

Application of Response Surface Methodology in the Development of Gluten-Free Bread

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ABSTRACT

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The formulation of gluten-free (GF) bread of high quality presents a formidable challenge as it is the gluten fraction of flour that is responsible for an extensible dough with good gas-holding properties and baked bread with good crumb structure. As the use of wheat starch in GF formulations remains a controversial issue, naturally GF ingredients were utilized in this study. Response surface methodology was used to optimize a GF bread formulation primarily based on rice flour, potato starch, and skim milk powder. Hydroxypropylmethylcellulose (HPMC) and water were the predictor variables. Analyses of the treatments from the design were made 24 hr after baking. Specific volume and loaf height increased

as water addition increased ($P < 0.01$). Crumb firmness decreased as water levels increased ($P < 0.01$). Significant interactions ($P < 0.01$) between HPMC and water were found for the number of cells/cm². The number of large cells (>4 mm²) decreased with increasing levels of HPMC and water. Optimal ingredient levels were determined from the data obtained. The optimized formulation contained 2.2% HPMC and 79% water flour/starch base (fsb) and measured responses compared favorably to predicted values. Shelf-life analysis of the optimized formulation over seven days revealed that, as crumb firmness increased, crust firmness and crumb moisture decreased.

Celiac disease (CD) is a chronic malabsorption disorder of the small intestine caused by exposure to gluten in the genetically predisposed individual (Laurin et al 2002; Porpora et al 2002). It is characterized by a strong immune response to certain amino acid sequences found in the prolamin fractions of wheat, barley, and rye (Fasano and Catassi 2001; Thompson 2001; Chirido et al 2002), resulting in damage to the mucosa of the small intestine and leading to the malabsorption of nutrients, thus adversely affecting all systems of the body (Pruessner 1998; Feighery 1999; Thompson 2001). Although traditionally the focus of attention in wheat has been directed on the prolamins (gliadins), recent work has also shown that glutenins contain toxic sequences (van de Wal et al 1999).

With the recent development of sensitive serological tests, it has become possible to evaluate the true prevalence of CD. It is now regarded as one of the most common genetic diseases, occurring in 1 of 130–300 of the European population (Horvath and Mehta 2000; Fasano and Catassi 2001). The disease is self-perpetuating in the continued presence of gluten, and patients are advised to follow a life-long gluten-free (GF) diet. The benefits of this include recovery of the villi of the small intestine and a reduced risk of malignant complications (Hansson 1999; Thompson 2001; Porpora et al 2002; Seraphin and Mobarhan 2002).

At present, many GF breads available on the market are of poor quality and flavor, with many exhibiting a dry crumbling texture (Ylimaki et al 1991; Arendt et al 2002; Gallagher et al 2003a). In addition, recommended breads for the GF diet are often based on wheat starch, which in principle should be free from gluten or gliadin. As it is very difficult to remove gliadin completely, wheat starch usually contains trace amounts of allergenic protein (Friis 1996). Residual gliadin can exacerbate CD and cause persistent symptoms in treated patients. Consequently, a diet naturally free of gluten should be followed (Ciclitira et al 1985; Thompson 2001; Sanchez et al 2002). Rice flour has unique attributes such as bland taste and hypoallergenic properties (Gujral et al 2003). However, it has relatively low amounts of protein and is devoid of the elastic-plastic properties that are indigenous characteristics of wheat gluten and essential for breadmaking (Kadan et al 2001). Therefore, GF

breads based on rice flour require polymeric substances that mimic the viscoelastic properties of gluten to provide structure and retain gas (Toufeili et al 1994). Gums and hydrocolloids like hydroxypropylmethylcellulose (HPMC) appear to improve gas retention and water absorbing characteristics usually supplied by wheat gluten (Gan et al 2001; Kadan et al 2001). HPMC has affinity for both the aqueous and nonaqueous 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 (Bell 1990). Rice breads with quality comparable to that of wheat bread have been obtained by incorporating HPMC (Ylimaki et al 1988; Cato et al 2001). HPMC has also been used as an improver in wheat bread, yielding better specific volume, softer crumb, and enhanced sensory characteristics (Collar et al 1999; Rosell et al 2001).

The incorporation of dairy ingredients in baked products has nutritional and functional benefits such as increased protein and calcium content, flavor and texture enhancement, and shelf-life extension (Mannie and Asp 1999; Kenny et al 2000). Gallagher et al (2003a) report on the use of dairy ingredients in a GF bread formulation and the resultant improvements in crust and crumb color, increased volume, and softening effect on crust and crumb.

