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Development and characterization of roll bread with partial replacement of wheat flour by malt bagasse[1]

Desenvolvimento e caracterização de pão tipo francês com substituição parcial da farinha de trigo por bagaço de malte

Desarrollo y caracterización del pan tipo francés con sustitución parcial de harina de trigo por bagasse de malta

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Abstract

The objective of this work was to develop roll bread with partial replacement of wheat flour by malt bagasse (MB) and to evaluate the changes in their physical-chemical characteristics and microbiological parameters. Four formulations of breads were elaborated: one control without MB, and 3 formulations with partial replacement of wheat flour varying the MB amount in 5, 10 and 15%. MB reduced the carbohydrate content of the dough; consequently, the calories of the bread rolls were also reduced. On the other hand, moisture, minerals and lipids contents were increased. The type of protein changed with the substitution of MB in the dough, but the amount of proteins was not altered. The lowest hardness values were observed in breads with 5% and 10% MB, being related to higher values of acidity in the dough. The increase in the mineral content of the dough was related to the darkening of the bread. All bread formulations showed microbiological quality within the limits established by Brazilian legislation for coliforms at 45 °C, *Bacillus cereus* and *Salmonella sp*. The formulation with 10% MB resulted in bread rolls with higher nutritional value, in terms of minerals, lower hardness and with a darker aspect all around when compared to the control bread, giving it an aspect of wholegrain bread.

Keywords: Beer by-product; Coloring; Functionality; Minerals.

Resumo

Objetivou-se desenvolver pão tipo francês com substituição parcial da farinha de trigo por bagaço de malte (BM) e avaliar as alterações nas suas características físico-química e nos parâmetros microbiológicos. Foram elaboradas 4 formulações de pães, sendo: um controle sem BM, e 3 formulações com substituição parcial da farinha de trigo variando o BM em 5, 10 e 15%. O BM reduziu o conteúdo de carboidratos da massa, e consequentemente as calorias dos pães. Por outro lado, aumentou os teores de umidade, minerais e lipídios. O tipo

de proteína mudou com a substituição do BM na massa, porém a porcentagem de proteína não foi alterada. Os menores valores de dureza foram observados em pães com 5% e 10% de BM, sendo relacionados com maiores valores de acidez na massa. O aumento do conteúdo de minerais da massa foi relacionado ao escurecimento do pão. Todas as formulações de pães apresentaram qualidade microbiológica dentro dos limites estabelecidos pela legislação brasileira para coliformes a 45 °C, *Bacillus cereus* e *Salmonella sp*. A formulação com 10% do BM resultou em pães com maior valor nutricional, em termos de minerais, menor dureza e com casca e miolo mais escuros quando comparado ao pão controle, dando um aspecto de pão integral.
Palavras-chave: Subproduto de cerveja; Coloração; Funcionalidade; Minerais.

Resumen

El objetivo fue desarrollar pan tipo francés con sustitución parcial de harina de trigo por bagazo de malta (BM) y evaluar cambios en sus características físico-químicas y parámetros microbiológicos. Se prepararon cuatro formulaciones de pan, siendo: un control sin BM, y 3 formulaciones con reposición parcial de harina de trigo, variando la BM en 5, 10 y 15%. BM redujo el contenido de carbohidratos de la masa y, en consecuencia, las calorías del pan. Por otro lado, aumentaron los contenidos de humedad, minerales y lípidos. El tipo de proteína cambió con el reemplazo de BM en la masa, sin embargo, el porcentaje de proteínas no cambió. Los valores más bajos de dureza se observaron en panes con 5% y 10% de BM, relacionándose con valores más altos de acidez en la masa. El aumento del contenido mineral de la masa estuvo relacionado con el dorado del pan. Todas las formulaciones de pan mostraron calidad microbiológica dentro de los límites establecidos por la legislación brasileña para coliformes a 45 ° C, *Bacillus cereus* y *Salmonella sp*. La formulación con 10% de BM dio como resultado panes con mayor valor nutricional, en términos de minerales, menor dureza y con una corteza y piel más oscura en comparación con el pan control, dando un aspecto de pan integral.
Palabras clave: Subproducto de cerveza; Colorante; Funcionalidad; Minerales.

