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Research article

Optimization of a novel, gluten-free bread's formulation based on chickpea, carob and rice flours using response surface design

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ABSTRACT

This study aimed to develop nutritious, gluten-free bread with high quality characteristics using a mixture of chickpea, carob and rice flours as substitutes of wheat flour. To optimize the bread formulation, a Box-Behnken experimental design was conducted to evaluate the effect of the corresponding flour blend addition, proofing time and water amount addition on the physicochemical, technological and sensory properties of the obtained formulated bread. The optimized formulation was calculated to contain 70% of mixture flour and 100% of water, with a proofing time of 40 minutes. This formulation produced bread with greater specific volume (3.73 ± 0.37 cm^3/g) and less baking loss ($22.98 \pm 0.94\%$) than those of control (+) bread (2.93 ± 0.21 cm^3/g and $31.65 \pm 0.72\%$, respectively). Findings proved that the mixture flour based on chickpeas, carob and rice represents a good alternative to make gluten-free bread with acceptable baking properties.

1. Introduction

Bread is one of the cereal products that is composed mainly of wheat flour. However, wheat is a main cause of several food allergic reactions (Kraft et al., 2021). The rapid increase in gluten-related diseases has led to a growing demand for gluten-free products in the market (Khemiri et al., 2020). Most gluten-free products (especially bread), which are typically produced from gluten-free flours and starches and devoid of gluten proteins, have poor sensory, textural and nutritional properties (Ua-Arak et al., 2017). Moreover, the total substitution of gluten protein in the formulation of gluten-free bread with a high quality is a big challenge, since gluten protein are the main responsible of the technological properties to the dough.

In order to present a dietary product that meets the needs of coeliac consumers, we propose a new formulation of a gluten-free food based on rice flour supplemented with chickpea and carob flours to improve the nutritional value of the suggested gluten-free bread.

Chickpea (*Cicer arietinum* L.) is a legume rich in protein, dietary fiber, carbohydrates, folate and minerals (Singh and Whelan, 2011). Carob (*Ceratonia siliqua* L.) is the fruit of a leguminous tree native to the Mediterranean basin. This species is used as a natural food additive, namely a thickener and a flavoring agent (Arribas et al., 2019). It is also a good source of dietary fiber (Valero-Muñoz et al., 2014). For this reason, carob and chickpea are receiving a lot of attention for being gluten-free functional foods that contain numerous phytochemicals and nutritional benefits (Aguilar et al., 2015); (Arribas et al., 2019). They can be an important alternative for rice fortification.

Arribas et al. (2019) investigated gluten-free extruded foods made from rice, beans and carob and reported that rice, bean and carob formulations were an excellent alternative for the development of new gluten-free products. Jagelaviciute and Cizeikiene (2021) studied the influence of non-traditional sourdough on the characteristics of gluten-free maize/rice bread. The authors reported that chia, hemp, and quinoa flour increased the acceptability of gluten-free maize/rice bread. Coronel et al. (2021) investigated gluten-free premixes and selected premixes made with buckwheat and chia flour. All these studies focused on adding vegetable flours to gluten-free breads. However, to our knowledge, no data is available in the literature on the formulation of gluten-free bread based on chickpea and carob flours and their combination with rice flour.

The main objective of this study is to develop nutritious gluten-free bread using a mixture of chickpea, carob and rice flours as substitutes of wheat flour. In order to optimize the best bread formulation, a Box-Behnken experimental design was established to evaluate the effect of

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the addition of corresponding flour mixture, proofing time and water amount addition on the physicochemical, technological and sensory properties of the formulated bread obtained in our work.

2. Materials and methods

2.1. Raw materials

Chickpea (*Cicer arietinum* L.), carob (*Ceratonia siliqua* L.) and rice flours were bought from a local market (Sfax region, Tunisia).

2.2. Physicochemical composition, technological parameters and color of flour

Moisture, ash, fat and protein contents were determined according to AOAC methods 935.29, 923.03, 920.85, and 920.87, respectively (AOAC, 2000). Protein content was calculated by multiplying the azote content (N) by a factor of 6.25. Total (TDF) and insoluble dietary fiber (IDF) expressed as g TDF or g IDF/100 g, were determined following the enzymatic–gravimetric AOAC method (method 985.29) (AOAC, 1991). The carbohydrate content was determined using the mass balance (Capitani et al., 2012). The water activity was measured at 25 °C using a laboratory aw meter (Novasina, swift aw, Switzerland).

The pH meter (Mettler Toledo, Greifensee, Switzerland) was used to measure the pH at 20 °C.

The swelling capacity of flours was determined according to the method suggested by Robertson et al. (2000). AACC (2000) method was applied to evaluate the Water Solubility Index (WSI). The water-holding capacity (WHC) and the Oil-holding capacity (OHC) were assessed according to the methods of McConnell et al. (1974) and Jorde and Linskens (1974), respectively.

Color measurement (L^*, a^*, b^*, $h°$ and ΔE) of the samples was carried out by colorimeter (Konica Minolta, Inc, Japan). The chroma (C^*) and hue angle ($h°$) indicating the saturation level and shade of the color, respectively, were calculated according to Saricoban and Yilmaz (2010) (Equations (1) and (2), respectively). The color change (ΔE) was calculated using the color values of control (–) (L_0^*, a_0^*, $b_0{}^*$) and bread (L^*, a^*, b^*) for each formulation (Equation (3)).

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$h^0 = \arctan\left(b^*/a^*\right) \quad (2)$$

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (3)$$

2.3. Gluten-free bread making procedure

All the ingredients used for the preparation of gluten-free bread have been purchased from a local supermarket. The preparation of Gluten-free bread included a mixture of flour the mixture percentages (%) in each formulation are optimized in section 2.4 below, i.e., 1.18% of salt, 1.14% of sugar, 2% of dry yeast, and 11% of sunflower oil.

The flour mixture was composed of 50% rice flour, 40% chickpea flour, and 10% carob flour. This flour mixture was developed not only on the basis of literature (Arribas et al., 2019), but also after preliminary experiments to obtain the best technological and nutritional properties. The control (+) bread was made from rice flour (100%) while the control (–) was made from wheat flour (100%).

The bread-making process is the direct fermentation of the dough, in which yeast and sugar were dissolved in the liquid phase and added to the remaining ingredients in a planetary mixer (Moulinex, Click and Mix 450, France), until complete homogenization. Kneading was performed for 1 min at 145 rpm then 5 min at 210 rpm in the same mixer and remaining at rest for 30 min. Each bread dough was placed in rectangular metal trays ($15 \times 7 \times 4$ cm^3) previously greased with soybean oil and placed in an oven for fermentation at 35 °C and 75% humidity for 60 min. Breads were then baked in an electrical oven (Jeio tech incubator, Korea) for 40 min at 200 °C. After that, they were cooled and stored in polyethylene packages at ambient temperature until further analyses. Each bread formulation was performed in triplicate.

