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Effect of partial substitution of wheat flour with okara flour in the elaboration of panettone

Sol Rodriguez[1], Víctor T. Ponce Aquino[1] and Jorge R. Uriarte Dávila[1]
[1] Facultad de ingeniería, Universidad Tecnológica del Perú, Perú, c19588@utp.edu.pe
[1] Facultad de ingeniería, Universidad Tecnológica del Perú, Perú, 1624156@utp.edu.pe
[1] Facultad de ingeniería, Universidad Tecnológica del Perú, Perú, 1627149@utp.edu.pe

Abstract*– "Okara" is an industrial residue generated from the production of soy milk. This residue can be transformed into okara flour, a higher value-added product that can be used in the baking industry. The main objective of this research is to determine the optimal substitution of wheat flour with okara flour without altering the process or the rheological characteristics in the preparation of panettone. As part of the methodology, the following technological characteristics were evaluated: fibre size, moisture percentage, height variations, final product weights, variation in beating times, acidity, and water activity. The results of this research showed that the optimal substitution of okara flour in the panettones is 4.2% (okara flour/wheat flour ratio of 0.05/1), resulting in characteristics similar to a standard panettone (without substitution), with a higher percentage of water retention that helps extend the product shelf-life. Hedonic scale evaluation demonstrated that panettones with 4.2% of okara flour did not present significant differences compared with panettones prepared with wheat flour only. In addition to the results obtained, it is shown that with greater substitution rates, there are technological variations in the size and formation of the gluten network. In conclusion, okara flour can be applied in the preparation of panettones with an optimal substitution of 4.2%, with 8.7% and 13.7% substitutions not recommended according to the tests carried out in the investigation.

***Keywords*— Okara flour, dough, panettone, wheat substitution.**

I. Introduction

Panettone is an Italian sweet bread consumed during the Christmas period in Italy and Latin-America. Especially, Perú is the major consumer of this product, reporting a consume of 1.10 kg per capita and ~150 million USD in sales [1]. Panettone is produced from mother dough (or sourdough), prepared with a mixture of wheat flour, fresh yeast, lactic acid bacteria and water; and dough can be mixed with optional ingredients such as candied fruits, raisins, almonds or chocolate with several steps of mixing and leaving [2]. The mother dough is spontaneously fermented, so it keeps the aroma and quality during storage. Fermentation is caused by bacteria (*Lactobacillus sanfranciscensis*, *Levilactobacillus brevis* and *Lactiplantibacillus plantarum*) and yeast (*Saccharomyces cerevisiae*, *Kazachstania exigua*, and *Kazachstania humilis*) at specific conditions. For example, Ref. [3] found that refreshment procedures maintain a stable and active microflora in industrial conditions. Further, Ref. [4] employed enzymes (amylase, xylanase and lipase) to improve physical characteristics of panettone (height, hardness and grain crumb structure). In opposition to the bread, panettone can be consumed until six months after being produced because of the maturation [5]. According to this, the main parameters associated with the quality of panettone are moisture, volatile compounds content, color, mechanical and superficial characteristics.

Mother dough in bakery is prepared with wheat flour (WF), however different alternatives have been explored for WF substitution such as sweet potato flour [6], mesquite flour [7], banana flour [8] or potato flour [9], demonstrating that partial substitutions (less than 35%) do not affect rheological properties nor the formation of gluten networks, but it can affect organoleptic attributes. Another alternative employed is okara flour, obtained as a byproduct of the production of soymilk. During the processing of soybeans, the crushed soybeans leave a residual yellowish paste consisting of an insoluble fraction [10], but after drying and milling processes, the paste can be converted in okara flour. In general, the soybean paste is discarded because its high moisture content and putrefaction susceptibility; however, okara contains beneficial components for functional and gluten-free products [11]. Moreover, okara flour has demonstrated to improve functional properties in bakery products such as viscosity, water and oil absorption, emulsifying and foaming capacity and gelling properties [12]. Previous studied have employed a mixture of okara flour and wheat flour for the elaboration of cookies [13] and bread [14] or for the elaboration of gluten-free flour [15].

