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ORIGINAL ARTICLE



### **Optimization of a novel improver gel formulation for Barbari flat bread using response surface methodology**

Amir Pourfarzad • Mohammad Hossein Haddad Khodaparast • Mehdi Karimi • Seyed Ali Mortazavi

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Abstract Nowadays, the use of bread improvers has become an essential part of improving the production methods and quality of bakery products. In the present study, the Response Surface Methodology (RSM) was used to determine the optimum improver gel formulation which gave the best quality, shelf life, sensory and image properties for Barbari flat bread. Sodium stearoyl-2-lactylate (SSL), diacetyl tartaric acid esters of monoglyceride (DATEM) and propylene glycol (PG) were constituents of the gel and considered in this study. A secondorder polynomial model was fitted to each response and the regression coefficients were determined using least square method. The optimum gel formulation was found to be 0.49 % of SSL, 0.36 % of DATEM and 0.5 % of PG when desirability function method was applied. There was a good agreement between the experimental data and their predicted counterparts. Results showed that the RSM, image processing and texture analysis are useful tools to investigate, approximate and predict a large number of bread properties.

Keywords Emulsifier  $\cdot$  Image processing  $\cdot$  Polyol  $\cdot$  Shelf life  $\cdot$  Texture analysis

#### Introduction

Flat breads are the main dietary staple in many Middle Eastern and North African countries. Freshly baked flat

M. Karimi Khorasan Razavi Agricultural and Natural Resources Research Center, Mashhad, Iran breads are soft, pliable and elastic, but when kept at room temperature, they stale within a few hours and become tough and rigid. From the consumers' point of view, high quality bread must have several characteristics including: suitable volume, low in density, fresh with attractive aroma, good taste, soft in crumb, crispy and brittle in crust, clean in color and long shelf life. However, bread produced using only the basic ingredients like bread flour, yeast, salt and water are insufficient to reach these characteristics. Thus, in today's demanding world, the use of bread improvers has become an indispensable part of enhancing the quality of bakery products. Bread improvers (also sometimes called dough conditioners) are technically sophisticated blends of functional ingredients, which if formulated correctly, will enhance the development of dough structure, facilitate trouble-free production and provide the desired result of consistent products having optimal quality at the lowest possible cost.

Among the functional food additives, polyols have been increasingly used to improve the quality and shelf life of bread. Gliemmo et al. (2006) showed that using polyols can depress the water activity and improve texture and mouthfeel. Polyols have been used successfully to extend the shelf life of ready to eat bread used by the military (Hallberg and Chinachoti 1992), Barbari bread fortified with soy flour (Pourfarzad et al. 2011), as well as flour tortillas (Suhendro et al. 1995).

Emulsifiers, a subset of surfactants, have been widely used by the baking industry. The function of surfactants, as crumb softening agents, is closely related to their interaction or complex formation with starch, particularly the linear amylose fraction, to retard bread staling. Emulsifiers may also slow the rate of bread firming by forming a complex with the amylopectin fraction within the starch granule (Kamel and Ponte 1993). The properties of the emulsifier are often discussed in terms of hydrophile–

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lipophile balance (HLB) values, according to Griffin (1949). but the physical state (molecular arrangement) of the emulsifier is equally important (Friberg and Wilton 1970). The physical state affects the solubility and mobility of the molecules. According to Krog (1981) surfactants, such as SSL and DATEM, that are the most efficient in breadmaking are able to form lamellar mesophases or gel structures in water. It has been shown that for polar lipids the lamellar liquid-crystalline phase is the most effective form in which this component can be added to bread dough in order to improve loaf volume (Eliasson and Tjerneld 1990). For example, the distilled saturated monoglycerides in the crystalline  $\beta$ -state do not melt until a temperature of 55–60 °C is reached. These non-spreadable particles do not have a surface active effect. If the  $\beta$ -crystals are melted in water and cooled again, an  $\alpha$ -crystalline emulsifier is formed, which consists of lipid bilayers separated by water between the hydrophilic groups (Krog and Sparsø 2004). This specific structure is thought to be useful for foam development because of its capacity to spread onto a surface although being firm enough to stabilize the surfaces towards coalescence (Richardson et al. 2004). The improvement in bread quality and rheological characteristics of dough with surfactant gels was reported by Azizi and Rao (2005a, b).

RSM can be utilized to study the relative significance of several affecting factors in the presence of complex interaction. RSM is an empirical statistical modeling technique employed for designing of experiments, developing models, considering the effects of several factors and evaluating optimum conditions for desirable responses (Nath et al. 2012).

The effectiveness of response surface methodology (RSM) in optimization of ingredient levels, formulations and processing conditions in food technology from raw to final products such as millet enriched biscuits (Chakraborty et al. 2011), production of shrimp waste protein hydrolysate (Dey and Dora 2011), radish fibre based snack (Gupta and Premavalli 2012), plantain and chickpea enriched biscuits (Yadav et al. 2012) and extraction of antioxidants from wheat bran (Singh et al. 2012) have been documented by different researchers.

