Optimization of *Aspergillus oryzae* S2 α -amylase, ascorbic acid, and glucose oxidase combination for improved French and composite Ukrainian wheat dough properties and bread quality using a mixture design approach

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Abstract A simplex-centroid experimental design was used for the optimization of both reducing and oxidizing improvers, namely *Aspergillus oryzae* S2 α -amylase (Amy), ascorbic acid (Asc), and glucose oxidase (GOD). This optimization was performed to enhance the dough and breadmaking qualities of soft French wheat flour and a composite counterpart that contained 30% Ukrainian wheat flour. Statistically significant correlations were calculated between the W index and textural parameters (e.g., dough chewiness and bread cohesiveness). The findings revealed that while the best mixture for French flour comprised 21.8% of Amy, 41.2% of Asc, and 37% of GOD, for the composite counterpart, it comprised 2.3% of Amy, 66% of Asc, and 31.7% of GOD. These optimized mixtures rearranged soft French wheat flour and its composite counterpart to a good quality and an improved flour texture, respectively. Additionally, they increased the loaf specific volumes of the breads made from soft French wheat flour and its counterpart by 25.8 and 45.43%, respectively, significantly decreased the breads' susceptibility to microbial contamination, and reclassified the breads as "good" in terms of sensory attributes.

Keywords: α -amylase, ascorbic acid, glucose oxidase, dough properties, bread quality

Introduction

Bread is an important element in human diets in both developed and developing countries worldwide. Dough quality is mainly influenced by the presence of disulfide cross-linking generated by the oxidation of gluten sulfhydryl residues, amylose amount, and starch particle size distribution and digestibility (1). Selecting the type and levels of mixed additives is a crucial step in the baking process. Alpha-amylases (EC 3.2.1.1) are endo-acting amylases that hydrolyse α -(1-4) glycosidic bonds of starch polymers, thereby resulting in oligosaccharides with varying lengths and α -limit dextrins. Note that α -amylase increases the amount of fermentable sugar and therefore enhances the yeast fermentation and the Maillard reaction products, which, in turn, strengthen the flavor and color of bread (2). Additionally, α -amylase increases the resistance, elasticity, and softness of the dough (3) and decreases crumb firmness and hardness (4-6). However, it decreases dough extensibility and stability; this may be ascribed to the high

amounts of amylase-reducing oligosaccharides (2). These amylase-reducing products cleave the gluten disulfide bonds and therefore involve the alteration of such wheat proteins. Consequently, incorporation of oxidizing improvers would be required (2).

Compared with other cereal proteins, wheat gluten proteins have high levels of water-binding capacity, distinctive amino acid composition, and distinct particle size distributions; this contributes to their unique viscoelastic properties (7). Wheat flour contains glutathione (GSH), which is capable of opening the disulfide bonds of gluten proteins. It can, therefore, cause depolymerization of its network (8).

Ascorbic acid (Asc) added to flour is firstly oxidized by the endogenous Asc oxidase (E.C. 1.10.3.3) to its major stable oxidized product, i.e., dehydroascorbic acid (DAsc) (8). Consequently, GSH is oxidized to oxidized glutathione (GSSG) using DAsc as a cosubstrate. Additionally, the GSSG produced is able to block free protein-associated thiol groups. Hence, the addition of Asc allows an increase in dough strength, hardness, and bread volume (9-10) and a decrease

in crumb firmness (10) and crust thickness (3). It also amplifies extensibility when combined to the alpha amylase (3).

The use of oxidative enzymes instead of chemical oxidants is a very interesting option because they are inactivated early in the baking process prior to the distinct gelatinization of starch (11). The glucose oxidase (GOD) enzyme (EC 1.1.3.4) has generally been used as an oxidizing agent in the breadmaking industry. In the presence of oxygen, it catalyzes the oxidation of α -D-glucose to gluconic acid and hydrogen peroxide (H_2O_2). H_2O_2 oxidizes the free thiol groups of gluten proteins and lead to the formation of arabinoxylan cross-linkings, phenolic linkages, and oxidative gelation of water-soluble pentosans, thereby modifying the rheological characteristics of the dough (12). Several authors have, therefore, suggested that GOD could be used as a partial substitute for Asc (13). The addition of 0.001% GOD has, for instance, been reported to increase dough tenacity, elasticity, and extensibility (12-14).

