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Response surface methodology assessment of the effect of whole wheat flour and fat replacer levels on bread quality

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Abstract

Bread is a staple of the human diet, and can be used as a vehicle to increase dietary fiber intake and decrease fat intake. Specific volume is one of the most important visual characteristics of breads that influence consumer choice, and, alongside hardness, contributes to quality assessment. Thus, this study aims to evaluate the effect of different amounts of whole-wheat flour and fat replacer (enzymatically modified corn starch) on quality parameters of breads produced on a pilot scale. Particle size analysis of flour and starch mixtures revealed uneven particle size, and physical-chemical analysis showed that bread containing higher levels of whole-wheat flour had high insoluble fiber, fat, ash, and protein content. Mathematical models were for specific volume and hardness were obtained and validated through response surface methodology. Model fit results showed a significant linear effect both for specific volume and for hardness, with an inverse trend between these parameters. Predicted values were very close to those obtained experimentally.

Introduction

Wheat is one of the ten most important commodities globally, with over 713 million tons being harvested annually (FAO, 2014). According to the Whole Grains Council (WGC, 2014), whole-wheat flour is characterized by its homogeneous particle size and preservation of the original proportions of the fractions present in the whole grain (endosperm, germ, and bran).

Whole-wheat flour consumption is on the rise, as reducing the production of wheat bran – a byproduct of white flour manufacturing – is a sustainable practice, whether for use in animal feed (FAO, 2014), in the increasing variety of healthy food products with high fiber content and bioactive/functional constituents offered by the food industry to consumers (EFSA, 2010), or to meet the demand for the health benefits derived from whole-wheat flour intake (Doblado-Maldonado *et al.*, 2012), which contains substantially more vitamins, minerals, antioxidants, and other nutrients than white flour, as these compounds are concentrated in the outer portions of the grain (Weaver, 2001).

The presence of bran in whole-wheat flour has adverse effects on the quality of bread (namely, on volume and hardness) (Rosell and Santos, 2010; Curti *et al.*, 2013); depending on its composition (Noort *et al.*, 2010) and particle size (Niu *et al.*, 2014), whole-wheat flour can reduce bread volume, increase its crumb hardness, and alter its flavor (Laurikainen *et al.*, 1998). Studies show that, during the bread-making process, fiber fraction adversely affect the formation and the physical properties of the gluten network, due to a combination of physical and chemical mechanisms. Physically, these adverse effects occur when an increase in particle surface size causes an increase in interaction with the reactive components of gluten, such as ferulic acid monomers bound to the cell wall, interacting with proteins. Chemically, they occur when components of the aleurone layer (ferulic acid monomers, glutathione, and phytate) interact with the gluten proteins (Noort *et al.*, 2010).

Baked goods are widely consumed on a daily basis and provide a convenient medium for delivery of dietary fiber and other healthy compounds to consumers' diets (Ktenioudaki and Gallagher, 2012). Nevertheless, acceptance of whole-wheat food products by the general public is limited due to the poor taste and texture profile of these products (Gan *et al.*, 1992), including bitter flavor, low volume,

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and high firmness (Ktenioudaki and Gallagher, 2012). This creates a need for technological efforts to improve the performance of products containing whole-wheat flour (Liu *et al*., 2015).

Additionally, there is growing demand for healthy products with reduced fat content, which encourages the use of fat replacers, such as those derived from starches, which provide quality parameters similar to those of products made with actual fat (Abbas *et al.*, 2010). Based on health concerns and on popular demand for foods with specific features that improve health, there has been increasing interest in so-called functional foods, which are consumed as part of the usual diet and which either provide physiological benefits or reduce the risk of chronic disease beyond meeting basic nutritional requirements (Shahidi, 2009). Diet and lifestyle can be modified to prevent and reduce risk factors for cardiovascular disease and diabetes, as well as for weight management (Stevenson, 2012). There is epidemiological evidence that healthy diets are rich in dietary fiber and low in saturated fats, trans fats, and cholesterol (Hu, 2002).