A literature survey indicated that no work has been carried out on the effect of a bread improver gel containing emulsifier, polyol, and water on the quality, shelf life and sensory characteristics of bread. Thus, the present study was designed: (a) to examine the effects of different emulsifiers (sodium stearoyl-2-lactylate and diacetyl tartaric acid esters of monoglycerides) and polyol (propylene glycol) on Barbari flat bread performance when used singly and in combination at different levels; (b) to determine the optimum formulations for Barbari flat bread improver; (c) to check the validity of RSM to analyze the additive, synergistic and/ or antagonistic effects of emulsifiers and polyols on the quality, shelf life and sensory properties; and (d) to obtain the relationship between quality, shelf life, sensory and image parameters.

#### Materials and methods

#### Materials

Commercial *Triticum aestivum* wheat flour (locally named Setareh) was procured from the AceeArd Co., Khorasan, Iran. Dried active yeast was obtained from Fariman Co., Khorasan, Iran. Propylene glycol (PG) was purchased from J.T. Baker Chemical Company (Phillipsburg, NJ). Sodium stearoyl-2-lactylate (SSL) and diacetyl tartaric acid esters of monoglycerides (DATEMs) were provided by Vista Tejarat Company (Tehran, Iran). Shortening was provided Jahan Company (Tehran, Iran). All other chemicals, reagents and solvents were of analytical grade.

#### Methods

#### Chemical characteristics

Moisture, ash, fat, wet gluten and falling number were determined according to standard AACC methods of 44–16 A, 08–07, 30–10, 38–11 and 56–81, respectively (AACC 2000). Flour protein was tested using a Kjeltec auto protein tester (model 1030, Tecator Co., Hoeganaes, Sweden). Three replications were taken for each characteristic.

#### Preparation of gels

Gels were prepared using emulsifier, polyol and water in the ratio of 1:1:4 using the SSL and DATEM as emulsifiers and PG as a polyol. First, dispersions were made according to Table 1, and then the dispersions, under continuous agitation, were heated to a temperature of 50 °C. Gels were prepared by cooling at 4 °C.

#### Bread making and evaluation of breads

The bread formula used for this kind of bread consisted of flour; compressed yeast (2 g/100 g flour); salt (2 g/100 g flour); sugar (1 g/100 g flour); shortening (1 g/100 g flour); water (based on water absorption at 400 BU). This consistency was found by experimentation to give the most reliable prediction of baking absorption (Maleki et al. 1981) when mixed with the dry ingredients at different speeds. Gels were mixed in the mixer (Electronic Stand Mixer, Hügel, Neuss, Germany) for 10 min at 100 rpm with the other ingredients of the bread formula. A baking technique, similar in principle to that of commercial procedure, was used for baking experimental loaves  $(15 \times 25 \times 2.5 \text{ cm})$ 

 Table 1
 Coded and uncoded addition levels of bread improver gel constituents, according to a central composite rotatable design

<sup>a</sup>variables were studied at five levels coded -1.6818, -1, 0, 1, and 1.6818

Independent variable	Symbol	Addition 1	evels <sup>a</sup> (g	g/100 g f	lour ba	sis)
		-1.6818	-1	0	+1	+1.6818
Sodium stearoyl-2-lactylate	SSL	0	0.13	0.32	0.5	0.63
Diacetyl tartaric acid esters of monoglycerides	DATEM	0	0.13	0.32	0.5	0.63
Propylene glycol	PG	0	0.13	0.32	0.5	0.63

having almost equal volumes. In this procedure, the ingredients were mixed for 10 min to optimum dough development obtained by farinograph. The dough samples were fermented in sealed steel containers at 30 °C and 75-85 % relative humidity for 60 min, and then divided into 200 g pieces and rounded by hand. The pieces were allowed to proof for 10 min in a sealed container placed in the proofing cabinet of oven (Minicombo rotor oven, Zucchelli, Trevenzuolo, Italy). The proofed dough pieces were passed through two pairs of sheeting rolls (gap of 2 mm). The ovalshaped dough pieces were then punched with a special hand puncher which inserted rectangular  $(1 \times 2 \text{ mm})$  holes into the sheeted dough. The dough pieces were then baked in a laboratory air impingement oven for 13 min at 260 °C to obtain the proper thickness and acceptable color and texture. After cooling, bread samples were packed in polyethylene bags of 20 mm thickness, stored at room temperature for 7 days and evaluated.

Quality analysis of fresh bread samples was carried out by measuring volume (rapeseed displacement), weight, specific volume and width/height ratio of the central slice (Mariotti et al. 2006). To determine these properties, slices of  $10 \times 10$  mm were cut from the center of the bread samples using a metal sharp template. Water activity (a<sub>w</sub>) was measured at 25 °C with a water activity meter (Novasina ms1aw, Axair Ltd., Pfaffikon, Switzerland) after calibration with standard salt. Bread moisture was determined according to AACC (2000) procedure 44-15A. Three replicates from three different sets of baking were taken for evaluation of bread characteristics.

#### Sensory evaluation

Sensory analysis was carried out using a 5-point ranking scale with scores ranging from 1 (least pleasure) to 5 (best pleasure). Sensory evaluation was performed by 10 trained panelists. Attributes of bread were selected according to the Iranian flat bread evaluation method, including bread form and shape; upper surface property; bottom surface property; cavity and porosity; firmness and softness of texture; chewiness; odor, flavor and taste. For each of the attributes, the average of the panelist scores was calculated. The overall quality score of bread was evaluated by considering the other sensory characteristics (Rajabzadeh 1991).

