

Formulation of Gluten-Free Pocket-Type Flat Breads: Optimization of Methylcellulose, Gum Arabic, and Egg Albumen Levels by Response Surface Methodology

IMAD TOUFEILI,^{1,2} SHAWKY DAGHER,¹ SOSSY SHADAREVIAN,¹ ABIR NOUREDDINE,¹ MAY SARA KBI,¹ and MOHAMMED T. FARRAN¹

ABSTRACT

Cereal Chem. 71(6):594-601

Response surface methodology was used to analyze the effects of methylcellulose, egg albumen, and gum arabic on the sensory properties of gluten-free pocket-type flat bread baked from formulas based on pregelatinized rice flour and pregelatinized corn starch with corn flour. A rotatable central-composite design consisting of three variables (methylcellulose, egg albumen, and gum arabic), in a five-level pattern (1.37, 2, 3, 4, and 4.63 g) with 20 runs (gluten-free formulations), was prepared over three blocks. This design was used to develop models for the different sensory responses. Responses were affected most by changes in methylcellulose and egg albumen levels, and to a lesser extent by gum arabic levels. Individual contour plots of the different responses were superimposed,

and regions meeting the maximum number of bread sensory attributes were identified. When 3 g of gum arabic was included in the bake mix, gluten-free breads comparable to regular wheat bread in the frequency of cracks, separation of layers, rollability, tearing quality, hardness, adhesiveness, and cohesiveness were obtained at methylcellulose levels ≥ 2.10 g and ≤ 4.12 g, and at egg albumen levels ≥ 2.18 g and ≤ 4.10 g. Higher levels (4.63 g) of gum arabic resulted in more cohesive products. Lower levels (1.37 g) of gum arabic produced loaves that were less cohesive and inferior to wheat bread in rollability. All breads possessed a perceptible corn flavor, a light-yellow crumb with apparent waxy patches, and a faster staling rate than that of regular wheat bread.

Wheat, pocket-type flat breads are the main dietary staple of people in the Middle East, the Nile Valley, and the Persian Gulf states. These breads are referred to as "Arabic", *balady*, *shami*, "Lebanese", and *maffood* in the Arab countries and as "pita" in the United States and Europe. The formulation, processing, and characteristics of these flat breads were recently reviewed by Faridi (1988). Reliable statistics on the prevalence of gluten intolerance in the aforementioned countries is lacking, or at best, difficult to locate. However, gluten intolerance has been reported in Northern Sudan (Suliman 1978), as well as in Middle Eastern countries such as Lebanon (Bitar et al 1970) and Iraq (Al-Hassany 1975). The principal treatment consists of removing gluten from the diet. Exclusion of gluten from the diet is a formidable task for dietitians, as wheat flour is present in a wide range of products including bread, biscuits, cakes, and pastas. The situation is particularly irksome in the case of bread, as flat bread consumption constitutes the cornerstone of dietary patterns for these populations.

Gluten-free breads require polymeric substances that mimic the viscoelastic properties of gluten in bread doughs. To this end, gluten-free pan breads have been successfully formulated by incorporating gums (Kim and De Ruiter 1968, Smith 1971), soy proteins (Ranhotra et al 1975), and egg white (Eggleston et al 1992) into nonwheat flours.

The effectiveness of response surface methodology (RSM) in the development and optimization of cereal products has been highlighted by different workers (Vaisey-Genser et al 1987, Shelke et al 1990, Malcolmson et al 1993). Recently, RSM was effectively utilized in mapping the levels of gums and water required for the production of sensorially acceptable gluten-free pan bread from a formula based on rice flour and potato starch (Yilmaki et al 1988, 1991).

This article reports on the use of RSM in defining the levels of methylcellulose, gum arabic, and egg albumen needed to formulate gluten-free pocket-type flat bread that is based on a bake mix of pregelatinized rice and corn starch, and that is compatible with regular wheat bread in key sensory attributes.

MATERIALS AND METHODS

Materials

Corn flour and corn starch (B. V. Meelunie, Amsterdam) and active dry yeast (Fermipan, Delft, Holland) were obtained from a local supplier. Methylcellulose (Methocel MC, 400 cP), gum arabic, and powdered sodium stearoyl-2-lactylate (Artodan SP 55) were purchased from Fluka (Buchs, Switzerland), Sigma Chemical Company (St. Louis, MO), and Grinsted Products (Brabrand, Denmark), respectively. Wheat flour, short-grain rice, eggs, sugar, and salt were procured from the local market.

Chemical and Physicochemical Analyses

Protein, fat, ash, moisture, and farinograph absorption were determined according to standard methods (AACC 1983). Degree of gelatinization was assayed as described by Chiang and Johnson (1977). Pasting temperature was determined from amylograms recorded with the 700 cm² cartridge for 8% (w/v) flour slurries, as reported by Rasper (1980). Particle size distributions were registered on a Malvern Mastersizer SB.0A (Malvern Instruments, England) at a focal length of 300 mm. Analyses were made in duplicate.

Experimental Flours and Dried Egg Albumen

Pregelatinized corn starch. Corn starch (1 kg) was soaked in water (12 L) for 15 min, and the suspension was heated in a steam-jacketed kettle at 95°C for 20 min. The slurry was cooled to room temperature, spread on stainless steel trays, and dried in an air-draft oven (Hotpack, Philadelphia, PA) at 110°C for 12 hr and then at 80°C for an additional 12 hr. The dried flakes were ground in a Wiley mill fitted with a 1-mm screen and then in an Alpine mill (Kollopex, 160Z, Augsburg, Germany) to minimize graininess. The flakes were sieved to pass through 300- μ m perforations and stored at 6°C until used. The powder composition was: protein (N \times 5.7), 0.44%; fat, 1.14%; moisture, 8.01%; ash, 0.36%; degree of gelatinization, 98.3%. It had a particle size distribution of 168.0 μ m maximum; 50% of the particles were ≤ 182.07 μ m.

Pregelatinized rice flour. Whole rice (1 kg) was hydrated in water (16 L) for 15 min, processed, and stored as described previously. The powder composition was: protein (N \times 5.95), 6.59%; fat, 1.98%; moisture, 6.04%; ash, 0.47%; degree of gelatinization, 96.7%. It had a particle size distribution of 159.20 μ m maximum; 50% of the particles were ≤ 133.71 μ m.

¹Departments of Food Technology & Nutrition, Biology, and Animal Science, American University of Beirut, Lebanon.

²Corresponding author.

