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*Corresponding authors: Guillermo Niño-Medina, Guadalupe Gutiérrez-Soto, Facultad de Agronomía, Universidad Autónoma de Nuevo León, Francisco Villa S/N, Col. Ex-Hacienda El Canadá, C.P. 66050, General Escobedo, Nuevo León, México
E-mail: guillermo.ninomd@uanl.edu.mx (G. Niño-Medina); ggutierrez0402@gmail.com (G. Gutiérrez-Soto)

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FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

Effect of laccase from *Trametes maxima* CU1 on physicochemical quality of bread

Guillermo Niño-Medina^{1*}, Guadalupe Gutiérrez-Soto^{1*}, Vania Urías-Orona² and Carlos Eduardo Hernández-Luna³

Abstract: The effect of laccase from *Trametes maxima* CU1 on physicochemical quality of bread was evaluated. Laccase treatment was 0.05% lower than the control in height, 0.33% higher in weight loss and reduced 17.71% the hardness of bread. In color, treatment with laccase was 18.72, 8.51 and 0.61% higher in L^* , h , and C^* , respectively. Chemical parameters of laccase treatment 12.00, 14.10 and 41.62% were higher than control in soluble arabinoxylans, protein and total phenols content, respectively. Based on the results obtained in the present study, laccase from *T. maxima* CU1 can be considered a good option for extraction and application as improver of the physicochemical quality of bread at industrial level.

Subjects: Breads, Cereals & Dough; Food Analysis; Food Biotechnology

Keywords: laccase; *Trametes maxima* CU1; physicochemical quality; bread

1. Introduction

Laccase (benzenediol: oxidoreductase oxygen, EC. 1.10.3.2) is part of a group of enzymes called polyphenol oxidases. It catalyzes the reduction of two electrons from molecular oxygen coupled to the oxidation of four hydrogens from the substrate to form water. It has the capacity to oxidize ortho and paradiphenols, aminophenols, polyphenols, polyamines, lignins and aromatic amines (Brijwani, Rigdon, & Vadlani, 2010; Rodríguez Couto & Toca Herrera, 2006). Laccase has been used in the

ABOUT THE AUTHORS

Guillermo Niño-Medina, is a full time professor of food industry engineer program in the Faculty of Agronomy of the Autonomous University of Nuevo Leon, Mexico. He is a Level I of the national researcher in the national research system of the Mexican National Council for Science and Technology. His research interests are focused in the baking technology, functional aspects (phenolic compounds and antioxidant capacity) of cereals and legumes and extraction of high value-added products (phenolics and dietary fiber) from cereal and legume industry byproducts.

Guadalupe Gutiérrez-Soto, is a full time professor in the Faculty of Agronomy of the Autonomous University of Nuevo Leon, Mexico. She is a candidate level of the national researcher in the national research system of the Mexican National Council for Science and Technology. Her research interests are focused in the production of enzymes from basidiomycetes (laccases, cellulases, xylanases) with application in food science and animal feed.

PUBLIC INTEREST STATEMENT

Enzymes have been used in the production of food products since ancient times. The scientific reports about the use of different enzymes to improve the quality of bread have largely increased in the last two decades. One of these enzymes is laccase, which has the ability to improve the quality of bread by oxidizing phenolic compounds and proteins present in flour during the mixing step of dough. The present work is the first report of the use of laccase from *Trametes maxima* CU1 in the improvement of physicochemical quality of bread having effects on hardness, color, protein and phenolics compounds.

treatment of environmental pollutants and dyes in the textile industry, modification of lignocellulosic materials in the paper industry and synthesis of new antibiotics in the pharmaceutical industry (Kudanga, Nyanhongo, Guebitz, & Burton, 2011). In the food industry, laccase has been used in the stabilization of wines and beers, juice clarification, determination of ascorbic acid and gelation of feruloylated polysaccharides (Minussi, Pastore, & Durán, 2002). In the last years, its application also has been implemented as a dough and bread improver in the baking industry using mainly commercial preparations (Caballero, Gómez, & Rosell, 2007; Renzetti, Courtin, Delcour, & Arendt, 2010) and also laccase obtained from *Trametes hirsuta* (Flander et al., 2008; Selinheimo, Autio, Kruus, & Buchert, 2007). The objective of this study was to evaluate the effects of laccase from *Trametes maxima* CU1 on the physicochemical quality of bread by testing the changes in height, weight loss, hardness, color crust, soluble arabinoxylans, protein and total phenols.