The present study was conducted to improve the breadmaking functionalities of French wheat flour and a composite counterpart that contains 30% Ukrainian wheat flour. The choice of a 30/70 mixture of Ukrainian and French wheat flour is justified by an economic reason. A mixture containing a high proportion of weak, low-cost flour and a small amount of strong, expensive flour is generally used in baking, especially in less developed countries. A simplex-centroid mixture design (15) was adopted to find optimum mixture proportions of α -amylase (Amy), Asc, and GOD for enhancing the dough properties and bread quality of soft French wheat flour and a composite counterpart.

Materials and Methods

Materials The mixtures used in the experimental design were prepared using GOD (Bakenzyme[®] GOP 10000BG; DSM, Delft, Netherlands) produced by *Aspergillus niger* 10,000 U/g, a crude extract of *Aspergillus oryzae* S2 118148.14 U/g, which did not contain aflatoxins (16) formulated according to the method described in a previous study (2), and Asc (Asc, Cargill Food Ingredients, São Paulo, Brazil). Two wheat flour samples were used. The first sample was French soft whole wheat flour containing 0.66% ash, 9.65% protein (N[°]5.70), 21.5% wet gluten, and 14.5% moisture. The second sample was a composite wheat flour containing 70% soft French wheat flour and 30% Ukrainian hard wheat flour with 0.64% ash, 10.24% protein (N[°]5.70), 24% wet gluten, and 14% moisture.

Methods

Wheat flour characterization: The dry matter and protein content (N[°]5.7) of the wheat flour samples were determined using the standard methods 44-15A and 46-10A, respectively (17). The ash content was evaluated via combustion at 550°C in a muffle furnace for 12 h. The amount of wet gluten was determined according to a method described in a previous study (18). Tests were performed in

triplicates.

Evaluation of dough and bread texture: The textural parameters of the dough and bread were determined using a texture analyzer (Texture analyzer, Lloyd Instruments, Fareham, UK). It was equipped with a 19-mm-diameter cylindrical aluminum probe and a 1000 (N) load cell. The detection range was 0.05 (N). The samples (2 cm in thickness) of dough portions or bread slices were compressed to 50% of their initial height at a speed of 10 mm/s, with a 30 s interruption between the first and second compression. The sample was cut by an electronic knife to obtain a regular shape. Primary parameters [springiness, hardness, and cohesion] and secondary parameters [chewiness and adhesion] were determined from the curves using the well-known texture profile analysis (TPA) method (19). Hardness was defined as the peak force of the primary compression cycle; adhesion was defined as the essential work required for pulling the compressing plunger away from the sample; and cohesiveness was defined as the quotient of the positive areas of the subsequent cycle to the initial cycle area. The height during the moment that elapses between the end of the first compression cycle and start of the second compression cycle is established as springiness. Chewiness is calculated as the product of cohesiveness, hardness, and springiness (20). Assays were performed after cooling for 2 h at room temperature. The values are the averages of three different determinations.

Alveograph testing: The alveograph measurements were obtained under conditions of constant dough water content and mixing times using the standard AACC method 54-30 (17). The dough samples were prepared using 100 g of flour, 58.5 mL of distilled water, and 2.2 g of salt (NaCl). They were then mixed in an alveograph mixer for 6 min at 60 rpm, laminated, and left to rest for 20 min in the alveograph compartment. The following alveograph parameters were automatically recorded by the computer software R-Design (Pullman, WA, USA): maximum over-pressure needed to blow the dough bubble (P; index of resistance to extension), average abscissa at bubble rupture (L; index of dough extensibility), dough elasticity (Ela; index; P_{200}/P , P_{200} : pressure at 4 cm from the beginning of dough bubble), deformation energy (W) index (dough strength), and P/L ratio, which indicates dough quality. Two curves were plotted for each sample, and the analysis was realized using the Chopin S.A. (Chopin, Tripette et Renaud, Villeneuve La Garenne, French) at 25°C.

