Optimization of gluten-free bread by response surface methodology

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RESUMEN: Los productos sin gluten presentan deficiencias de textura en comparación con los productos que contienen gluten. Se ha mejorado la textura de los productos sin gluten con la adición de hidrocoloides y almidones pregelatinizados. En este trabajo se estudiaron los efectos de la hidroxipropilmetilcelulosa (HPMC), la harina de plátano inmaduro pregelatinizada (HPI-P) y el agua sobre la calidad de un pan sin gluten. Se utilizó un diseño central compuesto y una metodología de superficie de respuesta. Se analizaron volumen específico, dureza, y se realizó un análisis de imagen de la miga. Los resultados mostraron que el volumen específico aumentó con la adición de HPMC y HPI-P, mientras que la dureza disminuyó. La adición de HPI-P y agua aumentó el número y el tamaño de los alvéolos y afectó la distribución de éstos en las migas. La distribución y el tamaño de los alvéolos afectaron las características físicas y de textura del pan. La harina de plátano inmaduro se puede usar como un ingrediente alternativo para preparar pan sin gluten que tiene características de buena calidad.

Palabras clave: Diseño central compuesto, pan, sin gluten.

ABSTRACT: Gluten-free products have some texture drawbacks compared with gluten products. The texture of gluten-free products is improved with the addition of hydrocolloids and pregelatinized starches. The effects of hydroxypropyl methylcellulose (HPMC), pregelatinized unripe banana flour (UBF-P) and water on the quality of gluten-free bread were studied. A composite central design and response surface methodology were used. The volume, specific volume, weight, and hardness were analyzed, and image analysis of the crumb was performed. The results showed that the volume and specific volume increased with the addition of HPMC and UBF-P, while the hardness decreased. The addition of UBF-P and water increased the number and size of alveoli and affected the distribution of alveoli in crumbs. The distribution and size of the alveoli affected the physical characteristics and texture of the bread. Unripe banana flour can be used as an alternative ingredient to prepare gluten-free bread that has good quality characteristics.

Keywords: Bread, composite central design, gluten-free.

Área: Desarrollo de nuevos productos

INTRODUCTION

Celiac disease (CD) is characterized by an autoimmune response with symptoms including inflammation of the small intestine. CD is mainly induced and maintained by the intake of gluten-containing products made with cereals, such as wheat, barley and rye; however, the availability of gluten-free products in many countries is limited because the development of gluten-free foods is a new area in the food industry (Gallagher *et al*., 2004). Hydrocolloids have been used to obtain viscoelastic properties similar to those in products with gluten (Mir *et al*., 2016). The hydrocolloid type influences the water retention capacity and interactions among the components of the food matrix, such as polysaccharides, lipids and proteins (Houben *et al*., 2012). Diverse flours have been used to produce gluten-free products, including pseudocereals (quinoa, amaranth, buckwheat), unripe fruits (bananas, apples) (Zandonadi *et al*., 2012), and legumes. The effect of the use of gluten-free flours on the quality and consumer acceptance of products, such as pasta, snacks, cookies, cakes, donuts and bread, has been evaluated (Lazaridou *et al*., 2007). Additionally, some studies have evaluated the use of pregelatinized gluten-free flours to improve the softness of the crumbs of bread with a decrease in the hardness of bread (Onyango *et al*., 2009). Unripe banana flour has a high level of indigestible carbohydrates (dietary fiber), including resistant starch (RS). Unripe banana flour mixed with other alternative flours has been used to prepare gluten-free products and increase the indigestible carbohydrate content; however, there have not been studies in which only unripe banana flour was used to make bread. Bread is consumed worldwide and is mainly made with wheat, wherein gluten gives functionality to the food matrix. The absence of gluten in bread produces a poor-quality product (Mondal and Datta, 2008). Breadcrumbs are an important quality characteristic that can be used to design new breads with alternative ingredients. The volume and specific volume of bread depend on the retention of gas by the matrix during fermentation and affect the bread quality (Ziobro *et al*., 2015). The aim of this study was to evaluate the effects of the levels of HPMC, pregelatinized unripe banana flour and water on the bakery quality of gluten-free bread. A composite central design and response surface methodology were used. The results showed that the volume and specific volume increased with the addition of HPMC and UBF-P, while the hardness decreased. The addition of UBF-P and water increased the number and size of alveoli and affected the distribution of alveoli in crumbs. Unripe banana flour can be used as an alternative ingredient to prepare gluten-free bread.