2. Materials and methods

2.1. Culture mediums and reagents

Culture mediums used were from BD Difco (Franklin Lakes, NJ, USA), while the rest of the chemicals were from Sigma-Aldrich (St. Louis, MO, USA). All of the mediums were sterilized at 121°C (15 lb/inch²) for 15 min. Wheat flour, dry yeast, salt and sugar used in this study were purchased in a local supermarket.

2.2. Biological material

T. maxima CU1 is a native strain isolated from the metropolitan area of Monterrey, Nuevo Leon. It is deposited in the Laboratory of Enzymology of the biological Sciences faculty of the Universidad Autónoma de Nuevo León. Strains were grown in YMGA medium (0.4% glucose, 1.0% maltose extract, 0.4% yeast extract and 1.5% bacteriological agar) for 5 days and later the liquid mediums were inoculated. Production medium was based on 2% Bran Flakes® in 60 mM at pH 6.0 potassium phosphate buffer. It was inoculated with a homogenized mycelium grown in YMGA at 2 g/100 mL of the medium in sterile conditions and cultures were grown at 28°C stirring at 170 rpm for seven days.

2.3. Enzymatic determination and protein quantification

The laccase activity was estimated using 20 mM DMP (2,6-Dimetoxifenol) in 200 mM sodium acetate at pH 4.5 (λ 468 = 49.6 1/mM 1/cm) (Abadulla et al., 2000). Protein concentration was determined by the Bradford method (Bradford, 1976), using Bio-Rad protein assay and bovine serum albumin as standard. Both, enzymatic activity and protein quantification were done in a Shimadzu UV1601 spectrophotometer (Kyoto, Japan) at room temperature.

2.4. Enzyme purification

Biomass was separated from liquid culture and supernatant was frozen at -20°C for 48 h to remove polysaccharides. Later, extracts were defrosted to be filtered through Whatman Schleicher & Schuell 520B filter paper. Supernatant was concentrated using Prep/Scale TFF-6 PLCC5 kD spiral cartridge (Merck Millipore, Germany) and a Millipore Amicon ultrafiltration system (Millipore, USA). Concentrated sample was applied in a DEAE-Sephacel column (2.5 cm × 40 cm) equilibrated with 20 mM potassium phosphate, pH 6.0. A 20–150 mM potassium phosphate lineal gradient was applied. Fractions with laccase activity were collected, concentrated and applied in a P-100 Biogel (2.5 cm × 65 cm), equilibrated and eluted with 20 mM potassium phosphate, pH 6.0. These laccase-activity fractions were collected, concentrated and stored at -20°C until the moment of its use.

2.5. Bread making procedure

Control was prepared by using 1,000 g of flour, 620 mL of water, 40 g of sugar, 20 g of salt and 30 g of commercial yeast, while enzymatic treatment included besides basic ingredients 1.5 laccase units per gram of flour. Solid ingredients were homogenized in a Globe SP 30 (Bridgeport, CT, USA) industrial mixer at low speed for 5 min, after that water was added and mixed for 7 min at maximum speed. Later, 140 g of dough were placed in aluminum mini loaf pans (14.5 cm × 8.4 cm × 4.7 cm),

left for fermentation at 45°C for 30 min and afterwards baking at 200°C for 15 min. Bread was cooled at room temperature for the analysis of physicochemical parameters.

