SCIENTIFIC NOTE

Effect of bacterial xylanase on the automated production of barley flour-enriched sandwich bread

Efecto de la xilanasa bacteriana en la producción automatizada de pan tipo molde enriquecido con harina de cebada

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ABSTRACT

The aim of this research was to determine the effect of bacterial xylanase on the automated baking of barley flour-enriched sandwich bread. For this purpose, xylanase (0.02% to 0.04%) was added to the basic formulation of sandwich bread enriched with 10% whole barley flour. The resulting bread slices were subjected to image analysis to determine colorimetric properties, cell count, and sensory evaluation using a 9-point hedonic scale. A 0.03% xylanase addition produced a higher number of cells compared to other treatments. Regarding the colorimetric properties, no significant differences were observed, and the bread with 0.03% xylanase addition received the highest preference score in the sensory evaluation. In conclusion, bacterial xylanase has positive effects on bread, improving its overall quality.

Key words: cell count, chromatic properties.

RESUMEN

El objetivo del presente estudio fue determinar el efecto de la xilanasa bacteriana en el horneado automatizado de pan tipo molde enriquecido con harina de cebada. Para tal efecto se realizaron adiciones de xilanasa (0,02 al 0,04%) a la formulación básica de un pan de molde enriquecida con 10% de harina de cebada integral. A los panes obtenidos se les realizaron análisis de imágenes para determinar las propiedades cromáticas y el número de células, y se evaluó sensorialmente cada muestra en una escala hedónica de 9 puntos. El 0,03% de adición de xilanasa presentó mayor número de células en contraste con los demás tratamientos; respecto a las propiedades cromáticas, no hubo diferencias significativas y el pan con adición de 0,03% de xilanasa presentó la mayor preferencia en la evaluación sensorial. En conclusión, la xilanasa bacteriana genera efectos positivos en el pan, mejorando su calidad.

Palabras clave: conteo de células, propiedades cromáticas.

Introduction

Barley is one of the most important crops in Peru, grown in the central highlands, southern highlands, and the Altiplano, thus becoming part of the Andean population's diet (MIDAGRI, 2024). It is also consumed in Europe, certain regions of Africa (north and south of the Sahara), central and southwestern Asia, as well as in the Andean regions of Ecuador and Bolivia. Industrially, it is used for animal feed, in the brewing industry, and as a coffee substitute (Esquisabel, 2022). There are improved varieties in Peru, such as INIA 411 and INIA 418, developed by the National Institute of Agrarian Innovation (INIA). These and other varieties are consumed in the form of grain and/or flour at the household level (INIA, 2018).

The marketing of barley as food in Peru is still for domestic consumption. In this country, industries import forage and malt barley from Argentina for use in the brewing and animal industries (Esquisabel, 2022; SENASA, 2021). Therefore, it is important to diversify the use of this product, since barley contains soluble and insoluble fiber, proteins, carbohydrates, minerals, and vitamins (Baltazar, 2024; Gupta *et al*., 2010). It also has a naturally high content of β-glucan, a polysaccharide comprising glucose residues made of 1,3-beta-d-glucopyranose (30% bonds) and 1,4-beta-d-glucopyranose (70% bonds) (Pontonio *et al*., 2020).

For bread production, wheat flour is the most important component due to its gluten content and ease of processing.

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However, other cereals like barley and rye, which contain high amounts of the amino acid proline (Sánchez & Esteban, 2022), can be utilized as flours in the bread industry. In ancient Egypt and in the Middle Ages, barley bread was made, but its consumption decreased as it was considered less smooth and digestible (Hidalgo López *et al*., 2023). For this reason, it is desired to incorporate enzymes to improve the technological and functional characteristics of barley bread.