TABLE I
Experimental Design and Amounts of Water in Different Formulations

Variables ^a	Coded Levels ^b			Water ^c (ml)
	X ₁ (Methylcellulose)	X ₂ (Gum Arabic)	X ₃ (Egg Albumen)	
Block 1	-1	-1	-1	91.5
	+1	+1	-1	101.5
	+1	-1	+1	101.5
	-1	+1	+1	94.3
	0	0	0	99.1
Block 2	0	0	0	96.7
	+1	-1	-1	100.0
	-1	+1	-1	93.5
	-1	-1	+1	93.1
	+1	+1	+1	104.2
Block 3	0	0	0	98.5
	0	0	0	97.2
	-1.633	0	0	91.3
	+1.633	0	0	100.2
	0	-1.633	0	92.2
	0	+1.633	0	95.0
	0	0	-1.633	93.3
	0	0	+1.633	96.1
	0	0	0	96.9
0	0	0	95.6	

^aBlocks and treatment combinations within a block were randomized.

^b-1.633 (1.37 g), -1 (2 g), 0 (3 g), +1 (4 g), +1.633 (4.63 g). Reported on 100-g flour mass basis.

^cAverage of two replicates on 100 g (flour mass basis). Differences did not exceed 4.5%.

Corn flour. Corn flour composition was: protein (N×5.7), 4.84%; fat, 2.18%; moisture, 14.13%; ash, 0.69%. The pasting temperature was 77°C. Particle size distribution was 258.61 μm maximum; 50% of the particles were ≤170.21 μm.

Dried egg albumen. Egg whites were separated manually from the yolks, mixed into a uniform mass, freeze-dried (Stokes 902-1-8, Penwalt, Philadelphia, PA), ground to a fine powder (Moulinex 241, France), sieved to pass 300-μm perforations, and stored at 6°C. Before use, the powder was reconstituted by adding 8 parts of water to 1 part solids and holding for 3 hr at room temperature, as described by Sultan (1969).

Experimental Design

A response surface design was used to study the relative contribution of different variables to bread quality and to determine optimum gluten-free product formulations. Methylcellulose, gum arabic, and egg albumen were chosen as variables for this study on the basis of data from preliminary screening experiments (Sarakbi 1985, Noureddine 1993). The gum and egg albumen levels were varied according to a rotatable central-composite design with three blocks and six replicates of the center point (Gacula and Singh 1984). The experimental design (Table I), which was replicated twice, consisted of three variables, a five-level pattern with 20 runs (bread formulations) prepared over three blocks (consecutive weeks). For the statistical analysis, the five levels of the three variables were coded as -1.633, -1, 0, +1, and +1.633.

Preparation of Bread Samples

The formula used in the preparation of experimental breads is shown in Table II. Breads were prepared by mixing the ingredients with simultaneous addition of water at speed 2 in Braun dough mixer (type 4122, Frankfurt, Germany) until a cohesive dough mass was obtained (~7-10 min). The amounts of water needed for optimum dough consistency (assessed kinesthetically) are shown in Table I. The resultant dough was fermented for 30 min in an incubator (GM, Precision) and maintained at 37°C. The fermented dough was divided and rounded into balls of 20 g each. The balls were covered with a wet cloth and fermented for 20 min at 37°C and then flattened into circular sheets of 1.7-mm thickness with a plexiglass roller. Throughout the dough-handling stages, corn flour was used as the dusting powder. The flattened sheets were proofed for 20 min and baked at 470°C to optimum crust color (~50-70 sec), as reported by Maleki and

TABLE II
Basic Gluten-Free Pocket-Type Flat Bread Formula

Ingredients	Mass (g)
Pregelatinized rice flour	100
Pregelatinized corn starch	50
Corn flour	50
Sodium stearyl-2-lactylate	0.5
Sugar	6.0
Salt	3.0
Yeast	4.0
Methylcellulose ^a	variable
Gum arabic ^a	variable
Egg albumen ^a	variable
Water ^b	

^aAmounts varied according to the experimental design (Table I).

^bOptimum dough consistency was assessed kinesthetically. Amounts needed for different formulations are shown in Table I.

Daghir (1967). After baking, the loaves were cooled for 10 min, placed in polyethylene bags to prevent moisture loss, and stored at -20°C until used.

A commercial bakers' flour (composition: protein [N×5.7], 10.83%; ash, 0.64%; moisture basis, 14.0%; farinograph absorption, 58.1%) (AACC 1983) was used in the preparation of the reference wheat bread. Dough preparation and baking were as described above. Reference bread samples were subjected to the same storage conditions as experimental breads.

Sensory Evaluation

Nine panelists (six female, three male, aged 22-26 years), who had completed a graduate course in sensory analysis, were trained in the profiling of pocket-type flat breads. The samples used for training included a range of wheat and rice-corn breads differing in formulation, processing, and storage conditions (time-temperature combinations). During the four weeks of training (twelve 1-hr sessions), panelists used group discussions to establish descriptive terms characterizing flavor and textural attributes of pocket-type flat breads. The attributes selected were: frequency of cracks in layers, separation of layers, crumb color, grain distribution, rollability, tearing quality, first bite hardness, adhesiveness, masticatory hardness, cohesiveness of mass, and corn flavor. Interestingly, rice flavor was not detected in rice-corn breads. Definitions of the aforementioned attributes were reported previously (Meilgaard et al 1987, Qarooni et al 1987, Williams et

al 1988).

Consistency of the panel was checked by subjecting data for the indicated attributes from five replicate ratings of five bread samples to principal component analysis (Kwan and Kowalski 1980, Powers 1984). The results (data not shown) revealed a cluster of seven assessors indicating agreement in evaluation, and two outliers. Consequently, sensory measurements were conducted with the "consistent" panel of seven assessors (five female, two male).

The attributes' descriptor ranges were: frequency of cracks (none—too many), separation of layers (fully separated— inseparable), crumb color (creamy white—yellow), grain distribution (continuous cellular structure—no cellular structure, waxy), rollability (loaf retains integrity—loaf cracks extensively), tearing quality (very easy—very difficult), first bite hardness (very soft—very hard), adhesiveness (not sticky—very sticky), masticatory hardness (very soft—very hard), cohesiveness of mass (very loose—very compact), and corn flavor (absent—very strong).

The sensory score sheet consisted of 15-cm unstructured line scales anchored at the extremes with the appropriate descriptors. In evaluating the breads, panelists were instructed to place an arrow across the line at the point that best quantified their response. The magnitude of the responses was scaled by measuring the distance from zero to the position of the arrow.

Samples, selected at random from the different treatments, were removed from storage 30 min before evaluation to equilibrate to room temperature. The breads were rated in comparison to regular wheat bread on two consecutive days. After equilibration, loaves (with three-digit codes) were presented to the panelists in a balanced random order and rated for continuity of surfaces, separation of layers, crumb color, grain distribution, rollability, tearing quality, first bite hardness, adhesiveness, masticatory hardness, cohesiveness, and corn flavor. On the second day, loaves from the same treatments were assessed for keeping quality on the basis of rollability, tearing quality, and cohesiveness after 36 hr of storage at room temperature. Panelists were instructed to rinse their mouths with water between samples. Four experimental breads, along with a regular wheat bread sample, were evaluated at each session. Apart from the first and last days, which included one session, two sessions per day were conducted, with the assessments completed over a 10-day period. The assessments, replicated twice, were conducted in partitioned booths equipped with white light.