2.6. Physicochemical evaluations of bread

The height of bread was measured with a Vernier caliper on the central part of the piece and it was reported in millimeters (mm). The weight loss percentage (%WL) was obtained with the next formula: $\%WL = ((WBB - WAB) / WBB) \times 100$, where: WBB = Weight Before Baking and WAB = Weight After Baking. Hardness was evaluated with a Stable Micro Systems TA.XT2i texture analyser (Surrey, UK) using a compression plate of 75 mm diameter at 5 mm/s speed and a final distance of 30 mm. Chromatic evaluations was done in the bread crust with a Konica Minolta CR-400 Chroma Meter (Tokyo, Japan), according to López-Contreras et al. (2015). Chromatic parameters were obtained using CIELAB (L^* , a^* , b^*) colour system where L^* defines Lightness (0 = black, 100 = white), a^* indicate red (positive a^*) or green value (negative a^*) and b^* indicate yellow (positive b^*) or blue value (negative b^*). In addition C^* (Chroma; saturation level of h) and h (hue angle; 0° = red, 90° = yellow, 180° = green, 270° = blue) were obtained using CIELAB colour values as $C^* = (a^{*2} + b^{*2})^{1/2}$ and $h = \arctan(b^*/a^*)$ (Commission Internationale de l'Eclairage, 2004). Colour view was obtained by online software ColorHexa colour converter using L^* , C^* and h values (ColorHexa, 2017). After physical evaluations, samples were dried at 40°C for 12 h, milled and sieved at a particle size smaller than 0.5 mm (mesh 35) and stored at -20°C until chemical evaluations.

Soluble arabinoxylans were extracted according to Hartmann, Piber, and Koehler (2005) with modifications by using α -amylase (163 U, pH 6, 80°C, 30 min), amyloglucosidase (75 U, pH 4.5, 60°C, 30 min) and alkaline protease (404 U, pH 7.5, 60°C, 30 min) for starch and protein elimination and the use of 66% ethanol volume for the precipitation of arabinoxylans. Yield of arabinoxylans was obtained with the next formula: $\%AX = ((IWB - WFDAX) / IWB) \times 100$ where: IWB = initial weight of bread and WFDAX = weight of freeze-died arabinoxylans. Protein quantification was evaluated by Bradford method (Bradford, 1976). Results were expressed as percentage of protein based on a calibration curve done with bovine serum albumin in concentrations from 0 to 0.3 mg/mL, lecture of samples was carried on a 595 nm. Extraction of phenolics was carried out by alkaline hydrolysis followed by diethyl ether extraction and evaporation, and finally dissolved in 80% methanol according to the procedure described by Kim, Tsao, Yang, and Cui (2006). Determination of total phenols was done following procedure described by Kyoung Chun and Kim (2004), based on the reaction of Folin-Ciocalteu reagent measuring samples at 750 nm. Results were expressed as micrograms of ferulic acid equivalents per hundred grams of arabinoxylans ($\mu\text{gFAE}/100 \text{ g AX}$) based on a calibration curve with ferulic acid in concentrations from 0 to 200 mg/L.

2.7. Statistical analysis

All of the results were expressed as mean values of three samples \pm standard deviation. Statistical significance among samples was evaluated by analysis of variance (ANOVA) followed by Tukey's test using Minitab 14.0 (Minitab 14 Statistical software, 2004). A level of probability of $p \leq 0.05$ (5%) was set as statistical significance.

3. Results and discussion

3.1. Laccase Purification

To evaluate the effect of native laccase from *T. maxima* CU1 on the physicochemical parameters of bread it was necessary to carried out a purification of the enzyme. After purification, a preparation with a specific activity of 121 U/mg was obtained (purification fold = 5) (Table 1).

3.2. Physicochemical evaluation of bread

Bread treated with laccase was 0.05% lower than control in height and 0.33% higher in weight loss, however there were not significant statistical differences ($p \leq 0.05$) in these two parameters. On the other hand, in the evaluation of hardness, treatment with laccase had a reduction of 17.71% presenting significant statistical difference ($p \geq 0.05$) respecting to the control (Table 2).