In this context, starch derivatives are indicated as fat replacers for use in bread for a variety of factors, including their low level of sweetness, which does not mask other flavors and aromas (Glueck *et al*., 1994); texture and mouthfeel similar to those of fats; ability to absorb and retain water in the product; similarity to fats in size and molecular weight (Alexander, 1995); and physical characteristics that successfully mimic fat, but with a lower energy content (Vanderveen and Glinsmann, 1992).

Bread dough can be considered a system. One of the methods used to evaluate the relative significance of a system affected by many factors, even in the presence of complex interactions, is response surface methodology (RSM). RSM is an empirical statistical modeling technique employed for multiple regression analysis using quantitative data obtained from properly designed experiments to solve multivariable equations simultaneously (Montgomery, 1991).

The aim of this work was to study the impact of whole-wheat flour and fat replacer at different levels on the specific volume and hardness of industrially manufactured bread loaves, by means of response surface methodology, and validate the resulting mathematical models.

Materials and Methods

Materials

Commercially white-wheat flour and whole-wheat flour for bread making, milled from wheat harvested in 2012 and stored at -18ºC, were provided by Cooperativa Agrária Agroindustrial, Guarapuava, state of Paraná, Brazil. The whole-wheat flour was made up of all parts of the grain, has uneven particle size and was produced by passage through stone mill, followed by roller mill. A representative sample of whole-wheat flour and white-wheat flour (refined wheat flour), both from the same batch of grain, was used to ensure standardization of variable parameters. Different blends of whole-wheat flour (WF) and white-wheat flour (IF) were prepared and encoded: 95.35WF (95.35WF+4.65IF); 85WF (85WF+15IF); 60WF (60WF+40IF); 35WF (35WF+65IF); and 24.64WF (24.64WF+75.36IF). The enzymatically modified cornstarch used in this study as a fat replacer (FR) was provided by Dutch Starches International, Netherlands, and is commercially available as Selectamyx C 150.

Bread samples

Bread samples were labeled according to the WF and IF blend employed, using the codes noted above, as experimental design of the Response Surface Methodology (RSM). Bread loaves were baked on a pilot scale and randomly selected two times according to the optimized straight-dough bread-making method (10-10B) (AACC, 2000), with a 60 min fermentation, using the following formulation: wheat flour (100%), sucrose (6%), instant active dry yeast (1.8%), sodium chloride (1.5%), fat (3%), and tap water (Flander *et al.*, 2007). Instead of fat, the aforementioned fat replacer. The amount of water used corresponded to 86% of the water absorption content as determined by Farinograph analysis (method adapted from Seyer and Gélinas (2009). The ingredients were mixed at speed setting 2 for 6 min (Flander *et al*., 2007; Oro, 2013) in a commercial mixer (RPD 25, Líder, Brazil). The temperature of the dough was kept at 28-29 °C after mixing. Subsequently, 500-g pieces of dough were placed in a proofing cabinet (CFC20, Perfecta, Brazil) at 30 °C and 85% relative humidity for 35 min. The dough was then kneaded once, fermented for 17 min, kneaded again, and fermented for a further 8 min. The dough was sheeted manually, placed into a rectangular mold (9.5 cm x 20 cm x 4.5 cm), and fermented for 24 min. Finally, the dough was baked in a revolving oven (Ventile, Líder, Brazil) for 24 min at 180°C and cooled for 1 hour at room temperature on metal racks.

Experimental design

Using the rotatable central composite design of RSM, with the percentage of whole-wheat flour (%WF) and the percentage of fat replacer (%FR) as independent variables. The rotatable central composite design yielded 14 experiments, as follows: four factorial treatments, in which the two factors were %WF (% whole-wheat flour) and %FR (% fat replacer), each with two levels coded to -1 and +1; four axial treatments including minimum and maximum level of each factor coded as -α and +α, where $\alpha=(2^2)^{1/4}$ = 1.414; and one central treatment repeated six times, to estimate the pure experimental error and calculate the reproducibility of the method, in which all factors are coded as zero. The real and encoded values of the two variables are shown in Table 1.

