European Journal of Nutrition & Food Safety

Volume 16, Issue 11, Page 177-194, 2024; Article no.EJNFS.122651
ISSN: 2347-5641

Physico-Chemical and Sensory Characterization of Biscuits Enriched with *Persea americana* (Avocado Pit Flour)

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Authors' contributions

This study was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ejnfs/2024/v16i111585

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/122651

Original Research Article

Received: 09/07/2024
Accepted: 11/09/2024
Published: 18/11/2024

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Cite as: *Júnior, Abel Alberto Massingue, Bernabé Bernardo Massie, Angélica Agostinho Machalela, António Elísio José, Enoque Bene Francisco Moiane, and Orlando Albertino Couve. 2024. "Physico-Chemical and Sensory Characterization of Biscuits Enriched With Persea Americana (Avocado Pit Flour)". European Journal of Nutrition & Food Safety 16 (11):177-94. https://doi.org/10.9734/ejnfs/2024/v16i111585.*

ABSTRACT

Biscuit is the product that essentially results from mixing wheat flour, egg, butter, milk and baking powder. Avocado pit flour is obtained through the milling process, contributes around 5% protein, 2% ash, 3% lipids and 3% crude fiber, as well as some functional properties, such as anti-cancer, anti-inflammatory, anti-diabetic and anti- hypertensive potential. The aim of this study was to assess the quality of biscuits produced with the partial addition of avocado pit flour, this flour promotes satiety and intestinal health, preventing diseases has anti-inflammatory proprieties. Four formulations (A), biscuits without avocado pit flour; (B), biscuits with 10% avocado pit flour; (C), biscuits with 20% avocado pit flour and (D), biscuits with 25% avocado pit flour were produced. Physico-chemical analyses were determined in terms of pH by potentiometry, titratable acidity by titrimetry, moisture content by desiccation at 105ºC, lipids by the Soxhlet method, proteins by the biuret method, ash by incineration at 550ºC, carbohydrates by difference calculation and calorific value by *artwater* conversion, and (ii) sensory analysis carried out with affective method, where 50 untrained tasters using 9-point hedonic scale. The results of physico-chemical parameters shown pH ranging from 6.39 to 6.93, titratable acidity from 1.20 to 2.24%, moisture around 8.03 to 8.50%, ash from 2.18 to 2.80%, lipids from 7.78 to 8.05%, protein from 6.53 to 7.11%, carbohydrates from 74.03 to 75.20% and calorific value ranging from 395.49 to 398.42 Kcal. In terms of taste, the intention buys shown that 66% of tasters would buy formulation (A) and 28% would buy formulation (B). The acceptability index shown that formulations A and B were accepted with averages ≥70% of around 85.8 and 73.6%, respectively. It can be concluded that the production of biscuits enriched with avocado pit flour is a viable and technological alternative for treating food waste.

Keywords: Biscuit; avocado pit flour; nutritional quality; sensory analysis.

1. INTRODUCTION

The avocado tree (*Peasea americana Mill*.), whose fruit is called avocado, has Mexican and American origins, belongs to the *Lauraceae* family, genus *Perseal* and comprises two genera: *Persea* and *Eriodaphe* (Bezerra et al.2018).

Its cultivation is carried out in various territories around the world, being predominant in tropical and subtropical regions of Mexico, South Africa, Hawaii, Israel, Tahiti, Australia and the United States (Ministries of Economy 2003).

Avocados are food with fat-soluble vitamins, proteins and high levels of unsaturated fatty acids and potassium. However, it is only consumed when the edible part (the flesh of the fruit) is used. Its stone (residue) accounts for an average of 25% of the fruit's total weight and is a source of interest for the development of new products, with substances of nutritional and commercial interest, such as fatty acids, antioxidant substances capable of fighting free radicals and antimicrobial and antibiotic substances (Freitas et al. 2021).

The avocado pit is a good source of carbohydrates, fat, protein, dietary fiber and bioactive compounds (tocopherols, carotenoids and polyphenols). It has greater antioxidant potential and a higher content of total phenolic compounds than the pulp. It has anti-inflammatory, antihypertensive, hypoglycaemic, hypolipidaemic and analgesic properties (Souza 2020).

By-products of the food industry consist of stones, seeds, pomace and peels. For some researchers, these by-products are often even more nutritious than the fruit itself, yet they are still wasted. Therefore, a nutrient-rich diet could be obtained from these by-products (Alexandre 2018).

The initialization of these by-products generates large quantities of organic waste, causing environmental and economic problems (Coelho 2022). Studies into the utilization of such waste have shown satisfactory results in terms of reducing waste and implementing new technologies, as well as having advantages in terms of reducing food costs, diversifying nutrients and adding nutritional value (Damiami 2020).

The waste of unconventional food parts can be associated with a lack of awareness of the nutritional value provided by these parts (Goubgou et al. 2021). One of the options for processing them is to produce flours and use them in the production of other foods, such as biscuits (Carnelossi et al. 2008). With this in mind, this study aims to carry out the physico-chemical and sensory characterization of biscuits enriched with avocado pit flour, with the aim of contributing to knowledge about technologies for reusing food waste as an alternative to adding nutritional value to biscuits. However, the consumption of biscuits enriched with avocado pit flour promotes satiety and intestinal health, the antioxidant components help fight free radicals in the body, aiding in the prevention of diseases, it has anti-inflammatory properties that reduce inflammation in the body and nutrients that include macronutrients such as protein, carbohydrates and fats, and micronutrients such as vitamins and minerals.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted at the laboratory of Higher Polytechnic Institute of Gaza (ISPG), located in the Chókwè district, in the province of Gaza, South-west of the right bank of Limpopo River, at a distance of 100 km from it and covering an area of 1595 km^2. bounded by Limpopo and Mazimechopes rivers, and by the districts of Bilene, Chibuto, Guijá, Mabalane in Gaza province and the district of Magude in Maputo province (Mohan et al. 2018).