Statistical Analysis

Data were analyzed as outlined by Ylimaki et al (1988, 1991). For each sensory response measured by the panel, analysis of

variance (ANOVA) was conducted to determine differences among the 15 treatment combinations. When the response showed significant differences, data were analyzed by multiple regression (MSTAT 1989) to estimate the effect of the variables on the sensory response. The effects were estimated by fitting the design to the second-order regression equations:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$

where X_1 = methylcellulose, X_2 = gum arabic, and X_3 = egg albumen, including linear, quadratic, and interaction effects. Coefficients of determination (R^2) were computed, and the adequacy of models was tested by separating the residual sum of squares into pure error and lack-of-fit (Gacula and Singh 1984). Contour plots, generated by Statgraphics (STSC 1991), were produced from the equations by holding the variable with the least effect on the response equal to a constant value and changing the other two variables. Each contour plot was compared to the corresponding reference wheat bread data. Areas on each plot representing rice-corn bread formulations that met reference standards were identified. Finally, contour plots for the responses were superimposed to locate gluten-free bread formulations that met regular wheat bread standards for the indicated sensory characteristics.

RESULTS AND DISCUSSION

The ranges of mean sensory scores, over all treatment combinations, along with the reference wheat bread, are shown in Table III. ANOVA determined that differences were not significant in frequency of cracks, separation of layers, and corn flavor. Comparison of the mean sensory scores for these responses to those of the reference revealed that the scores of cracks and separation of layers fell within the range of the reference. Accordingly, gluten-free breads were considered to have met the reference standards for the aforementioned attributes. All gluten-free formulations had stronger corn flavor than did the reference bread.

ANOVA determined that differences were significant in crumb color, grain distribution, first day rollability (Rollability₁), first day tearing quality (Tearing₁), first bite hardness, adhesiveness, first day cohesiveness (Cohesiveness₁), second day rollability (Rollability₂), second day tearing quality (Tearing₂), and second day cohesiveness (Cohesiveness₂). Accordingly, regression equations were computed for those attributes and are presented in Table IV.

Coefficients of determination (R^2) indicated that regression equations accounted for 43–96% of the variance in sensory responses. The low R^2 values of 0.43 for Cohesiveness₂, 0.57 for crumb color, and 0.63 for grain distribution suggested that factors other than the levels of gums and egg albumen affected the responses. More specifically, the apparent passivity of gums and egg albumen to changes in Cohesiveness₂ point to the dominating role of starch in shaping the textural properties of bread during storage. Moreover, the unexplained variability in crumb color scores may have resulted from the inherent instability of the quality scores and the complexity of the judging task. Individual loaves often exhibited pronounced variations in the intensity of color in the inner layers. Furthermore, the marginal effects of gums and egg albumen on the grain distribution are in line with previous observations in regular wheat breads where grain characteristics were affected mainly by fermentation variables (Qarooni et al 1989). Each regression equation was tested for lack-of-fit. Responses for masticatory hardness, crumb color, Rolling₂, Tearing₂, and Cohesiveness₂ showed significant lack-of-fit. The lack-of-fit of second-order models was attributed to 1) the heterogeneity of the error term utilized in the calculation of lack-of-fit between the center point and the other design points (Ylimaki et al 1988); 2) the nonlinearity of the responses over the surveyed area (Mora-Escobedo et al 1991); 3) the low sensitivity of the responses to changes in the levels of the independent variables (Chow et al 1988). In spite of their significant lack-of-fit and

TABLE III
Range of Mean Sensory Response Scores for Gluten-Free Bread and Reference Wheat Bread^a

Response	Range of Mean Sensory Response Scores ^a	Range of Mean ^b Reference Bread Scores
Frequency of cracks	2.5–4.3	0.5–3.1
Separation of layers	13.2–15.0	13.5–15.0
Crumb color	7.2–11.1*	0.9–1.2
Grain distribution	6.3–9.3*	9.5–11.3
Rollability ₁ ^c	6.3–13.5*	12.0–14.5
Tearing ₁ ^c	6.1–8.9*	7.3–8.5
First bite hardness ^c	3.8–8.6*	5.3–6.7
Adhesiveness ^c	6.2–10.9*	6.7–8.1
Masticatory hardness ^c	4.5–12.5*	6.1–8.1
Cohesiveness ₁ ^c	6.6–9.7*	8.2–10.7
Rollability ₂ ^d	2.4–7.4*	12.2–14.5
Tearing ₂ ^d	1.7–4.5*	5.6–7.2
Cohesiveness ₂ ^d	1.9–6.1*	6.7–9.2
Corn flavor	4.1–6.2	0

^aRange over 20 design points. N = 14 (7 panelists × 2 replicates). Maximum score = 15. * = significant differences among scores determined by ANOVA ($P < 0.05$).

^bN = 70 (7 panelists × 10 replicates).

^cSensory attributes judged on fresh bread samples (first day).

^dSensory attributes judged on aged bread samples (second day).

low R^2 values, second-order models have been effectively utilized in optimization studies of cereal-based formulations (Shelke et al 1990, Malcolmson et al 1993), presumably due to the exploratory nature of RSM. Thus, contour plots for responses that showed significant differences by ANOVA were generated by holding the value of the variable with the least effect on the responses constant at -1.633 , 0 and $+1.633$, and varying the levels of methylcellulose and egg albumen. Each contour plot was compared to the reference standards. Areas on the contour plots that represented gluten-free formulations, predicted to meet the range of reference response values for that specific response were identified.

Gluten-free formulations had a decidedly yellow crumb color when compared to the creamy white crumb of the reference bread, presumably due to the carotenoid pigments present in corn flour (Weber 1987). Inclusion of sodium stearoyl-2-lactylate into the bake mix enhanced the whiteness of the crumb. However, the level of addition was limited to 0.25% (flour mass basis), because higher concentrations of the surfactant led to a marked deterioration in the textural attributes of experimental breads (Noureddine 1993). Gluten-free formulations were less grainy than the reference, with waxy (no cellular structure) patches apparent in the inner surfaces of both layers of the loaf. An effect (albeit non-significant) was noted for gum arabic on the grain distribution of the experimental breads. Thus, while none of the formulations met standards at the medium level of gum arabic, regions predicted to fall in the range of the reference were identified at the low and high levels of the gum. Grain distribution was predicted to meet that of the reference at methylcellulose-to-egg albumen levels of ≤ 4.2 to ≥ 3.9 g and ≥ 4.3 to ≤ 4.3 g at the -1.633 (1.37 g) and $+1.633$ (4.63 g) levels of gum arabic, respectively.

