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PHYSICAL AND SENSORY PROPERTIES OF FRESH BREAD WITH ADDITION OF THE ENZYMES LACCASE, XYLANASE, AND LIPASE

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ABSTRACT

The aim of this investigation was to evaluate some physical and sensory properties of bread made with a combination of different enzymes such as laccase, xylanase, and lipase in order to propose a baked good product without chemical additives. The methodology included determining volume and specific volume, analyzing the internal characteristics of bread and internal bread color in accordance with IRAM Norm 15858-1. A sensory analysis was performed using a triangular test consisting of 38 untrained judges. The significant differences between results were analyzed by Bengtsson's tables with a significance level of 95%. The main results included that the volume of the bread obtained with different formulations ranged between 476cm3 and 784 cm3, and the specific volume obtained for the formulation of bread composed of laccase-xylanase-lipase was 5.23 cm3/g. As for the sensory analysis, there were no significant differences reported regarding the acceptability of bread made in this study versus traditional bread with chemical additives. It can be concluded that the combination of the three enzymes used (laccase, xylanase, and lipase) yielded a baked good product with specific characteristics.

KEYWORDS: bread, laccase, xylanase, lipase, physical properties, sensory analysis.

PROPIEDADES FÍSICAS Y SENSORIALES DE UN PAN FRESCO, CON LA ADICIÓN DE LAS ENZIMAS LACASA, XILANASA Y LIPASA

RESUMEN

El objetivo de la presente investigación fue evaluar algunas propiedades físicas y sensoriales de un pan elaborado con la combinación de las enzimas lacasa, xilanasa y lipasa, con el fin de proponer un producto panificable sin aditivos químicos. La metodología incluyó la determinación del volumen, volumen específico y análisis de características internas del pan como color de miga según la Norma IRAM 15858-1. El análisis sensorial se realizó mediante una

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prueba triangular compuesta por 38 jueces no entrenados, las diferencias significativas de los resultados se analizaron mediante las tablas Bengtsson's con un nivel de significancia del 95 %. Como resultado principal se obtuvo que el volumen de los panes obtenidos con las diferentes formulaciones varió entre 4,76 cm^3 y 7,84 cm^3, en tanto que el volumen especifico obtenido para la formulación de un pan compuesto por lacasa-xilanasa-lipasa fue de 5,23 cm^3/g. En cuanto al análisis sensorial, no reportó diferencias significativas la aceptabilidad del pan formulado en esta investigación versus un pan con aditivos químicos tradicionales. Se puede concluir que la combinación de las tres enzimas utilizadas dio un lugar a un panificado con características propias del producto.

PALABRAS CLAVE: pan, enzimas, lacasa, xilanasa, lipasa, propiedades físicas, análisis sensorial.

AS PROPRIEDADES FÍSICAS E SENSORIAIS DO PÃO FRESCO COM A ADIÇÃO DA ENZIMA LACASE, XILANASE E LIPASE

RESUMO

O objetivo deste estudo foi avaliar algumas propriedades físicas e sensoriais de um pão feito com a combinação das enzimas lacase, xilanase e lipase, a fim de propor um produto de padaria, sem aditivos químicos. A metodologia incluiu a determinação do volume, o volume específico e análise de recursos internos, tais como cor do miolo de pão de acordo com a norma IRAM 15.858-1. A análise sensorial foi realizada utilizando um teste de triângulo composto por 38 juízes não treinados, as diferenças significativas nos resultados foram analisados por tabelas do Bengtsson com um nível de significância de 95%. O principal resultado foi obtido, o volume dos pães obtidos com diferentes formulações variou entre 4,76 e 7,84 cm3 cm3, enquanto que o volume específico obtido para o desenvolvimento de um pão composto por lacase-xilanase - lipase foi de 5,23 cm3 / g. em quanto ao análise sensorial, não teve diferenças significativas a aceitabilidade de pão feito nesta pesquisa contra o tradicional pão com aditivos químicos. Pode concluir-se que a combinação das três enzimas utilizadas deu origem a um bom produto cozido com as suas próprias características.

PALAVRAS-CHAVE: pão, enzimas, lacase, xilanase, lipase, propiedades físicas, analise sensorial.

1. INTRODUCTION

Bread is one of the most widely consumed products worldwide. Germany is the country which consumes the most, at approximately 120kg per capita. In Latin America, the leading consumer is Chile, at 96kg per capita. Colombia is in fifth place at 23kg per capita (Fenalco, 2013). It has also been shown that prohibited additives such as bromate and potassium are used in bread-making in Colombia, making this product a potential health risk (Vega et al.: 2010). Given the above information and bread's importance for nourishment, it is necessary to undertake studies that allow us to obtain new baked good products based on new raw materials or natural additives that benefit consumer health. The food industry is constantly seeking raw materials since consumers are looking for healthy foods with fewer chemical additives, and the bread industry is no exception.