2.4. Experimental design and optimization

A Box-Behnken factorial design (Box and Behnken, 1960) with three independent factors (amount of water added, fermentation time, and percentage of flour mixture in the total formulation) and three levels for each factor. Three experiences were done at the center point, in order to fit a second-order-response-surface model (Equation (4)). A total of 15 experiments has been conducted, with three low, middle, and high coded values levels of 1, 0 and −1, respectively, that are designated for the variables. The studied responses were presented in Table 1.

$$\hat{Y} = a_0 + \sum_{i=1}^{3} a_i \cdot x_i + \sum_{i=1}^{3} \sum_{i \neq j}^{3} a_{ij} \cdot x_i \cdot x_j + \sum_{i=1}^{3} a_{ii} \cdot x_i^2 \quad (4)$$

$\hat{Y}$: estimated response;
a_0, a_i, a_{ij}, and a_{ii}: estimated model coefficients;
x_i: coded variables (factors).

All experiments were performed in triplicate and the mean values ± standard deviation is given in Table 1.

2.5. Bread characteristics analysis

The moisture content of gluten-free bread was determined by drying the sample (5 ± 0.01 g) to a constant weight at 103 ± 2 °C, and it was expressed as a percentage of the initial sample weight (AOAC, 1991).

Bread specific volume (V_{SP}) was calculated as the ratio between the cake volume and its weight (Equation (5)) (Mill, 1982) with a volume accuracy of ±10 cm^3.

$$\text{Specific volume} = \frac{\text{Cake volume}}{\text{Cake weight}} \quad (5)$$

The baking loss (%) was determined by weighing each batter before baking (W_0) and each bread after baking and cooling (W_f) using Equation (6) (Coelho and de las Mercedes Salas-Mellado, 2015). Bread baking loss was calculated using six independent bread samples from each type, 24 h after baking.

$$\%\ \text{Baking loss} = \frac{W_f - W_0}{W_0} \times 100 \quad (6)$$

The Textural Profile Analysis (TPA) was applied on bread samples to evaluate the different texture parameters i.e. hardness and masticability. The texture Analyser (LLOYD instruments, Fareham, England) connected to a computer provided the force-time curve for a two cycle compression (Ammar et al., 2021). All measurements have been carried out in a controlled room at 25 °C. The measurements were carried out on 60 mm-width × 60 mm-length × 40 mm-height bread samples. An aluminum cylinder probe was used. The compression was done with a displacement speed of 40 mm/min and to 50% (20 mm) of the original height of the bread. Parameters were registered on three independent bread samples from each type, 2 and 24 h after baking.

The colors of the crumb and crust were determined using the procedure described in section 2.2 above. The determination of crumb color was performed on the central portion of three slices, whereas crust color was analyzed on six pre-selected locations of the crust of each bread sample.

2.6. Sensory analysis

Sensory analysis of gluten-free bread was done by 60 subjects (aged between 22 and 35) 18 h after baking. The taste, color, aroma, texture, shape, masticability, pores' structure and overall acceptability were

Table 1. Box-Behnken experimental design and the obtained physicochemical and techno-functional properties of different gluten-free bread formulas as response.

F	Water (g/100 g of flour)	Fermentation time (min)	Flour percentage (/total mixture)	a_w	pH (24 h)	Dough moisture (%)	Bread moisture (24 h) (%)	Bread Vsp (cm^3/g)	Baking loss (%)	Hardness (24 h) (N)	Masticability (24 h) (N·mm)
1	85	30	75	0.912 ± 0.01^{d}	5.37 ± 0.04^{a}	48.26 ± 0.63^{a}	34.71 ± 0.84^{d}	1.76 ± 0.44^{f}	9.4 ± 0.49^{f}	5.48 ± 0.12^{a}	9.39 ± 0.36^{a}
2	100	30	75	0.926 ± 0.01^{c}	5.28 ± 0.04^{b}	45.37 ± 2.02^{b}	40.3 ± 1.56^{b}	2.18 ± 0.21^{e}	12.39 ± 0.65^{e}	2.25 ± 0.62^{c}	3.72 ± 0.55^{e}
3	85	50	75	0.957 ± 0.01^{a}	5.24 ± 0.07^{b}	41.83 ± 0.3^{c}	35.4 ± 0.3^{d}	2.84 ± 0.11^{e}	10.02 ± 0.41^{e}	2.73 ± 0.69^{c}	4.3 ± 0.12^{d}
4	100	50	75	0.915 ± 0^{d}	5.06 ± 0.03^{d}	48.73 ± 1.18^{a}	37.9 ± 0.59^{c}	3.43 ± 0.36^{d}	16.68 ± 0.77^{b}	1.9 ± 0.22^{d}	3.19 ± 0.08^{e}
5	85	40	50	0.917 ± 0^{d}	5.22 ± 0.06^{b}	40.23 ± 0.51^{d}	32.5 ± 0.4^{e}	3.24 ± 1.02^{d}	13.48 ± 0.5^{d}	2.02 ± 0.81^{c}	3.18 ± 0.19^{e}
6	100	40	50	0.927 ± 0^{c}	5.14 ± 0.11^{c}	45.72 ± 1.31^{b}	33.04 ± 1.14^{e}	4.1 ± 1.13^{c}	16.69 ± 0.72^{b}	1.55 ± 0.09^{d}	1.72 ± 0.51^{f}
7	85	40	100	0.921 ± 0^{c}	5.13 ± 0.01^{c}	46.77 ± 0.21^{b}	43.66 ± 0.5^{a}	3.68 ± 0.19^{d}	11.68 ± 0.28^{e}	3.71 ± 0.97^{b}	5.97 ± 0.11^{c}
8	100	40	100	0.921 ± 0.01^{c}	5.13 ± 0.03^{c}	46.86 ± 0.72^{b}	31.36 ± 0.79^{e}	4.18 ± 0.22^{c}	15.42 ± 0.43^{c}	2.95 ± 0.26^{c}	2.64 ± 0.73^{f}
9	92.5	30	50	0.915 ± 0.01^{d}	5.27 ± 0.02^{b}	46.11 ± 0.9^{b}	32.79 ± 0.48^{e}	4.58 ± 0.26^{b}	17.83 ± 0.52^{a}	3.87 ± 0.83^{b}	5.96 ± 0.3^{c}
10	92.5	50	50	0.918 ± 0.01^{d}	4.98 ± 0.1^{d}	42.5 ± 0.25^{c}	33.25 ± 1.35^{e}	5.84 ± 0.25^{a}	18.06 ± 0.47^{a}	3.02 ± 0.44^{b}	4.42 ± 0.1^{d}
11	92.5	30	100	0.911 ± 0.01^{d}	5.02 ± 0.07^{d}	45.65 ± 0.42^{b}	37.27 ± 0.56^{c}	4.84 ± 0.36^{b}	13.78 ± 1.61^{d}	5.17 ± 0.15^{a}	5.88 ± 0.12^{c}
12	92.5	50	100	0.906 ± 0^{d}	4.9 ± 0.1^{d}	48.12 ± 0.42^{a}	34.95 ± 0.52^{d}	5.61 ± 0.37^{a}	18.32 ± 0.37^{a}	3.33 ± 0.5^{b}	5.89 ± 0.16^{c}
13	92.5	40	75	0.917 ± 0.01^{d}	5.27 ± 0.03^{b}	48.25 ± 0.82^{a}	37.01 ± 0.27^{c}	5.79 ± 0.42^{a}	15.98 ± 1.56^{c}	3.09 ± 0.65^{b}	5.15 ± 0.19^{c}
14	92.5	40	75	0.917 ± 0.01^{d}	5.27 ± 0.03^{b}	48.25 ± 0.82^{a}	37.01 ± 0.27^{c}	5.79 ± 0.42^{a}	15.98 ± 1.56^{c}	2.54 ± 0.42^{c}	6.59 ± 0.15^{b}
15	92.5	40	75	0.917 ± 0.01^{d}	5.27 ± 0.03^{b}	48.25 ± 0.82^{a}	37.01 ± 0.27^{c}	5.79 ± 0.42^{a}	15.98 ± 1.56^{c}	2.54 ± 0.42^{c}	6.59 ± 0.15^{b}