Response surface methodology (RSM) is a statistical technique that has been successfully applied in the development and optimization of cereal products (Ylimaki et al 1988; Toufeili et al 1994; Gallagher et al 2003b). The relative contribution of predictor variables to product characteristics is evaluated and allows optimum ingredient levels to be determined (Crowley et al 2001).

The objective of the current study was to develop an optimized GF rice bread formulation using RSM. Optimum levels of HPMC and water were determined. A formulation from Nishita et al (1976) was adapted and modified. Preliminary baking trials were conducted evaluating the addition of potato starch, dairy ingredients, and gums. The ultimate changes to the formulation were the inclusion of potato starch, skim milk powder, and the replacement of carboxymethylcellulose with HPMC.

MATERIALS AND METHODS

Ingredients

Commercial rice flour and potato starch were obtained from Healy Chemicals Ltd. (Dublin, Ireland). Additional ingredients used were skim milk powder (Irish Dairy Board, Dublin, Ireland),

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vegetable oil, sugar, salt, and HPMC (Food Ingredient Technology Ltd., Bedfordshire, UK). Fresh yeast was obtained from Yeast Products Ireland (Dublin, Ireland).

Design Generation and Bread Production

Response surface methodology (RSM) was used to study the simultaneous effects of HPMC and water additions on GF bread. After preliminary baking tests, the upper and lower limits for these variables were established. A central composite design was prepared and the HPMC levels were 0.5–2.5% fsb and the water levels were 70–95% fsb. Five levels of each variable were chosen (Table I) with analysis of 13 combinations of these variables being performed. Assessment of error was derived from five replicates of one treatment combination (1.5% HPMC and 82.5% water, fsb). 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 ($P < 0.05, 0.01, \text{ or } 0.001$) effects, whereas the lack-of-fit should be insignificant. R^2 is 0–1 and should be close to 1. Where contradictions between these three requirements existed, the best overall solution was chosen. The coefficients for all terms in the model (all linear terms for the linear model, all linear, and squared terms; and two-way interactions for the quadratic model) were tested for significance (H_0 coefficient = 0 vs. H_A coefficient $\neq 0$), and significance statements in the text are based on these tests.

Numerical optimization was based on the desirability function

$$D = (d_1^{r_1} \times d_2^{r_2} \times \dots \times d_n^{r_n})^{\frac{1}{\sum r_i}}$$

where d_i are the desirability indices for each response ($d_i = 0$ least desirable; $d_i = 1$ most desirable) and r_i the relative importance of each response. The greatest overall desirability was searched.

TABLE I
Experimental Design

Treatment	Coded Levels ^a	
	HPMC	Water
1	-1	-1
2	+1	-1
3	-1	+1
4	+1	+1
5	-1.414	0
6	+1.414	0
7	0	-1.414
8	0	+1.414
9	0	0
10	0	0
11	0	0
12	0	0
13	0	0

^a Variable levels (flour/starch basis): HPMC: -1.414 = 0.50%, -1 = 0.79%, 0 = 1.50%, +1 = 2.21%, +1.414 = 2.50%; Water: -1.414 = 70%, -1 = 73.66%, 0 = 82.50%, +1 = 91.34%, +1.414 = 95%.

TABLE II
Gluten-Free Bread Formulation

Ingredient	Flour and Starch Base (%)
Rice flour	50
Potato starch	50
Skim milk powder	10
Vegetable oil	6
Yeast	5
Sugar	5
Salt	2

Optimization was based on the +1 and -1 variable levels of the experimental design (Table I): HPMC at 0.79–2.21% fsb and water at 73.66–91.34% fsb.

In a first attempt (optimization 1), the responses used for optimization were loaf specific volume (maximize $r_i = 5$); loaf height (maximize $r_i = 5$); crumb firmness (minimize $r_i = 3$); number of small cells ($0.05 < x < 4.00 \text{ mm}^2$, maximize $r_i = 5$); and number of large cells (minimize $r_i = 3$). Optimization 2 used the responses crumb firmness (minimize $r_i = 1$), number of small cells (maximize $r_i = 5$); number of large cells (minimize $r_i = 5$). Eight baking trials were then performed for both the evaluation of the optimized formulation and for the short-term shelf-life study on the optimized formulation. RSM was performed with Design-Expert 5 (Stat-Ease Corporation, Minneapolis, MN).

