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SELECTED STRUCTURAL CHARACTERISTICS OF HPMC-CONTAINING GLUTEN FREE BREAD: A RESPONSE SURFACE METHODOLOGY STUDY FOR OPTIMIZING QUALITY

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Gluten free (GF) breads require a gluten replacement to provide structure and gas retaining properties in the dough and mimic the viscoelastic properties of gluten. Hydroxypropylmethylcellulose (HPMC), which forms thermoreversible gel networks on heating had proved the most effective in structuring baked products. Response surface methodology was used to optimize a GF bread formulation based on ingredients such as maize starch and rice flour, which are naturally GF. (HPMC) and water (W) were the predictor variables (factors) and loaf specific volume, crumb firmness, and overall acceptability were the dependent variables (responses) used to assess the product quality. The optimal formulation, determined from the data, contained 1.5 kg/100 kg HPMC and 88.7 kg/100 kg Water, corn starch-rice flour blend basis (sfb). The developed mathematical models for the measured responses could be successfully used for their prediction during baking. Shelf life study of the optimized formulation revealed that bread stored under modified atmosphere packaging (MAP) exhibited lower crumb firmness and moisture content values, thus remained softer through storage. Scanning electron microscopy of the crumb showed continuum matrix between starch and HPMC, in the optimized formulation, obtaining a more aerated structure.

Keywords: ***Celiac Disease, Gluten free, HPMC, Bread, Optimization.***

INTRODUCTION

Celiac disease (CD) is a chronic disorder of the small intestine caused by exposure to the gluten fraction of wheat, rye and barley in the genetically predisposed individuals.[1,2] In CD patients, ingestion of gluten leads to inflammation and mucosal damage of the small intestine, ending in the malabsorption of several important nutrients including iron, folic acid, calcium and fat-soluble vitamins.[3,4] This can lead to associated diseases such as osteoporosis, anemia, and type I diabetes. CD is now regarded as one of the most common genetic diseases, occurring in 1 of 130–300 of the global population.[5,6] The only effective treatment is strict adherence to a gluten-free (GF) diet throughout the patient's lifetime, which results in clinical and mucosal recovery. Although there is

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a high demand for GF breads for CD patients currently, many GF products available on the market are of low quality, exhibiting low loaf volume, poor color and crumbling crumb.[7–9] Bakery products addressed to celiac patients require the development of complex matrixes with sufficient viscoelastic properties for holding the carbon dioxide released during fermentation and enable to keep the structure during the expansion along baking. There are two established ways of creating a stable network by heat (as in baking). One is to use a thermosetting protein; an example is egg white in meringues or cakes. The second is by using hydrophobically substituted polysaccharides such as hydrocolloids.

Several studies have been carried out showing the potential use of hydrocolloids in GF formulations.[10,11] Hydrocolloids have been applied to act as polymeric substances that mimic the viscoelastic properties of gluten, inducing dough strengthening and increasing the dough's water absorption and gas retaining ability. With this addition, the texture and structure of GF bread could be much improved and made comparable to wheat-flour bakery products. Among them, high molecular weight HPMC that form thermoreversible gel networks on heating and exhibit lower variability than hydrocolloids from natural sources had proved the most effective in structuring baked products.[12]

HPMC is obtained by the addition of methyl and hydroxypropyl groups to the cellulose chain, leading to a polymer with a high surface activity and unique properties regarding its hydration characteristics in the solution state and during temperature changes. In addition, despite the presence of hydrophobic groups in the HPMC chain, this polymer partially maintains the hydrophilic properties of the cellulose.[13] Those properties allow the HPMC to act as emulsifier, strengthen the crumb grain, and increase the moisture content of the crumb.[14,15]

HPMC has been used as an improver in wheat bread, yielding better specific volume, softer crumb and enhanced sensory characteristics.[12,16,17] Sivaramakrishnan[18] found that the rice dough containing HPMC had similar rheological properties as that of wheat flour dough. The use of HPMC also confers good quality properties to GF bread based on 70% sorghum flour, and 30% potato starch.[19] Rice bread of comparable quality to wheat bread has been obtained by incorporating HPMC.[20–22]

Response surface methodology (RSM) is an effective statistical technique used to optimize processes or formulations.[23–26] The relative contribution of predictor variables to product characteristics is evaluated and allows optimum ingredient levels to be determined.[27] Successful application of RSM in the development and optimization of different types of GF bread has been reported.[10,20,28–30] However, there are not any examples in the literature concerning the application of RSM to optimize the recipe of HPMC containing GF bread in order to promote its standardization as well as shelf-life extension.