Means with different superscripted letters in the same column were significantly different according to Duncan's test ($p < 0.05$).

Table 2. Physicochemical, technological and color parameters of rice, carob, chickpea, and mixture flours.

		Rice	Carob	Chickpea	Mixture flour
Physicochemical properties					
pH		5.6 ± 0.36^a	5.21 ± 0.05^a	5.29 ± 0.11^a	5.29 ± 0.16^a
a_w		0.381 ± 0^c	0.51 ± 0^b	0.59 ± 0^a	0.5 ± 0^b
Moisture (%)		10.75 ± 0.15^d	11.22 ± 0.48^c	14.22 ± 0.73^a	12.08 ± 0.2^b
Protein (% DM)		9.02 ± 0^c	7.23 ± 0.01^d	27.01 ± 0.02^a	16.37 ± 0.02^b
Fat (% DM)		3.17 ± 0.83^b	2.06 ± 0.17^c	6.41 ± 0.17^a	3.59 ± 0.5^b
Ash (% DM)		0.25 ± 0.08^c	3.64 ± 0.15^a	2.94 ± 0.52^b	2.37 ± 0.4^b
Carbohydrate (% DM)		85.21 ± 0.91^a	83.67 ± 0.47^b	59.67 ± 0.58^c	73.27 ± 0.86^c
Fibers (% DM)	Total Fiber	19.64 ± 0.36^a	8.67 ± 0.11^d	14.54 ± 0^c	16.87 ± 0.03^b
	Insoluble Fiber	11.57 ± 0.42^a	4.85 ± 0.12^d	6.4 ± 0^c	9.21 ± 0.79^b
	Soluble Fiber	8.07 ± 0.39^a	3.83 ± 0.11^c	8.31 ± 0.04^a	7.66 ± 0.41^b
Technological properties					
Swelling capacity (cm^3/g)		0.13 ± 0.13^c	0 ± 0^c	0.63 ± 0.13^b	0.75 ± 0.25^a
Water solubility index (%)		15 ± 0.3^d	55 ± 0.15^a	28.5 ± 0.5^c	32.5 ± 1^b
Water-holding capacity (g/100 g)		114.8 ± 1.5^d	218.8 ± 0.6^a	206.35 ± 0.95^b	159.75 ± 0.75^c
Oil-holding capacity (g/g)		1.70 ± 0.18^c	3.08 ± 0.01^a	2.58 ± 0.11^b	2.52 ± 0.08^b
Color parameters					
L^*		93.55 ± 0.02^a	51.43 ± 0.17^d	79.02 ± 0.4^c	89.65 ± 0.28^b
a^*		1.47 ± 0.01^b	6.43 ± 0.02^a	1.48 ± 0.04^b	0.48 ± 0.03^c
b^*		4.63 ± 0.02^c	16.27 ± 0.12^b	21.23 ± 0.04^a	5.98 ± 0.11^c
C^*		4.85 ± 0.01^d	17.48 ± 0.12^b	21.28 ± 0.04^a	6.00 ± 0.11^c
$h°$		107.67 ± 0.16^a	68.45 ± 0.19^c	86.01 ± 0.1^b	85.39 ± 0.21^b

Means with different superscripted letters in the same row were significantly different according to Duncan's test ($p < 0.05$). DM: dry matter.

assessed using a 5-point hedonic scale, where point 5 means 'like extremely' and point 1 denotes 'dislike extremely'. Samples without crust were cut in slices (thickness about 1 cm). Water was provided for rinsing between testing samples. These experiments have been conducted according to established ethical guidelines, and informed consent was obtained from all participants prior to conducting the tasting tests.

2.7. Statistical analysis

All experimental analyses and measurements were performed in triplicate and were mentioned as mean values ± standard deviation. The Duncan's procedure was used to assess the significant differences between samples ($p < 0.05$) using the SPSS statistics 19. The design, the mathematical modeling and all statistical tests of the experimental design using Box-Behnken design were done using Minitab (version 16, Minitab Inc, Launcher).

3. Results and discussion

3.1. Physicochemical and technological properties of the flour samples

The flour mixture is composed of 50% rice, 40% chickpea, and 10% carob flours. The physicochemical composition, technological properties and color parameters of the three flours are summarized in Table 2.