Nowadays, according to many reports in 2022 [16]–[19], the world is suffering a food crisis related to shortage of grains, mainly corn and wheat. Many reasons have been considered such as Ukraine's ports blockaded, the shortage of fertilizers and the uncertain of China's harvest. Additionally, COVID-19 induced lockdown impacted the food market. For example, several reports indicated a 15% increase in agricultural prices in India and Africa, an increase of 4-7% in dairy products and a decreasing demand for meat (7-18%) worldwide [20]. On the other hand, food commodities experimented an increase of prices by almost 30% during 2020 due to the increase of strategic food reserves (wheat, rice, and other main commodities). Besides, Ref. [21] mentioned that international grain and cereals prices are more resilient to COVID -19 pandemic than other products [22]. Concluding, the post-covid world is facing new challenges associated with climate change, water scarcity, fragile states and population displacements. For those reasons, it is necessary to find alternative sources for wheat flour for bakery products such as breads, cakes and panettones.

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Hence, the aim of the present work is to investigate the influence of using okara flour as a partial substitute of wheat flour for the elaboration of panettone. Physicochemical aspects and quality requirements were evaluated in order to compare and analyze the substitution rate of wheat flour.

II. Methodology

A. *Materials*

Okara flour was obtained as a by-product of the production of soy milk. After been grounded in water, grounded soybeans were separated from the liquid (soy milk) and the precipitated formed a soybean paste, known as "okara". In order to obtain okara flour, soybean paste must be dried and milled [10]. In our case, recovered soybean paste was dried at 150°C for 6 hours under oven conditions and cooled at 40°C for 1h until constant moisture (4.5%). Then, dried okara was milled in a hammermill until get a fine powder of okara flour (mesh #140) and, finally, stored until use. For panettone formulations, wheat flour, fresh yeast, white sugar, anhydrous milk fat, milk powder, eggs, salt, raisins and candied fruit were also used. Other chemicals used for this study were analytical grade.

B. *Elaboration of panettone*

For the processing of panettones, five stages were stablished and the proportions of okara flour and wheat flour is shown in Table 1.:

- Mixing I: The natural yeast (obtained in a previous fermentation) wheat flour and the other ingredients (white sugar, wheat gluten, margarine, flour treatment agents, emulsifiers, and water) are kneaded for 8 to 15 minutes at 24°C - 32°C until gluten development (homogeneous texture).
- Fermentation: After mixing, the dough is transferred to a resting chamber where it remains for 2.0 to 3.0 hours at room temperature. In this stage, dough volume increases. An acidity control is carried out, which must have a maximum value of 0.45% lactic acid.
- Mixing II: After resting, ingredients are added again (wheat flour, wheat gluten, margarine, salt, egg yolk, preservatives, anhydrous milk fat, white sugar, flour treatment agents, skimmed milk powder, essences, colorants, emulsifiers, stabilizers, antioxidants, candied fruit, candied orange and raisins) and mixed until an elastic dough is achieved. Mixing is done for a time of 8 to 25 minutes. An acidity control for dough is carried out, which must have a maximum value of 0.45% lactic acid.
- Molding: It consists of providing the fractionated mass with a compact and spherical shape, it is done manually; then the rounded dough is deposited in the mold, to then be taken to the fermentation chamber.
- Fermentation II: It is carried out in a fermentation chamber and consists of obtaining the increase to the desired volume through fermentation, under controlled conditions of temperature from 30 to 36 °C and relative humidity from 70 to 93%, for a time between 3.0 to 9.0 hours.
- Baking: Molded dough is baked from 120°C to 220°C for 60 minutes. In this stage, the dough size increases and panettone dough is browning in the oven due to caramelization
- Cooling: The temperature in the over decreases until 32°C under natural air-flow condition for 4 hours. It is important to note that condensation due to air humidity must be avoided.
- Quality control: Finally, panettone is evaluated to detect ferrous and non-ferrous metals by using sensors. Then, panettone is packaged and encoded. For packaging, plastic bags were employed and panettones were stored at 20-25°C

A process flowchart is shown in Fig. 1.

