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Effect of black sesame (*Sesamum indicum*) and flaxseed flour (*Linum usitatissimum*) on bread quality using surface response methodology

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Abstract

The study aimed to evaluate the effect of black sesame and flaxseed flour addition in different proportions on bread quality. The bread formulations were developed through the Rotational Central Composite Design (RCCD). The independent variables were: black sesame and flaxseed flour which incorporated in amounts from 25.85% to 54.15%. The effect of the ingredients on bread quality was evaluated through physical parameters (specific volume, expansion index and volume produced). Statistical analysis was performed using response surface methodology and the two formulations with better technical performance were submitted to proximate composition (moisture, protein, fat, ash, carbohydrates and calcium), crumb structure, scanning electron microscopy (SEM) and sensorial analysis. It has been found that black sesame promotes greater reductions in the mass expansion capacity than flax meal. There was an improvement in the nutritional value of the loaves by increasing the protein content, ashes, fat and calcium, in addition to the reduction of carbohydrates. The breads developed had good acceptability in all evaluated attributes. In this way, the inclusion of black sesame and flaxseed flour as ingredients in bread formulations promotes products with technological and sensorial quality.

1. INTODUCTION

A major nutritional limitation of the wheat bread, an inexpensive staple food is its low nutritional quality. Keeping aside the animal protein, with protein contents double than other cereal crops, oil crop and legumes have been emerged as an economical and environmentally sustainable protein source having potential to improve the nutritional value of breads (Xiao et al., 2015). An increasing demand for nutritionally enriched breads with whole grains and different seeds has emerged because of their positive effects on blood cholesterol and their dietary fiber content (Fukumitsu et al., 2010).

Sesame (*Sesamum indicum L.*) is an important edible oil crop for humans and has a long history of cultivation (Ji et al. 2019), is one of the most widely consumed food either in fresh or processed from. It is also an important component of many processed food products due to its excellent flavor, attractive color and high levels of many macro- and micro-nutrients (Yoshida and Takagi, 2015).

Antioxidants substances like sesamol, sesamolin and sesaminol can be found in *S. indicum L.* (Wichitsranoi et al. 2011). Kiran and Asad (2008) showed that seeds and oil of *S. indicum* L. have considerable healing activity when administered orally or topically. The black sesame pigment obtained by removal of the fat content using dichloromethane and hydrolytic protocol displays considerable antioxidant and antinitrosant effects (Panzella et al. 2012). It has been shown that the black sesame cream presents considerable antioxidant potential (Silva et al. 2011). The sesame cortex also displays conspicuous antioxidant capacity (Shahidi et al. 2006).

Flaxseed (*Linum usitatissimum*, L.) belonging to Linaceae family, is known to be an attractive material in production of functional food. It is also considered as a source of soluble and insoluble fiber, lignan and α-linolenic acid (Oomah and Mazza, 1998). Flaxseed, containing high oil level, is low in saturated fat (9 g/100 g) and rich in polyunsaturated fatty acids. α-Linolenic acid (ALA, 18:3, ω-3) constitutes almost 51–55 g/100 g of the total fatty acids of flaxseed oil, introducing flaxseed as a leading plant source of omega-3 fatty acids (Bloedon and Szapary, 2004). Flaxseed has an average of 30% lipids (50%–55% as ALA and 15%–18% as Linolenic acid - LA). Furthermore, a seed contains 28% fibers, 21% protein, and 4% minerals (MAÍRA, et al., 2018). These observations suggest that flaxseed flour could be a promoter of bone health in adults.

Alpaslan and Hayta (2006) suggested that flaxseed flour could be added to a typical snack formulation up to levels of 10% with a reasonable acceptance offering promising nutritious and healthy alternative to consumers.

Thus, the objective of this work was to study the incorporation of black sesame and flaxseed flour in different proportions and to evaluate the physical and nutritional bread quality.

2. MATERIAL AND METHODS

2.1. Materials

Black sesame and flaxseed flour, as well as the other ingredients for the bread production were purchased from the local market of Fortaleza, Brazil.

