 6.68% in relation to the three tea samples. In the work by Carneiro et al, (2022), when evaluating the quality of peppermint (*Mentha piperita*) samples sold in Feira de Santana-Bahia, he obtained values ranging from 17.53% to 22.91% ash for the 4 mint samples.

The protein content was the one that stood out the most among the compounds analyzed, obtaining a value of 18.68%, assuming that propolis contains approximately 5% pollen and that geopropolis has a similar percentage (Vargas, et al. 2014), contributing to this result. Esmelindro et al, (2002), obtained a maximum value of 13.45% when analyzing the processed yerba mate.

The table above shows the moisture value of 6.5% in the dry extract of geopropolis and mint. According to Brasil (1998), the maximum moisture content is 12%, which is the same value for the topic of mixed tea, so the product elaborated in the present work remains within the established range. This fact is justified by the fact that it is a vegetable raw material that has undergone the drying process, reducing its moisture levels

Diniz (2013), when evaluating new strategies for simultaneous classification of the type and geographic origin of teas, obtained an average of 6.95%, while for other types of black tea, such as Argentine and Srilanquensa, he obtained results of 7.61% and 9.07%, respectively. These values varied according to the nature of the material, i.e., the drying process carried out in the plant, in addition to the storage conditions of the product.

As for the lipid content, there is no parameter for mixed tea or for mint tea proposed by Brasil (1998), only values for essential oil. In this work, a value of 1.22% of lipids was found, probably originating from geopropolis, which is formed by resins mixed with wax and

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other compounds (Paiva, 2020). In this way, the value obtained may be a reflection of these compounds extracted through the extract and that they added to the mint.

In Roberto's (2018) work, when evaluating the nutrients and bioactive compounds of fresh, dehydrated rosemary, basil and mint and their hot and cold infusions, he obtained an average concentration of lipids in mint of 0.33%, a value below when compared to the study. Esmelindro et al, (2002), obtained fat values between 5.57% and 9.10% when analyzing processed yerba mate.

As for the flavonoid contents, 98.59mg/L, phenolic compounds of 38mg/L and vitamin C of 22.5 mg/100g were obtained. They are not mentioned in ordinance No. 519, of June 26, 1998, however, they are desired compounds in the sample, since both the extract and the mint have phenolic and antioxidant compounds. According to Silva et al. (2013), in propolis, some types of phenolic compounds are found, these being flavonoids, in addition, vitamin C or ascorbic acid, which is a water-soluble antioxidant, which act in the interruption of free radicals (Angelo; Jorge, 2007). In the work of Firmino and Miranda, (2015), on total polyphenols and flavonoids in samples of green tea (*Camellia sinensis L.*) of different brands marketed in the city of Salvador - BA obtained values of up to 28.93mg/g.

RESULTS OF MICROBIOLOGICAL ANALYSES

The present study indicated the absence of *Escherichia coli and Salmonella sp*., which is in agreement with Brazil (2022). The dry extract of geopropolis and mint came from a hydroalcoholic solution, and the alcohols have bacterial action (Wiest, 1984). In addition, the extract of geopropolis, which is rich in compounds that make it antifungal and antibacterial, as reported in the research by Torres (2018), where it points to antimicrobial activity against gram-negative bacteria, such as *Escherichia coli.*

Oliveira et al. (2016) who evaluated the quality of chamomile, mint and carqueja teas *obtained the highest rates of Escherichia coli contamination* in the samples (92.08%).

Vieira et al. (2017) in samples of chamomile and fennel, detected the absence of Salmonella, as well as Negrão and de Paiva (2020) in industrialized teas in Pouso Alegre, MG.

According to Silva et al. (2020), quality control is an indispensable part of the process for the effectiveness of phytotherapy as a safe and effective practice, and must be established in the entire production process, from planting to the ready-to-consume

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product. The importance of proper packaging of the herb to avoid the proliferation of fungi and bacteria and the hygiene of the handler and utensils is emphasized, considering that it can be a means of contamination by coliforms.

RESULTS OF MICROSCOPIC ANALYSES

The results of the microscopic analysis based on Ordinance No. 519, of June 26, 1998, of the Ministry of Health are shown in Table 4.

Table 4: Results of microscopic analyses on tea based on geopropolis and mint extract (*Menta piperita L)*.

Characteristics	Quantity	Sample
Fragments of insects typical of the crop	Maximum 15/10g	Absent
Fragments of insects typical of the culture (in black tea, boldo tea, carqueja and mint)	Maximum 30/10g	Absent
Fragments of insects typical of the culture (in tea of whole flowers and green mate)	Maximum 100/10g	Absent
Fragments of other insects	Absence in 10g	Absent
Live insects and mites	Absence in 10g	Absent
Crop insects and whole dead mites	Maximum 2/10g	Absent
Crop insects and whole dead mites (in whole flower teas and green mate)	Maximum 6/10g	Absent
Animal droppings	Absence in 10g	Absent
Rodent Birds	Absence in 10g	Absent
Extraneous histological elements	Absence in 5g	Absent
Strange starches	Absence in 5g	Absent
Heavy Subjects	Maximum 150mg/10g	Absent
Other foreign matter	Absence in 10g	Absent

Source: Authors, 2024

All microscopic evaluations showed absence of the fragments mentioned in the regulation. The absence of these materials reflects the fact that the geopropolis extract goes through the filtration process and does not present solids and that the process of manual selection of dried mint leaves to be mixed with the extract is carried out. Different result from that found by Marreiro; Teixeira; Souza (2010) when analyzing the types of foreign materials found in tea sachets sold in the city of Teresina-Pi where in the same work the presence of black dots, dust and fragments in tea samples was identified.