Flour particle size

Determination of granularity of the WF+IF flour blends and of the fat replacer (FR) was performed in three determinations, based on AACC Approved Method nº 66-20 (AACC, 2000), with adaptations. For this purpose, was used a set of sieves (30, 40, 60, 80 and 100 mesh, with mesh openings of 600, 425, 250, 180 and 150 µm, respectively) coupled to a vibratory sieve shaker (D-55743 model, Fritsch, Germany). Briefly, 3 metal balls were placed in each sieve and the materials were sieved for 5 minutes.

Of the 14 total experiments (bread loaves) with 6 repetitions of the central treatment, 12 experiments with 4 repetitions of the central treatment were evaluated, adopting an orthogonal block design (Fisher, 1918), which aims to eliminate the effect of heterogeneity present in experimental units on the comparison of treatments. Its purpose is to make homogeneous groups of the unit of analysis (blocks), each of which receives a repetition of all treatments.

Chemical composition of the loaves

The chemical characteristics of the bread loaves were measured in duplicate by AACC Approved Methods (AACC, 2000). Moisture (44-15A), lipids (30-25), ash (08-01), total starch (76-13), insoluble and soluble dietary fiber (IDF, SDF) (32-07), and protein were measured using the Kjeldahl method (46-10; the nitrogen to protein conversion factor was 5.76 for bread loaf flour blends).

Volume and specific volume measurement

Analysis of volume and calculation of specific volume were performed in quadruplicate. The volume of the bread loaves was determined using a modified standard rapeseed displacement method (10-05) (AACC, 2000), using sesame seeds instead of rapeseeds (Kittisuban *et al.*, 2014). The specific volume (cm^3/g) was calculated based on the average weight of rolls baked from approximately 30 grams of dough (Aplevicz, 2013).

Table 1. Bread composition.

Loaf	Treatment	Independent variables			
		%WF		%FR	
		Real	Encoded	Real	Encoded
1	Factorial	35.00	-1	0.60	-1
2		35.00	-1	2.60	+1
3		85.00	+1	0.60	-1
4		85.00	+1	2.60	+1
5	Axial	24.64	- α	1.60	0
6		95.35	+ α	1.60	0
7		60.00	0	0.18	- α
8		60.00	0	3.00	+ α
9	Central	60.00	0	1.60	0
10		60.00	0	1.60	0
11		60.00	0	1.60	0
12		60.00	0	1.60	0
13		60.00	0	1.60	0
14		60.00	0	1.60	0

WF: whole-wheat flour; FR: fat replacer (enzymatically modified corn starch).

Briefly, the volume of the loaves was measured in a container with known volume (KV). The container was filled with seeds, the loaf was removed, and the volume of seeds recorded (VR). The loaf volume (LV) was calculated according to Equation 1.

$$LV\ (cm^3) = KV - VR \qquad (1)$$

All loaves were measured and weighed (W) using a digital scale (g). The specific volume (SV) of the bread loaves was calculated according to Equation 2.

$$SV\ (cm^3/g) = LV/W \qquad (2)$$

Hardness measurement

Hardness is the force required to obtain a deformation (Szczesniak, 2002). Hardness assessment, one of the components of texture profile analysis (TPA), was performed on eight 25-mm cubes obtained from the center of each loaf (Ulziijargal *et al.*, 2013), in accordance with method 74-10A (AACC, 2000). Hardness was measured with a TA.HD Plus texture analyzer (Stable Micro System, Surrey, UK) equipped with a 50-kg load cell and a 36-mm aluminum cylindrical probe, which tests compression up to 40% penetration of the original height (distance of 10 mm) at a crosshead speed of 2 mm/s (Oro, 2013).

Statistical methods

Were used RSM to analyze the effect of the independent variables WF and FR on the responses of interest. The second-order regression model is represented by Equation 3 (Montgomery, 1991), where: z = estimated results for the response variables (specific volume and hardness); x = whole-wheat flour content (%WF); y = fat replacer content (%FR); and b_0, b_1, b_{11}, b_2, b_{22}, b_{12} = estimates of the regression coefficients.

$$z = b_0+b_1x+b_{11}(x)^2+b^2y+b_{22}(y)2+b_{12}xy \quad (3)$$

The ideal model should have good significance ($p\leq0.05$), high reliability (data within the 95% confidence interval, i.e., irrelevant residuals), and low variability ($R^2 \geq 70\%$ and $\leq 20\%$ CV).