Baking test: The bread was prepared using the following formula (percentages on a flour weight basis): 100 g of flour, 3% of dry baker's yeast, 2.2% of salt, and 58.5% of water, Amy, Asc, and GOD. A separate control was set up with the same protocol and without the supplementation of enhancers for the two wheat flours. All the ingredients were mixed, and the dough was left to rest for 15 min at 30°C. The dough was then divided into 100-g portions, hand-molded, kept at 30°C and 96% of moisture for 30 min, and baked in an electric oven at 200°C for 18 min.

Sensory evaluation: The breads were chosen for sensory assessment on Day 8 of storage. The evaluation was conducted using the scoring system reported by Gomes-Ruffi *et al.* as the reference (6). The

scores (a maximum of 100 points) were determined for the following attributes: outside parameters (volume, crust color, shred, and symmetry), inside parameters (crust characteristics, crumb color, crumb structure, and crumb texture), and taste and aroma. This score was converted into a global concept evaluated as follows: very good (>90), good (80–90), regular (70–80) and detestable (<70). The breads were evaluated by a team of five trained panelists (three females and two males) selected from the graduate students and administrative staff of the University of Sfax, Tunisia, according to the following criteria: nonsmokers aged between 20 and 46 who consume bakery products regularly. These panelists had previously participated in training sessions to get accustomed to the sensory characteristics of bread. The sensory evaluation of each attribute was conducted by assigning scores as follows. Volume: 1=low extended and 20=high extended. Crust color: 1=extremely pale/ extremely dusky brown and 10=golden brown. Shred: 1=very coarse and 5=very fine. Symmetry: 1=with low end or overlapping and 5=symmetrical. Crust characteristics: 1=flat and uneven and 5=convex shape. Crumb color: 1=brown and 10=white. Crumb structure 1=small and closed pores and 10=foam-like structure. Crumb texture 1=doughy/sticky and 10=easy breakdown and leaves the mouth clean. Aroma 1=not at all intense and 15=very intense.

Water activity determination: Water activity was evaluated using a water activity measurement apparatus (water activity, AW SPRINT TH-500; Axair Ltd., Pfäffikon, Switzerland).

Microbiological analysis: A microbiological test was conducted to enumerate the flora that contaminate bread after eight days of storage. Plate count agar (PCA) and plate dextrose agar (PDA) media were used to enumerate bacterial and fungal flora, respectively. One gram of the bread was homogenated with 10 mL of (0.9%) sterile saline solution, and sequential dilutions (10^1 , 10^2 , 10^3 , and 10^4) were performed. Additionally, 1 mL of each dilution was plated in triplicate on PCA and PDA plates and incubated for 24 h at 37°C and 48 h at 30°C, respectively. The plates with 30–300 colonies were chosen to evaluate the viable cell concentrations expressed in the colony forming units (CFU)/g of the sample (21). The assay was conducted in

triplicate.

Bread specific volume: The bread specific volume was evaluated via rapeseed displacement, as described in a previous study (2). The specific volume was the quotient of bread volume (cm^3)/bread weight (g). Improvement in the bread loaf specific volume was calculated as follows:

$$\text{Improvement} = \left[\frac{(\text{specific volume of bread test})}{(\text{specific volume of control sample})} \right] \times 100$$

All experiments were performed in triplicates, and the results were calculated as mean values.

Experimental design: A simplex-centroid experimental design (15) was employed to investigate the appropriate concentration and effects of the three components in the mixture (Table 1), namely Amy (x_1), Asc (x_2), and GOD (x_3). For 10 kg of wheat flour, x_1 varied from 4 to 7 mg, x_2 from 0.4 to 1 g, and x_3 from 0.5 to 1.5 mg. The wheat flour corresponding to each experiment was assayed with the total enhancer mixture weight kept constant. The variations selected for each component were based on the standard values accepted by the bread quality legislation.

