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The development of protein biscuits for pets containing proteinrich baru residue (*Dipterix alata Vog*)

Desenvolvimento de biscoitos proteicos para animais de estimação contendo resíduo de baru rico em proteínas (*Dipterix alata Vog*)

El desarrollo de galletas proteicas para mascotas que contienen residuo de barú rico en proteínas (*Dipterix alata Vog*)

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Zamira Abel de Jesus Maria
Master's Degree in Food Science and Technology
Institution: Universidade Federal de Goiás
Address: Goiânia, Goiás, Brazil
E-mail: zamiradejesus9@gmail.com

Maria Carolina de Almeida
PhD in Food Science and Technology
Institution: Universidade Federal de Goiás
Address: Goiânia, Goiás, Brazil
E-mail: maria_carolina@ufg.br

Júlio Cesar Briceno Colivet
PhD in Food Engineering
Institution: Universidade Federal de Goiás
Address: Goiânia, Goiás, Brazil
E-mail: julio_colivet@ufg.br

Flávio Alves da Silva
PhD in Food Engineering
Institution: Universidade Federal de Goiás
Address: Goiânia, Goiás, Brazil
E-mail: flaviocamp@ufg.br

Fernando Pereira de Sá
PhD in Chemistry
Institution: Instituto Federal de Educação, Ciência e Tecnologia de Goiás
Address: Inhumas, Goiás, Brazil
E-mail: fernando.fpsa@ifg.edu.br

Márcio Caliari
PhD in Food Technology
Institution: Universidade Federal de Goiás
Address: Goiânia, Goiás, Brazil
E-mail: macaliari@ufg.br

Tatianne Ferreira de Oliveira
PhD in Chemical Engineering
Institution: Universidade Federal de Goiás
Address: Goiânia, Goiás, Brazil
E-mail: tatianne_ferreira_oliveira@ufg.br

ABSTRACT

The objective of this work was to evaluate the chemical composition of by-product flours for the development of dog biscuits. 5 formulations were prepared with 0%, 5%, 10%, 15% and 20% replacement of baru by-product flours. Defatted pie flour contains high levels of protein (25.93 g 100 g^{-1}) and lipids (24.89 g 100 g^{-1}), but low in total fiber (6.15 g 100 g^{-1}). Baru bark flour had low lipid (1.0 g 100 g^{-1}) and protein (5.34 g 100 g^{-1}) contents, but high total fiber content (59.67 g 100 g^{-1}). The levels of total phenolic compounds vary from (14525 and 12532 mg EAG g 100 g^{-1}) with emphasis on baru peel flour. The predominant nutrients in the cookies were carbohydrates (56.49 to 51.20 g 100 g^{-1} for cookies made with baru bark flour (BCB) 52.78 and 49.62 for cookies made with degreased pie flour baru (BTDB), followed by lipids (21.67-23.53 g 100 g^{-1} for BTDB and 19.05-22.33 g 100 g^{-1} for BCB) and protein (12.10-15.05 for BTDB g 100 g^{-1} and 11.23 and 12.19 for BCB g 100 g^{-1}), the study highlights the importance of using by-products in the development of dog food products.

Keywords: *Dipteryx alata* Vog. By-Products. Food Formulation. Food Technology. Full Use of By-Products.

RESUMO

O objetivo deste trabalho foi avaliar a composição química de farinhas de subprodutos para o desenvolvimento de biscoitos caninos. Foram elaboradas 5 formulações com 0%, 5%, 10%, 15% e 20% de substituição de farinhas de subprodutos de baru. A farinha de torta desengordurada contém altos níveis de proteínas (25,93 g 100 g^{-1}) e lipídios (24,89 g 100 g^{-1}), mas baixo teor de fibra total (6,15 g 100 g^{-1}). A farinha de casca de baru apresentou baixo teor de lipídeos (1,0 g 100 g^{-1}) e proteínas (5,34 g 100 g^{-1}), mas elevado teor de fibra total (59,67 g 100 g^{-1}). Os teores de compostos fenólicos totais variam entre (14.525 e 12.532 mg EAG g 100 g^{-1}) com destaque para a farinha de casca de baru. Os nutrientes predominantes nos biscoitos foram carboidratos (56,49 a 51,20 g 100 g^{-1} para biscoitos feitos com farinha de casca de baru (BCB) 52,78 e 49,62 para biscoitos feitos com farinha de torta de baru desengordurada (BTDB), seguidos de lipídios (21,67-23,53 g 100 g^{-1} para BTDB e 19,05-22,33 g 100 g^{-1} para BCB) e proteína (12,10-15,05 para BTDB g 100 g^{-1} e 11,23 e 12,19 para BCB g 100 g^{-1}), o estudo destaca a importância do uso de subprodutos no desenvolvimento de rações para cães.

Palavras-chave: *Dipteryx alata* Vog. Subprodutos. Formulação de Alimentos. Tecnologia Alimentar. Aproveitamento Total de Subprodutos.

RESUMEN

El objetivo de este trabajo fue evaluar la composición química de harinas subproductos para la elaboración de galletas para perros. Se prepararon 5 formulaciones con 0%, 5%, 10%, 15% y 20% de sustitución de harinas de subproducto barú. La harina para tarta desgrasada contiene altos niveles de proteínas (25,93 g 100 g^{-1}) y lípidos (24,89 g 100 g^{-1}), pero bajo contenido de fibra total (6,15 g 100 g^{-1}). La harina de cáscara de barú tuvo un bajo contenido de lípidos (1,0 g 100 g^{-1}) y proteínas (5,34 g 100 g^{-1}), pero un alto contenido de fibra total (59,67 g 100 g-1). Los niveles de compuestos fenólicos totales varían entre (14,525 y 12,532 mg EAG g 100 g^{-1}) con énfasis en la harina de cáscara de barú. Los nutrientes predominantes en las galletas fueron los carbohidratos (56,49 a 51,20 g 100 g^{-1} para galletas elaboradas con harina de corteza de barú (BCB) 52,78 y 49,62 para galletas elaboradas con harina de pastel de barú desgrasada (BTDB), seguido de los lípidos (21,67-23,53 g 100 g^{-1} para BTDB y 19,05-22,33 g 100 g^{-1} para BCB) y proteínas (12,10-15,05 para BTDB g 100 g^{-1} y 11,23 y 12,19 para BCB g 100 g^{-1}), el estudio destaca la importancia del uso de subproductos en el desarrollo del perro alimento.

Palabras clave: *Dipteryx alata* Vog. Subproductos. Formulación de Alimentos. Tecnología Alimentaria. Aprovechamiento Total de Subproductos.

1 INTRODUCTION

Brazil stands out as the world leader in canine populations, housing approximately 57 million dogs as pets, according to data from the Brazilian Association of the Pet Products Industry (ABINPET, 2022). During the COVID-19 pandemic, there was a significant increase of 30% in the purchase and adoption of pets in Brazil, according to a report by the Companion Animal Commission (COMAC, 2021). As a direct result of this phenomenon, the food market for pets also experienced significant growth, registering an increase of 24% between 2019 and 2020 (ABINPET, 2021). This expanding scenario has aroused the interest of manufacturers, who aim to provide products enriched with nutrients to promote a healthy diet for animals. Currently, new natural alternative sources are being investigated, notably those rich in minerals, fatty acids, and proteins (Lirio *et al.*, 2018).