The trend in this industry is to use of new flours and enzymes or design baked good products for people who suffer from celiac disease. Some of these developments have been published by (Mahmoud et al., 2013; Perez et al.,2012; Gamonpilas et al., 2014; Van der Goot et al., 2011; Laureati et al. 2012; Korus et al., 2013; Korus et al. 2010), as well as in studies that aim to replace traditional additives, such as bromate and potassium, which are harmful to health (Ribotta et al., 1999; Solito & Pavesi, 2003).

Studies worldwide report the use of new raw materials other than wheat to make bread, as well as the use of new additives such as enzymes, prebiotic or probiotic products, added vitamins and minerals, and even green tea (Chen et al., 2014; Batifouliera et al., 2005; Morais et al., 2013; Mihhalevski et al., 2013; Demigne et al., 2006; Rosell et al., 2012; Zhou et al., 2007).

However, the use of new raw materials affects the physiochemical, sensory, textural, and rheological properties of bread products. On this matter, various authors have determined some of these properties of bread with regards to the raw materials used (Shittu et al., 2007; Abdelrahman et al., 2012; Bovell-Benjamin et al., 2008; Haros et al., 2013), reporting the valuing of properties, such as volume and specific volume, and determining the rheological, microbiological, and micro-structural parameters of breads made from yucca, wheat, garbanzo, sweet potato, and amaranth flours. Regarding sensory parameters, Mishra et al. (2012) and Arasaratnam et al. (2010) found these parameters for breads made from millet, wheat, and rice malt flours. Other studies in the same vein evaluating physical and sensory properties include those by Baiano et al. (2009); Vodovotz & Lodi (2008); Mandala (2005), Kim et al. (2013); Noor et al. (2013); and Arendt et al. (2010).

In addition, the search for natural additives or those which do not leave residuals in foods has led to the use of enzymes in food design. In the case of bread, studies have been performed on the use of different enzymes and their effect on this food's properties. Jiang et al. (2010) evaluated the effect of the enzyme xylanase derived from *Chaetomium* sp. on the volume of steamed bread, and Selinheimo et al. (2006) evaluated the effect of combining the enzymes laccase and xylanase on the rheological properties of bread dough. Likewise, Caballero et al. (2007) studied the different combinations of enzymes such as alpha-amylase, xylanase, protease, transglutaminase, glucosidase, and laccase to improve the rheological properties of bread dough and the effect of these combinations on bread's shelf life. Rosell & Singh (2004) improved the quality of bread made with rice flour by adding the enzyme glucose oxidase. Other studies, such as those by Madamwar et al. (2006), Stojceska et al. (2012), Schoenlechner et al. (2013), and Vega et al. (2010) determined physical sensory and fermentation properties for breads made with different types of enzymes, such as xylanase amylase, lipase, and gluco-oxydase transglutaminase and laccase.

The goal of this study was to formulate bread based on three enzymes - laccase, xylanase, and lipase - and evaluate different physical properties such as the bread's volume, weight, and symmetry, as well as its sensory characteristics (bread color, texture, crust, structure, aroma, and taste). This formulation is an alternative that moves toward creating healthy baked good products, that is, products without chemical contents. The physical and sensory characteristics of the breads formulated are compared to the formulation of bread with traditional additives, such as ascorbic acid and azidocarbonamide.

2. MATERIALS AND METHODS

2.1. Raw materials

To obtain the flour used in this study, wheat harvested in the municipalities of Azul and 25 de Mayo, Buenos Aires Province (Argentina) was milled. The environmental and geographical conditions in Azul and 25 de Mayo are: 137 masl with an average annual temperature of 15oC located at 36º46'39"S - 59º51'48"W and 58 masl with an average annual temperature of 18oC located at 35°25'41"S - 60°10'27"W, respectively. The wheat was stored for 24 hours in concrete silos and before milling, it was brought to a humidity of 16% from its initial humidity of 11%. After grinding, the product obtained was mixed to obtain 000 flour.

The enzymes were provided by different companies. The laccase was donated by Atime S.A., and the brand was Muhlenchemie (Alphamalt PPO MC9901001). The enzyme was synthesized using the fungus Myceliophthera thermophila produced by submerged fermentation in a genetically modified strain of Aspergillus orizae. The xylanase was acquired from Puratos Argentinos with the denomination 20ARG, and the lot number was 25014. The lipase was acquired from Granotec Argentina under the name Emulzime, and the lot number was 2006127017. Finally, the oxidizing agents, ascorbic acid (ASC) and azidocarbonamide (ADA), were acquired from Epecuen S.A, and their lot numbers were 7042605 and 70524001, respectively.