The breads' first day rollability (Rollability_1) was affected more by changes in methylcellulose concentration than by changes in egg albumen levels. The response was affected slightly by gum arabic; a deterioration in rollability was noted at the extreme levels. Thus, while none of the formulations met reference standards at low levels of gum arabic, treatment combinations approached the lower limit of the reference range at the high level of the gum. Gluten-free formulations satisfied the reference standards for Rollability_1 at the medium level of gum arabic when levels of methylcellulose between 1.37 and 3.30 g and egg albumen concentrations between 2.18 and 4.40 g were included in the bake mix (Fig. 1, top).

Experimental breads met tearing quality standards at all levels of gum arabic and at specific combinations of methylcellulose and egg albumen. At the $+1.633$ (4.63 g) level of gum arabic,

bread with scores for Tearing_1 similar to that of the reference were obtained in the complement of the subset defined by methylcellulose levels of 2.55 – 4.63 g, and egg albumen concentrations of 1.90 – 4.63 g, in the region of methylcellulose ≥ 2.18 g and egg albumen ≥ 1.60 g. Combinations described by the elliptical curve located in the region of methylcellulose levels of ≥ 2.60 and 2.18 g \leq egg albumen ≤ 4.20 g satisfied standards at the 0 (3 g) level of gum arabic. At the lowest level of gum arabic, gluten-free formulations met the reference criterion for Tearing_1 over a wide range of methylcellulose and egg albumen levels. Regions with minimum responses were identified in the uppermost left and lowermost right quadrants at egg albumen levels ≥ 2.18 and ≤ 1.58 g, respectively (Fig. 1, middle).

The first bite hardness of breads met reference standards over a wide range of egg albumen levels at methylcellulose concentrations ≥ 2.18 g. At the low and medium levels of gum arabic, formulations with high-low and low-high combinations of methylcellulose and egg albumen that were compatible with the reference for the indicated response were identified in the region of methylcellulose levels of ≥ 3.18 g, and at all levels of egg albumen. Combinations of high levels of methylcellulose and egg albumen invariably resulted in excessively hard bread. At the $+1.633$ (4.63 g) level of gum arabic, optimum first bite hardness was realized at 2.38 g \leq methylcellulose levels ≤ 4.10 g over the full range of egg albumen levels (Fig. 1, bottom).

Apart from the curvature at the low levels of methylcellulose, contour plots for masticatory hardness were similar to those of first bite hardness. Again, combinations of high and low levels of methylcellulose and egg albumen that satisfied standards were obtained at a wide range of egg albumen when methylcellulose concentrations of ≤ 4.1 g were present in the formula, irrespective of gum arabic levels. Low-low and high-high combinations of methylcellulose and egg albumen yielded products with excessive softness and hardness, respectively.

Adhesiveness responses were affected by changes in the levels of the three variables; the effect of gum arabic was significant at $P < 0.05$. Combinations meeting the reference standards for adhesiveness were obtained over a wide range of methylcellulose and egg albumen at the different levels of gum arabic. Increasing concentrations of gum arabic in the bake mix allowed for optimum responses to be achieved in narrower regions of methylcellulose and egg albumen (Fig. 2, top).

The response surface for Cohesiveness_1 was saddle-shaped, and similar values were encountered at various combinations of minimum and maximum levels of the independent variables (Fig.

TABLE IV
Regression Analysis^a of Sensory Responses of Gluten-Free Pocket-Type Flat Bread

	Crumb Color	Grain Distribution	Rollability ^b	Tearing ^b	First Bite Hardness ^b	Adhesiveness ^b	Masticatory Hardness ^b	Cohesiveness ^b	Rollability ^c	Tearing ^c	Cohesiveness ^c
b_0	6.005	7.400	12.732	7.972	5.696	6.938	5.969	8.596	4.257	3.635	2.680
b_1	0.118	0.594***	-1.362**	0.582**	1.120**	1.040	1.879**	0.689**	-0.962**	0.309*	-0.093
b_2	0.239	-0.098	-0.237	0.130	0.182	0.303*	0.347	0.040	0.454*	0.371*	0.006
b_3	0.148	0.315	-0.168	-0.097	0.601*	0.315**	0.835**	0.008	-0.387	0.593**	-0.327
b_{11}	0.342	0.406	-1.145**	-0.427**	0.319	1.128**	0.886**	-0.388**	0.523*	-0.173	0.714
b_{22}	0.316	0.162	-0.483	0.276	-0.103	0.028	0.260	-0.558**	0.055	-0.144	0.394
b_{33}	0.550	-0.016	-0.946**	-0.569**	-0.003	0.815**	0.320	0.289*	-0.215	-0.234	0.639
b_{12}	1.512**	0.306	-0.509	0.267	0.256	-0.053	0.169	0.454**	0.177	0.469*	0.459
b_{13}	0.154	-0.269	-0.864*	0.347	0.286	0.380*	0.214	0.164	0.357	-0.269	-0.086
b_{23}	0.227	-0.081	-0.834*	0.202	-0.119	0.247	0.197	0.057	-0.310	-0.147	-0.216
R^{2d}	0.57	0.63	0.86	0.86	0.78	0.96	0.94	0.91	0.82	0.80	0.43
Equation significance			**	**	*	***	***	***	**	*	
Lack-of-fit	*						*		*	*	*

^a $Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3$, where x_1 = methylcellulose, x_2 = gum arabic, x_3 = egg albumen.

^bSensory attributes judged on fresh bread samples (first day).

^cSensory attributes judged on aged bread samples (second day).

^dCoefficient of determination.

*, **, ***, significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively.

2, bottom). Gum arabic notably affected cohesiveness of bread samples. Thus, while none of the formulations met the reference Cohesiveness₁ at the low level of gum arabic, the response was satisfied between methylcellulose levels of ≥ 2.90 and ≤ 2.18 g over the full range of egg albumen, and in the range of 2.80 g \leq methylcellulose levels ≤ 4.40 g, with egg albumen concentrations ≤ 1.80 g, and at methylcellulose levels ≥ 2.80 g and egg albumen ≥ 3.82 g, at the 0 (3 g) level of gum arabic. At the +1.633 (4.63 g) level of gum arabic, Cohesiveness₁ met reference standards at methylcellulose levels of ≥ 3.81 g, egg albumen levels of ≤ 2.90 g, and in the region defined by methylcellulose and egg albumen levels ≥ 3.15 g and ≥ 2.80 g, respectively.

Although the rollability, tearing quality, and cohesiveness after overnight storage of the experimental breads were predicted to

be realized with specific combinations of the independent variables (plots not shown), none of the formulations met the reference criteria for the indicated attributes (Table II). All gluten-free formulations cracked extensively upon application of stress, had a markedly reduced ability to resist shearing forces, and were notably less cohesive when compared to regular bread aged for a similar interval of time.