The increase in food production has resulted in a greater generation of agri-food waste. These residues, as pointed out by Freitas *et al.* (2021), represent a valuable source of several essential nutrients for health. The inclusion of these residues in pet food formulation not only promotes environmental sustainability but also contributes to economic sustainability by increasing the number of competitively priced ingredients available to pet food formulators (Acuff *et al.*, 2021).

Despite the growing interest in this field, the literature still lacks comprehensive studies on the use of these residues in animal feed, making further investigations imperative. The correct use of these residues in diet formulations for dogs and cats depends on having knowledge of their benefits, requiring studies to evaluate the chemical composition, nutrient availability, and effects on animal metabolism (Abud; Narain, 2009).

Biscuits, considered as snacks intended for domestic animals, have the function of pleasing or rewarding and may have specific characteristics (Brasil, 2009). These cookies are often made from residues from meat processing, such as viscera and bones, associated with cereals such as soybean bran and hulls. Given this context, it is imperative to promote research that explores the use of agri-food residues in the formulation of dog food. A study conducted by Andrade *et al.* (2019), which investigated feed production using papaya and orange residues, obtained satisfactory results concerning biomass. However, it was found that a small part of the nutritional needs were met but required supplementation with other products.

Baru (*Dipteryx alata* Vog.) by-products, such as residual peel and defatted pie, have aroused interest as ingredients in the formulation of new products in the food industry. The residual bark, especially the baru pulp (mesocarp), has high fiber content, but lower protein content (Egea *et al.*, 2023). On the other hand, defatted pie, due to its composition, which includes the presence of proteins, minerals, amino acids, fatty acids, and phenolic compounds from almonds, has been the subject of several studies to evaluate possible beneficial effects of baru on health and the development of new products (Lima *et al.*, 2022).

Incorporating baru by-products into dog food formulation has emerged as a valuable source for sustainable development and mainly in the reduction of waste of animal origin In this context, the objective of this work was to develop and characterize dog biscuits with partial replacement of baru by-product flour (*Dipteryx alata* Vog.).

2 METHODOLOGY

Ingredients of notable relevance originating from the Cerrado biome were used to form the biscuits, particularly from the state of Goiás. The residual bark by-product (*Dipteryx alata* Vog) used in this study was supplied by baru nut producers from the community of Caxambú de Pirenópolis-GO. The partially defatted cake was acquired in collaboration with Florir de Sal Natural Products Industry, established in Goiânia-GO.

2.1 PREPARATION OF THE FLOURS

The baru bark was washed to remove dirt, and then dried in an oven at 70 ± 2 ºC for 72 hours. After the drying process, the baru bark was ground in a knife mill and passed through a 0.25 mesh sieve. The defatted cake was roasted in an oven (Tecnal brand, model TE-395) at 120 °C for 20 minutes, and crushed in a blender (Figure 1) (Mondialbrand*,* model L66 – 10 speeds) (Coutinho, 2020).

Figure 1. (a) Baru peel flour; (b) Defatted baru pie flour.

(a) (b)

Source: Prepared by the authors.

2.2 BISCUIT PREPARATIONS FROM BARU BY-PRODUCTS

The biscuits were developed in the pilot baking laboratory at the Federal University of Goiás, School of Agronomy (UFG). Biscuit processing was performed according to the methodology of Abud, Narain (2009). Initially, a biscuit reference formulation composed entirely of wheat flour was prepared, as described in (Table 1). Subsequently, the other formulations were developed, partially incorporating the baru by-product flour, the residual baru shell, and the defatted cake, in varying concentrations of 0.0%, 5%, 10%, 15%, and 20% in relation to the initial weight of the wheat flour.

Table 1. Ingredients used in the formulation of dog biscuits with the addition of baru by-product flours *(Dipteryx alata* Vog).

Ingredients	Percentage level of replacement of wheat flour by baru by-product flour								
	FC	FCB[1]				FTDB[2]			
	0	5%	10%	15%	20%	5%	10%	15%	20%
Wheat flour (g)	130	123	117	110	104	123	117	110	104
Egg (g)	51	51	51	51	51	51	51	51	51
Butter (g)	50	50	50	50	50	50	50	50	50
Milk (mL)	50	50	50	50	50	50	50	50	50
Bovine liver[1]	10	10	10	10	10	10	10	10	10
Vanilla essence (g)	2	2	2	2	2	2	2	2	2
Salt (g)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Bicarbonate (g)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
CB (g)	-	7	13	20	26	-	-	-	-
TDB (g)	-	-	-	-	-	7	13	20	26

Subtitle: FCB, baru peel flour; FTDB; Defatted baru cake flour; FC control formulation (without addition of baru by-product flour); [1] FCB, baru bark formulation; [2]FTDB, defatted caked formulation from Baru.

Source: Prepared by the authors.

The selection of ingredients, homogenization, mixing, and dough formation was conducted per the Good Manufacturing Practices (GMP) standards, as established in RDC No. 216/04 (BRASIL, 2004). The ingredients were weighed individually, starting the first stage of the mixture, which involved the egg, butter, and milk. Then the vanilla essence was incorporated into the composition, being manually mixed for 5 minutes. Subsequently, the gradual addition of liver, salt, bicarbonate, and flour was carried out, to achieve homogeneity in the dough.

After completion of the mixing process, the resulting mass was placed on an aluminum countertop. For the cookie molding process, a stainless metal mold in the shape of a pet bone was used. Then, the biscuits were placed in aluminum trays previously coated with butter and flour to avoid sticking. These measures were taken to ensure the desired shape and texture during cooking.

The biscuits were then placed in an electric oven preheated to 120 ºC. As the internal temperature reached 150 ºC, the biscuits were baked for a period of 25 minutes, ensuring the desired texture and appropriate coloring were obtained. At the end of the cooking process, the biscuits were left to cool to room temperature for 10 minutes, before being packed in plastic packaging, to preserve their organoleptic properties and maintain quality.

2.3 PHYSICOCHEMICAL CHARACTERIZATION OF FLOURS AND BISCUITS

2.3.1 Centesimal Composition and Total Energy Value

The proximal composition of the sample was determined as recommended by AOAC (2016). Moisture content was quantified using the oven drying method and was calculated based on mass loss after constant weight (method 925.45b). Lipids were quantified by extraction with petroleum ether based on mass after solvent evaporation (method 920.39).

The nitrogen content was determined by digestion, distillation, and titration of the defatted sample, and the conversion factor of 6.25 (method 921.20) was used to transform N into crude protein. The fixed mineral residue was determined by complete carbonization of the defatted sample in muffle and calculated based on the mass after carbonization (method 923.03). The determination of carbohydrates was performed by difference, taking into account the values of the components previously determined. The determination of the total fiber was performed according to AOAC standard No. 985.29. All attempts were performed in triplicate and the results were expressed on a dry basis. The total energy value was calculated using the coefficients of Atwater and Woods (1896), where digestible carbohydrates (total carbohydrates - insoluble crude fiber) contain 4.0 kcal g^{-1}, lipids 9.0 kcal g^{-1}, and protein 4.0 kcal g^{-1}.