For all baking experiments, ingredients were weighed according to the formulation, and the levels of HPMC and water required per treatment (Tables I and II). Dry ingredients were mixed for 1 min at speed 1 in a three-speed mixer (model A120, Hobart, UK). The yeast was dissolved in water at 35°C before being added with the oil during mixing at speed 1 for a further 1 min. All ingredients were mixed at speed 2 for 2 min, 450 g of batter or dough was scaled into 454-g tins ($17 \times 8.5 \times 8 \text{ cm}$) and placed in a proofer at 40°C until the batter had reached the top of the tin, and proof time was noted. The batter or dough was baked at 230°C for 25 min in a deck oven (Tom Chandley Ovens, Manchester, UK). The loaves were cooled to room temperature and heat-sealed in polyethylene bags. For shelf-life analysis, breads were packed in a modified atmosphere of 60% N_2 /40% CO_2 (A300 packaging machine, CVP Systems Ltd., UK). All breads were stored at ambient temperature until required for testing.

Loaf Tests

Analyses of breads from the 13 experimental treatments (Table I) and on breads from the subsequent optimized formulation were performed 24 hr after baking. Four loaves from each treatment were tested. For short-term shelf-life analysis, two loaves from each replicate were tested on days 1, 4, and 7 after baking.

Loaf volume was measured using rapeseed displacement. Loaf weight was recorded and loaf specific volume (mL/g) calculated. The height of three slices from each loaf was measured using electronic digital calipers. Crust and crumb color were measured using a chromameter (Minolta CR-100, Osaka, Japan) recording $L^*a^*b^*$. Each value was the average of six measurements per loaf. Bread was sliced transversely using a slice regulator to obtain uniform 10-mm slices and a 10-mm sample of crust was cut from the base of the loaf. Crust and crumb characteristics were assessed using a texture analyzer (TA-XT2i, Stable Micro Systems, Surrey, UK). Crust was penetrated using a 6-mm cylindrical aluminum probe and a test speed of 1 mm/sec. A 20-mm cylindrical perspex

TABLE III
Batter Consistency and Proof Time

Treatment	Batter Consistency ^a	Proof Time (min)
1	1	30
2	3	30
3	1	25
4	2	40
5	1	30
6	2	30
7	3	35
8	1	30
9	2	30
10	2	30
11	2	35
12	2	30
13	2	30

^a Batter: 1 = thin batter, poured into tins; 2 = firm batter, spooned into tins; 3 = dough, molded by hand.

probe and a 40% compression rate at a test speed of 2 mm/sec were used for crumb texture profile analysis (TPA). Crumb moisture was determined using a single-stage drying process for 2 hr at 130°C (Xu et al 1992). Crumb grain was assessed using a digital image analysis system (Crowley et al 2000). The crumb grain parameters evaluated in this study were number of small cells ($0.05 < x < 4.00 \text{ mm}^2$), number of large cells ($>4.00 \text{ mm}^2$), number of cells/cm² (including small and large cells), and mean cell area in mm². Both sides of three central slices of each loaf were used for crumb grain measurements (60 mm × 60 mm cropped scanned images of the crumb), yielding 24 images per formulation in the optimization experiment and 12 images per replicate in the shelf-life trial. Sensory analysis of the optimized formulation was conducted at one sitting using an untrained panel of 20 members. Panelists were asked to assess the breads for acceptability using a 5-cm line (0 = unacceptable, 5 = very acceptable).

RESULTS AND DISCUSSION

Effects of Water and HPMC on Bread Quality

Variations were observed in the consistency of the batters obtained, which were categorized as either batter or dough (Table III). Two treatments, one with the lowest level of water (70% fsb) and one with high HPMC and low water (2.2 and 74% fsb, respectively) resulted in a dough that was moldable by hand but did not resemble dough of wheat flour, as it lacked viscoelastic character. This is in agreement with the findings of Haque and Morris (1994). Proof times were generally lower (Table III) than those reported for wheat loaves. Besides a relatively high yeast concentration in the formulation, the absence of a viscoelastic gluten network might account for this. It appears plausible that while the gluten network requires comparatively high pressure to be extended by the carbon dioxide of the yeast, GF systems can be expanded much more easily and rapidly, in a manner similar to that of soap micelles. However, the drawback is that the soft, foam-like, GF systems lack stability.

