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Challenges of using ora-pro-nóbis (*Pereskia aculeata* Miller) Leaf mucilage as an ingredient. An example of the bitter taste in sugar-reduced muffins

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Abstract

The high sugar consumption in Brazil, linked to the rise in chronic diseases, underscores the need for strategies that promote a reduction in sugar consumption among the population. Reducing sugar in food formulations can compromise important technological characteristics, such as texture. The use of ora-pro-nóbis mucilage has proven to be a promising technological alternative. However, its bitter taste represents a sensory challenge. The aim of this work was to evaluate the effects of applying ora-pro-nóbis mucilage to sugar-reduced muffins on the perception of bitter taste. Quantification of compounds responsible for the bitter taste in ora-pro-nóbis mucilage was obtained. A sensory panel to evaluate bitter taste was selected and trained. Dilution and ranking tests were applied. Hedonic and word association tests were used to determine the preferred formulation. The free amino acid profile and the content of tannins, catechins, and total flavonoids indicate a mucilage with potential for a sensory perception of bitterness, as confirmed by the sensory panel. The reduced-sugar muffin with ora-pro-nóbis added was accepted by consumers. The muffin with 0.2% ora-pro-nóbis mucilage had an acceptance rate of 88.7%, and no «Bitter» or negative mentions appeared in the word association test.

Keywords: acceptance; consumer; emulsifier; free amino acids; polyphenols; sensory panel.

Practical Application: To reduce sugar consumption, new food ingredients emerge as an important strategy.

1 INTRODUCTION

In food products, sugar offers multiple functions beyond providing sweetness. Its contribution primarily affects texture, color, and osmotic pressure, which consequently helps reduce microbial growth. Although its technological importance is recognized, there is increasing pressure to reduce sugar in processed food products, since excessive consumption is directly linked to obesity, diabetes, and cardiovascular diseases (Zhao et al., 2024).

Given this scenario, a voluntary agreement was signed in 2018 between the Brazilian government (Ministry of Health) and associations in the food sector with the aim of reducing the amount of sugar in processed foods (Brasil, 2023). Currently, it is known that this process is ongoing, and companies have been seeking alternatives, such as sugar substitutes and functional ingredients, to improve texture (Agência Nacional de Vigilância Sanitária [Anvisa], 2024).

Within this context, natural food ingredients are gaining increasing prominence not only for their technological functionality in food, but also for their nutritional value, or for being non-harmful to the human body (Lise et al., 2021; Ohtaki et al., 2023; Sbardelotto et al., 2025).

It turns out that these natural ingredients, mostly derived from plant sources (Oliveira et al., 2023). Often, they present some kind of limitation or difficulty in their use by the food sector (Bordim et al., 2021; Carocho et al., 2018; Ohtaki et al., 2023). These limitations are related to stability (Carocho et al., 2018), the presence of unpleasant tastes and flavors, known as off-flavors (Bordim et al., 2021; Ohtaki et al., 2023), and inherent colorations of the ingredient (Bordim et al., 2021; Queiroz & Mitterer-Daltoé, 2023). Studying strategies to reduce these limitations is of fundamental importance to facilitate the use of these natural ingredients by the food industry. Therefore, developing formulations from natural ingredients that achieve technological, nutritional, and sensory quality is a challenge for the sector.

The mucilage of Pereskia aculeata Miller (ora-pro-nóbis [OPN]) has been investigated as a potential functional ingredient for enhancing food texture (Lise et al., 2021; Mazon et al., 2020). The mucilage extracted from the leaves is a hydrocolloid composed of arabinogalactans linked to protein components in its structure (Martin et al., 2017). The presence of this hydrocolloid makes it possible to use OPN mucilage as a gelling, thickening, and emulsifying agent. The mucilage can simulate the texture conferred by sugar, aiding in the restructuring of the food. However, its bitter taste, conferred by the presence of compounds characteristic of plant-based foods, represents a sensory challenge. Plant-based foods are characterized by specific sensory attributes, such as bitterness and astringency, due to the presence of polyphenols (Pagliarini et al., 2021) and free amino acids (Su et al., 2023). Therefore, the aim of this work was to evaluate the effects of applying OPN mucilage to sugar-reduced muffins on the perception of bitter taste.

