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Development of fibre-enriched gluten-free bread: a response surface methodology study

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Abstract
The enrichment of gluten-free (GF) baked products with dietary fibre (DF) seems to be necessary since it has been reported that coeliac patients have generally a low intake of DF due to their GF diet. Response surface methodology was used to optimize a fibre-enriched GF bread formulation based on corn starch, rice flour and hydroxypropylmethyl cellulose. Maize fibre and water were the predictor variables (factors), and loaf specific volume, crumb firmness and crumb *L* value were the dependent variables (responses) used to assess the product quality. The optimal formulation, determined from the data, contained 6.5% maize fibre and 102.5% water, starch/flour base. 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 exhibited lower crumb firmness and moisture content values, and thus remained softer through storage. Scanning electron microscopy of the crumb showed a continuum matrix between starch and maize fibre, in the optimized formulation, obtaining a more aerated structure.

Keywords: *Coeliac disease, dietary fibre, enrichment, bread, optimization*

Introduction

Coeliac 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 (Laurin et al. 2002; Hamer 2005). In CD patients, ingestion of gluten leads to inflammation and mucosal damage of the small intestine, ending in the malabsorption of several important nutrients. This can lead to associated diseases such as osteoporosis, anaemia and type I diabetes. CD is now regarded as one of the most common genetic diseases, occurring in one of 130–300 of the global population (Fasano and Catassi 2001; Fasano et al. 2003). The only effective treatment for CD is strict adherence to a gluten-free (GF) diet throughout the patient's lifetime, which results in clinical and mucosal recovery. However, there are growing concerns over the nutritional adequacy of the GF dietary pattern because it is often characterized by an excessive consumption of energy, proteins, and fats, and a reduced intake of complex carbohydrates and dietary fibre (DF) (Thompson 2001; Thompson et al. 2005).

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The term dietary fibre is referred to polysaccharides, oligosaccharides and their hydrophilic derivatives, which cannot be digested by the human digestive enzymes to absorbable components in the upper alimentary tract (Thebaudin et al. 1997). The physiological actions promoted by fibre addition in foods are depicted in the following: maintenance of gastrointestinal health, reduction of intestine transit time, protection against colon cancer, lowering of total and low-density lipoprotein cholesterol in the blood serum, reduction of postprandial blood glucose levels, increase of the calcium bioavailability and reinforcement of the immunological system (Thebaudin et al. 1997; Tungland and Meyer 2002). DF can also provide a multitude of functional properties when incorporated in food systems. Thus, fibre addition contributes to the modification and improvement of the texture, sensory characteristics and shelf-life of foods resulting in their water-holding capacity, gel forming ability, fat mimetic, antisticking anticlumping, texturizing and thickening effects (Gelroth and Ranhotra 2001).

The GF baked goods are consequently low in fibre, as wheat flour is mainly substituted with commercial starches, which usually do not contribute to DF content. Therefore, the enrichment of GF baked products with DF seems to be necessary, since a typical CD patient diet should not usually assure the recommended 25–38 g DF intake per day (Mayer et al. 1991; Lohiniemi et al. 2000; Grehn et al. 2001). Several studies have been carried out showing the potential enrichment of wheat-based bread with DF (Wang et al. 2002; Gomez et al. 2003; Rosell et al. 2006; Rao et al. 2007). Enriched GF products are not common (Taylor and Parker 2002; Kiskini et al. 2007; Marco and Rosell 2008), but such products have been suggested to improve the quality of the GF diet (Kupper 2005). The GF formulation used in this study was completely devoid of gluten and was based on ingredients such as maize and rice, which are naturally gluten-free. In a previous study, maize fibre (MF) proved the most promising potential, from other cereal fibres used, in the development of fibre-enriched GF bread (Sabanis et al. 2008).

Response surface methodology (RSM) is an effective statistical technique used to optimize processes or formulations (Malcolmson et al. 1993; Bas and Boyaci 2007). The relative contribution of predictor variables to product characteristics is evaluated and allows optimum ingredient levels to be determined (Crowley et al. 2001). Successful application of RSM in the development and optimization of different types of GF bread has been reported (Ylimaki et al. 1991; Toufeili et al. 1994; Sanchez et al. 2002, 2004; McCarthy et al. 2005). However, there are no examples in the literature concerning the application of RSM to enhance the recipe of enriched GF breads in order to promote their standardization as well as shelf-life extension.