The dependent variables or responses of interest were specific volume and hardness. Analysis of variance (ANOVA) and Tukey's test (significance level $p\leq0.05$) were used to determine the significance of the data. All statistical analysis and graphical representations were performed in STATISTICA 7.0®.

Validation of RSM results

To validate the mathematical model obtained with RSM, a bread formulation (50%WF/1.5%FR) was chosen at random from the intervals studied in experimental design. Experimental and predicted values were compared and prediction errors (in %) were calculated to determine the validity of the models using Equation 4 (Skara *et al*., 2013).

Predicted error = (experimental – predicted)/predicted x 100% (4)

Results and Discussion

Flour particle size

Table 2 shows the results of particle size analysis of the flour and fat replacer mixtures. The results of particle size analysis of the mixtures of whole-wheat and white-wheat flour and fat replacer (Table 2) show that increasing whole-wheat flour content is associated with increased percentage of retained material with a larger particle size (600 µm sieve), as observed in samples 3 (85WF+0.6FR), 4 (85WF+2.6FR), and 6 (95.35WF+1.6FR), with retention of 63.86, 41.34, and 63.86% of particles respectively. This result is consistent with a previous study of whole-wheat and white-wheat flour blends by Oro (2013), in which particle size varied according to the increase in whole-wheat flour content, mainly due to the presence of fragments derived from the outer layers of the grain.

According to Curti *et al.* (2013), the presence of outer grain fractions in whole-wheat flour affects the properties of the resulting bread, mainly by dilution of the gluten-starch matrix by components that are not part of the endosperm (Gan *et al.*, 1992). The outer fractions of the grain (pericarp and germ) act on the dough through different compositions, resulting in smaller, harder bread, and through different particle sizes, which, conversely, have no significant impact on volume or hardness.

Table 2. Particle size analysis of flour and fat replacer mixtures.

Sample	Blends		Tyler/Mesh					
			30	40	60	80	100	Catch
	WF	FR	Sieve (µm)					
			600	425	250	180	150	< 150
	(%)	(%)	Material retained (%)					
1	35.00	0.60	41.34	5.58	5.83	5.60	10.69	31.08
2	35.00	2.60	19.19	2.06	2.38	16.09	13.57	46.68
3	85.00	0.60	63.86	9.27	9.20	4.06	2.05	11.50
4	85.00	2.60	41.34	5.58	5.83	5.60	10.69	31.08
5	24.64	1.60	19.19	2.06	2.38	16.09	13.57	46.68
6	95.35	1.60	63.86	9.27	9.20	4.06	2.05	11.65
7	60.00	0.18	41.34	5.58	5.83	5.60	10.69	31.08
8	60.00	3.00	40.17	5.49	6.77	11.53	8.83	26.63
9	60.00	1.60	39.87	6.02	8.39	12.76	9.40	23.73
10	60.00	1.60	41.41	5.37	7.37	11.31	11.14	23.19
11	60.00	1.60	42.29	5.49	6.97	12.12	8.02	25.08
12	60.00	1.60	42.19	5.00	5.82	10.11	10.88	25.85
9-12*	60.00	1.60	41.44	5.47	7.14	11.57	9.86	24.46

Results expressed as mean of three determinations. *Average of loaves 9 to 12.

By contrast, as whole-wheat flour content decreased and fat replacer content increased, the percentage of material retained with a smaller particle size (sieve < 150 µm) also increased, as seen between samples 1 (35WF+0.6FR) and 2 (35WF+2.6FR), with respective retention rates of 31.08 and 46.68% of particles, and between samples 3 (85WF+0.6FR) and 4 (85WF+2.6FR), with retention of 11.50 and 31.08% of particles, probably because the fat replacer is retained in the catch pan. The fat replacer used in this study was an enzymatically modified corn starch, which justifies retention in the receiving pan of the sieve set, as, according to Chen and Zhang (2012) and Ma *et al*. (2006), enzymatic hydrolysis causes the native starch granules to decrease in size.