Loaf specific volume (Fig. 1) and loaf height (Fig. 2) exhibited similar trends in that both increased as water addition increased ($P < 0.01$). Similar increases in loaf volume with increased water addition have been reported by Gallagher et al (2003a). The effects of HPMC in the present study were less clear: a slight decrease in specific volume (Fig. 1), but at the same time a slight increase in height (Fig. 2) were found with increasing HPMC in

the range of $\approx 0.8\text{--}2.2\%$ fsb. Varying its dosage in this range caused much smaller effects than varying the amount of water. It is noteworthy that the treatments yielding the highest specific volumes and loaf heights gave poor quality breads with very large gas cells, thereby not truly reflecting specific volume. This agrees with the findings of Nishita et al (1976) and Haque and Morris (1994) on rice bread, both of whom reported loaves with large volume containing large air pockets when excessive water was used. Haque and Morris (1994) used a combination of HPMC and milled seed husk from *Plantago ovata* Forsk (ispaghula) as polymer additives, and reported a trend to higher specific volumes with increasing polymer concentration. At the same time, Haque and Morris (1994) data show that HPMC on its own caused an increased specific volume up to a dosage of 2%, but at the highest level of 4% it had adverse effects. These authors also pointed out that the right dough consistency, as adjusted by the water content, was a central problem. The overall findings reported in this section seem to indicate that there are complex interactions in GF breads between type and level of hydrocolloid used and water level. Together, these parameters determine rheological properties and gas-holding capability. Up to a certain level, a soft consistency, as promoted by high water addition and only limited amounts of hydrocolloid, seems to be advantageous, allowing for a larger increase in volume. However, pushing this too far seems to cause the bubbles to become unstable, resulting in large holes. More research is needed to fully understand such GF systems and the specific effects of hydrocolloids.

Rice breads tend to have a whiter crumb than wheat bread (Ylimaki et al 1988; Cato et al 2001). Crumb L^* values in the current study were 81–86 (100 is the maximum) for most treatments and increased as HPMC increased (data not shown). One treatment had an L^* value of 71. This particular treatment also had a low specific volume and dense crumb following low levels of HPMC and water inclusions (0.79 and 73.66% fsb, respectively). As the chromameter measures the reflectance of light, the dense crumb structure could explain the subsequent low L^* value.

One of the most undesirable characteristics of GF rice breads is a firm and crumbly texture due to the starch base (Cato et al 2001). In the current study, crumb firmness was 179–771 g, which is considerably higher than the value of 125 g reported for wheat bread by Keehan et al (2002) using the identical instrumental setup. A significantly softer crumb was observed as water addition increased ($P < 0.01$, Fig. 3). This can be partly attributed to the

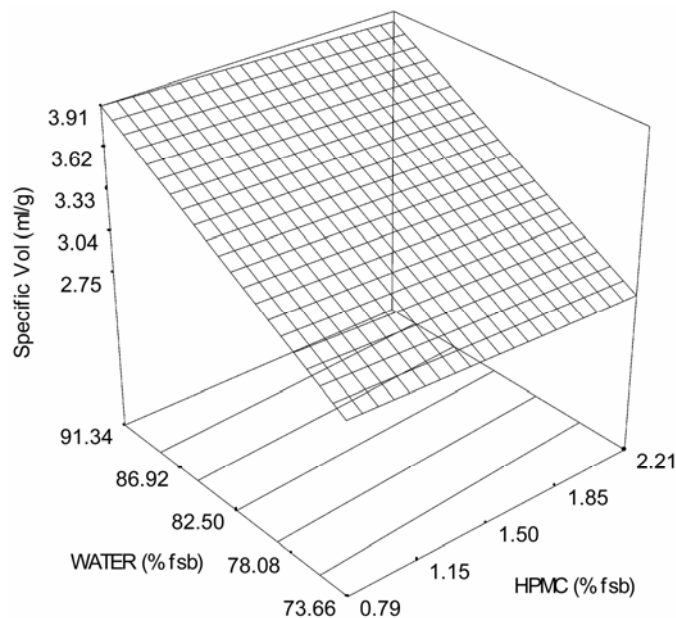


Fig. 1. Effect of HPMC and water addition on loaf specific volume.