The total energy value (TEV) was calculated by the following (Equation 1):

$$\text{TEV kcal} = 4 \times \text{carbohydrates} + 4 \times \text{proteins} + 9 \times \text{lipids} \qquad (1)$$

2.3.2 Color Determination

The color of the samples was evaluated by determining the CELAM parameters (L*, a* and b*) using a Color Quest II colorimeter (Hunter-Lab, Reston, Virginia, USA) according to the method described by Paucar-Menacho *et al.* (2008), where L* defines the luminosity (L* = 0 black and L* = 100 white), a* and b* define chromaticity (+ a* red and -a* green, +b* yellow and -b* blue).50 repetitions of color analysis were performed per sample.

2.4 CHARACTERIZATION OF BIOACTIVE COMPOUNDS IN FLOURS

2.4.1 Preparation of Extracts

The extracts were prepared from 2.5 g of the baru by-product flours, and 40 mL of ethanol was added. The samples were mixed on a magnetic stirrer (SL-91/A) at room temperature (25 ºC ± 2 ºC) for 1 h, according to the methodology proposed by Tabart *et al.* (2007) and adapted by Michiels *et al.* (2012) with changes. Then the mixture was filtered with 12 cm diameter Qualitative Filter Paper and a second extraction with the residue under the same conditions was completed. The extract was filtered and stored in amber flasks at -18 °C until use. Extracts were characterized for total phenolic content (CFT) and their antioxidant activity (AA).

2.4.2 Determination of total phenolic compounds

Total phenolic compounds were determined according to the method proposed by Zielinski, Kozlowski, (2000) with modifications, 2.0 mL of the intermediate solution of the extracts (triplicate), 1.0 mL of 1N Folin-Ciocalteureagent, and 1.0 mL of sodium bicarbonate solution (25% w/v) were added to test tubes with aluminum foil. The samples remained at rest for 30 minutes, protected from light and at room temperature. Then, a spectrophotometer (BEL SP 2000 UV Photonics) was read at a wavelength of 760 nm. A gallic acid standard curve was constructed ($y = 0.9665 x + 0.0131$, $R^2 = 0.9993$) and the total phenolic content was expressed as mg gallic acid equivalent (Gae) per 100 grams of material.

2.4.3 Determination of Total Flavonoids

Total flavonoids were determined according to the methodology proposed by Kim; Jeong; Lee (2003), with modifications, with an aliquot of 1 ml of the extract added to the amber flask containing 4 mL of distilled water. At time zero, 0.3 mL of 5% $NaNO_2$ was added to the flask. After 5 min, 0.3 mL of 10% $AlCl_3$ was added. At 6 min, 2 mL of 1 M NaOH was added to the mixture. The reaction flask was diluted immediately with the addition of 2.4 ml of water and mixed well. The absorbance of the mixture was determined at 510 nm, and the results were expressed based on fresh weight in mg 100 g^{-1} catechin equivalents (EC). Samples were analyzed in triplicates.

2.4.4 Determination of Antioxidant Capacity - Antioxidant Power of the Free Radical $ABTS^+$

The determination of antioxidant capacity by the $ABTS^+$ radical elimination method was performed according to the methodology described by Rufino *et al.* (2007), with modifications. It was prepared from the reaction of 5 mL of the 7 mM ABTS reagent solution with 88 μL of the 140 mM potassium persulfate solution.

Then kept in the dark for 16 hours. Following this, 1 mL of this mixture was diluted in ethyl alcohol until it reached an absorbance of 0.7 nm ± 0.05 nm at 734 nm. 30 µL of each dilution of the extract was transferred to an amber flask in 3 mL of the ABTS $^{\bullet+}$ radical and homogenized in a vortex mixer. Ethyl alcohol was used for the negative control and 2 mM Trolox antioxidant was used for the positive control. After 6 minutes in the dark, a reading was performed at 734 nm. The percentage inhibition (%) of Trolox for ABTS^{+} free radicals was calculated using the expression below (Equation 2):

% inhibition = (final control Abs - Abs of Trolox mixture + control ABTS Abs) (2).

2.5 GC-MS ANALYSIS OF BARU (*DIPTERYX ALATA VOG*) BY-PRODUCT FLOURS

The flours composed of baru by-products (Dipteryx *alata* Vog) were analyzed with Headspace-HRGC-MS to identify the chemical compounds, and the analyses were performed in a gas chromatograph coupled to a mass spectrometer (Shimadzu Nexis GC2030), equipped with a capillary column, SH-Stabilwax-ms (30 m, 250 µm id, 0.25 µm). Samples were previously heated via headspace (AOC-6000 autosampler) at 80 ºC for 45 min and a quantity of 2.0 mL was injected into the chromatograph. Split mode was employed with a ratio of 10:1 with an equilibrium time of 3 minutes. The programming of the oven temperature was initially set at 40 °C, maintained for 1 minute, then the heating ramp was increased by 5 °C min^{-1} to 160 °C, and then by 10 °C min^{-1} to 250 °C and maintained for 15 min. The total time taken for the analyses was 49 minutes. Helium 5.0 was used as a carrier gas, with a pressure of 4.7 psi, a flow rate of 0.79 mL min^{-1}, and a linear speed of 32.4 cm s^{-1}. The temperature of the injector, interface, and ion source was maintained at 250 °C. The mass spectrometer operated in scan mode by recording ions in the range of 20 to 400 *m/z* with a scan time of 150 ms and was compared to the reference compounds from the NIST 17 library.

2.6 MICROBIOLOGICAL ANALYSIS OF FLOURS

The flours were submitted to microbiological analysis for the presence of *Salmonella* sp, *Bacillus cereus*, and *Escherichia coli*, according to the methodology of the American Public Association (APHA, 2001), and obeying the standards established by Brazilian legislation through the Collegiate Board Resolution - RDC No. 724, and Normative Instruction - IN No. 161, of July 1, 2022, which establishes a microbiological standard for flours, indicated in item 19, and establishes a maximum count for *Bacillus cereus* $< 10^3$ CFU g^{-1}, *Escherichia coli* $< 10^2$ CFU g^{-1} and absence of *Salmonella* sp in 25 g of the product.

2.7 TEXTURE PROFILE

The hardness of the biscuits was evaluated on a *TA-XT2* Texture Technologies Corp software Texturometer (Exponent Stable Micro Systems), using a rectangular steel blade (Warner Bratzter, reversible, HDP/BSK probe) and the HDP/90 platform, in which the biscuits were arranged horizontally and cut in half. The analysis was performed with twelve cookie samples of each formulation, and the results were expressed in Newton (N) and represented the arithmetic mean of twelve breaking force determinations for samples from the same batch. The parameters used in the tests were: pre-test speed = 1.5 mm s^{-1}; test speed = 2.0 mm $s^{-1;}$ post-test speed = 2.0 mm s^{-1}; contact force = 20 g and distance 30.0 mm, Data Acquisition Route of 400 pps.

2.8 WATER ACTIVITY (AW), PH, TOTAL ACIDITY

Water activity was evaluated according to the instructions of the AquaLab® equipment (3TE Series, Pullman, WA). The pH determination was performed using a potentiometer, following method No. 943.71 of AOAC (2016). Total acidity was measured by potential metric volumetry, according to method No.937.05 of AOAC (2016).

2.9 STATISTICAL ANALYSIS

Afterward, the validated data underwent the analysis of variance (ANOVA). The Fisher test was applied for multiple comparisons between means, at a significance level of 5%, using statgraphics centurion software version 19.06.01.

3 RESULTS AND DISCUSSIONS

Table 2 shows the results of the proximate composition of baru by-product flours, which were used as raw material in the production of biscuits for dogs.