Physical and chemical composition

Table 3 shows an analysis of the physical and chemical composition of the loaves. For total starch, sample values ranged from 54.03 to 61.46%, with significant differences only between sample 5 (24.64WF+1.60FR), which had a greater white-wheat flour content, and samples 3 (85WF+0.6FR) and 4 (85WF+2.6FR). For protein, the samples ranged from 12.56 to 14.71% ($p\leq0.05$). As whole-wheat flour content decreased, so did protein content, as both whole-wheat flour and white-wheat flour came from the same batch of grain.

For ash, sample content ranged from 2.13 to 2.61% ($p\leq0.05$). Again, as whole-wheat flour content decreased, so did ash content; this was expected, as, according to Chiang *et al.* (2006), wheat ash is basically located in the bran. For lipids, sample content ranged from 0.07 to 0.60% ($p\leq0.05$). As

Table 3. Analysis of physical and chemical composition, specific volume and hardness of baked loaves.

Bread	Total starch (%)	Protein (%)	Ash (%)	Lipid (%)	Soluble fiber (%)	Insoluble fiber (%)	SV (cm^3/g)	Hardness (N)
1	57.72^{ab} ± 3.80	12.95^{g} ± 0.23	2.14^{d} ± 0.07	0.10^{e} ± 0.00	1.50^{b} ± 0.17	4.54^{cde} ± 0.09	2.49^{a} ± 0.23	11.56^{f} ± 0.95
2	58.40^{ab} ± 0.81	12.57^{g} ± 0.23	2.18^{d} ± 0.01	0.07^{e} ± 0.00	1.53^{b} ± 0.11	3.90^{def} ± 0.01	2.37^{ab} ± 0.23	11.21^{f} ± 1.50
3	54.33^{b} ± 1.10	14.49^{abcd} ± 0.23	2.50^{ab} ± 0.00	0.44^{bcd} ± 0.02	1.43^{b} ± 0.39	5.01^{bcd} ± 0.28	1.81^{d} ± 0.21	29.56^{a} ± 1.13
4	54.03^{b} ± 0.49	14.26^{cde} ± 0.23	2.45^{bc} ± 0.03	0.38^{cd} ± 0.03	1.24^{b} ± 0.00	6.29^{a} ± 0.04	2.03^{cd} ± 0.13	22.77^{b} ± 2.12
5	61.46^{a} ± 1.82	12.56^{g} ± 0,23	2.13^{d} ± 0.00	0.16^{e} ± 0.00	1.51^{b} ± 0.14	2.81^{f} ± 0.12	2.46^{a} ± 0.13	8.40^{f} ± 1.31
6	55.14^{ab} ± 1.48	14.71^{a} ± 0.23	2.61^{a} ± 0.06	0.45^{abcd} ± 0.01	1.41^{b} ± 0.11	5.88^{ab} ± 1.04	1.79^{d} ± 0.17	29.86^{a} ± 2.61
7	60.48^{ab} ± 0.26	14.55^{abc} ± 0.23	2.46^{bc} ± 0.02	0.54^{ab} ± 0.03	1.95^{ab} ± 0.28	5.03^{abcd} ± 0.08	2.03^{cd} ± 0.18	19.17^{cd} ± 2.49
8	55.34^{ab} ± 0.22	14.65^{ab} ± 0.23	2.42^{bc} ± 0.02	0.58^{ab} ± 0.05	2.36^{a} ± 0.20	5.35^{abc} ± 0.28	2.13^{bc} ± 0.25	16.53^{de} ± 1.67
9	55.76^{ab} ± 1.91	13.81^{f} ± 0.00	2.44^{bc} ± 0.01	0.33^{d} ± 0.01	1.52^{b} ± 0.25	4.29^{cde} ± 0.07	1.96^{bcd} ± 0.08	15.58^{e} ± 2.51
10	54.36^{b} ± 2.78	14.33^{bcde} ± 0.06	2.44^{bc} ± 0.03	0.15^{e} ± 0.02	1.49^{b} ± 0.15	4.30^{cde} ± 0.08	2.22^{abc} ± 0.27	16.06^{de} ± 2.14
11	59.40^{ab} ± 0.66	14.61^{ab} ± 0.07	2.37^{bc} ± 0.06	0.47^{abcd} ± 0.02	1.56^{b} ± 0.16	3.34^{ef} ± 0.05	2.08^{bcd} ± 0.16	20.42^{bc} ± 2.69
12	59.63^{ab} ± 0.28	14.07^{ef} ± 0.06	2.42^{bc} ± 0.03	0.50^{abcd} ± 0.09	1.49^{b} ± 0.06	3.40^{ef} ± 0.07	2.10^{bcd} ± 0.19	20.46^{bc} ± 1.36
13	57.32^{ab} ± 1.90	14.18^{de} ± 0.03	2.35^{c} ± 0.00	0.60^{a} ± 0.08	1.31^{b} ± 0.07	3.44^{ef} ± 0.43	2.07^{bcd} ± 0.25	16.91^{de} ± 0.99
14	58.08^{ab} ± 1.18	14.06^{ef} ± 0.06	2.41^{bc} ± 0.01	0.50^{abc} ± 0.03	1.24^{b} ± 0.20	3.58^{ef} ± 0.22	2.01^{cd} ± 0.25	17.21^{de} ± 1.52
9-14*	57.43^{ab} ± 0.84	14.18^{de} ± 0.00	2.41^{bc} ± 0.00	0.46^{abcd} ± 0.05	1.44^{b} ± 0.00	3.73^{ef} ± 0.08	2.07^{bcd} ± 0.11	17.77^{cde} ± 0.84