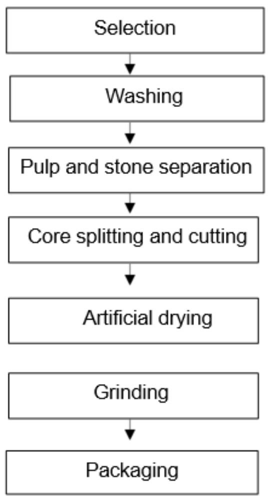
2.2 Purchase of Raw Materials

20kg of avocado was bought at Zimpeto wholesale market, located in the municipality of Maputo. 4kg of wheat flour, 2kg of sugar, 1kg of sodium chloride, eggs, 2kg of butter (Rama), 500mL of condensed milk (*promex*) and 250g of chemical yeast (*Royal*) were purchased from the Limpopo supermarket in Chókwè city. Using the probabilistic model based on simple random sampling, the products were placed in polypropylene bags and taken to the laboratory of the Higher Polytechnic Institute of Gaza for production of biscuits and for physico-chemical and sensory analyses.

2.3 Production of Avocado Pit Flour

The Flowchart 1 shows the production stages for avocado pit flour.

The selection of fruit was carried out by organoleptic characteristics, seeking to separate vocados that were damaged and/or unsuitable for processing due to their stage of ripeness. Next, was selected fruit that showen characteristics of optimum ripeness, (i) dark color and (ii) close to wine color and (iii) sensitivity to touch. The washing process was carried out washing in running water to reduce impurities adhered to the fruit. Next, the pulp and core were separated manually using a knife, making a circular cut around the fruit to make it easier to separate the pulp and core. The core was split by hand using a knife and cut into 2 mm thick slices to facilitate the drying process. The drying process was carried out as recommended by Nascimento et al. (2016), in an oven at a temperature of 60ºC for a period of 24 hours in order to remove free water. The dehydrated kernels were reduced in size using a wooden pestle to grind them into flour, which was then sieved through a 250 µm sieve. The biscuits were packed in 750g glass containers and stored at room temperature ±25ºC.



Flow chart 1. Production of avocado pit flour.
Source: Júnior et al.

2.4 Preparation of Biscuits with Partial Addition of Avocado Stone Flour

Table 1 shows the formulations used to produce avocado pit flour biscuits. The standard formulation used 200 grams of whole wheat flour without Avocado Pit Flour (APF), and the others used 10, 20 and 25% APF to replace the wheat flour.

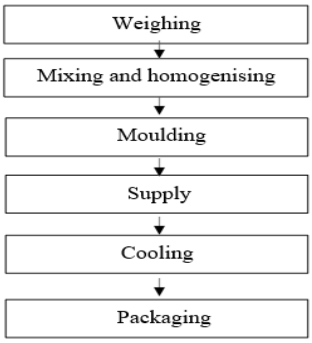
2.4.1. Biscuit production process

The stages of biscuit production are shown, according to Freitas et al. (2008) as shown in Flowchart 2.

Table 1. Formulations of biscuits produced with partial substitution of wheat flour by Avocado Pit Flour (APF)

	Formulations			
Ingredients (%)	A	B	C	D
Wheat flour	50	40	30	20
Avocado Pit Flour	0	10	20	25
Sugar	8	8	8	8
Sodium Chloride	2	2	2	2
Egg	15	15	15	15
Butter	8	8	8	8
Condensed milk	15	15	15	15
Chemical yeast (Royal)	2	2	2	2

(A), biscuits without addition of avocado pit flour; (B), biscuits with 10% addition of avocado pit flour; (C), biscuits with 20% addition of avocado pit flour and (D), biscuits with 25% addition of avocado pit flour.
Source: Authors.



Flowchart 2. Production of biscuits enriched with avocado pit flour
Source: Authors.

The ingredients were weighed on an analytical balance model (BIOBASE) with a precision of around 0.001 grams. Mixing and homogenizing consisted of adding the ingredients in stages and then homogenizing them in a *Depoint* HM945B mixer. To do this, the sugar, margarine, egg and milk were first mixed and homogenized. Next, the flours and baking powder were added, constantly homogenizing. The previously homogenized dough was placed in metal molds to give the biscuits their final shape. The molded dough was then placed in the oven to bake. This process consisted of baking at 160ºC for 30 minutes. The biscuits were placed in stainless steel bowls and cooled at room temperature (±25ºC) for 20 minutes. The biscuits were packed in 750g glass containers until the physico-chemical analyses and/or sensory evaluation were carried out.

2.5 Determining Flour Yield

The yield of avocado stone flour was determined using expression 1, following the methodology referenced by Santana (2014).

$$R = \frac{PF}{PCI} * 100 \quad (1)$$

Where:

R - Yield (%);
PF - Flour weight (g);
PCI - Raw kernel weight (g).

2.6 Physico-Chemical Analyses

Physico-chemical analyses were carried out for pH, titratable acidity, moisture, ash, protein, fat, lipids, calorific value and carbohydrates following the procedures recommended by IAL (2008) and Rigo et al. (2016).

2.6.1 Hydrogen Potential (pH)

The pH of the samples was determined in triplicates. To do this, 5g of the sample was weighed using a Biobase analytical balance in a 150ml elermeyer flask and diluted in 95ml of water. The sample was read directly on an APEX pH meter, previously calibrated with pH 4.0, 7.0 and 10.0 buffer solutions.

2.6.2 Titratable acidity

The titratable acidity of the samples was determined in triplicate. With the aid of an analytical balance, 5 g of the sample was weighed and diluted in 95 mL of distilled water in a 250 mL elermeyer, to which 3 drops of phenolphthalein solution were added and with the aid of a burette, 0.1 M sodium hydroxide solution (NaOH) was added, homogenizing until the turning point was reached. The results were obtained according to expression 2.

$$Acidity \% = \frac{V * F * M}{P} \quad (2)$$

Where:

V - number of ml of sodium hydroxide solution used in the titration;

F - correction factor for the sodium hydroxide solution;

P - sample mass in g or pipetted volume in ml;
M - molarity of the sodium hydroxide solution.

2.6.3 Moisture

Moisture was determined by desiccation in an oven at 105ºC. Flour and biscuits were sampled (5g) in previously desiccated Petri dishes and dried in an oven for 2 hours at a temperature equivalent to 105ºC and cooled to room temperature. The determinations were carried out in triplicates and the results expressed using Equation 3.

$$Moisture\ \% = \frac{100 * N}{\mathrm{P}} \tag{3}$$

Where:

N - number of grams of moisture;
P - number of grams of sample.