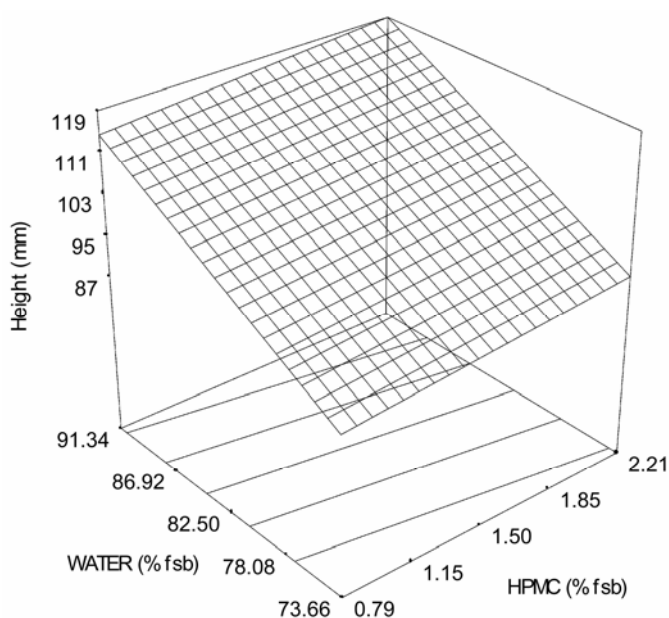


Fig. 2. Effect of HPMC and water addition on loaf height.

higher specific volume and less dense crumb structure of these breads. Using low water levels yielded smaller specific volumes, denser crumb, and subsequently higher crumb firmness values. HPMC reduced crumb firmness in wheat bread (Armero and Collar 1998; Rosell et al 2001). This effect went along with an increased loaf specific volume in the study of Rosell et al (2001). In the current study, crumb firmness increased as HPMC increased, most likely because HPMC had such a high water-binding capacity and at the same time did not increase volume. Furthermore, it is noteworthy that in both studies with wheat bread (Armero and Collar 1998; Rosell et al 2001), dough consistency was standardized to 500 BU, and in Rosell et al (2001) a strongly increased water absorption relative to the control was reported when HPMC was added. In contrast, in the current study, the independent effects of HPMC and water were evaluated. Crumb firmness decreased as water levels increased ($P < 0.05$, data not shown). Softer crust and crumb textures have been reported with increased water addition for wheat-starch-based GF bread (Gallagher et al 2003a).

As expected, higher crumb moisture levels were detected with increased water addition ($P < 0.0001$). Overall, the bread moisture levels were higher than those for wheat bread and this is due to higher levels of water added. The need for high water levels in GF breads to improve loaf volume is described above. Consequently, the treatments with the highest crumb moisture also had the highest loaf specific volume and the lowest crumb firmness.

Crumb grain analysis showed significant interactions ($P < 0.01$) between water and HPMC for the number of cells/cm² (Fig. 4). The number of small cells ($0.05 < x < 4.00$ mm²) exhibited a similar trend ($P < 0.01$, data not shown). Up to a certain limit, the number of cells/cm² increased as HPMC and water increased. The combination of high levels of both, however, decreased the cells/cm² (Fig. 4). This reduction in the number of cells/cm² may be due to the presence of large gas cells in breads with high volume, as described above. The combination of minimum water and maximum HPMC levels resulted in the highest number of cells/cm² (Fig. 4) and also the lowest volume (Fig. 1). This is in agreement with Cato et al (2001), who reported a more uniform cell size and structure in breads with a smaller specific volume. The number of large cells (>4.00 mm²) decreased as water and HPMC increased (data not shown), which may be attributed to the coalescence of many gas cells into one large cell. This was observed in the breads with the highest specific volume.

Optimization

Although in breadmaking the perception of product quality is very personal (Cauvain 2003), widely accepted quality criteria for white pan breads are large volume, soft crumb, and a uniform crumb grain. Other reports specifically state that, in general, crumb cells of relatively small size ($\approx 1\text{--}2$ mm) are required in bakery products and that large voids are undesirable (Cauvain 1998; Cauvain et al 1999). Therefore, in a first attempt (optimization 1), we tried to maximize the responses for loaf specific volume, loaf height, and the number of small cells, and to minimize crumb firmness and the number of large cells. These levels were 0.8% HPMC and 91% water (fsb), which are identical to those of treatment 3 from the experimental design. However, this bread was of poor quality. The very high relative importance assigned to specific volume and height resulted in a calculated optimum that yielded breads with very large gas cells. Therefore, the procedure was repeated with most importance assigned to a maximum number of small cells and a minimum number of large cells (optimization 2). Two solutions were obtained: the first was identical to treatment 3, and the second was 2.2% HPMC and 79% water (fsb). Despite lower calculated desirability (0.67 vs. 0.73), optimization 2 yielded bread of notably better quality and was subsequently analyzed to compare predicted responses with measured values.