2.2. Bread preparation

The control bread formulation was comprised of 300.0 g wheat flour, 120.0 g water, 30.0 hydrogenate vegetable fat, 15.0 g refined sugar, 11.0 g dry yeast, 2.0 g salt and the inclusion of black sesame and flaxseed flour was based on Central Composite Rotational Design (CCRD) as presented in Table 1. A second order design matrix used for the evaluation of the process variables effects on some physical properties of dough and bread. The amounts of the independent variables used were based on a preliminary study in relation to the dough expansion capacity. Ingredients were mixed in a Lieme BP06 spiral mixer (Lieme, Brazil) for 7 minutes. The dough formed after mixing was placed in a baking pan and proofed for 90 min at 28 °C and 75% relative humidity (RH). The dough was molded in a dough molder (Paniz, Brazil), so that had a length of 15 cm, a height of 4 cm and a width of 3 cm, weighing approximately 250 g. The dough was placed in lightly greased pans and set for final proofing for another 36 min at 28 °C ± 2 °C and 75% RH. After final proofing, the bread dough was baked at 220 °C for 20 min in electric oven (Electrolux, Brazil). The loaf was removed from the pans and cooled at room temperature (T = 25 ± 2 °C). Bread characteristics were tested two hours after the loaves were removed from the oven.

Table 1. Experimental design matrix with coded and real values.

	Independent Variables	
Formulations	Black Sesame (g/100 g wheat flour) – X_1	Flaxseed Flour (g/100 g wheat flour) – X_2
1	30.0 (-1)	10.0 (-1)
2	30.0 (-1)	15.0 (+1)
3	50.0 (+1)	10.0 (-1)
4	50.0 (+1)	15.0 (+1)
5	25.85 (-1.41)	12.5 (0)
6	54.15 (+1.41)	12.5 (0)
7	40.0 (0)	8.5 (-1,41)
8	40.0 (0)	17.0 (+1,41)
9	40.0 (0)	12.5 (0)
10	40.0 (0)	12.5 (0)
11	40.0 (0)	12.5 (0)

2.3. Physical analysis

2.3.1. Specific Volume

The bread was weighed after cooling and its volume (mL) was determined by the rapeseed displacement method. The specific volume (mL/g) was calculated as loaf volume/bread weight (AACC, 2000).

2.3.2. Expansion Index

A portion of the prepared dough was separated to achieve the expansion index. This portion was molded into spherical shape, specifically for this analysis, to allow measurements of the diameter and height. Expansion Index of the bread was calculated using equation 1:

$$Expansion\ Index = \frac{\frac{(Dp + Hp)}{2}}{\frac{(Dm+Hm)}{2}} \qquad (1)$$

Where: Hp and Dp = Diameter and height of the dough after baking (cm); Hm and Dm = Diameter and height of the molded dough (cm).

2.3.3. Volume produced during Fermentation

Dough portions of approximately 15 g were placed in sterilized beakers of 100mL. Volume produces during fermentation was calculated as the difference between the final volume produced after 90 minutes of fermentation and the initial volume.

2.3.4. CCRD statistical analysis

The CCRD was performed to obtain a second order model to predict the quality of the dough and bread as a result of black sesame and flaxseed flour in the bread formulations. This model can be observed in the following equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X^2 + \beta_{22} X^2 + \beta_{12} X_1 X_2 + \varepsilon \qquad (2)$$

Where y is the predicted response (dough and bread quality variables), β_0 is the global mean, β_1 and β_2 is the linear coefficient, β_{12} is the coefficient of interaction, β_{11} and β_{22} is the quadratic coefficient, ε is the error of the model, and X_1 and X_2 are the coded values of the independent variables, such as, black sesame and flaxseed flour, respectively.

The experimental data were analyzed using Statistica software, version 9.0 (Statsoft, Inc., Tulsa, OK, USA). Analysis of variance (ANOVA) tables was generated, and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The significances of the dependent variables were judged statistically according to the *p*-value, which was set at a 5% significance level. The quality-of-fit of the equation model was expressed by the coefficient of determination (R^2), and its statistical significance was determined using the F-test. For validation of the statistical results, the observed values of bread dough and quality variables were compared with the predicted values obtained by the experimental models. The independent variables optimized were X_1 (Black sesame), X_2 (Flaxseed flour) for dependent response, Y_1 (Specific volume), Y_2 (Expansion index), and Y_3 (Volume produced during fermentation). Besides explaining the behavior of variables with surface plots, the models created in this study were used for modeling purposes, using point prediction post-analysis to optimize the black sesame and flaxseed flour content in the bread, which could be while maintaining high-quality traits. This process includes converting each response variable into a function of three independent variables. The predicted and experimental values were compared and analyzed using a one-way analysis of variance (ANOVA).