2.6.4 Ash

Ash was determined by incineration. This analysis proceeded by weighing 5g of previously crushed biscuits and placing them in porcelain crucibles, carbonized and calcined on a magnetic plate until the ash turned white or greyish. Subsequently, the crucibles with the samples were incinerated in a muffle furnace at 550º C for 4 hours until a constant weight was obtained, after which the crucibles were removed using tweezers and transferred to an oven with circulating air at 105ºC for 30 minutes. The determinations were carried out in triplicates and the results expressed using Equation 4.

$$Ash\ \% = \frac{(m2 - m)}{(\mathrm{m1} - \mathrm{m})} \tag{4}$$

Where:

m - Crucible eight;
m1 - Weight of crucible with wet sample;
m2 - Weight of crucible with ash.

2.6.5 Proteins

The protein content was determined using the Biuret method, where 300 µL of each extract (prepared in the ratio: 10g of sample and 90 mL of water) was mixed with 2000 µL of Biuret reagent and left in a dark place for 30 minutes to give a purple complex color, then the sample was transferred to a cuvette and the absorbance was read at 540 nm on a spectrophotometer previously zeroed with distilled water.

2.6.6 Fat determination

The fat content was determined using the Soxhlet method. This analysis was carried out by weighing 5g of the sample, which had previously been crushed in a mortar, placing it in the cartridge and attaching it to the extractor, which was then connected to a flat- bottomed flask (previously dried in an oven with air circulation at 105ºC). 250 mL of petroleum ether was added to the flask and placed in the hood at (60ºC), starting the fat extraction process for 4 hours and the fat was deposited in the extractor. After extraction, the extracted fat was taken to an oven at 105ºC for 30 minutes and cooled to room temperature (±25ºC). Expression 5 was used to determine the fat percentage.

$$Fat\ content\ \% = \frac{(weight + fat) - balloon\ weight)}{sample\ Weight * 100} \tag{5}$$

2.6.7 Calorific value

The total energy value in 100g of biscuit was determined according to estimated calorific values from the *Atwater* conversion, multiplying the available carbohydrate and protein values by four and the lipid value by nine. The sum of the products constituted the total energy value. The determinations were carried out in triplicates and the results expressed using Equation 6.

$$\text{Calorific value} = \%\ \text{Protein} + \%\ \text{Carbohydrates} * 4 + \%\ \text{Fat content} * 9 \tag{6}$$

2.6.8 Carbohydrates

The quantification of total carbohydrates was based on the difference calculation method, where based on equation 6, the percentages of moisture, fat, protein and ash in 100g of the sample were added and subtracted from the maximum percentage (100%) and determined using Equation 7.

$$\text{Carbohydrates (\%)} = 100 - (\%\text{Moisture} + \%\text{Ash} + \%\text{Protein} + \%\text{Fat}) \tag{7}$$

2.7 Sensory Analysis

The sensory analysis was carried out using the affective method. They were selected at random. 50 untrained tasters were randomly selected on the campus of Higher Polytechnic Institute of Gaza (ISPG). For this purpose, the taster was asked to express their emotional state or affective reaction towards the avocado stone flour biscuits, using a 9-point hedonic scale, with the extremes "9 - liked very much" and 1 - disliked very much". The sample was delivered on disposable plates coded with three digits, the evaluation form and a glass of water so that the flavor of the previous sample would not interfere with the evaluation of the next sample. Attributes were assessed in terms of: appearance, color, texture, flavor, crunchiness, aroma, aftertaste and overall assessment, considering the methodology proposed by Oliveira et al. (2021). The product is considered accepted if it is equal to or greater than 70% of the acceptability index (AI) (Dutcosky 2019). Expression 8 was used to determine the acceptability index of the biscuits:

$$IAC = \frac{A*100}{B} \quad (8)$$

Where:

IAC - Purchase Intention Index;
A - average score obtained for the product;
B - maximum grade given to the product.

2.7.1 Purchase intention test

The purchase intention was determined by the total number of a sample's purchase preference over the total value of the number of tasters that made up the analysis panel. The result was expressed using Equation 9.

$$IAC = \frac{A}{B*100} \quad (9)$$

Where:

IC - Purchase intention;
A - Total number of preferences in the sample;
B - Total number of tasters.

2.8 Statistical Analysis

The experiment was set up in a Completely Randomized Design (CRD) with 4 biscuit formulations consisting of (A), biscuits without addition of avocado pit flour; (B), biscuits with 10% addition of avocado pit flour; (C), biscuits with 20% addition of avocado pit flour and (D), biscuits with 25% addition of avocado pit flour in triplicate replicates for the physico-chemical analyses. On the other hand, the Randomized Block Design (RBD) was used to analyze the sensory attributes. The analysis of variance (ANOVA) was carried out using the Minitab 18.1 statistical package at a 5% significance level, and the means (*p<0.05*) were compared using the Tukey test.

3. RESULTS AND DISCUSSION

3.1 Avocado Pit Flour

3.1.1 Yield of avocado pit flour

Table 2 shows the yields of avocado pit flour.

At the beginning, the avocados had a total weight of 19.5kg. After pulping, the weight of the pit was around 16.48kg, equivalent to 84.53% of the fruit's weight.

The avocado pits weighed a total of 3.016 kg, corresponding to 15.45% of the total weight of the fruit. They were dried in an oven for 24 hours at 60ºC and then ground into flour, with a final weight of 1.01 kg, giving a final yield of 33.49%. This may be intrinsically linked to losses during the production process, particularly water loss during the drying process.

In the study conducted by Marques et al. (2018) found yield of 32.99%, when carried out the characterization of avocado pit flour, this result is close to that found in this research.

Higher values than those obtained in this research were reported by Dos Silva et al. (2019) when determining the physicochemical properties of avocado pit starch, obtaining a yield of 36.5%, while Cordeiro (2020) found a 36% yield when characterizing avocado seed flour and brewing residue. Santos et al. (2017) studying the physicochemical characterization of avocado stone flour (*Persea americana*), obtained a yield of 46.28%. This variation may be associated with the varieties used and the agro-climatic growing conditions.

3.1.2 Physico-chemical composition of avocado pit flour

The physico-chemical composition of avocado stone flour is shown in Table 3.

Table 2. Yield of avocado stone flour

Raw materials	Stage	Mass (Kg)	Losses (Kg)	IP (%)	%(R)
	Reception	19.5	-	-	-
Avocado pit	Pulping	3.016	16.48	84.53	-
	Drying	1.026	1.99	-	-
	Grinding	1.01	0.016	1.55%	33.49

(R), Yield as a percentage and (IP), Loss Index as a percentage.
Source: Authors

Table 3. Physico-chemical characterization of avocado pit flour

Parameters	Results
pH	5.06±0.02
Titratable acidity (%)	4.5±0.30
Moisture (%)	7.26±0.06
Ash (%)	3.65±0.07
Lipids (%)	3.9±0.03
Protein (%)	4.10±0.27
Carbohydrates (%)	81.1±0.16
Calorific Value (Kcal/100g)	375.71±0.47

Source: Authors

The study showed a pH of 5.06 in the characterization of avocado pit flour, indicating that the flour is moderately acidic as well as the existence of organic acids.