Table 1. Scheme of laccase purification from *Trametes maxima* CU1

Step	Total units (U)	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Purification (fold)
Concentrate 10 K	6,418	266	24	100	1
DEAE Sephacel	10,796	119	91	168	4
BiogelP-100	13,337	110	121	208	5

Results obtained in height are different to data reported by Renzetti et al. (2010), who evaluated the effect of laccase in formulations based on oat flour, using 0.1 and 1.0 units of laccase per gram of flour and observed an increase in height of 8.4–9.8%. On the other hand, the results obtained on the evaluation of hardness are in agreement with data reported previously by several authors; Selinheimo et al. (2007) observed a 19.04% in the reduction of hardness by using a partially purified laccase from *T. hirsuta* using 60 units of enzyme per gram of flour. Flander et al. (2008) found a 14.28% in the reduction of hardness by using partially purified laccase from *T. hirsuta* using 0.83 units of enzyme per gram of flour in bread made with mixture of oat-wheat flours (51:49 p/p). Renzetti et al. (2010) reported a decrease from 9.14 to 14.59% in the hardness of oat bread by using treatment of commercial laccase. Only Caballero et al. (2007) did not found significant statistical difference in hardness of bread using a treatment with 20 µL of commercial laccase (10,500 U/g) for every 100 g of flour.

Regarding to color parameters, laccase treatment resulted in an increase of 18.17% in L^* and 8.51% in h showing significant statistical differences ($p \geq 0.05$) against control (Table 2). On the other hand, it was observed an increase of 0.61% in C^* with no significant statistical differences ($p \leq 0.05$) between treatments (Table 2). None of the previously studies in which laccase was used for bread improvement have reported the effect of the enzyme treatment on the color of crust. Results of chromatic evaluation in the present work indicated according to the h value, that color of both treatments are located in the orange region of the hue color circle, being classified the color of the control and enzymatic treatments as “Dark Moderate Orange” and “Slightly Desaturated Orange”, respectively. The crust color of both treatments showed the same level C^* , however treatment with laccase has a higher levels of L^* and h resulting in a bread with a higher lightness and a higher orange tone than control in the crust.

In the chemical parameters, laccase treatment was higher than control in 12.00, 14.10 and 41.52% in the content of soluble arabinoxylans, protein and total phenols, respectively and showed significant statistical differences in those evaluations (Table 3). Nowadays there are no reports on chemical evaluations of bread from dough treated with laccase. However, some of these parameters have been evaluated in bread made under traditional process; Hartmann et al. (2005) reported 0.70% of soluble arabinoxylans in bread, which is higher than both treatments of the present work. Furthermore, these authors also evaluated ferulic acid content of arabinoxylans and reported 550 µg/kg, being this result higher than control and lower than laccase treatment of our work.

Table 2. Effect of the use of laccase from *Trametes maxima* CU1 in physical parameters of bread

Treatment	Height (mm)	Weight loss (%)	Hardness (N)	Color			
				L^*	C^*	h	View
Control	59.65 ± 0.77 ^a	18.14 ± 0.2 ^a	33.02 ± 0.41 ^a	49.58 ± 0.61 ^b	32.31 ± 0.37 ^a	63.18 ± 0.72 ^b	
Laccase	59.62 ± 0.53 ^a	18.20 ± 0.1 ^a	27.17 ± 0.53 ^b	60.59 ± 0.21 ^a	32.51 ± 0.38 ^a	69.06 ± 0.67 ^a	

Notes: Values with different superscript letters within a column are significantly different ($p \leq 0.05$); Data are expressed as means values of three samples ± standard deviation.

