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Study of the total replacement of egg by white lupine protein, emulsifiers and xanthan gum in yellow cakes

Abstract The possibility of a total substitution of egg proteins in yellow cakes by vegetable proteins isolated from white lupine seeds was studied. The effects of several components on hardness, moisture content, volume, and shape characteristics of lupine-cakes were evaluated, using response surface methodology. Baking powder, soy lecithin, and mono- and diglycerides (MDG), were studied through a central composite rotatable experimental design and an optimization of the leavening agent proportion was achieved; however, an undesirable collapse of the cakes was observed. This problem was solved by the combined use of emulsifiers (MDG) and hydrocolloids (xanthan gum) in a second experimental design. Xanthan gum improved the internal structure of crumb, reducing shrinkage and increasing the cake height. Optimum contents for the lupine-gum-emulsifier system were: 2.2% baking powder, 4.5% MDG, and 0.55% xanthan gum, all relative to the total weight of flour and sugar.

Keywords Cakes · Egg · White lupine proteins · Xanthan gum · Emulsifiers · Volume · Height · Hardness

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Introduction

Eggs are the most costly ingredients in some cakes. In yellow cakes, eggs are a significant source of cholesterol. The use of vegetable proteins for partial or total substitution of eggs in cake formulations appears, therefore, to be an interesting objective, and especially so for the people with specific dietary needs or restrictions (vegans, vegetarians, high cholesterol people, etc.). The almost unique foaming, emulsifying, and heat coagulation properties of egg proteins confer them a very important functional role in the definition of cake characteristics, namely volume and texture. This makes it extremely difficult to replace eggs successfully by a different source of proteins, even by the use of several types of additives, such as hydrocolloids, in cakes.

Good results have been obtained by using bovine blood plasma to substitute egg whites at various levels [1, 2, 3]. Other protein sources, such as egg white, egg yolk, skimmed milk, whey powder, and a soy protein isolate have been employed in the study of volume and textural characteristics of rice cakes [4]. On the other hand, other studies have suggested the use of xanthan gum to partially replace egg white content in cakes [5]. In some cases [6], the cakes obtained after the inclusion of xanthan gum in the batter formulation showed similar or better characteristics, in terms of volume, height and shrinkage, than those of the control cakes.

Vegetal proteins, isolated from lupine (*Lupinus albus*) seeds, have demonstrated good foaming and emulsifying properties [7, 8]. The aim of this work was to analyze the possibility of total substitution of egg proteins in small-ratio yellow cakes by this lupine protein isolate. The optimum leavening agent, emulsifiers, and xanthan gum levels in this system were also studied.

Materials and methods

Cake ingredients

A white lupine protein isolate (Lupi E, Innovationszentrum Kreislaufpolymere GmbH, Freising, Germany) with a protein content

(Dumas) of 92% (wb) was used to prepare a 20% protein content aqueous solution (w/w). This solution was employed to substitute egg (with an estimated protein content of 15%) in the control formulation. Commercial wheat flour (55% extraction, 8.0% protein, 14.5% moisture, 0.6% ash), sucrose (Sidul, Alcantara Refinarias Açucares S.A., Portugal), vegetal shortening (Vaqueiro, Fima, Portugal), non-fat dry milk (Molico, Nestlé Portugal S.A.), and synthetic commercial vanilla flavor were used in all treatments. Baking powder (Royal, Royal Brands Portugal S.A.), soy lecithin (Leciflor 100, Muhlenchemie, Germany), MDG – a mixture of mono- and diglycerides of edible fatty acids and polyglycerol esters (Emulpals 102, Palsgaard, Denmark), and xanthan gum (Keltrol T, Nutrasweet Kelco Co., UK) were added in different proportions.