1. Introduction

Roll bread is one of the best-selling items in Brazilian bakeries (Cavalcante & dos Santos, 2019). This type of bread is characterized by a crispy, golden brown crust, spongy and soft crumb of cream-white coloration, and typical flavor (Torrezan et al., 2017). Roll bread is essentially produced with wheat flour, salt, yeast, water, fat and bread improver (Alcântara et al., 2020). Currently, there is a great tendency for the development of bakery products with partial or total replacement of wheat flour by other flours, usually gluten-free and rich in nutrients (Marchini et al., 2020). Despite the socioeconomic profits associated with the production and sale of roll bread (Cavalcante & dos Santos, 2019), research on the partial or total replacement of wheat flour by other flours is scarce for this type of bread (Alcântara et al., 2020).

On the other hand, Brazil is one of the largest producers of beer in the world, consequently generating a huge amount of by-product, in which malt bagasse is the most representative, reaching 85% of these by-products (Jacometti et al., 2015). Malt bagasse is a solid material resulting from the filtration of wort before boiling in the beer production process. It consists mainly of leftover malt husks and pulp, grains, such as rice, corn and wheat, as well as some additives (Santos, Silveira, Santos, Quatrin, & Rosa, 2017).

Malt bagasse is rich in fiber, protein and lipids (Kuiavski, Bezerra, Teixeira, & Rigo, 2020). The most common destination of this by-product is for animal feed (Jacometti et al., 2015). Its use by the food industry represents an alternative for the development of functional foods and nutrient recovery, in addition to mitigating environmental impacts associated with its uncontrolled disposal, providing economic benefits to the brewing industry (Kuiavski et al., 2020).

One of the options found is the partial replacement of wheat flour by malt bagasse flour in the production of baking products, such as bread and cake (Kuiavski et al., 2020; Santos et al., 2017). The high value of fibers, protein residue and sugars, makes the malt bagasse an excellent ingredient for food enrichment, especially in relation to dietary fibers, which provide benefits to consumer health, playing an important role in preventing constipation, high cholesterol and ulcerative colitis (Stojceska, 2019).

In addition, malt bagasse can be used to improve the color and texture of the breads, presenting a high index of acceptability, associated with sensory quality. However, the optimal proportion for the substitution depends on the type of flour in the formulation and the desired quality (Kuiavski et al., 2020). The substitution can affect physical properties such as volume, texture, water absorption, mass development time and mass stability (Wirkijowska et al., 2020).

In view of this, researchers have sought to study the ideal condition for this partial substitution of wheat flour by other types of flour from the by-products of the food and beverage industries, for the enrichment of bakery products (Aparecida et al., 2019; Santos et al., 2017; Wirkijowska et al., 2020). To this date, scientific papers about the application of malt bagasse flour in bakery products and its impacts on the final quality of roll bread are scarce.

In this context, the objective of this work was to evaluate the effect on the physical-chemical (proximal composition, pH, acidity, color and texture) and microbiological (coliforms, Bacillus cereus and Salmonella sp.) properties after the partial replacement of wheat flour by malt bagasse in roll breads.

2. Methodology

The present study was performed through a quantitative methodology of a laboratory research (Pereira, Shitsuka, Parreira, & Shitsuka, 2018), and the experimental data were analyzed by statistical softwares and their results expressed in table and graphs. These are discussed through comparative analyses of data available in the literature.

2.1 Obtaining malt bagasse

Malt bagasse (MB) was obtained from bock-type beer processing at the Laboratory of Research and Development of Fermented and Distilled Beverages of the Center for Human, Social and Agrarian Sciences of the Federal University of Paraiba (CCHSA/UFPB). The wet MB, without residues of hops, adjuncts or additives was crushed in multiprocessor (Oster, MPR870 680 W) for 3 minutes before use.

2.2 Production of roll breads

For the production of roll breads, four formulations were elaborated, one being a control formulation, with 100% wheat flour, and 3 other formulations with partial replacement of wheat flour for 5, 10 and 15% of MB (Table 1).

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Table 1 - Formulation of bread rolls with partial replacement of wheat flour by malt bagasse.

Ingredients	Control	F1	F2	F3
Wheat flour (%)	100.00	95.00	90.00	85.00
Malt bagasse (%)	-	5.00	10.00	15.00
Yeast (%)	1.50	1.50	1.50	1.50
Water (%)	50.00	52.00	55.00	60.00
Sugar (%)	1.00	1.00	1.00	1.00
Salt (%)	1.80	1.80	1.80	1.80
Bread improver (%)	1.00	1.00	1.00	1.00
Vegetable fat (%)	1.00	1.00	1.00	1.00

Source: Authors.