The deterioration in rollability, tearing quality, and cohesiveness of breads upon storage have been used by different workers as indicators of the rate of staling (Qarooni et al 1987, Quail et al 1991). The relatively small differences in rollability, tearing, and cohesiveness in the reference wheaten sample after overnight storage that were observed in this study may have resulted from its simultaneous rating with the experimental breads, rather than

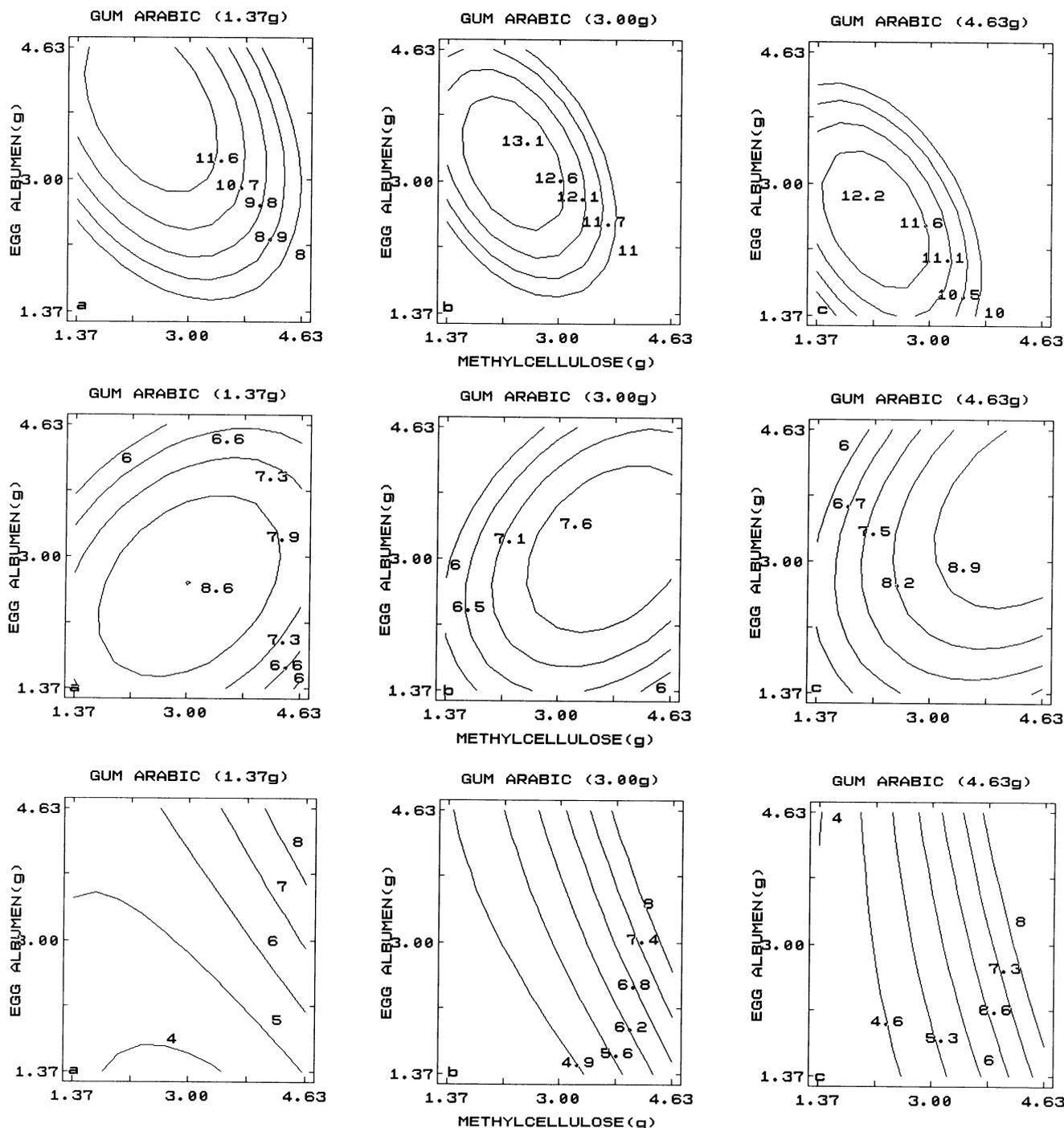


Fig. 1. Contour plots for gluten-free pocket-type flat bread made with low (left), medium (center) and high (right) levels of gum arabic. **Top row:** First day rollability scores. Range of wheat bread scores is 12.0–14.5. **Middle row:** First day tearing quality scores. Range of wheat bread scores is 7.3–8.5. **Bottom row:** First bite hardness (first day) scores. Range of wheat bread scores is 5.3–6.7.

its fresh analogue, and the inevitable use of rating scales as relative (not absolute) measuring devices by sensory judges (Lawless and Malone 1986). Gluten-free samples staled at a decidedly faster rate as reflected by the marked decrease in the scores of the aforementioned attributes. This acceleration in the rate of changes associated with the staling phenomenon lends support to the role of gluten in modulating the textural properties of baked goods during storage (Willhoft 1973, Maleki et al 1980, Davies 1986). Moreover, the detrimental effects of high levels of pregelatinized starch (25% pregelatinized corn starch and 50% pregelatinized rice flour [flour mass basis]) on the keeping quality of gluten-free bread are consistent with the positive relationships between staling rate and the degree of starch gelatinization reported in Arabic bread (Toufeili et al 1993), Egyptian *baladi* bread (Faridi and Rubenthaler 1984), and western pan bread (Martin et al 1991). Response contour plots for the significant sensory responses, at the three levels of gum arabic, were superimposed; regions meeting the maximum number of reference standards simultaneously were identified (Fig. 3).

At the lowest level of gum arabic, breads with methylcellulose levels between 2.46 and 4.16 g and egg albumen levels between 2.00 and 4.13 g met standards for Tearing₁, first bite hardness, masticatory hardness, and adhesiveness (Fig. 3, left). At the 0 (3 g) level of gum arabic, combinations of 2.10 g ≤ methylcellulose ≤ 4.12 and 2.18 g ≤ egg albumen ≤ 4.10 g yielded gluten-free products that satisfied standards for Rollability₁, Tearing₁, first bite hardness, adhesiveness, masticatory hardness, and Cohesiveness₁ (Fig. 3, center). At the highest level of gum arabic, loaves baked from formulations with methylcellulose levels between 1.71 and 3.16 g and egg albumen levels between 1.90 and 3.18 g were compatible with those of wheat bread in Rollability₁, Tearing₁, first bite hardness, adhesiveness, and masticatory hardness (Fig. 3, right). In these regions, breads are expected to be less rollable

and less cohesive on the first day than the reference bread at the -1.633 (1.37 g) level of gum arabic, and more cohesive at the +1.633 (4.63 g) level of gum arabic. Moreover, in the indicated regions, formulations would yield breads devoid of rice flavor that were comparable to the reference bread in those responses that showed no significant differences due to treatments. The bread had mean scores that met standards for frequency of cracks and extent of layer separation. However, loaves baked from formulations in the indicated regions would have stronger corn flavor, a more yellow and less grainy crumb, and an inferior keeping quality when compared to wheaten pocket-type flat breads.