The optimized formulations were evaluated for the proximate composition, bread crumb structure porosity and scanning electron microscopy.

2.3.4. Proximate Composition

Bread proximate composition was determined according to the official method as described by AOAC (1990). Oven drying (AOAC method 977.11), Kjeldahl's (Nx6.25) (AOAC method 955.04), Soxhlet (AOAC method 960.39), dry ashing (AOAC method 923.03) were used to analyze moisture, crude protein, crude fat and ash contents, respectively and Total fiber (AOAC method 912.05). Carbohydrate content was calculated by difference.

2.3.5. Bread Crumb Porosity

The bread crumb porosity was realized according to (Martínez, Román & Gómez, 2018) methodology.

The bread was sliced transversely with a slicing machine. The images were captured using a color camera (NIKON P610, Nikon Inc., Japan) with a resolution of 4272 × 2848 pixels. The camera was located at 20 cm overhead on the sample platform to obtain the best image of the samples of bread slices throughout the experiment. The samples were illuminated with two 36 W fluorescent lights (model: T8 G13, Phillips, Brazil) and enclosed in a black cardboard box with a door (Al-Rahbi et al., 2013). The inside wall of the box was concealed with black paper to reduce the reflectance, and the roof was covered with white paper to enhance light scattering and reduce the shadow (Al-Ohali, 2011). The sliced bread samples were positioned on a black background to provide higher contrast between the background and the sample. The camera was connected to a computer, which contained remote shooting software Nikon Wireless Mobile Utility (WMU) (version 1.5.0, Nikon INC, Japan) through which the digital images were acquired.

The digital images were made using ImageJ software (National Institute of Health, Bethesda, MD, USA). The image was split into color channels. The contrast was enhanced and, finally, the image was binarized after applying a grey scale threshold. This was performed with the aim of dividing images into regions of cells and surrounding cell wall material. The analysis was performed on a slice area of 15 × 17 mm. Porosity measurements were run in triplicate.

2.3.6. *Scanning electron microscopy (SEM)*

The dough and bread surface were observed using a Quanta FEG 450 Scanning Electron Microscope (FEI, Hillsboro, OR, USA) at a low energy of 10 kV. The samples were deposited on carbon tapes and coated with gold, using vapor deposition techniques. The surface was scanned using magnification between 5.000 and 40.000×.

2.3.7. *Sensory Analysis*

Sensory evaluation for bread was carried out, under white light, at room temperature, by a panel of 64 untrained tasters from the students and the staff members of the Federal University of Ceará (UFC), who habitually consume bread. Samples were analyzed 24 h after baking. Before analysis, the samples were sliced into equally sized pieces (2 cm thick), coded with three-digit numbers and then randomly served. Breads were evaluated based on acceptance of their appearance (visual aspect), crust colour, smell, taste, texture, and overall acceptability on a nine-point scale where 1, "dislike extremely"; 5, indifferent; 9, "like extremely".

3. RESULTS AND DISCUSSION

Fig. 1 shows the specific volume, expansion index and volume during fermentation results in function with different levels of black sesame and flaxseed flour added in breads.

The specific volume decreases with increasing black sesame and flaxseed flour levels. This decrease was observed by Dhen et al. (2018), and this behavior is often attributed to the dilution of gluten, however, the presence of the fibers and minerals in black sesame and flaxseed flour also can promote discontinuity and porosity to gluten. The generated models, only with the significant coefficients, were: Specific volume = $2.27 - 0.58X_1 + 0.15X_1^2 - 0.50X1 + 0.09X2^2 + 0.27\ X_1X_2$ ($R^2 = 0.9422$); Expansion index = $1.13 - 0.10X_1 - 0.02X_1^2 - 0.13X_2$ ($R^2 = 0.9166$); Volume produced = $17.33 - 2.38X_1 - 3.60X_2$ ($R^2 = 0.8849$).