The formulation of the cakes was, based on 51.2% of wheat flour; 48.8% of sucrose plus 15.3% of shortening; 14.0% of milk; 5E – 4% of vanilla flavor with 28.0% of whole egg in the case of the control cakes (based on the weight of flour and sucrose). For the lupine cakes, 21% of the lupine solution, and several other baking ingredients, according to the two experimental designs, replaced the egg protein.

Experimental design

Two central, composite, rotatable experimental designs were performed using to the response surface methodology (RSM). In the first design, the independent variables considered were the baking powder (x_1), soy lecithin (x_2), and MDG (x_3) contents. Dependent variables were volume, moisture content, and hardness and the target was to maximize the volume and minimize hardness. Once the optimum content of baking powder was fixed, a second experimental design was carried out, with MDG (x_1) and xanthan gum (x_2) as independent factors. In this design, besides the previous dependent variables, maximum diameter, central height, and peripheral height of the cakes were also determined in order to evaluate the shrinkage upon cooling.

Cake preparation

The mixing and baking procedures were held constant over the experimental designs. A multi-stage mixing method was selected. This consisted of creaming the sucrose with the shortening by mixing during 5 min, followed by the addition of lupine solution (3 min), then the milk (30 s), and at last the dry ingredients: wheat flour, baking powder, soy lecithin, MDG, and xanthan gum (5 min). A Krups 3 Mix 7007 Pro mixer (Robert Krups GmbH & Co., Solingen, Germany) was used always at its lowest speed. Approx. 350 g of batter was prepared for each formulation; 50±0.1 g of batter was poured into each of the six oven-proof cake molds. Baking was carried out at 180 °C for 30 min in an electrically heated, natural convection oven (Werner & Pfleiderer, Stuttgart, Germany). The cakes were allowed to cool at room temperature for about 1 h before being individually wrapped in polyvinyl plastic bags. Analysis of cakes was performed after 72 h storage at room temperature for equilibration.

Cake analysis

After weighing the cakes, their volume was determined by rapeseed displacement. A digital caliber was used to measure the cake heights and diameters. Cake moisture content was determined by weight loss after 24 h at 104 °C. Hardness was calculated from a texture profile analysis (TPA), using a TA-XT2 device (Stable Microsystems). An 11 mm diameter cylindrical stainless steel probe which was set to penetrate twice to 30% depth of the average cake height, at a 1 mm s^{-1} speed, with a crosshead load of 25 kg, and with a 5 s delay between first and second penetration. Five texture profiles were obtained from each cake, and five cakes were analyzed from each formulation, therefore the presented data are the average calculated from 25 measurements.

Table 1 Experimental design 1: RSM matrix, and mean values for response variables

x_{11}, x_{12}, x_{13} (%)	Hardness (g)	Moisture (%)	Volume (cm^3)
0.6, 0.4, 1.6	562.5	20.79	80
0.6, 0.4, 6.4	462.6	19.03	88
0.6, 1.6, 1.6	504.9	22.49	75
0.6, 1.6, 6.4	554.0	20.97	83
2.4, 0.4, 1.6	200.7	17.09	113
2.4, 0.4, 6.4	215.7	16.25	104
2.4, 1.6, 1.6	235.0	17.74	103
2.4, 1.6, 6.4	363.7	17.46	116
0.0, 1.0, 4.0	846.6	21.80	80
3.0, 1.0, 4.0	311.7	16.84	112
1.5, 0.0, 4.0	273.7	16.25	128
1.5, 2.0, 4.0	299.5	17.49	106
1.5, 1.0, 0.0	319.6	19.20	103
1.5, 1.0, 8.0	352.4	18.54	99
1.5, 1.0, 4.0	266.1	18.19	107
1.5, 1.0, 4.0	293.3	16.57	119
1.5, 1.0, 4.0	337.9	18.43	112
1.5, 1.0, 4.0	337.8	18.00	106

Table 2 Experimental design 1: Regression coefficients of the terms involved in each response equation, with significance; R-squared statistic and the mean absolute error