The aim of the present study is to maximize fibre-enriched GF breads desirability: (1) by finding adequate models (i.e. prediction equations) to predict characteristics of the product as a function of the independent variables levels, and (2) by determining the optimum levels of the independent variables for overall product quality. Also to examine the staling profile of GF bread, stored either in air or in a modified atmosphere. Preliminary baking trials were conducted evaluating the addition of MF, rice flour and hydroxypropylmethyl cellulose (HPMC).

Materials and methods

Raw materials

The GF formulation contained corn starch (Roquette; Chemicotechnica SA, Athens, Greece) with moisture, protein and ash contents of 13.2%, 0.4% and 0.1% (dry basis) respectively, rice flour (Medireranean Farm SA, Athens, Greece) with moisture, protein, ash and fat contents of 12.8%, 7.8%, 0.68% and 2% (dry basis) respectively, and HPMC (Methocell E-464, Dow Chemical Company). The MF added was obtained from Astron Chemicals SA (Athens, Greece) and its characteristics (according to the manufacturer) are presented in Table I. The formulation contained also dried yeast (Yiotis Company, Athens, Greece), sunflower oil (Minerva SA, Schimatari, Greece), sucrose (Hellenic Sugar Industry, Larissa, Greece) and salt (Kallas Company, Athens, Greece).

Fibre characterization

The moisture and ash content of MF were determined in accordance with American Association of Cereal Chemists (AACC) methods 44-15 and 08-01, respectively (AACC 2000). The bulk density and the water binding capacity were determined in accordance with DIN 53468 and AACC method 56-30, respectively (AACC 2000). The total and insoluble DF content of the fibre was determined in accordance with AACC method 32-07 (AACC 2000), and its particle size distribution by sieve analysis in accordance with DIN 53724. The colour was determined using a Minolta CR200 tristimulus chromatometer (Minolta Company, Osaka, Japan). Readings were displayed as a, b and L colour parameters according to the CIELAB system of colour measurement. The a value is a measure of greenness ranging from -100 to $+100$, the

Table I. Characteristics of the maize fibre used in the study.

Fibre characteristic	MF
Chemical composition (%)[a]	
Moisture	8.35
Protein	0.4
Ash	2.4
Physical data	
Bulk density (g/l)	94
Water binding capacity (g water/g solid)	8.0
Colour L value	74.29
Colour a value	-0.55
Colour b value	11.71
Nutritional composition (%)[a]	
Total DF	97.0
Insoluble DF	94.0
Particle size distribution (%)	
>200 μm	0.4
100–200 μm	12.7
32–100 μm	73.9
<32 μm	13

[a] Dry basis.

b value ranges from -100 (blueness) to $+100$ (yellowness), while the *L* value indicates the measure of lightness and ranges from 0 (black) to 100 (white) (Hutchings 1994). The chromatometer was calibrated using a white reference plate, which is the standard of reflectiveness. The *L, a* and *b* values for the reference plate were 97.47, -0.29 and -3.83, respectively.

Dough/bread formulation

For all baking experiments, ingredients were weighed according to the levels of MF and water required per treatment and the formulation (Tables II and III, respectively). The MF level is the amount of maize fibre (g) added per 100 g starch and flour mix during dough/bread formulation, and the water level is the amount of water (g) added per 100 g starch and flour mix during dough/bread formulation. The dry ingredients were placed in a seven-speed spiral mixer (Model KM 400; Kenwood, London, UK) and mixed for 2 min at 90 rpm. The yeast was dissolved in warm water (35°C) and 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. Then 400 g of the resultant dough were placed in aluminium baking pans (measuring $17 \times 8.5 \times 8$ cm^3) 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, sensory and firmness 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% nitrogen/30% carbon dioxide using a BOSS (Bad Hamburg, Germany) packaging machine and stored at 25°C for 6 days.

Table II. Worksheet of the central composite experimental design.