Similar values to those obtained in this study were reported by Oliveira (2018), around 5.62 pH, when produced and evaluating gluten-free biscuits with flour from fruit by-products, and Bezerra et al. (2018) when preparing avocado pit flour to use waste, found pH value of 5.66.

Ministries of Economy (2003) when carrying out the physico-chemical and functional characterization of avocado seed flour and its viability for making a food product, reported a pH of 5.70.

The titratable acidity showed a level of around 4.5% acidity. Lower value was reported by Silva et al. (2018) when carried out the physico-chemical and centesimal characterization of the fruit of the jatobá-do-cerrado (*Hymenaea stigonocarpa* Mart.), around 0.41% in the jatobá-do-cerrado kernel flour.

Silva (2019) when determining the nutritional, physical, chemical and technological functional properties of Baru (*Dipteryx alata* Vogel) flour as a function of different fruit drying conditions, reported 4.47% of acidity value, result similar to that obtained in this study.

The study found that avocado stone flour had a moisture content of around 7.26%. This was in line with the recommendations of Decree-Law no. 65/92 and Ministerial Order no. 254/2003, which stipulate a moisture content of less than 14.5% for flours used in bakery products (Moreira 2020).

Freitas et al. (2021) when they studied the obtaining and physicochemical characterization of avocado pit flour for addition to cakes, found a moisture content of 6.75 in avocado pit flour. Higher moisture (15.38% and 27.55%) values were referenced by Oliveira et al. (2021) when studying the elaboration and evaluation of gluten-free biscuits with flour from fruit by- products, obtained moisture around 15.38%, and Siol & Sadowska (2023) in their study entitled sensory evaluation of *cookie-type* biscuits made with sweet potato flour, found 27.55% moisture. According to Novelina et al. (2022) foods with a high moisture content favor the development of microorganisms.

The ash content found in avocado stone flour was 3.65%. This value is higher than that recommended by Decree-Law no. 65/92 and Ordinance no. 254/2003, which stipulates an ash content of less than 2.5% for flours used in bakery products (Moreira 2020).

Lower values were found by Marques et al. (2018) when characterizing avocado pit flour, with an average of 2.83%. This comparison indicates a greater amount of inorganic residue, mostly composed of minerals in the samples in this study.

Siol and Sadowska (2023) when characterizing albedo, pomace and orange peel flours, an average of 3.8%, which may be associated with the high concentration of mineral content in the orange peel compared to the other parts (albedo (3%) and pomace (2.4%). Freitas et al. (2021) when carrying out the production and physicochemical characterization of avocado stone flour, observed 2.33% of ash. This is probably due to the variation in the mineral content of the varieties studied.

Results close to those of the present research were reported by Ramos et al. (2018) when studying the physicochemical characterization of avocado seed extract flour (*Persea americana*), obtained a fat content of 2.20%, similarly, Ministries of Economy (2003) when carrying out the physicochemical and functional characterization of avocado seed flour and its viability for the preparation of a food product, obtained 2.48% of fat.

Avocado pit flour had a protein value of 4.10%. A higher result was reported by Siol Sadowska (2023) when they studied the preparation and sensory analysis of a *cookie* made from avocado pit flour and obtained 5.17% of protein. This is probably due to the variation in varieties, the ripeness of the avocado, and processing, since processes involving high temperatures (drying) can result in protein loss through denaturation.

Lower values were obtained by Souza (2020) when they studied the physicochemical and bioactive properties and its use as a functional food, reporting a protein content of around 3.4%, and by Ministries of Economy (2003) when studying the physicochemical and functional characterization of avocado seed flour and its viability for making a food product, with a protein content of around 1.50%.

The carbohydrate content obtained in this study was 81.1%. A result close to this study was described by Freitas et al. (2008) when they characterized avocado stone flour for addition to cakes, obtaining 82.55% carbohydrates.

Nyakang'i et al. (2023) when determining the centesimal composition and minerals of avocado pit flour, found 63.8% carbohydrates and, on the other hand, (Instituto Adolfo Lutz – IAL 2008) when determining the nutritional composition and acceptability of avocado pit sweets, found 44.7% of carbohydrates, lower than the results of this research. This was possibly linked to the variety used, the level of ripeness of the fruit, the climate and soil conditions.

This study revealed a calorific value of 375.71 Kcal. Marques da Silva et al. (2018) studying the physicochemical characterization of avocado (*Persea americana*) seed extract flour, found 367.343Kcal. Freitas et al. (2021) obtained 378.93 Kcal, when carried out the physical and chemical characterization of avocado pit flour for use in cakes, which is similar to the results of this study. Substantially lower value was reported by Siol & Sadowska (2023) when characterizing avocado pit flour, found calorific value of 328.27Kcal.

3.2 Physico-Chemical Composition of Biscuits Enriched with Avocado Pit Flour

Table 4 shows the results of the analyses of the centesimal composition of the biscuits made by partially replacing wheat flour with avocado flour.

3.2.1 pH

The results shown pH values ranging from 6.39 to 6.90. Higher pH values were observed in formulations A (6.90) and B (6.84), while lowest values were obtained in formulations C (6.68) and 6.39. Statistically, formulations A and B did not differ significantly from each other (p>.05). Significant differences (p< .05) were evident in formulations C and D in relation to formulations A and B. This difference can be explained by the higher concentration of organic acids (myristic, palmitic, palmitoleic, stearic, oleic and linoleic) in avocado stone flour, resulting in a reduction in pH as a result of their addition.

Higher values were described by Piovesana et al. (2011) in their study on the preparation and quality of *cookie-type* biscuits enriched with *Caryocar coriacium Wittm* almond press cake, by adding 2, 4 and 6% of *Caryocar coriacium Wittm* almond flour to the biscuits, with a pH of 8.03 8.34. This is probably due to the low concentration of acids in the almond flour.

