Optimisation of the manufacturing of processed cheese without added fat and reduced sodium

The authors

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Abstract

The main causes of cardiovascular diseases are obesity and hypertension, both of which are associated with high fat intake and high fat and sodium intake, respectively. *Requeijão cremoso* – a type of processed cheese that is an important part of the eating habits of average Brazilians – is a source of fat and salt (sodium chloride), as are most cheeses. In view of the high consumption of this cheese in Brazil, and the current demand for healthier foods, no-fat added reduced-sodium *requeijão* (NFARSR) would be an alternative to meet the needs of the changing market. The objective of this study was to optimise the use of emulsifying salts (JohaS9+JohaB50) so as to reduce the level of sodium of an existing no-fat added *requeijão* formulation developed earlier at the Instituto de Tecnologia de Alimentos-ITAL. In this study, the fat in *requeijão* was replaced by whey protein concentrate (WPC34), and sodium reduction was achieved by partially (40%) substituting potassium chloride for sodium chloride and by replacing part of the traditional sodium phosphate-based emulsifying salt (JohaS9) by a sodium-and-potassium-based emulsifying salt (JohaB50) containing 85% less sodium compared to S9. To this purpose, a 2^2 factorial design with two factors (JohaS9 and JohaB50) and two levels (+1, -1) was used, resulting in 11 experimental trials. The results were evaluated by response surface methodology to assess physical-chemical, sensory and instrumental texture parameters. Analysis of the response surface graphs showed that R5 – made with 1.0% JohaS9 and 1.2% JohaB50 – was the NFARSR formulation that best met the pre-set specifications.

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Cardiovascular diseases are the main cause of death in Brazil and the world and may be directly associated with diets rich in fat and sodium. The consumer of today has a better understanding of the relationship and interactions between diet and health, and important changes have been observed in the consumers' attitudes toward foods with reduced levels of fat and/or sodium. *Requeijão cremoso* is a typical Brazilian dairy product widely consumed throughout the country, which is the reason why it is one of the main targets of research efforts aimed at developing functional foods. However, like most cheeses, *requeijão cremoso* is a source of fat and sodium chloride. A no-fat added reduced-sodium version of *requeijão* would be an alternative to meet the needs of a growing and dynamic consumer market for functional and healthy foods. The fat in *requeijão* can be replaced by whey protein concentrate (WPC34), and the original sodium level can be reduced by partially substituting potassium chloride for sodium chloride and replacing part of the traditional, sodium phosphate-based emulsifying salt by a sodium-and-potassium-based emulsifying salt. The objective of this study was to develop an optimised blend of emulsifying salts that allows a reduction in the sodium content of a no-fat *requeijão* (RZC) developed earlier at Instituto de Tecnologia de Alimentos-ITAL, and to evaluate the physical-chemical, microbiological, instrumental texture and sensory characteristics of the resulting product.

Materials and methods

A total of 11 experimental manufacturing runs (R1 to R11) of no-fat added reduced-sodium *requeijão cremoso* (NFARSR) were performed at Instituto de Tecnologia de Alimentos-ITAL using an adapted version of the method described by Bosi (2008). The main steps of the manufacturing process of NFARSR were: (1) heat-acid coagulation by adding 1:9 water-diluted lactic acid directly to heated milk; (2) holding of the fresh curd for 10 min; (3) draining of the curd; (4) moulding and pressing of the base curd; (5) weighing and milling of the curd; (6) addition of emulsifying salts, sodium chloride and light salt (mixture of sodium chloride and potassium chloride) to the base curd; (7) cold homogenisation of the blend; (8) adding calcium caseinate, water, WPC34, whitening and anti-foaming agents; melting of the blend; hot-fill packing in vacuum-sealed glass containers fitted with an easy-open lid; (9) cooling; and (10) cold storage (4±2°C).