Mean of duplicate ± standard deviation in the same column followed by different superscript letters denotes significant difference (p≤0.05), with SV in quadruplicate and hardness measured from four determinations. Results are expressed on a dry weight basis. N x 5.76. WF, whole wheat flour; FR, fat replacer; SV, specific volume. *Average of 9 to 14 loaves.

whole-wheat flour content decreased, so did lipid levels.

Soluble fiber content ranged from 1.24 to 2.36% (p≤0.05), and insoluble fiber content, from 2.81 to 6.29% (p≤0.05). As whole-wheat flour content increased, so did insoluble fiber content. The results of chemical composition analysis in the present study are consistent with the findings published by Borges *et al.* (2011), in a study in which bread was made from linseed flour.

Specific volume

Table 3 shows the results of assessment of the quality parameters of interest (specific volume and hardness) in loaves baked with different blends of whole-wheat flour and fat replacer. Figure 1A shows the effect of the percentage of whole-wheat flour and fat replacer on specific volume.

As shown in Table 3 and Figure 1A, the specific volume of bread samples ranged from 1.79 to 2.49 cm^3/g. Similar results were obtained in breads in which white-wheat flour was replaced with another type of flour rich in fiber, such as: 10% fiber mixture (Rosell and Santos, 2010); 40% amaranth flour (Sanz-Penella *et al.*, 2013); or 7.5% peas, 15% buckwheat, and 15% teff (Eragrostis tef, a cereal common in Ethiopia) (Collar *et al.*, 2014).

According to the model proposed by RSM, the parameters estimated for the experimental data regarding specific volume can be described by Equation 5 ($R^2 = 0.9113$): where: SV, specific volume (cm^3/g); WF, whole-wheat flour (%); FR, fat replacer

(%).

$$SV = 3.24735 - 0.02362WF + 0.00007WF^2 - 0.24413FR + 0.02245FR^2 + 0.00336WFFR \quad (5)$$

The R^2 coefficient of determination expresses model fit, and denotes that the specific model may account for 91.13% of the variability in response. Furthermore, while the use of fat replacer had no significant effect on specific volume, whole-wheat flour showed a negative linear association with this parameter (data not shown).

Figure 1A shows that breads baked with lower whole-wheat flour content, regardless of the fat replacer content, have a higher specific volume. Similar values for specific volume (2.46 and 2.55 cm3/g) were obtained by Skendi *et al*. (2010) in breads made only from white-wehat flour. In other words, as the whole-wheat flour content in the bread mixture increased, the specific volume of the resulting loaves decreased (Figure 1A). In the present study, fiber – in the form of whole-wheat flour – may have acted both as a diluent and as a competitor, because it had both a diluting effect on the gluten network, interrupting the gluten-starch matrix, decreasing gas retention, and reducing bread volume (Mandala *et al.*, 2009), and an effect of competing for water, gluten, and starch from wheat flour, possibly hindering sufficient hydration and thus reducing the volume of the baked loaves (Lai *et al*., 1989).