CONCLUSIONS

Gluten-free pocket-type flat breads, compatible with regular wheat bread in key textural attributes, could be formulated from a bake mix based on pregelatinized rice flour and corn starch that incorporated methylcellulose, gum arabic, and egg albumen as a gluten replacement. Sensory responses were tolerant to changes in gum arabic levels and exhibited wide variations with changes in methylcellulose and egg albumen concentrations. While gum arabic had a relatively minor effect on the sensory properties of the final product, its presence in the bake mix imparted "strength" to the dough and improved its tolerance to mechanical handling. Methylcellulose and egg albumen were identified as the major determinants of the product's sensory quality. Inclusion of 3 g of gum arabic (with methylcellulose levels ≥ 2.10 g and ≤ 4.12 g and egg albumen levels of 2.18–4.10 g) in the bake mix yielded gluten-free breads that were compatible with regular wheat bread in the frequency of cracks, separation of layers, rollability, tearing quality, first bite hardness, masticatory hardness, adhesiveness, and cohesiveness. Higher levels (4.63 g) of gum arabic

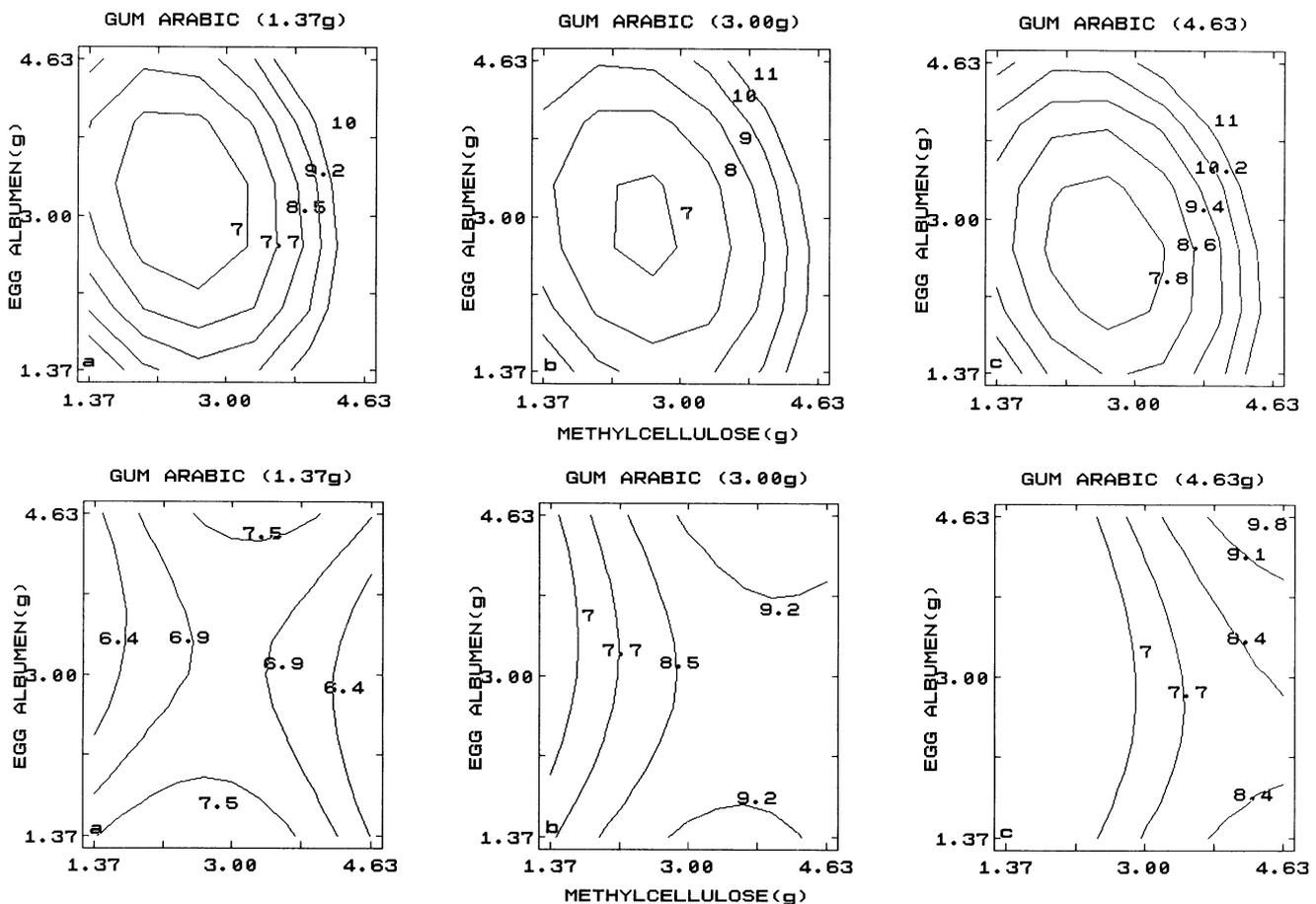


Fig. 2. Contour plots for gluten-free pocket-type flat bread made with low (left), medium (center), and high (right) levels of gum arabic. **Top row:** First day adhesiveness scores. Range of wheat bread scores is 6.7–8.1. **Bottom row:** First day cohesiveness scores. Range of wheat bread scores is 8.2–10.7.