The ingredients were mixed in domestic mixer. Then, the masses were divided into 27 g portions, modeled, rested for 2 h in a fermentation chamber at 30 °C and 80% RH. The bread rolls were baked in a turbo oven at 190 °C for 16 min, with steam in the first moments of bakingThe bread rolls removed from the oven were allowed to cool at room temperature (25 °C) for 1 h before being packed in polypropylene bags. Then they were stored at room temperature until the time of analysis.

2.3 Physicochemical characterization

2.3.1 Proximal composition

The analyses of proximal composition of the malt bagasse and bread formulations were performed in triplicate, by the official methods of the AACC - American Association of Cereal Chemists (2000): moisture (method 44-10) by drying in an oven without air circulation at 105 °C; proteins by the Kjeldahl method (method 46-13) using correction factor 5.83 for farinaceous; ash (method 08-01) by incineration in muffle furnace at 550 °C. The lipids extracted in Soxhlet with petroleum ether followed AOAC - Association of Official Analytical Chemistry (2005) method 920.39C. The total carbohydrates were quantified by difference [100 – (moisture + proteins + ash + lipids)]. The results were expressed in g/100g on a wet basis. The total energy value was expressed in kcal/100 g, estimated from Atwater conversion factors: kcal = (4 x g protein) + (4 x g carbohydrates) + (9 x g lipids) (Merril & Watt, 1973).

2.3.2 pH e acidity

The pH and titratable acidity of the bread rolls were determined in triplicate by the methodology described in the manual of the Adolfo Lutz Institute (IAL, 2008). The pH of the bread rolls was quantified at 25 °C using digital potentiometer (Quimis), previously calibrated with pH buffer solutions at pH 7.0 and 4.0. The acidity of the bread was determined by titration with NaOH mol/L solution until consumed by 10 g of bread (Robert et al., 2006).

2.3.3 Colorimetry

The crust and crumb of the bread rolls were analyzed in triplicate, in spectrophotometer (GretagMacbeth Color-Eye 2180), using the CIELAB system with D65 illuminator and observation angle of 10° and with a vision area of one square inch. Readings were made to the color parameters, where L^* is the luminosity, a^* varies from green (-) to red (+) and b^* varies from blue (-) to yellow (+). The total color difference (ΔE) was calculated according to Equation 1, between the control formulation and the formulations with partial replacement of the MB.

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad \text{(Equation 1)}$$

2.3.4 Compression tests

The texture analysis of the bread rolls was performed in triplicate in the four formulations using the TPA (Texture Profile Analysis) evaluation method in texturometer TA.XT Express Enhanced of 10 kg, measuring hardness (g). The P/1.5l probe with aluminum cylinder with 2 mm diameter was used to apply a compression of 20 mm to the samples, considering the ratio of sample height measurements before and after compression (Chen et al., 2019).

2.4 Microbiological characterization

The breads were analyzed for the microbial load of coliforms at 45 ºC/g, *Bacillus cereus*/g and *Salmonella* sp/25 g, in compliance with the requirements of RDC 331, of

December 23, 2019, ANVISA (Brazil, 2019). The analyses followed the methodology of the American Public Health Association - APHA (2001).

2.5 Statistical analysis

The data were submitted to analyses of variance and the comparison between means performed by the Tukey test at 5% probability. The data were represented by the mean and standard deviation. Pearson's correlation analysis was applied to assist in understanding the direct and indirect effects of malt bagasse on the quality of breads. All analyses were performed with the aid of software R (Core Team, 2018).

3. Results and discussion

3.1 Characterization of malt bagasse

The MB was characterized as to proximal composition and microbiological quality before being used in breads. The proximal composition of MB showed the possible feasibility of using this by-product of beer production as an ingredient in bakery products due to high nutritional value. It presented protein (23.53±0.51 g/100 g), carbohydrates (37.67±0.24 g/100 g), lipids (9.42±0.40 g/100 g), ash (5.42±0.38 g/100 g), and moisture (23.96±0.08 g/100 g).

The high protein content in MB ensures that, even with the decrease in the concentration of wheat protein, the viscoelastic structure of the gluten network will be established by the combination of protein with water, through mixing (Kuiavski et al., 2020). The high carbohydrate content may be associated with the content of dietary fiber (Saraiva, Agustinho, Vital, Staub, & Pintro, 2019), providing a greater functionality of this by-product as a functional ingredient, combined with the content of minerals.