	Independent variables[a]		Dependent variables		
Trial	MF, X_1	Water, X_2	Specific volume (ml/g), Y_1	Firmness (N), Y_2	Crumb *L* value, Y_3
1	-1	-1	2.92	5.6	69.12
2	$+1$	-1	3.14	5	54.75
3	-1	$+1$	3.16	4.1	67.76
4	$+1$	$+1$	3.17	4.1	51.81
5	-1.414	0	3.2	4.8	71.34
6	$+1.414$	0	3.15	5.3	52.23
7	0	-1.414	3.02	6	62.14
8	0	$+1.414$	3.18	3.8	58.56
9	0	0	3.28	4	62.08
10	0	0	3.3	4.1	61.78
11	0	0	3.22	4.2	63.89
12	0	0	3.23	4.2	64.54
13	0	0	3.28	4	62.65

[a]Variable levels (starch/flour basis): MF: $-1.414 = 2.0\%$, $-1 = 2.88\%$, $0 = 5.0\%$, $+1 = 7.12\%$, $+1.414 = 8.0\%$; Water: $-1.414 = 80\%$, $-1 = 84.4\%$, $0 = 95\%$, $+1 = 105.6\%$, $+1.414 = 110\%$.

Table III. Gluten-free bread formulation.

Ingredient	Starch/flour base (%)
Maize starch	75
Rice flour	25
HPMC	1.5
Sunflower oil	4
Yeast	2
Sugar	3
Salt	2
Water	Variable[a]
Maize fibre	Variable[a]

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

Bread quality assessment

Breads were weighed (g) and then their loaf volume (ml) was determined by rapeseed displacement (Hallen et al. 2004). 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 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. The crumb colour of the baked samples was measured using a Minolta CR200 tristimulus chromatometer (Minolta Company, Osaka, Japan). Readings were displayed as a, b and L parameters according to the CIELAB system of colour measurement as described above. Only the L value was considered in the experimental design.

Crumb firmness was evaluated by the Texture Analyzer (TA-XTi2; Stable Microsystems, Surrey, UK). The bread samples were, thereafter, sliced in the middle using a double-blade knife (fabricated in house) to obtain uniform slices of 1 cm thickness. A two-cycle 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 AACC method 74-09 (AACC 2000). The shelf-life of the optimized formulation was evaluated by determining crumb firmness over a 6-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.

Sensory evaluation

Overall acceptability was carried out by a panel of 50 consumers (ages 18–35 years, both sexes) that were recruited from the student community. They were not CD patients but they were aware of tasting starch-based GF bread from previous studies (Sabanis and Tzia 2007a). A nine-point hedonic scale was used to evaluate the overall acceptability of the breads; the panellists 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 panellists cleansed their palates between samples with water and unsalted crackers. Samples were served at room temperature ($25 \pm 1^\circ$ 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 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 800x. 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, OK, USA) was used to determine significant differences among the factors (maize fibre 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 and statistical analysis

In designing this experiment by RSM, a central composite design was employed (Table II). Two quantitative controllable factors (independent variables) were used: level of MF (X_1) and level of water (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 crumb L value (Y_3). After preliminary baking tests, the upper and lower limits for the independent variables were established. MF levels were 2–8% starch/flour base and the water levels were 80–110% starch/flour base. Five levels of each variable were chosen and 13 baking trials (Table II) were performed for the evaluation of the optimized formulation. Five replicates (Trials 9, 10, 11, 12, and 13) at the centre of the design were used to allow for estimation of the pure error at the sum of the square.

To establish predictive models for the bread properties from the varying MF and water content used in baking, the experimental data for each response variable were fitted to the following equation (the regression parameters for the equations are presented in Table IV):

$$Y_0 = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \qquad (1)$$

where X_1 is the MF level; X_2 is the water level, including linear, quadratic and interaction effects; b_0 is the value of the fitted response at the centre point of the design, that is point (0, 0); b_1 and b_2 are linear regression terms; b_{11} and b_{22} are quadratic regression terms; and b_{12} 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, 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

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

Coefficient	Specific volume (Y_1)	Firmness (Y_2)	Crumb L value (Y_3)
b_0	−6.0534	42.271	−31.62
b_1	NS	NS	−0.2571***
b_{11}	−0.0119*	0.0903***	NS
b_2	0.1713**	−0.6584***	2.2937*
b_{22}	−0.0008**	0.0029***	−0.0122*
b_{12}	−0.0023*	0.0067*	NS
R^2	0.85	0.92	0.98
Lack of fit	$P>0.05$	$P<0.05$	$P>0.05$

[a]$Y_0 = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2$, $X_1 =$ MF, $X_2 =$ 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 $P<0.001$.

data. It ranges between 0 and 1, where 1 is obtained for perfect models. Where contradictions between these three requirements existed, the best overall solution was chosen.