Terms	Hardness (g)	Moisture (%)	Volume (cm^3)
Constant	896.8	23.0	55.9
x_1: Baking powder	–508.7***	–4.5***	40.7***
x_2: Lecithin	–91.2 NS	0.9*	
x_3: MDG	22.8 NS	–0.8 NS	7.6 NS
x_1^2	116.0***	0.9**	–9.2**
x_3^2		0.1 *	1.0 *
$x_2.x_3$	22.8*		
R^2	94.1	86.6	76.5
MSE	29.7	0.6	5.9

***: significant at P=0.001, **: significant at P=0.01, *: significant at P=0.05, NS: not significant (if the quadratic or a interaction effect of any independent variable is significant at P=0.05, the term corresponding to the linear effect is include in the equation, although it is not significant).

Statistical analysis

Multiple regression and optimization of the response surface was performed with the Statgraphics Plus version 2.0 software package.

Results and discussion

Mean values for cake hardness, moisture content, and volume of experimental design 1 formulations are presented in Table 1. After a stepwise elimination of not significant effects, response predicting models were obtained (Table 2). Considering the R-squared statistics, better results were obtained for hardness, followed by moisture and volume. Table 2 and Figs. 1 and 2 clearly show that baking powder was the independent variable with the largest effect on the response variables. A drastic decrease of cake hardness and moisture was produced when the baking powder proportion was increased. Just

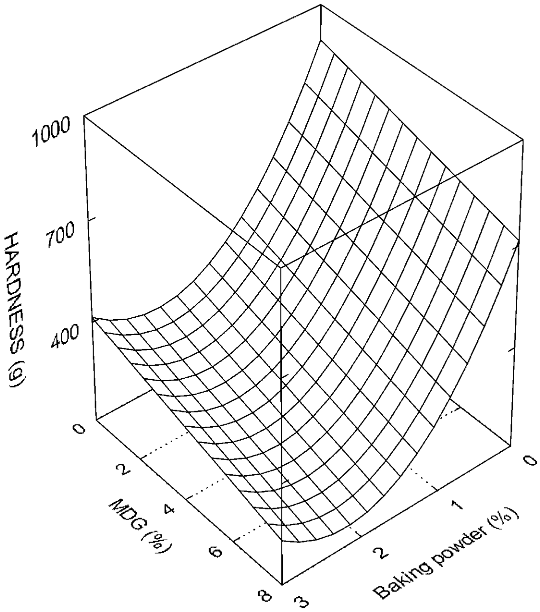

Fig. 1 Response surface showing the effect of MDG and baking powder on cake hardness

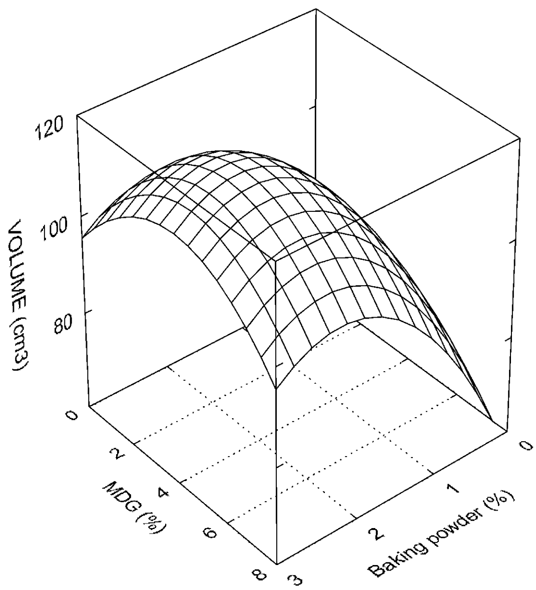

Fig. 2 Response surface showing the effect of MDG and baking powder on cake volume

the opposite was observed for the volume. This suggested that the three phenomena were related. The volume of cakes was directly related with the leavening action of the baking powder. A higher volume implies a less compact cake structure, therefore a lower value for cake hardness. On the other hand, a more intense leavening process should cause an increase of water loss during baking, resulting in a lower final moisture content.