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1.1 Relevance of the work

Natural food ingredients have been gaining prominence not only for their technological functionality in food, but also for their nutritional and safety benefits to the human body. However, it often presents some sensory limitations for the food sector. This work explored the ora-pro-nóbis leaf mucilage as a new natural ingredient for sugar-reduced muffin formulations. With previously unexplored results, it was possible to characterize the ingredient regarding the chemical compounds responsible for the bitter taste, define the mucilage concentration to be used in muffins with good acceptance, and characterize the sensory profile.

2 MATERIALS AND METHODS

2.1 Mucilage obtaining

The leaves of the OPN plant were harvested in Pato Branco, southwest of the Paraná state (26° 13′ 42″ S 52° 40′ 14″ W; altitude: 769 m), in the spring/summer seasons in Brazil. The leaves used in the extraction are registered by the exsiccate HPB 959.

The mucilage from OPN was obtained in a manual-mechanical mixed process with water as described by Lise et al. (2021).

2.2 Quantification of compounds responsible for the bitter taste in ora-pro-nóbis mucilage

The characterization of the free amino acid profile of the OPN mucilage was performed using high-performance liquid chromatography (HPLC), following Lise et al. (2021), White et al. (1986), and Hagen et al. (1989). The quantification of catechin was performed using CLAE and followed the protocol described in the Natura Integrated Normative System—Determination of Methylxanthine and Catechin by HPLC—MA-858/Rev.00. Tannin quantification was performed by colorimetry, following the Brazilian Compendium of Animal Feed, 2013, method 52. Total flavonoid quantification was performed by Ultraviolet-Visible spectrophotometry, following the protocol of Chabariberi et al. (2009). The analyses were performed in triplicate.

2.3 Elaboration of muffins with ora-pro-nóbis mucilage added

The preparation of the muffins followed the operational processes described in chronological order as follows: The ingredients used in the muffin formulation were wheat flour (33.9%), egg (12.1%), whole milk (33.9%), soybean oil (7.3%), sugar added (12.1%), baking powder (0.7%), and OPN mucilage (0.0, 0.1, 0.2, 0.3, 0.4, and 0.5%). To achieve 100% coverage of the total ingredients in the formulation, the variation in the percentage of mucilage added was compensated for by the flour ingredient. The amount of sugar was based on the agreement signed between the Brazilian government and the food industry (Brasil, 2023). For cakes, the planned reduction is approximately 32%. Therefore, based on industrially produced muffins that contain approximately 44% sugar, the planned amount was 12.1%.

The preparation was based on the method described by Karimi et al. (2024). First, whole eggs were whipped and mixed with milk and soybean oil. A batter was then made by adding powder ingredients to the foamy mixture. After filling muffin cups, they were baked for 20 min at 180 °C. Upon baking, the samples were allowed to cool and then stored at ambient temperature in plastic zipper bags for further tests.

2.4 Sensory tests

The study was approved by the Ethics Committee under Certificate of Presentation of Ethical Assessment No. 77358924.3.0000.0177. The sensory tests were conducted in the Food Technology Laboratory of Federal University of Technology—Parana, which is equipped with three individual booths suitable for sensory analysis. Participants were provided with an informed consent form at the beginning of the tests.

2.4.1 Selection of assessors for identifying basic tastes

The selection of assessors was carried out in accordance with ABNT NBR ISO 8586 (Associação Brasileira de Normas Técnicas [ABNT], 2012). A total of 50 participants took part in this stage. The invitation was extended to academics and staff of UTFPR.

Solutions of sucrose (10 g/L), citric acid (0.3 g/L), caffeine (0.3 g/L), sodium chloride (2 g/L), and monosodium glutamate (0.6 g/L) representing the basic tastes of sweet, sour, bitter, salty, and umami, respectively, were prepared 24 h before. The participants received a total of 10 samples, consisting of duplicates of each prepared solution, packaged in cups containing approximately 20 mL. All samples were identified by being coded with three random digits and presented monadically, and water was provided for palate cleansing between evaluations.