Table 2. Physicochemical characterization of baru *(Dipteryx alata* Vog*)* by-product flours used in the formulation of dog biscuits.

Parameter (g ^{100} g $^{-1}$)	FCB[1] (b.s.)	FTDB[2] (b.s.)
Humidity	10.44±0.17^{A}	3.68 ± 0.09^{B}
Ash	3.50 ± 0.29^{A}	4.11 ± 0.01^{B}
Lipids	1,00 ± 0.06^{A}	24.89 ± 0.60^{B}
Proteins	5.34 ± 0.05^{A}	25.93 ± 0.04^{B}
Carbohydrates	79.72^{A}	41.39^{B}
Total fibers	59.67^{A}	6.15^{B}
Total energy value (kcal g 100g^{-1})	349.24 ^{A}	498,49^{B}
Water activity	0.470 ± 0.004B	0.440 ± 0.02^{A}
Microbiological analysis		
E. coli	< 10 CFU/g	< 10 CFU/g
Bacillus cereus	< 10 CFU/g	< 10 CFU/g
Salmonella sp.	Absence in 25 g	Absence in 25 g

Subtitle: Data expressed mean ± SD. Different letters in the same row indicate differences ($p < 0.05$) measured by Fisher's LSD test, [1]FCB; baru bark flour, [2]FTDB, baru degreasing pie flour.
Source: Prepared by the authors.

When comparing the results of the physicochemical characterization of the two flours, it was observed that there are statistically significant differences ($p < 0.05$) between them, especially concerning moisture, ash, protein, fiber, and lipid contents. According to the guidelines of the Resolution of the Collegiate Board (RDC) No. 711, July 1, 2022, issued by the National Health Surveillance Agency (ANVISA), the maximum value allowed in flour materials (Brasil, 2022) is stipulated as 15 g 100 g^{-1} The moisture contents presented in this work are in full compliance with current legal standards.

As for the ash content, the value was more expressive in the CRB flour (3.50 g 100 g^{-1}), lower than that found by Abud, Narain (2009), in the flour of umbu pulp processing residues (*Spondias tuberosa* Arruda Câmara) and passion fruit (*Passiflora edulis*), at 12.50 and 4.41 g 100 g^{-1} respectively. The mineral content of TDB flour was lower than that reported by Moreira *et al.*, (2022), for baru cake flour (3.54 g 100 g^{-1}), and higher than that obtained by Borges *et al.* (2019), for European sweet chestnut flour (*Castanea sativa* Mill) 1.67 g 100 g^{-1}. With regard to protein and lipid contents, it is notable that TDB flour showed as about eight times greater in both parameters than CB flour. In an analysis of the proximate composition of partially defatted baru flour (*Dipteryx alata* Vog*)* carried out by Caetano *et al.* (2017), significantly high levels of lipids were found. However, it is important to note that the protein content in this flour was relatively low from the value reported in this study, at (56.12 and 12.67 g 100 g^{-1}, respectively), which differs slightly from the values of Borges *et al.* (2022), who obtained high lipid and protein contents of 25.12 and 34.42 g 100 g^{-1}. Moreira *et al.* (2022), justify the high lipid content in baru cake flour as being attributed to the mechanical pressing system, which was not able to remove all the oil content present in the almond.

The protein and lipid content of BC flour was lower compared to the results obtained by Viana *et al.* (2023), which showed substantial levels of proteins and lipids, of between 10.05 and 1.79 g 100 g^{-1} for baru pulp flour. In addition, BC flour also showed lower levels of these parameters concerning apple and silver banana peel flour (*Musa spp)*, which showed protein levels of 9.97 and 7.10 g 100 g^{-1}, and lipids of 8.49 and 11.67 g 100 g^{-1} respectively (Castilho; Alcantara; Clemente, 2014). The proximate analysis of the flours reveals statistically significant differences ($p < 0.05$) in carbohydrate and fiber contents, as shown in (Table 2). BC flour stood out for its high carbohydrate and fiber contents (80.32 and 59.67 g 100 g^{-1} respectively), higher than those reported by Santiago *et al.*, (2018), for baru bark at 51.05 and 24.1 g 100 g^{-1}, and olive pomace flour (*Olea europaea* L) 11.71 and 43.75 g 100 g^{-1} (Trindade *et al.*, 2023). While TDB flour had low levels for these parameters (41.39 and 6.15 g 100 g^{-1}), Pagliarini *et al.*

(2018) found low carbohydrate levels and a significantly high amount of fiber in baru almond flour, with values of 18.51 g and 15.68 g 100 g^{-1}, respectively.

On the other hand, Clerici *et al.*, (2013) observed high levels of carbohydrates and fibers in defatted sesame flour (*Sesamum indicum*), with values of 37.26 and 32.41 g 100 g^{-1} respectively. Carbohydrates provide energy and aid in the digestion and assimilation of other nutrients, Ehiabhi *et al.* (2012) and can be correlated with dietary fiber content, the consumption of fiber that has been associated with beneficial effects on human and animal health, sometimes considered useful for the prevention of obesity due to the induction of good intestinal digestion (Borges *et al.*, 2008; Fustier *et al.*, 2009). The total energy value of CB flour was significantly lower when compared to that of TDB flour (347.94 g and 493.29 g 100 g^{-1}) respectively. This disparity can be attributed to the low lipid and protein content in BC flour, confirming the advantage of this low-energy flour as a potential reducer of risks associated with obesity and chronic diseases such as hypertension, diabetes, and metabolic disease. These can be used for the development of new food products that promote health.

The water activity values obtained in the flours of baru by-products was below 0.60, demonstrating the ability to delay the undesirable actions of microorganisms and enzymes. This result gives these flours a chemical stability, making them suitable for use in food formulation.

Considering the microbiological analysis of the flour, it was carried out in compliance with the standards established by Brazilian legislation, through the Resolution of the Collegiate Board of Directors - RDC No. 724, and Normative Instruction - IN No. 161, of July 1, 2022, and presented values of < 10 CFU g^{-1} for *Escherichia coli*, *Bacillus cereus* and absence for *Salmonella* in 25 g for baru by-product flours. These results attest to a satisfactory quality, meaning that the flours derived from the by-products are suitable to be incorporated into food formulations.

3.1 TOTAL PHENOLIC COMPOUNDS, TOTAL FLAVONOIDS, AND ANTIOXIDANT CAPACITY OF BARU BY-PRODUCT FLOURS

Table 3 shows the results of total phenolic compounds, total flavonoids, and antioxidant activity by the ABTS^{+} assay of baru by-product flours.

Table 3. Total phenolic compounds, total flavonoids, and antioxidant capacity of baru bark flour (FCB) and baru defatted pie (FTDB).

Bioactive Compounds.	FCB	FTDB
^{1}CFT	14525 ± 1114 ^{A}	12532 ± 2049^{B}
^{2}FT	442.72 ± 12.86^{B}	1751.82 ± 19.28^{A}
^{3}ABTS+	2.173 ± 0.07^{A}	1.885 ± 0.15^{B}

Subtitle: Data expressed mean ± SD. Different letters on the same line indicate differences ($p < 0.05$) measured by Fisher's LSDtest.1 mg EAG 100 g^{-1}; ^{2}mg EC 100 g $^{-1}$; ^{3} Troller µmol L$^{-1;}$
Source: Prepared by the authors.