#### Measurement of crumb firmness (texture)

Staling phenomenon and its changes were evaluated by penetration test. A QTS texture analyzer (CNS Farnell, Hertfordshire, UK) was used to measure the force required for penetration of a round-bottom (2.5 cm diameter  $\times$  1.8 cm height) probe at a velocity of 30 mm/min into the bread samples. The probe descended 30 mm (a sufficient distance to pass through the slice of 10  $\times$  10 cm of bread) and the trigger force was set at 0.05 N (Pourfarzad et al. 2011). Hardness was evaluated after 1 hour, 3, 5 and 7 days.

#### Image analysis

In the cereals industry, image processing has been applied in widely different ways for assessment of the appearance and quality of product (Mariotti et al. 2006; Yamsaengsung et al. 2010).

For each bread loaf, three slices were obtained from the central region and images were captured using a flatbed HP Scanjet G4010 Photo Scanner (Hewlett-Packard, Palo-Alto, CA, USA) supporting Desk Scan II software (Hewlett Packard, USA). A single 60 mm×60 mm square field of view (FOV) was evaluated for each image. Brightness was adjusted to150 units and contrast to 170 units. Images were scanned full-scale in 256 grey levels at 150 dots per inch (dpi) each comprising 355 columns by 355 rows of picture elements (pixels) (Crowley et al. 2002). JPEG image file format were analyzed with ImageJ 1.4 g (National Institute of Health, USA).

Color images were converted to 8-bits 256 gray level images. The thresholding method (conversion to a binary image) of the 256 gray level digital images was used for image segmentation according to Otsu (1979). The selected crumb grain features were the mean cell area, porosity (cell to total area ratio) and cell density (number of cells/cm<sup>2</sup>) that were analyzed in triplicate for each sample (Angioloni and Collar 2011).

#### Color analysis

The CIE  $L^*a^*b^*$  (or CIELAB) color model was used for determination of the crumb and crust color. The three parameters of such model represent the lightness of color (L\*) which ranges from 0 to 100 (black to white), its

position between red and green (a\*, negative values indicate green while positive values indicate red) and its position between yellow and blue (b\*, negative values indicate blue and positive values indicate yellow) (Sciarini et al. 2010). Since images were acquired in the RGB color space by scanning the bread samples, space conversion were carried out to obtain CIE L\*a\*b\* model parameters. The average values of L\*, a\*and b\* colors describing the outer crust and inner crumb regions were obtained from all 20 baked samples. Color analysis was done in triplicates for each sample.

#### Statistical analysis

A central composite design was constructed using the software Design Expert Version 6.0.10 (Stat-Ease Corporation, Minneapolis, MN, USA) and was used for sampling of different combination of gel constituents (Table 1). Maximum and minimum ingredient levels were chosen by carrying out preliminary screening tests and according to the literature reports and economic aspects. The design consisted of eight factorial points, six axial points and six replicates of the central point. To study three factors (predictor variables) at five levels coded -1.6818, -1, 0, 1, and 1.6818 were required 20 gel formulations. For each of the response variables, multiple linear regression analysis was used and the data were fitted as linear or quadratic models. The cubic model was aliased because there were not enough points for this type of model. From this information, the most accurate model was chosen via the sequential F-tests, lack-of-fit tests and other adequacy measures. Three-dimensional response surface plots were generated for each quality parameter. In this study, predictor variables were permitted to be at any level within the range of the design. Statistical experimental design was used to optimize the gel formulation which was checked with respect to effect on Barbari bread quality, shelf life, sensory and image properties. All experiments were carried out in triplicate. Quadratic equation for the variable was as follows:

$$Y: \beta_0 + \Sigma \beta_i X_i + \Sigma \beta_{ii} X_i X_i + \Sigma_i \Sigma_j \beta_{ij} X_i X_j$$

Where Y is the predicted response;  $\beta_0$  a constant;  $\beta_i$  the linear coefficient;  $\beta_{ii}$  the squared coefficient; and  $\beta_{ij}$  the cross-product coefficient. In addition, Lack of fit, coefficients of determination (R<sup>2</sup>), adj-R<sup>2</sup>, coefficient of variation (CV) and significant probabilities were calculated to check the model adequacy. The above quadratic equation was used to build surfaces for the variables. The software Design Expert Version 6.0.10 was used to analyze the results. By keeping one variable at the central point, three-dimensional plots of two factors versus evaluated properties were drawn, and corresponding contour plots were obtained. Multivariate correlation matrix was performed by using Minitab 15 software (Minitab Inc., State College, PA, USA).

#### Optimization and verification procedures

Besides explaining the behavior of variables by the contour curves, the models fitted in this study could also be utilized for optimization purposes using the desirability function. Optimization was based on generation of the best results for quality, shelf life, sensory and image properties of Iranian Barbari flat bread. The calculation of the optimal levels of ingredients to be used was performed using a multiple response method called desirability (a multiple response method). This optimization method incorporates desires and priorities for each of the variables.

The quality, shelf life over 7 days, sensory and image properties of Barbari bread were determined after gel production under optimal formulation. In order to determine the validity of the model, the experimental and predicted values were compared by paired *t*-test using Minitab 15 software.

#### **Results and discussion**

Chemical quality characteristics of wheat flour

The characteristics of the flour are in the range of typical values of medium strong flour, suitable for Iranian Barbari flat bread. The flour had  $10.52\pm0.36$  % moisture,  $10.8\pm0.24$  % protein,  $1.76\pm0.5$  % fat,  $0.79\pm0.006$  % ash,  $26.7\pm0.55$  % wet gluten,  $82\pm1.5$  % extraction rate and falling number of  $407\pm3$  s.