The aim of the present study is to maximize GF breads desirability (1) by finding adequate models (i.e., prediction equations) to predict characteristics of the product as a function of the levels of the independent variables, and (2) by determining the optimum levels of the independent variables for overall product quality. Also to develop an optimized GF formulation using RSM and examine its staling profile, stored either in air or in a modified atmosphere. The GF formulation used in the study was completely devoid of gluten and was based on ingredients such as maize and rice that are naturally GF.

MATERIALS AND METHODS

Materials

The GF formulation contained corn starch (Roquette, Chemicotechnica SA, Greece) with moisture, protein and ash contents of 13.2, 0.4, and 0.1 g/100 g dry solids, respectively, rice flour (Mediterranean Farm SA, Greece) with moisture, protein, ash and fat contents of 12.8, 7.8, 0.68, and 2 g/100 g dry solids respectively and HPMC (Methocell E-464, Dow Chemical Company). The formulation also contained dried yeast (Yiotis Company, Greece), sunflower oil (Minerva SA, Greece), sucrose (Hellenic Sugar Industry, Greece), and salt (Kallas Company, Greece) as 2, 4, 3, and 2 g/100 g formulation, respectively.

Dough/Bread Formulation

For all baking experiments, ingredients were weighed according to the formulation and the levels of HPMC and W required per treatment (Table 1 and 2, respectively). HPMC level is the amount of HPMC (g) added per 100 g of corn starch and rice flour mix during dough/bread formulation and W level is the amount of water (g) added per 100 g of corn starch and rice flour mix during dough/bread formulation. The dry ingredients were placed in a seven-speed spiral mixer (Model KM 400, Kenwood, UK) and mixed for 2 min at 90 rpm. The yeast was dissolved in warm water (35°C), the resulted solution was added to the dry ingredients, and finally the oil was added. The mixture was blended at 90 rpm for 2 min and following at 180 rpm for 6 min. 400 g of the resultant dough were placed in aluminum baking pans (measuring $17 \times 8.5 \times 8$ cm) and fermented at an incubation chamber (Bekso EB 1N, Bekso, Brussels, Belgium) set at 35°C and 80% relative humidity for 35 min. Baking for each sample was conducted in a laboratory oven with air circulation (Thermawatt TG103, Thermawatt, Athens, Greece) at 200°C for 30 min. The loaves were removed from the pans and cooled at room temperature. Baking, firmness, and sensory characteristics of the loaves were tested 1 h after their removal from the oven (day 0). For shelf-life analysis of the optimized loaves they were packed either in polyethylene bags or in a modified atmosphere of 70% N2/30% CO2 using a BOSS (Bad Hamburg, Germany) packaging machine and stored at 25°C for 6 days.

Table 1 Gluten free bread formulation.

Ingredient	Starch/flour base (kg/100 kg)
Maize starch	75
Rice flour	25
Sunflower oil	4
Yeast	2
Sugar	3
Salt	2
HPMC	Variable[a]
Water	Variable[a]

[a]Amounts varied according to the experimental design (Table 2).

Table 2 Worksheet of the central composite experimental design.

	Independent variables[a]		Dependent variables		
Trial	HPMC X1	Water X2	Specific volume (ml/g) Y1	Firmness (N)Y2	Overall acceptability Y3
1	−1	−1	2.35	5.7	4.8
2	+1	−1	2.63	3.1	6
3	−1	+1	2.28	6	4.5
4	+1	+1	2.34	3.4	6.4
5	−1.414	0	2.4	4.5	6
6	+1.414	0	2.18	5	4.9
7	0	−1.414	2.36	6.4	4.4
8	0	+1.414	2.71	3	7
9	0	0	2.92	4.9	6.6
10	0	0	3.12	5	6.8
11	0	0	3	5.1	6.3
12	0	0	3.02	5	6.5
13	0	0	2.95	5.1	6.5

[a]Variable levels (starch/flour basis): HPMC: –1.414 = 0.5 kg/100 kg, –1 = 0.79 kg/100 kg, 0 = 1.5 kg/100 kg, +1 = 2.21 kg/100 kg, +1.414 = 2.51 kg/100 kg; Water: –1.414 = 70 kg/100 kg, –1 = 73.66 kg/100 kg, 0 = 82.5 kg/100 kg, +1 = 91.34 kg/100 kg, +1.414 = 2.5 kg/100 kg.

Bread Quality Assessment

Breads were weighed (g) and then their loaf volume (mL) was determined by rapeseed displacement.[31] Specific volume (mL/g) was calculated by dividing volume by weight. The moisture content of bread crumb and crust was determined by drying 5–6 g of sample in a forced convection oven at 105°C for 24 h. The samples were cooled in desiccators and weighed by an analytical balance (sensitivity, 0.01 mg). Results were expressed on a wet weight basis.