Calculation of the optimal formulation recipe was performed using a multiple response method called desirability. This optimization method incorporates desires and priorities for each of the quality variables. Derringer and Suich (1980) 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 the predictor variables levels 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 variables were obtained from responses by multiple regression analysis (Table IV) and the achieved mathematical models are shown below:

$$\text{Specific volume } (Y_1) = -6.05 - 0.0119X_1^2 + 0.1713X_2 - 0.0008X_2^2 - 0.0023X_1X_2 \quad (2)$$

$$\text{Crumb firmness } (Y_2) = 42.271 + 0.0903X_1^2 - 0.658X_2 + 0.0029X_2^2 + 0.0067X_1X_2 \quad (3)$$

$$\text{Crumb } L \text{ value } (Y_3) = -31.62 - 0.2571X_1 + 2.293X_2 - 0.0122X_2^2 \quad (4)$$

Once the models were obtained, analysis of variance was applied to verify their capability to represent the data. The analysis of variance for all four responses is presented in Table IV. In all cases the values of the R^2 coefficient were high and ranged between 0.85 and 0.98, confirming that these models were sufficiently accurate for predicting each corresponding response.

For crumb firmness the lack-of-fit test was significant, which means that the order of the regression was not secondary (the model may not have included all appropriate functions of independent variables or the experimental region may be too large for a quadratic model was used). It was pointed out that when a large amount of data was included in the analysis, a model with significant lack of fit could still be used (Box and Draper 1987). We considered the high coefficients R^2 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 a three-dimensional view. The surfaces predicted by Equations (2)–(4) are presented in Figures 1–3.

Effect of maize fibre and water on loaf specific volume

Analysis of variance for each response (Table IV) shows that a significant effect ($P < 0.05$) exists for specific volume, with regard to MF (X_1^2) and water (X_2) variables as well as their interaction (X_1X_2). Both variables affect rheological properties and gas-holding capability of the GF dough. The response surface plot (Figure 1) shows that, up to a certain limit, the specific volume increased as MF and water increased. 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 MF, seems to be advantageous, allowing the product to have a larger increase in volume. Similar increases in loaf volume with increased water addition have been reported by Gallagher et al. (2002a, 2003) in GF and by Rosell et al. (2001) and Rao et al. (2007) in wheat breads. The improving effect of MF in loaf volume may be attributed to the presence of insoluble matters in MF and the formation of

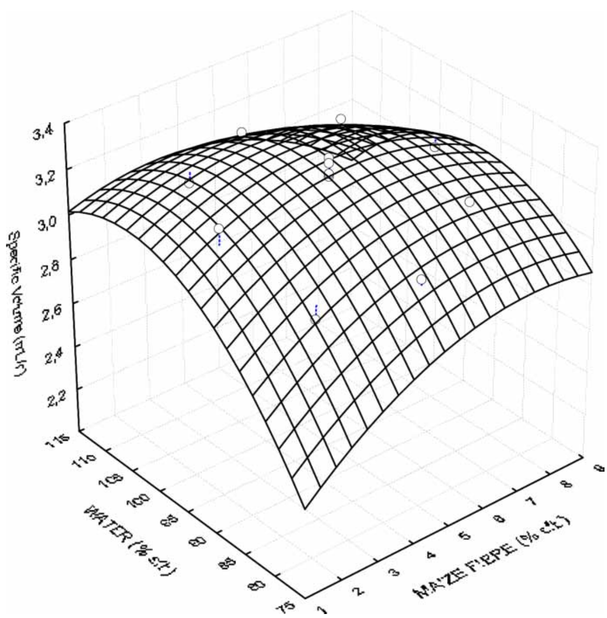

Figure 1. Response surface plot: effect of maize fibre and water addition on loaf specific volume. sfb, starch/flour base.