#### Quality and shelf life

The estimated regression coefficients of the quadratic polynomial models for the response variables of quality properties are given in Table 2. The values of the regression coefficients give an idea as to what extent the control variables affect the responses quantitatively. Analysis of variance (ANOVA) shows that the selected quadratic models adequately represented the data obtained for Barbari quality. The regression models were highly significant for all quality properties with satisfactory coefficient of determination  $(R^2)$ that varied from 0.83 to 0.99. Moreover, coefficient of variation (CV) describes the extent to which the data were dispersed. The CV for each quality property was within the acceptable range. Since CV is a measure of expressing standard deviation as a percentage of the mean, the small values of CV give better reproducibility. In general, a high CV indicates that variation in the mean value is high and does not satisfactorily develop an adequate response model (Daniel 1991). The lack-of-fit tests, which measure the fitness of the model, did not result in a significant F-value, indicating that the model is sufficiently accurate for predicting the quality of Barbari bread.

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Table 2         Regression	coefficients of predicted	quadratic polynomial	models for quality	and shelf life properties
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Source	Specific volume (cm <sup>3</sup> /g)	Moisture content (%)	Water activity	Hardness (1 day) (N)	Hardness (3 day) (N)	Hardness (5 day) (N)	Hardness (7 day) (N)
Constant	4.25***	26.93***	0.915***	2.36***	2.52***	2.69**	3.04**
А	$0.48^{***}$	4.74***	$0.017^{***}$	$-1.90^{***}$	$-0.98^{***}$	$-1.37^{*}$	-1.26*
В	0.35***	3.20***	0.025***	$-1.12^{***}$	-1.15***	$-0.90^{**}$	-1.16**
С	ns	$-1.52^{**}$	$-0.047^{***}$	$-0.37^{***}$	$-0.59^{***}$	$-0.76^{*}$	$-0.66^{*}$
AB	1.26*	ns	$-0.022^{**}$	ns	-0.43**	ns	ns
AC	ns	ns	ns	$-0.35^{**}$	ns	ns	ns
BC	ns	ns	$0.046^{***}$	$1.08^{***}$	1.5***	$1.50^{*}$	1.53**
AA	ns	ns	$0.011^{*}$	1.41***	1.16***	1.64**	1.42**
BB	ns	ns	$-0.011^{*}$	0.64***	$0.64^{**}$	ns	ns
CC	ns	ns	0.032***	ns	ns	ns	ns
Lack of fit	0.5228 <sup>ns</sup>	0.0504 <sup>ns</sup>	0.5154 <sup>ns</sup>	0.2601 <sup>ns</sup>	0.2439 <sup>ns</sup>	0.6584 <sup>ns</sup>	0.1855 <sup>ns</sup>
fit R <sup>2</sup>	0.8622	0.9063	0.9930	0.9904	0.9902	0.8351	0.8875
Adj-R <sup>2</sup>	0.8364	0.8888	0.9878	0.9848	0.9845	0.7762	0.8473
CV	2.02	1.43	0.089	0.98	1	4.37	3.42

ns no significant effect at level <0.05

\*p<0.05

\*\**p*<0.01

\*\*\*p<0.001

CV coefficient of variation

A: sodium stearoyl-2-lactylate (SSL)

B: diacetyl tartaric acid esters of monoglycerides (DATEM)

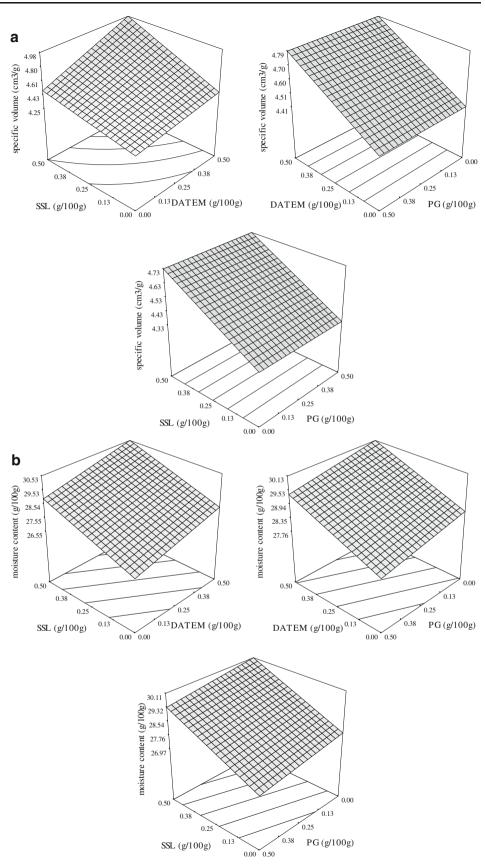
C: propylene glycol (PG)