Crumb firmness was evaluated by the Texture Analyzer (TA-XTi2 Stable Microsystems, Surrey, UK). The bread samples were sliced in the middle using a double blade knife (fabricated in house) to obtain uniform slices of 1 cm thickness. A two cycles crumb compression test was performed using the SMS P/45 aluminum platen probe (probe diameter 36 mm, probe surface area 10 cm^2, test speed 3 mm/s, penetration distance 15 mm). The peak force of compression was reported as firmness (N) in accordance with the AACC method 74–09.[32] The shelf life of the optimized formulation was evaluated by determining crumb firmness over a six day period. While testing the various bread properties the room temperature was 25°C and the relative humidity 60%. The average value of three measurements is presented. In the case of bread analysis, the replicates were from the same baking process but from different bread pieces.

Consumer Acceptability

Overall acceptability was carried out by a panel of 50 consumers (ages 18–35, both sexes), which were recruited from the student community. They were not CD patients but they were aware of tasting starch based GF bread from previous studies.[33] A nine-point hedonic scale was used to evaluate the overall acceptability of the breads; the panelists scored on a scale of 1 (dislike extremely) to 9 (like extremely). The samples were presented separately and in a random sequence as slices, 1-cm high, in coded dishes. The evaluation was conducted in a climate-controlled sensory evaluation laboratory equipped with separately partitioned booths. The panelists cleansed their palates between samples with water and unsalted crackers. Samples were served at room temperature (25 ± 1°C) and analyses were performed under normal lighting conditions. The experiment was designed so that three replicates were obtained for each type of bread.

Scanning Electron Microscopy Analysis

For scanning electron microscopy (SEM) analysis, bread crumb samples of a control non fibre containing GF and the optimized formulation were prior dried and powdered. A Quanta 200 (FEI, Czech Republic) scanning electron microscope was used. Samples were observed at a magnification level of 800×. Higher levels of magnification could not be applied to the samples as they caused excessive heating and deformation.

Statistical Analysis

The software package STATISTICA release 7, statistical software (Statsoft 224 Inc., Tulsa, USA) was used to determine significant differences among the factors (HPMC and water content), fit second-order models and generate response surface plots. Significant differences among the factors were identified by F-tests ($P < 0.05$, 0.01, or 0.001).

Experimental Design

In designing this experiment by response surface methodology (RSM), a central composite design was employed (Table 2). Two quantitative controllable factors (independent variables) were used: level of HPMC (X_1) and level of W (X_2). Three dependent variables were selected as responses for representing the main parameters of GF bread quality: Loaf Specific Volume (Y_1), crumb firmness (Y_2), and overall acceptability (Y3). After preliminary baking tests, the upper and lower limits for the independent variables were established. HPMC levels were 0.5–2.5 kg/100 kg and the W levels were 70–95 kg/100 kg. HPMC level is the amount of HPMC (g) added per 100 g of corn starch and rice flour mix during dough/bread formulation and W level is the amount of water (g) added per 100 g of corn starch and rice flour mix during dough/bread formulation. Five levels of each variable were chosen and thirteen baking trials (Table 2) were performed for the evaluation of the optimized formulation. Five replicates (trials 9, 10, 11, 12, 13) at the centre of the design were used to allow for estimation of the pure error at sum of the square.

To establish predictive models for the bread properties from the varying HPMC and W content used in baking, the experimental data for each response variable were fitted to the following Eq. (1). The regression parameters for the equations are shown in Table 3.

$$Y_i = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1{}^2 + b_{22}X_2{}^2 + b_{12}X_1X_2, \tag{1}$$

where X_1 = HPMC level; X_2 = Water level, including linear, quadratic and interaction effects. b0 is the value of the fitted response at the centre point of the design, that is, point (0,0); b1 and b2 are linear regression terms; b11 and b22 are quadratic regression terms; and b12 is the cross-product regression term. Model selection (mean = no model, linear or quadratic) for each response was made on the basis of the sequential model sum of squares (SMSS), lack-of-fit tests and the multiple correlation coefficient (R^2). In SMSS, the highest degree model should be selected, for which the F-tests show significant ($P < 0.05$, 0.01,

Table 3 Regression equations[a] coefficients and analysis of variance for the different responses.[b]

Coefficient	Specific volume (Y1)	Firmness (Y2)	Acceptability (Y3)
b0	−19.957	−2.177	−43.355
b1	2.032*	1.303**	3.2335*
b11	−0.7168***	−0.3575**	−1.1632**
b2	0.51*	0.2881***	1.059***
b22	−0.003**	−0.0026**	−0.0058**
b12	ns	ns	ns
R^2	0.97	0.99	0.93
Lack of fit	P > 0.05	P > 0.05	P > 0.05