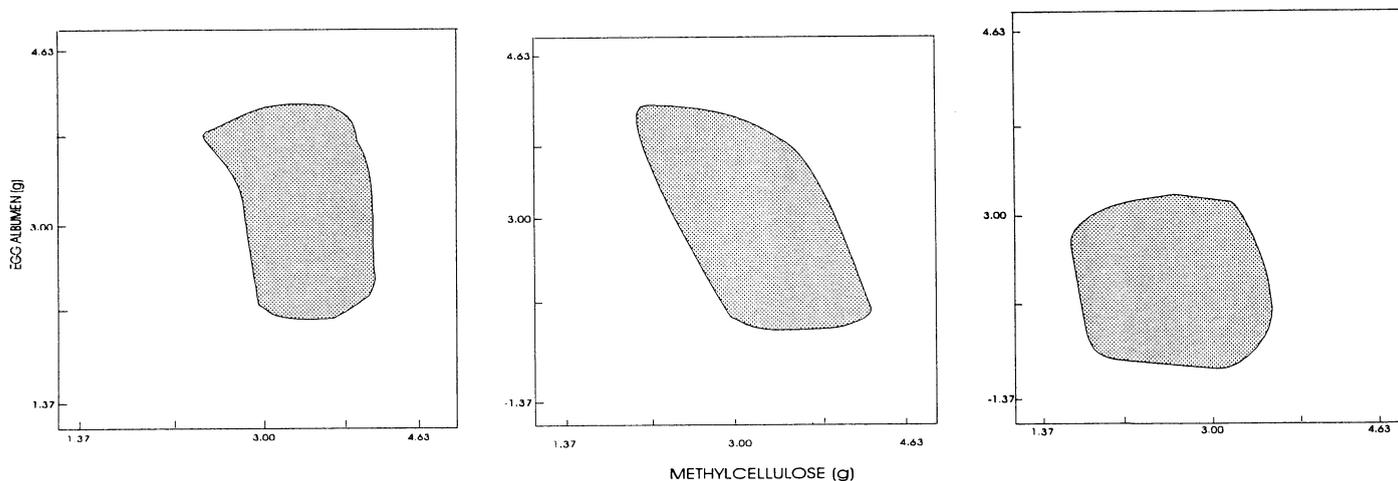


Fig. 3. Superimposed contour plot for the sensory responses of gluten-free pocket-type flat bread made with gum arabic. **Left:** 1.37 g of gum arabic. Shaded region met reference standards for continuity of surfaces, separation of layers, adhesiveness, and first day tearing quality, first bite hardness, and masticatory hardness. **Center:** 3 g of gum arabic. Shaded region met reference standards for continuity of surfaces, separation of layers, adhesiveness, and first day tearing quality, rollability, cohesiveness, first bite hardness, and masticatory hardness. **Right:** 4.63 g of gum arabic. Shaded region met reference standards for continuity of surfaces, separation of layers, adhesiveness, and first day tearing quality, rollability, first bite hardness, and masticatory hardness.

resulted in more cohesive products. Lower levels (1.37 g) of the gum arabic resulted in loaves that were less cohesive and inferior to wheat bread in rollability. All breads possessed a perceptible corn flavor, a light-yellow crumb with apparent waxy patches, and a faster rate of staling than that of regular wheat bread.

ACKNOWLEDGMENT

We gratefully acknowledge the help of I. Van Damme (Reading University Scientific Services, UK) with the viscoamylograph and particle size distributions studies, and the URB of AUB for the financial support.

LITERATURE CITED

- AL-HASSANY, M. 1975. Celiac disease in Iraqi children. *J. Trop. Pediatr.* 21:178-179.
- AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC, 8th ed. Methods 08-01, 30-10, 44-15A, 46-12, 54-21. The Association: St. Paul, MN.
- BITAR, G. J., SALEM, A. A., and NASR, A. T. 1970. Celiac disease from the Middle East. *Leban. Med. J.* 23:423-444.
- CHIANG, B.-Y., and JOHNSON, J. A. 1977. Measurement of total and gelatinized starch by glucoamylase and *O*-toluidine reagent. *Cereal Chem.* 54:429-435.
- CHOW, E. T. S., NEIL, L. S., DEVOR, R. E., and STEINBERG, M. P. 1988. Performance of ingredients in a soybean whipped topping: A response surface analysis. *J. Food Sci.* 53:1761-1765.
- DAVIES, A. P. 1986. Protein functionality in bakery products. Pages 89-104 in: *Chemistry and Physics of Baking*. J. M. V. Blanshard, P. J. Frazier, and T. Galliard, eds. J. W. Arrowsmith Ltd.: Bristol, England.
- EGGLESTON, G. O., OMAKA, P. E., and IHEDIOHA, D. O. 1992. Development and evaluation of products from cassava flour as new alternatives to wheaten breads. *J. Sci. Food Agric.* 59:377-385.
- FARIDI, H. A. 1988. Flat breads. Pages 457-506 in: *Wheat: Chemistry and Technology*, Vol. 2, 3rd Ed. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
- FARIDI, H. A., and RUBENTHALER, G. A. 1984. Effect of baking time and temperature on bread quality, starch gelatinization, and staling of Egyptian baladi bread. *Cereal Chem.* 61:151-154.
- GACULA, M. C., JR., and SINGH, J. 1984. *Statistical Methods in Food and Consumer Research*. Academic Press: New York.
- KIM, J. C., and DE RUITER, D. 1968. Bread from non-wheat flours. *Food Technol.* 22:867-878.
- KWAN, W.-O., and KOWALSKI, B. R. 1980. Data analysis of sensory scores. Evaluations of panelists and wine score cards. *J. Food Sci.* 45:213-216.
- LAWLESS, H. T., and MALONE, G. J. 1986. A comparison of rating scales: Sensitivity, replicates and relative measurements. *J. Sens. Stud.* 1:155-174.
- MALCOLMSON, L. J., MATSUO, R. R., and BALSHAW, R. 1993.

- Effects of drying temperature and farina blending on spaghetti quality using response surface methodology. *Cereal Chem.* 70:1-7.
- MALEKI, M., and DAGHIR, S. 1967. Effect of baking on retention of thiamine, riboflavin, and niacin in arabic bread. *Cereal Chem.* 44:483-487.
- MALEKI, M., HOSENEY, R. C., and MATTERN, P. J. 1980. Effects of loaf volume, moisture content and protein quality on the softness and staling rate of bread. *Cereal Chem.* 57:138-140.
- MARTIN, M. L., ZELEZNAK, K. J., and HOSENEY, R. C. 1991. A mechanism of bread firming. I. Role of starch swelling. *Cereal Chem.* 68:498-503.
- MEILGAARD, M., CIVILLE, G. V., and CARR, B. T. 1987. *Sensory Evaluation Techniques*. CRC Press: Boca Raton, FL.
- MORA-ESCOBEDO, R., PAERDES-LOPEZ, P., and DOMINGUEZ, J. 1991. Optimization of a germination procedure by response surface methodology. *Lebensm. Wiss. Technol.* 28:518-522.
- MSTAT. 1989. *User's guide: A microcomputer program for the design, management, and analysis of agronomic research experiments*. Version 5.0. Michigan State University: East Lansing.
- NOUREDDINE, A. 1993. Optimization of structure-supporting agents in gluten-free pocket-type flat-breads by response surface methodology. MS thesis. American University of Beirut: Lebanon.
- POWERS, J. J. 1984. Using general statistical programs to evaluate sensory data. *Food Technol.* 38:74-82, 84.
- QAROONI, J., ORTH, R. A., and WOOTTON, M. 1987. A test baking technique of arabic bread quality. *J. Cereal Sci.* 6:81-90.
- QAROONI, J., MISKELLI, Y. D., and WOOTTON, M. 1989. Factors affecting the quality of arabic bread: Fermentation variables. *J. Sci. Food Agric.* 48:99-109.
- QUAIL, K. J., McMASTER, G. J., and WOOTTON, M. 1991. Flour quality tests for selected wheat cultivars and their relationship to arabic bread quality. *J. Sci. Food Agric.* 54:99-110.
- RANHOTRA, G. S., LOEWE, R. J., and PUYAT, L. V. 1975. Preparation and evaluation of soy-fortified gluten free bread. *J. Food Sci.* 40:62-64.
- RASPER, V. 1980. Theoretical aspects of amylographology. Pages 1-6 in: *The Amylograph Handbook*. W. C. Shuey and K. H. Tipples, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.
- SARAKBI, M. 1985. Formulation and nutritive value of arabic bread prepared for uremia, cirrhosis and gluten intolerance diseases. MS thesis. American University of Beirut: Lebanon.
- SHELKE, K., DICK, J. W., HOLM, Y. F., and LOO, K. S. 1990. Chinese wet noodle formulation: A response surface methodology study. *Cereal Chem.* 67:338-342.
- SMITH, E. B. 1971. Gluten free breads for patients with uremia. *J. Am. Diet. Assoc.* 59:572-574.
- STSC. 1991. *Statgraphics*. Version 5.0. Statistical Graphics Corporation: Rockville, MD.
- SULIMAN, G. E. 1978. Celiac disease in Sudanese children. *Gut* 19:121-125.
- SULTAN, W. J. 1969. *Practical Baking*, 2nd ed. Avi Publishing: Westport, CT.
- TOUFEILI, I., SLEIMAN, G., and ABU SALMAN, R. 1993. Correlation

of selected biochemical, chemical and physicochemical parameters and sensory perception of staling in arabic bread. *Lebensm. Wiss. Technol.* 26:34-37.