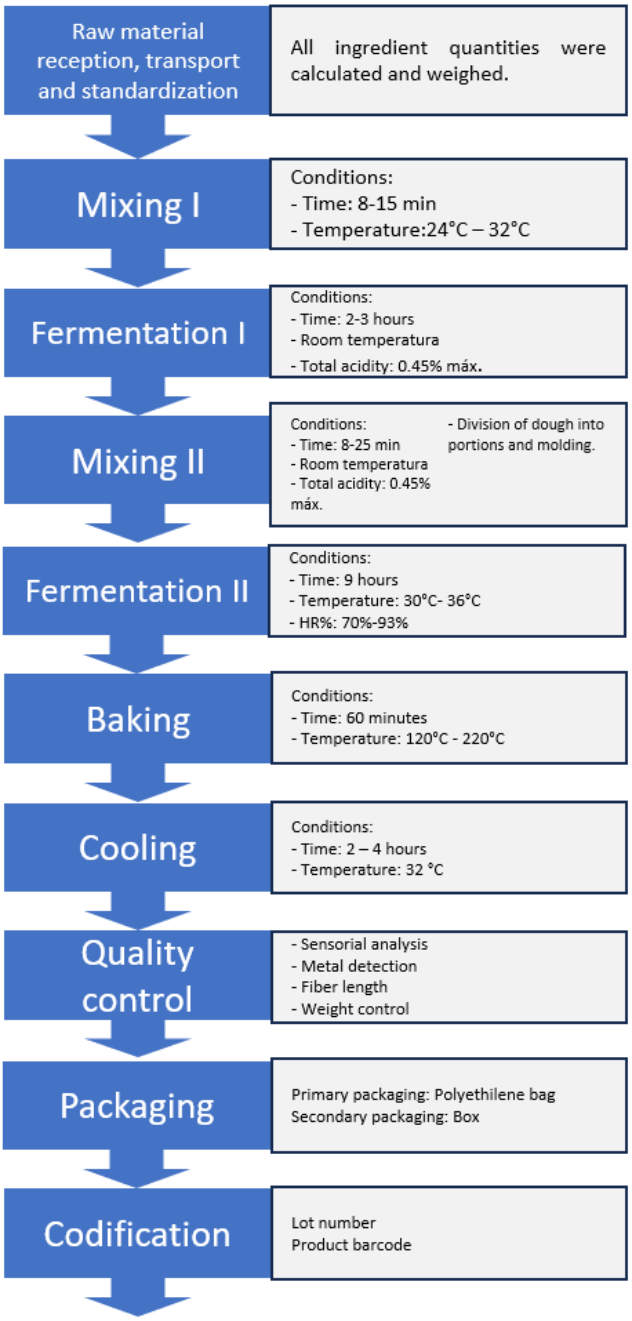


Fig. 1. Process flowchart of panettone elaboration.

Table 1. Proportion of okara flour employed in each formulation. Other ingredient quantities remain unchanged.

Code	Okara flour proportion in mother dough (%)	Proportion of flour Okara/Wheat
P0	0.0	0
P1	4.2	0.05
P2	8.7	0.10
P3	13.7	0.15

C. Analysis and characterization techniques

Physicochemical analyses were conducted monthly to evaluate the characteristics of the panettone and determine its shelf life. Parameters such as moisture content (%), total acidity (%), fibre size (cm), height (cm), and water activity were measured. Moisture content was assessed according to NTP 209.004:1968 (2011) using a Lab Balance OHAUS MB45, while total acidity was determined following NTP 206 guidelines. Water activity (AW) was measured using a water activity meter. Total titratable acidity was determined through titration with NaOH (0.1N) and expressed as % lactic acid.

Additionally, a survey was conducted for sensory evaluation of the prepared panettone. Ten trained participants were employed to assess different characteristics such as external color, internal color, smell, taste, and texture. Subsequently, a statistical study was performed to assess the perception and acceptance of panettone across different formulations.

III. Results and Discussions

To produce okara flour, soybean residues were collected, dried, and milled. The initial okara flour contained 85.45% moisture, and after drying, the moisture content reduced to 4.85% (Fig. 2). The okara flour was ground and passed through a 140-mesh sieve. Subsequently, the okara flour was stored under desiccator conditions. The yield was approximately 8.5% based on the raw okara. According to different authors, okara flour must not exceed 15% in moisture content [12], however, okara flour is highly hygroscopic and the moisture level could be around 800g/kg [15].