Han et al. (2018) attributed the friction capacity of ash and fiber particles with the protein chains that result in the thinning and breakage of the protein filaments. Hsieh et al. (2017) studied the whole grain flours incorporation in chinese bread and verified a reduction in the specific volume with the increase of these flours. According to Sivam et al. (2011) the addition of soluble dietary fibers at a low level strengthened the dough structure and consequently improved the bread quality; however, excess amounts of insoluble dietary fibers destroyed the gluten network formation (Ahmed et al. 2013). The same analogy can be made for non-gluten-forming flours. It was found that the area to maximize the specific volume comprised the incorporation amounts of 20 to 35 g of flaxseed flour and up to 25 g of black sesame with specific volumes greater than 2.20 mL/g. The effects

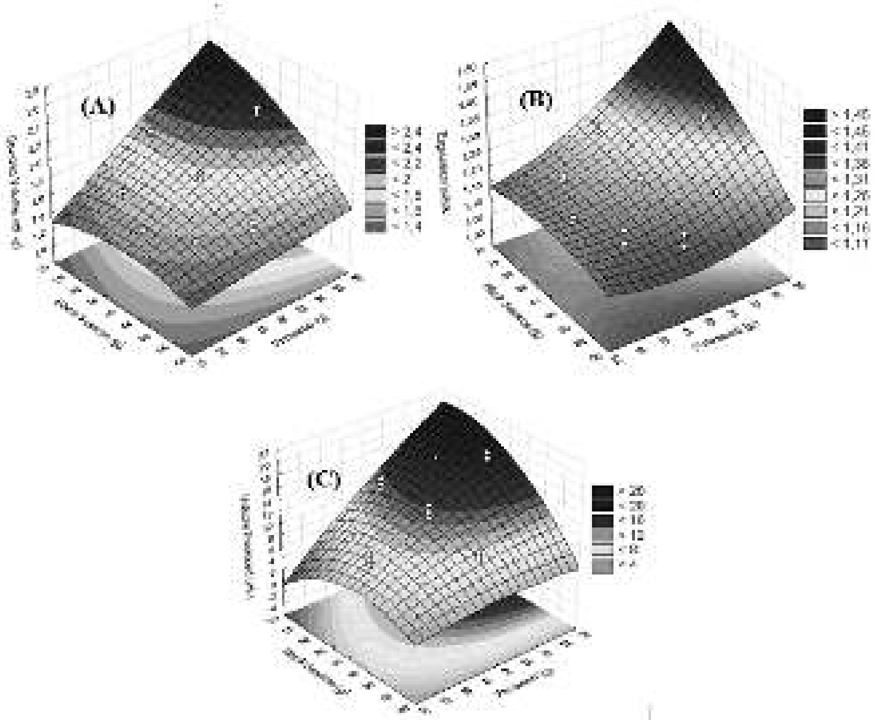

Figure 1. Bread physical properties with different content of black sesame and flaxseed flour. (A) Specific volume; (B) Expansion Index and (C) Volume produced.

analysis showed that the black sesame (-1.044) promoted greater reduction when compared to flaxseed flour (-0.553).

Kajihausa et al. (2014) found values near to 10% of fiber and 4% of ash to black sesame seed flour. Machado et al. (2017) related that black sesame cake has 19.8% of insoluble fibers and 8% of ash. According to Ellleuch et al. (2007) the total fibers present in sesame seeds vary from 19.33% to 42.03%, which may cause the expansion reduction and increased bread and crumb density.

On the other hand, Kaur et al. (2017) obtained results of, approximately, 3.5% ash and 8.0% fiber. Čukelj et al. (2017) reported a significant increase in the content of biscuit fibers incorporated with flax meal. In this way, it can be observed that, by its composition, black sesame may be more harmful to gluten than flaxseed.

Similar behavior occurred to expansion index and volume during fermentation. The highest values were obtained when the addition of both ingredients did not exceed 30%. When the ingredients incorporation to levels above 40 g the bread specific volume was reduced to less than 1.80 mL/g producing therefore a poor bread quality.

