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Full Length Research paper

Optimisation of raw tooke flour, vital gluten and water absorption in tooke / wheat composite bread, using response surface methodology (Part II)

Florence I. Muranga[1, 2]*, Martin Mutambuka[1], Fabian Nabugoomu[3] and M. G. Lindhauer[4]

[1]Department of Food Science and Technology, Makerere University, Kampala, Uganda,
[2]Presidential Initiative on Banana Industrial Development (PIBID), Kampala, Uganda.
[3]Faculty of Science, Uganda Christian University, Mukono, Uganda.
[4]Federal Institute for Food and Nutrition (Bundesforschungsanstalt für Ernährung und Lebensmittel, BFEL), Detmold, Germany.

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The objective of this study was to optimise raw tooke flour-(RTF), vital gluten (VG) and water absorption (WA) with respect to bread-making quality and cost effectiveness of RTF/wheat composite flour. The hypothesis generated for this study was that *optimal substitution of RTF and VG into wheat has no significant effect on baking quality of the resultant composite flour.* A basic white wheat bread recipe was adopted and response surface methodology (RSM) procedures applied. A D-optimal design was employed with the following variables: RTF (x_1) 0-33%, WA (x_2) -2FWA to +2FWA and VG (x_3) 0 - 3%. Seven responses were modelled. Baking worth number, volume yield and cost were simultaneously optimized using desirability function approach. Models developed adequately described the relationships and were confirmed by validation studies. RTF showed the greatest effect on all models, which effect impaired baking performance of composite flour. VG and Farinograph water absorption (FWA) as well as their interaction improved bread quality. Vitality of VG was enhanced by RTF. The optimal formulation for maximum baking quality was 0.56%(x_1), 0.33%(x_2) and -1.24(x_3) while a formulation of 22%(x_1), 3%(x_2) and +1.13(x_3) maximized RTF incorporation in the respective and composite bread quality at lowest cost. Thus, the set hypothesis was not rejected.

Key words: Raw tooke flour, composite bread, baking quality, response surface methodology, Farinograph water absorption, vital gluten.

INTRODUCTION

Raw tooke flour (RTF) is a novel, value added product from *matooke,* a triploid Acuminate East African Highlands (AAA-EA) cooking banana cultivar (Muranga, 1998) developed to improve shelf stability of the fruit and to find alternative uses. The flour has found potential use in the baking and confectionery industries, showing potential to composite with wheat flour in biscuits (Nayiga et al., 2004), bread (Luwangula et al., 2004) and cakes (Waiswa et al., 2004). Research interest in composite flours has been on the rise in the recent past, driven by the desire to find non-wheat bread making alternatives in order to reduce dependence on imported wheat by non-wheat producing countries (Pacheco-Delahaye and Testa, 2005; Mepba et al., 2007).

However, just like most non-wheat flours, RTF is devoid of gluten (the protein that confers the unique bread making properties to wheat) and its addition impairs bread making quality of wheat flour. Luwangula et

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*Corresponding author. E-mail: director@pibid.org. Tel: +256772363271, +256312265789, +256414371050. Fax: +256414340413.

al. (2004) reported a significant decrease in volume and overall acceptability and an increase in crumb firmness in RTF/wheat composite bread at substitution rates greater than 12% RTF. Mepba et al. (2007) and Pacheco-Delahaye and Testa (2005) reported similar effects with plantain (*Musa paradisiaca*) flour at even lower substitution rates (> 5% and > 7%plantain flour respectively).

On the other hand, vital wheat gluten, a by-product of wheat starch extraction process, has been shown to improve bread making properties of weak flours (Stenvert et al. 1981, Wiepert and Lindhauer, 1999), improve loaf volume, crumb grain, texture, softness and shelf life. For flours substituted with modified starches, Miyazaki et al. (2006) recommended that the starch content should not exceed 20% and amount of vital gluten added should be 8% of the weight of starch substitution, a weight almost equal to the percentage of insoluble wheat protein, glutenin and gliadin combined in wheat flour.

Other technologies that improve composite bread baking include: intensive dough mixing, sponge and dough system, chemical/activated dough development, short proof times and use of dough conditioners and bread improvers (Ruiter, 1978). Beside, Luwangula et al. (2004) identified water absorption as a critical parameter in RTF/wheat composite bread baking which could potentially improve bread quality when optimized. However, up-to-date, no effort has been made to optimise the composite bread system, with or without improvers.