The raw materials for the bread-making include: milled flour (see number 1); compressed or dehydrated yeast: 3% or 1%, respectively, in relation to the flour; sugar: 2.5% in relation to the flour; salt: 1% in relation to the flour; water for analysis. Subsequently, a sugar and salt solution is prepared: for each test, 1 gram of salt and 2.5 grams of sugar are dissolved in 23.1 milliliters of water. For suspension of the yeast, 3 grams of yeast are weighed and mixed with 22.5 ml of water as homogeneously as possible.

The equipment used to make the bread includes a ± 0.01 gram scale, a semi-rapid dough mixer which operates at 90v/min, and a mechanical laminator and former. For the bread's form, metal pans with two 1.5mm layers of sheet steel were used. Their measurements were: 9.5cm x 5.5cm at the base, 10.5cm x 7cm at the open top and 5.5cm high. Regarding the fermentation process, an automatic regulation chamber for humidity (80 ± 5%) and temperature (30 ± 1oC) was used, and for the baking process, an electric oven with a resistant floor was used.

2.2. Bread baking

To bake the bread, IRAM Norm 15858-1 of December 1996, "Cereals; experimental bread-making test" was used. This method is used in wheat improvement programs. Before describing the bread-making process, it must be clarified that 7 formulations were made, as is described below:

F1: 000 flour;

F2: 000 flour + ADA + ASC;

F3: 000 flour + laccase;

F4: 000 flour + xylanase + ADA + ASC;

F5: 000 flour + laccase + xylanase;

F6: 000 flour + xylanase + lipase + ADA + ASC;

F7: 000 flour + laccase + xylanase + lipase.

In each of the formulations, 15ppm of ADA, 4ppm of ASC, 200ppm of lipase, 100ppm of xylanase, and 50ppm of laccase were used.

The comparisons made in this study were based on the role each additive or enzyme played in the formulation. As such, F2 was compared to F3 since both the chemical and enzyme additives play an oxidation and rheological role in the bread dough, in this case the chemical additives ADA and ASC (Quaglia, 1991; Solito & Pavesi, 2003), and for laccase (Selinheimo et al., 2006). Given that oxidizing agents tend to decrease the dough's elasticity, an enzyme or agent is required to improve this property. Therefore, a new formulation is made with the addition of xylanase to improve the dough's machinability (Williams & Pullen, 1998; Callejo, 2002). As such, formulations F4 and F5 are compared. Finally, it was decided that lipase would be added to improve the system's emulsion after a final comparison of formulations F6 and F7. F1 is bread without any kind of additives.

To make the bread, 100g of flour are mixed with 25 milliliters of the yeast suspension, the salt solution, and the sugar, along with 16.5ml of water, and it is kneaded for 3.5 minutes. The dough's final temperature should be 27 ± 1oC. The pre-fermentation is then done at 30 ± 1oC and 80 ± 5% for 85 minutes. During this part of the process, two punches, or soft hand kneadings, must be done to remove the carbon dioxide. The first punch is done 45 minutes after finishing mixing, and the second punch is done fifteen minutes after the first. Then the bread is formed, removing the ball of dough to flatten and smooth it with a wooden rolling pin until it forms a disc with a thickness of approximately 8mm. The bread is then formed.

For the final fermentation, the pan is taken to the fermentation chamber at 30oC and 80% for 75 minutes. It is then taken to baking at 225oC with the addition of water vapor through an injector. The bread is baked for 15 minutes in the pan, then it is taken out of the pan, and baking continues until 30 minutes are completed.

2.3. Determination of the bread's properties

The bread's properties were determined based on IRAM Norm 15858-1 of December 1996, "Cereals; experimental bread-making test," a method used in wheat improvement programs.

Bread volume: The bread's volume is measured one hour after baking using the volume measurement apparatus, which includes a recipient for the bread. The measurement is made of the canola or turnip seeds displaced by the space the bread occupies. The bread's specific volume is calculated as the ratio between the bread's net volume and its weight.

General evaluation: In order to evaluate the bread dough, it is allowed to cool and is weighed. Regarding the bread's exterior appearance, the bread's shape was evaluated: whether it is symmetrical, its color, and the shine of the crust. Regarding its interior appearance, this was evaluated two hours after removal from the oven. The bread is sliced and its texture, structure, and the interior color are evaluated.