Mahawan et al. (2015) when carrying out the physicochemical and microbiological characterization of biscuits made with fruit waste flour, observed a pH of 6.01 to 6.36, values close to those of the present study. Silva et al. [19], when carrying out the physical and chemical characterization of a savoury biscuit enriched with flour from carrot processing waste and

Table 4. Centesimal composition of biscuits made with partial substitution of wheat flour by avocado flour

Parameters	Formulations			
	A	B	C	D
pH	6.90±0.03^{a}	6.84±0.04^{a}	6.68±0.02^{b}	6.39±0.05^{c}
Titratable acidity (%)	1.20±0.00^{d}	1.48±0.09^{c}	1.96±0.06^{b}	2.24±0.06^{a}
Moisture (%)	8.04±0.03^{b}	8.03±0.06^{b}	8.50±0.17^{a}	8.22±0.06^{b}
Protein (%)	6.53±0.29^{b}	6.71±0.04^{ab}	7.00±0.13^{a}	7.11±0.11^{a}
Lipids (%)	7.78±0.02^{b}	7.86±0.04^{b}	7.97±0.02^{a}	8.05±0.04^{a}
Ash (%)	2.80±0.06^{a}	2.18±0.04^{b}	2.29±0.09^{b}	2.45±0.22^{b}
Carbohydrates (%)	74.83±0.38^{ab}	75.20±0.18^{a}	74.21±0.18^{b}	74.16±0.34^{b}
Calorific Value (Kcal)	395.50±0.28^{b}	398.42±0.24^{a}	396.67±1.04^{ab}	397.56±0.96^{a}

Means ± standard deviation followed by the same letter on the same line do not differ significantly from each other at the 5% significance level. (A), biscuits without addition of avocado pit flour; (B), biscuits with 10% addition of avocado pit flour; (C), biscuits with 20% addition of avocado pit flour and (D), biscuits with 25% addition of avocado pit flour. Source: Authors

spices, reported that the pH of the biscuits gradually decreased as a result of the addition of fiber-rich flours, justifying the reduction in pH due to the avocado stone flour.

3.2.2 Acidity

The results showed titratable acidity ranging from 1.20 to 2.24 %. The highest value was observed in formulation D (2.24). Lower values were observed in formulations A, B and C with values of around 1.20, 1.48 and 1.96 % respectively. Statistically, all the formulations showed differences ($p< 0.05$) between each other. This may be due to the high concentration of fibers in avocado stone flour, resulting in a gradual increase in acidity as a result of its incorporation.

Consistent results were reported by Araujo (2019) around (1.24 to 3.84) % acidity, when he developed a *cookie-type* biscuit based on the percentage substitution of pineapple peel perola and maracujá rubi cerrado flour.

Lower values than those obtained in this research were reported by Tanssini (2016) who developed a pequi almond biscuit and obtained 0.22 to 0.69% acidity, and by Paulo et al. (2020), when carrying out studies on the development, physicochemical characterization and sensory evaluation of a *cookie* with passion fruit peel flour added to *whey protein*, reported acidity of 0.12 to 0.16%. This is probably due to the low concentration of organic acids in pequi and passion fruit kernel flour.

3.2.3 Moisture

The moisture content showed results in the 8.03 to 8.50% range. The lowest values were observed in formulations A (8.04%) and B (8.03%). Statistically, formulation C was statistically different ($p< .05$) from the others. This phenomenon is related to the water retention capacity of avocado stone flour, which caused the variation in moisture content.

Nascimento et al. (2019) when studying the physicochemical and sensory characteristics of biscuits made with avocado as a fat substitute, reported moisture contents ranging from 5.56 to 11.39%, Silva et al. (2020) in their study on the elaboration and sensory analysis of *cookie-type* biscuits made from avocado stone flour, found that the greater the addition of avocado stone flour, the lower the moisture percentage, ranging from 13.87 to 18.44%.

Ifesan (2015) when determining the physicochemical and sensory properties of biscuits produced by partially replacing margarine with avocado puree, reported a moisture content of 11.13 to 14.60 %, results that differ from those of the present research. This is due to the fact that the puree is incorporated in its natural form and the proportion of ingredients.

3.2.4 Proteins

The protein value ranged from 6.53 to 7.11 %. Formulation D (7.11%) had the highest average, followed by formulation C (7%). Formulations B, C and D did not differ significantly ($p>.05$) from each other. On the other hand, formulation A differed ($p< .05$) from formulations C and D. This variation may be linked to the availability of protein in avocado pit flour.

Results lower than those of this research were described by Oliveira et al. (2021) in his study on the elaboration and evaluation of gluten-free biscuits with fruit by-product flour, obtaining 4.42% protein, and by Okoyeuzu et al. (2023) with 3.66 to 4.73% protein, when determining the use of avocado pit flour (*Persea americana*) as a mixture of modified cassava flour in the manufacture of biscuits. This is probably due to the low protein content of the fruit by-products, as well as the proportions incorporated.

Tanssini (2016) when studying the development of a *cookie* made from pequi kernel flour, obtained 8% protein, results close to those obtained in this research.

3.2.5 Lipids

The fat content ranged from 7.78 to 8.05 %. The highest content (8.05%) was evident in formulation D. Statistically, formulations A and B differed ($p < .05$) from formulations D and C. This variation may be associated with the concentration of lipids in the avocado pit flour, confirmed by the increase in lipids as a function of the concentration of avocado pit flour in the biscuits.

Similar values to those obtained in this research were reported by Soares et al. (2018) when developing a pequi kernel biscuit, around 8% fat, by Siol & Sadowska (2023) in their study entitled elaboration and sensory analysis of a *biscuit* made from avocado stone flour, obtained 8.0%.

Results above those found in this research were described by Hussein et al. (2020) when he developed biscuits enriched with mango stone flour, obtaining 10.14 to 13.02% fat. This is possibly associated with the high concentration of mango flour incorporated, with formulations containing 50 and 75 % mango flour.

3.2.6 Ashes

The ash content ranged from 2.18 to 2.80 %. The highest averages were found in formulations A (2.80 %) and D (2.45 %). Statistically, formulation A showed significant differences ($p < .05$) in relation to formulations B, C and D. This differentiation is possibly associated with the higher concentration of minerals in the wheat flour.

Oliveira (2020) when determining the consumer acceptability and quality characteristics of cookies produced from wheat flour and banana and/or avocado peels, reported values in the range of 2.06%, which is within the range of the results obtained in this study.