Response surface methodology (RSM) is an effective tool for optimizing industrial food processes and ingredient combinations (Hu, 1999). It relates product properties by using regression equations that describe interrelations between input variables and product properties. The main objectives of this study, therefore, were first to model RTF/wheat composite bread system and establish critical factors affecting bread quality and cost and secondly to determine optimal levels of ingredients for maximum bread quality and minimum cost of RTF/wheat composite bread.

MATERIALS AND METHODS

Material preparation

RTF from Nandigobe an indigenous triploid Acuminate East African Highlands banana variety (Musa AAA-EA) was prepared according to patent No AP/P/2005/003308 (Muranga, 2005). All other baking ingredients were procured locally in Detmold, Germany.

Flour chemical composition

Moisture, crude protein, crude ash were determined according to standard ICC procedures: No. 110, 167 and 104, respectively (ICC, 2001). Crude fat content was determined by soxhlet extraction using petroleum ether while starch content was determined by an enzymatic hydrolysis procedure using alpha amylase and amyloglucoamylase. The reducing sugars content was determined by the enzymatic bio assay methods developed by R-BIOPHARM (Cat. No. 0716251 and Cat.No.10716260035).

Dough rheological properties

Dough rheological properties were tested using a Barbender Farinograph (model 820600; Barbender OHG, Duisburg, Germany) according to ICC standards No. 114 (ICC, 2001).

Baking test

A straight dough procedure, making use of a basic white wheat bread recipe, was used (BFEL, 1994). The recipe used (based on 100% flour portion) was, Flour (100%), Fresh yeast (5%), Sugar (1%), Salt (1.8%), Fat (1%), Ascorbate (0.1%) and Farinograph water absorption (FWA) (according to ICC standard No. 115) (ICC, 2001). The dough was moulded with a mechanical moulder (Frilado,Typ 2; F.Laureck, Backerei Machinen, Dortmund, Germany) and proofed for 60 minutes in a proofing cabinet (Ehret, Type KMB 6; Dipl.Ing. W. Ehret GmbH, Emmendingen, Germany) at 32°C and 80% RH. The dough was baked at 240°C for 30 min in a multi level oven (Daub Backmeister, Type BOSX; Daub Hamburg GmbH; Hamburg, Germany) with steam injection before and after inserting the tinned dough. After baking, loaves were glazed with a little water, left to cool at room temperature and stored in air tight cabinets. Evaluation of loaf characteristics was done after 24 h.

Volume yield, baking worth number and pore

Volume yield and baking worth number were determined according to BFEL standard procedures (BFEL, 1994). The pore picture was estimated by comparison with the local Dallman scale (Moritz Schäfer Publishers, 1988).

Bread firmness

The firmness of the test bread was determined using a texture analyser (TA-XT2, Stable Micro Systems Ltd, UK.) according to the AACC (74-09) standard method (AACC, 2001); using a 25mm cylinder probe. 25 mm thick loaves were compressed to 40% of slice thickness at 1.7 mm/s test speed. Crumb firmness was measured as the force (in Newtons) required to compress the bread slice from a force-time curve that is area under the curve.

Crumb and crust colour

Crumb and crust colour were determined using a chromameter (Konica Minolta, Osaka, Japan) on L*a*b* colour system. The crust colour was taken as the average of the top, side and bottom colour readings. Crumb colour was taken on a freshly mechanically cut surface.

Cost of composite bread

Wholesale cost prices of bread ingredients were obtained from local bakeries in Kampala, Uganda and multiplied by the amount of ingredient required for producing a gram of dough to get the total cost per gram of bread/dough as an independent response. The cost per gram of dough was then entered as a response in the optimisation design.

Table 1. D-Optimal design used to optimise levels of the key ingredients in composite bread.