Figure 1a-c shows the response surfaces of the specific volume, moisture content and hardness (at first day) of the Barbari bread as functions of the improver gel components. As it can be seen in Fig. 1a and Table 2, the response surfaces showed that the SSL and DATEM had linear and interactive significant effects on specific volume. However, the effect of PG on the specific volume was not significant (p < 0.05). The moisture content and water activity of the Barbari bread increased with the elevation of the concentration of SSL and DATEM and decreased with the increase in the concentration of PG (Fig. 1b). DATEM and SSL are both hydrophilic emulsifiers and can interact to a greater degree with polar water molecules and hold moisture, hence increasing the moisture level. This leads to an increase in moisture content (Conforti and Smith 1998). The ability of the polyols to decrease the bread moisture content might be attributed to the fact that less water is required to produce the suitable dough in their presence (Suhendro et al. 1995). Hardness is commonly used as an index to determine bread quality and shelf life. According to Table 2, increasing the concentration of gel constituents resulted in significant decreases in the bread hardness on day one and over the storage time. On the other hand, crossed and quadratic effects of improver components were experimented in some cases. For example, positive effects of crossed DATEM – PG and SSL – SSL were observed on water activity and hardness at evaluated times. These results are in accordance with data for surfactant gel supplemented breads (Azizi and Rao 2005a) and wheat tortillas treated with polyols (Suhendro et al. 1995). Interactions between emulsifiers and gluten proteins strengthen the gluten protein network and contribute to improved gas retention, better texture and increased volume of baked product (Chin et al. 2007). Pommet et al. (2005) reported that plasticizing effect of PG resulted in hardness reduction because of lowering the amount of cross-links in retrograded starch molecules.

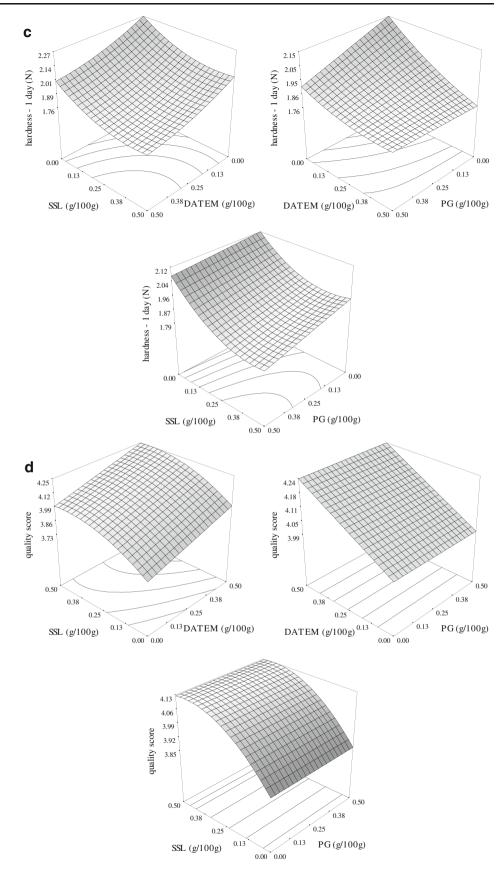
#### Sensory properties

The results of ANOVA for sensory properties with the corresponding coefficients of multiple determinations ( $R^2$ ) for the gel constituents are shown in Table 3. In general, the regression models were highly significant for sensory properties with satisfactory coefficient of determination ( $R^2$ ) that varied from 0.71 to 0.99. Moreover, the CV for each sensory parameter was within the acceptable range and the lack-of-

Fig. 1 Response surface plots of specific volume (a), moisture content (b), hardness at 1 day (c), quality score (d), crust L\* (e) and crumb porosity (f) SSL: sodium stearoyl-2-lactylate, DATEM: diacetyl tartaric acid esters of monoglycerides, PG: propylene glycol; other variables are held at medium level

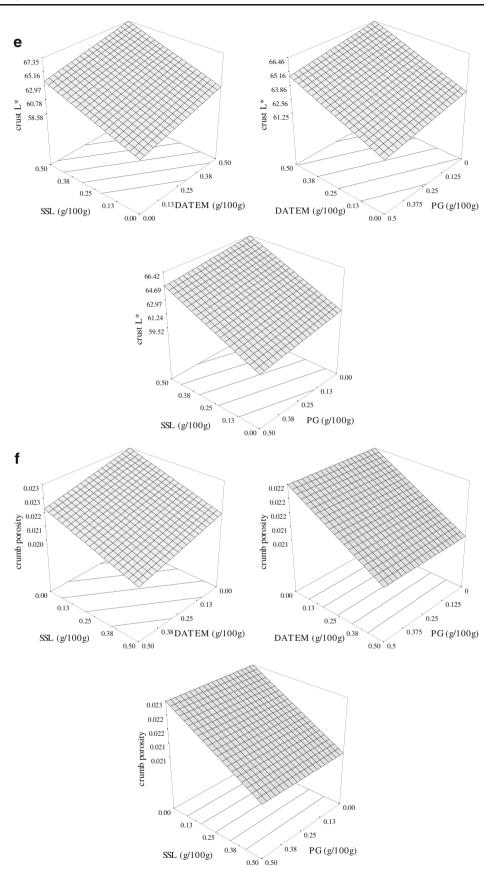


#### Fig. 1 (continued)



## Author's personal copy

#### Fig. 1 (continued)



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#### Table 3 Regression coefficients of predicted quadratic polynomial models for sensory properties