TABLE 1. VALUES OF SOME OF THE BREAD'S PROPERTIES. Source: IRAM Norm 15858-1

Property	***Maximum score***
Volume	25
Crust	15
Texture	15
Interior color	15
Structure	10
Aroma	10
Taste	10

The bread interior's texture is classified on a scale that includes the following upper and lower limits: soft and elastic, rough and rigid. Regarding the structure, the bread is checked for homogenous small alveoli with thin walls, which receive the optimal evaluation. Finally, the color must be a cream white, which receives the optimal evaluation. Each of these parameters is evaluated according to the scores established by IRAM Norm 15858-1 of December 1996.

2.4. Sensory analysis

This analysis was done using a triangular test which consists of presenting the evaluator with three samples, of which two were the same. The taster must decide which is the different sample. The significant difference in the results is evaluated using Bengtsson's tables as a reference with a level of significance of 95%.

This evaluation was made with two panels of untrained evaluators totaling 38 judges. Panel 1 was made up of 31 evaluators and was divided into two groups of 15 and 16 evaluators, respectively. Panel 2 was made up of 7 evaluators. Also, three additional questions were asked of the evaluators to inquire about the difficulty of identifying the sample and the intensity with which each evaluator distinguished the different sample. Finally, each evaluator was asked to indicate which sample he or she preferred.

The evaluation charts for panel 1 were divided into two groups. For group 1 of the evaluators, the following notations were used: A: bread with laccase + xylanase + lipase; B and C: bread with xylanase + lipase + ADA + ASC. For group 2 of panel 1, the following notations were used: A: bread with xylanase + lipase + ADA + ASC; B: bread with laccase + xylanase + lipase. For panel 2, the following notations were used: A: bread with laccase + xylanase + lipase; B and C: bread with xylanase + lipase + ADA+ASC. The identification of each type of sample was done using random number tables. For each repetition, different charts were used, as shown in **Tables 2**, **3**, and **4**.

TABLE 2. CONTROL CHART FOR GROUP 1 OF EVALUATOR PANEL NO. 1

Evaluator no.	*Sample 1*		*Sample 2*		*Sample 3*	
1	A	785	B	137	C	377
2	A	416	C	161	B	446
3	C	490	A	714	B	141
4	C	743	B	437	A	378
5	B	358	C	529	A	537
6	B	181	A	814	C	149
7	A	430	C	309	B	979
8	B	345	A	450	C	57
9	C	527	B	373	A	228
10	B	684	A	969	C	342
11	C	565	B	537	A	591
12	A	663	C	732	B	104
13	C	590	A	778	B	429
14	C	116	B	805	A	906
15	B	965	C	769	A	485

TABLE 3. CONTROL CHART FOR GROUP 2 OF EVALUATOR PANEL NO. 1

Evaluator no.	*Sample 1*		*Sample 2*		*Sample 3*	
16	B	908	A	445	C	467
17	A	664	C	14	B	511
18	A	328	B	642	C	567
19	C	628	B	114	A	140
20	B	798	A	319	C	240
21	A	876	B	660	C	537
22	A	135	C	410	B	831
23	C	49	A	59	B	37
24	C	15	B	8	A	72
25	B	73	C	29	A	91
26	B	64	A	55	C	37
27	A	10	C	93	B	86
28	B	51	A	18	C	20
29	C	96	B	73	A	28
30	B	69	A	99	C	58
31	C	49	B	31	A	94

TABLE 4. CONTROL CHART FOR EVALUATOR PANEL NO. 2

Evaluator no.	*Sample 1*		*Sample 2*		*Sample 3*	
32	A	79	A	83	B	19
33	A	62	B	81	A	14
34	B	32	A	86	A	42
35	A	96	B	77	C	53
36	B	57	C	84	A	75
37	C	69	A	29	B	91
38	B	71	A	23	A	12

2.5. Statistical analysis

A free randomized experiment was designed taking a single factor, the bread's formulation, which had 7 levels. Response variables included the bread's volume in cm^3, its specific volume in cm^3/g, and the bread's color. The data was analyzed using an ANOVA table with a level of significance of 95% in order to establish whether there were significant differences between the groups. In order to establish between which groups there were differences, an LSD analysis was done. The software used was STATGRAPHICS Centurion XVI.I.

3. RESULTS AND DISCUSSION

Table 5 below shows the values obtained for each of the items.