Run no.	Actual values			Coded values		
	RTF(% of flour portion)	Gluten (% of flour portion)	Water(+/-FWA)	RTF	Gluten	FWA
1	11	2	+2	-0.33	1	0.33
2	16.5	1.5	0	0	0	0
3	11	1	-2	-0.33	-1	-0.33
4	33	1	-1	1	-0.5	-0.33
5	0	2	-1	-1	-0.5	0.33
6	0	1	1	-1	0.5	-0.33
7	22	1	-2	0.33	-1	-0.33
8	22	1	+2	0.33	1	-0.33
9	16.5	1.5	0	0	0	0
10	16.5	1.5	0	0	0	0
11	11	2	-2	-0.33	-1	0.33
12	22	3	-1	0.33	-0.5	1
13	11	0	-1	-0.33	-0.5	-1
14	11	3	+1	-0.33	0.5	1
15	22	3	-1	0.33	-0.5	1
16	0	1	+1	-1	0.5	-0.33
17	11	3	+1	-0.33	0.5	1
18	33	2	+1	1	0.5	0.33
19	22	0	+1	0.33	0.5	-1
20	33	2	+1	1	0.5	0.33

Experimental design

Table 1 shows the multivariate, D-optimal design generated by design-expert statistical soft ware (Version 6.0, Stat-Ease, Inc., MN, USA; 2003) used in the study. It consisted of 20 runs, 10 minimum model points, 4 points for estimation of lack of fit, 4 replicates and two additional centre points. This methodology enables elicitation of individual and interactive effects of factors on desired responses across the specified design space. In the design, the three ingredients were varied simultaneously as follows: RTF (x_1) varied from 0 - 33%, WA (x_2), -2FWA to +2FWA and VG (x_3) 0 - 3%. The ranges of RTF and VG explored in this study were based on recommendations proposed by Miyazaki et al. (2006). Ranges of FWA were based on pre trials in order to obtain workable doughs.

Attaining optimum conditions

The desirability function approach (DFA) was used to simultaneously optimise baking worth number and volume yield. The two responses were chosen as the most important product quality parameters and were maximised simultaneously by calculating optimal ingredient combinations for maximum performance of the composite flour. (Table 2) shows the optimal formulations maximising volume yield and baking worth number. The above method incorporates desires and priorities for each of the variables with desirability of 1 being a maximum. The optimisation criteria were set as follows: Cost per gram of dough at minimum (≤ 87.5 UShs/kg, 0.072 $/kg), baking worth number and volume yield at maximum (≥ 200 and ≥ 630 cc/100 g of flour, respectively). The optimisation criteria was chosen to reflect product characteristics at 10% RTF substitution rate as the upper limit beyond which product characteristics are significantly different from wheat bread (Luwangula, 2004; Mepba et al., 2007).

Validation of regression models

Tables 2 shows the validation trial runs used to test the adequacy of the predicative models. The two verification trials outside the optimal formulation range were chosen because one of the goals of this study was to maximise RTF without significantly affecting product quality. Therefore, formulations containing 10.8% and 20% RTF at their respective predicted optimum levels of vital gluten and FWA were chosen. Run 1, the control formulation consisted of 0% matooke, 0% vital gluten and FWA, run 2 formulation was composed of 10.8% matooke, 0% vital gluten, 0.24FWA, while run 3 contained 20% matooke, 2.7% vital gluten and 1 FWA.

Data analysis

The design-expert soft ware, version 6.0 computer package (Stat-Ease, Inc., MN, USA; 2003) was used to analyse the data. Statistical parameters used to relate input variables to responses are p-value, R^2 and lack of fit of the models. All models presented were significant at $p < 0.001$.

RESULTS

Dough properties

Table 3a shows the dough properties of the dough corresponding to the different runs. Dough surface feel was normal for the majority of the experimental runs. However, runs which high levels of RTF and water

Table 2. Validation trial runs for validating significant models.

Run	1		2		3	
Ingredient composition						
RTF (%)	0		10.8		20	
VG (%)	0		0		2.7	
FWA	0		0.24		1	
Responses						
	Predicted value (99% CI)	**Measured value**	**Predicted value (99% CI)**	**Measured value**	**Predicted value (99% CI)**	**Measured value**
Baking worth number	250.0 ± 23.9	247	200.8 ± 15.8	204	200.3 ± 13.0	203
Volume yield (cm^3/100g of flour)	684.4 ± 42.3	687	644.2 ± 24.6	635	656.8 ± 22.1	657
Crumb color l* value	77.1 ± 0.7	78.53	74.3 ± 0.4	69.85	71.7 ± 0.52	72.86
Crust color l* value	58.1 ± 1.0	58.1	55.59 ± 0.62	57.15	53.44 ± 0.62	56.93

Table 3a. Results of responses obtained from the baking experiment –Dough properties.