Source	Form and shape	Upper surface property	Bottom surface property	Cavity and porosity	Firmness and softness of texture	Chewing ability	Odor, flavor and taste	Overall Quality score
Constant	4.12 <sup>ns</sup>	4.14***	3.94*	3.95***	3.49***	2.95***	3.89 <sup>ns</sup>	3.73***
А	ns	0.30***	ns	0.53***	2.06**	2.15**	ns	1.26*
В	ns	$0.48^{***}$	ns	0.34***	1.96***	1.72***	ns	$0.49^{**}$
С	ns	0.35***	ns	ns	$0.40^{**}$	$0.67^{**}$	ns	ns
AB	ns	ns	ns	$0.98^*$	$0.64^{*}$	ns	ns	ns
AC	ns	ns	ns	ns	$0.88^{**}$	ns	ns	ns
BC	ns	ns	ns	ns	$-1.97^{**}$	-1.54**	ns	ns
AA	ns	ns	ns	ns	-2.91***	-2.65***	ns	-1.43**
BB	ns	ns	ns	ns	$-1.09^{**}$	-0.83*	ns	ns
CC	ns	ns	ns	ns	$0.44^{*}$	ns	ns	ns
Lack of fit	0.4648 <sup>ns</sup>	0.0558 <sup>ns</sup>	0.3292 <sup>ns</sup>	0.7292 <sup>ns</sup>	0.6322 <sup>ns</sup>	0.0966 <sup>ns</sup>	0.5890 <sup>ns</sup>	0.2832 <sup>ns</sup>
R <sup>2</sup>	0	0.9654	0	0.8906	0.9941	0.9571	0	0.7157
Adj-R <sup>2</sup>	0	0.9589	0	0.8700	0.9888	0.9373	0	0.6623
CV	5.98	0.61	6.39	1.76	0.79	1.84	4.19	2.49

ns no significant effect at level <0.05

\**p*<0.05

\*\*p<0.01

\*\*\*p<0.001

CV: coefficient of variation

A: sodium stearoyl-2-lactylate (SSL)

B: diacetyl tartaric acid esters of monoglycerides (DATEM)

C: propylene glycol (PG)

Table 4 Regression coefficients of predicted quadratic polynomial models for image properties<sup>1</sup>

Source	Crust			Crumb					
	L*	a*	b*	L*	a*	b*	Mean cell area (mm <sup>2</sup> )	Cell density (cells/cm <sup>2</sup> )	Porosity
Constant	59.42***	16.83 <sup>ns</sup>	32.92*	77.33***	2.92*	30.47*	0.55***	43.60***	0.023***
А	10.46***	ns	ns	8.76***	ns	ns	$-0.084^{***}$	4.93***	$-0.004^{***}$
В	7.07***	ns	ns	6.37***	ns	ns	$-0.069^{***}$	3.59***	-0.003***
С	-3.35**	ns	ns	ns	ns	ns	ns	ns	ns
AB	ns	ns	ns	$26.48^{*}$	ns	ns	ns	14.95*	ns
Lack of fit	0.0511 <sup>ns</sup>	0.2031 <sup>ns</sup>	0.3292 <sup>ns</sup>	$0.5020^{\mathrm{ns}}$	0.2217 <sup>ns</sup>	0.3147	0.5165 <sup>ns</sup>	0.5012 <sup>ns</sup>	$0.7990^{\rm ns}$
$\mathbb{R}^2$	0.9070	0	0	0.8418	0	0	0.8097	0.8413	0.8337
Adj-R <sup>2</sup>	0.8895	0	0	0.8121	0	0	0.7873	0.8116	0.8141
CV	1.42	9.85	6.39	2.34	6.76	6.42	2.16	2.34	1.94

ns no significant effect at level <0.05

\**p*<0.05

\*\*p<0.01

\*\*\**p*<0.001

CV coefficient of variation

A: sodium stearoyl-2-lactylate (SSL)

B: diacetyl tartaric acid esters of monoglycerides (DATEM)

C: propylene glycol (PG).

<sup>1</sup> The results for AC, BC, AA, BB and CC were not significant

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÷	aw	SV	MC	USP	ц	Ь	CA	QS	IH	H3	H5	H7	LMB	LST	MCA	CD
Specific ( volume	0.634**															
(SV) Moisture content	0.846***	0.793***														
(MC) Upper surface properties		0.728***	0.587**													
	0.446* 0.696**	$0.692^{**}$ $0.989^{***}$	$0.634^{**}$ $0.842^{***}$	0.799*** 0.702 <sup>**</sup>	0.679**											
	$0.449^{*}$	0.677**	$0.642^{**}$	0.776***	0.988***	0.666**										
	0.496*	0.571**	$0.611^{**}$	0.76***	0.875***	0.597**	0.877***									
score (QS) Hardness -		$-0.67^{**}$	$-0.615^{**}$	-0.786***	-0.995	$-0.658^{**}$	$-0.984^{***}$	-0.87***								
1 day (H1) Hardness-		$-0.712^{***}$	$-0.629^{**}$	$-0.784^{***}$	-0.98***	$-0.697^{**}$	$-0.967^{***}$	-0.859***	0.983***							
3 day (H3) Hardness-		$-0.647^{**}$	$-0.488^{*}$	$-0.751^{***}$	$-0.904^{***}$	$-0.6^{**}$	$-0.873^{***}$	$-0.68^{**}$	$0.901^{***}$	$0.904^{***}$						
	-0.479*	$-0.66^{**}$	$-0.636^{**}$	$-0.801^{***}$	-0.955***	$-0.645^{**}$	$-0.944^{***}$	-0.796***	0.955***	$0.946^{***}$	$0.862^{***}$					
H7) *	0.654**	0.985***	0.809***	0.685**	$0.686^{**}$	0.977***	0.673**	$0.564^{*}$	-0.668**	-0.709***	$-0.64^{**}$	$-0.659^{**}$				
_	$0.846^{***}$	0.794***	1***	0.588**	0.635**	0.843***	$0.642^{**}$	$0.611^{**}$	$-0.616^{**}$	$-0.629^{**}$	$-0.489^{*}$	$-0.636^{**}$	0.81***			
	$-0.613^{**}$	$-0.994^{***}$	-0.772***	-0.745***	$-0.701^{**}$	$-0.982^{***}$	$-0.687^{**}$	$-0.575^{**}$	$0.679^{**}$	0.716***	0.659**	$0.667^{**}$	$-0.961^{***}$	-0.773***		
area (MCA) Crumb cell ( density	0.654**	0.985***	0.809***	$0.684^{**}$	0.686**	0.977***	0.673**	0.563*	-0.668**	-0.709***	$-0.64^{**}$	-0.659**	1***	0.81***	-0.961	
rumb porosity (CP)	-0.654**	-0.966	-0.797***	-0.723***	-0.671**	-0.977	-0.657**	-0.593**	0.647**	0.682**	0.592**	0.632**	-0.918***	-0.798	0.982***	-0.918***