The phenolic compounds and antioxidant activity of baru by-product flours were analyzed to explore their beneficial properties to apply them optimally in the production of dog biscuit. Statistical differences were identified between the flours of baru by-products analyzed in this study ($p > 0.05$). All samples revealed significant amounts of phenolic compounds, with flour extracts ranging between 12532.2049 and 14525 mg EAG 100 g^{-1}. It is noteworthy that BC flour presented a high content of total phenolic compounds, 14525.1114.4 mg EAG 100 g^{-1}, approximately double compared to TDB flour, higher than the content of total phenolic compounds obtained in lychee peel powder (*Litchi chinensis)* 1578 mg EAG 100g^{-1} per Vine *et al.* (2021**),** and Omena *et al.* (2012) found lower levels of phenolic compounds for umbum peel flour (*Spondias tuberosa L*) 5250.0 mg EAG 100 g^{-1}, but also higher than those reported for Cavendish banana peel flour at different stages of maturation (*Musa* AAA), (590-2900 mg EAG 100 g^{-1}) (Khawas; Deka, 2016; Rebello *et al*., 2014).

The CFT content found for TDB flour was 12532.2049 mg EAG 100 g^{-1}, ten times higher than that obtained by Burbano and Correa, (2021) for defatted walnut flour (*Juglans regia* L) 10.9 mg EAG 100 g^{-1}, partially defatted baru pie flour 121.34 mg EAG 100 g^{-1} observed by De Oliveira Pineli *et al.*, 2015), Kornsteiner; Wagne; and Elmadf, (2006); Yang; Liu; and Halim (2009), reported lower CFT contents for almond flour (*Prunus dulcis* Mill) from 130 to 459 mg EAG

100 g^{-1} and from 326 to 552 mg EAG 100 g^{-1} for peanuts (*Arachis hypogaea* L). Phenolic compounds are secondary metabolites found in plants, playing a crucial role as a defense mechanism against predators. They tend to be produced in greater quantities in the aerial parts of plants, such as leaves and bark. This may explain why flour derived from baru bark had a higher content of these compounds. The consumption of foods rich in phenolic compounds can help in the treatment or prevention of different chronic diseases in animals and humans. Diseases such as diabetes, cardiovascular diseases and neurodegenerative diseases (Garcia-Conesa; Larosa, 2020; Luca *et al.*, 2020).

Regarding the content of total flavonoids, the results showed to be contrary to that of the total phenolic compounds, the TDB flour presented a high content of total flavonoids 1751.45 mg CE 100 g^{-1} , Carocho *et al.* (2012), found lower levels of total flavonoids of 234 mg CE 100 g^{-1} for freeze-dried Portuguese nut flour (*Castanea sativa* Mil), Yang *et al.* (2009), also obtained lower content of total flavonoids for Brazil nuts and macadamias with 107 and 140 mg CE 100 g^{-1} respectively.

The results of total flavonoids of BC flour were higher than those obtained by Vella; Cautela; Laratta (2019), for melon bark flour (*Cucumis melo* L) 15.19 mg CE 100 g^{-1}, lower than that reported by Correira *et al.* (2012), for acerola powder (*Malpighia emarginata*), Jambolão (*Syzygium cumin)*, Pitanga (*Eugenia uniflora*) and caja-umbu (*Spondias* sp*)* 8839.33.775267, 4253.17 and 518.63 mg CE 100 g^{-1} respectively. The defatted baru pie flour had a significantly high content of total flavonoids, which can be attributed to the pressing process. This method can influence the degradation of compounds present in the oil and in the defatted baru cake itself, or even facilitate its volatilization (Savoire; Lanoisellé; Varobiev, 2013).

When evaluating the radical scavenging capacity by the ABTS assay, the TDB flour extract obtained an antioxidant capacity of 1.885 Trolex µmol L^{-1}, slightly higher than that obtained by Ramos *et al.*, (2021) in pequi almond (*Caryocar brasiliense* Camb) extracts 1.59 Trolex µmol L^{-1}, plus five times lower than that observed in walnut *(Brosimum alicastrum)* extracts 92.55 Trolex µmol

L^{-1} and walnut (*Juglans regia* L) extracts 92.1 Trolex µmol L^{-1}, as reported by (Ozer, 2017).

3.2 GC-MS ANALYSIS OF BARU (*DIPTERYX ALATA* VOG) BY-PRODUCT FLOURS

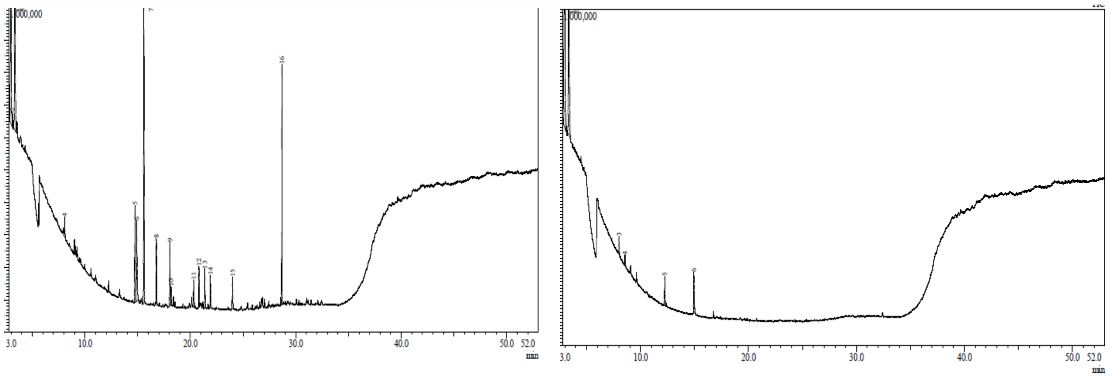
The results of the characterization and identification of the compounds present in the flours of baru by-products (*Dipteryx alata* Vog*)* determined by GC/MS analysis (Table 4) and (Figures 1, and 2) are presented. The total of eighteen peaks were identified, representing several classes of organic compounds (Table 4). The major compound detected was ethanol, present in concentrations of (49.1%) in TDB flour and (21.53%) in CB flour. The presence of ethanol in food is commonly associated with fermentation, a process that often occurs in fruits during their spoilage process. However, it was observed that this substance has been previously identified in fresh fruits (Zhu *et al.*, 2003).

Table 4. List of active constituents of baru *(Dipteryx alata Vog)* by-product flours.

No.	Name of compounds	Molecular Formula	Relative % of each flour	
			FCB	FTDB
1	Acetic acid	CH_3COOH	6.15	5.16
2	Methyl Alcohol	CH_3OH	14.83	39.92
3	Alpha -Cubebene	$C_{15}H_{24}$	6.08	-
4	Butanal, 3-methyl-	$C_5H_{10}O$	1.23	-
5	1- Butanol, 3-methyl-	$C_5H_{12}O$	-	1.09
6	Copaene	$C_{15}H_{24}$	19.00	-
7	Cyclohexane, 1-ethenyl-1-methyl-2.4-bis (1-methylethenyl	$C_{15}H_{24}$	3.85	-
8	Caryophyllene	$C_{15}H_{24}$	1.07	-
9	Gamma -Muurolene	$C_{15}H_{24}$	1.64	-
10	D-limonene	$C_{10}H_{16}$	1.08	1.72
11	Ethanol	C_2H_6O	21.53	49.1
12	Germacrene D	$C_{15}H_{24}$	2.46	-
13	1-Hexanol	$C_6H_{14}O$	--	2.96
14	Hexanoic acid	$C_6H_{12}O_2$	1.93	-
15	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo	$C_{15}H_{24}$	3.88	-
16	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7	$C_{22}H_8O$	10.50	-
17	(1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methy	$C_{15}H_{22}$	2.27	-
18	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4.7-dimethyl-	$C_{15}H_{24}$	2.48	-

Source: Prepared by the authors.