Run no.	Moisture content (%)	Flour weight, g (14%WG)	Water, ml,(%flour)	Dough yield	Practical dough weight (g)	Wt of dough in baking tin (g)	Dough surface	Dough elasticity
1	13.1	990	67.0	166	173.4	867	Normal	Somehow elastic
2	13.1	990	65.2	164.2	171.6	858	Normal	Somehow short, good condition
3	13.1	990	63.0	162	169.6	848	Normal	Somehow short
4	13.1	990	65.0	164	171.6	858	Normal	Very short
5	13.1	990	61.6	160.6	168.2	841	Normal	Normal
6	13.1	990	63.2	162.2	170	850	Normal	Normal
7	13.1	990	64.0	163	170.6	853	Normal	Short
8	13.1	990	68	167	174.6	873	Somehow wet	Somehow short
9	13.1	990	65.2	164.2	171.6	858	Normal	Somehow short, good condition
10	13.1	990	65.2	164.2	171.8	859	Normal	Somehow short, good condition
11	13.1	990	63	162	170	850	Normal	Somehow short
12	13.1	990	65.8	164.8	173	865	Wettish	Somehow short
13	13.1	990	64.1	163.1	170.8	854	Normal	Somehow short
14	13.1	990	66.8	165.8	173.6	868	Wettish	Still normal
15	13.1	990	65.8	164.8	172.8	864	Wettish	Somehow short
16	13.1	990	63.2	162.2	170	850	Normal	Normal
17	13.1	990	66.8	165.8	173.4	867	Wettish	Still normal
18	13.1	990	67.4	166.4	174.2	871	Wettish	Short
19	13.1	990	66.5	165.5	173.2	866	Wettish	Somehow short
20	13.1	990	67.4	166.6	174.4	872	Wettish	Short

absorption tended to produce wet dough surfaces. Water absorption showed the highest influence on dough surface feel. Indeed all formulations with more water than FWA produced dough with wet surfaces except where water absorption was adequately compensated by matching vital gluten levels (Runs 1 and 16). Dough elasticity was poor in runs incorporating RTF but dough properties were improved by vital gluten and high FWA below 22% RTF incorporation. Beyond 22% incorporation of RTF the dough became progressively short. Stability to fermentation (difference in form between bread fermented for 60 and 70 min) was good for formulations

Table 3b. Results of responses obtained from the baking experiment –bread properties.

Run no.	Pore picture	Volume yield (cm^3/100g of flour)	Baking worth no.	crumb colour l*	crust colour l*	Crumb firmness (N)
1	656	6	210	75.26	56.56	237.46
2	628	6	189	74.34	55.93	272.93
3	636	6	190	74.85	57.62	228.72
4	458	5	59	71.24	52.68	-
5	682	6.5	242	76.78	58.15	187.77
6	668	6.5	237	77.35	58.1	180.16
7	568	5	107	72.61	52.2	364.95
8	618	5	145	72.39	-	279.84
9	632	6	190	72.62	54.41	299.05
10	626	6	197	71.98	53.91	288.55
11	646	6.5	210	73.49	54.2	284.74
12	644	6	195	69.97	50.62	335.43
13	632	6	193	73.42	54.6	237.17
14	680	6.5	231	74.07	53.62	207.19
15	642	5.5	184	69.83	53.62	323.52
16	684	6.5	241	77.96	58.58	185.94
17	682	6	220	73.39	55.29	219.76
18	578	5	127	65.97	48.36	466.56
19	620	5	150	69.8	50.59	277.59
20	580	5	129	67.12	49.97	435.85

with up to 16.5% RTF. For formulations with higher levels of *matooke* flour, higher levels of vital gluten and water absorption improved stability to fermentation.

Loaf properties

Results of bread quality characteristics used in the optimisation procedure are as shown in (Table 3b). The form of the loaf (after 60 min of fermentation was good for all formulations save for those with less water than the FWA and 22-33% RTF. The loaf form was apparently dependant on interaction between RTF and water such that high levels of RTF with low water levels resulted in flat loaves. These formulations were also generally unstable to longer fermentation (> 75 min). Crust browning intensity increased with increasing levels of RTF. While vital gluten interacted with RTF in its impact on crust colour.