2.4.2 Training of selected assessors to identify bitter and sweet tastes

The selected evaluators participated in training to identify bitter and sweet tastes. Paired comparison tests (ABNT, 2017) were performed for these two tastes, using caffeine and sucrose at threshold concentrations (0.2 g/L caffeine and 6 g/L sucrose). For the test, participants were given 10 sets of two coded samples with three random digits. One set contained the caffeine/sucrose solution, and the other contained distilled water. Candidates were asked to taste the sample from left to right and identify the sweeter/bitter sample.

2.4.3 Dilution test for evaluating muffin formulations

The dilution test was conducted using a paired comparison test applied to 25 trained assessors (ABNT, 1994, 2017). Each assessor received five pairs of muffin sample combinations in a plastic dish, each coded with a three-digit random number. For each pair, one sample was standard (without added mucilage), and the other was a sample with added mucilage at a concentration ranging from 0.1 to 0.5%. The assessor tasted each pair of samples and, based on sensory perception, indicated which one had a greater intensity of bitter and sweeter taste, marking the corresponding code on the sensory sheet.

2.4.4 Preference ranking test

The ranking test was based on the (ABNT, 2015). Sixty consumers simultaneously received the five muffin samples with mucilage of OPN added in a plastic dish, each coded with a three-digit random number. After tasting, participants ranked the samples from most to least preferred.

2.4.5 Word association and acceptance tests

Word association (WA) and acceptance tests were conducted using the sample defined by the ranking test. The muffin with 0.2% of OPN added was selected as the most preferred. Sixty consumers (Bordim et al., 2021) participated in the evaluations. Consumers received the sample and were asked: "*You are receiving a muffin prepared with ora-pro-nóbis mucilage added. Write the first four words or sensations that come to mind about the product.*" Consumers also rated how much they liked or disliked the product using a nine-point hedonic scale (1 = dislike very much; 9 = like very much).

2.5 Data analysis

Data from the free amino acid profile of OPN mucilage were expressed as mean ± standard deviation and evaluated by analysis of variance and the Tukey test.

The results of the selection and training of assessors were evaluated by sequence analysis, according to the graphical method (ABNT, 2020). The decision system was obtained through hypothesis testing Ho: $p1 \leq p0$ and using the values $p0 = 0.33$ (probability of a correct response when no perceptible difference exists) $p1 = 0.66$ (probability of a correct response when a perceptible difference does exist) for α risk = 0.05 (probability of concluding that a perceptible difference exists when one does not) and β risk = 0.10 (probability of concluding that no perceptible difference exists when one does).

The dilution test was calculated as the concentration corresponding to 75% of the correct answers obtained from the total number of assessments (ABNT, 1994).

For the ranking test, results corresponded to the sum of ranks assigned by assessors to each sample. Differences between samples were evaluated using the Friedman test, with significance determined according to the Newell and MacFarlane table (ABNT, 2015).

WA data were analyzed following Bordim et al. (2021). Terms were grouped into categories and subsequently into broader dimensions. The classification was performed independently by three researchers, who then met to verify agreement. Final categories and labels were defined by consensus, and only those mentioned by more than 5% of participants were included in the analysis. Differences among categories were evaluated by Cochran' Q test (Eidelwein et al., 2025).

The acceptance rate was calculated from the average score, with the maximum value on the nine-point scale (9) corresponding to 100% acceptance.

3 RESULTS AND DISCUSSION

3.1 Quantification of compounds responsible for the bitter taste in the mucilage of ora-pro-nóbis leaves

Table 1 presents the free amino acid profile of the OPN mucilage. Twenty free amino acids have been identified, of which eight are associated with the bitter taste. It is worth noting that the amino acids Arg, Ile, Leu, Phe, Val, and Met are known for activating bitter taste receptors and were identified in the mucilage in low amounts (0.13 of 0.91%).