Figure 2. a) Chromatogram of baru bark flour (FCB); and b) Chromatogram of the degreasing cake flour of the baru.



(a) (b)

Source: Prepared by the authors.

The second predominant compound was methanol, with concentrations of 39.9% in TDB flour and 14.83% in CB flour. Methanol is known to be an odoriferous and toxic compound, affecting the central nervous system. Plants generate methanol intracellularly through the demethylation reaction of macromolecules such as DNA, RNA, and proteins (Dorokhov *et al.*, 2018). Estimates of food derivatives indicate the presence of methanol in humans, with 7.9 mg/kg in beans, lentils, and peas (National Toxicology Program, 2003). However, a study by Gürler *et al.* (2022) revealed high levels of methanol, reaching 150 mg kg^{-1} in vegetables such as tomatoes, eggplant, and pickles, and 130-390 mg kg^{-1} in legumes such as beans, corn, and peas, possibly due to food processing. The presence of alcohol in fruits is associated with aroma and may result in off flavors, related to burning and pungency notes (Senesi *et al.*, 2005).

The third major compound was copaene, also known as α-Copaene, a non-oxygenated sesquiterpene present predominantly in BC flour, with a concentration of 19%. This compound is recognized as one of the main constituents of copaiba oil, known for its antimicrobial, anti-inflammatory, and healing properties (Brito *et al.*, 2005). Studies have shown that copaiba oil can inhibit the growth of bacteria and fungi (Veiga, Pinto, 2002).

3.3 PHYSICAL-CHEMICAL CHARACTERIZATION OF COOKIES

3.3.1 Centesimal Composition of Biscuits

Table 5 shows the results of the analysis of the proximate composition of biscuits prepared with baru bark flour (CB) and defatted baru pie (TDB). In all cookies analyzed, the predominant component was carbohydrates, followed by lipids, protein, and moisture.

The moisture content of the biscuits prepared with the replacement of baru by-product flours, peel, and degreased pie ranged from 10.48 to 13.34 g 100 g^{-1}, and from 9.49 to 13.37 g 100 g^{-1}, while the control was 9.11 g 100 g^{-1}.

A significant highlight can be noted in the increase in the moisture content of cookies when incorporated into flour (CB), especially at concentrations of 10% and 15%. This increase in moisture content can be attributed to the nature of the fiber present in the flour, foods rich in fiber, whose property is the ability to absorb more water, forming gel due to the presence of soluble fiber in its composition (Agama-Acevedo *et al.*, 2012; Sangnark; Noomhorm, 2004; Cortat *et al.*, 2015).

Table 5. Centesimal composition, (b.s.) and total energy value of cookies prepared with replacement of baru bark flour (CB) and baru degreasing pie (TDB) in different concentrations (%).

	Replacement levels of wheat flour with baru flour					
Parameter (g 100 g^{-1})	Cookies	0,0	5%	10%	15%	20%
Humidity	CB^{1}	9,11±0,07^{C}	10,48±0,05^{B}	13,10±0,09^{A}	13,34±0,18^{A}	11,81±0,16^{E}
	TDB^{2}	9,11±0,07^{C}	12,21±0,004^{F}	13,37±0,12^{A}	9,49±,0,40^{D}	10,48±0,10^{B}
Ash	CB	1,37±0,08^{D}	2,75±0,05^{F}	2,20±0,07^{E}	2,50±0,05^{C}	2,47±0,01^{C}
	TDB	1,37±0,08^{D}	1,24±0,07^{AB}	1,34±0,03^{A}	1,21±0,03^{B}	1,31±0,06^{A}
Proteins	CB	11,68±0,35^{A}	11,23 ±0,32^{A}	11,93±0,12^{A}	11,45±0,11^{A}	12,19±0,65^{A}
	TDB	11,68±0,35^{A}	12,10±0,02^{A}	13,93±0,79^{B}	14,05±0,70^{B}	15,06±0,09^{C}
Lipíds	CB	17,49±0,07^{D}	19,05±1,19^{E}	21,83±0,09^{A}	21,38±0,08^{A}	22,33±0,12^{AB}
	TDB	17,49 ±0,07^{D}	21,67±0,21^{A}	21,61±0,11^{A}	22,64±0,07^{BC}	23,53±0,37^{C}
Carbohydrates	CB	60,38^{C}	56,49^{B}	50,94^{A}	51,33^{A}	51,2^{A}
	TDB	60,18^{C}	52,78^{B}	49,75^{A}	52,61^{B}	49,62^{A}
Total energy value (kcal g 100 g^{-1})	CB	455,65^{D}	442,33^{A}	447,95^{B}	443,54^{A}	454,53 ^{D}
	TDB	455,65^{D}	454,55 ^{D}	449,21^{B}	470,40^{A}	470,45^{A}

Data expressed mean ± DP. Different letters on the same line indicate differences ($p < 0.05$) measured by Fisher's LSD test.^{1} Baru shell biscuits; ^{2}Baru defatting pie biscuits.

Source: Prepared by the authors.

In line with the results of the present study, Silva, Andrade and Gomes (2019) reported similar moisture contents in cookies prepared with 10% and 20% replacement of avocado stone flour, recording humidity levels of 14.04 and 13.87 g 100 g^{-1}, respectively. However, Queiroz *et al.* (2017) obtained higher moisture contents in cookies made with coconut flour, at concentrations of 5% and 10%, recording humidity levels of 16.23 and 23.73 g 100 g^{-1} respectively.

In the cookies made with flour (TDB), there was a tendency to reduce the moisture content, especially in the cookies in which the flour was replaced, especially the concentrations of 15% and 20%, which can be explained by the low moisture content obtained in the defatted cake flour. These observations highlight the diversity in moisture contents related to different sources of meal substitution, underlining the complexity in the formulation of food products. As established by ANVISA Resolution RDC No. 263, of September 22, 2005, the humidity of cookies and biscuits must be, at a maximum, 14 g 100 g^{-1} (BRASIL, 2005). Therefore, it is worth noting that the cookies produced comply with current legislation.

The ash content in the biscuit formulations ranged from 1.24 to 2.75 g 100 g^{-1}, so the results point to a significant increase in the ash content in biscuits made with (CB) flour compared to the control formulation ($p < 0.05$). This result is in line with the findings of Mariani *et al.* (2015), who also recorded a significant increase in the ash content in cookies as the rice bran concentration in the formulation was increased, ranging from 1.56% to 4.23%.

Clerici *et al.* (2013) corroborated these results in their study on cookies made with 10% replacement of defatted sesame flour with wheat flour, resulting in ash contents of 1.83 g 100 g^{-1}. In addition, the literature points out that a high ash content in dog food, as indicated by Johndon *et al.* (1998), may restrict the absorption of other nutrients.