Fig. 2. Okara flour at different moisture content. After the initial extraction, the okara flour shows large-sized lumps, which disappear during the drying and pulverization stages.

Panettone elaboration remained unchanged in the process; the only modification was the substitution of wheat flour. As shown in Table 2, the process conditions (temperature, beating time, and total acidity) remained constant during Mixing I stage. In Mixing II stage, it was observed that acidity slightly increased, but water absorption rose abruptly with a greater substitution of wheat flour. On the other hand, the application of okara flour in baked products has been reported because it is a rich source of nutrients (dietary fiber, fat, protein) and could be used to improve nutritional quality of bread-like products [14], [23], [24]

Table 2. Conditions for the mixing stages.

Condition	P0	P1	P2	P3
Mixing 1				
Beating time (min)	12	12	12	12
Acidity (% lactic acid)	0.30	0.30	0.30	0.30
Temperature (°C)	31.3	31.3	31.4	31.3
Mixing 2				
Beating time (min)	13	13	13	13
Acidity (% lactic acid)	0.31	0.32	0.35	0.35
Temperature (°C)	28.9	29.0	29.7	28.5
Water absorption (g)	0	30.0	50.0	110.0

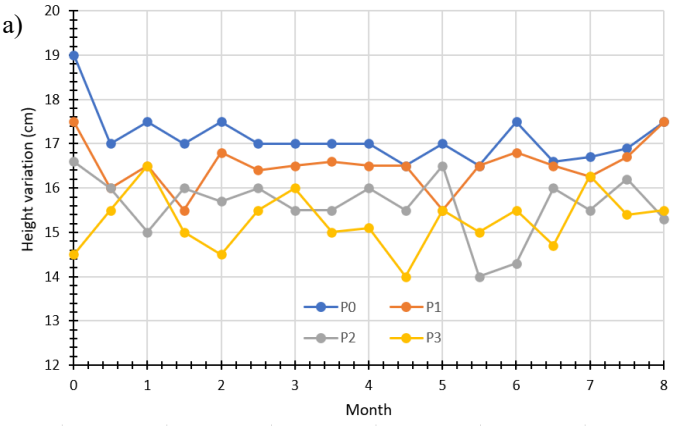
The characteristics of panettones were evaluated to determine the optimal rate of wheat flour substitution. Fig. 3 shows the relationship between the height of panettones and the substitution rate over 8 months. As depicted in Fig. 3a, the height of panettones decreased during the first month (except for the P3 formulation). Subsequently, the height remained constant for control P0 and P1. However, P2 and P3 exhibited significant variations after the fifth month. By the eighth month, the height of P1 was similar to that of control P0. Fig. 3b illustrates an example of the effect of panettone shrinkage at higher rates of wheat flour substitution.

According to Fig. 4, the standard formulation P0 exhibited a porous structure due to the characteristic gluten network. However, the gluten network appeared to shrink and compact with the addition of okara flour. Similar results were reported by Ref. [25], which indicated that cupcakes made with soybean flour showed a dense crumb due to soybean proteins. Additionally, Ref. [23] reported the same effect in biscuit elaboration. Okara flour dough demonstrated lower stickiness compared to wheat flour dough due to the higher water absorption capacity of okara, resulting in increased adhesion.

Another quality parameter for panettone and most cakes is fiber size. This test aims to evaluate gluten network formation in cakes. In our case, panettone is considered a moist cake, and gluten networks could help extend the shelf life and retain key volatile compounds. In Fig. 5, the fiber size of different formulations was evaluated. As observed, P0 exhibited the highest fiber size for the first 6 months, and then the fibre size of P0 became similar to that of P1. On the other hand, values for P2 and P3 showed a lower fiber size. As described above, okara flour is rich in protein (34,33% [14]), the main effect is to harden the mother dough and increase water absorption. For other flours like oat, Ref. [26] found that the higher content of protein and fibre in oat flour hardened the dough mass and, as

a result, the fibre size tend to reduce. This result can be attributed to the rheological behavior of the dough, where a loss of elasticity and higher viscosity may lead to variations in firmness and specific volume[13], [25].

a)