Regression equations allowed an estimation of the optimum baking powder level to obtain cakes with appropriate characteristics. These theoretically optimum values were: ca. 2.2% to minimize hardness and maximize volume, and almost 2.6% to minimize moisture content.

Main and quadratic effects of baking powder could explain, respectively, 94, 87, and 76% of the cake hardness, moisture, and volume variability. Also MDG and soy lecithin showed a small role in the definition of these variables. Nevertheless, MDG presented a desirable effect in the reduction of moisture and volume increase, with an optimum level, in both cases, of about 4%. Soy lecithin had no influence to explain the cake volume variability and induced a slight augmentation of cake moisture. While baking powder showed an absolute independent effect in the three response variables, a significant interaction between MDG and soy lecithin in relation to cake hardness was observed. When the soy lecithin level was high, increasing the concentration on MDG produced a hardness increase. The opposite happened at low lecithin content. Therefore, lecithin was considered to be an unsuitable ingredient for these cakes.

Nevertheless, a structural deficit was observed in these lupine cakes. During baking the leavening action of baking powder caused the expansion of the batter, but when cakes were taken out from the oven, they collapsed. The batter expanded, but the foam was not stable. The characteristic heat coagulation of egg proteins was not compensated for by the denaturation of the lupine proteins, therefore the internal structure of the crumb collapsed when the cakes were allowed to cool.

In order to avoid this undesirable effect, the combined use of emulsifiers and hydrocolloids was considered [9, 10], and a second experimental design was carried out, with xanthan gum and MDG as independent factors. Response variables included hardness, moisture, volume, and three dimensions of the cakes to evaluate the shrinkage effect on cooling: central height (h_c), peripheral height (h_e), and maximum diameter (d_m). The ratio h_c/h_e was regarded as a good variable to evaluate the shrinkage of cakes. Values of this expression equal to or above 1 should indicate that no collapse has occurred, while values below 1 should mean that the structure of the cake collapsed to a certain degree. Average values and regres-

Table 3 Experimental design 2: RSM matrix, and mean values for response variables

x_{21}, x_{22} (%)	Hardness (g)	Moisture (%)	Volume (cm^3)	h_c (cm)	d_m (cm)	h_c/h_e
1.2, 0.12	227.0	16.34	114	2.6	7.7	0.68
1.2, 0.68	498.8	17.57	112	3.7	6.7	0.99
6.8, 0.12	367.7	17.62	113	3.2	7.3	0.84
6.8, 0.68	449.3	16.04	122	4.0	6.7	1.03
0.0, 0.40	464.5	17.15	108	3.2	7.0	0.85
8.0, 0.40	442.9	17.31	114	3.7	6.8	0.96
4.0, 0.00	297.6	17.08	120	2.9	7.7	0.76
4.0, 0.80	443.7	18.39	124	4.2	6.7	1.05
4.0, 0.40	304.0	15.95	132	3.9	7.1	0.98
4.0, 0.40	334.1	17.51	124	3.6	7.1	0.93
4.0, 0.40	360.3	17.16	123	4.0	6.9	1.00

Table 4 Experimental design 2: Regression coefficients of the terms involved in each response equation, with significance; R-squared statistic and the mean absolute error.

Terms	Hardness (g)	Volume (cm^3)	h_c (cm)	d_m (cm)	h_c/h_e
Constant	250.7	109.51	2.11	7.6	0.69
X_1:MDG	–26.4 NS	7.2 NS	0.29**		0.02*
X_2:xanthan	485.1***		3.23**	–1.3***	0.40***
X_1^2	6.3**	–0.9**	–0.03***		
X_2^2			–2.00*		
$x_1.x_2$	–59.4*				
R^2	87.8	65.7	95.2	80.1	85.1
MSE	21.9	3.3	0.08	0.12	0.03

***: significant at P=0.001, **: significant at P=0.01, *: significant at P=0.05, NS: not significant (if the quadratic or a interaction effect of any independent variable is significant at P=0.05, the term corresponding to the linear effect is include in the equation, although it is not significant).