Analyses of the cheeses R1 to R11 manufactured for the purpose of this study were performed 1-3 days after manufacture according to official methods. Physical-chemical analyses were performed according to Instituto Adolfo Lutz (2005) (fat, fat in dry matter-FDM); International Dairy Federation (1964, 1982) (total protein – TP, total dry matter – TDM), Horwitz (2005) (ash) and Horwitz and Latimer Jr. (2006) (sodium – Na). Microbiological analyses were conducted according to the procedures of Bergère and Sivelä (1990) (determination of mesophilic and psychrotrophic anaerobic sporeforming bacteria); Frank and Yousef (2004) (determination of mesophilic and thermophilic aerobic sporeforming bacteria and total yeast and mould count) and Davidson *et al*. (2004) (number of total and thermotolerant coliforms).

Sensory analysis was done by a panel of 51 regular consumers of *requeijão cremoso*, who evaluated the samples for overall acceptance, consistency on the spoon, spreadability and flavour, by assigning scores on a 9-point hedonic scale (9: like extremely; 5: neither like nor dislike, 1: dislike extremely) (Meilgaard 2006).

Texture profile analysis was performed using a Universal Texture Analyser TA-XT2 and the method recommended by Van Dender (2006).

To optimise the combination of Joha S9 and Joha B50 emulsifying salts to reduce the sodium level of a no-fat-added requeijão, a 2^2 factorial design with two factors (JohaS9 and JohaB50) and two levels (+1, -1) was used, resulting in 11 experimental trials (Table 1).

The results were statistically evaluated by Analysis of Variance (ANOVA) and the Tukey test at the 5% level of significance to compare the mean values of the physical-chemical, textural and sensory parameters and by response surface analysis to optimise the formulation with regard to the best emulsifying salt combination (Statistica version 5.5 – StatSoft, 2000).

Results and discussion

Microbiological assessment of requeijão samples

The microbiological evaluation of *requeijão* samples R1 to R11 showed that all may be considered microbiologically stable and safe, mainly because of the absence of psychrotrophic anaerobic sporeforming bacteria and low counts of yeasts and moulds.

Physical-chemical composition of requeijão samples

The results of the physical-chemical composition of *requeijão* samples R1 to R11 are depicted in Table 2.

The fat levels of the *requeijão* samples (R1 to R11) varied from 0.22% to 0.58%. Although no additional fat was added (cream) during processing, the curd contained 0.5% fat. This is explained by the fact the skim milk used in the experiments may contain, according to legislation, up to 0.5% fat (Brasil 2002).

With regard to TDM and TP, the treatments differed significantly from each other, with all samples exhibiting values greater than those obtained by Bosi (2008) for *requeijão* made without the addition of fat (20.07 and 15.12%, respectively). As for the ash levels, significant differences were observed among the *requeijão* samples, which probably reflects the use of different emulsifying salt combinations in the formulations investigated.

Statistical evaluation of physical-chemical, sensory and textural parameters

The best combinations of emulsifying salts Joha S9 and Joha B50 to formulate no-fat added reduced-sodium *requeijão* were selected based on the results of statistical analysis (Tukey test and response surface methodology) obtained for the physical-chemical parameters FDM and sodium, and all sensory and instrumental texture parameters. Table 3 shows the equation models and determination coefficients (R^2) generated for each parameter.

Statistical evaluation of physical-chemical parameters

The most important physical-chemical parameter values to be taken into account in formulating no-fat added reduced-sodium *requeijão* are a low percentage value for fat in dry matter and a minimum reduction of 25% in the sodium level compared to the control sample (no-fat added *requeijão* – RZC).

Table 1: Experimental factorial design at two levels for the independent variables S9 and B50.