Overall, the measured responses compared favorably to the predicted values (Table IV). Lower specific volumes have been reported for rice bread when compared with wheat breads (Yilmaki et al 1988; Haque and Morris 1994). However, this may not be objectionable as it is more realistic to regard rice bread as a different product (e.g., for consumers requiring a special diet) (Kadan et al 2001). In the current study, a specific volume of 3.03 mL/g was obtained. This specific volume was higher than that of the wheat-starch-based GF bread described by Gallagher et al (2003a), which yielded 2.57 mL/g. Crumb firmness was the only response to show a substantial deviation from the predicted value: it was 32% lower than the predicted value of 461 g. The optimized formulation yielded a softer crumb at 24 hr postbaking than a wheat-starch-based GF bread with a firmness value of ≈ 350 g, measured with the same TPA system (Gallagher et al 2003a). The number of small cells at 773 was lower than the 1,187 found by Crowley et al (2000) in wheat bread, applying the same digital image analysis system. Thus, the fine pore structure of a wheat bread could not be reached completely.

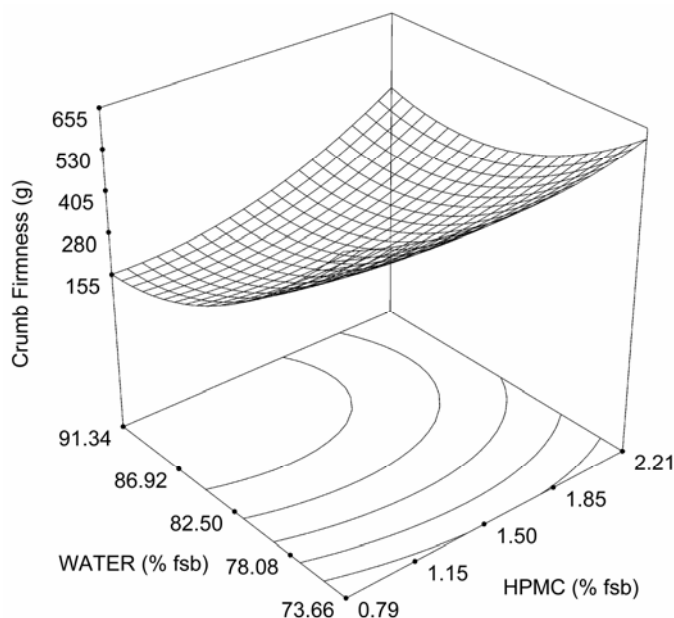


Fig. 3. Effect of HPMC and water addition on crumb firmness.

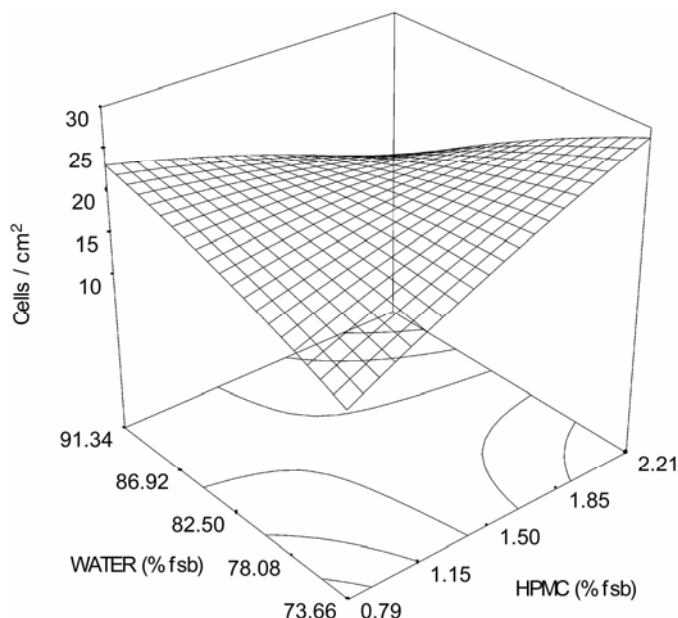


Fig. 4. Effect of HPMC and water addition on the number of cells/cm².