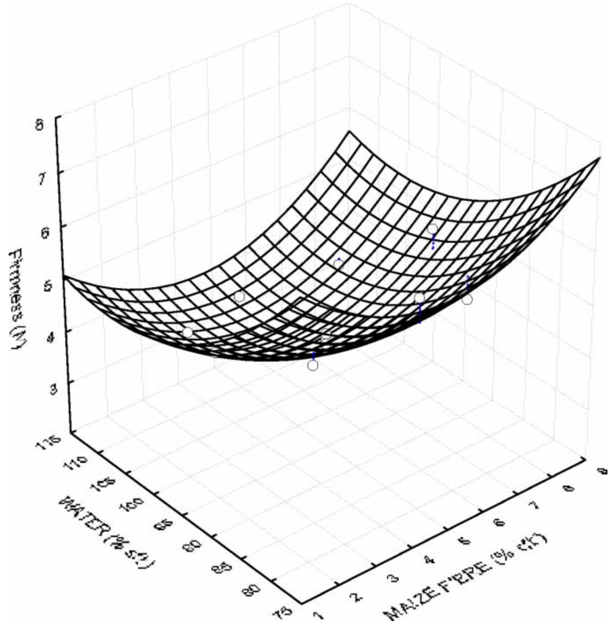

Figure 2. Response surface plot: effect of maize fibre and water addition on crumb firmness. sfb, starch/flour base.

networks comprised of hydrated cellulose and hemicellulose. However, extreme levels of MF (8%) and water (110%) decreased the specific volume. This reduction may be due to the interaction of fibre 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. (2008) have also reported lower specific volume in rice cake with low consistency index values compared with that of medium consistency dough. Haque and Morris (1994) pointed out that the right dough consistency, as adjusted by the water content, is a central baking problem.

Effect of maize fibre and water on crumb firmness

The resistance of the bread crumb to deformation is the textural attribute referred to as firmness; it is an important factor in bakery products strongly correlated with consumers' perception of bread freshness (Ahlborn et al. 2005). Some GF breads due to their high moisture content exhibit soft texture during the baking day. In the current study, crumb firmness ranged from 3.8 to 6 N, which is lower than the value of 6.64 reported for wheat bread (Sabanis and Tzia 2007b) using the identical instrumental setup.

Use of low water levels (Trials 1, 2, 7) yielded smaller specific volumes, denser crumb, and subsequently higher crumb firmness values than non-fibre GF bread. Earlier studies (He and Hoseney 1990; Every et al. 1998) have shown an inverse relationship between bread loaf volume and firmness. This was related to more entanglements, and interactions occurred between the more densely packed polymers

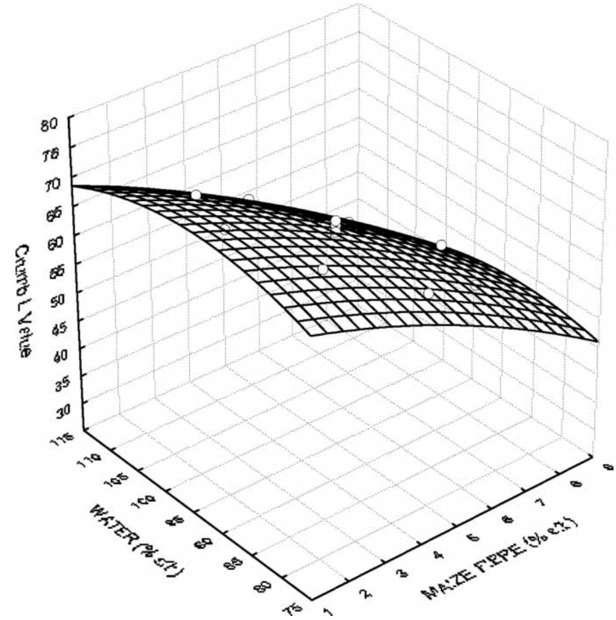

Figure 3. Response surface plot: effect of maize fibre and water addition on crumb *L* value. sfb, starch/flour base.