Manufacturing run	Code	Coded variables		Actual variables		Sum
		Joha S9	Joha B50	Joha S9	Joha B50	S9 + B50
1	R1	-1	-1	0.8	0.8	1.6
2	R2	-1	0	0.8	1	1.8
3	R3	-1	1	0.8	1.2	2
4	R4	0	-1	1	0.8	1.8
5	R5	0	1	1	1.2	2.2
6	R6	1	-1	1.2	0.8	2
7	R7	1	0	1.2	1	2.2
8	R8	1	1	1.2	1.2	2.4
9	R9	0	0	1	1	2
10	R10	0	0	1	1	2
11	R11	0	0	1	1	2

Table 2: Results of physical-chemical characterisation of no-fat added reduced-sodium *requeijão cremoso* NFARSR 1-3 days after manufacture (R1 to R11).

NFARSR	TDM %	Fat %	FDM %	TP %	Ash %	Sodium mg/100g
R1	23.81 ± 0.08 abc	0.24 ± 0.01 d	1.02 ± 0.05 e	20.15 ± 0.42 a	2.63 ± 0.05 cd	341.33 ± 7.95 c
R2	22.65 ± 0.40 abc	0.38 ± 0.01 c	1.70 ± 0.04 cd	19.37 ± 0.32 a	2.53 ± 0.01 d	333.73 ± 5.96 c
R3	22.08 ± 0.08 bc	0.41 ± 0.00 bc	1.86 ± 0.01 bc	18.96 ± 0.35 ab	2.62 ± 0.08 cd	332.26 ± 17.61 c
R4	23.14 ± 0.05 abc	0.24 ± 0.02 d	1.05 ± 0.09 e	18.97 ± 1.22 ab	2.67 ± 0.08 cd	357.72 ± 6.47 abc
R5	21.94 ± 2.31 c	0.22 ± 0.01 d	1.02 ± 0.14 e	19.23 ± 0.08 ab	2.67 ± 0.03 cd	362.85 ± 10.58 abc
R6	22.21 ± 0.07 abc	0.46 ± 0.01 b	2.08 ± 0.04 b	17.64 ± 0.40 b	2.75 ± 0.02 bc	359.03 ± 4.40 abc
R7	24.34 ± 0.24 a	0.58 ± 0.01 a	2.38 ± 0.04 a	19.04 ± 0.90 ab	2.90 ± 0.02 b	376.03 ± 29.50 ab
R8	24.47 ± 0.10 a	0.25 ± 0.01 d	1.04 ± 0.05 e	19.35 ± 0.40 a	3.13 ± 0.01 a	406.47 ± 8.86 a
R9,R10,R11	24.21 ± 0.11 ab	0.38 ± 0.04 c	1.57 ± 0.17 d	19.89 ± 0.17 b	2.89 ± 0.09 b	378.27 ± 6.65 ab
L.S.D. (5%)	2.26	0.05	0.25	1.67	0.15	31.61

L.S.D.: least significant difference of the Tukey test at the 5% probability level.

The samples (mean ± standard deviation) followed by the same lower case letters within a column do not differ at the 5% level.

The surface analysis of the FDM parameter (Figure 1A) found that the lowest values for this attribute were obtained with the following combinations: concentrations of Joha B50 ranging from 0.75% and 0.8% and Joha S9 concentrations between 0.75% and 1% or Joha B50 concentrations between 1.15% and 1.25% and Joha S9 concentrations varying from 0.95 to 1.15%.

Since the studies conducted by Bosi (2008) show that the sodium level of traditional no-fat added *requeijão* should be approximately 536 mg/100 g sample, in order to achieve a reduction of at least 25%, the no-fat added reduced-sodium *requeijão* samples of this study should exhibit sodium values not greater than 402 mg/100 g. Assuming this value as the maximum allowable level for sodium, analysis of Figure 1B shows that formulations containing between 1.15% and 1.25% Joha B50 and 1.16% to 1.25% Joha S9 are not viable, as is the case of formulation R8 (1.2% B50 and 1.2% S9).