During fermentation, yeast cells produce CO2, which is retained within the dough by a complex matrix consisting of a viscoelastic gluten protein-starch network (Jayaram et al. 2013).

The dough volume during the fermentation process may have been affected by the presence of the black sesame and flaxseed flour components, due to the weakening of the gluten network. Most probably the yeasts continued to consume the sugar present in the medium and produced carbon dioxide, however, the dough lost gasification power.

The rheological properties of dough are known to be intrinsically linked to the final quality of the baked product, consequently, the non-gluten-forming flours inclusion alters the dough rheological profile.

From the results obtained with the physical analyzes it was possible to estimate the optimal incorporation values of black sesame and flaxseed flour. Thus, two formulations of breads were developed: A – 25.85% black sesame/13.9% flaxseed flour and B – 31.40% black sesame/15.5% flaxseed flour and were compared with control formulation (without black sesame and flaxseed flour).

Table 2 presents the proximate composition of control and optimized breads added of black sesame and flaxseed flour. The ingredients inclusion did not promote significant changes in the bread moisture content. The standardization of baking time and temperature contributed to this result.

Table 2. Proximate composition of control and optimized breads.

Samples	Control	A	B
Moisture (%)	31.6^{a}±0.3	31.9^{a}±0.2	31.2^{a}±0.1
Protein (%)	7.63^{c}±0.4	9.41^{b}±0.3	13.66^{a}±1.0
Fat (%)	6.16^{c}±0,3	8.61^{b}±0.2	11.72^{a}±0.7
Ash (%)	2.27^{b}±0,05	3.34^{b}±0,02	4.51^{a}±0,01
Carbohydrates (%)	52.34^{a}±0.4	46.74^{b}±0.4	38.31^{b}±1.3
Total Fibers (%)	2.55^{c}±0.10	8.33^{b}±0.08	11.78^{a}±0.06
Calcium (mg/100 g)	80.58^{c}±0.3	134.94^{b}±0.6	149.78^{a}±0.3

[a] Values represent the means. Small letters in the same row denote significant differences according to Tukey's test (p ≤ 0.05).

However, for the other variables of proximal composition the inclusion of black sesame and flaxseed flour significantly modified the parameters. The bread content almost doubled. Comparing the control and B bread formulations, it was found an increase of 6.3%. The same behavior was obtained to fat and ash content. According to Tenyang et al. (2017) the proximate composition of black sesame: 2.82% moisture; 6.03% ash; 49.63% fat; 23.11% protein and 13.60% carbohydrates. Kaur et al. (2017) determined the proximal composition of flaxseed flour and obtained the following results: 8.76% moisture; 3.16% ash; 20.33% protein and 35.17% fat. Kim et al., (2014) analyzed various species of black sesame and the average protein content was around 30% and the lipids content showed values near to 45%.

In this way, the inclusion of these ingredients in bread formulations proves to be beneficial from the nutritional point of view, with increasing amount of micro- and macronutrients. Therefore, formulation B which had the highest content of black sesame and flaxseed flour presented the best nutritional value in relation to these components. Thus, it also presented the lowest carbohydrate content (38.31%) and the highest calcium content (149.78 mg/100 g of bread). Marpalle et al. (2014) also observed an increase in the bread nutritional quality when they added flaxseed flour up to 15% on wheat flour weight, however, the nutritional gain was lower than that evidenced in this study. The total fibers of bread was elevated as the increase in the black sesame and flaxseed flour levels. The calcium content in breads formulations containing black sesame and flaxseed flour confirms the results obtained by Souza et al. (2018), where they studied the amount of different minerals in the flaxseed seeds and black sesame. For calcium, the values found were 1.20% and 1.50%, respectively. Bolaños et al. (2016) reported that the linseed and sesame samples contained high concentration of Ca, K, Mg and P, normally higher than 2000 µg g^{-1}, and lower concentrations of Cu, Fe, Mn, Mo, Na and Zn. Elleuch et al., (2007) developed Halaweh of black sesame waste and found calcium content of 105.4 mg/100 g, below the value found in this study. Table 3 show the sensory analysis results.