in samples derived from low-volume breads. Table IV and Equation (3) show that water addition promoted a decrease in crumb firmness having negative linear and positive quadratic significant effect. Gallagher et al. (2003) have also reported softer crust and crumb texture with increased water for wheat-starch-based GF bread. The response surface plot (Figure 2) shows that addition of MF induced a decrease of crumb firmness when added at low levels (up to 5% flour-starch blend basis). The water binding capacity of these fibres that prevents water loss during storage as well as the possible hydrogen bonding between fibre and starch that would delay the starch retrogradation may explain the former observation. The decrease of crumb firmness upon addition of fibres in both wheat and GF bread has been previously reported (Gallagher et al. 2002b; Wang et al. 2002). On the other hand, excessive addition of MF (Trial 6) increased crumb firmness, probably because MF had a high water-binding capacity, and at the same time did not increase volume. It is well known that DF addition increases the flour water absorption (Pomeranz et al. 1977; Wang et al. 2002). The extent of the increase depends upon the structure of the fibre and it is probably caused by the great number of hydroxyl groups existing in the fibre molecules, which allow more water interaction through hydrogen bonding. Gomez et al. (2003) reported an increase in crumb firmness upon addition of wheat fibre in wheat bread attributed to the thickening of the walls surrounding the air bubbles in the crumb.

Effect of maize fibre and water on crumb colour

Colour is an important characteristic for baked products because, in addition to texture and aroma, it contributes to consumer preference. Estelle and Lannes (2008) reported that bread colour depends on the characteristics of the dough (water content, pH, reducing sugars and amino acid content) and on the operating conditions applied during baking (temperature, relative humidity, modes of heat transfer). Crumb L values in the current study were 60–70 for most treatments and decreased significantly as MF increased (Figure 3, $P<0.001$) due to the colour of MF. A similar decrease in crumb lightness upon addition of fibre in wheat flour has been reported previously (Knuckles et al. 1997; Kaark et al. 2006). According to Gomez et al. (2003), the original colour of the fibre had no influence on the crust colour because this is mainly associated with Maillard and caramelization reactions. In contrast, the colour of crumb is usually similar to the colour of the fibre because the crumb does not reach such high temperatures as the crust. Water addition promoted a decrease of crumb L value, having a positive linear and negative quadratic significant effect (Table IV).

Optimization

Based on the above-described results, it can be asserted that the quality of the fibre-enriched GF bread is 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 in 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. Thus, the criteria of optimization must be selected; that is, a variable response may either be maximized or minimized. Although the perception of bread quality is very personal, widely accepted quality criteria are the large volume, the soft crumb and a uniform crumb colour (MacDougall 1983; Cauvain 2003). Therefore, the objective was to maximize the responses for loaf-specific volume and to minimize crumb firmness and the crumb L value. The obtained darkening of the crust and crumb colour due to the inclusion of fibre was desirable as GF breads usually tend to have a lighter colour in respect to wheat breads (Gallagher et al. 2002a).

As a result of the optimization step, the best conditions that were attained for the expected response values were a MF level of 6.5% and water level of 102.5%. This formulation had a calculated desirability of 0.745 and the resulted bread exhibited good quality, which was subsequently analysed in order to compare predicted response to measured values. The responses variables can be predicted by using the achieved mathematical models (Equations (2)–(4)). Overall, the measured responses compared favourably with the predicted values (Table V). In the current study a specific volume of 3.20 ml/g was obtained. This specific volume was higher than that of the GF bread described by McCarthy et al. (2005) and Gallagher et al. (2003), 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 14% lower than the predicted value of 4.07 N. The optimized formulation yielded a softer crumb 1 h post baking than a GF bread that had a firmness value of approximately 11 N, measured with the same system (Lazaridou et al. 2007). The crumb L value for the optimized formulation was 57.12, lower than the 71.67 presented by Lazaridou et al. (2007) and the 86 presented by McCarthy et al. (2005)—thus the GF bread in this study exhibited a more desirable dark colour.