Currently, various methods, including biotechnology, have been used to optimize and improve the quality of bread, with special attention to enzymes. For example, this included the use of pentosanase and glucose oxidase in a bread with sprouted wheat, where these enzymes reduced the formation time and increased the stability of the mass, thus improving the technological functionality of the mass (Tran *et al*., 2024). The use of amylolytic enzymes such as exoamylases and debranching enzymes improves the texture and freshness of bread (Zhao *et al.,* 2022). The combination of α-amylase and lipases in a steamed potato bread reduced its toughness and improved the volume of the bread (Ma *et al*., 2022). The cocktail of enzymes α-amylases, xylanases and cellulases intensified the brown color of the bread crust and improved the rheology of the dough and properties of the bread (Hmad *et al*., 2024). The use of an enzyme cocktail can produce breads of better quality, but, in this research, emphasis was placed on one of these enzymes. Endo-1,4-xylanases (xylanases; EC 3.2.1.8) are hydrolytic enzymes that catalyze the degradation of xylans by randomly breaking the β-(1-4) glycosidic bonds of xylose chains present in the hemicellulose of plant cell walls, producing oligosaccharides of different sizes, both soluble and insoluble. The action of these enzymes in bread is to solubilize insoluble arabinoxylan to yield soluble, high molecular weight arabinoxylans. This action removes insoluble arabinoxylan that interferes with gluten network formation in the dough, making the dough more elastic and increasing its viscosity (Kim & Yoo, 2020; Sheikholeslami *et al*., 2021). Thus, they contribute to the baking industry by providing technological benefits (Obando Garzón, 2013), such as improving the dough's rheological properties, specific volume of the bread, and crumb firmness, resulting in a softer texture (Butt, 2008). Xylanolytic activity during the baking process begins during kneading, modifying the dough's viscoelastic characteristics, and continues during fermentation and the initial minutes of baking until denaturation occurs due to the high baking temperatures (Caballero *et al*., 2007).

The aim of this study was to determine the effect of bacterial xylanase on the automated production of barley flour-enriched sandwich bread.

Materials and methods

Raw materials

For the bread-making process, Nicolini brand commercial wheat bread flour and barley flour with a particle size of 300 μm were acquired, along with other ingredients such as sea salt, Famosa brand vegetable shortening, Fleischmann's instant yeast, and sugar. As for the bacterial xylanase enzyme, it was purchased from Polifood Perú, food-grade with 80% purity.

Bread formulation

For the bread formulation, a standard bread recipe was used with the addition of 10% barley. Additionally, experiments were conducted with three levels of bacterial xylanase addition. The formulations for each experiment and the control sample can be observed in Table 1.

TABLE 1. Formulations used for bread making.

Ingredients	Levels of xylanase addition			
	Control	0.02%	0.03%	0.04%
Wheat flour (%)	90	90	90	90
Barley flour (%)	10	10	10	10
Sugar (%)	8	8	8	8
Salt (%)	1	1	1	1
Vegetable shortening (%)	9	9	9	9
Yeast (%)	2	2	2	2
Powdered milk (%)	5	5	5	5

Making bread

To prepare the bread, all ingredients were placed into a Blackline® BM-6301 bread maker (Blanik, Chile) programmed for basic bread setting with dark crust baking.

Image acquisition system

The image acquisition system in this study (Fig. 1) had the following specifications: (1) the walls had a dimension of 30 cm and a matte black internal color, (2) equilateral white light bulbs with a 10 cm edge, (3) a 13-megapixel image resolution camera, (4) a computer with an Intel Core i3 processor with 4 GB of RAM. The collected samples were recorded through images in *.jpg format.