The rice, carob and chickpea flours used in this study have very close pH values ($p > 0.05$) of 5.6 ± 0.36, 5.21 ± 0.05 and 5.29 ± 0.11, respectively (Table 2). These values are in the same range given by Jagelaviciute and Cizeikiene (2021). Water activity values vary significantly ($p < 0.05$) between 0.38 ± 0 for rice flour, 0.51 ± 0 for carob flour and 0.59 ± 0 for chickpea flour. These values have led to values <0.495 for the flour mixture, thereby ensuring its preservation. Significant differences ($p < 0.05$) are obtained for protein contents of the studied flours as shown in Table 2. Indeed, chickpea flour had the highest protein content of 27.01 ± 0.02% DM, while the lowest content was attributed to carob flour of 7.23 ± 0.01% DM, which was comparable to the findings of Youssef et al. (2013). However, rice flour is found to have an average protein content of 9.02 ± 0% DM. This last value is higher than that mentioned by Ciqual (2019). Moreover, protein content of the optimized flour mixture shows a significant value (16.37 ± 0.02% DM) which is higher ($p < 0.05$) than that obtained for wheat flour (9.31 ± 0.86% DM) (Ammar et al., 2021).

Fat content in chickpea flour is significantly higher ($p < 0.05$) than those in rice and carob flours (6.41 ± 0.17 *vs* 3.17 ± 0.83 and 2.06 ± 0.17, respectively). Ash content indicates the purity of the flour. This latter could be affected significantly the rate of extraction and the mineralization of milled grains (Colas, 1998; Feillet, 2000). The studied mixture of flours contains an ash content of 2.37 ± 0.4% DM, which is lower than that found by Arribas et al. (2017). In addition, Emire and Tiruneh (2012) reported that for human food, it is better to use a flour with low ash content. Fiber content of mixture flour is 16.87 ± 0.03% DM, higher than the 15.03% reported in Arribas et al. (2017). High crude fiber is nutritionally appreciated and is favorable for use in formulating foods lacking this nutrient. The proximate composition results demonstrate that mixture flour could replace gluten proteins in food products.

The color of flour is an important criteria that affects the hedony of new developed food. In fact, chickpea, carob and rice flours are markedly different in terms of color (Table 2). The lightness of flour is affected by the ash content (Kim and De-Ruiter, 1968). Rice flour has the highest L^* value (93.55 ± 0.02, $p < 0.05$), with low ash content indicating the whiter appearance of this flour. Carob flour, has the lowest L^* value (51.42 ± 0.17, $p < 0.05$) with high ash content, indicating a significantly darker flour. The coordinate a^* is significantly higher for the carob flour ($p < 0.05$), reflecting a more reddish hue for this flour, while the coordinate b^* was significantly higher for the chickpea flour, indicating a more yellowish hue, related to its greater lightness ($p < 0.05$). Overall, the mixture flour showed bright color with less intensity of the a^* and b^* values, making it appropriate for gluten-free bread formulation. WHC and WSI are important functional properties required in food formulations especially those involving dough operating. There are differences between WHC, WSI and swelling capacity of the flours which may reflect differences in the amount and nature of hydrophilic constituents (Olatunji et al., 1992). Carob flour has yielded significantly higher WHC and WSI values than rice and chickpea flours as shown in Table 2 ($p < 0.05$). This can be related to its higher fiber content.

3.2. Technological properties of the bread samples

The formulated breads exhibited a_w levels higher than 0.650 (Table 1), which makes these products vulnerable to microbiological alterations. Our results are comparable to those reported by Cappa et al. (2016) for the formulation of gluten-free breads. Significant differences ($p < 0.05$) are obtained for pH values between different bread formulations. A maximum pH value of 5.37 ± 0.04 corresponds to F1 while the

Table 3. Color parameters of different gluten-free bread formulations.

F	Crumb					Crust				
	L^*	a^*	b^*	C^*	$h°$	L^*	a^*	b^*	C^*	$h°$
1	53.08 ± 0.88^b	4.26 ± 0.02^c	14.82 ± 0.45^c	15.42 ± 0.43^c	73.93 ± 0.01^a	51.34 ± 0.84^a	9.39 ± 0.79^d	25.71 ± 0.95^b	27.38 ± 1.15^b	69.96 ± 0.9^a
2	54.15 ± 0.79^b	4.76 ± 0.07^b	15.21 ± 0.28^b	15.94 ± 0.26^c	72.6 ± 0.4^b	48.62 ± 0.87^b	10.88 ± 1.2^b	26.46 ± 0.84^b	28.62 ± 1.24^b	67.71 ± 1.54^a
3	51.55 ± 0.19^c	4.99 ± 0.22^a	15.33 ± 0.28^b	16.12 ± 0.29^b	72.05 ± 0.58^b	42.24 ± 2.04^c	13.62 ± 1.05^a	24.62 ± 1.67^c	28.17 ± 1.46^b	62.75 ± 0.17^c
4	54.21 ± 0.11^b	4.59 ± 0.19^b	16 ± 0.26^a	16.64 ± 0.29^b	74 ± 0.47^a	48.37 ± 0.33^b	10.08 ± 0.34^c	24.65 ± 2.64^c	26.74 ± 0.36^c	60.99 ± 0.27^d
5	58.04 ± 0.29^a	4.14 ± 0.16^c	15.68 ± 0.27^b	16.22 ± 0.26^b	75.2 ± 0.63^a	52.75 ± 0.42^a	11.53 ± 0.23^b	28.33 ± 0.61^a	30.65 ± 1.47^a	68.02 ± 0.5^a
6	60.26 ± 0.2^a	4.06 ± 0.11^c	15.6 ± 0.38^b	16.12 ± 0.4^b	75.39 ± 0.07^a	47.15 ± 1.41^b	14.8 ± 1.28^a	29.61 ± 1.07^a	33.12 ± 1.37^a	63.47 ± 0.35^c
7	50.31 ± 0.69^c	4.96 ± 0.22^a	15.29 ± 0.12^b	16.07 ± 0.18^b	72.1 ± 0.67^b	41.17 ± 0.98^d	13.45 ± 0.29^a	24.06 ± 0.43^c	27.56 ± 0.29^b	60.78 ± 1.63^d
8	50.27 ± 0.69^c	4.89 ± 0.14^a	14.7 ± 0.46^c	15.48 ± 0.48^c	71.6 ± 0.25^c	43.12 ± 1.16^c	10.94 ± 0.21^b	23.84 ± 0.67^d	26.23 ± 0.53^c	65.32 ± 0.88^b
9	56.4 ± 0.22^b	4.37 ± 0.04^c	15.13 ± 0.1^b	15.74 ± 0.09^c	73.88 ± 0.2^b	48.24 ± 2^b	14.44 ± 1.23^a	29.55 ± 0.45^a	32.91 ± 0.53^a	63.98 ± 1.02^c
10	56.49 ± 0.72^b	4.79 ± 0.2^b	16.46 ± 0.4^a	17.14 ± 0.43^a	73.76 ± 0.32^b	53.53 ± 0.8^a	8.83 ± 0.83^d	26.7 ± 1.69^b	28.12 ± 1.86^b	71.73 ± 2.11^a
11	49.87 ± 0.12^d	4.9 ± 0.1^a	14.75 ± 0.32^c	15.54 ± 0.34^c	71.61 ± 0.1^c	43.58 ± 1.62^c	11.24 ± 0.15^b	24.28 ± 0.73^c	26.76 ± 0.69^c	65.16 ± 0.66^b
12	45.75 ± 0.31^d	4.98 ± 0.14^a	10.1 ± 5.91^d	14.95 ± 0.42^c	70.55 ± 0.65^c	39.55 ± 1.34^d	12.54 ± 0.66^b	22.49 ± 1.4^d	25.76 ± 1.46^c	60.83 ± 0.64^d
13	52.49 ± 0.43^c	4.85 ± 0.04^a	15.01 ± 0.08^b	15.74 ± 0.11^c	72.07 ± 0.21^b	47.96 ± 1.41^b	10.32 ± 0.96^c	25.76 ± 1.49^b	27.76 ± 1.71^b	63.98 ± 1.13^c
14	54.73 ± 0.19^b	4.57 ± 0.29^b	15.41 ± 0.5^b	15.89 ± 0.21^c	73.45 ± 0.13^b	47.46 ± 1.41^b	10.30 ± 1.06^c	25.78 ± 0.34^b	27.79 ± 0.67^b	68.2 ± 1.73^a
15	54.73 ± 0.19^b	4.57 ± 0.29^b	15.41 ± 0.5^b	15.89 ± 0.21^c	73.45 ± 1.34^b	47.46 ± 1.41^b	10.30 ± 1.06^c	25.78 ± 0.35^b	27.79 ± 0.67^b	68.2 ± 1.73^a