[a]$Yi = b0 + b1X1 + b2X2 + b11X1^2 + b22X2^2 + b12X1X2$, X1 = HPMC, X2 = Water. [b]Only values of significant coefficient are presented (95% confidence level); ns: no significant effect at the 5% level. *Significant at P < 0.05, **Significant at P < 0.01, ***Significant at P < 0.001.

or 0.001) effects, whereas the lack-of-fit should be insignificant. The multiple correlation coefficient R^2 represents the power of fit; it is a measure of how well the regression model fits the raw data. It ranges between 0 and 1, where 1 is for perfect models. Where contradictions between these three requirements existed, the best overall solution was chosen.

Calculation of optimal formulation recipe was performed using a multiple response method called desirability. This optimization method incorporates desires and priorities for each of the variables. Derringer and Suich[34] developed a procedure for specifying the relationship between predicted responses on a dependent variable and the desirability of the responses. Their procedure involved transforming scores on each of the dependent variables into desirability scores that could range from 0.0 for undesirable to 1.0 for very desirable. After transforming the predicted values of the dependent variables at different combinations of levels of the predictor variables into individual desirability scores, the overall desirability of the outcomes at different combinations of levels of the predictor variables can be computed as the geometric mean of the individual desirabilities.

RESULTS AND DISCUSSION

Statistical Analysis

Estimated regression coefficients for dependent variable were obtained from responses by multiple regression analysis (Table 3) and the achieved mathematical models are shown below:

$$\text{Specific volume (Y1)} = -19.957 + 2.032X_1 - 0.7168X_{1^2} + 0.51X_2 - 0.003X_{2^2} \quad (2)$$

$$\text{Crumb firmness (Y2)} = -2.177 + 1.303X_1 - 0.357X_{1^2} + 0.288X_2 - 0.0026X2^2 \quad (3)$$

$$\text{Acceptability (Y3)} = -43.35 + 3.233X_1 - 1.16X1^2 + 1.059X_2 - 0.0058X_{2^2} \quad (4)$$

where X_1 = HPMC level, X_2 = Water level, including linear, quadratic and interaction effects. Once the models were obtained, ANOVA was applied to verify their capability to represent the data. The ANOVA for all four responses is presented in Table 3. In all cases the values of the R^2 coefficient were high and ranged between 0.89 and 0.99 and the lack of fit test was not significant (P > 0.05) confirming that these models were sufficiently accurate for predicting each corresponding response. The high coefficients R^2 was considered as evidence of the applicability of the regression model between the ranges of variables included.

Response Surface Plots

A helpful tool for a better understanding of the link between each factor and response is given by the response surface plots, in which the effect of two factors on one specific response is displayed in 3-D view. The surfaces predicted by Eqs. (2)–(4) are presented in Figs. 1–3.

Effect of HPMC and Water on Loaf Specific Volume

Analysis of variance for each response (Table 3) shows that a significant effect (P < 0.05) was found for specific volume, with regard to HPMC and W. Both variables exhibited positive linear and negative quadratic significant effect. Together, these variables determine rheological properties and gas-holding capability of the GF dough. Response surface plot (Fig. 1) shows