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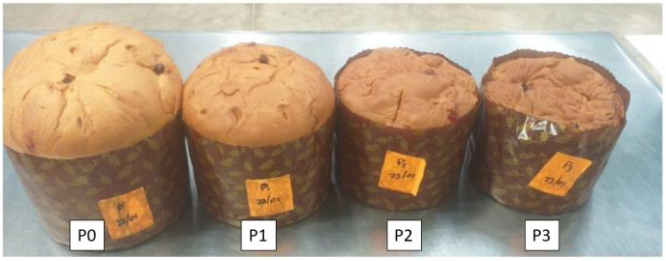

Fig. 3. a) Evaluation of panettones' height for 8 months. b) Panettones from different formulations.

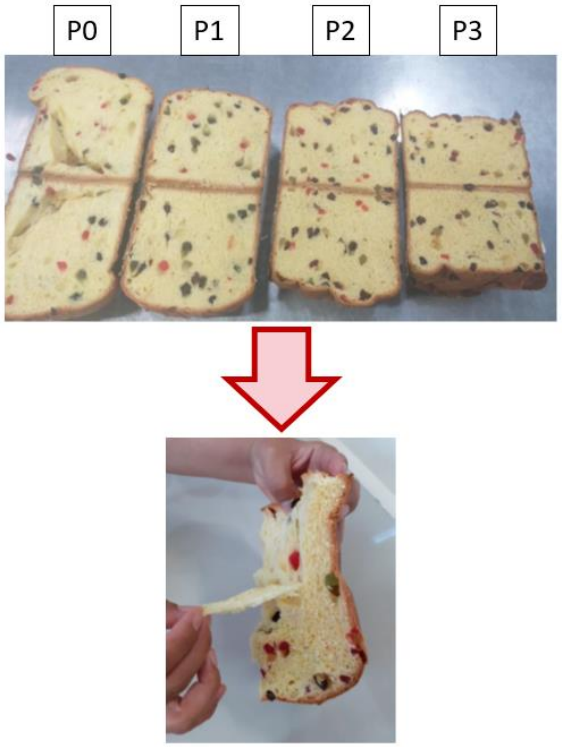

Fig. 4. Above: Cross-section of the panettones. A reduction in porosity is observed for the P2 and P3 formulations. Below: Example of fibre size test.

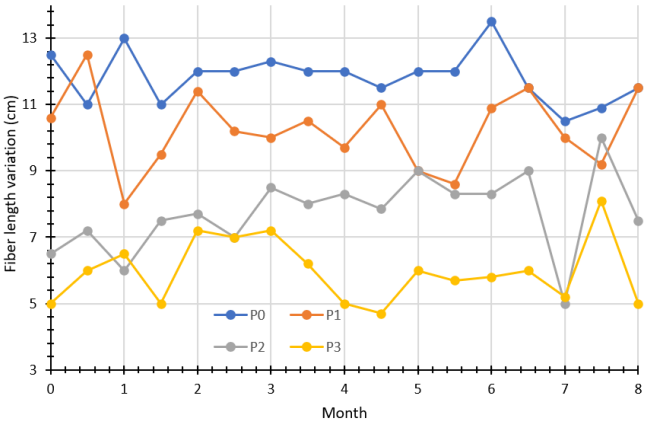

Fig. 5. Evaluation of panettones' fibre size for 8 months.

In Fig. 6 and Fig. 7, the weight loss and moisture content of panettone were assessed. Moisture content (Fig. 6) decreased during the first month, with an ~8% loss for all formulations. Similarly, weight loss was consistent across all formulations, around 2% in the second month and 5-7% in the eighth month. (Fig. 7). The formulation P3 presented higher water absorption because the higher content of okara flour. This phenomena could be related to the hydration of okara proteins, that could represent the third part of okara weight [23]. Furthermore, the formation of a gluten network promotes water retention, distributes volatile compounds, and allows the diffusion of CO_2 [15], [25]. Consequently, okara flour induces compaction in the final panettones (Fig. 3a) and increases water retention. Additionally, weight loss is a consequence of moisture evaporation. Panettone is considered a moist cake, and its moisture content helps conserve freshness until consumption [2]. However, moisture migration between the inner crumb and the external crust is inevitable. In our case, all formulations exhibited the same phenomenon, but it is concluded that okara flour increases water retention.