fit tests did not result in a significant F-value, indicating that the model is sufficiently accurate for predicting the sensory aspects.

Positive linear effects of SSL, DATEM and PG were observed on upper surface property, firmness and softness and chewing ability. Positive linear effects of SSL and DATEM and crossed quadratic effect of SSL-DATEM were observed on cavity and porosity. Figure 1d shows the change in quality score of Barbari bread with respect to the improver gel ingredients. It is clear from Fig. 1d that the overall quality score was increased by SSL and DATEM, but decreased by quadratic effect of SSL. Results on positive effects of SSL and DATEM addition on bread overall quality score are in line with those previously reported by Azizi and Rao (2005a). On the other hand, PG had no significant effect (p < 0.05) on the overall quality score and this was consistent with the finding of Pourfarzad et al. (2011). Among sensory parameters, form and shape, bottom surface property, odor, flavor and taste were not affected by improver constituents.

#### Image processing

Quadratic models were fitted for image properties of crust and crumb (Table 4). Analysis of variance (ANOVA) shows that the selected quadratic models are well adjusted to the experimental data of crust and crumb. The regression models were highly significant for all image properties with satisfactory coefficient of determination ( $\mathbb{R}^2$ ) that varied from 0.80 to 0.95. Moreover, coefficient of variation (CV) for each image property was within the acceptable range. The lack-of-fit tests did not result in a significant F-value, indicating that the model is sufficiently accurate for predicting the color and texture.

The surface color of bread is an important quality feature, associated with aroma, texture, and appearance characteristics. In fact, color is an essential attribute of bread, contributing to consumer preference. Brown crust in bread is a result of non-enzymatic browning reaction (Maillard type) between amino acids and reducing sugars (Kent and Evers 1994). Figure 1e reveals that crust L\* was increased by SSL and DATEM, but decreased by PG. It may be related to moisture content increment by SSL and DATEM that lead to sugar and amino acid dilution in the dough and subsequently crust lightness enhancement. PG decreased the moisture content of the dough: hence the increased crust darkness. Crumb L\* was increased by SSL, DATEM and crossed quadratic effect of SSL-DATEM. This is probably due to better light reflection from more uniform fine gas cells (Chin et al. 2007). However, no significant difference was observed in the a\* and b\* of crust and crumb because of additive addition.

Visual characteristics of crumb such as mean cell area, cell density and porosity are elements of the quality of the final product. Almost every scientist who has studied

Table 6	Predicted and experimental values of the response variables at
optimun	1 formulation

Response variables	Predicted values	Experimental values
Specific volume (cm <sup>3</sup> /g)	4.77	4.81±0.33
Moisture content (%)	29.45	$28.92{\pm}1.26$
Water activity	0.920	$0.918{\pm}0.021$
Form and shape	4.12	$4.21\!\pm\!0.23$
Upper surface properties	4.61	$4.50{\pm}0.18$
Bottom surface properties	3.94	$3.09 {\pm} 0.52$
Cavity & porosity	4.45	$4.37 {\pm} 0.61$
Firmness and softness of texture	4.60	$4.62 {\pm} 0.44$
Chewing ability	3.95	$4.01 \pm 0.63$
Odor, flavor and taste	3.89	$3.91 \pm 0.29$
Overall quality score	4.21	$4.34 {\pm} 0.53$
Hardness - 1 day (N)	1.78	$1.64 {\pm} 0.04$
Hardness- 3 day (N)	1.91	$1.93 {\pm} 0.13$
Hardness- 5 day (N)	1.99	$2.04 {\pm} 0.23$
Hardness- 7 day (N)	2.33	$2.42 {\pm} 0.11$
L* - Crust	64.98	$65.04{\pm}1.53$
a* - Crust	16.83	$15.95 {\pm} 1.05$
b* - Crust	48.42	$46.23 {\pm} 2.98$
L* - Crumb	87.27	$85.43 \pm 1.73$
a* - Crumb	2.68	$3.54 {\pm} 0.63$
b* - Crumb	27.30	$29.15 \pm 1.76$
Mean cell area (mm <sup>2</sup> )	0.49	$0.55 {\pm} 0.31$
Cell density (cells/cm <sup>2</sup> )	49.20	$49.75 \pm 1.55$
Porosity	0.021	$0.019 {\pm} 0.003$

texture and cellular structure of bread crumb has indicated that the structure plays a predominant role in the textural properties of bread crumb (Scanlon and Zghal 2001). Major negative effects on mean cell area and porosity of crumb (Fig. 1f) were provided by SSL and DATEM. The cell density significantly increased as the amount of SSL or DATEM increased.