MATERIALS AND METHODS

Materials

UBF was purchased from Mi Ranchito Bananas S.P.R. of R.L. (Colima, Mexico) (3.15% protein, 0.37% lipids, 2.44% ash, 5.99% fiber all on a dry basis, and 4.83% moisture). UBF-P was obtained by adding boiling water (96 °C) to UBF, and the dough was mixed until the lumps were removed by stirring for 10 min. Then, the mixture was cooled to 40 °C. Hydroxypropyl methylcellulose (HPMC-K4M) (Rettenmaier Mexicana S.A de C.V., Estado de México, México) and fresh eggs (CFE, Huevo San Juan, Jalisco, México) were used as substitutes for gluten. The rest of the ingredients used for bread-making were instant dry yeast, sugar, and salt, which were purchased from a local market (Morelos, México), and water.

Experimental design and statistical analysis

Response surface methodology (RSM) using a central composite face-centered design (CCF) was used for the analysis (Farris and Piergiovanni, 2009). The independent variables were as follows: HPMC (-1=2.0 and +1=6.0%), UBF-P (-1=5.0 and +1=45 g/100 g of UBF) on a dry basis and water (-1=46 and -1=100 mL). The minimal and maximal levels of the independent variables were selected based on preliminary trials. The dependent variables were selected as responses for representing the parameters of quality: specific volume (cm^3/g) and analysis of crumb (alveolar area (%), large alveoli and largest alveoli). The experimental conditions were 6 replicates at the center point, 6 axial points or stars, and 8 intermediate points, leading to a total of 20 experiments (Table I). For the regression model, it was assumed that i mathematical functions, fz (z=1,2…i), exist for each response, Yz, function of l independent factors, and Xk (k=1,2,…l) such that: $Y_z = f_z(X_1, X_2, \dots X_l)$
where i=6 and l=3. Finally, the fz functions were assumed to be approximated by a second-degree polynomial equation of:

$$Y_z = b_{z0} + \sum_{k=1}^{l=3} b_{zk}X_K + \sum_{k=1}^{l=3} b_{zkk}X_k^2 + \sum_{k\neq j}^{l=3} b_{zkj}X_kX_j + \varepsilon$$

Where:
bz0= response value with all factors set at medium level (center point);
bzk= linear regression coefficient;
bzkk=quadratic regression coefficient;
bzkj=interaction regression coefficient; and
ε=residual response variation not explained by the model.
The regression model was obtained using a multiple regression program (Design-Expert software version 10, Stat-Ease, Inc. Minneapolis, USA). The results were validated experimentally. The bread sample produced by the optimized model was evaluated in terms of specific volume, and analysis of

the crumb. One sample T-test (p≤0.05) was used to compare the optimal bread and the predicted values using the Sigma-Stat statistical software version 11.0 Jandel Corp., California, USA.

Gluten-free breadmaking procedure
Bread formulation were prepared following the methodology proposed by Demirkesen *et al*., (2013).

Specific volume and hardness
The specific volume was calculated as the ratio of the bread volume (cm^3) to its mass (g), and four replications were performed for each formulation. The hardness (N) of the bread was measured following the methodology by Liu *et al*., (2018).

Crumb digital image analysis
An image analysis of crumb was carried out following the methodology proposed by Sánchez-Pardo *et al*., (2008).

RESULTS AND DISCUSSION

Diagnostics checking of the models
The data were adjusted to a quadratic model (data don't show), with values of R^2 greater than 0.90. In contrast, Adequate Precision (adeq precision) ensures that values above 4 have a high adjustment; therefore, the predicted values will be very close to the experimental, as with the low coefficients of variation (less than 12%).