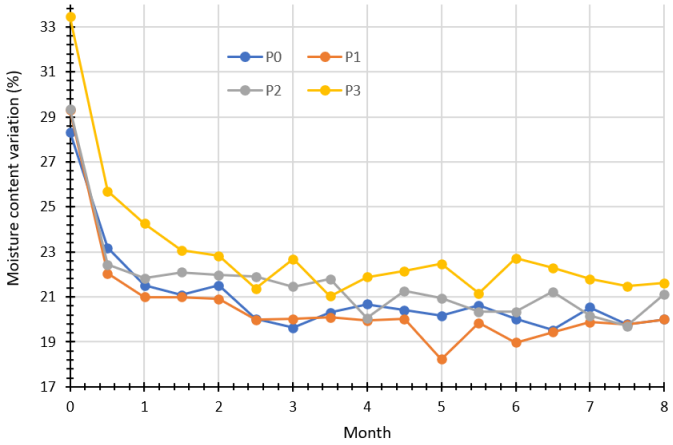

Fig. 6. Evaluation of moisture content of panettones for 8 months.

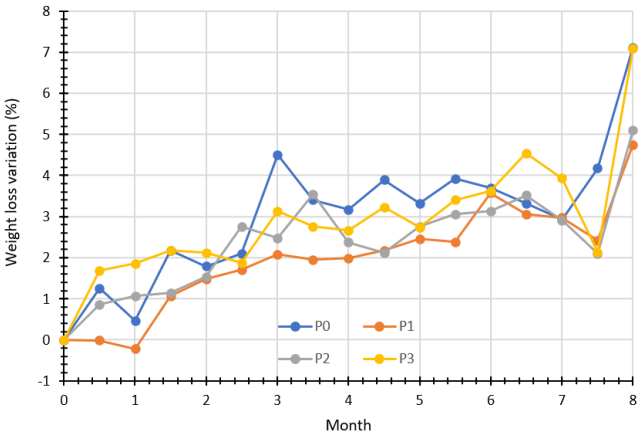

Fig. 7. Evaluation of weight loss (%) of panettones for 8 months.

In Fig. 8, total acidity was evaluated. This parameter is crucial because typical dough has a pH range of 5-6.5 (slightly acidic) due to the presence of organic acids produced by the fermentation process. Lactic acid was monitored because its presence is known to be conducive to the production of desired aromas or aroma precursors [3]. In all cases, the total acidity increased until the fourth month, after which it decreased. By the end of the test, P1 and P0 presented similar values of total acidity (0.44%), slightly higher than at the beginning. However, for P2 and P3, the values were 0.53% and 0.58%, respectively.

Furthermore, water activity (Fig. 9) was evaluated because this parameter indicates the type of water interaction with other components of the flour. During the evaluation period, all samples showed a reduction in AW, but the values of P1 and control P0 appeared similar, while the values of P3 were higher. As mentioned earlier, this behavior is linked to the water retention produced by okara proteins. Additionally, the presence of water is a critical parameter for ensuring the durability of panettone. Higher AW values could indicate the presence of free water, potentially diminishing the antifungal effects of preservatives in panettones. [27], [28].

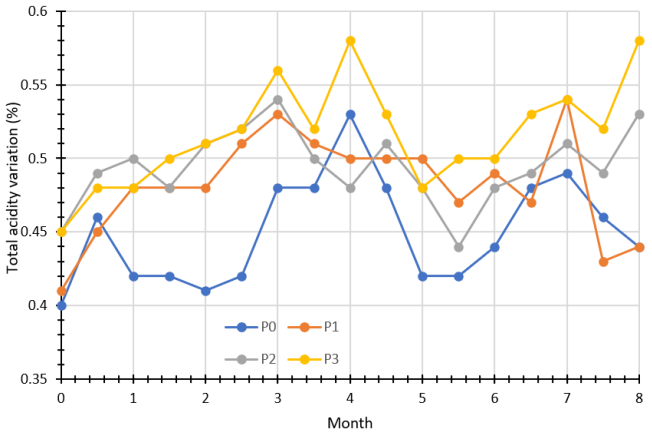

Fig. 8. Evaluation of total acidity of panettones for 8 months.