Comparing the data obtained in this study to those found in the bibliography, Chen et al. (2014) found values for specific weight between 2.5 and $3.75cm^3/g$ for bread made with Bifidobacterium lactis Bb12. These values are lower than those reported in this study. However, this could be due to the fact that the baking time in the study by Chen et al. (2014) was 12 minutes compared to 15 in this study. Baking time influences this variable since a longer baking time leads to a greater specific volume. Jiang et al. (2010) and Caballero et al. (2007) give specific weight values between $2.04\text{-}2.54cm^3/g$ and $3.22\text{-}4.17cm^3/g$ for breads made with xylanase and other enzymes such as transglutaminase, glucose oxidase, and laccase, respectively. Authors like (Rosell & Singh, 2004; Madamwar et al., 2006; Stojceska et al., 2012; and Schoenlechner et al., 2013) found values between 1.6-3.73 cm^3/g for specific volume, all based on the bread's formulation. For gluten-free breads, Gamonpilas et al. (2014) report values of 1.85 to 2.84cm^3/g.

For bread volume, Noor et al. (2013), Korus et al. (2010), and Shittu et al. (2007) obtained value ranges of 544-804 cm^3, 600-800 cm^3, and 440 cm^3-920 cm^3 for breads made with banana and wheat with different types of maltodextrin and concentrations of the same formulations made with yucca and wheat flours.

Madamwar et al. (2006) determined the volume of bread made with xylanase and wheat bran, obtaining a value of 460 cm^3 with regards to the control bread, which was 300cm3. This result is in agreement with the results reported in this study since with the addition of the enzyme xylanase, that is, treatments F4 and F5 (see **Table 5**), the bread's volume increased due to the fact that xylanase improves the dough's extensibility and it can therefore retain more gas, which leads to a greater bread volume. Finally, Mishra et al. (2012) report volumes of 139-165 cm^3 for breads made with millet flour.

TABLE 5. VALUES OF PHYSICAL AND SENSORY PROPERTIES FOR EACH FORMULATION

DOUGH CHARACTERISTICS	***F1***	***F2***	***F3***	***F4***	***F5***	***F6***	***F7***
Dough weight	819	827	829	816	836	834	824
Bread weight	691.7	703.74	707.46	714.76	701.04	690.22	676.52
Bread volume in cm^3	560	476	480	676	652	792	784
Yield per kg	38.34%	40.75%	41.49%	42.95%	40.21%	38.04%	35.30%
Water loss in oven	15.54%	14.90%	14.66%	12.41%	16.14%	17.24%	17.90%
% of water loss	38.29%	36.79%	36.28%	30.22%	40.29%	42.92%	44.35%
EXTERNAL CHARACTERISTICS							
Volume	12.15	10.14	10.17	14.19	13.95	16.08	15.69
Symmetry	A*	S*	S	S	S	S	S
Crust color	8	8	9	12	12	10	13
INTERNAL CHARACTERISTICS							
Texture	10	7	10	12	11	10	12
Structure	5	5	8	10	9	11	10
Taste	10	10	10	10	8	9	8
Aroma	5	10	7	10	10	10	10
Color	5	8	8	10	8	11	10
Specific volume in cm^3/g	4.05	3.38	3.39	4.73	4.65	5.36	5.23
Specific weight in g/cm^3	0.25	0.3	0.29	0.21	0.22	0.19	0.19
FINAL SCORE	**60.15**	**66.14**	**70.17**	**88.19**	**80.95**	**77.08**	**78.69**

The variance analyses show that for the specific volume response variable, there were significant differences (P< 0.05) for the different formulations, except between F2 and F3. The same behavior was shown by the bread volume response variable, for which F2 and F3 did not show significant differences. However, for the other formulations, the value of p was < 0.05. Regarding color, the ANOVA table showed a p value of < 0.05, and the analysis of the minimum significant differences found homogeneity between F2, F3, and F5. These results can be seen in **Figure 1**, which shows the LSD graphs for these response variables.

Compared to formulation F1, formulations F2 and F3 decreased bread volume by approximately 14.5%. This is probably due to the increase in tenacity and, in the case of the chemical oxidants, also to the increase in elasticity. A decrease in specific volume was also observed. Virtanen et al. (1999) reported that laccase from Myceliophthora thermophila produced an increase in bread volume, which is contrary to the results obtained for F3. In that study, the reported work pH is 7.0, while the conditions for this study included a pH of 5.9, and it is probable that this had an influence on the reported differences.

There is a difference from the perspective of bread interior quality: the treatment with laccase formed a moister and spongier interior with small alveoli. However, with the addition of ADA-ASC, the bread interior lacked elasticity, the alveoli were very irregular, and some of them were large, in agreement with what is described in Ribolta & León (1999). This final point can be observed in the photograph of the slices of each bread (**Figures 2A** and **2B**). In the same figure, it can also be observed that the use of chemical oxidants created a thicker crust in comparison to the bread made with flour with added laccase. **Figure 2** shows the difference in size of the alveoli and the bread's interior characteristics. This can indicate that the bread made with the enzyme laccase is better than that made with chemical additives, which is shown in the scores assigned by the judges.