Specific volume of bread
The response variables were used to determine the quality characteristics, the specific volume was affected by the increase in water and UBF-P and the decrease in HPMC (Fig. 1a). The highest specific volume values were related to the intermediate water content (73 mL), gum (4 g/100 g) and UBF-P (25 g/100 g). The specific volume is related to the network produced by the gum and starch of the UBF and by the water via the formation of hydrogen bridges. A similar pattern was reported in gluten-free breads made with rice flour and cassava starch (Crockett *et al*., 2011; Sabanis and Tzia, 2011), and the researchers concluded that the increases in volume and specific volume were because the structure of the gum (HPMC) contained hydrophilic groups (hydroxypropyl) that produced hydrogen bridges with OH- groups of starch and water. The increasing the amount of water and UBF-P resulted in a decrease in specific volume. Increasing the amount of water in the formulations resulted in a dilution effect of the components, generating weak networks unable to resist the pressure steam and CO_2 during baking, obtaining matrices with low specific volume (Föste *et al*., 2017).

Table I. Central composite design to optimize the content of gum, pregelatinized immature banana meal and water from baked products

Formulation	Independent variable			Dependent variable	Hardness (N)
	Concentration of HPMC (g/100g)	Concentration of UBP-P (g/100g)	water (mL)	Volume (cm3)	
F1	4 (0)	25 (0)	73 (0)	65.09±1.46	4.02±0.47b
F2	2 (-1)	5 (-1)	46 (-1)	46.98±1.18	78.19±4.88a
F3	6 (+1)	45 (+1)	100 (+1)	56.46±0.92	1.77±0.38h
F4	2 (+1)	5 (-1)	100 (-1)	46.18±0.95	56.75±5.83a
F5	4 (0)	45 (0)	73 (+α)	59.44±0.88	2.43±0.54fg
F6	4 (+α)	25 (0)	100 (0)	71.86±1.54	3.97±0.95bcd

F7	4 (0)	25 (0)	73 (0)	74.31±0.87	2.85±0.54de
F8	2 (0)	25 (-α)	73 (0)	59.44±0.88	3.32±0.50cf
F9	6 (-1)	5 (+1)	46 (-1)	44.38±1.27	75.87±1.26a
F10	4 (0)	25 (0)	73 (0)	85.15±1.12	2.30±0.37gh
F11	2 (+1)	45 (-1)	100 (+1)	43.00±0.68	3.64±0.70bce
F12	2 (-1)	45 (-1)	46 (+1)	50.04±0.81	3.14±0.42cg
F13	4 (-α)	25 (0)	46 (0)	64.56±0.88	4.14±0.51bc
F14	4 (0)	25 (0)	73 (0)	67.03±1.17	2.25±0.45f
F15	4 (0)	25 (0)	73 (0)	71.66±1.72	2.47±0.46ef
F16	4 (0)	25 (0)	73 (0)	66.03±0.88	4.83±0.70b
F17	6 (-1)	45 (+1)	46 (+1)	69.80±2.35	2.58±0.34ef
F18	4 (0)	5 (0)	73 (-α)	57.26±1.91	67.61±2.23a
F19	6 (0)	25 (+α)	73 (0)	90.54±3.62	4.33±0.49bc
F20	6 (+1)	5 (+1)	100 (-1)	54.10±1.23	55.55±4.85a

α =1, *100g of unripe banana flour, HPMC= hidroxipropilmetilcelulosa, UBF-P= pregelatinized unripe banana flour, RS=resistant starch. Values are mean ± standard deviation, n = 6 breads, different letters in each column represent statistical difference.

Hardness

The texture of bread is an important quality variable because a soft crumb is desirable by consumers as it is related to freshness (Crockett *et al*., 2011). The highest hardness (55-78 N) was observed in formulations with a low content of UBF-P; however, the increase in HPMC also influenced the hardness (Table I). This pattern was related to the higher gum concentration due to producing a rigid network and preventing the loss of water, and the water present in the network prevents an association among starch chains, which is responsible for bread staling and, consequently, for a hard texture (Sabanis and Tzia, 2011).