MB is a very unstable by-product and susceptible to rapid microbial deterioration, mainly due to high water content (Costa et al., 2020). However, the MB used in this study presented microbiological quality for coliforms at 45 ºC, *Bacillus cereus* and *Salmonella* sp., within the limits established by Brazilian legislation (Brazil, 2019). Together, these results ratify the great potential of MB as a source of nutrients for the food industry, especially bakery, for products such salty or sweet dough bread, as well as in cakes and cookies.

3.2 Physicochemical characterization of roll bread formulations

The visual aspect of the bread rolls showed small differences in the volume and crust after baking. The bread rolls with MB presented a smaller volume and a rougher crust. They had irregular shape and surface irregularities; an aspect already observed at the end of the fermentation period (Fig. 1).

Figure 1 - Roll bread without malt bagasse (A) and with malt bagasse in the concentrations of 5% (B), 10% (C) and 15% (D) at the end of the fermentation period.

(A) (B) (C) (D)

Control (0% MB) F1 (5% MB) F2 (10% MB) F3 (15% MB)

Source: Authors.

These changes were proportional to the increase in MB concentration in the bread dough. In addition, it was observed that the addition of MB increased the time required for the development and growth of the dough. This could be associated with the change of the type of protein, from wheat to malt.

Table 2 shows the mean values of the parameters of proximal composition, pH and acidity of the bread rolls, as well as the energy value of the control formulation and other formulations containing the MB.

Table 2 – Results of proximal composition, pH and acidity for roll breads (control and with MB substitutions).

Parameters	**Control (0% MB)**	**F1 (5% MB)**	**F2 (10% MB)**	**F3 (15% MB)**
pH	5.73±0.35 [a]	5.67±0.11 [a]	5.76±0.10 [a]	5.69±0.18 [a]
Acidity (0.1 N/10 g)	4.43±0.01 [a]	3.78±0.40 [b]	3.76±0.40 [b]	2.72±0.37 [b]
Moisture (g/100g)	24.84±0.95 [b]	28.70±0.74 [a]	29.32±0.63 [a]	29.20±1.03 [a]
Ash (g/100g)	1.96±0.06 [b]	2.05±0.06 [b]	2.51±0.41 [ab]	2.98±0.08 [a]
Protein (g/100g)	9.23±0.38 [a]	8.70±0.53 [a]	9.02±0.56 [a]	9.35±0.70 [a]
Lipid (g/100g)	1.60±0.08 [b]	1.97±0.04 [a]	1.96±0.06 [a]	1.93±0.05 [a]
Total carbohydrates (g/100g)	62.37±1.07 [a]	59.48±0.72 [b]	57.19±0.57 [c]	55.87±0.78 [c]
Energetic value (kcal/100g)	300.80	290.45	282.48	278.25

* Values are mean ± SD (n=3). **Different superscript letters in the same row indicate significant differences (p<0.05) by Tukey test. Source: Authors.

In turn, independent on the concentration, the addition of MB reduced the acidity and increased the moisture and lipid content of the bread dough. The reduction of acidity may be associated with the changes observed in the process of mass growth. The higher moisture content of the dough indicates that MB addition resulted in a dough with greater water absorption capacity. The importance of the water content for the proper development of the mass is already well known. In general, water optimizes the development of the gluten network, affects the consistency of the dough, and may favor the growth and quality of bread (Parenti et al., 2019).

The increase in the lipid content of the mass was due to the higher lipid content of MB (9.42 g/100 g) in relation to the lipid content normally observed in wheat flour (0.23 g/100 g) (Guilherme & Jokl, 2005). Although wheat flour contains free lipids that aid in the development of the dough, different sources of lipids are added during the production of bread to accelerate the development of the dough, facilitate the incorporation of air during mixing, stabilize gas cells during baking, and improve the appearance, texture and flavor of bread (Pareyt, Finnie, Putseys, & Delcour, 2011).