The free amino acids responsible for the umami taste, Glu and Asp, together accounted for almost 50% of the total amino acids. Umami is one of the five basic tastes recognized by the human palate, described as causing a "savory" sensation and producing a flavor-enhancing and lingering effect in the mouth. The presence of umami enhances the overall flavor of foods, which is considered positive for culinary applications and is widely utilized in the food industry (Dutta et al., 2022). However, it cannot be ruled out that, even with a predominance of umami-free amino acids, their umami intensity can be suppressed by the bitterness intensity.

Possibly, the greatest influence on the unpleasant taste is related to the presence of polyphenols (Table 2). These compounds contribute to the bitter taste and the sensation of astringency.

Table 1. Free amino acid profile of ora-pro-nóbis mucilage.

Free amino acids	Mean ± standard deviation (%)	Flavor *
Aspartic acid (Asp)	0.190 ± 0.000 [a]	Umami
Glutamic acid (Glu)	0.305 ± 0.005 [a]	Umami
Glutamine (Gln)	0.100 ± 0.028 [c]	Sweet
Serine (Ser)	0.025 ± 0.005 [d, e]	Sweet
Glycine (Gly)	0.010 ± 0.000 [e]	Sweet
Proline (Pro)	0.045 ± 0.005 [d]	Sweet
Alanine (Ala)	0.030 ± 0.000 [d, e]	Sweet
Threonine (Thr)	0.020 ± 0.000 [e]	Sweet
Arginine (Arg)	0.080 ± 0.014 [c]	Bitter
Valine (Val)	< 0.01 *LQ	Bitter
Isoleucine (Ile)	< 0.01 *LQ	Bitter
Leucine (Leu)	0.020 ± 0.000 [e]	Bitter
Phenylalanine (Phe)	0.015 ± 0.005 [e]	Bitter
Histidine (His)	0.015 ± 0.005 [e]	Bitter
Methionine (Met)	< 0.01 *LQ	Bitter
Tyrosine (Tyr)	< 0.01 *LQ	Bitter
Cystine (Cys)	0.020 ± 0.010 [e]	Tasteless
Lysine (Lys)	0.020 ± 0.000 [e]	Tasteless
Asparagine (Asn)	0.020 ± 0.000 [b]	Tasteless
Hydroxyproline (Hyp)	< 0.01 *LQ	Tasteless
Sum of free amino acids	0.910 ± 0.071	

*LQ: limit of quantification. *Assigning flavor to amino acids (Sissons et al., 2022). Means followed by equal letters in the column do not show any significant difference, using the Tukey test ($p \leq .05$).

Table 2. Polyphenols of ora-pro-nóbis mucilage.

Polyphenols	Mean ± standard deviation
Tannins (%)	0.466 ± 0.17
Catechin (mg/kg)	652.9 ± 194.5
Total flavonoids (mg/kg)	5.978.48 ± 1811.84

Phenolic compounds are secondary metabolites produced by plants as defense mechanisms; chemically, they can confer color, taste, aroma, and texture to ensure survival. These compounds are classified as flavonoids and non-flavonoids, and catechin belongs to the flavonoid group (Soares et al., 2013)

Tannins, which belong to the polyphenol group, have the ability to interact with proteins, including salivary proteins; this interaction occurs with TAS2R receptors, which may explain part of the bitter sensation, in addition to causing residual astringency. The same sensory behavior is found in catechin (Soares et al., 2020). The presence of a large amount of flavonoids in the chemical composition of OPN mucilage has already been reported by Kobayasi et al. (2023), supporting the idea that the significant quantities of these compounds in the mucilage have a greater impact on bitter sensory perception.