For formulations F4 and F5, the addition of xylanase along with the oxidants created a more extensible dough, favoring an increase in volume and improving the bread's interior texture with more uniform alveoli.

Figure 1. LSD analysis p< 0.05 for color, volume, and specific volume for the breads.

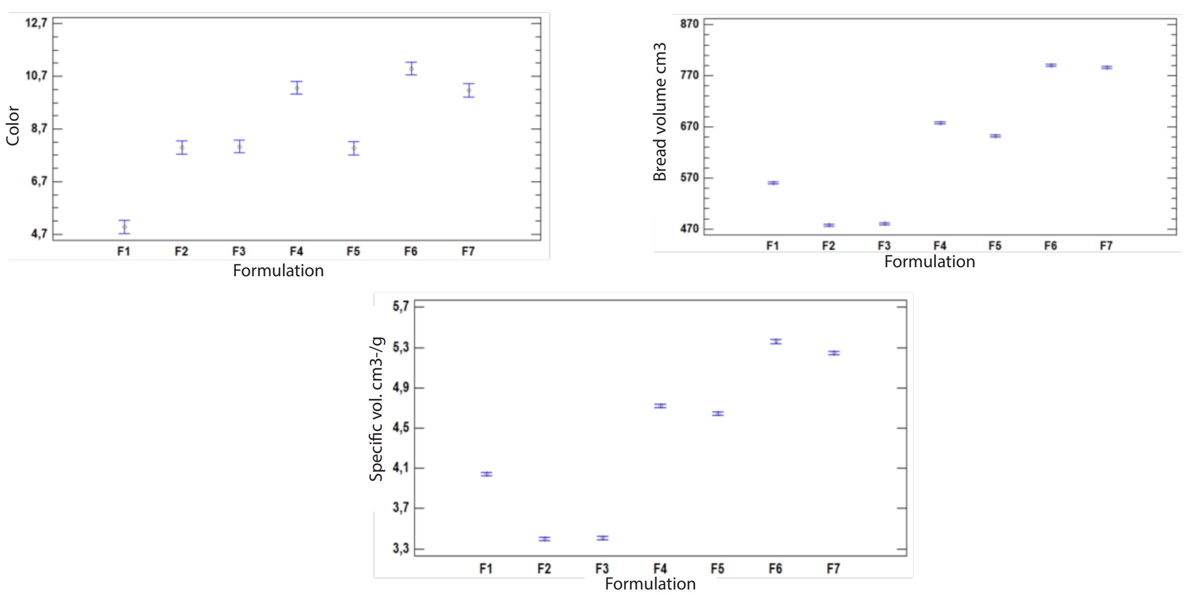


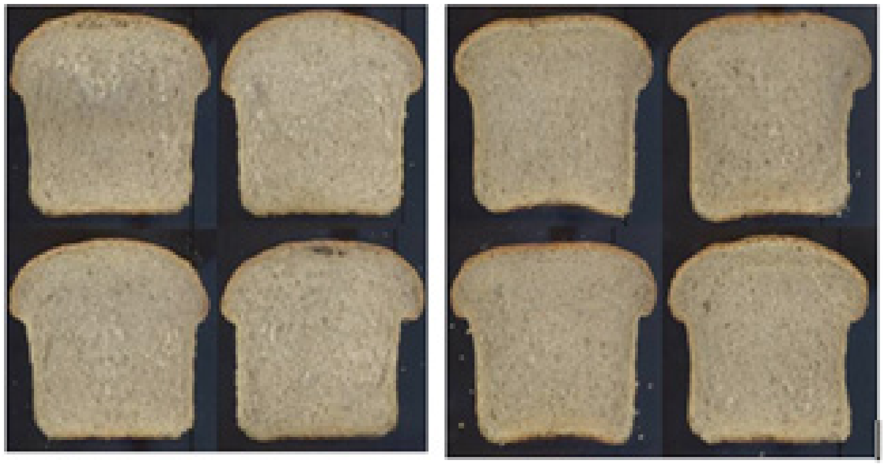
Figure 3 shows photographs of formulations 4 and 5, which illustrate the information given above. It is important to note that the differences in final score are due to the differences in the bread's interior quality. However, these differences may be due to an inhibition of the effect between the two enzymes, which is reported by Selinheimo (2006). Nonetheless, and in accordance with the scores assigned by the judges, the breads obtained with this new formulation have a score of more than 80 points.