With the increase in MB percentage, there was an increase in mineral content and a reduction in carbohydrates of the dough. In this sense, the higher ash content in MB (5.42 g/100 g) may represent an increase in the mineral content for the dough of the breads, since

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the wheat flour has a much lower ash content (0.8 g/100 g) (Guilherme & Jokl, 2005). The reduction in the carbohydrate content of the dough can be attributed to the lower carbohydrate content of MB (37.67 g/100 g), compared to wheat flour that has a content higher than 85 g/100 g (Guilherme & Jokl, 2005). The high moisture of MB (23.96 g/100 g) can result in dilution of the carbohydrates of the flour in the dough.

With the addition of MB in the formulations, there was a decrease in bread roll calorie content, with the control formulation being the one with the highest value (300.80 kcal/100 g) and the formulation with 15% of MB the lowest value (278.25 kcal /100 g).). The MB made the buns less caloric, possibly associated with their lower carbohydrate content.

3.3 Roll bread crust and crumb colorimetry

In Table 3, the values of the color parameters for the crust and crumb of the control and the other formulations with MB are expressed.

Table 3 - Results of the colorimetry of the crust and crumb of roll breads (control and with MB substitutions).

	Parameters	Control (0% MB)	F1 (5% MB)	F2 (10% MB)	F3 (15% MB)
Crust	*L*	72.93 ± 3.12^{a}	70.90 ± 1.01^{ab}	67.68 ± 1.55^{b}	59.33 ± 0.57^{c}
	*a**	5.91 ± 0.51^{c}	6.45 ± 0.78^{bc}	7.50 ± 0.58^{ab}	8.57 ± 0.30^{a}
	*b**	33.44 ± 1.71^{a}	33.14 ± 1.08^{a}	34.97 ± 0.04^{a}	35.37 ± 0.61^{a}
	ΔE	-	2.12	5.69	13.99
Crumb	*L*	74.67 ± 2.34^{a}	71.84 ± 0.78^{a}	59.50 ± 5.66^{b}	54.48 ± 1.21^{b}
	*a**	-1.58 ± 0.36^{b}	-1.76 ± 0.18^{b}	-1.89 ± 0.11^{b}	-2.97 ± 0.04^{a}
	*b**	14.92 ± 1.27^{c}	16.37 ± 0.63^{c}	18.62 ± 0.47^{b}	22.41 ± 0.34^{a}
	ΔE	-	3.18	15.62	21.58

* Values are mean ± SD (n=3). **Different superscript letters in the same row indicate significant differences ($p<0.05$) by Tukey test. Source: Authors.

The higher proportions of MB resulted in lower *L** and *a** values in the crust and crumb of the bread, indicating a darker coloration, giving an aspect of "whole grain". Similar results were reported for sliced bread, which despite the darker appearance, obtained good acceptance by tasters (Kuiavski et al., 2020).

On the other hand, parameter b^* had no difference between the control and the formulations with MB in the bark, however, there was a change in the crumb with higher values in the formulations F2 and F3.

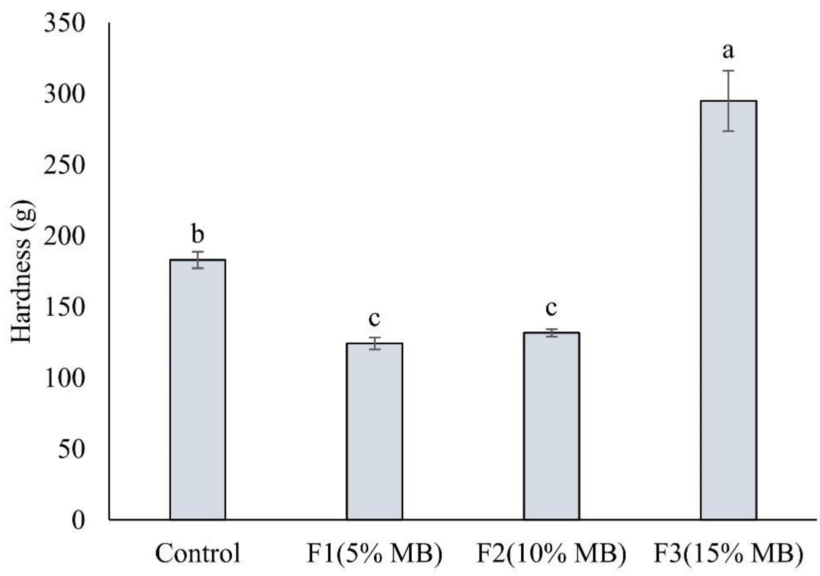
Color differences of the rolls were very evident in the parameter ΔE obtained in relation to the color of control rolls. For this parameter, the higher the MB percentage, the higher the ΔE value, which ranged from 2.12 to 13.99 in the crust and from 3.18 to 21.58 in the crumb, confirming the aspect of "whole grain".