3.2 Sensory tests

3.2.1 Selection of assessors with acuity to identify basic tastes

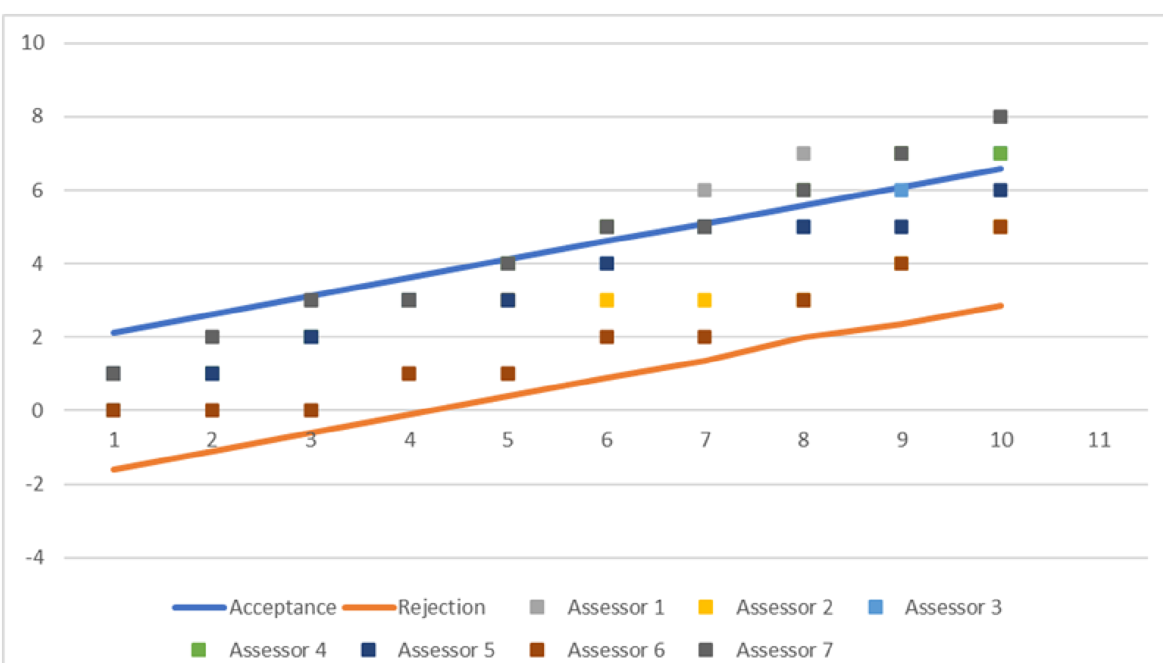
Figure 1 shows the results of selecting some candidates with acuity to detect different basic tastes, as assessed by the Wald Sequential Analysis. The graph illustrates the individual trajectories of the first seven assessors in relation to the decision lines for acceptance and rejection. Straight lines of acceptance and rejection that were obtained through the sequential test for the selection of assessors were: An: 2.0994 + 0.4948n and Rn: −1.6412 + 0.4948n. Each accumulated point represents a correct answer over the 10 attempts. Assessors whose trajectories exceeded the acceptance threshold were considered eligible for the next stage. Those whose trajectories remained below the rejection threshold were disqualified. The remaining evaluators were analyzed until a decision criterion was met. Based on this approach, it was possible to select 25 participants out of the 50 candidates. These individuals were then referred for specialized training in the perception of sweet and bitter tastes.

3.2.2 Training of selected evaluators for bitter taste

Through the paired comparison test, the 25 previously approved assessors participated in the training stage to identify bitter taste. The evaluation of the training for the selected candidates was also conducted using Wald Sequential Analysis. Figure 2 shows the path of the five assessors along the decision lines for acceptance and rejection. The 25 selected assessors were trained and approved to proceed to the next phase. The use of this group of assessors as a measurement tool is compared to the use of any scientific instrument to obtain measurements of specific parameters for products under study (Lawless & Heymann, 1998).