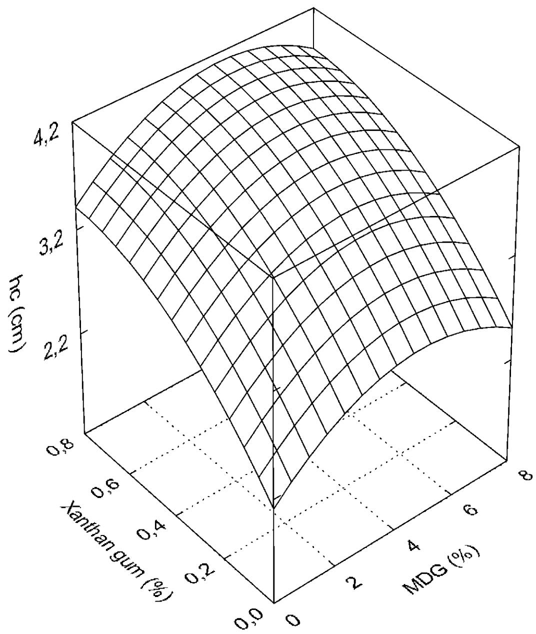

Fig. 3 Response surface showing the effect of xanthan gum and MDG on cake central height

sion coefficients obtained for each variable are presented in Tables 3 and 4, respectively.

The variability of moisture was very small, which explains the fact that no predicting model cold be achieved. Moisture content of cakes was always close to 17%, a correct value in terms of the shelf life of this kind of foodstuff.

Poor results were obtained for the prediction of cake volume. Less than 66% of its total variability was explained by the independent variable MDG, that weakly improved cake volume, and for which the optimum value was 4%, the same value as obtained with the first design. Contrary to our expectations, xanthan gum did not show any significant effect on cake volume.

Nevertheless, xanthan gum played a decisive role into the definition of the height and shape of these cakes, and

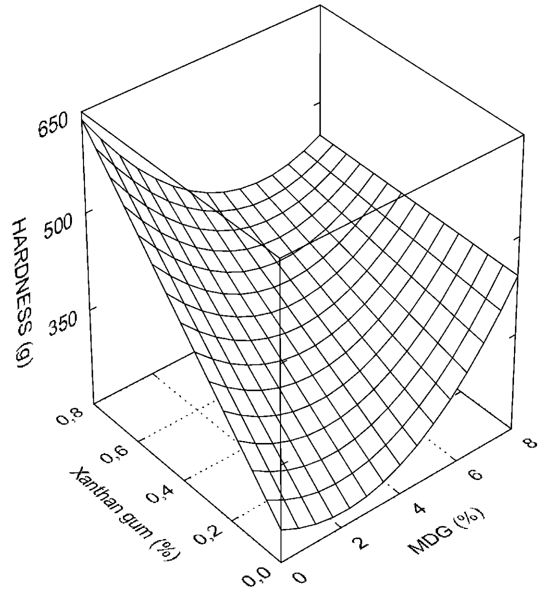

Fig. 4 Response surface showing the effect of xanthan gum and MDG on cake hardness

in overcoming the problem of their collapse. The R-squared statistic value of the central height regression equation was 95%. Except for the interaction between both independent variables, all the possible effects were significant. As can be seen in Fig. 3, xanthan gum substantially improved cake height. On the other hand, the maximum diameter of the cakes was inversely related with their xanthan gum concentration. This agrees with results reported by several authors [6, 11]. They showed a significant increase of height and volume, and a shrinkage decrease of white layer cakes when xanthan gum was added. In this case, it was clearly observed that increasing the xanthan gum content thickened the cake batter. Then, during baking, the batter suffered a vertical, but not lateral, expansion. One effect compensated the other, and that explains the absence of xanthan in the predictive model of cake volume. Xanthan gum provided well-structured, non-collapsed lupine cakes, with appreciable height, but not with large width. The optimum level of xanthan gum, theoretically maximizing the central height of these cakes, was above the highest experimental level (0.8%). MDG showed a positive, but secondary, role in the development of cake height, and its optimum level was 5.3%.