3.4 Compression test

The texture of the bread was analyzed by TPA, being expressed through the hardness parameter, used to investigate the hardening of bread, which refers to the force necessary to deform the sample to a certain extent (Chen et al., 2019). Lower hardness values indicate softer breads, although it also indicates a less crispy crust (Parenti et al., 2019).

The control bread presented higher hardness when comparing with bread rolls containing 5% and 10% MB (Fig. 2). However, the highest hardness value was observed for breads with 15% MB. It is clear that concentrations of MB up to 10% improves the softness of roll bread, while, values equal to 15% MB makes the bread harder, and can impact the perception of crispness felt by consumers, a characteristic of roll bread.

Figure 2 - Hardness of roll breads (control and with MB substitutions).



* Values are mean ± SD (n=3). **Different superscript letters in bars indicate significant differences (p<0.05) by Tukey test. Source: Authors.

3.5 Microbiological characterization

Roll bread with partial replacement of wheat flour by MB presented microbiological quality within the limits established by Resolution RDC 331 of December 23, 2019 for the number of coliforms at 45 ºC, *Bacillus cereus* and *Salmonella* sp. (Table 4).

Table 4 - Microbiological quality of roll breads (control and with MB substitutions).

Formulation	Coliforms (45°C) CFU.g^{-1}		*Salmonella sp.* 25g^{-1}		*Bacillus cereus* CFU.g^{-1}	
	Dough	Bread	Dough	Bread	Dough	Bread
Control (0% MB)	< 1 x 10^1	< 1 x 10^1	Absent	Absent	<10	<10
F1 (5% MB)	< 1 x 10^1	< 1 x 10^1	Absent	Absent	1 x 10^3	<10
F2 (10% MB)	< 1 x 10^1	< 1 x 10^1	Absent	Absent	1 x 10^3	<10
F2 (15% MB)	< 1 x 10^1	< 1 x 10^1	Absent	Absent	1 x 10^3	<10
EV	Max 1 x 10^2		Absent in 25 g		Max 1 x 10^3	

*CFU/g: Colony forming unit per gram. EV: Established Value (RDC 331/2019). Source: Authors.

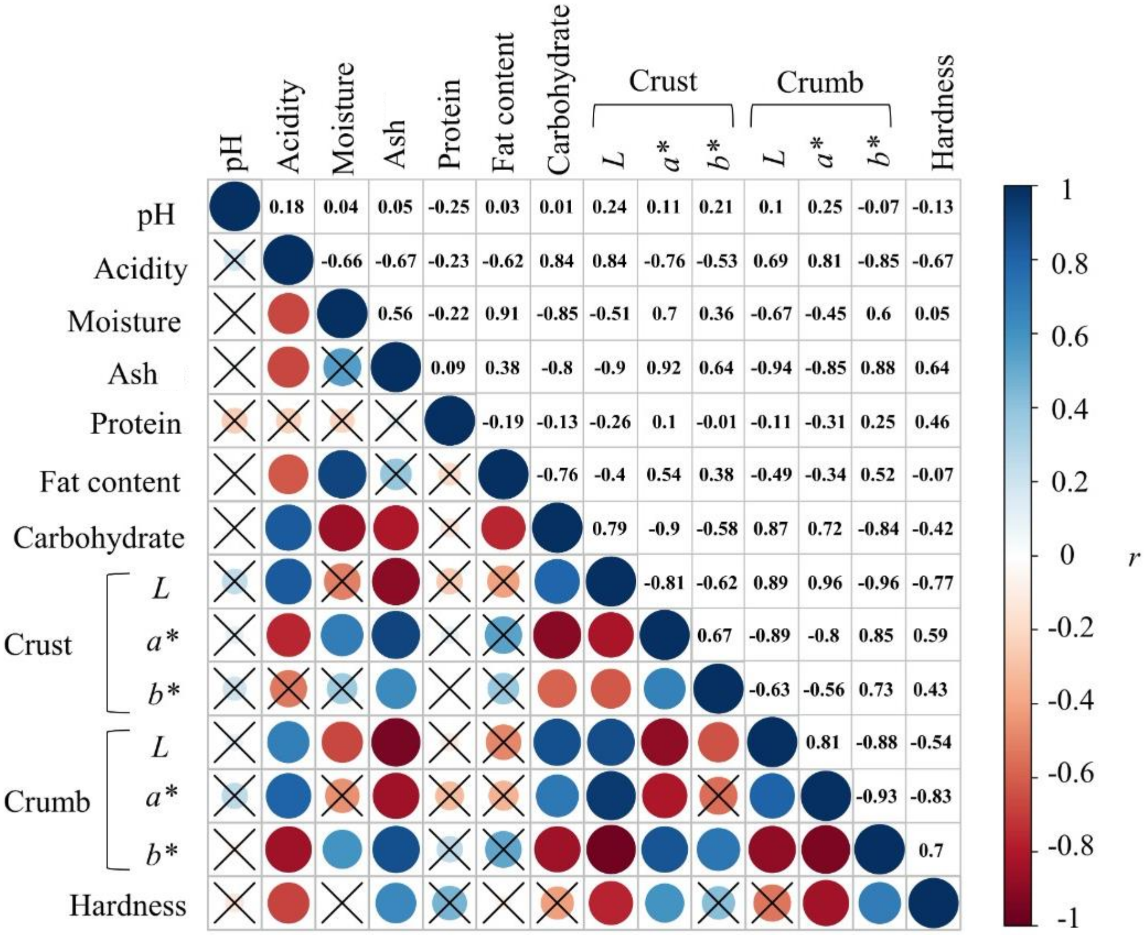
The presence of these microorganisms in breads can provide information on the probable occurrence of contamination of fecal origin or on the potential deterioration of food, being a risk to the health of consumers (Fazio, Campana & Geromel, 2020). The result indicates that MB is a safe raw material from the microbiological point of view for the production of breads for human consumption.