Means with different superscripted letters in the same column were significantly different according to Duncan's test ($p < 0.05$).

minimum pH value was 4.9 ± 0.1 for F12 (Table 1). This can be explained by protein denaturation and the decrease in their solubility due to heat treatment, which directly affects the pH (Aguilera et al., 2009). This acidification results from the release of acid compounds during fermentation, which leads to a decrease in the pH values of the bread samples. The moisture content of gluten-free breads ranges from $31.36 \pm 0.79\%$ to $43.66 \pm 0.5\%$ (Table 1). This significant variation ($p < 0.05$) is correlated by the added water content in each formulation. In addition, moisture content of gluten-free bread is positively affected by moisture content of the flours used. Indeed, when the water content added in the recipe reaches the maximum (100 g/100 g of flour), the moisture of the final bread reaches an optimal value of 37.9±0.59%, which is the case of F4 (Table 1). This may be explained by the fact that higher amounts of water cannot be absorbed by the fibers; thus, an increase in the moisture content of the crumb is expected. Similar results have been obtained by other researchers. Indeed, Vittadini and Vodovotz (2003) report a decrease in the water content of soy-containing bread when increasing the amounts of soya flour. Tsatsaragkou et al. (2014) reveal that the moisture content of gluten-free model doughs ranges between 31.5 and 39.1%.

F10 bread (50:50 rice and mixture flours) yields the highest specific volume values (5.84 ± 0.25, $p < 0.05$). The volume of bread is strongly influenced by the amount of gas retained by the dough during the kneading stage. The higher the gas retention capacity of the dough is, the greater the volume of loaves becomes (Balla et al., 1999). The addition of various flours to gluten-free doughs generally increases the specific volume of bread. However, the interaction between the flour types, the concentrations used and the amount of water added can all affect the specific volume results. Azarbad et al. (2019) found that a gluten-free bread formulation including 17.8% rice flour, 67.2% sorghum flour and 15% millet flour results in a specific volume of about 2.56 cm^3/g, which is lower than those obtained in the current study (Table 1). Baking loss is important for the structural processing of the breads. From the obtained results (Table 4), the baking loss rate significantly and positively depends on both the amount of water added ($p < 0.001$) and the fermentation time ($p < 0.01$). Similar observation has been obtained by de la Hera et al. (2013) for a formulation of a gluten-free bread based on rice flour.

Color parameters of both the crust and the crumb of the studied bread formulations were measured after 24 hours of making (Table 3). For the crust, a significant difference is noted in the values of the $h°$ between the fifteen experiments ranging from $60.78 \pm 1.63°$ to $71.73 \pm 2.11°$ ($p < 0.05$). This variation in tone is accompanied by a significant increase in the crumb L^* ($p < 0.05$) from 45.75 ± 0.31 to 60.26 ± 0.2 for the different bread making tests, resulting in a decrease in the crust L^* from 39.55 ± 1.34 to 53.53 ± 0.8 (Table 3). In addition, a^* values are lower than b^* values for the crumb and the crust. Therefore, the variation in $h°$ indicates that the color of the bread samples is yellow. This behavior is consistent with the visual observation of the bread samples (Fig. 1a).

F3 bread returns the whitest crumb among all the bread samples tested. The crumb color for the different formulations tends to be yellow. This color is a result of the flours color used in the formulation (Tables 2 and 3). Fig. 1a depicts the color parameters of the gluten-free bread formulations. The crumb color of the bread is significantly lighter ($p < 0.05$) than that of the crust (Table 3 and Fig. 1a). The a^* value is significantly higher for the crust than for the crumb of all bread formulations ($p < 0.05$). The b^* value of the mixture flour significantly decreases for the crust ($p < 0.05$), thereby indicating a lower intensity of yellow color. As for the crumb, the flour color is the single most important factor that affects the crust color. The F1 and F11 based breads exhibit significantly higher ($p < 0.05$) hardness of all other bread formulations tested (Table 1). There is a negative correlation between the water amount added and the hardness (the corresponding coefficient $a_1 = -0.765$ is determined as very significant, $p < 0.01$; Table 4).

3.3. Study of the optimized conditions of bread formulation

Based on the Box-Behnken experiment design, the optimal levels for water amount, proofing times, and flour mixture amount were calculated among the determined models carried out. Table 4 shows the corresponding coefficients for each dependent variable (a_w, pH (24 h), dough moisture, bread moisture, bread specific volume, baking loss, hardness (24 h), masticability (24 h)). All models fit very well the experimental data of dependent variables since the *p-values* is ≤0.001 (and obviously the other statistical coefficients: R^2, R^2_{Adj} and RMSE), except for pH and masticability.