Olaoye et al. (2019) when characterizing the physicochemical and sensory properties of biscuits made with avocado as a fat substitute, reported 1.37 to 1.78% ash and Nyakang'i et al. (2023) when studying the physicochemical properties of avocado seed extract, obtained 1.82% ash.

3.2.7 Carbohydrates

Carbohydrates ranged from 74.21 to 75.20%. The highest average was observed in formulation B (75.20%). The lowest average was observed in formulations D (74.1%) and C (74.21%). Statistically, no significant differences ($p > 0.05$) were observed between formulations A, C and D. Differences ($p < .05$) were observed in formulation B in relation to formulations C and D. This is possibly associated with the higher concentration of macronutrients in the wheat flour.

Divergent results to those of this research were reported by Ifesan (2015) when determining the physicochemical and sensory properties of biscuits produced by partially replacing margarine with avocado puree, around 58.86 to 62.22%, and by Viera et al. (2015) around 42.15 to 49.12%, when characterizing buttery biscuits free of added sugar made partially with jackfruit pulp and seeds. Guidone da Hussein et al. (2020) when developing biscuits enriched with mango stone flour, observed a range of 56.83 to 67.23% carbohydrates. This may be due to the low concentration of macronutrients as well as the proportions of flour incorporated into the biscuits.

3.2.8 Calorific value

The calorific value ranged from 395.50 to 398.42Kcal. The highest value was measured in formulation B (398.42Kcal), followed by formulation D (397.56Kcal). Statistically, formulations B, C and D did not differ ($p > .05$) from each other. These calorie levels are probably due to the qualitative concentration of the macronutrients.

Higher values than those of the present research were described by Silva et al. (2019) in their study on the physical and chemical characterization of a savoury biscuit enriched with flour from carrot processing residues and spices, obtaining 419.49 Kcal, and by Viera et al. (2019) around (467kcal), when carrying out the development and characterization of a biscuit made from bacuri flour (*Platonia insignis*, Mart). This is possibly due to the high concentration of macronutrients in these flours.

In the evaluation carried out by Oliveira (2020) in their study seeking to determine consumer acceptability and quality characteristics of *cookies* produced from wheat flour and banana and/or avocado peel, they obtained 354.31 to 351.41 kcal when incorporating 5 and 10% avocado puree.

3.3 Sensory Analysis

The results of the sensory analysis of the biscuits enriched with avocado coconut flour are shown in Table 5 on a 9-point hedonic scale.

3.3.1 Appearance

The appearance attribute ranged from 5.78 to 7.02, at the extremes of "neither liked nor disliked to moderately liked". The highest averages were seen in formulations A (7.02) and B (6.68). Lower values were noted in formulations C and D with averages of around 6.06 and 5.78. Differences ($p < 0.05$) were observed in formulations A and B in relation to formulations C and D. This variation may be associated with the amount of avocado stone flour added to the biscuit, since the more it is incorporated, the color of the biscuit changes.

Similar results to those obtained in this study were reported by Siol & Sadowska (2023) who carried out a study on the preparation and sensory analysis of a *cookie* made from avocado pit flour and reported scores ranging from 6.59 to 7.49 for biscuits containing 6, 12 and 24% avocado pit flour, and by Freitas et al. (2021) in a study on obtaining and physicochemical characterization of avocado pit flour for addition to cakes, reported values of 7.52 to 7.47.

Peccin et al. (2022) in their study on determining consumer acceptability and quality characteristics of *cookies* produced from wheat flour and banana and/or avocado peels, reported values ranging from 6.1 to 7.4, results close to those of this study.

3.3.2 Color

Color scores ranged from 5.94 to 7.60. Higher scores were noted for formulations A (7.60) and B (6.70). However, lower average scores were recorded for formulations C and D, respectively. Significant differences ($p < .05$) were observed between formulation A and the others. This difference is possibly due to the amount of flour added, which has a totally different color to wheat flour (dark brown). The greater the amount added, the greater the change in color of the biscuits due to the anthocyanin content of the flour.

Vasconcelos et al. (2018) when making a functional buttery biscuit based on cowpea flour (*Vigna unguiculata* L. *Walp*), obtained an average of 6.10 to 6.92, similar to the values found in this study.

Similar results to those found in this study were reported by Nascimento et al. (2023) when carrying out their research into the sensory analysis and protein content of vegan cake made with pumpkin seed flour (*Cucurbita moschata*), they obtained averages between 7.46 and 7.78. For their part, Nascimento et al. (2019) when studying the physicochemical and sensory characteristics of biscuits made with avocado as a fat substitute, reported results ranging from 7.06 to 7.4 for the color attribute.

3.3.3 Texture

The highest value was found in formulation A (7.24) and the lowest in formulation B (5.06). There were no significant differences ($p > .05$) between formulations A and B, but they did differ from formulations C (5.08) and D (5.06). This may be associated with the variation in wheat flour due to the addition of avocado stone flour, which influences the availability of gluten, which is primarily responsible for the formation and structure of the biscuit dough.

Similar results to those obtained in this research were described by Orloski et al. (2016) in a study on the sensory acceptability of cereal bars made with papaya seed flour, obtained averages between 5.26 and 6.80, by Ifesan (2015) when determining the physicochemical and sensory properties of biscuits produced by partially replacing margarine with avocado puree, obtained scores of 6.85 to 7.50.

Divergent values were reported by Okoyeuzu et al. (2023) when determining the use of avocado stone flour (*Persea americana*) as a mixture of modified cassava flour in the manufacture of biscuits, ranging from 3.32 to 4.16.

3.3.4 Flavor

Flavor scores ranged from 4.28 to 6.80 in the "slightly dislike to slightly like" range. The highest values were observed in formulations A (6.80) and B (5.98). There were no significant differences (p> .05) between formulations A and B. This is due to the characteristic flavor of avocado stone flour, resulting from the phenolic compounds such as tannins and flavonoids that can be transferred to the biscuits, the eating habits of the tasters and the expectation error, if the tasters expected a similar flavor to the traditional biscuit.

Barros et al. (2020) in their study on the physicochemical and sensory evaluation of *cookie-type* biscuits enriched with açaí stone and pulp flour, obtained scores from 4.56 to 6.73, results similar to those obtained in this research. On the other hand, Vieira et al. (2015) evaluating the effect of replacing trio flour in the development of gluten-free biscuits, obtained scores ranging from 6.20 to 6.48 for enriched biscuits, results in agreement with those of the present research. According to Oliveira et al. (2021) the low flavor scores may be due to the influence of the residual flavor of the flours that are transferred to the biscuits.