Table 3. Sensory analysis of control and optimized breads.

Samples	Control	A	B
Colour	6.91^a±0.2	7.28^a±0.2	6.83^a±0.3
Smell	7.00^a±0.3	7.00^a±0.2	6.96^a±0.2
Taste	7.16^a±0.2	7.01^a±0.3	6.43^b±0.3
Texture	7.05^a±0.2	7.33^a±0.3	6.88^b±0.2
Overall Impression	7.10^a±0.3	7.18^a±0.4	6.88^a±0.3

^{a}Values represent the means. Small letters in the same row denote significant differences according to Tukey's test ($p \leq 0.05$).

In general, the inclusion of black sesame and flaxseed flour reduced numerically the sensory parameters. However, the formulation A, did not differ significantly from the control sample. The sample B differed significantly from control only in the taste and texture attributes, most likely due to the characteristic taste of black sesame. According to the obtained results, we can infer that the black sesame and flaxseed flour addition in bread formulations had more influence on the bread physical parameters than the sensorial attributes, being within the range of positive acceptability of the hedonic scale of 9 points. Therefore, the product has acceptability if marketed as an alternative to conventional bread and allowing consumers to obtain a product with better nutritional value through the addition of these ingredients. Lodhi and Verma (2014) developed bread supplemented of garlic powder and flaxseed up to 15%. Flaxseed decreased the hedonic points of the samples. Marpelle et al. (2014) found hedonic values within the acceptable range (~ 7.00) in functional breads incorporated with flaxseed.

The increase of the hedonic values for the texture attribute in sample A compared to the control and sample B may be related to the lipid content of the two ingredients. According Daun et al. (2003), flaxseed is made up approximately 45% lipid and 55% meal on a dry basis. Flaxseed contains a high proportion of polyunsaturated fatty acids, such as ALA (about 55%) and linoleic acid (about 14%), and a moderate proportion of monounsaturated fatty acid, being mainly oleic acid (18%) (Carter, 1993). In this way, the lipids present in flaxseed flour could contribute to the lubrication of the gluten network (Carr et al. 2009) and minimize the discontinuity caused to the protein network and favor the product texture. However, with the increase of the amount of non-gluten-forming flours in formulation B, this effect was not verified, justifying the reduction of the hedonic value for the bread texture. Fig. 2 show the overall shape, crumb and crust structure.

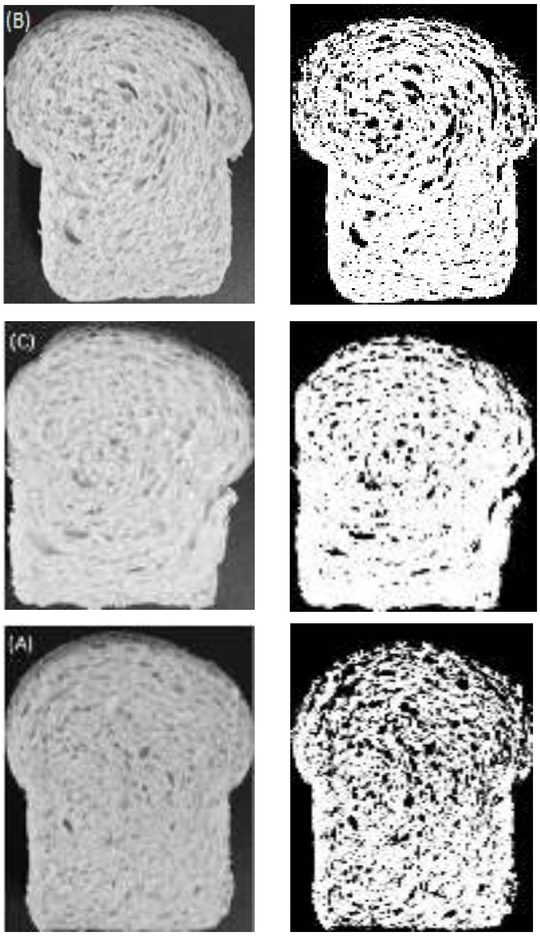

Figure 2. The 2D images of bread crumb. (A) Control; (B) Sample A; (C) Sample B.