The amount of water added positively influences on (Table 4): pH ($a_1 = 0.042$), dough moisture (1.200), bread specific volume Vsp (0.229) and baking loss (2.602). However, it has a negative impact on the other responses: bread moisture ($a_1 = -0.46$), hardness (-0.765) and masticability (-0.278). Likewise, fermentation time presents also positive influences on some responses (a_w; bread specific volume; baking loss) and negative influences on others (pH; dough moisture; bread moisture) (Table 4). We can see also that the percentage of flour mixture presents practically the same behavior with those independent variables: positive influences on dough moisture, bread moisture, hardness and masticability; and negative influences on the others (Table 4). It is clear that there are some positive and negative values of the quadratic terms (a_{kk}, $k = 1, 2, 3$) of independent variables. Indeed, the negative value of a_{kk} means that the corresponding dependent variable presents a parabolic form with maximum as function of the corresponding variable (x_k), and the positive ones present the same form but with a minimum. About factor interactions, there are the two types of influences for all cases.

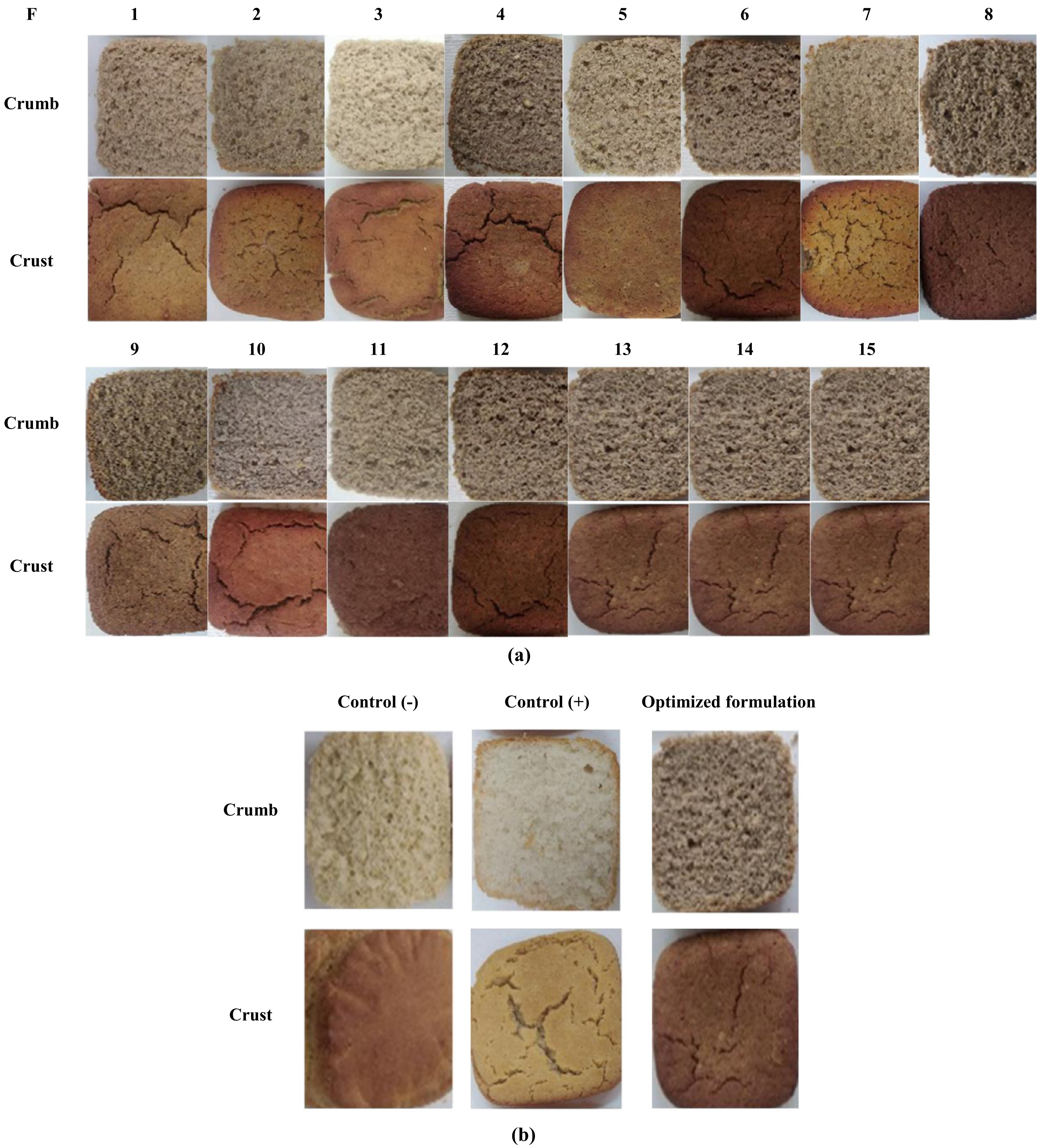

Fig. 1. Photos of gluten-free breads of the different studied formulations (a). Photos of optimized gluten-free bread in comparison with control (+) and control (−) breads (b).

Table 4. Model coefficients ($\hat{Y} = a_0 + \sum_{i=1}^{3} a_i \cdot x_i + \sum_{i=1}^{3} \sum_{i \neq j}^{3} a_{ij} \cdot x_i \cdot x_j + \sum_{i=1}^{3} a_{ii} \cdot x_i^2$) and regression parameters for the tested physicochemical and technological properties of gluten-free breads designed by Box-Behnken experimental design.

Response \ Coefficient	a_w	pH (24 h)	Dough moisture (%)	Bread moisture (24 h) (%)	Bread Vsp (cm^3/g)	Baking loss (%)	Hardness (24 h) (N)	Masticability (24 h) (N·mm)
a_0	0.926***	5.147***	47.352***	37.01***	3.478***	16.112***	2.743***	3.366***
a_1	−0.002	0.042	1.200*	−0.46	0.229**	2.602***	−0.765**	−0.278
a_2	0.004	−0.072*	−0.526	−0.45	0.477***	1.363**	−0.470	−0.147
a_3	−0.002	−0.051	1.607**	1.96	−0.240**	−1.479**	0.606*	1.307***
a_{11}	0.006	−0.009	−1.000	0.32	−0.731***	−1.510*	0.152	−0.224
a_{22}	−0.004	−0.046	−0.302	−0.25	−0.179	0.729	1.087**	0.221
a_{33}	−0.009**	−0.005	−1.455	−2.19	0.155	2.323**	0.150	−0.116
a_{12}	−0.014***	0.073	2.449**	−0.77	0.055	1.223*	0.586	0.168
a_{13}	−0.002	−0.006	−1.352*	−3.21*	−0.121	0.176	−0.143	−0.363
a_{23}	−0.002	−0.026	1.518*	−0.70	−0.165	1.437*	−0.105	−0.045
p-value	0.001**	0.08	< 0.001***	< 0.001***	< 0.001***	< 0.001***	< 0.001***	0.066
R^2	56.20%	35.42%	60.61%	26.47%	82.93%	74.55%	48.97%	36.54%
R^2_{Adj}	43.88%	17.26%	49.54%	5.79%	78.13%	67.39%	34.62%	18.69%
RMSE	0.011	0.136	2.254	5.021	0.308	2.058	1.198	1.591

a_1: coefficient of amount of water added (coded variable); a_2: coefficient of fermentation time (coded variable), and a_3: coefficient of percentage of flour mixture (coded variable); a_{ii}: second order coefficients; a_{ij}: interaction coefficients.