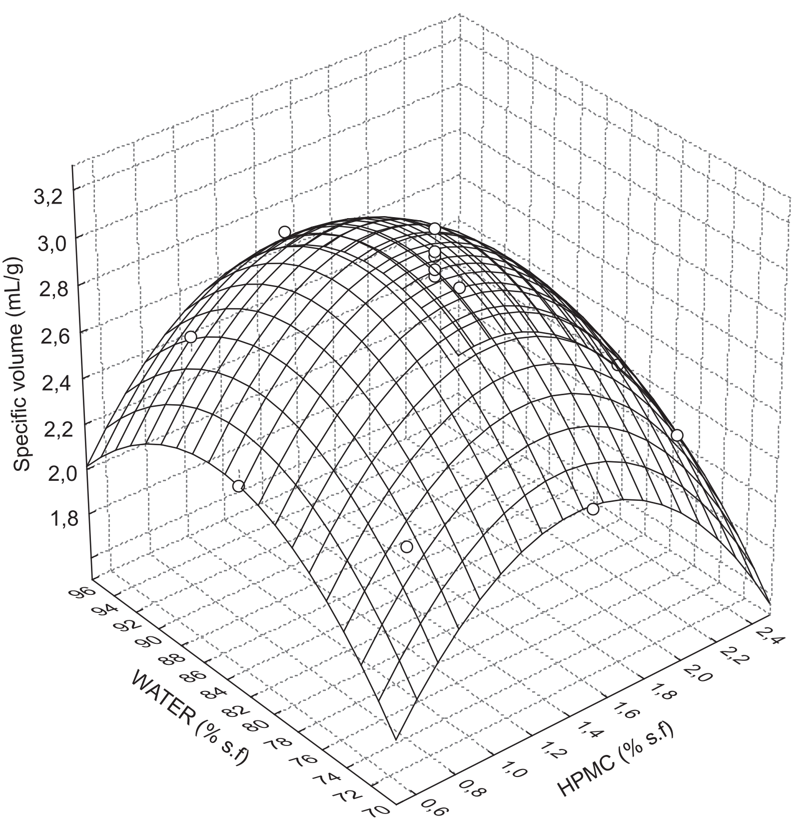

Figure 1 Response surface plot: effect of HPMC and water addition on loaf specific volume. sfb: starch/flour base.

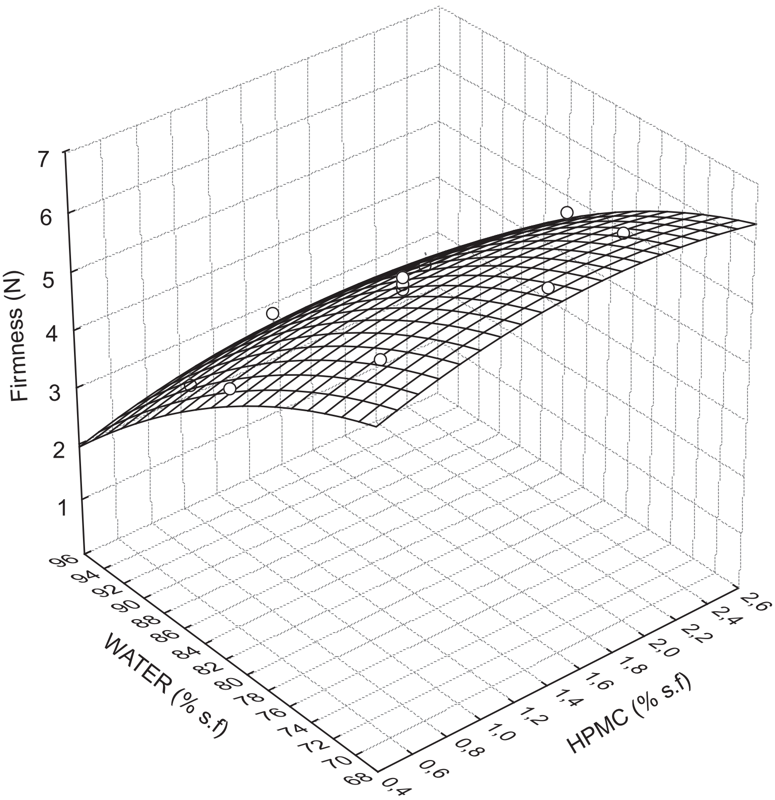

Figure 2 Response surface plot: effect of HPMC and water addition on crumb firmness. sfb: starch/flour base.

that up to a certain limit specific volume increased as HPMC and W increased. Similar increases in loaf volume with increased water addition have been reported by Gallagher et al.[35,36] in GF and Rosell et al.[16] and Rao et al.[37] in wheat breads.

The combination of medium levels of both factors resulted in the highest specific volume. The soft consistency, as promoted by high water addition and limited amounts of HPMC, seems to be advantageous, allowing for a larger increase in volume. An earlier study indicated the improving effect of HPMC in GF loaf volume.[38] It was also found that HPMC enhanced gas retention in bread made with rice flour.[39,40]

HPMC has affinity for both the aqueous and non-aqueous phases of a dough system, therefore maintaining uniformity and stability. However, during baking the HPMC polymers lose their affinity for water and instead gel with one another. This causes an increase in viscosity, strengthens gas cell walls and prevents excess moisture loss. The gel network does not linger after cooling and there are no adverse effects on the texture of the final product.[14]

Addition of extreme levels of HPMC (2.5 kg/100 kg starch-flour blend basis) and W (95 kg/100 kg starch-flour blend basis) decreased specific volume. This reduction may be due to the interaction of HPMC with starch thus to a decrease of gas retention capacity and also to the lower consistency of the dough and high plasticity of the structure because of the extra water. The lower consistency causes the bubbles to become unstable, resulting in large holes and the high plasticity results in collapse of structure. Turabi et al.[41]