Equations modeling: The experimental results obtained via instrumental determinations were applied to achieve the regression models (Table 1). On the basis of the experimental design, the cubic model could be fitted in case the lacks of fit of the quadratic or linear models were statistically significant ($p < 0.05$).

$$y_i = b_1 * x_1 + b_2 * x_2 + b_3 * x_3 + b_{12} * x_1 x_2 + b_{13} * x_1 x_3 + b_{23} * x_2 x_3 + b_{123} * x_1 x_2 x_3;$$

where y_i is the evaluated response, b_i represents the coefficients estimated using the least square method, x_1 is Amy content, x_2 is Asc content, and x_3 is GOD content; x_i refers to the dependent variables, i.e., $1 \geq x_i \geq 0$ and $\sum x_i = 1$. The quality of the fit of the models was determined via analysis of variance (ANOVA), in which the repetition supplied the degree of freedom to obtain the pure error. The calculations and the graphics were carried out by NEMRODOW (NEMRODOW, Version 2000, Marseille, France).

Table 1. Centroid-simplex experimental design for the actual and coded values

Proportion of each compound in the mixture ($x_1/x_2/x_3$)		Values of each compound (g) in 5 g of mixture ²⁾				
		Amy(x_1) (mg)	Asc (x_2) (mg)	GOD(x_3) (mg)	Base ³⁾ (mg)	Total ⁴⁾ (mg)
Assay ¹⁾	(0/0/0)	-	-	-	5,000.0	5,000
Mix 1	(1/0/0)	7	400	0.5	4,592.5	5,000
Mix 2	(0/1/0)	4	1,000	0.5	3,995.5	5,000
Mix 3	(0/0/1)	4	400	1.5	4,594.5	5,000
Mix 4	(0.5/0.5/0)	5.5	700	0.5	4,294.0	5,000
Mix 5	(0.5/0/0.5)	5.5	400	1	4,593.5	5,000
Mix 6	(0/0.5/0.5)	4	700	1	4,295.0	5,000
Mix 7	(0.333/0.333/0.333)	5	600	0.8	4,394.2	5,000

¹⁾parameters observed without any mix addition

²⁾Amy, *Aspergillus oryzae* S2 α -amylase; Asc, ascorbic acid; GOD, glucose oxidase

³⁾Wheat flour was used to keep the total enhancer weight constant.

⁴⁾Five grams of the total mixture were added to 10 kg of wheat flour.

Results and Discussion

The substitution of 30% of French whole wheat flour by Ukrainian wheat flour slightly enhanced the moist gluten content from 21.5% to 24%. The mix of a high proportion of weak, low-cost flour with a low amount of strong, expensive flour did not give a satisfactory result. In fact, Hrušková *et al.* (22) reported that moist gluten in flour must be in the acceptable range of approximately 27–37%, with 25 and 45% corresponding to weak and hard flours, respectively. French whole wheat flour and the substituted one were still noted to be weak flours. They were enriched by the addition of Amy, Asc, and GOD.

The effects of the interactions between these additives on the bread quality and dough properties were considered in the seven experiments of the simplex-centroid mixture design. The dough parameters and the bread quality were evaluated using improvers at each experimental point.

Alveographic analysis of composite and whole wheat flour The experimental values obtained for the alveographic dough parameters according to the experimental design for the substituted and French whole wheat flours are presented in Table 2. They fitted well into the equations, with regression coefficients and correlation values for adjusted cubic models having high determination coefficients (R^2) and, hence, a high quality of fit.

Dough elasticity (Elas index) was maximal when the two oxidant compounds (Mix 6) were added to the two wheat flours. This result was visualized in the contour plot of the elasticity response, where the highest values were given on the edge of Asc–GOD (Fig. 1A and 1B).