TABLE IV
Model Quality and Comparison of Predicted vs. Measured Values for Optimized Formulation

Parameter	Model	Model Quality ^a			Predicted Value	Measured Value ^b
		SMSS	Lack-of-Fit	R ²		
Specific volume (mL/g)	Linear	$P < 0.01$	$P > 0.05$	0.67	3.08	3.03 ± 0.16
Loaf height (mm)	Linear	$P < 0.05$	$P < 0.001$	0.60	99	104 ± 10
Crumb firmness (g)	Quadratic	$P > 0.05$ (0.065)	$P > 0.05$	0.82	461	313 ± 49
Number of small cells ^c	Quadratic	$P < 0.05$	$P < 0.05$	0.79	851	773 ± 37
Number of large cells ^d	Linear	$P < 0.05$	$P > 0.05$	0.51	21	24 ± 2

^a Best model (linear or quadratic) based on 1) sequential model sum of squares (SMSS, highest degree model should be selected, for which *F*-tests show significant effects); 2) lack-of-fit (should be insignificant); 3) multiple correlation coefficient (*R*² should be close to 1).

^b Average ± standard deviation.

^c $0.05 < x < 4.00 \text{ mm}^2$.

^d $x > 4.00 \text{ mm}^2$.

TABLE V
Color, Moisture, Texture Profile Analysis (TPA), and Crumb Grain Analysis of Optimized Bread

Parameter	Value ^a
Crust <i>L</i> [*]	67 ± 5
Crumb <i>L</i> [*]	86 ± 1
Crumb moisture (%)	47.6 ± 0.5
TPA crumb springiness	0.80 ± 0.03
TPA crumb resilience	0.33 ± 0.02
Crust firmness (g)	521 ± 73
Cells/cm ²	22 ± 1
Mean cell area (mm ²)	1.05 ± 0.11

^a Average ± standard deviation.

TABLE VI
Texture Profile Analysis of Optimized Bread Measured Over Seven Days

Parameter	Days		
	1	4	7
Springiness	0.81 ± 0.03	0.77 ± 0.03	0.75 ± 0.04
Resilience	0.36 ± 0.03	0.30 ± 0.04	0.27 ± 0.03

The results for the other tests are outlined in Table V, and will be compared with results of Keehan et al (2002), Gallagher et al (2003a), and the digital image analysis data of Crowley et al (2002), all of whom used methods and instruments identical to those of the present study. Color (*L*^{*}) measurements for both crust and crumb color show considerable differences in both wheat breads and wheat-starch-based GF breads. The optimized bread yielded a lighter crust than both wheat and GF bread, which had *L*^{*} values of 45 and 60, respectively (Keehan et al 2002; Gallagher et al 2003a). Substantial differences in crumb color were also observed. Gallagher et al (2003a) reported an *L*^{*}-to-*b*^{*} ratio (white-to-yellow ratio) of ≈4.3 for wheat-starch-based bread. The *L*^{*}-to-*b*^{*} ratio of 7.4 (*L*^{*} 86) for the optimized bread was closer to the 6.7 (*L*^{*} 82) reported for wheat bread by Keehan et al (2002). Due to the higher level of water addition in the GF bread, crumb moisture was higher than that of wheat bread. At 521 g, crust firmness was lower than the value of 838 g reported for wheat bread (Keehan et al 2002) and 800 g for wheat-starch-based GF bread (Gallagher et al 2003a). This was attributed to the higher moisture level of the optimized GF bread, which resulted in a less crisp crust.

Crumb grain analysis showed that the mean cell area of 1.05 mm² is comparable to the 1.01 mm² measured in wheat bread 26 hr postbaking by Crowley et al (2002). The gas cell walls of the optimized bread appeared to be thicker than those in wheat bread and, consequently, the 22 cells/cm² finding was lower than the 32 cells/cm² finding in wheat bread (Crowley et al 2002).

Shelf Life of GF Breads from Optimized Formulation

Bread has a short shelf life largely due to extensive textural and organoleptic modifications such as loss of softness, moist texture,

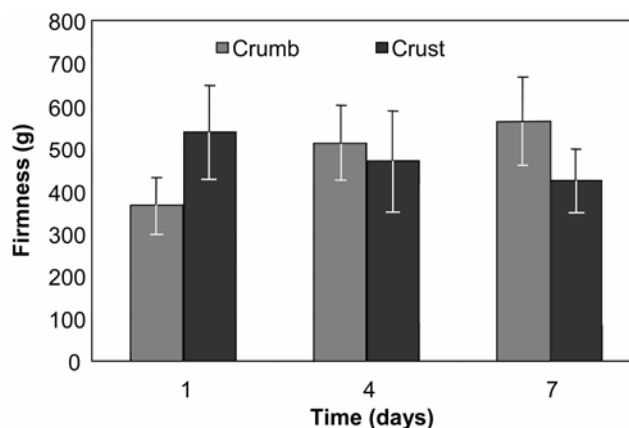


Fig. 5. Textural changes of the optimized gluten-free (GF) bread over a storage period of seven days. Error bars represent standard deviation.