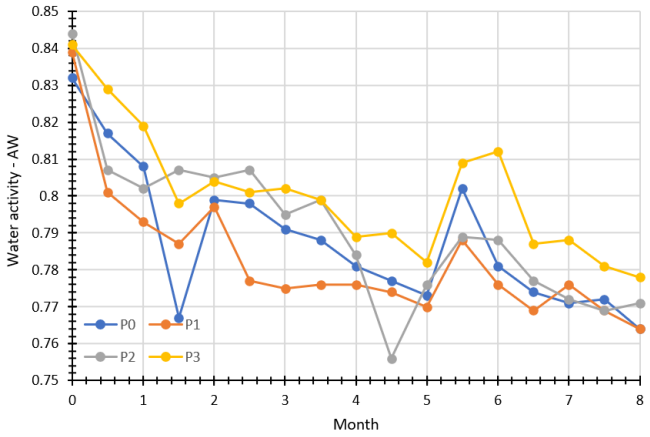

Fig. 9. a) Evaluation of water activity (AW) of panettones for 8 months.

Finally, a survey was conducted to gather perceptions of panettones with varying okara flour content. The data is presented in Table 3. As observed, the P0 control exhibits minimal changes in sensory characteristics. Formulation P1 shows the most similarities to P0. In both cases, even after 8 months, the citrus aroma is preserved, although some dryness is noticeable while chewing. In contrast, formulations P2 and P3 develop strong aromas from fermentation by the eighth month. Notably, the P3 formulation exhibits inconspicuous visual characteristics, including a slightly spongy crumb, smaller size, and dryness during chewing. This aligns with the water retention characteristic of okara flour. Excessive water can lead to yeast proliferation, subsequent fermentation of starches and organic acids, contributing to acidic flavors and aromas not typical of panettone.

This study aims to assess the use of okara flour as a substitute for wheat flour. Okara flour possesses intriguing properties, including higher protein content and water retention capacity. Moreover, as a by-product of the soymilk process, okara flour offers a sustainable alternative, potentially reducing reliance on wheat flour. Existing literature explores various applications of okara flour. Ostermann et al. utilized okara flour in gluten-free cookies for individuals with celiac disease [15]. Ref. [29] incorporated okara flour into bread, highlighting increased dietary fiber and protein content at a 7.5% substitution level with wheat flour. Ref. [30] replaced meat with okara flour, noting structural benefits in burgers, cholesterol reduction, enhanced juiciness, and no adverse effects on sensory properties. In our case, formulation P1 demonstrated higher similarities to the P0 control, suggesting that using okara flour at a 4.2% mass or 5% substitution of wheat flour preserves the main properties of panettones. However, higher okara content may lead to panettone deterioration due to fermentation, emitting undesirable smells and acidic tastes. While okara flour proves suitable due to its protein content, the water retention aspect could impact shelf-life and sensory properties. Hence, further research is essential to explore novel alternatives to wheat flour, bearing in mind

that total substitution is not a universal solution. Different substitution rates may be necessary for different products.

Table 3. Perception of sensorial properties of panettones after the survey.