Overall acceptability evaluation depicted that the optimized bread exhibited fine taste, uniform crumb texture, brown colour and fresh appearance. It was rated with a 7.7 score, far superior to the score of the non-fibre control GF that was rated with 6.8

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

	Optimum conditions	
Independent variables (responses)	Measured value	Predicted value
Specific volume (ml/g)	3.20±0.15	3.22
Firmness (N)	3.5±0.44	4.07
Crumb *L* value	57.12±1.5	55.88

on a nine-point scale (Sabanis and Tzia 2007a). The reduced crumb firmness and fat mimetic effect of DF probably affected mouthfeel, flavour release and texture perception during consumption. It was observed that the crumb of the optimized bread had medium size air pores and good uniformity. It must be noted that the panellists commented that this bread 'looked more like real bread' and that the loaves had 'loaf volume and crust colour similar to wheat bread'.

Shelf-life of gluten-free breads from optimized formulation

The shelf-life of bread is mainly influenced by loss of moisture, staling and microbial deterioration (Willhoft 1971). Of these, staling is the main shelf life-limiting factor. The optimized formulation was baked, stored under a conventional and modified atmosphere, and tested over a 6-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).

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 Figure 4. Crumb moisture of the optimized bread decreased over the 6-day period (Figure 5), which signifies the migration of moisture from crumb to crust. The gluten network in wheat bread slows the movement of water while the absence of gluten in GF bread can result in accelerated moisture migration from crumb to crust, respectively (Roach and Hoseney 1995; Gallagher et al. 2003).

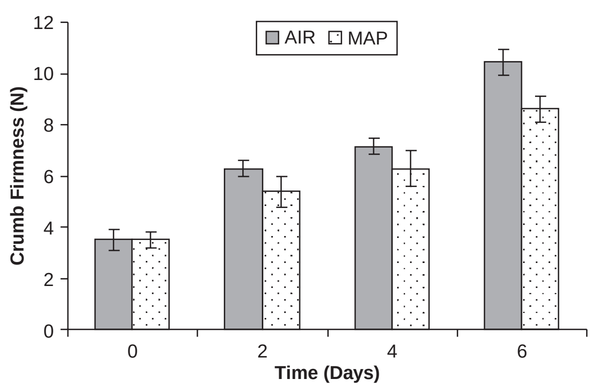

Figure 4. Effect of storage on crumb firmness of the optimized gluten-free bread. Mean values±standard deviation of three replicates. Values followed by different letters in the same column are significantly different ($P<0.05$). MAP, modified atmosphere packaging.

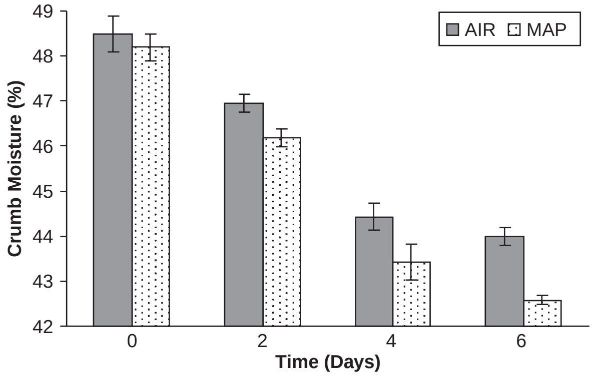

Figure 5. Effect of storage on crumb moisture of the optimized gluten-free bread. Mean values ± standard deviation of three replicates. Values followed by different letters in the same column are significantly different ($P < 0.05$). MAP, modified atmosphere packaging.

The optimized bread stored in modified atmosphere packaging proved softer and moister than the one stored in air and exhibited a lower staling rate.

Nutritional aspects

The nutritional importance of DF has been demonstrated in many studies. A typical western diet contains less than 20 g/day, whereas the recommended daily intake has been set at 38 g for men and 25g for women (Trumbo et al. 2002). At the 6.5% MF level (optimized formulation), the DF content of the loaves was 5.2%. This was in contrast to the 2.2% of gluten-free non-fibre-containing loaf and the 3.7% of an ordinary wheat bread loaf (Figure 6). According to Abdul-Hamid and Luan (2000), soluble DF could be hydrolysed by yeast enzymes and lost during baking while insoluble DF suggested by the present study had more promising potential in the development of fibre-enriched GF bread in order to increase the daily fibre intake.