The addition of lipase for formulations F6 and F7 produced softness in the bread's interior and a loosening of the dough, making possible the formation of a more uniform alveolar structure and a greater volume compared to the results obtained for formulations F4 and F5. It is important to note that the texture achieved with formulation F7 (laccase-xylanase-lipase) was spongier and more elastic. It received a score of 12 regarding the specifications of IRAM Norm 15858, which is the highest score, while formulation 6 (ADA-ASC-xylanase-lipase) received a score of 10.

Figure 2. Photographs of the breads obtained with the ADA-ASC formulation F2 (2a) and the formulation with laccase F3 (2b).

Figure 3. Photographs of the breads obtained with formulation F4 (3a) and formulation F5 (3b).

Figure 4. Photographs of the breads obtained with formulation F6 (4a) and formulation F7 (4b).



4a 4b

Regarding color, a somewhat darker bread interior was observed for F7 compared to F6. This may be due to the fact that chemical oxidants, in general, yield a whiter dough. Except for the two above considerations, no great difference was found between the last two determinations. Therefore, it can be concluded that F7 yields an excellent bread product.

Figure 4 shows the quality of the breads corresponding to F6 and F7, where a uniform interior, color, and crust can be seen. These photographs correspond to the scores assigned by the judges.

3.1. Sensory analysis

The results of the sensory analysis performed by the two panels of evaluators are presented in **Table 6**. An analysis of this type was implemented only to contrast formulations F6 and F7 in accordance with the results previously obtained.

Of all 38 evaluators, judges number 2, 17, 20, and 28 did not select a different sample. To correct the error, the evaluation was made with 34 evaluators. For the results obtained, no significant differences were found between the bread formulated with laccase-xylanase-lipase and that formulated with xylanase-lipase-ADA-ASC. There were no significant differences because for a level of 5% and for 34 judges, it was necessary that a minimum of 17 identify the different sample, and in the tasting session in this study, only 11 judges identified the different sample. The judges that identified the different sample were: 1, 6, 13, 14, 16, 18, 22, 25, 32, 36, and 38. Regarding preference according to the formulation of the bread, 39% of the judges preferred the bread formulated with laccase, 43% preferred the formulation without laccase, and the remainder indicated no preference. In themselves, the preferences were very similar, as can be seen on the graph in **Figure 5**, which makes bread formulated with laccase acceptable.

Regarding the ease of identifying the sample, 42% of the judges stated that it was easy to identify the different sample, while 29% answered that identification of the sample was difficult. 13% stated that it was very difficult, 11% found identifying the sample almost impossible, and, finally, 5% of the judges found it very easy to find the different tasting, as is shown graphically in **Figure 6**.

With regards to the intensity with which the judges perceived the different sample, 41% stated that this perception was weak, 29% perceived it with moderate intensity, 16% of the judges responded that the intensity was very weak and, finally, 11% and 3% found the perception of difference clear and intense (**Figure 7**).

TABLE 6. RESULTS OF THE SENSORY ANALYSIS TEST FOR FORMULATIONS F6 AND F7

Evaluator no.	*Sample 1*		*Sample 2*		*Sample 3*		*Diff. Sample*	*Inten. Dif.*	*Ident. Dif.*	*M. Prefer.*
A: bread with laccase, B and C: bread with ADA and ASC										
1	A	785	B	137	C	377	785	I	F	785
2	A	416	C	161	B	446		D	Cl	416
3	C	490	A	714	B	141	141	MD	Md	141
4	C	743	B	437	A	378	437	MD	Cl	
5	B	358	C	529	A	537	358	MD	Di	537
6	B	181	A	814	C	149	814	D	Di	181
7	A	430	C	309	B	979	309	MD	F	309
8	B	345	A	450	C	57	345	D	MF	450
9	C	527	B	373	A	228	527	MD	Cl	
10	B	684	A	969	C	342	342	D	Di	684
11	C	565	B	537	A	591	537	D	Di	565
12	A	663	C	732	B	104	732	D	Di	104
13	C	590	A	778	B	429	778	MD	Md	778
14	C	116	B	805	A	906	906	D	Di	
15	B	965	C	769	A	485	965	MD	Cl	965
A: bread with ADA and ASC; B and C: bread with laccase										
16	B	908	A	445	C	467	445	M	F	
17	A	664	C	14	B	511		D	Di	664
18	A	328	B	642	C	567	328	C	F	642
19	C	628	B	114	A	140	628	D	F	628
20	B	798	A	319	C	240		C	F	240
21	A	876	B	660	C	537	660	D	Di	876
22	A	135	C	410	B	831	135	D	F	135
23	C	49	A	59	B	37	37	D	F	
24	C	15	B	8	A	72	8	D	Md	
25	B	73	C	29	A	91	91	M	Di	73
26	B	64	A	55	C	37	37	C	F	64
27	A	10	C	93	B	86	93	M	F	93
28	B	51	A	18	C	20		C	MF	20
29	C	96	B	73	A	28	96	MD	Md	
30	B	69	A	99	C	58	69	M	F	69
31	C	49	B	31	A	94	49	M	Md	31
A: bread with laccase, B and C: bread with ADA and ASC										
32	A	79	A	83	B	19	19	D	F	79
33	A	62	B	81	A	14	14	M	F	81
34	B	32	A	86	A	42	42	M	F	32
35	A	96	B	77	C	53	77	D	F	77
36	B	57	C	84	A	75	75	M	Di	57
37	C	69	A	29	B	91	91	M	F	91
38	B	71	A	23	A	12	71	D	Di	71