In general, the addition of fiber to wheat flour has a negative effect on bread volume (Tuncel *et al*., 2014), as shown in Tables 4 and 5, considering fiber in the form of whole-wheat flour. Particle size analysis (Table 2) showed that increasing whole-wheat flour content increased the amount of material with larger particle size retained in the 600-µm sieve, due to the presence of fragments derived from the outer layers of the grain (bran and germ). In this context, it is appropriate to point out that wheat flour, the object of this study, is widely commercially accepted in Brazil, since the Brazilian legislation does not specify particle size limits for whole-grain flour. Furthermore, the higher the whole-wheat flour content used in the bread mixture, the higher the levels of soluble fiber, insoluble fiber, ash, and lipids and the lower the total starch and protein content of the finished loaves (Table 3), which confirms the chemical composition of the characteristic fractions of the wheat grain (pericarp, endosperm, and germ), since both the white-wheat flour and the whole-wheat flour came from the same batch of grain.

Also regarding specific volume, a comparative of breads made with similar, high whole-grain flour

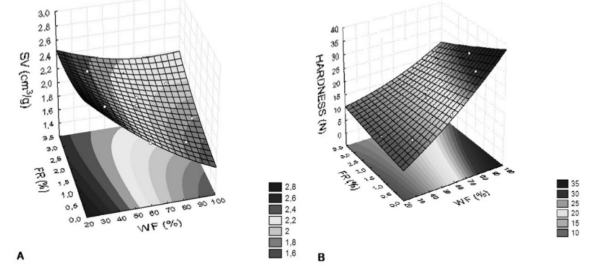

Figure 1. Effect of the percentage of whole-wheat flour and fat replacer on the: A - specific volume, B - hardness of baked loaves.
SV: specific volume; FR: fat replacer; WF: whole wheat flour.

contents but with different levels of fat replacer revealed a trend toward an increase in specific volume proportional to the increase in fat replacer content, as visible in Figure 1A, although Table 3 shows no significant numeric difference in specific volume. This is probably because of the fat replacer used in this study conferred some stability to the dough system interface, providing additional strength to gas cells during baking, as reported in a similar study which used hydrocolloids (Rosell *et al*., 2001).

Hardness

Figure 1B shows the effect of the percentages of whole-wheat flour and fat replacer on bread hardness. As shown in Table 3 and Figure 1B, hardness measurements of the bread samples ranged from 8.40 to 29.86 N, similar to those found in breads enriched with different sources of fibers, including cereals, pseudocereals, and vegetables (8.7 to 25.3 N) (Collar *et al*., 2014).

According to the model proposed by RSM, the parameters estimated for the experimental data regarding hardness can be described by Equation 6 ($R^2 = 0.9508$): where hardness (N); WF, whole wheat flour (%); FR, fat replacer (%).

$$Hardness = 0.38252 + 0,25833WF + 0,00120WF^2 + 2,18162FR + 0,10059FR^2 - 0,06440WFFR \quad (6)$$

The coefficient of determination (R^2), which expresses model fit, was 0.9508, indicating that this model may account for 95.08% of the variability in response.

Fat replacement had no significant effect on bread hardness; however, whole-wheat flour content had a positive linear effect on this parameter (data not shown). In other words, for the same whole-wheat flour content, varying the percentage of fat replacer did not result in variations in bread hardness; therefore, only the contribution of the percentage of

Table 4. Model-predicted and actual (experimental) values

Variable	Values predicted by the full model	Values predicted by the simplified model	Experimental values	Error (%)
SV	2.18 (cm^3/g)	2.07 (cm^3/g)	2.40 ± 0.27 (cm^3/g)	10.0
Hardness	14.97 (N)	13.30 (N)	11.42 ± 1.02 (N)	23.7

Mean ± standard deviation ($p \leq 0.05$). SV: specific volume; N: Newton.

whole-wheat flour could account for the hardness behavior of the baked loaves. However, from a review of the hardness results of samples 3 (85WF+0.6FR) and 4 (85WF+2.6FR) (Table 3), 29.56 and 22.77 N, respectively, it appears that increasing fat replacer content decreased bread hardness. This was probably because the fat replacer interacts with the starch present in the flour, reducing the swelling and solubilization thereof, thereby causing less surface area to be exposed to gluten, weakening and reducing cross-linking bonds to protein and, consequently, decreasing crumb hardness (Martin *et al.*, 1991).