A significant increase in the protein content in the biscuits was observed as the concentration of defatted baru pie flour (TDB) was increased. This was attributed to the protein richness of this flour. Biscuits made with 20% TDB flour and residual baru shell (CB) showed significantly higher values when compared to the other formulations. Melini *et al.* (2017) observed lower protein content in pet biscuits made with locust bean flour and cotyledons, ranging from 10.7 to 13.3 g 100 g^{-1} of protein. In contrast, Poppi *et al.* (2023) reported high protein levels in dog biscuits made with different fish meals and chicken offal, recording values between 18.24 and 18.89 18 g 100 g^{-1}. Regarding lipid content, significant differences were identified between the formulations ($p < 0.05$). An increase in lipid content was observed as the level of substitution of wheat flour for the flours of both baru by-products was increased. The biscuits of the controlled formulation and those with 5% residual baru shell flour (CB) had low lipid contents, of 17.49 and 19.05 g per 100 g, respectively. Another aspect to consider was that biscuits added with 20% baru defatted pie flour (TDB) exhibited high lipid contents.

Coradini *et al.* (2018) found lower lipid contents, equivalent to 6.06%, in dog biscuits made with fish meal. However, De Souza *et al.* (2021) obtained lower lipid contents, ranging from 0.52 to 1.75, in treats for dogs prepared with different concentrations (0%, 10%, 20%, and 30%) of tilápia head carcass flour. According to Mariani *et al.* (2015), they state that the lipid content of foods can vary according to the amount and type of ingredients used. These authors mentioned that different proportions of rice flour, soybean flour, and rice bran in biscuit formulation contributed significantly to increases in the lipid content of these products. The European Federation of the Pet Food Industry (FEDIAF, 2020) establish as a minimum recommendation for daily nutritional needs, a content of 18 g of protein and 5.5 g of lipids per 100 g^{-1}. Protein stands out as the most crucial nutrient, playing a key role in body development (Cuesta *et al.*, 2015). Essential for the proper functioning of digestive processes, energy generation, blood coagulation, and muscle contraction, its lack in the diet can result in reduced body weight (Kępińska-Pacelik; Biel, 2021). Generally, biscuits are offered to animals as a way to please them, and they don't need to be products with a high content of these macronutrients. However, they may represent an additional way of complementing the daily feeding of the animal.

The carbohydrate content was significantly higher in the biscuit of the control formulation, at 60.38 g 100 g^{-1}, while the lowest carbohydrate content was observed in the biscuit of the formulation with the highest concentration of TDB flour, which may be related to the high protein and lipid content, 15.06 and 23.53

g 100 g^{-1} respectively. It was found that as the level of substitution of wheat flour for flour from both baru by-products increased, a decrease in the carbohydrate content in the biscuits was observed. Osama *et al.* (2023) reported the opposite effect, in biscuits enriched with *Neolamarckia cadamba* fruit flour. The carbohydrate content increased significantly with the level of replacement of the fruit flour, and they presented higher values of 56.76 to 59.46 g 100 g^{-1} of this macronutrient when compared to this study.

Regarding the caloric value content, the high content of this component was found in biscuits with maximum replacement of baru by-product flours at 20% with 470.45 kcal g 100 g^{-1} for cookies added TDB flour and 454.53 kcal g 100 g^{-1} for cookies made with CB flour, a difference of 16 g 100 g^{-1}. However, the substitution of TDB flour in the formulations contributed to the increase in the energy value of the cookies compared to the CB formulation. This effect is attributed to the high lipid content present in the TDB flour.

3.3.2 Instrumental Biscuit Color

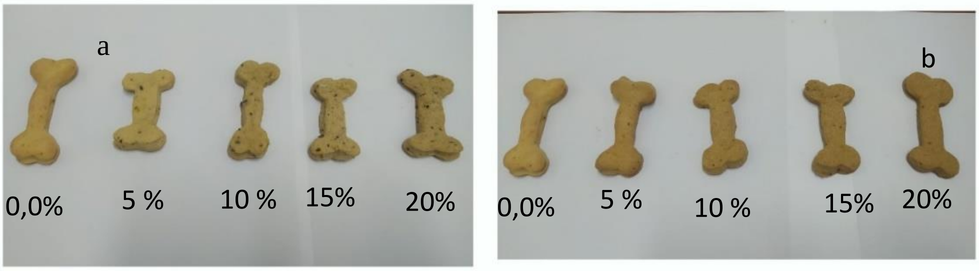
The color results of the cookies are shown in Table 6. The results revealed that as wheat flour was replaced by CB and TDB flour, there was a decrease in the values of L*, a *, and b*. This resulted in cookies that incline towards darker shades, with nuances of red and yellow (Figure 3). The cookies of the control treatment showed higher values of L* and b* (57.83 and 35.84).

Table 6. Instrumental color.

	Replacement levels of wheat flour with baru flour					
Color	Cookies	0.0	5%	10%	15%	20%
L*	CB	57.83 ± 3.30^{E}	52.70 ± 0.44^{Cd}	50.06 ± 1.75^{B}	45.89 ± 1.69^{A}	45.49 ± 0.47^{A}
	TDB	57.83 ± 3.30^{E}	54.56 ± 2.30^{D}	53.10 ± 1.90^{Cd}	54.20 ± 2.13^{D}	51.20 ± 2.31^{Bc}
a*	CB	11.32 ± 1.09^{C}	11.77 ± 051^{Cd}	11.26 ± 0.52^{C}	12.49 ± 0.84^{D}	11.22 ± 0.36^{C}
	TDB	11.32 ± 1.09^{C}	9.80 ± 0.55^{B}	8.15 ± 0.40^{A}	8.07 ± 0.54^{A}	9.50 ± 1.77^{B}
b*	CB	35.84 ± 1.20^{E}	33.47 ± 0.84^{D}	31.45 ± 1.12^{Bc}	30.17 ± 1.10^{B}	27.96 ± 0.70^{A}
	TDB	35.84 ± 1.20^{E}	31.78 ± 1.65^{Cd}	28.09 ± 2.08^{A}	27.92 ± 2.04^{A}	26.30 ± 1.12^{A}

Data expressed mean ± SD. Different letters on the same line indicate differences ($p < 0.05$) measured by Fisher's LSD test. Luminosity, a* and b* represent the chromaticity coordinates (c*).
Source: Prepared by the authors.

Figure 3. (a) Biscuits with replacement of wheat flour with the flour of the defatted baru pie; (b) Biscuits with replacement of wheat flour with the flour of the baru shell (b).



(a) (b)

Source: Prepared by the authors.

The results obtained are similar to those observed by Gurram and Sharma (2019), who studied cookies prepared with wheat flour and millet, fortified with orange peel powder (with substitution ranging from 3% to 15%), and found that, as the concentration of orange peel powder increased, the L* value decreased, from 91.57 (with 3% orange peel powder) to 53.85 (with 15% orange peel powder). Comparatively, the study by Masmoudi *et al.* (2021) on cookies enriched with jujube flour (*Zizyphus lotus* L) at concentrations of 5%, 10%, and 15% also revealed similar results. There was an increase in the a* coordinate values and a decrease in the b* coordinate values as the concentration of jujube flour increased. The a* chromaticity values ranged from 5.39, 6.65, and 7.43, while the b* coordinate values ranged from 26.57, 24.10, and 23.23, respectively.

The color (L*, a*, b*) of cookies is predominantly developed in the final stages of the baking process, resulting from the Maillard reaction, a reaction that occurs between amino acids or proteins and a reducing carbohydrate, providing a golden appearance to food after baking (Souza *et al.*, 2023). In addition to the composition of the ingredients, several factors, such as cooking time and atmospheric conditions, can impact color characteristics (Kulthe *et al.*, 2017).