It is clearly seen that, surfactant addition results in an increase in the crumb cell density while simultaneously inducing decreases in crumb porosity and mean cell area. These results are in agreement with Junge et al. (1981) and Chin et al. (2007) who found that surfactants such as SSL and DATEM impart a fine grain in the finished product by forming more and smaller air cells during mixing.

Relationships between quality, shelf life, sensory and image parameters in Barbari bread

Multivariate data handling of gel formulation variables provided information on the significantly correlated properties of Barbari bread (Table 5). Using Pearson correlation analysis, a range of correlation coefficients (r) (from 0.449 to 1) was obtained for the relationship between quality, shelf life, sensory and image parameters. Within quality characteristics, moisture content and water activity highly and positively correlated (r: 0.846). This result was in concordance with Cauvain and Young (2000) who indicated that there is a direct link between moisture content and water activity of a product. Higher percentage of specific volume in Barbari breads corresponded to breads with higher moisture content (r: 0.793) and cell density (r: 0.985) but lower mean cell area (r: -0.994) and crumb porosity (r: -0.966). Harder samples (at 1 day of storage) strongly corresponded to those with higher mean cell area (r: 0.679) and crumb porosity (r: 0.647) but lower crumb cell density (r: -0.668), specific volume (r: -0.67) and moisture content (r: -0.615). Hardness during storage (up to 7 days of storage) also followed the same trend. Sensory properties deserved a special attention. Upper surface properties positively correlated with moisture content (r: 0.587) and crust L\* (r: 0.588). High significant negative correlations were observed between firmness score and hardness at 1 day and during storage (r: -0.904 to -0.995). Cavity and porosity correlated positively with cell density (r: 0.977) and negatively with mean cell area (r: -0.982) and crumb porosity (r: -0.972). Within sensory characteristics, quality score showed many significant relationships. These results show that the image processing and texture analysis can potentially be used to estimate different properties of bread. This development may have significant potential to optimize and improve product quality, shelf life, image parameters and sensory aspects and reduce time and costs by minimizing experiments.

#### Optimization procedure and verification of results

Multiple response optimizations were performed to measure the optimum levels of independent variables to achieve the desired response goals. Specific volume and sensory aspects were desired maximal whereas hardness as an indicator of shelf life was specified as minimum desirable. It is well known that the moisture content of bread crumb is a major contributor to the perception of product freshness and that, within limits, the higher the moisture content, the fresher the bread will be perceived by the consumer. Conversely, if the water activity is sufficiently low, microbial growth rates can be extremely slow and in practice are considered to have been stopped (2000). Thus, moisture content and water activity were specified as maximum and minimum level desirable, respectively. Cauvain and Young (2000) indicated that the porosity of bakery components influences the rate of moisture migration between components. In general, the less porous the product structure, the slower will be moisture migration. Besides, as can be concluded from relationships within bread parameters, most of investigated parameters such as specific volume, hardness during storage and sensory aspects had a negative linear correlation with mean cell area and crumb porosity whereas they had a positive linear correlation with crumb cell density. Therefore, mean cell area and porosity were specified as minimum while cell density was determined as maximum level desirable. People generally prefer to consume bread that has a golden brown crust and a creamy white crumb (Baiano et al. 2009). Thus, crumb L\* was nominated maximal and crumb a\* and b\* and crust L\*, a\* and b\* were fixed to intermediate level. Then, the optimal conditions were extracted by Design Expert software.

The final result for this optimization suggested that a mixture containing 0.49 % of SSL, 0.36 % of DATEM and 0.5 % of PG in gel formulation could be a good mixture of these three improver compounds in order to achieve the best quality, shelf life, sensory and image properties of Barbari bread. This new mixture was submitted to the same experimental procedures applied as those from the beginning of this study (Table 6). There was no significant difference between the estimated and observed values (P < 0.05), suggesting a good fit between the models to the experimental data.

#### Conclusion

Response surface methodology was an efficient statistical tool to model the influence of SSL, DATEM and PG on quality, shelf life, sensory and image properties of Barbari bread. These results also suggested that by modifying the proportion of these additives, a large range of variations may be obtained. SSL and DATEM both improved quality, shelf life, sensory and image properties. PG had major improving effect on quality and shelf life properties, but sensory and image properties were less influenced. Based on these models, the optimum improver gel formulation was obtained. The effectiveness of the proposed formula was tested on an industrial plant, yielding satisfactory results. It was determined that the average improvement of bread quality, shelf life and sensory parameters due to addition of the optimized improver gel was about 7 %, 13 % and 25 %, respectively. In addition to establishing best formulation, the image processing and texture analysis have been shown to be useful tools to investigate, approximate and predict a large number of bread properties. This study was preliminary and needs to be studied at molecular level in detail.

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