3.6 Correlation analysis between the evaluated parameters

According to correlation analysis (Fig. 3), the pH had no effect on other quality attributes. This result can be attributed to the absence of significant variance of this variable. The hardness of the bread showed moderate negative correlation (r = -0.67) with acidity and moderate positive correlation (r = 0.64) with the mineral content of the rolls. The lowest values of hardness of the bread were related to higher values of acidity. The decrease in the hardness of the bread was favored by the higher content of organic acids in the dough. Su et al. (2019) reports that the increase in the content of organic acids in the dough also favors greater volume to bread rolls, and these effects are related to the increase in yeast activity and positive regulation of proteolysis and amylolysis reactions. However, this increase in dough volume was not observed in bread rolls with addition of MB.

Figure 3 expresses Pearson's correlation results between the different physical-chemical and physical characteristics of roll bread.

Figure 3 - Pearson's correlation results.



*'X' indicates a non-significant correlation using the t test (p ≥ 0.05). Source: Authors.

Regarding the color of the crust and crumb of the bread, significant correlations were observed with the content of carbohydrates, minerals, acidity and moisture of the rolls. It is important to highlight that the relationships between mineral content and *CIELab* color parameters are exactly opposites of the relationships of carbohydrate content and acidity with the same color parameters. In other words, while the higher carbohydrate content and/or higher acidity of the dough favored lighter and brighter bread rolls, the higher mineral content in the dough caused darker color of the bread. These results are supported by recent research, where high mineral contents in wheat flour resulted in bread with dark color (Ariyantoro, Amanto, & Kristin, 2020).

4. Final Considerations

The physical-chemical and microbiological properties of roll bread with partial replacement of wheat flour by malt bagasse showed that this substitution favors roll bread in terms of reducing calories and increasing the content of minerals. The substitution made possible developing a more nutritious bread with visual aspect of a wholegrain bread, with a darker shade and a softer texture without losing the crispy characteristic of the bread. Therefore, it is concluded that the objective of the research was achieved completely.

The formulation with 10% of malt bagasse is the best in terms of nutritional value, for the high ash content, lower hardness and with darker crust and crumb when compared to the control bread, giving an aspect of wholegrain bread. The increase in the ash content of the dough with the increase in the concentration of malt bagasse was the main resulting factor for breads with darker coloration. All bread formulations showed microbiological quality within the limits established by Brazilian legislation for coliforms at 45 °C, *Bacillus cereus* and *Salmonella* sp.

As recommendation for future work, a sensory analysis of the developed breads and the use of malt bagasse as an ingredient in other types of bakery products, such as cake and biscuits are advised.

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