Crumb properties

The texture of the crumb was woolly for formulations with only up to 11% RTF. At the latter level of RTF incorporation, the crumb became softer with increasing water levels. Vital gluten effects at this level were not significant well as water absorption effects were significant at all levels. Formulations with 16.5 - 22% *matooke* flour produced 'tender' crumb structure, while formulations with 33% *matooke* flour produced coarse/rough textured crumbs. High levels of water absorption softened the crumb texture. Pore consistency and crumb elasticity were rather even in most trials across the board, that is they did not depend on any of the variables. Crumb colour, as subjectively determined by the baker, was only normal for formulations without RTF. Crumb colour intensity increased with increasing levels of RTF. Crumb colour did not apparently depend on water absorption and vital gluten. Crumb structure was 'normal' to 'good' up to 16.5% RTF. Above 16.5% RTF, it became short but was slightly improved by increasing water absorption. Vital gluten played no significant role in influencing crumb structure.

Predicative models

The significant ($p < 0.001$) models (Equations 1 - 6 below were expressed as unscaled variables.

$$Y_1=249.98-3.81x_1-2.17x_2-5.33x_3-0.07x_1^2 3.70x_3^2+1.05x_1x_2+0.64x_1x_{3,} (\mathbf{R}^{2=} 0.99) \quad (1)$$

Where; x_1-RTF, x_2-Vital gluten, x_3-FWA;

Equation 1 defines the significant predicative model for baking worth number ($p < 0.001$). In this model all variables showed significant linear effects. Only RTF and

Plate 1. Cross sectional view of loaves baked from model validation trials; (Left to Right) 100% wheat, 10.8% RTF, 0% gluten, 0.24FWA and 20% RTF, 2.7% VG and 1FWA.

FWA showed significant quadratic effects. The interactive effects were only significant between RTF /vital gluten and RTF/FWA. All the linear and quadratic effects of the three variables on baking worth number were negative while all interactive effects were positive.

$$Y_2 = 684.38 - 2.52x_1 - 4.89x_2 - 6.55C - 0.12x_1^2 + 1.30\, x_1 x_2 + 1.04\, x_1 x_{3,} (\mathbf{R}^{2} = 0.96) \quad (2)$$

In Equation 2, for the predicative model for volume yield all three ingredients showed significant negative linear effect on volume yield. Only RTF showed a quadratic effect, which was also negative. Interactive effects between *matooke* flour and vital gluten and, *matooke* flour and FWA were significant and had a positive effect on volume yield.

$$Y_4 = 152.4 + 3.0\, x_1 + 53.5x_2 - 11.8x_3 - 0.1x_1^2 - 15.9x_2^{2,} (\mathbf{R}^{2} = 0.97) \quad (3)$$

In Equation 3, the predicative model for crumb firmness showed the three variables had linear effects, while only RTF and vital gluten showed significant quadratic effects. The linear effects of RTF and vital gluten were positive, while the linear effect of FWA and all significant quadratic effects were negative.

$$Y_5 = 77.06 - 0.26x_1 + 0.73x_3 - 0.05x_1x_3 (\mathbf{R}^{2} = 0.94) \quad (4)$$

The relationship between X_1, X_2, X_3 and crumb colour (l* values) is shown by equation 4 and resulted in significant linear models with negative effects.

$$Y_5 = 58.12 - 0.23x_{1,} \quad (R^2 = 0.83) \quad (5)$$

The relationship between X_1, X_2, X_3 and crust colour l* values is shown in equation 5 and resulted in a linear model in which, the effect of *matooke* flour was negative on crust l* value.

$$Y_{12} = 6.38 - 0.053x_1 + 0.16x_2 - 0.057x_3 (\mathbf{R}^2 = 0.87) \quad (6)$$

Equation 6 is a linear model describing the relationship between X_1, X_2, X_3 and crumb pore picture. *Matooke* flour and FWA showed negative linear effects while vital gluten showed a positive linear effect. There was no significant interactive effect between any of the three variables. Maximum pore picture reading was predicted to correspond to minimum FWA and *matooke* flour and maximum vital gluten. (Table 2) and plate 1 shows results from the three trial runs employed to validate the models developed. The results showed that the models ably predicted the responses with in the design space 99% of the time. The relationships between input variables and bread quality characteristics are depicted in (Figures 1 - 3). Taking baking worth number as a single figure measure of baking performance of a flour, results in (Figure 1) show that maximum effect of VG was achieved at intermediate FWA. From (Figure 2), it can be seen from constructed response surfaces that at medium VG levels, a firmer crumb is obtained as compared to both upper and lower extremes. (Figure 3) shows overlay plot showing possible optimal ingredient combinations. Overall, the optimal formulation was 0.56% RTF, 0.33% VG and -1.24 FWA. In order to maximise RTF at minimum cost, a formulation of 22% RTF, 3% VG and 1.13 FWA was optimal.