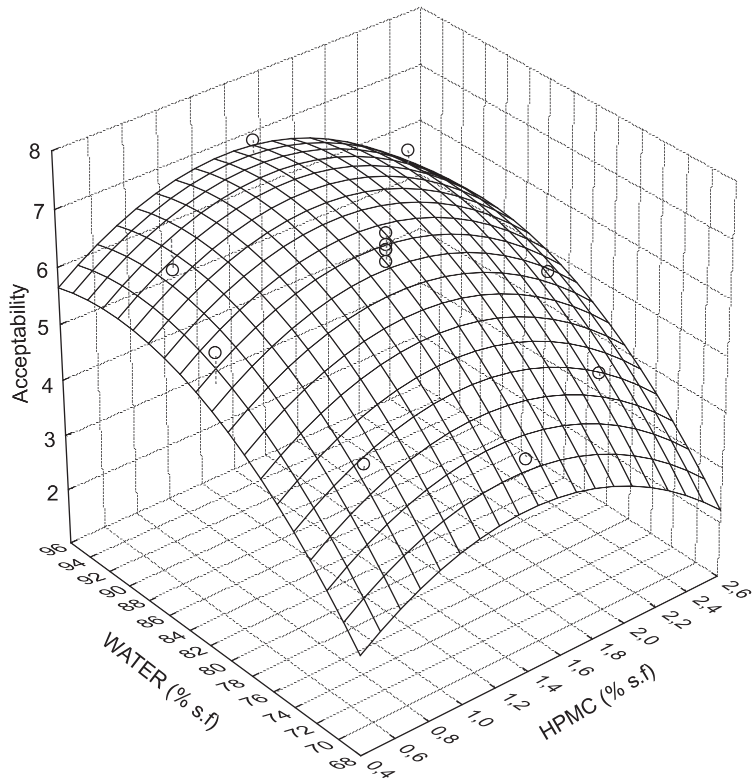

Figure 3 Response surface plot: effect of HPMC and water addition on crumb L value. sfb: starch/flour base.

have also reported lower specific volume in rice cake with low consistency index values compared with that of medium consistency dough. The volume depressing effect upon addition of excessive amount of HPMC (more then 2%) has been also reported by Haque and Morris[38] which pointed out that the right dough consistency, as adjusted by the water content, is a central baking problem.

Effect of HPMC and Water on Crumb Firmness

The resistance of the bread crumb to deformation is the textural attribute referred to as firmness and it is an important factor in bakery products since it is strongly correlated with consumers' perception of bread freshness.[42] Some GF breads due to their high moisture content exhibited soft texture during the baking day. In the current study, crumb firmness ranged from 3.1–6.4 N, which is lower than the value of 6.64 reported for wheat bread[41] using the identical instrumental setup.

Analysis of variance (Table 3) shows that a significant effect ($P < 0.05$) is found for crumb firmness, with regard to HPMC and W. Both variables exhibited positive linear and negative quadratic significant effect. Use of low water levels (trials 1, 2, 7) yielded smaller specific volumes, denser crumb, and subsequently higher crumb firmness values. Earlier studies have shown an inverse relationship between bread loaf volume and firmness.[44,45] This was related to more entanglements and interactions that occur between the more densely packed polymers in samples derived from low-volume breads. Gallagher et al.[35] have also reported softer crust and crumb texture with increased water addition for wheat-starch-based GF bread. Response surface plot (Fig. 2) shows that addition of HPMC induced a decrease of crumb firmness when added at low levels (up to 1.5 kg/100 kg starch -flour blend basis). This result may be due to the water binding capacity of HPMC that avoids water loss during storage and with the possible hydrogen bonding between HPMC and starch that would delay the starch retrogradation. The decrease of crumb firmness upon addition of HPMC in wheat and in GF bread has been previously reported.[16,17,33,38]

Excessive addition of HPMC (trial 6) increased crumb firmness most likely because HPMC had such a high water-binding capacity and at the same time did not increase volume. As reported by several researchers HPMC addition increases the flour water absorption.[16,46] The extent of the increase is likely caused by the great number of hydroxyl groups existing in the HPMC molecules, which allow more water interaction through hydrogen bonding.

Effect of HPMC and Water on Bread Acceptability

Analysis of variance (Table 3) shows that a significant effect ($P < 0.05$) is found for bread overall acceptability with regard to HPMC (X_1) and W (X_2). Both variables exhibited positive linear and negative quadratic significant effect. The formulations containing low levels of water (trials 1, 3, 7) and high level of HPMC (trial 6) were considered unacceptable since they received scores lower than 5. These breads exhibited low loaf volumes and high crumb firmness values. Moreover, breads containing higher amounts of HPMC were rated low due to their powdery taste and sharp flavor. Response surface plot (Fig. 3) shows that up to a certain limit acceptability increased as HPMC and W increased. The combination of medium levels of both factors resulted in the highest scores.