The increase in dough elasticity (Elas index) upon the addition of GOD and Asc was the result of a mixed oxidative system. The ameliorative effect of Asc could be ascribed to the aggregative aspect of GSSG and gluten proteins resulting from the oxidation of free thiol groups (9). GOD, on the other hand, could have allowed the generation of H_2O_2 , which oxidizes gluten thiol groups to form disulfide bonds and therefore improves dough elasticity (12). This synergistic effect of the two oxidant compounds on elasticity suggests that the H_2O_2 molecule could have contributed to the oxidation of Asc in addition to exerting a direct action on the gluten SH residues. Further studies are, however, needed to clarify this plausible mechanism.

Moreover, the Elas index was noted to increase in the mixture containing GOD and Amy (Mix 5) and the one containing Asc and Amy (Mix 4) for the substituted French whole wheat flour (Table 2). The improvement in elasticity due to the action of Amy could be explained via the starch network hydrolysis due to swelling, which lead to a dough with reduced strength (23). The W index, which reflects bread strength, showed the highest values on the edge of GOD–Asc and Amy–Asc–GOD center for French whole wheat flour and on the Asc vertex for the substituted French whole wheat flour. French whole wheat flour showed more possibilities of mixture applications to maximize this response variable.

With regard to these findings, we can conclude that the individual use of Asc as an oxidant can substitute the effect GOD–Asc in French whole wheat flour, but the combination of GOD–Asc is favorable to the dough rheological properties in the substituted one. These results indicate that oxidizing improvers differently altered the protein parts (gliadins, glutenins, globulins, or albumins) according to their special action processes (24–25). These results also show that

Table 2. Evaluation of the alveographic parameters and textural parameters of French soft whole wheat flour (1) and the substituted one (2). The regression and determination (R^2) coefficients were evaluated according to the adjusted models

		W index (*10 ⁻⁴ Joules)		P/L		Elas index		Dough chewiness (Nmm)		Bread cohesiveness	
		1	2	1	2	1	2	1	2	1	2
Experimental assays	Control ¹⁾	160±1 ^b	250±1 ^b	0.36±0.01 ^a	0.44±0.01 ^a	61±1 ^b	66±1 ^a	1.203±0.03 ^e	1.180±0.02 ^e	0.133±0.03 ^a	0.167±0.01 ^a
	Mix 1	130±2 ^a	230±2 ^a	1.08±0.12 ^e	0.73±0.04 ^c	57±1 ^a	76±1 ^b	2.045±0.04 ^f	1.921±0.027 ^f	0.117±0.01 ^a	0.148±0.02 ^a
	Mix 2	250±3 ^e	280±1 ^d	0.68±0.02 ^{bc}	0.71±0.03 ^c	81±0.5 ^e	88±2 ^c	0.481±0.01 ^{ab}	0.816±0.04 ^c	0.386±0.03 ^d	0.396±0.03 ^c
	Mix 3	230±2 ^d	260±1 ^c	0.72±0.01 ^{bc}	0.69±0.05 ^c	71±0.7 ^d	92±1 ^d	0.705±0.02 ^c	1.000±0.03 ^d	0.310±0.03 ^c	0.320±0.01 ^b
	Mix 4	210±1 ^c	310±3 ^f	0.63±0.03 ^b	0.56±0.03 ^b	63±0.2 ^c	95±2 ^d	0.887±0.05 ^d	0.511±0.06 ^a	0.230±0.01 ^b	0.522±0.03 ^d
	Mix 5	210±1 ^c	280±2 ^d	0.78±0.04 ^{cd}	0.71±0.01 ^c	71±0.4 ^d	94±3 ^d	0.836±0.06 ^d	0.816±0.01 ^c	0.240±0.02 ^b	0.396±0.03 ^c
	Mix 6	250±2 ^e	310±2 ^f	0.83±0.06 ^d	0.7±0.01 ^c	95±1 ^f	98±1 ^e	0.506±0.012 ^b	0.512±0.06 ^a	0.381±0.03 ^d	0.520±0.02 ^d
	Mix7	270±1 ^f	290±2 ^e	0.67±0.07 ^b	0.71±0.02 ^c	80±1.1 ^e	89±1 ^c	0.412±0.06 ^a	0.606±0.012 ^b	0.429±0.02 ^e	0.489±0.02 ^d
Regression coefficients	b1	130	230	1.08	0.73	57	76	2.04	1.92	0.11	0.14
	b2	250	280	0.68	0.71	81	88	0.48	0.81	0.38	0.39
	b3	230	260	0.72	0.69	71	92	0.70	1	0.31	0.32
	b12	80	220	-1	-0.64	-24	52	-1.50	-3.41	-0.08	1
	b13	120	140	-0.48	0	28	40	-2.15	-2.57	0.10	0.64
	b23	40	160	0.52	0	76	32	-0.34	-1.57	0.13	0.64
	b123	1081.01	-659.19	-1.34	1.92	39.24	-272.77	-6.05	5.46	4.01	-1.28
	R^2	0.921	0.925	0.989	0.941	0.998	0.901	0.95	0.95	0.95	0.95