*The cell titled different simple refers t the number of the simple selected by the evaluator as different. W: Weak, I: Intense; VW: Very weak; M: Moderate; C: Clear. E: Easy; Di: Difficult; VD: Very difficult; AI: Almost impossible.

The conclusion of the sensory analysis is that it did not report significant differences between the bread formulation with laccase-xylanase-lipase and the bread formulated with xylanase-ADA-ASC-lipase.

Figure 5. Porcent of preference for each bread according to formulation.

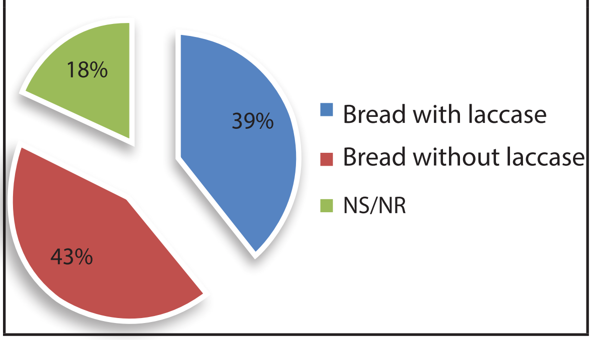


Figure 6. Percentage of the degree of difficulty with which the evaluators identified the different sample.

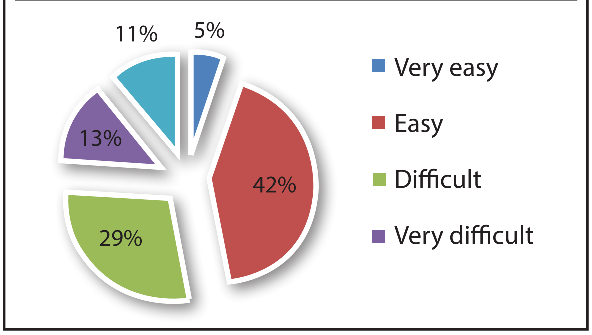
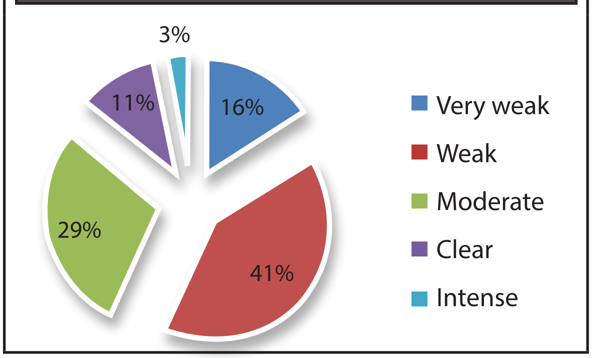


Figure 7. Degree of intensity with which evaluators perceived the different sample.



4. CONCLUSIONS

The addition of a mix of enzymes, laccase-xylanase-lipase, to a dough composed of wheat flour, yeast, salt, sugar, and water generates a good "oven spring," obtaining an increase in volume in the oven. In addition, the internal characteristics of the bread have a homogenous alveolar distribution and a spongy texture. Given these characteristics, a sensory analysis was performed on the product obtained. This analysis demonstrated that the mixture proposed yields a product with good quality and acceptability for the consumer. It is therefore concluded that with a strategy of enzyme combination, a bread flour can be made of comparable quality to that obtained with chemical and enzyme additives currently used. The results so far obtained are promising in the search for enzyme substitutes for chemical additives in bread-making. It can therefore be concluded that the combination of the three enzymes used (laccasa, xylanase y lipase) yielded a bread product with good characteristics.

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