Optimization

Based on the above-described results, it can be asserted that the quality of the GF bread was not dependent on a single main factor and both independent variables were important in defining the characteristics of the bread. So, the next step involved the detection of the best combination of factors that are able to produce the expected characteristics of the final product. All comments arising from the response surface plots were taken into account in the optimization, considering that the optimal solution arises from a compromise among the different responses. In this phase, the criteria of optimization must be selected, that is, a variable response may either be maximized or minimized. In breadmaking, the perception of product quality is very individualistic; however, for widely accepted breads quality criteria are large volume, soft crumb, and high sensory acceptability.[47] Therefore, the objective was to maximize the responses for loaf-specific volume and acceptability and to minimize crumb firmness. As a result of the optimization step, the best conditions, which were attained for the expected response values, were: HPMC level: 1.5 kg/100 kg starch–flour blend basis and W level: 88.75 kg/100 kg starch–flour blend basis. The calculated desirability for this formulation was 0.73 and resulted bread of good quality, which was subsequently analysed in order to compare predicted responses to measured values. The response variables of the bread can be predicted by using the achieved mathematical models (Eqs. 2–4). Overall, the measured responses compared favorably to the predicted

Table 4 Predicted and observed data for the responses at optimum conditions.

	Optimum conditions	
Independent variables (responses)	Measured value	Predicted value
Specific volume (mL/g)	3.04 ± 0.8	2.96
Firmness (N)	3.5 ± 0.4	4.03
Acceptability	7.0 ± 0.2	6.91

values (Table 4). In the current study a specific volume of 3.05 mL/g was obtained. This specific volume was higher than that of the GF bread described by Mc Carthy et al.[30] and Gallagher et al.,[35] which yielded 3.03 and 2.57 mL/g, respectively. Crumb firmness was the only response to show a substantial deviation from the predicted value: it was 13% lower than the predicted value of 4.03 N. The optimized formulation yielded a softer crumb 1 h post baking than a GF bread which had a firmness value of approximately 11 N, measured with the same system.[11] Overall acceptability evaluation depicted that the optimized bread exhibited fine taste, uniform crumb texture, brown color and fresh appearance. It was rated with 7.7 score far superior to the score of the non HPMC containing GF bread which was rated with 6.8 on a nine point scale.[33] The improving effect of HPMC in sensory characteristics of bread has been previously reported.[17] It was observed that the crumb of the optimized bread had medium size air pores and good uniformity. Panelists commented that this bread "looked more like real bread" and that the loaves had "loaf volume and crust color similar to wheat bread."

Shelf Life of GF Breads from Optimized Formulation

The shelf life of bread is mainly influenced by loss of moisture, staling, and microbial deterioration.[48] Of these, staling is the main shelf life-limiting factor. The optimized formulation was baked, stored under conventional and modified atmosphere and tested over a six-day period. GF bread products exhibit faster rates of staling when compared to related wheat products.[10,39,49] As GF breads typically have higher moisture levels than wheat breads, starch retrogradation may progress more rapidly during storage of GF breads. The textural changes in crumb are illustrated in Fig. 4. The crumb firmness of the optimized formulation during the third day of storage was 6.28 N, which is lower than the value of 17N reported for non HPMC containing GF bread under similar conditions.[11] Cellulose derivatives, mainly HPMC had proved to increase water absorption and to give softer doughs and breads with longer keepability.[40] The optimized bread stored in MAP was approximately 15% softer than the one stored in air and exhibit lower staling rate. Crumb moisture of the optimized bread decreased over the 6 d period (Fig. 5), which signifies the migration of moisture from crumb to crust. The gluten network in wheat bread slows the movement of water and therefore, the absence of gluten in GF bread can result in accelerated moisture migration from crumb to crust.[35,50] The optimized bread stored in air was slightly moister than the one stored in MAP.

Microstructure of Gluten Free Bread Crumbs

Scanning electron microscopy was used in order to investigate the structural integrity of the optimized GF bread crumb. The structure of the non HPMC containing bread

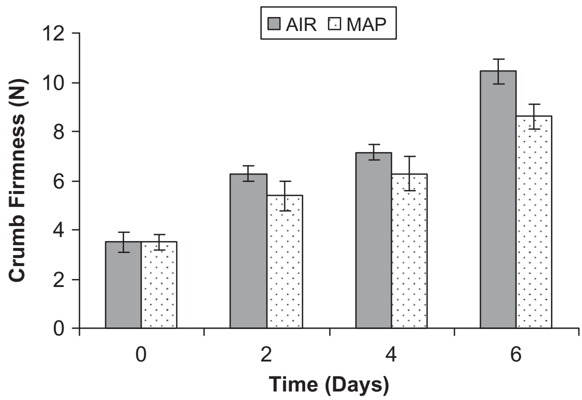

Figure 4 Effect of storage on crumb firmness of the optimized gluten free bread. Mean values ± standard deviation of 3 replicates; Values followed by different letters in the same column are significantly different ($P < 0.05$).