Data expressed as means±SD. $n=3$. Means followed by the same letter within a column are non significantly different (* $p<0.05$).

¹⁾parameters observed in substituted soft French wheat flour without any mix addition.

Fig. 1. Mixture contour plot (left) and surface plot (right) of the effects of Amy, Asc, and GOD on dough elasticity. Part (A) corresponds to French soft whole wheat flour, and part (B) corresponds to the substituted one.

these oxidant agents with dissimilar biochemical activities could not replace each other for achieving an optimistic effect on bread behavior or product quality in the substituted French wheat flour. The P/L variable, which reflects the elasticity–extensibility balance parameters, showed highest values on the GOD–Amy edge for the substituted and French whole wheat flours, respectively.

Optimization of the alveographic parameters The models fitted in this study could also be used for optimization. Accordingly, the procedure was conducted to simultaneously maximize the three responses (Elas index, P/L, and W index). This optimization assay suggests that while a mixture containing 21.8% of Amy, 41.2% of Asc, and 37% of GOD could offer optimum alveographic parameters for French whole wheat flour, a mixture comprising 2.3% of Amy, 66% of

Asc, and 31.7% of GOD could be optimal for the substituted one. Applying the optimal conditions, the rheological dough parameters of French whole flour and the substituted one were significantly enhanced (Table 3). In fact, W (10^4 J), P/L, and the Elas index values were substantially higher than those of the control test. These optimized conditions induced dough elasticity increases of 39.3% and 46.9% for the substituted and French whole wheat flours, respectively. They also increased the P/L by 86.11% and 65.9%.

Textural profile analysis of the substituted French wheat flour The experimental values obtained for the textural parameters of the dough and bread for the substituted French wheat flour under investigation are presented in Table 2. The results showed that the highest values of bread cohesiveness were achieved via the

individual chemical or enzymatic oxidant treatment (Table 2). Compared with individual oxidant treatment, a mix of the oxidative system considerably increased bread cohesiveness. In fact, cohesiveness (26) was influenced by the abundance of gluten disulfide bridges induced by the number of oxidant improvers.

The study of textural parameter optimization involved maximizing bread cohesiveness and minimizing dough chewiness. The alveographic and textural parameter optimizations obtained for the composite and whole wheat flour coincided with that of the same mixture. We observed good correlations between the Windex and final texture values ($r > 0.92$).

Accordingly, the texture study showed that the incorporation of improvers had a significant effect on dough properties ($p < 0.05$). The most notable effect of additives on textural crumb bread was detected when a mix of Amy, GOD, and Asc was added. An optimistic effect of these improvers was observed on textural responses of dough. The mix of additives (Mix 7) had a significant effect on the textural parameters; this resulted in an optimum improvement of the dough rheological behavior. From these findings, we can deduce that the combination of various gluten modifying oxidants among Amy could be an excellent option to upgrade the overall quality of baked products. The optimal conditions allowed a significant enhancement of the textural parameters of French whole wheat flour and the substituted one compared with those of the control test. Indeed, these optimized conditions allowed an increase of 3.23 and 3.17% in bread cohesiveness fold for French whole wheat flour and the substituted one, respectively. They also decreased dough chewiness by 72.56 and 57.62%.