*: significant influence or regression ($p < 0.05$).

**: very significant influence or regression ($p < 0.01$).

***: very highly significant influence or regression ($p < 0.001$).

Table 5. Physicochemical parameters of bread samples optimized in comparison with control (+) and control (−) breads.

Parameter		Optimized formulation		Control (−)		Control (+)	
Moisture (%)	Dough	49.85 ± 1.1^b		48.98 ± 1.07^c		52.14 ± 0.85^a	
	Bread	41.32 ± 1.27^a		32.38 ± 0.88^c		33.40 ± 2.46^b	
pH		5.10 ± 0.08^c		5.58 ± 0.07^a		5.42 ± 0.2^b	
a_w		0.931 ± 0^a		0.956 ± 0^a		0.918 ± 0^a	
Vsp (cm^3/g)		3.73 ± 0.37^b		5.02 ± 0.64^a		2.93 ± 0.21^c	
Baking loss (%)		22.98 ± 0.94^c		27.62 ± 0.13^b		31.65 ± 0.72^a	
		2 h	24 h	2 h	24 h	2 h	24 h
Crumb color	L^*	53.63 ± 0.56^c	49.88 ± 0.7^d	50.43 ± 0.36^d	47.99 ± 0.24^e	64.66 ± 0.8^b	67.71 ± 0.24^a
	a^*	4.75 ± 0.09^d	7.85 ± 0.46^c	15.85 ± 0.74^a	16.04 ± 0.8^a	8.8 ± 0.33^b	9.15 ± 0.28^b
	b^*	15.24 ± 0.33^e	22.7 ± 0.4^d	28.5 ± 0.3^b	26.9 ± 0.47^c	32.28 ± 0.9^a	32.4 ± 0.26^a
	C^*	15.96 ± 0.31^b	15.88 ± 0.85^b	32.61 ± 0.49^a	31.33 ± 0.6^a	33.57 ± 0.21^a	33.68 ± 0.53^a
	$h°$	72.68 ± 0.54^b	71.07 ± 0.16^b	60.92 ± 0.08^c	59.18 ± 0.94^c	74.85 ± 0.8^a	74.29 ± 0.6^a
	ΔE	17.58 ± 0.37^a	9.28 ± 0.51^b				
Crust color	L^*	46.86 ± 0.94^e	54.44 ± 0.33^d	70.32 ± 0.06^c	68.90 ± 0.66^c	85.29 ± 0.1^a	76.89 ± 0.69^b
	a^*	9.19 ± 0.28^a	4.83 ± 0.09^b	0.99 ± 0.14^c	1.06 ± 0.1^c	0.13 ± 0.14^d	0.27 ± 0.09^d
	b^*	24.48 ± 0.15^a	15.59 ± 0.55^c	19.31 ± 0.69^b	19.46 ± 0.16^b	8.82 ± 0.49^e	13.81 ± 0.51^d
	C^*	26.16 ± 0.53^a	16.08 ± 0.74^c	19.99 ± 0.22^b	18.54 ± 0.93^b	9.06 ± 0.17^e	15.2 ± 0.51^d
	$h°$	69.54 ± 0.7^d	72.78 ± 0.58^c	60.92 ± 0.08^e	86.89 ± 0.32^b	74.85 ± 0.8^c	88.91 ± 0.34^a
	ΔE	25.39 ± 0.9^a	15.43 ± 0.5^b				
Hardness (N)		1.84 ± 0.37^d	3.37 ± 0.07^c	0.81 ± 0.12^e	0.65 ± 0.18^e	3.55 ± 0.83^b	4.41 ± 0.49^a
Masticability (N mm)		2.5 ± 0.24^c	3.01 ± 0.45^b	3.28 ± 0.43^b	2.86 ± 0.27^c	6.76 ± 0.13^a	2.93 ± 0.21^b

Means with different superscripted letters in the same row were significantly different according to Duncan's test ($p < 0.05$).

Moreover, it can be seen that some independent variables more or less significantly affect all the dependent variables studied ($p < 0.05$). For example, the fermentation time influences: (1) very significantly dough moisture, specific volume of bread, and baking loss ($p < 0.01$), and (2) very highly significantly masticability ($p < 0.001$).

For all those behaviors, it is necessary to use the optimizer tool of Minitab software in order to determine the most appropriate formulation which is chosen to produce the best quality properties such as hardness and specific volume. The optimized formulation was calculated to contain 70% of mixture flour, 100% water and a proofing time of 40 minutes.

The optimized formulation results in a significantly ($p < 0.05$) higher moisture content than control (+) and control (−) breads (Table 5). Moisture content is influenced by the added and retained water levels. It depends on the starch/flour base as well as on the type and level of dietary fiber in the formulation (Capriles and Arêas, 2014).

On the other hand, the control (−) bread registers the highest Vsp by far among all the tested samples ($p < 0.05$, Table 5). This can be due to both the quality and quantity of gluten proteins, which can contribute to the increase in Vsp, thus the establishment of a homogeneous alveolar structure. The amount of gas production rises with the increase of fermentation time (Saad et al., 2015). During bread-making, these proteins form an impermeable, three-dimensional network capable of retaining carbon dioxide at the time of dough fermentation and forming a fine and regular alveolar structure after gas expansion at the time of baking (Fould-Springer and Bellamy, 1996). The optimized formulation shows a sufficiently higher Vsp of 3.73 ± 0.37 than that of control (+) bread (2.93 ± 0.21) ($p < 0.05$, Table 5). The obtained result of the current study is relatively higher compared to that reported by Santos et al. (2021) (3.73 *vs* 2.53 cm^3/g) who have investigated the effects of chickpea flour and psyllium on gluten-free bread quality.