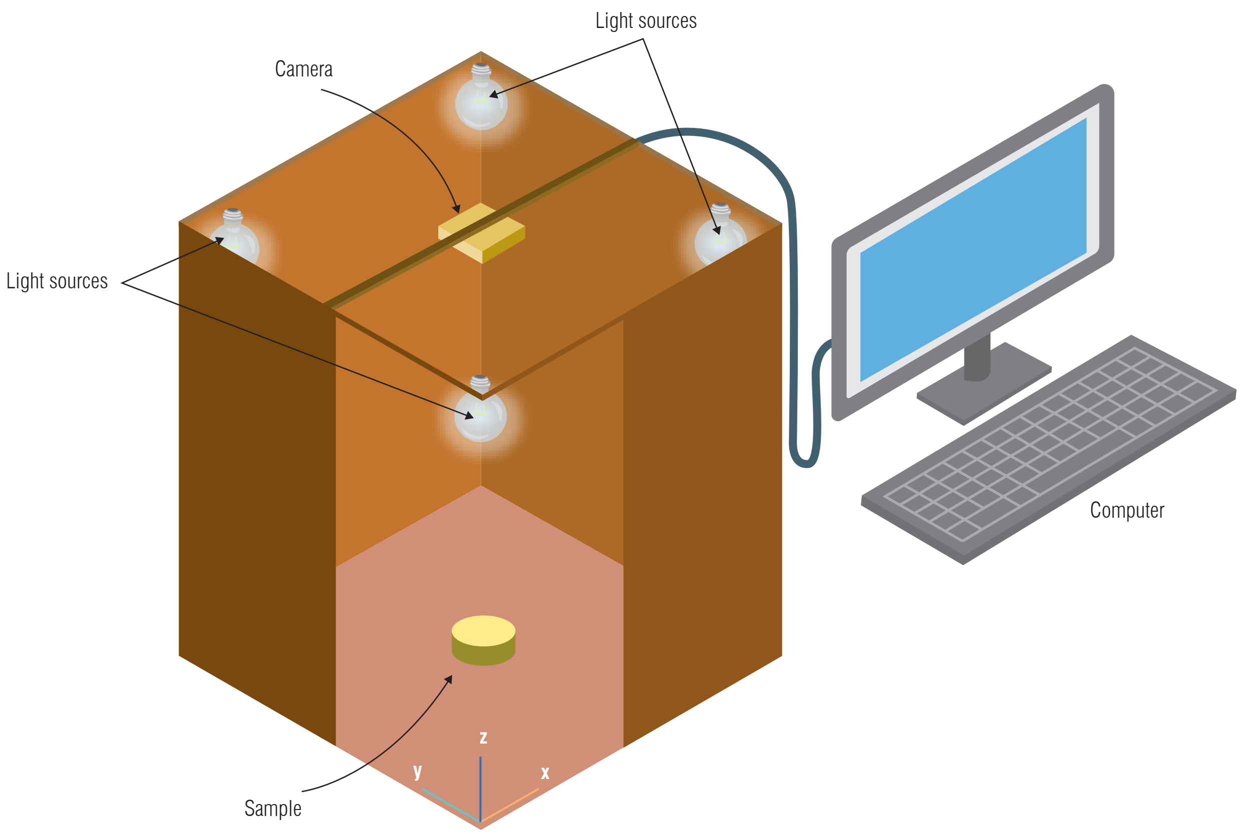

FIGURE 1. Image acquisition system (reproduced from Chambi-Rodriguez *et al*. (2023), with permission by Agronomía Colombiana).

Cell analysis and chromatic properties

The experiments involved segmenting samples into sections (slices) of 1 cm thick; subsequently, each section was subjected to the image acquisition system (Fig. 1). The resulting images were analyzed using ImageJ software to determine the number and size of cells present in each section. To assess chromatic properties in the crust and crumb, including luminosity (L*) and redness level (a*/b* ratio, where a* denotes the red-green hue and b* denotes the yellow-blue hue), RGB information was captured from regions of interest (ROI). Subsequently, chromatic information was calculated using CIELab coordinates derived from these data. These analyses were conducted in three replicates to ensure result consistency. The transformation from RGB to CIELab involves a mathematical procedure that facilitates color representation in a three-dimensional space based on human perception (Castro *et al*., 2017). The formulas used in this process were as follows:

$$L* = 116 * f\left(\frac{Y}{Y_n}\right) - 16 \quad (1)$$

$$a* = 500 * \left[f\left(\frac{X}{X_n}\right) - f\left(\frac{Y}{Y_n}\right)\right] \quad (2)$$

$$b* = 200 * \left[f\left(\frac{Y}{Y_n}\right) - f\left(\frac{Z}{Z_n}\right)\right] \quad (3)$$

In these equations, L* represents the luminosity, while a* and b* denote the chromaticity coordinates. Y stands for the relative luminance of the sample in RGB, and Yn represents the relative luminance of a reference white color, such as D65. Additionally, X, Y, and Z represent the trichromatic coordinates of the sample in RGB, and X_n, Y_n, and Z_n represent the trichromatic coordinates of a reference white color, also exemplified by D65. Furthermore, f(t) refers to a nonlinear function employed to adjust the human eye's response to various wavelengths (Chambi *et al*., 2023).

Using these coordinates, the purity (c*) was derived through the following equation:

$$c* = \sqrt{(a*)^2 + (b*)^2} \quad (4)$$

Sensory analysis

The control sample and the breads made with the addition of xylanase were evaluated by 50 untrained judges of both sexes aged between 18 and 30 years. The assessed properties included odor, color, taste, texture, and overall appearance. A 9-point hedonic scale was employed for this evaluation (1 = Extremely dislike to 9 = Extremely like). The results were displayed on a radar chart (Garcia *et al.*, 2022).