Effects of optimized mixture addition on volume, microbiological properties, and water activity of bread To validate the predicted results, dough and bread were prepared using the optimum mixtures for the two studied flours. The predicted and experimental results are presented in Table 3. The findings showed no significant differences between the theoretical and observed alveographic and textural parameters, thereby suggesting a good agreement between the predicted and experimental data. With regard to the Windex

parameter, and according to Bordes *et al.* (27), the optimized mixture additions rearranged French whole wheat flour to a good quality flour and the substituted French wheat flour to an improved flour.

Controlling the unwanted microorganisms in bread after cooking is an interesting approach because these microorganisms might proliferate through the commercial life-cycle of the bread; deteriorate the nutritional, textural, and sensorial properties; and cause several diseases (28). We had evaluated the potentiality of the optimized mixture to inhibit molds and bacteria growth after eight days of bread storage. Results presented in Table 4 indicate that these optimized additions significantly decreased the bread's susceptibility to microbial contamination. The analysis of these results shows that mold and bacteria counts determined via tests significantly differ from that of the control test for $p < 0.05$. The inhibitory effect of molds and bacteria spoilage could be ascribed to the significant lower water activity values (Table 4) of bread with added optimum enhancer mixtures. This inhibitory effect is slightly more pronouncing in the substituted wheat flour. The inhibitory effect of microorganism spoilage could be ascribed to the lower free-water content of the bread with added optimized improvers toward the control test. The decrease in the free-water content could be attributed to the ability of pentazans to attract large water content in the process of gelation after the addition of GOD (29). The results obtained through the evaluation of bread quality, which was conducted by a team of five bakery product specialists using the scoring system described by Gomes-Ruffi *et al.* (6), are presented in Table 4. The findings show that breads with optimized ameliorant proportion from assays of French whole wheat flour (B) and the substituted one (D) exhibit a better crumb quality than the control counterparts (A, D). The total scores from sensory attributes were 83.5 and 89.7% for B and D assays, respectively; this classifies the breads as good, according to Gomes-Ruffi *et al.* (6) and based on the results of alveographic and instrumental texture. The crumb qualities under the optimal conditions validated for both wheat flours are shown in Fig. 2. The corresponding crumb showed well-dispersed pores, thereby demonstrating good gas retention. The results also revealed that the application of the new optimized enhancer formulations for the two

Table 3. Estimated and observed textural and alveographic values for French soft whole wheat flour (1) and the substituted one (2) added with the corresponding optimized mixtures compared with that of the control counterparts

	Parameters	Control	Estimated value	Observed value	r (Windex-textural parameter)
1	W index ($\times 10^{-4}$ joules)	160 $\pm$ 1	275.32	270 $\pm$ 1	-
	Elas index	61 $\pm$ 1	85.06	85 $\pm$ 1	-
	P/L	0.36 $\pm$ 0.01	0.66	0.67 $\pm$ 0.01	-
	Dough chewiness (Nmm)	1.203 $\pm$ 0.003	0.34	0.33 $\pm$ 0.01	-0.958*
	Bread cohesiveness	0.133 $\pm$ 0.003	0.44	0.43 $\pm$ 0.02	0.972*
2	W index ($\times 10^{-4}$ joules)	250 $\pm$ 1	307.17	310 $\pm$ 3	-
	Elas index	66 $\pm$ 1	95.45	97 $\pm$ 2	-
	P/L	0.44 $\pm$ 0.01	0.70	0.73 $\pm$ 0.01	-
	Dough chewiness (Nmm)	1.180 $\pm$ 0.002	0.52	0.50 $\pm$ 0.01	-0.941*
	Bread cohesiveness	0.167 $\pm$ 0.001	0.50	0.53 $\pm$ 0.03	0.968*

Correlation coefficients between the Windex and textural parameters (r) were evaluated (* $p < 0.05$).