On the other hand, an undesirable consequence of the crumb structure induced by xanthan gum and the emulsifier was observed. Cake hardness increased with increasing concentrations of both components. A significant cross-interaction was observed between them. The lower the level of one factor, the higher was the effect on hardness of the other (Fig. 4).

It was, therefore, decided that 4–5% MDG, and 0.5–0.6% xanthan gum, both given relative to the total weight of flour and sugar, defined the optimum experimental range, in order to obtain cakes with no collapse,

Table 5 Response variable mean values and 95% confidence intervals for 10 replicates, for the control and optimum cakes characteristics

Variable	Control egg cakes	Optimum lupine cakes[a]
Hardness (g)	241.9±14.9	345.2±17.8
Moisture (%)	18.98±0.53	17.43±0.25
Volume (cm^3)	138±2	126±2
h_c (cm)	4.30±0.08	3.87±0.07
d_m (cm)	7.66±0.09	6.70±0.05
h_c/h_e	1.04±0.02	1.05±0.02

[a] 4.5% MDG, 0.55% xanthan gum.

adequate volume and shape (compromise of height and diameter), and without a hard crumb.

Finally, differences between control cakes (traditional recipe with egg) and lupine cakes with 4.5% MDG and 0.55% xanthan gum can be observed in Table 5. The volume of control cakes was much higher than that of the lupine cakes. Control cakes showed both large central height and maximum diameter, which did not occurred in lupine cakes, as was explained above. This lower volume, due to the thickening effect of xanthan gum, may be the reason why the lupine-cakes were harder than the controls.

Conclusions

Although lupine proteins have good foaming and emulsifying properties, they did not possess the typical egg-heat coagulation capacity. The undesirable volume collapse of the lupine cakes was reduced by the use of emulsifiers, with the addition of 4–5% MDG, and hydrocolloids, by adding 0.5–0.6% of xanthan gum, as predicted by the RSM technique.

This work made possible the development of an alternative, egg-free cake, using lupine protein and moderate quantities of baking additives. The cakes produced with this vegetable protein, are very rich (>10%) in the amino acid arginine, and can be useful for children or athletes, who need this supplement, as well as for vegetarians, vegans, and people with high cholesterol levels for other obvious reasons.

The new lupine cakes developed using this RSM method can be further improved and sensory evaluated for target groups or consumer acceptance.

References

1. Lee CC, Johnson LA, Love JA, Johnson S (1991) Cereal Chemistry 68:100–104
2. Lee CC, Love JA, Johnson LA (1993) Cereal Chemistry 70: 18–21
3. Raeker MO, Johnson LA (1995) Cereal Chemistry 72: 299–303
4. Mohamed S, Hamid NS (1998) J Sci Food Agric 76:464–468
5. Miller LL, Setser C (1983) Cereal Chemistry 60:62–65
6. Miller RA, Hoseney RC (1993) Cereal Chemistry 70:585–588
7. Raymundo A, Empis J, Sousa I (1998) Z Lebensm Unters Forsch A 207:91–96
8. Franco JM, Raymundo A, Sousa I, Gallegos C (1998) J Agric Food Chem 46:3109–3115
9. Mettler E, Seibel W (1993) Cereal Chemistry 70:373–376
10. Mettler E, Seibel W (1995) Cereal Chemistry 72:109–114
11. Lee CC, Hoseney RC (1982) Cereal Chemistry 59:392–395