Statistical analysis

A one-way analysis of variance (ANOVA) was applied at a significance level of $P<0.05$, adding bacterial xylanase at different concentrations (0.02%, 0.03%, or 0.04%) followed by a comparison of means (Tukey´s test).

Results and discussion

Cell analysis and chromatic properties

In Figure 2, it can be observed that the number of cells in the samples with 0.03% xylanose was higher than in the other samples. This is followed by the sample with 0.04% xylanose. The control and the 0.02% xylanase addition samples have the lowest numbers of cells. These are considered statistically equal at a significance level of 0.05.

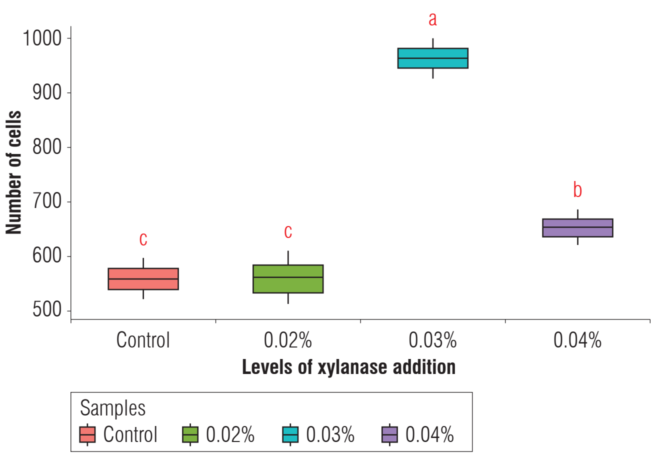

FIGURE 2. Boxplot of the number of cells in the 1 cm-thick bread sample with added xylanase enzyme. Different letters indicate significant differences according to the Tukey´s test ($P<0.05$).

The results obtained for the chromatic coordinates (L*, a*, and b*) of bread containing 10% barley and bacterial xylanase at different concentrations (0.02%, 0.03%, and 0.04%) indicated no significant differences in the luminosity, red-green hue, or yellow-blue hue of the bread crumb compared to the control bread. All values remained within ranges similar to those of the control bread, suggesting that the addition of bacterial xylanase did not significantly influence the chromatic characteristics of the evaluated bread crumb.

In terms of the bread crust, a similar trend was observed. The chromatic coordinates for the crust (L*, a*, and b*) remained close to control values across different xylanase concentrations. Specifically, the luminosity (L*) of the crust decreased slightly with the increase in xylanase concentration, indicating a darker crust, but the differences were not substantial enough to be considered significant. The red-green hue (a*) and yellow-blue hue (b*) values also remained within the same range as in the control samples, suggesting no significant impact on the crust's color. Therefore, the addition of bacterial xylanase at the evaluated concentrations did not cause any meaningful changes in the chromatic properties of the bread crust either.

Sensory analysis

Figure 3 presents the sensory evaluation of barley-containing bread samples with different levels of xylanase addition. Five aspects were assessed: odor, color, flavor,

TABLE 2. Chromatic properties of bread with xylanase addition.

Levels of xylanase addition	Chromatic properties			
	L*	a*	b*	c*
		Crust		
Control	39.70 ± 4.47	14.09 ± 1.61	33.127 ± 0.21	35.99 ± 6.89
0.02%	27.61 ± 6.64	14.08 ± 1.65	33.81 ± 1.86	36.64 ± 6.89
0.03%	34.15 ± 5.65	34.81 ± 1.63	33.95 ± 1.74	48.63 ± 6.89
0.04%	36.36 ± 3.43	12.59 ± 0.90	30.39 ± 0.76	32.89 ± 6.89
		Crumb		
Control	45.45 ± 2.69	0.86 ± 0.20	11.86 ± 0.76	11.89 ± 1.25
0.02%	38.25 ± 5.53	1.31 ± 0.25	14.35 ± 1.33	14.41 ± 1.25
0.03%	39.46 ± 3.09	0.91 ± 0.23	11.72 ± 1.02	11.76 ± 1.25
0.04%	36.54 ± 6.19	0.85 ± 0.33	12.12 ± 1.50	12.15 ± 1.25

Note: All data are expressed as means ± SD (n=3).

texture, and overall appearance, using a 9-point hedonic scale. It is observed that the odor tended to increase slightly with xylanase addition, reaching its peak in the sample with 0.03% xylanase. The color remained intense in all samples, with a slight decrease in samples with 0.02% and 0.04% xylanase. Regarding the flavor, a gradual increase was observed in samples with xylanase addition, being the highest in the sample with 0.03% xylanase. However, the texture appeared to decrease slightly with xylanase addition, with the lowest score in the sample with 0.04% xylanase. The overall appearance tended to improve with xylanase addition, reaching its highest score in the sample with 0.03% xylanase. In conclusion, xylanase addition variably affected the sensory characteristics of bread, enhancing its flavor and overall appearance at certain addition levels, but potentially negatively impacting the texture at higher concentrations.