3.3.5 Crunchiness

Crunchiness showed results ranging from 4.94 to 7.08. The highest values were seen in samples A (7.08) and B (6.50). On the other hand, samples C and D had a crunchiness of around 5.48 and 4.94, respectively. Statistically, samples A and B did not differ significantly (*p >0.*05) from each other. This is associated with the structural properties of avocado stone flour, which differs from wheat flour in aspects such as the absence of gluten, which influences dough growth and the final texture, resulting in less crunch.

Similar results were observed by Oliveira et al. (2021) when determining the consumer acceptability and quality characteristics of *biscuits* produced from composite wheat flours and banana and/or avocado peels, they reported scores ranging from 6.6 to 7.4, by Hussein et al. (2020) when developing biscuits enriched with mango stone flour, obtaining a range from 5.58 to 7.19, and by Ifesan (2015) when determining the physicochemical and sensory properties of biscuits produced by partially replacing margarine with avocado puree, reported averages of 6.65 to 7.85.

Marques da Silva et al. (2018) revealed that the crunchiness decreases as a result of the increase in avocado stone flour due to the decrease in gluten that supports the structure of the biscuit.

3.3.6 Aroma

The highest average corresponded to formulation A (6.74), followed by formulation B (6.10), respectively. There were no significant differences (*p>.05*) between formulations B, C and D. This variation is possibly linked to the characteristic of avocado stone flour and the increase in its quantity resulted in greater sensitivity on the part of the tasters.

Similar results to those of this study were reported by Oliveira et al. (2021) in his study on the elaboration and evaluation of gluten-free biscuits with flour from fruit by-products, obtaining averages ranging from 5.52 to 6.05.

Silva et al. (2019) in their sensory evaluation of *cookie-type* biscuits made with sweet potato flour, obtained averages ranging from 6.97±1.45 to 7.58, results that were higher than those obtained in this study. On the other hand, Rapina (2017) when characterizing cupuaçu seed flours and using them in the production of cookie-type biscuits, reported aroma results ranging from 7.28 to 7.64. This is possibly associated with the eating habits of the tasters.

Silva et al. (2019) in their sensory evaluation of *cookie-type* biscuits made with sweet potato flour, obtained averages ranging from 6.97±1.45 to 7.58, results that were higher than those obtained in this study. On the other hand, Rapina (2017) when characterizing cupuaçu seed flours and using them in the production of cookie-type biscuits, reported aroma results ranging from 7.28 to 7.64. This is possibly associated with the eating habits of the tasters.

3.3.7 Aftertaste

The aftertaste showed an average ranging from 4.46 to 6.74 at the extremes of "slightly disliked to neither liked nor disliked". The highest score (6.74) was seen in formulation A, followed by formulation B (5.60). The lowest

Table 5. Sensory analysis of biscuits enriched with avocado pit flour

	Attributes							
Samples	**Appearance**	**Color**	**Texture**	**Flavor**	**Crunchiness**	**Aroma**	**Aftertaste**	**Overall assessment**
A	7.02±1.8[a]	7.60±1.29[a]	7.24±2.03[a]	6.80±2.37[a]	7.08±1.98[a]	6.74±1.92[a]	6.74±2.08[a]	7.72±1.62[a]
B	6.68±1.9[ab]	6.70±1.61[b]	6.38±2.09[a]	5.96±2.52[a]	6.50±1.85[a]	6.10±2.20[ab]	5.60±2.53[b]	6.62±2.24[b]
C	6.06±2.12[b]	6.62±1.63[bc]	5.08±2.17[b]	4.78±2.40[b]	5.48±1.96[b]	5.80±1.92[b]	4.88±2.45[bc]	5.68±2.27[c]
D	5.78±2.25[b]	5.94±2.17[c]	5.06±2.57[b]	4.28±2.64[b]	4.94±2.43[b]	5.48±2.28[b]	4.46±2.47[c]	5.14±2.56[c]

Means ± standard deviation followed by the same letter in the same column do not differ significantly from each other at the 5% significance level. (A), biscuits without addition of avocado pit flour; (B), biscuits with 10% addition of avocado pit flour; (C), biscuits with 20% addition of avocado pit flour and (D), biscuits with 25% addition of avocado pit flour.

Source: Authors.

average was observed in formulation D (4.46). There were no significant differences (*p >0.*05) in formulations B and C. This may be associated with the acidity present in avocado pit flour, caused by organic compounds such as myristic, palmitic, palmitoleic, stearic, oleic and linoleic.

Results close to those of the present research were reported by Peccin et al. (2022) when they carried out studies on the preparation of cream cracker biscuits with added flaxseed flour and reduced sodium content, they obtained scores ranging from 5.80 to 6.70, by Guidone et al. (2016), when determining the effect of three types of oil and their level of incorporation on the sensory quality of sorghum biscuits, they reported lower scores of around 3.27 to 4.04. This is due to the peculiar characteristics of the raw materials used, as well as the consumption habits of the tasters.

Higher results were described by Oliveira et al. (Oliveira 2018, De Souza 2015, Dos Santos 2016) when evaluating the quality attributes of biscuits mixed with pineapple juice, tamara flour and wheat, with aftertaste ranging from 6.20 to 7.40. According to Dutcosky (2019), aftertaste is a complement to flavor and should be above average 5, a value achieved by formulations A and B.

3.3.8 Overall assessment

The overall assessment ranged from 5.14 to 7.72. Formulations A and B had the highest scores of around (7.72) and (6.62) respectively. Statistically, no significant differences (p>0.05) were observed in formulations C and D. Differences were evident in formulations A and B. This is probably due to the conditions in which the analysis was carried out, the color, crunchiness, flavor and aroma of the product.

Oliveira et al. (2021) when determining the consumer acceptability and quality characteristics of *cookies* produced from composite wheat flours and banana and/or avocado peels, reported scores ranging from 6.7 to 7.6, results allied to those of the present study. This may be associated with the characteristic flavor of the fruit peels.

Higher values were observed by Freitas et al. (2021) when they developed cakes with the partial addition of avocado stone flour, obtaining averages of 7.08 to 7.80. However, (Vicentini 2015, Gomes 2018, Gouvea et al. 2021) when characterizing cupuaçu seed flours and using them in the production of *cookie-type* biscuits, reported overall evaluation results ranging from 7.48 to 7.76. This was probably due to differences in the raw materials used.