The bread prepared with 100% rice (control +) and the bread with 100% wheat flour (control −) yield significantly higher ($p < 0.05$) baking loss than the optimized formulation. This behavior could be due to the weakness of the starch network due to the presence of fibers as reported for gluten-based and gluten-free matrices (Cabrera-Chávez et al., 2012). Interestingly, the optimized formulation has resulted in the lowest baking loss. Therefore, it can be concluded that the optimized formulation is able to maintain the integrity of the bread during baking.

Color parameter is important because of its great relationship with sensory properties of bakery products. Thus, two periods (2 and 24 h) were fixed to evaluate this attribute for both the crust and the crumb of the tested bread samples. For the crumb, the L^*, ΔE value of the

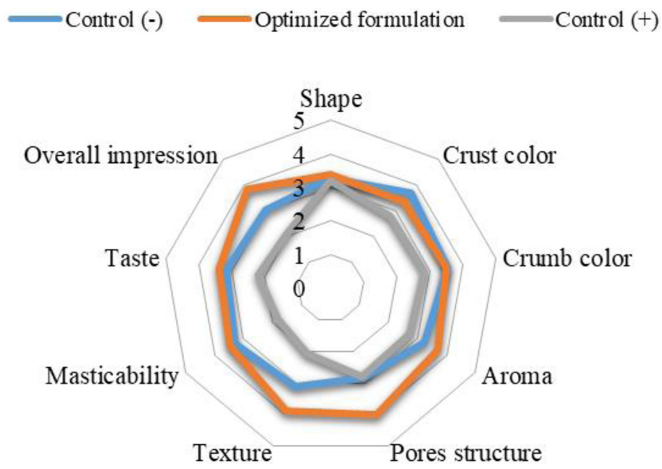

Fig. 2. Sensory evaluation of optimized Gluten-free bread in comparison with control (+) and control (−) breads.

bread decreased, *a*, *b* values increased. The color of the optimized formulation had an absolute dominance of the carob color, which led to the decrease in the brightness of the bread (Fig. 1b). Upon baking at 240 °C, the crust' darkness could be explained by the Maillard reaction between amino acids and reducing sugars. The use of mixture flour would contribute to this reaction by leading to an increase in protein and sugar contents of the bread. Indeed, the higher the sugar contents of the flour are, the more pronounced the Maillard reaction is. The a^* and b^* values of the crumb are significantly higher for the control (−) and control (+) breads at 2 and 24 h. On the other hand, a^*, b^* and C^* of the crust have increased for the optimized sample, indicating a higher color intensity. The color improvement caused by mixture flour is advantageous since the darker color is desirable by consumers compared to pale color (Campos et al., 2016).

Textural parameters are one of the main characteristics that determines the acceptability of new formulated food products. Generally, for gluten free bread, in particular, quick staling is one of the main problems since the presence of gluten could delay staling in the starch matrix (Ahlborn et al., 2005). Interestingly, the optimized sample of bread exhibited ($p < 0.05$) lower hardness that is significantly than that of the bread prepared with 100% rice flour (control +) (Table 5) at 2 and 24 hours after baking, which demonstrated that the optimized formulation can help reduce the hardness effects and improve the overall appearance of the bread. The reduced hardness compared to the control sample may be due to the water binding capacity of the fibre present in the mixture flour, preventing the moisture loss during storage and delay the retrogradation of starch. A similar explanation has been offered by O'shea et al. (2015) for the results observed on the softening effects of crumb in a gluten-free formulation enriched with orange pomace.

Moreover, the control bread containing only rice flour had a low volume, coupled with a hard texture, which confirms the negative correlation between the Vsp of the bread and its hardness (Table 4). Masticability was higher for the control (+) bread and lower than that of the control (−) bread for 2 h, but they are in the same range for 24 h. These results differ from those of Pessanha et al. (2021) and Torbica et al. (2019) who have developed breads from pseudo cereals and found that all breads made from gluten-free flours are harder and less elastic.

3.4. Sensory evaluation

The evaluation of sensory properties (aroma, taste, masticability, texture, color, shape, pores' structure and overall impression) of gluten-free breads prepared from the optimized formulation compared to control (+) and control (−) is shown in Fig. 2. The optimized gluten-free breads are liked by the consumers since their overall acceptability is in line with the minimum acceptance that a product requires in the market, which according to Spehar and Santos (2002) must be equal to or greater than 70%. Moreover, the color for either the crumb or the crust seemed to be liked with the same score (3.5) as the control breads judged by the participants. No significant difference has been noticed by the tasters between the studied bread samples tested for taste and aroma, with greater acceptability for the optimized bread (Fig. 2). The color of the optimized bread is more intense than the control breads, which is a very positive sign for the consumers. In fact, dark colored bread is much more often preferred by consumers due to the increasing conscience of the presence of health-promoting compounds (Campos et al., 2016). However, the control (+) bread with 100% of rice flour had the lowest scores across all attributes. As seen from Fig. 1.b, control (+) had a cracked crust and light color. The crumb color results showed that the control (+) were less desirable than control (−) and the optimized formulation. In contrast, the optimized formulation had less cracks and it has dark crust. Accordingly, the smooth surface and brown-colored bread were the most preferred by consumers. Overall the optimum gluten-free bread had the highest scores. The improvement effect of mixture flour on the sensory quality of bread could be due to its influence on the crumb softer and crust color.

4. Conclusion

The mixture of rice, carob and chickpea flours may be used in gluten-free bread formulations to produce bread with acceptable baking properties. The response surface design was successfully applied to the optimization of the gluten-free bread formulation and process conditions. The optimized formulation was set to contain 70% of mixture flour, 100% water and proofing time of 40 minutes. This formulation produced bread with higher specific volume, lower baking loss and lower hardness. In addition, the optimized bread was the most acceptable by the tasting participants. In general, findings indicated that the mixture flour based on chickpea, carob and rice represents a viable alternative to produce gluten free bread with acceptable baking properties. Accordingly, future studies should be undertaken to deeply investigate the nutritional aspect of the selected bread and its microstructure.

Declarations

Author contribution statement

Imène Ammar, Imène Felfoul: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper. Haifa Sebii, Takwa Aloui: Performed the experiments; Contributed reagents, materials, analysis tools or data. Hamadi Attia: Contributed reagents, materials, analysis tools or data; Analyzed and interpreted the data. Bilel Hadrich: Conceived and designed the experiments; Analyzed and interpreted the data.

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Data availability statement

Data included in article/supp.material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

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Additional information

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