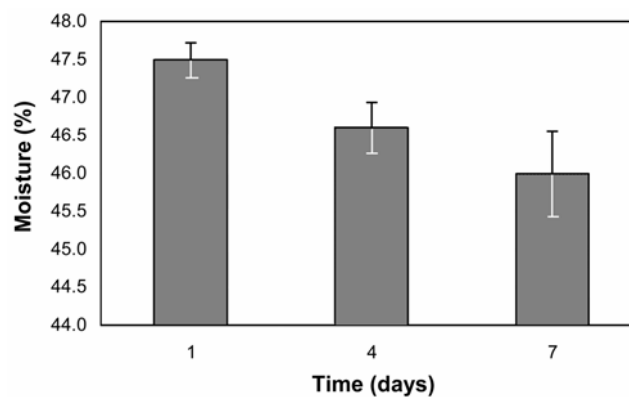


Fig. 6. Changes in crumb moisture of the optimized gluten-free (GF) bread over a storage period of seven days. Error bars represent standard deviation.

and flavor (Piazza and Masi 1995; Baik and Chinachoti 2000, 2002). The optimized formulation was baked and tested over a seven day period. GF bread products exhibit faster rates of staling when compared with related wheat products (Nishita et al 1976; Toufeili et al 1994; Kadan et al 2001) and the textural changes in crust and crumb are illustrated in Fig. 5. In wheat bread, crust penetration force values decrease over time due to a reduction in crust firmness or crispness (O'Brien et al 2000). Crust firmness also decreased in the current study and a concurrent increase in crumb firmness was observed. This is attributed to moisture equilibration within the loaf during storage (Crowley et al 2002), and increasing crumb firmness beyond that of starch retrogradation (see below). Texture profile analysis showed that crumb springiness decreased over time (Table VI), which is indicative of an increase in brittleness (Kadan et al 2001; Moore et al 2004). This was also

reflected in the tendency of the bread to crumble when sliced on day 7. Crumb firming and an increase in crumbliness have a negative impact on the eating quality of bread (Keetels et al 1996a,b). Resilience decreased during storage (Table VI), which, along with increasing crumb firmness, suggests the onset of starch retrogradation (Kadan et al 2001); the rate of starch retrogradation in wheat bread increases as moisture content is increased (Rogers et al 1988). As GF breads typically have higher moisture levels than wheat breads, starch retrogradation may progress more rapidly during storage of GF breads. Furthermore, the absence of a gluten network may render changes in the starch phase more important with regard to the overall crumb texture. It was concluded earlier that a continuous elastic structure of denatured gluten strands surrounding the starch might mask some of the changes originating from starch retrogradation (Moore et al 2004). Crumb moisture decreased over seven days (Fig. 6), 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 (Roach and Hosenev 1995; Gallagher et al 2003a).

Sensory Analysis

The optimized GF bread was rated 1.8 cm on a scale with end points of 0 (unacceptable) and 5 (acceptable). However, it must be noted that for nonceliac panelists, most would be unfamiliar with breads based on rice flour and potato starch. Thus, their expectations of the characteristics of bread would be based entirely on products made from wheat flour (Haque and Morris 1994). This may account partly for the low score. Using a panel of CD patients would be more appropriate as a person with wheat intolerance has a limited choice of baked products and would be more familiar with the organoleptic properties of starch-based products and therefore may not be as critical as a person without CD or a wheat allergy when judging bread quality characteristics (Nishita et al 1976).

CONCLUSIONS

Response surface methodology was successfully applied to optimize HPMC and water levels in a GF bread that was not wheat-starch-based. Of the two variables employed in the study, water had a greater effect on the quality of GF bread. Increasing water levels significantly increased loaf specific volume and height, and decreased crumb firmness. HPMC and water showed significant interactions in their effect on crumb grain structure. When incorporated into the formulation, the optimized levels of 2.2% HPMC and 79% water yielded good quality bread.

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