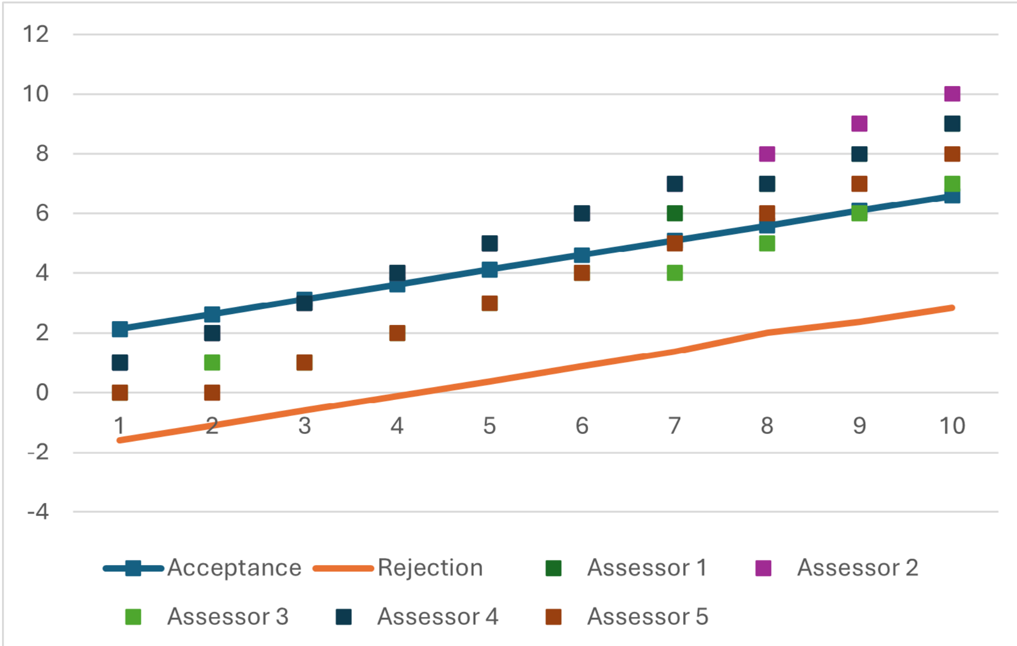
3.2.3 Dilution test

A dilution test is a technique that determines the maximum amount of test material that cannot be detected when mixed with a standard material (ABNT, 1994). For the present research, the application of this test aimed to identify the muffin formulation with OPN mucilage added and reduced sugar (32% reduction) that does not present a significant difference from the standard sample (without mucilage and with reduced sugar) in terms of bitterness. For this purpose, the paired comparison technique (ABNT, 2017) was applied to the trained assessors. Based on the responses, it was found that none of the formulations tested, with mucilage concentrations between 0.1 and 0.5% and reduced sugar content, showed significant similarity to the standard sample when evaluated by the trained team. In other words, even at the lowest concentration (0.1%), the perception of a bitter taste emerged.



y: right responses; x: répétitions.

Figure 1. Wald sequential analysis for selection of assessor.



y: right responses; x: répétitions.

Figure 2. Wald sequential analysis for training assessors.

The relationship between natural ingredients (from plants) and the bitter taste perceived is reported in the literature (Bordim et al., 2021; Ohtaki et al., 2023). Bordim et al. (2021) used moringa leaf extract as a naturally colored antioxidant in three different food product categories (bread, yogurt, and pate). For consumers, the addition of this ingredient (0.5%) to bread resulted in a bitter taste, as well as a bitter residual. Ohtaki et al. (2023) used ultra-refined yerba mate as a naturally colored food ingredient in cake (4.32%), and "Bitter" was an attribute used by consumers to characterize the cake.

Both studies emphasize the significance of understanding the interaction between basic tastes. The researchers affirm that for the use of some types of natural ingredients (such as those derived from plants) in food products, it is necessary to plan formulations that also contain large amounts of salt and sugar, which are recognized as bitter taste suppressors (Keast & Breslin, 2002). However, for this study, the OPN mucilage is being applied in a formulation characterized by reduced sugar content, which therefore makes its use even more challenging.

Although it was not possible to define the formulation through the dilution test, since all of them showed significant differences from the standard in terms of bitterness, it is worth highlighting that the results discussed are discriminative, not affective. In other words, it is necessary to investigate whether a formulation, even with a perceptible bitter taste, would be well accepted or not. Therefore, the next stage of the research aimed to select a preferred formulation through a preference ranking test and subsequently verify its acceptance and characterization based on that selection.

3.2.4 Preference ranking test

The ranking test facilitates the assessment of differences among several samples based on the intensity of a single attribute or an overall impression. It is indicated for assessing products, such as descriptive criteria or hedonic preferences (ABNT, 2015). Table 3 lists the results of the ranking test completed by sixty consumers. According to the results, formulation B (0.2% OPN mucilage added) was the preferred formulation, as it was the sample most frequently ranked with a preference order of 1 (increasing order of preference). Once the preferred formulation was defined, the next step was to evaluate its acceptance and provide a holistic description of the formulation.