Table 3. Effect of the use of laccase from *Trametes maxima* CU1 in chemical parameters of bread

Treatment	Soluble arabinoxylans (g/100 g of bread)	Protein (g/100 g of AX)	Total phenols ($\mu\text{gFAE/g}$ of AX)
Control	0.40 ± 0.04^b	4.18 ± 0.2^b	495 ± 6.36^b
Laccase	0.43 ± 0.03^a	4.75 ± 0.1^a	848 ± 9.72^a

Notes: Values with different superscript letters within a column are significantly different ($p \leq 0.05$); Data are expressed as means values of three samples \pm standard deviation.

Laccase acts as an oxidizing agent of dough components and its improve effect in weight loss, hardness, soluble arabinoxylans, protein and total phenols is due to three principal factors: (a) formation of crosslinking in arabinoxylans chains through ferulic acid dimers as a product of the oxidation of ferulic acid linked to the arabinoxylans (Figuerola-Espinoza & Rouau, 1998), (b) crosslinking of gliadins fractions from the oxidation of tyrosine mainly and in lesser extent, tryptophan and cysteine (Mattinen et al., 2006; Selinheimo et al., 2007), (c) crosslinking of arabinoxylans and proteins in flour through the interaction ferulic acid-tyrosine (Piber & Koehler, 2005). These three factors generate a better absorption and better distribution of water in dough and thus volume of bread increases, crumb structure improves and thereby a softer bread product it is obtained (Minussi et al., 2002).

On the other hand, the color of the bread crust is the result of the Maillard reaction, which on initial stages it leads to the formation of Amadori and Heyns rearrangement products (Helou, Jacolot, Niquet-Léridon, Gadonna-Widehem, & Tessier, 2016). Previously, it has been reported that free radical scavenging capacity of ferulic acid against intermediary compounds of Amadori or Heyns rearrangement, is related to alterations in Maillard reaction in baking model systems (Jiang, Chiaro, Maddali, Prabhu, & Peterson, 2009). In addition, some authors have reported that ferulic acid dimers obtained by catalytic action of laccase have a higher free radical scavenging capacity than ferulic acid in its monomer form (Adelakun et al., 2012; Aljawish et al., 2014), so that they could have a high effect on the Maillard reaction and thus modify color development of the bread crust, as observed in the present work.

4. Conclusions

Except for height and chroma (C^*), the rest of the physicochemical parameters evaluated were positively affected in bread treated with laccase from *T. maxima* CU1. The results obtained were in agreement with data reported by other authors who used laccases from other fungal sources and in some cases using a higher enzyme activity than our work. The present work is the first evaluation of the effect of laccase obtained from *T. maxima* CU1 on the physicochemical quality of bread. Based on the results obtained in the present study, laccase from *T. maxima* CU1 can be considered a good option for extraction and application as improver of the physicochemical quality of bread at industrial level.

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Competing Interests

The authors declare no competing interest.

Author details

Guillermo Niño-Medina¹
E-mail: guillermo.ninomd@uanl.edu.mx
ORCID ID: <http://orcid.org/0000-0003-3316-2316>

Guadalupe Gutiérrez-Soto¹

E-mail: ggutierrez0402@gmail.com

Vania Urías-Orona²

E-mail: vania.uriaso@uanl.mx

Carlos Eduardo Hernández-Luna³

E-mail: carloshlmx@yahoo.com

¹ Facultad de Agronomía, Universidad Autónoma de Nuevo León, Francisco Villa S/N, Col. Ex-Hacienda El Canadá, C.P. 66050, General Escobedo, Nuevo León, México.

² Facultad de Salud Pública y Nutrición, Universidad Autónoma de Nuevo León, Av. Dr. Eduardo Aguirre Pequeño y Yuriria, C.P. 64460, Col. Mitras Centro, Monterrey, Nuevo León, México.

³ Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Av. Pedro de Alba esq. Av. Manuel Barragán, Ciudad Universitaria, C.P. 66451, San Nicolás de los Garza, Nuevo León, México.

[†] Guadalupe Gutiérrez-Soto and Guillermo Niño-Medina contributed equally in writing the manuscript.

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