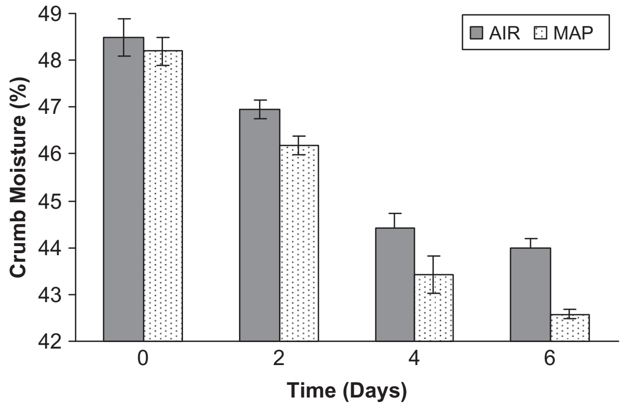

Figure 5 Effect of storage on crumb moisture of the optimized gluten free bread. Mean values ± standard deviation of 3 replicates; Values followed by different letters in the same column are significantly different ($P < 0.05$).

(Fig. 6A) was based on starch and HPMC addition disturbed this structure. Addition of HPMC (Fig. 6B) resulted in a more continuous HPMC-starch matrix exhibiting a more aerated structure.

Marco and Rosell reported that the addition of HPMC resulted in dough with improved structure, since less number and bigger size of cell gas were observed.[51] The HPMC component within the highly developed protein-HPMC-starch network may increase the starch-protein binding and hence explain the decrease in bread firmness. However, HPMC alone do not seem to be enough to stabilize gas cells and produce a web like structure when it is used in starch based, GF breads, indicating the importance of the proteins to form a continuous phase.[42,51] An attempt to incorporate a combination of proteins and HPMC in a GF formulation will be carried out in a future work.

CONCLUSIONS

Response surface methodology was successfully applied to optimize HPMC and water levels in GF bread. The two variables employed in the study had a great effect on the

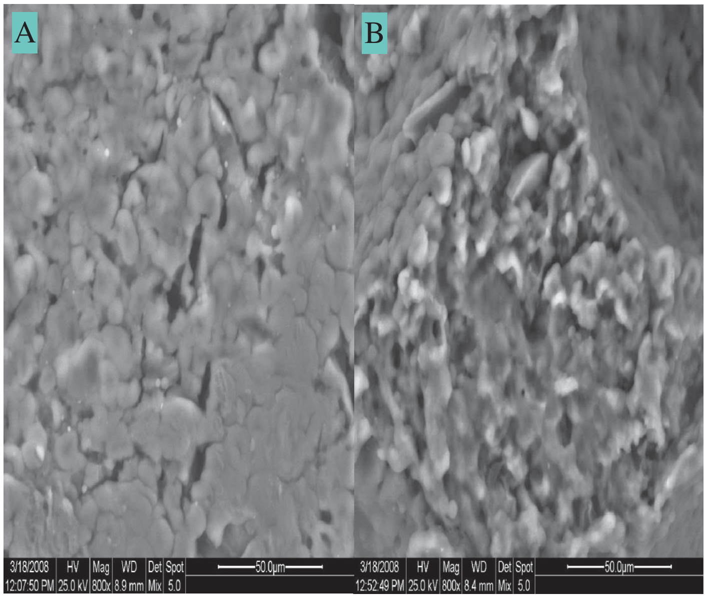

Figure 6 Scanning electron micrograms (800×) of the crumb of gluten free bread. (a) Control –non HPMC; and (b) optimized with HPMC.

quality of GF bread. Up to a certain limit of HPMC addition, the specific volume increased and crumb firmness decreased. The statistical approach allowed the achievement of the optimized recipe which contained 1.5 kg/100 kg HPMC and 88.75 kg/100 kg water (corn starch-rice flour blend basis) and yielded good quality bread with an overall acceptability 13% higher than that of non-HPMC GF bread. Moreover, the modeling of experimental data allowed the generation of useful equations for general use, to predict the behavior of the system under different factor combinations. Within the modified atmosphere package, it appeared that the GF bread staled at a lower rate.

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