DISCUSSION

The negative effect of RTF on baking quality of composite flour can be related to its lack of gluten

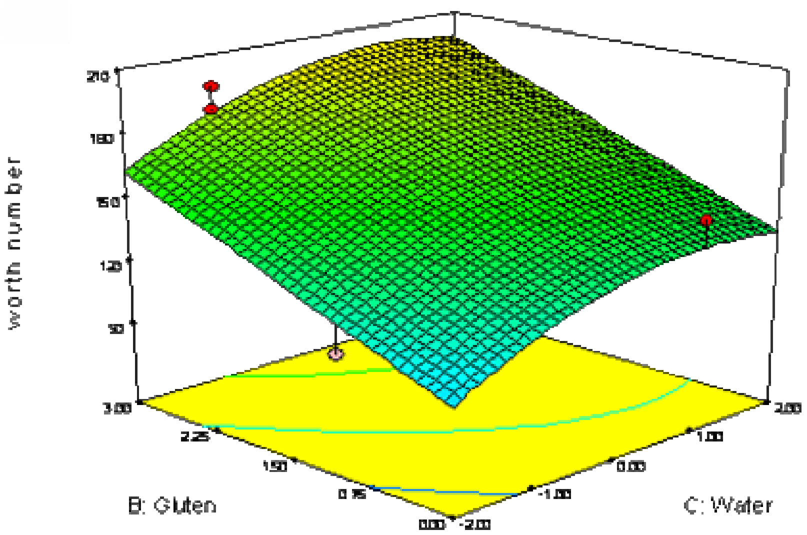

Figure 1. Response surface showing relationship between vital gluten, FWA and Baking worth number at 22% RTF.

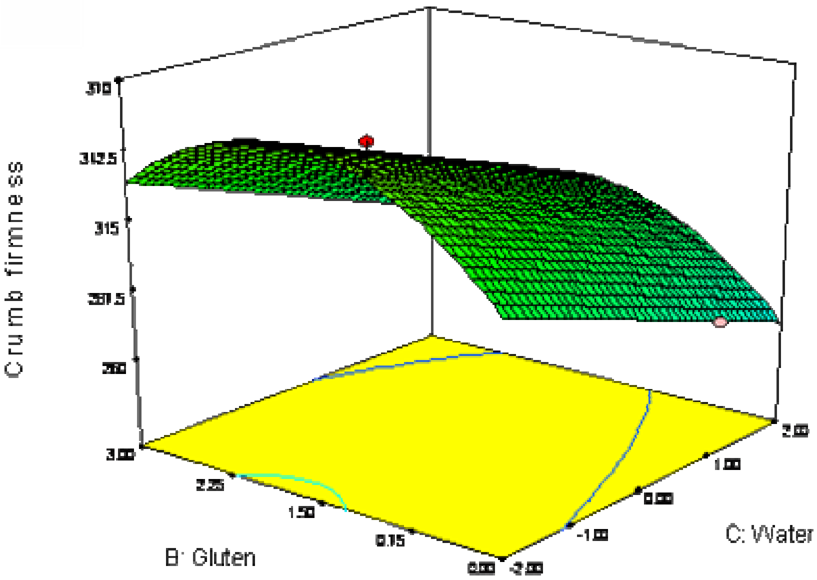

Figure 2. Response surface showing relationship between vital gluten, FWA and Crumb firmness at 22% RTF.

proteins (gliadin and glutenin) as well as due to its starch properties. Gluten proteins are necessary for formation of viscoelastic dough capable of trapping air to form well aerated bread products. A minimum protein content of 11.0% in wheat flour has been proposed as a basic requirement for production of yeast-leavened bread (Zeleney, 1971).

Conversely, non-wheat starch and proteins have been shown to have deleterious effects on bread making quality of wheat (Sandstedt, 1961; D'Appolonia and Giles, 1971; Hoseney et al., 1971; Rasper et al., 1974; Ciacco and D'appolonia, 1977; Defloor and Delcour, 1993). This effect has been suggested to be a result of dilution, rapture or disruption of the gluten matrix. During proofing, non-wheat flours have been shown to break-down dough structure (Ruiter, 1978). Finally, pasting properties of non-wheat starch have also been implicated in reducing baking quality (Ciacco and D'appolonia, 1977).