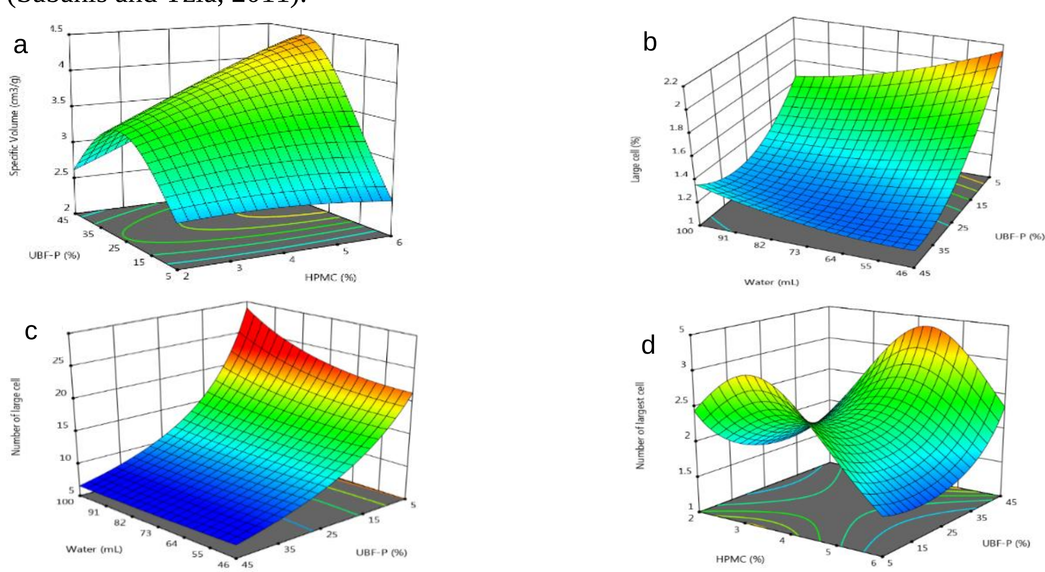

Figure 1. Response surface plot the effect of specific volume (cm^3/g): **a**, UBF-P & HPMC; large cell (%): **b**, Water & UBF-P; number of large cell: **c**, Water & UBF-P; number of largest cells: **d**, HPMC & UBF-P

Analysis of the crumb

Analysis of the crumb was performed by image analysis to examine the quality of bread (Demirkesen *et al*., 2013). The alveoli distribution in the crumb was related to the water content and UBF-P (Fig. 1b). Bread with a medium level of UBF-P (25 g/100 g) presented the lowest alveoli number, but the mean area of the alveoli in the slice was higher (24-40 mm^2, results not shown). This pattern was related to the gum stabilizing the alveolar structure produced during the fermentation step (Mariotti *et al*., 2013). During the fermentation step, large alveoli were produced with high gas retention, but during baking, gas quickly expanded, and the crumb collapsed from the effects of the pressure generated by the gas. Bread with small alveoli homogeneously distributed in the crumb is related to a soft texture, but this was not found in this study. In general, the size of the alveoli was related to the contents of UBF-P, water and HPMC (Fig. 1c,d). Breads with a low content of UBF-P had the highest number of smaller alveoli. The better alveolar area distribution and alveoli number of center slices were obtained in breads with level medium of water and UBF-P.

Improvement of the formulation and bread quality parameters

The optimization of the formulation was carried out through the Design-Expert program by maximizing specific volume, and decreasing the presence of the large alveoli, the largest alveoli (area greater than 20 mm^2) and the alveolar distribution and desirability (0.85). The optimal formulation was produced (duplicated) and compared with the predicted values (Table II). The actual values were equal to the predicted values. Thus, the confirmative test validated the experimental results. The presence of statistically significant ($p < 0.05$) differences between the mean value and the estimated values from the model was investigated by the one sample T–test. No statistically significant difference ($p>0.05$) was found between the results obtained from the validation test, indicating that the model obtained with the optimization was experimentally successful.

CONCLUSIONS

The central composite design and response surface methodology helped to optimize the formulation of a bread made with UBF, obtaining better characteristics of specific volume and hardness. It is possible to develop bread with good quality characteristics using alternative ingredients, such as UBF.

Table II. Comparison of optimum point verification test result with experimental values from model

Dependent variable	**Predicted values**	**UBF bread**	**p-value**
Specific Volume(cm^3/g)	4.04	4.29±0.16	0.234
Large cell (%)	1.12	0.95±0.35	0.608
Number of large cell	7.09	6.17±4.17	0.944
Number of largest cell	1.39	1.47±0.78	0.718

Mean ± standard deviation, $p < 0.05$ was considered statistically significant, RS= resistant starch, UBF= unripe banana flour. RS= resistant starch.

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