3.2.5 Acceptance and word association tests

Hedonic scales are used to quantify the affective dimension of food perception by consumers and its commercial success (Peryam & Pilgrim, 1957).

The muffin formulation (0.2%) had an acceptance rate of 88.7%. This result is particularly satisfactory within the context of a food characterized by bitterness, according to the trained assessors.

It is suggested that the bitterness is of low intensity, sufficient to be perceived by the trained panel, but insufficient to compromise the acceptance of the muffin. The lack of perception of bitterness by consumers can be corroborated by the results of the word association test, since the category "Bitter" did not emerge spontaneously during the test (Table 4). According to (Roininen et al., 2006), when dealing with food products, the associations that first come to mind may be the most relevant for consumer decisions related to product purchase.

In previous research, such as that conducted by Bordim et al. (2021) and Ohtaki et al. (2023), when applying the WA test to foods with natural ingredients added, the term "Bitter" emerged, highlighting consumers' perception of bitterness. In the present study, in addition to the word "Bitter" not

Table 3. Preference rankings assigned by consumers.

Consumers	F1 (0.1%)	F2 (0.2%)	F3 (0.3%)	F4 (0.4%)	F5 (0.5%)
1	4	1	2	3	5
2	4	1	5	3	2
3	4	2	3	5	1
4	2	1	3	4	5
...	...	...	...	...	...
60	4	5	2	3	1
TOTAL	163ab	146a	195b	198b	198b

Different letters mean a difference in the total sum of orders for the preference ranking test. DMS: 47.2

Table 4. Frequency of categories mentioned for the muffin with ora--pro-nóbis mucilage added.

Category	Number of mentions
Tasty	33 A
Soft	31 A
Mild sweet	16 B
Dense texture	9 B
Childhood	9 B
Coffee	5 B
Humidity	5 B
Home	5 B
Grandmother	5 B
Natural	4 B
Nostalgia	4 B

Different letters in the column indicate a significant difference at 5% by Cochran's Q test.

appearing, terms of a very positive nature stood out: taste and soft. According to Antmann et al. (2011), the most mentioned terms can be considered as most relevant to consumers and most commonly used by them to describe the characteristics of food products. In this sense, it can be stated that the WA test corroborated the high acceptance rate of the muffin. Furthermore, for consumers, the muffin formulated with reduced sugar and with OPN added can be described as tasty, soft, mildly sweet, and moist. Moreover, it evokes a sense of something natural, bringing nostalgia and reminding one of home, childhood, and grandmother, often paired with coffee. Although mentioned by a small percentage of participants, the *Natural* category was marked and deserves attention. Consumers' awareness of synthetic food additives has been documented in previous studies (Mitterer-Daltoé et al., 2021; Sbardelotto et al., 2025), and the development of food products with natural ingredients is a trend in the food sector (Bezerra et al., 2023; Colares et al., 2025).

4 CONCLUSIONS

This study explored the bitter taste as a challenge to using OPN mucilage as a food ingredient. To this end, a chemical characterization of OPN mucilage was conducted, and a study on the development of reduced-sugar muffins was undertaken, incorporating various sensory tests applied to both trained assessors and consumers. The free amino acid profile and the content of tannins, catechins, and total flavonoids indicate a mucilage with potential for a sensory perception of bitterness. This perception was confirmed on the reduced-sugar muffins with OPN mucilage added.

It is noteworthy that the perception of bitterness was identified only by trained assessors. Although the drawback of bitterness is acknowledged as a challenge, the reduced-sugar muffin with OPN added was not rejected by consumers. The muffin with 0.2% OPN mucilage had an acceptance rate of 88.7%, and no «Bitter» or negative mentions appeared in the WA test.

Although natural ingredients have significant health benefits and are a core topic in the food sector, they face limitations related to the challenges of their use. Specifically regarding OPN mucilage, not only bitterness but also stability, texture, and color are examples of issues for future research.

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