VG improved baking quality by becoming an integral part of the endogenous gluten thus strengthening the protein matrix and improving viscoelasticity (Stenvert et

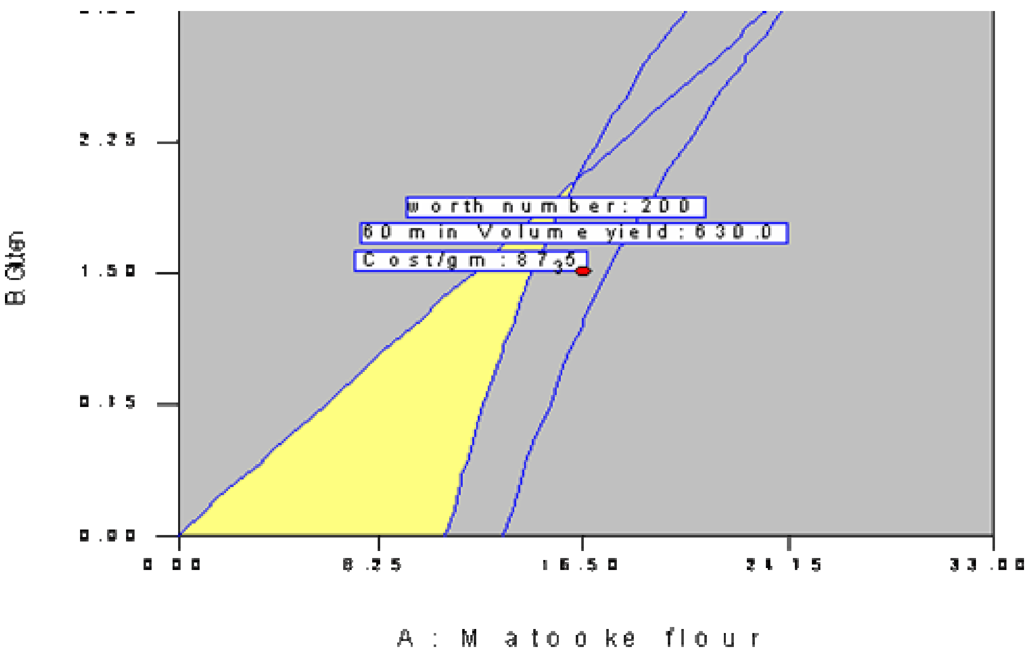

Figure 3. Overlay plot showing optimal formulation region (yellow) satisfying maximum worth number and volume yield and minimum cost at 1.13 FWA.

al., 1981). VG has been shown to improve dough elasticity of slack dough's to normal status, improve loaf volume, crumb grain, texture and softness and prolong shelf life (Wiepert and Lindheaur, 1999). A high FWA improved baking quality by improving gelatinisation of starch. The higher the water content the greater the extent of starch gelatinisation and thus the softer the crumb (Sluimer, 2005). Efficient gelatinisation also leads to improved loaf form and crumb texture. Also, non-wheat flours have been shown to require higher FWA for optimal baking performance (Ruiter, 1978). However, the interactive effect between FWA and VG was negative showing that gluten required reduced water levels to facilitate its setting during baking.

The positive RTF × VG interactive effect on bread baking can be attributed to a factor in RTF which was apparently capable of improving vitality of dry gluten. Presence of residual sodium metabisulfite in RTF (used as an anti-browning agent during RTF production) could help explain this phenomenon. Stenvert et al. (1981) showed that adding up 35 ppm sodium metabisulfite, a reducing agent, significantly improved the baking performance of poor quality vital gluten. Sodium metabisulfite acts by 'softening' dry vital gluten so that it attains optimal development and thus gas retaining capacity at the same time as the endogenous gluten. This principle is employed in activated dough development process in which balanced blends of reducing and oxidising agents are used to bring about dough maturation (Ruiter, 1978). However, this possible effect of residual sodium metabisulfite in RTF would suggest that the RTF/wheat composite dough should be fermented for shorter periods of time to avoid breakdown of the dough structure.

Conclusion

RSM procedures satisfactorily modelled RTF/wheat composite bread systems in which VG proved to be an effective improver of RTF/wheat composite bread. Vitality of VG is enhanced by RTF and a higher FWA than predicted by the farinograph was needed for optimal baking quality of RTF/wheat composite flour. Optimal composite flour baking formulation for maximum RTF and minimum cost was 22% RTF, 3% VG and 1.13 FWA. The hypothesis was therefore not rejected.

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