SENSORY EVALUATION RESULT

Figure 1 shows the averages attributed to each attribute of the tea based on geopropolis extract and mint.

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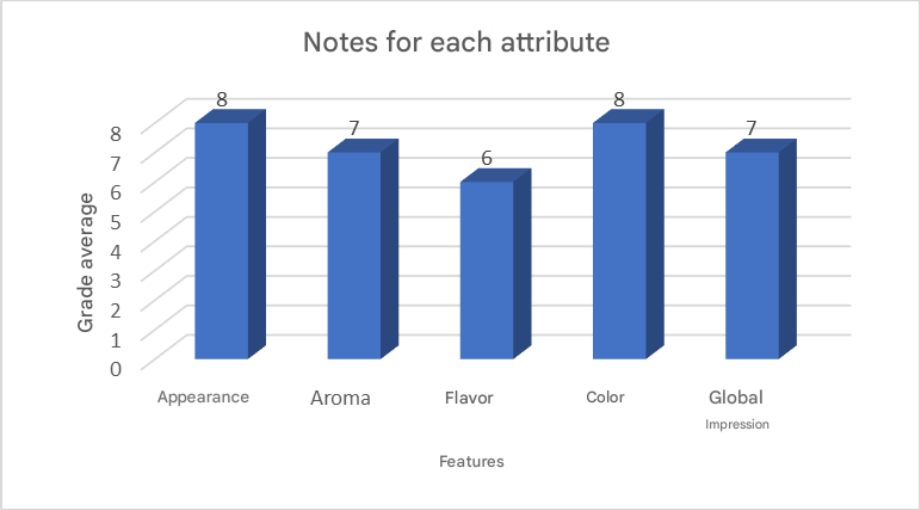
Figure 1: Average of the scores of each attribute of the tea based on geopropolis extract and mint.



Source: Authors, 2024

An overall average score of 7 (global impression) was found in the score defined on the hedonic scale, which gives the tea a rating: "I liked it moderately" and 77.77% acceptance.

In the work by Vicentin et al, (2023) when preparing kombuchas based on mint tea and green tea infusions, 70% of the judges obtained marking the overall grade of the tea between 6 and 9. In the work by Andrade et al., (2024), on the chemical and sensory characterization of fermented hibiscus tea beverage (hibiscus sabdariffa, L.) flavored with pineapple, ginger, and mint, an average of 6 and 8 was obtained for the attributes color, aroma, flavor, viscosity, and overall impression, in addition to obtaining good acceptance by tasters.

As for purchase intention, the result is described in figure 2.

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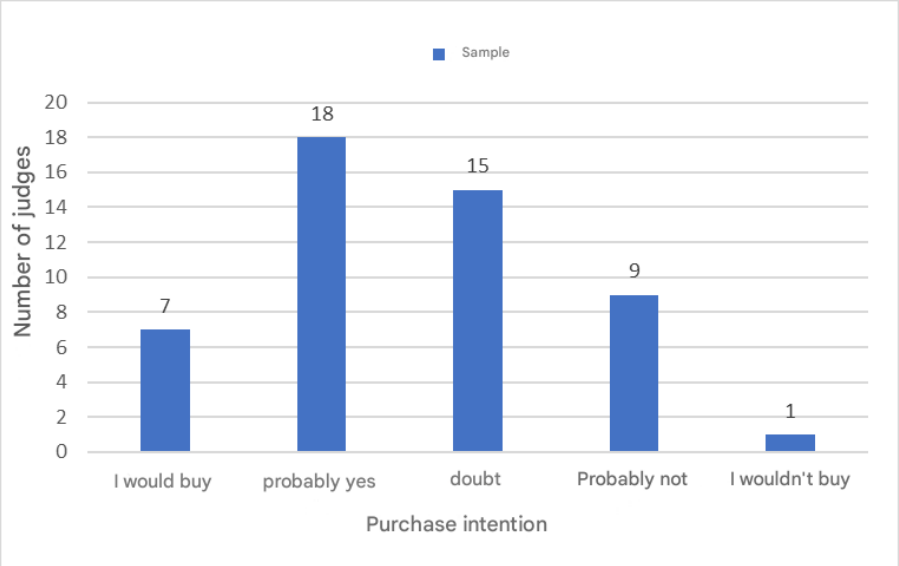
Figure 2: Purchase intention of tea based on geopropolis extract and mint.



Source: Authors, 2024

Regarding the sample, a total of 25 (50%) tasters would buy the sample and 10 (20%) probably would not, this fact can be attributed to the feeling of bitterness reported by one of the candidates in their evaluations, in addition to observations that they would opt for the product if the tea had a lower degree of bitterness, which disfavors the purchase of this product. Also in the work of Vicentin et al, (2023) when preparing kombuchas based on infusions of mint tea and green tea, 75.5% of the judges would buy the sample.

CONCLUSION

It can be concluded that by adding the geopropolis extract to the mint, a tea with excellent physicochemical characteristics was obtained, such as the presence of bioactive compounds, high protein content, in addition to verifying good acceptability, absence of microorganisms such as *Salmonella* sp. and *Escherichia coli* and in accordance with the regulations in relation to dirt.

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