The results depicted in Table 2 attest that formulation R8 could not be considered a reduced-sodium *requeijão*, since it contained 406.47 mg Na/100 g sample. This table also shows that of all the other formulations investigated, the samples with the lowest FDM percentages were R1 (0.8% B50 and 0.8% S9), R4 (0.8% B50 and 1.0% S9) and R5 (1.2% B50 and 1.0% S9).

Statistical evaluation of sensory parameters

The results of statistical evaluation of the sensory data for *requeijão* samples R1 to R11, generated by response surface methodology and Analysis of Variance (ANOVA) and the Tukey test, are depicted in Figure 2 and Table 4, respectively.

The results of response surface analysis for the parameter overall acceptance (Figure 2A) showed that the highest overall acceptance was achieved with the use of combinations of Joha B50 concentrations between 1.05% and 1.25% and Joha S9 concentrations between 0.95% and 1.2%. With regard to the parameters spreadability (Figure 2C) and flavour (Figure 2D) the results were similar to those for overall acceptance.

As for consistency (Figure 2B), higher scores correlate with the use of combinations of 1.05% to 1.25% emulsifying salt B50 and 0.95 to 1.25% emulsifying salt S9.

The results of Table 4 indicate that the *requeijão* samples that achieved the highest scores for overall acceptance and flavour were the following: R5 (1.2% B50 and 1% S9), R8 (1.2% B50 and 1.2% S9) and R9-R10-R11 (1% B50 and 1% S9), the values of which did not statistically significantly differ among each other ($p \leq 0.05$). Also for the attributes consistency and spreadability, most well accepted were once again *requeijão* samples R5 (1% S9 and 1.2% B50), R8 (1.2% S9 and 1.2% B50) and R9-R10-R11 (1% S9 and 1% B50), in addition to R7 (1% B50 and 1.2% S9).

Statistical evaluation of instrumental texture parameters

The results of statistical analysis of the instrumental texture data for *requeijão* samples R1 to R11, achieved by response surface methodology and Analysis of Variance (ANOVA) and the Tukey test, are depicted in Figure 3 and Table 5, respectively.

Instrumental hardness of the *requeijão* samples may be correlated with the sensory attributes consistency (as assessed with a spoon) and spreadability. Thus, extrapolating the usage level range of emulsifying salts Joha B50 and Joha S9 that yielded the best liking scores for consistency (from 1.05% to

Table 3: Equation models and R^2 values obtained for the attributes used to select the best requeijão formulations using combinations of emulsifying salts Joha S9 and Joha B50.

Attribute	Model	R^2
FDM	$-12.5352 - 10.8013xS9 + 11.6437xS9^2 + 38.4497xB50 - 13.4617xB50^2 - 11.7231xS9xB50$	0.9233
Sodium	$191.993 + 278.630xS9 - 259.999xS9^2 - 66.949xB50 - 125.009xB50^2 + 353.208xS9xB50$	0.8538
Overall acceptance	$-12.5022 + 36.5230xS9 - 19.7239xS9^2 - 2.6926xB50 - 1.0965xB50^2 + 5.8824xS9xB50$	0.9440
Consistency	$-28.3169 + 51.5390xS9 - 32.0562xS9^2 + 10.6729xB50 - 12.6935xB50^2 + 17.5245xS9xB50$	0.8560
Spreadability	$-27.4280 + 51.6370xS9 - 28.6249xS9^2 + 11.7350xB50 - 9.5072xB50^2 + 9.1912xS9xB50$	0.8291
Flavour	$-15.0746 + 37.8969xS9 - 18.0212xS9^2 + 0.5603xB50 - 0.1290xB50^2 + 0.6127xS9xB50$	0.9031
Hardness	$1810.47 - 2903.11xS9 + 755.03xS9^2 - 1141.76xB50 - 164.84xB50^2 + 1695.41xS9xB50$	0.8674
Adhesiveness	$-3914.10 + 6966.67xS9 - 1767.18xS9^2 + 1964.99xB50 + 849.68xB50^2 - 4220.87xS9xB50$	0.8832
Cohesiveness	$0.6981 - 0.3175xS9 + 0.5576xS9^2 + 0.5957xB50 + 0.1410xB50^2 - 0.8375xS9xB50$	0.7726
Gumminess	$1481.92 - 2457.81xS9 + 665.19xS9^2 - 854.90xB50 - 169.64xB50^2 + 1377.92xS9xB50$	0.8731