VAISEY-GENSER, M., YLIMAKI, G., and JOHNSON, B. 1987. The selection of canola oil, water, and an emulsifier system in cake formulations by response surface methodology. *Cereal Chem.* 64:50-54.

WEBER, E. J. 1987. Lipids of the kernel. Pages 311-349 in: *Corn: Chemistry and Technology*. S. A. Watson and P. E. Ramstad, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.

WILLHOFT, E. M. A. 1973. Mechanism and theory of staling of bread and baked goods, and associated changes in textural properties. *J.*

Texture Stud. 4:292-322.

WILLIAMS, P. C., EL-HARMEIN, F. J., NELSON, W., and SRIVASTAVA, P. J. 1988. Evaluation of wheat quality for the baking of Syrian-type two-layered flat breads. *J. Cereal Sci.* 7:195-207.

YLIMAKI, G., HAWRYSH, Z. J., HARDIN, R. T., and THOMSON, A. B. R. 1988. Application of response surface methodology to the development of rice flour yeast breads: Objective measurements. *J. Food Sci.* 53:1800-1805.

YLIMAKI, G., HAWRYSH, Z. J., HARDIN, R. T., and THOMSON, A. B. R. 1991. Response surface methodology in the development of rice flour yeast breads: Sensory evaluation. *J. Food Sci.* 56:751-755.

[Received January 24, 1994. Accepted July 12, 1994.]

SOFT WHEAT PRODUCTS

Association of Sugar-Snap Cookie Quality with High Molecular Weight Glutenin Alleles in Soft White Spring Wheats¹

E. SOUZA,^{2,3} M. KRUK,² and D. W. SUNDERMAN^{2,4}

ABSTRACT

Cereal Chem. 71(6):601-605

High molecular weight glutenins (HMW-*Glu*) affect the quality of leaven breads produced from wheat (*Triticum aestivum* L.) flour. However, effects of these proteins on pastry quality are poorly understood. Sugar-snap cookie quality was compared to HMW-*Glu* alleles of soft white spring wheat breeding lines and cultivars from multiple trials over four years at Aberdeen, ID. Sugar-snap cookie quality was affected less by the composition of the flour protein than by the quantity of flour protein. Individual alleles did not have significant effects on cookie diameter, except for the 13+19 allele of the *Glu*-1B locus, which was associated with smaller cookie diameters. The glutenin strength of alleles at the three HMW-*Glu* loci was estimated using a glutenin rank sum (GRS)

derived from previously published research. The glutenin strength (GRS score) of cultivars and breeding lines was negatively correlated to cookie diameter ($b = 0.02 \text{ cm unit}^{-1}$; $P < 0.05$). The negative correlation between GRS score and cookie diameter was greatest in the year with the lowest average flour protein content and least in the year with the highest average protein content. The effect of allelic variation was probably masked in the years with high average protein content due to the overriding effects of total protein content. Selection for cultivars with low GRS scores may produce cultivars with better and more predictable sugar-snap cookie quality.

Quality of pastry wheat (*Triticum aestivum* L.) can be assessed through indirect tests such as alkaline water retention or particle size index, or directly by baking test products such as standardized cookies, crackers, sponge cakes, or udon noodles (Hoseney et al 1988). The protein content of a soft wheat flour is also used as a predictor of the flour quality (Finney et al 1987). Phenotypic correlation of protein percentage and pastry quality is strongly negative. However, the genotypic correlation between a cultivar's flour protein and pastry quality is generally poor within a population of improved soft wheat cultivars. Patterson and Allan (1981) identified genotypes with quite high protein content and good pastry characteristics. Strength of the gluten developed by a flour's protein is negatively associated with pastry quality, as measured by sugar-snap cookies. Alveograph dough strength (P value) has been found to be negatively correlated to cookie quality and could be used to predict residual effects from a simple linear model of flour protein content estimation of cookie spread (Bettge et

al 1989). The composition of the flour protein, therefore, may determine, in part, the intrinsic pastry quality of a cultivar.

The high molecular weight glutenin alleles (HMW-*Glu*) are a class of genes that can influence gluten strength. Three homeo-allelic loci for HMW-*Glu* are located on chromosomes 1A, 1B, and 1D (respectively *Glu*-1A, *Glu*-1B, and *Glu*-1D). Each locus is complex, conferring zero to two distinct proteins (Graybosch 1992). Allelic variation is noted by numbering each HMW-*Glu* sequentially, based on mobility in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Payne 1987).

The effect of HMW-*Glu* alleles on end-use quality of soft or pastry-type wheats has had little attention previously. However, the HMW-*Glu* allelic variation in hard wheats can significantly influence bread-baking quality (Payne 1987). Cressey et al (1987), for example, compared two allelic variants for the *Glu*-1D locus, 2+12 and 5+10, and found an average of 5% greater loaf volume in cultivars with the 5+10 allele than in cultivars with the 2+12 allele. Payne et al (1984) summarized previous work on the general effects of HMW-*Glu* loci on bread wheat quality. At the *Glu*-1A locus, the null allele is inferior to the 2* and 1 allele, with the 2* and 1 alleles approximately equal in effect ($\text{null} < 2^* = 1$). The alleles of the *Glu*-1B locus with known effects on bread quality are: (in order of increasing favorable effects) 6+8 < 7 < 7+9 < 17+18 = 13+16 = 7+8. The third locus, *Glu*-1D, has two common alleles, 2+12 and 5+10, of which 5+10 is the more favorable for bread quality. The alternate alleles at the *Glu*-1D locus 3+12 and 4+12 have been found to be less favorable for bread quality than either the 2+12 or 5+10 alleles. Payne et al

¹Manuscript 94708 of the Idaho Agricultural Experiment Station. Research supported in part by Hatch Project H962.

²Dept. of Plant, Soils, and Entomological Science, University of Idaho, Aberdeen Research and Extension Center.

³Corresponding author.

⁴Retired.