Table 4. Sensory evaluation, microbiological analysis, and water activity of (A) French soft whole wheat flour, (B) validated condition corresponding to French soft whole wheat flour, (C) the substituted one, (D) validated condition corresponding to the substituted one

Sensory evaluation		Assays			
Parameters	Maximum value	A	B	C	D
Volume	20	12±0.1	18±0.1	13±0.2	18.7±0.1
Crust color	10	4±0.1	8±0.2	4.5±0.1	9±0.1
Shred	5	2±0.1	4±0.1	3±0.2	4±0.1
Symmetry	5	2±0.1	3.5±0.2	2.5±0.3	4±0.1
Crust characteristics	5	2±0.2	4±0.1	2±0.1	4±0.1
Crumb color	10	6±0.2	8±0.2	5±0.1	9±0.2
Crumb structure	10	4±0.1	9±0.2	4.5±0.1	9±0.2
Crumb texture	10	5±0.1	8±0.1	5±0.1	9±0.1
Aroma	10	5±0.1	8±0.1	6±0.2	9±0.1
Taste	15	6±0.2	13±0.2	6±0.2	14±0.1
Total score	100	48±0.13	83.5±0.15	51.5±0.16	89.7±0.12
Microbiological analysis					
Mold (CFU/g of bread)		8.2×10 ⁴ ±0.457×10 ⁴	4.4×10 ² ±0.292×10 ²	8×10 ⁴ ±0.457×10 ⁴	4×10 ² ±0.292×10 ²
Bacteria (CFU/g of bread)		9.1×10 ⁴ ±0.125×10 ⁴	1.3×10 ³ ±0.132×10 ³	9×10 ⁴ ±0.125×10 ⁴	1×10 ³ ±0.132×10 ³
Water activity		0.84±0.01	0.64±0.01	0.82±0.01	0.68±0.02
Loaf specific volume (cm ³ /g)		4.88±0.09	6.14±0.05	5.04±0.05	7.33±0.4

Water activity of bread was evaluated after eight days of storage. Means followed by the same letter within a line are non significantly different (**p*<0.05). All values given are means of three determinations; ±SD, Standard deviation of three replicates.

Fig. 2. Crumb aspects of (A) the control of French soft whole wheat flour, (B) the validated condition corresponding to French soft whole wheat flour, (C) the control of the substituted one, and (D) validated condition corresponding to the substituted one.

wheat flours gave satisfactory crust color and bread appearance. Regarding the sensory evaluation, the optimized mixture gave a more golden brown crust color and whiter crumb color with French whole wheat flour and with the substituted one compared with the control counterparts. The optimized conditions allowed an increase of 25.8 and 45.43% in the bread volume for French whole wheat flour and the substituted one, respectively. The breads obtained with these optimized mixtures exhibit a marked improvement in crumb structure. The resulting loaves are characterized by a soft, fine crumb structure (30). We also verified a beneficial effect on the sensory attributes of bread, including aroma and taste.

In this study, we aimed to optimize both reducing and oxidizing improvers for enhancing dough and bread qualities of soft French wheat flour and a composite counterpart. We found that Amy increases dough resistance; however, it reduces dough extensibility

and stability possibly because of the gluten protein alteration due to high amounts of amylase-reducing polysaccharides. Considering that breadmaking performances are strongly related to the interplay between dough resistance and extensibility, the incorporation of oxidizing improvers would, consequently, be required. For 10 kg of French whole wheat flour and the substituted one, a 5-g mixture containing 21.8% of Amy, 41.2% of Asc, and 37% of GOD and 2.3% of Amy, 66% of Asc, and 31.7% of GOD, respectively, could impart optimum bread qualities. These optimized mixtures rearranged soft French wheat flour and its composite counterpart to good quality and an improved flour, respectively, and increased loaf specific volumes by 25.8% and 45.43% and dough elasticity by 39.3 and 46.9%, respectively. Moreover, a satisfactory crumb structure (texture and porosity) were achieved.

Ethical statements This article does not contain any studies performed by any of the authors involving human participants or animals.

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