Parameter	Beginning	4th month	8th month
External color (crust)	- P0: dark brown color with a uniform gold hue. - P1: dark brown color with a uniform gold hue. - P2: dark brown color with a uniform gold hue. - P3: dark brown color with a uniform gold hue.	- P0: dark brown color with a uniform gold hue. - P1: dark brown color with a uniform gold hue. - P2: dark brown color with a uniform gold hue. - P3: dark brown color with a uniform gold hue.	- P0: light brown color with a uniform gold hue. - P1: dark brown color with a uniform gold hue. - P2: dark brown color with a uniform gold hue. - P3: dark brown color with a uniform gold hue.
Internal color (crumb)	- P0: Egg yolk yellow color. - P1: Egg yolk yellow color. - P2: Egg yolk yellow color. - P3: Egg yolk yellow color.	- P0: Egg yolk yellow color - P1: Egg yolk yellow color. - P2: Egg yolk yellow color. - P3: Egg yolk yellow color.	- P0: Egg yolk yellow color - P1: Egg yolk yellow color. - P2: Slightly dark yellow. - P3: Egg yolk yellow color.
Smell	- P0: Characteristic of panettone, soft pleasant citrus aroma. - P1: Characteristic of panettone, soft pleasant citrus aroma. - P2: Characteristic of panettone, soft pleasant citrus aroma. - P3: Characteristic of panettone, soft pleasant citrus aroma.	- P0: Characteristic of panettone, soft pleasant citrus aroma. - P1: Characteristic of panettone, soft pleasant citrus aroma. - P2: Characteristic of panettone, soft pleasant citrus aroma. - P3: Characteristic of panettone, soft pleasant citrus aroma.	- P0: Characteristic of panettone, soft pleasant citrus aroma. - P1: Characteristic of panettone, soft pleasant citrus aroma. - P2: Strong, penetrating smell of ferment. - P3: Non-characteristic panettone smell, strong citrus aroma.
Taste	- P0: Pleasant sweet, characteristic of panettone. - P1: Pleasant sweet, characteristic of panettone. - P2: Pleasant sweet, characteristic of panettone. - P3: Pleasant sweet, characteristic of panettone. -	- P0: Pleasant sweet, characteristic of panettone. - P1: Pleasant sweet, characteristic of panettone. - P2: Little sweet, pleasant crumb, characteristic of panettone. Slightly dry when chewing. - P3: Little sweet, pleasant, characteristic of panettone.	- P0: Pleasant sweet, characteristic of panettone. - P1: Little sweet, pleasant, characteristic of panettone. Dry when chewing. - P2: Sour, bitter flavor, not characteristic of panettone. Dry when chewing. - P3: Little sweet, not pleasant, not characteristic of panettone. An acidic taste is perceived.
Texture	- P0: Soft, sharp to the cut, spongy to external pressure, soft crumb, moist to the touch. - P1: Soft, sharp to the cut, spongy to external pressure, soft crumb, moist to the touch. - P2: Soft, sharp to the cut, spongy to external pressure, soft crumb, moist to the touch. - P3: Soft, little fiber when cut, spongy to external pressure, soft crumb, moist to the touch.	- P0: Soft, sharp to the cut, spongy to external pressure, soft crumb, moist to the touch. - P1: Soft, short fiber, spongy to external pressure, soft crumb, slightly dry to the touch. - P2: Soft, little fiber when cut, little spongy to external pressure, little moisture to the touch. - P3: Porous crumb, short fiber, not very spongy to external pressure, hard crumb, dryness when chewing.	- P0: Soft, short fiber, little spongy to external pressure, dough dry to the touch. Sensation of dryness when chewing. Wrinkled bark. - P1: Soft, short fiber, slightly spongy to external pressure, soft crumb, dry to the touch. Bark with a wrinkled appearance. - P2: Compact mass. Little fiber, slightly soft crumb, moist to the touch. The crumb disintegrates when chewing. Keke appearance. - P3: Porous crumb, short fiber, not very spongy to external pressure, hard crumb, dry when chewing and to the touch. Appearance: wrinkled crust, shrunken panettone.

IV. Conclusions

In this study, panettone was prepared by incorporating okara flour as a partial substitute for wheat flour in three formulations (P1, P2, and P3). Formulation P1, with an Okara flour/wheat ratio of 0.05/1, demonstrated superior performance in terms of ease of dough molding, closely resembling the characteristics of the finished product to the P0 standard (without substitution). The increase in acidity observed with the use of okara flour is attributed to greater fermentation activity resulting from the bioavailability of short-chain carbohydrates. This characteristic presents the potential for producing panettone with lower doses of yeast. As the substitution of wheat flour increased, so did water absorption, given that okara flour functions as a moisture-retaining fiber. However, P2 and P3 panettones exhibited undesirable sensory characteristics after eight months, including an acidic flavor and fermentation smell, likely associated with the higher moisture content. For formulations P2 and P3, it is recommended to add gluten as a dough-strengthening agent to enhance the formation of the gluten network. Additionally, considering a lower yeast dose for fermentation and controlling acidity is suggested. For future studies, the authors recommend evaluating different wheat flour substitution rates for various bakery products.

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