3.3.3 pH, Total Acidity, Water Activity (aw) and Texture

Table 7 shows the results regarding pH, water activity, total acidity, and texture of the cookies produced by replacing wheat flour with baru by-product flour.

Table 7. pH, total acidity (TA) and water activity (Aw) of biscuits made with baru (*Dipteryx alata* Vog) baru husk (CB) and defatted baru pie (TDB) by-product flour.

		Replacement levels of wheat flour with baru flour				
Parameters	Cookies	0.0	5%	10%	15%	20%
pH	CB	6.34 ± 0.08^{C}	6.32 ± 0.04^{C}	6.32 ± 0.11^{Bc}	6.45 ± 0.12^{D}	6.21 ± 0.02^{AB}
	TDB	6.34 ± 0.08^{C}	6.10 ± 0.03^{A}	6.48 ± 0.02^{D}	6.47 ± 0.04^{D}	6.46 ± 0.03^{D}
AT[1]	CB	3.41 ± 0.002^{B}	3.41 ± 0.005^{B}	3.79 ± 0.32^{CD}	3.97 ± 0.001^{D}	3.97 ± 0.32^{D}
	TDB	3.41 ± 0.002^{B}	3.98 ± 0.003^{D}	$2,84\pm0.002^{A}$	3.41 ± 0.002^{B}	$3.60\pm0.33B^{C}$
Aw	CB	0.629 ± 0.04^{A}	0.667 ± 0.01^{B}	0.781 ± 0.003^{DE}	0.792 ± 0.001^{DEf}	0.723 ± 0.03^{C}
	TDB	0.629 ± 0.04^{A}	0.798 ± 0.005^{Ef}	0.807 ± 0.006^{F}	0.722 ± 0.007^{C}	0.771 ± 0.01^{D}
Hardness[2]	CB	49.40 ± 13.9^{A}	51.16 ± 11.2^{A}	54.46 ± 11.98^{AB}	54.35 ± 8.27^{AB}	57.77 ± 10.45^{AB}
	TDB	49.40 ± 13.9^{A}	55.77 ± 16.12^{Ab}	$61.52\pm17,3^{B}$	51.95 ± 9.73^{AB}	52.28 ± 10.45^{AB}

Data expressed mean ± SD. Different letters on the same line indicate differences ($p < 0.05$) measured by Fisher's LSD test, [1]Total acidity in molar solution % (V/m) 0.1NaoH, [2]N.

Source: Prepared by the authors.

Within the scope of the study in question, the replacement of baru by-product flour with wheat flour did not have a significant impact on the pH of the biscuits.

However, it was observed that the pH of the cookies elaborated in the present study approaches the neutrality range. Following the classification proposed by Azeredo *et al.* (2012), biscuits developed with baru by-product flour can be considered low-acid foods, with a pH between 6.10 and 6.48. Ferreira *et al.* (2020), obtained similar pH values of 6.25 and 6.63 for cookies made with 20% baru pulp flour pretreated and not treated with acetic acid, respectivel.

The pH of the biscuits in this study is within the range conducive to the growth of most microorganisms, highlighting the need for strict control during storage to ensure food quality and safety.

With regard to the total acidity content (TA), an inversion of behavior in cookies made with CB and TDB flour is remarkable. With the replacement of baru by-product flours, there was an increase in TA levels for cookies produced with CRB, while there was a decrease in TA values for cookies made with TDB flour. The determination of acidity can provide important data to assess the conservation status of the product since a decomposition process by hydrolysis or oxidation often changes the sensory and nutritional characteristics of the product (Ferreira *et al.*, 2015).

Regarding the parameter of water activity (aw) of the biscuits, there was a significant increase ($p < 0.05$) as the replacement levels of baru by-product flours were progressively increased. Activity contents were considerably high in biscuits formulated with TDB flours at concentrations of (5% and 10%) at 0.798 and 0.807 respectively, followed by biscuits prepared with BC flour at percentages of (10% and 15%) at 0.781 and 0.792 respectively. The results of water activity (aw) obtained in this study contrast with that observed by Hikpah *et al.* (2023), who reported that biscuits intended for human consumption and with replacement of shea pulp flour (*Vitellaria paradoxa*) at concentrations of 5% and 25% showed a decrease in water activity values, from 0.348 to 0.185, respectively.

Water activity (W_A) is an important parameter of the quality of baked goods due to its influence on texture, and product shelf life after cooking and during storage (Korese *et al.*, 2021; Malawi *et al.*, 2022). High levels of water activity in food is not desirable, as they favor the proliferation of molds and yeasts. This condition becomes particularly concerning when most of the water is available for enzymatic reactions and microbiological growth. Note that the water activity of the biscuits produced in this study is slightly below the threshold at which enzymatic changes are likely to occur (0.85) and above the limit conducive to microbial growth (0.6) (Safefood360, 2014).

The results of the hardness analysis indicate that the inclusion of baru by-product flours in the biscuit formulations did not show significant differences ($p > 0.05$) between them. The cookies of the control formulation obtained the lowest hardness value (49.40 N), while the increase in the concentration levels of baru flour showed an increase in hardness. Biscuits formulated with 5% and 10% flour (TDB) showed high levels of hardness (55.77 and 61.52 N), followed by biscuits formulated with (CB) flour at the percentage levels of 10% and 20%, which obtained hardness values of 54.46 and 57.77 N, respectively.

The increase in biscuit hardness can be attributed to interactions in the formation of the gluten network, influenced by the presence of fibers in the flours of baru by-products. They tend to confer greater rigidity when combined with other ingredients used in the formulation of cookies (Laganà *et al.*, 2022).

Different behavior was observed in the study by Bick *et al.* (2014), who observed that the increase in portion meal (*Chenopodium quinoa*) in gluten-free human biscuits at the percentage levels of 10% 20%, and 30%, favor the significant decrease in this physical parameter, of (12.4, 10.9 and 8.5 N) probably caused by the weakening of the gluten network.

Gluten proteins form a continuous network within the structure of the dough after hydration during kneading. When heated with water, the starch granules swell and gelatinize, losing crystallinity and molecular order. This phenomenon contributes to the formation of the mass matrix (Santos *et al.*, 2021). During the cooling and storage of gelatinized starch, retrogradation occurs, which involves changes such as the reassociation and crystallization of the starch molecule, contributing to the increase in hardness (Sasaki *et al.*, 2008).

4 CONCLUSION

Flours from baru by-products appear as promising ingredients in the formulation of dog food, standing out for their physicochemical properties, driven by the content of bioactive compounds, fibers, proteins, and lipids. This composition of the flours played a crucial role in the development of a new product. It was observed that biscuits made with 15% of baru by-product flours compared to the 0.0% control formulation biscuits stood out for their high lipid, protein, and low carbohydrate content.

The substitution of baru by-product flours in the preparation of dog biscuits revealed measurable effects on the parameters of water activity, color, and hardness. More specifically, the concentrations of 10%, 15%, and 20% for both flours influenced the increase in water activity contents and biscuit hardness.

These results not only ratify the feasibility of including these flours in pet products, but also emphasize the direct influence of these substitutions on the chemical and physical characteristics of the biscuits, providing a promising prospect for innovation in the pet food industry.

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