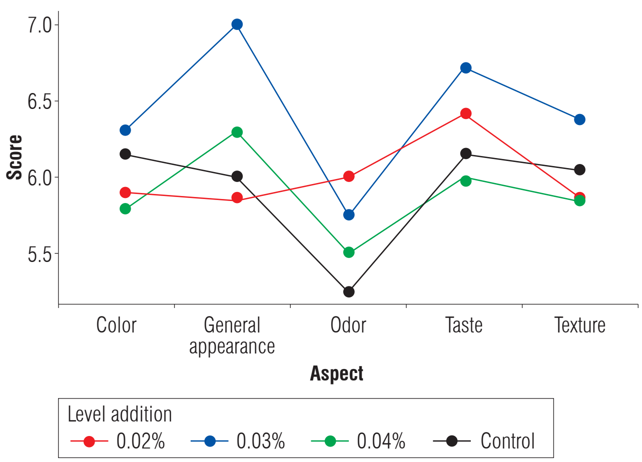

FIGURE 3. Sensory attribute scoring of barley-containing bread samples with different levels of xylanase addition (0.02%, 0.03%, and 0.04%).

Discussion

Regarding the cell size (Fig. 2), the results obtained are supported by previous research, such as that conducted by Vega Castro *et al.* (2015) on the influence of enzymes on bread production. They observed that samples treated with xylanase had an increase in volume as well as greater uniformity in cell size. Furthermore, according to Moreno-Araiza *et al.* (2018), at an addition concentration of 0.02%, the samples showed higher volume and a greater number of cells compared to those treated with a concentration of 0.04%. In the study by Wang *et al.* (2023) on wheat sprout-incorporated bread, xylanase addition resulted in higher specific volume and lower bread hardness. Similarly, in the study by Belyavskaya *et al.* (2022) on rye and flaxseed bread, xylanase application resulted in increasing specific volume and improving crumb resilience. This finding is consistent with data obtained in our study. However, our study demonstrated better performance with an addition of xylanase at 0.03%, where a significant increase in the number of cells was observed. According to Liu *et al.* (2022), the volume increase, consequently leading to an increase in cell number and bread quality, is attributed to the action of xylanase on arabinoxylans present in the flour.

Regarding the chromatic properties (Tab. 2) of the samples analyzed, no marked differences were found. However, previous studies by Altuna *et al.* (2015) identified discrepancies among samples, attributing this phenomenon to the degradation of hemicellulose to xylose, a reducing sugar that exhibits greater reactivity than hexoses and disaccharides.

Finally, the samples subjected to sensory evaluation presented significant differences for at least one sample with the concentration of 0.03% showing the highest level of acceptance. However, some studies did not detect significant differences (Altuna *et al.*, 2015). The color of bread is due to Maillard reactions; the more reducing sugars there are, the darker the color will be. This effect is seen when amylases are introduced, while xylanases produce it but at a lower scale (Kim & Yoo, 2020).

Conclusions

The addition of 0.03% xylanase resulted in the highest cell count, followed by 0.04%, while the control and 0.02% showed the lowest count, with statistically similar values. The chromatic properties (L^*, a^*, b^*) did not significantly differ between control and the bread samples with xylanase at 0.02%, 0.03%, and 0.04%. Sensory evaluation highlighted improved taste and appearance of the bread with 0.03% xylanase, but a potential decline in texture at higher concentrations. These results emphasize the importance of precise xylanase usage to enhance bread quality.

Conflict of interest statement

The authors declare that there is no conflict of interests regarding the publication of this article.

Author's contributions

ADCR: conceptualization, methodology, formal analysis, research, writing of original draft, editing. LVCT: writing of original draft, editing. PSS: data curation. AMTJ: writing of original draft, editing, research, validation. All authors reviewed the final version of the manuscript.

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