Microstructure of gluten-free bread crumbs

Scanning electron microscopy was used in order to investigate the structural integrity of the optimized fibre-enriched GF bread crumb. The structure of the non-fibre-containing bread (Figure 7a) was based on starch and HPMC, and fibre

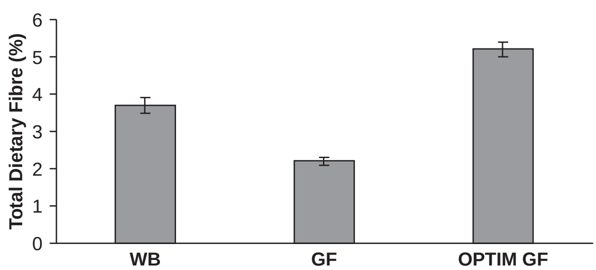

Figure 6. Total DF contents of optimized gluten-free bread with added maize fibre (OPTIM GF) compared with wheat flour bread (WB) and gluten-free non-fibre-containing bread (GF). Curves are means of three replicates for each bread formulation. Error bars represent the standard deviation.

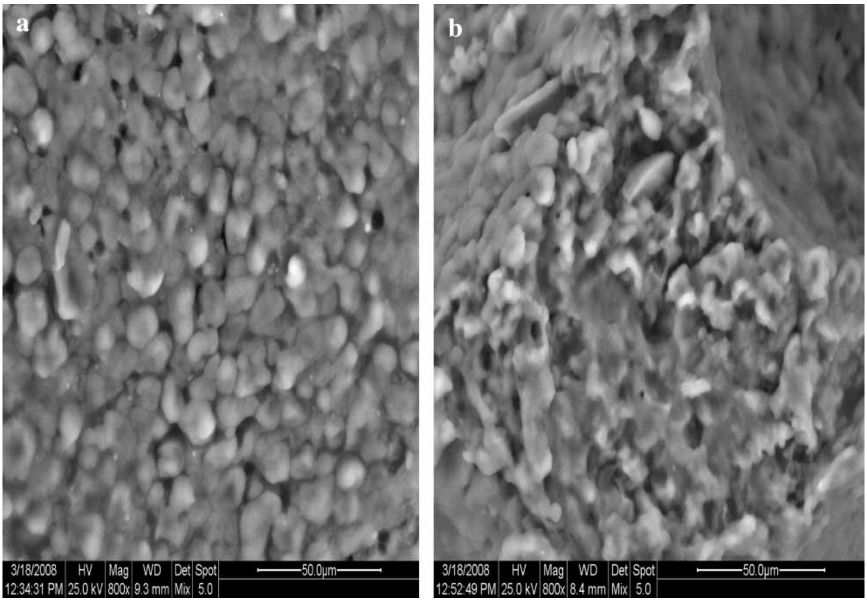

Figure 7. Scanning electron micrograms (800x) of the crumb of gluten-free bread (scale bar = 50 μm): (a) control—non-fibre, (b) optimized with maize fibre.

addition disturbed this structure. Addition of MF (Figure 7b) resulted in a more continuous fibre–starch matrix exhibiting a more aerated structure. The fibre component within the highly developed protein–fibre–starch network may increase the starch–protein binding and hence explain the decrease in bread firmness. Similar finding were presented by Tudorica et al. (2002) with the elasticity of fibre-enriched pasta. However, even MF cannot produce a web-like structure when it is used in starch-based, gluten-free breads, indicating the importance of the proteins to form a continuous phase since the HPMC and fibre alone do not seem adequately to stabilize gas cells (Ahlborn et al. 2005; Marco and Rosell 2008). An attempt to incorporate a combination of proteins, HPMC and MF in a gluten-free formulation will be carried out in a future work.

Conclusion

RSM was successfully applied to optimize maize fibre and water levels in gluten-free bread. The two variables employed in the study had a great effect on the quality of gluten-free bread. Up to a certain limit of MF addition, the specific volume increased and crumb firmness decreased. Crumb *L* values decreased as the MF level increased, while crumb firmness decreased as the water content increased ($P < 0.05$). The statistical approach allowed the achievement of the optimized recipe, which contained 6.5% maize fibre and 102.5% water and yielded good quality bread with a total DF content 40% higher than that of ordinary wheat bread. Moreover, the modelling of experimental data allowed the generation of useful equations for general use, to predict the behaviour of the system under different factor combinations. Within the modified atmosphere package, it appeared that the bread staled at a lower rate.

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