As shown in Figure 1B, there was a trend toward increasing hardness with increasing whole-wheat flour content, regardless of the fat replacer content in the mixture. This behavior was similar to that reported in a prior study in which crumb hardness increased with partial substitution of whole-wheat flour made with intermediate wheat grain fractions in place of white-wheat flour (Blandino *et al.*, 2013).

Model fit

According to the results obtained, the percent content of whole-wheat flour (%WF) was associated with the only significant linear effect in the model, both for specific volume and for hardness ($p \leq 0.05$). Hence, the non-significant effects were removed and the model was adjusted for simple linear regression, represented by Equation 7: where z, estimated results for the response variables (specific volume and hardness); x, whole-wheat flour (%); b_0 and b_1, estimates of regression equation coefficients.

$$z = b_0 + b_1 x \quad (7)$$

Therefore, an inverse trend was observed between specific volume and hardness (Figures 1A and 1B, respectively), as shown in a previous study (Skara *et al.*, 2013).

Loaf volume and crumb hardness are bread quality attributes (Skendi *et al.*, 2010). However, the use of whole-grain flour results in less bread volume, which, in turn, limits acceptance of the product by the public, despite the beneficial health effects (Rosell *et al.*, 2009). In addition, a food can be characterized as "whole-grain" when at least 51% of its ingredients are whole grains (WGC, 2013). As shown in Figures 1A and 1B, loaves prepared with about 60% WF, regardless of FR content, had higher specific volume readings (close to 2.00 cm^3/g) and intermediate hardness values (18.00 N).

Similar results were obtained in breads made with the bioingredient *Lactobacillus brevis* and in which 20% of the white-wheat flour content was replaced with wheat bran (2.46 cm^3/g specific volume and 1385.5 g hardness) (VALERIO *et al.*, 2014). In a previous study of breads made from white-wheat flour in which 10% of the refined flour content was replaced with wheat bran and an additional 10% with rice bran, the specific volume was measured as 2.65 cm3/g and the hardness as 18.72 N. These breads were considered acceptable on sensory analysis, as demonstrated by acceptability scores (Tuncel *et al.*, 2014). In view of the results obtained for the quality parameters specific volume and hardness in the present study, we conclude that, by analogy, the experimental bread made with 60%WF would be acceptable from a sensory standpoint, regardless of %FR.

Validation of mathematical models

Table 4 shows the predicted values and experimental values obtained with mathematical models for the variables of interest (specific volume and hardness) in randomly selected loaves made with 50% whole-wheat flour and 1.5% fat replacer. As shown in Table 4, the predicted values were very close to those obtained experimentally, especially for the SV variable, since some experimental error is likely to have occurred for the hardness parameter. Although the study conditions were kept constant, there may have been changes in physical variables such humidity, microorganism activity for fermentation, and room temperature.

Conclusions

The combined addition of different amounts of whole-wheat flour and fat replacer for pilot-scale manufacturing of fat-free bread with a fiber content suitable for human intake was beneficial, as it enabled analysis of quality parameters. Use of the response surface methodology yielded mathematical models for specific volume and hardness. Model fit revealed an inverse trend between specific volume and hardness. The only linear significant effects found were of whole-wheat flour (containing bran and germ) on specific volume and hardness.

The model was validated, as the results of the experimental values obtained for the response variables (specific volume and hardness) were very close to the values predicted for bread making, as selected randomly within the initial range. In future research, we suggest that variation levels be altered to yield an $+\alpha > 3.0$ %, so as to enable a more robust evaluation of the effect of fat replacer content on the quality parameters specific volume and hardness.

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