From the figures, it is possible to verify that the larger addition of the ingredients was, the smaller bread volume was. The number of present alveoli was also significantly reduced, providing evidence of gluten network discontinuity and dough inability to retain the carbon dioxide produced during fermentation. According Galvão et al. (2018), the non-gluten-forming flours have an influence on the gluten network, promoting cracks, which reduces the carbonic gas holding capacity within the dough and expands the number of pores formed during the mixing step. The number and size of pores in the bread crumb was greatly increased and some of the pores became stacked, giving the dough a disrupted structure, as can be observed in sample B. According Martínez et al. (2018), the bread specific volume was inversely correlated with its hardness.

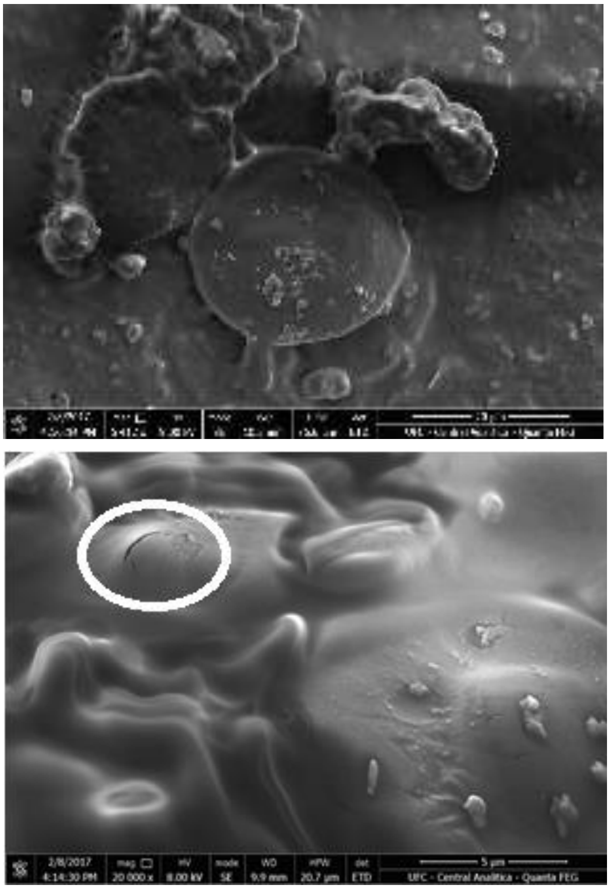

Figure 3. Dough surface SEM of Sample B. (5412x and 20000x magnification).

The same behavior was reported by Gomez et al. (2008), and it was attributed to the denser crumb (higher cell density) and more compact cells (smaller in size) in low volume breads, what justifies the bread corresponds to sample C to present the lowest hedonic values of texture, differentiating significantly from the other samples.

The gluten network discontinuity and the presence of the ingredients between the protein and starch chains can be visualized through SEM in fig. 3. Carlson and Bohlin (1978) demonstrated that up to 10% of gas gets occluded into the wheat flour dough during its mixing. Jha et al. (2017) verified that the increase in resting time was found to increase the dough porosity. This may be due to due to influx of CO2 in the pre-existing pores created during mixing, yeast produces carbon dioxide gas and as a result the porosity of the dough increases. This occurs because the gas will occupy the alveoli formed in the mixture. However, with the addition of black sesame and flaxseed flour, the network weakens and fissures, as SEM image shows, allowing carbon dioxide to escape. This escape makes the bread smaller and denser, reducing its aeration.

4. CONCLUSIONS

In view of the obtained results, it could be concluded that black sesame and flaxseed flour constitute good ingredients to be added together in bread formulations up to 30% black sesame and 15% flaxseed flour without impairing the physical properties of the breads. There was a significant increase in the nutritional quality of breads with black sesame and flaxseed flour, mainly in the increase of the protein, fat and ashes content and in the reduction of carbohydrates, in addition to the contribution of calcium. The developed breads presented good sensory acceptability for the attributes of color, smell, taste and texture. Through the analysis of image and scanning electron microscopy it was possible to observe the increase of the bread crumb density as a function of black sesame and flaxseed flour, as well as discontinuity to the gluten network.

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