3.4 Purchase Intention

Graph 1 shows the results of the purchase intention index for biscuits enriched with avocado pit flour.

The purchase intention test showed that 66% of the tasters reported that they would buy formulation A, (28%) formulation B. On the other hand, only 4 and 2% said they preferred formulations C and D, respectively. This is probably due to the varying preferences of the tasters, their unfamiliarity with the flour used to make the biscuits and their eating habits.

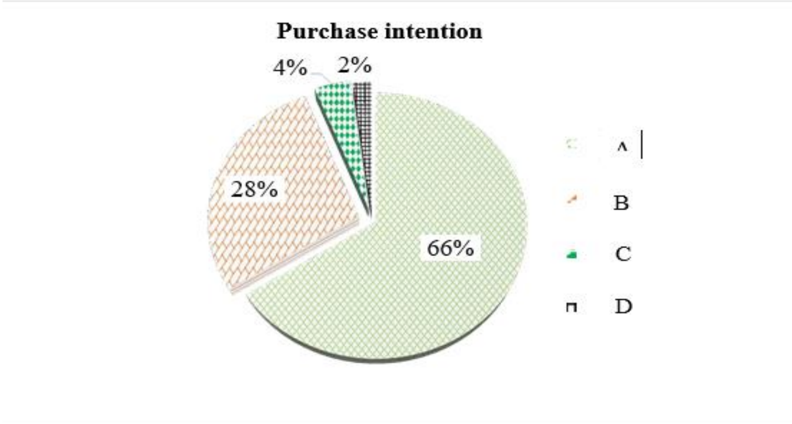
The purchase intention test showed that 66% of the tasters reported that they would buy formulation A, (28%) formulation B. On the other hand, only 4 and 2% said they preferred formulations C and D, respectively. This is probably due to the varying preferences of the tasters, their unfamiliarity with the Peccin et al. (2022) reported that 64.40 to 73.33% of the tasters would buy the biscuit, in their study entitled the elaboration of a cream *cracker* biscuit with added flaxseed flour and reduced sodium content, and (Rigo et al. 2016, Lima et al. 2019, Medeiros 2017) when determining the acceptability of a cookie enriched with jatoba flour, found that 88% of the tasters would buy their biscuit.

3.5 Acceptability Index

Graph 2 illustrates the acceptability index of the biscuits enriched with avocado pit flour.

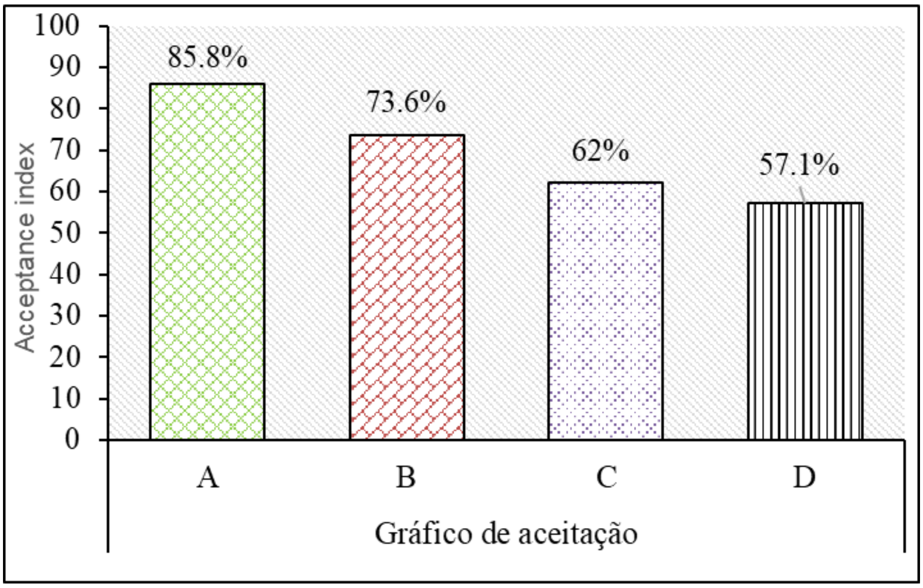
According to Dutcosky (2019) in order for a product to be considered accepted in terms of its sensory properties, it needs to have an Acceptability Index (AI) of at least 70 %, which was observed in formulation B, with the addition of 10 % avocado pit flour.

Santos and André (2021) and (Rapina 2017, Rivera et al. 2019) in their study seeking to determine the physical, chemical and sensory characteristics of biscuits made with a mixture of flours, reported an acceptability index ranging from 55.56 to 83.5%, and Santana et al. (2014,



Graph 1. Purchase intention index

(A), biscuits without addition of avocado pit flour; (B), biscuits with 10% addition of avocado pit flour; (C), biscuits with 20% addition of avocado pit flour and (D), biscuits with 25% addition of avocado pit flour. Source: Authors



Graph 2. Acceptability of biscuits with partial addition of avocado pit flour

(A), biscuits with no avocado pit flour added; (B), biscuit with 10% avocado pit flour added; (C), biscuit with 20% avocado pit flour added and (D), biscuit with 25% avocado pit flour added. Source: Authors

The acceptance index values ranged from 57.1 to 85.8 %. The highest value was observed in formulation A (85.8) followed by formulation B with 73.6 %, the lowest indices were observed in formulations C and D with 62 and 57.1 %.

Silva 2017) when carrying out the physicochemical and sensory evaluation of *cookie-type* biscuits added with malt bagasse flour as a source of fiber, reported an acceptability index ranging from 70 to 78%, results in agreement with those of the present research.

Soares et al. [35], when studying the acceptance of *cookie-type* biscuits added with pequi flour (*Caryocar Brasiliense*), reported an acceptance rate of 88.89%.

4. CONCLUSION

The physicochemical parameters of the biscuit showed similarities in terms of moisture content, pH, proteins, lipids, carbohydrates and calorific value. Differences were seen in acidity and ash. The standard biscuit made with 100 % wheat flour and 10 % avocado pit flour performed best, achieving sensory acceptance ratings of 85.8 % and 73.6 % respectively. The results obtained show that there are alternatives for reusing waste to partially replace wheat flour with FCA and that it is ideal for making biscuits.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The authors declare that no generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

ACKNOWLEDGEMENTS

We express with love and affection, our gratitude to all those who directly or indirectly provided their preponderant help, whether moral or material, for the development of this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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