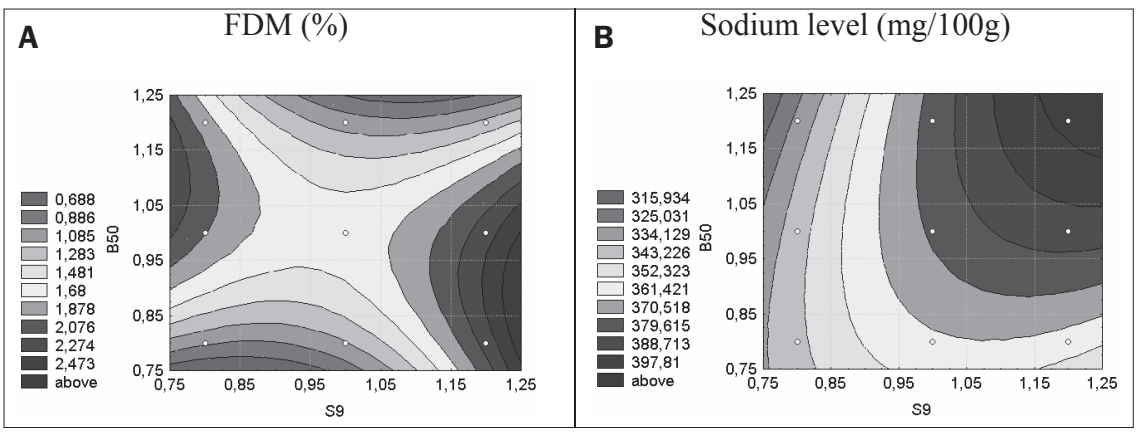

Figure 1: Contour plot of the influence of different combinations of emulsifying salts B50 and S9 used in the manufacture of *requeijão* samples R1 to R11 on the physical-chemical parameters fat in dry matter (FDM) (A) and sodium level (B).

1.25% B50 and from 0.95% to 1.25% S9) and spreadability (1.05% - 1.25% B50 and 0.95% - 1.2% S9) to Figure 3A, allows us to conclude that the ideal hardness values would fall within the 61.562 gf to 259.621 gf range. This range of hardness values lies above that reported by Bosi (2008), who found an ideal hardness value of 35 gf for *requeijão* manufactured without the addition of fat (RZC).

On the other hand, the instrumental texture parameters adhesiveness, cohesiveness and gumminess may be correlated with the sensory attribute spreadability. Extrapolating the usage level range of emulsifying salts Joha B50 and Joha S9 that yielded the highest liking scores for spreadability (1.05% to 1.25% B50 and 0.95% to 1.2% S9) to Figures 3B, 3C and 3D, allows us to conclude that the best values for adhesiveness, cohesiveness and gumminess would fall, respectively, between -107.766 gf.s to -610.815 gf.s; 0.835 to 0.871 and 51.98 to 214.245. Bosi (2008), who studied *requeijão* made without added fat, reported ideal adhesiveness, cohesiveness and gumminess values of -75 gf.s; 0.84 and 29, respectively.

Conclusions

To optimise the formulation of no-fat added reduced-sodium *requeijão* based on physical-chemical, sensory and instrumental texture parameters, the ideal combination of emulsifying salts Joha B50 and Joha S9 would fall within the range of 1.15% to 1.25% Joha B50 and 0.95% to 1.15% Joha S9. Of all combinations tested for the purpose of this study, the formulation *requeijão* R5 (1.2% Joha B50 and 1.0% Joha S9) would provide the best finished product.

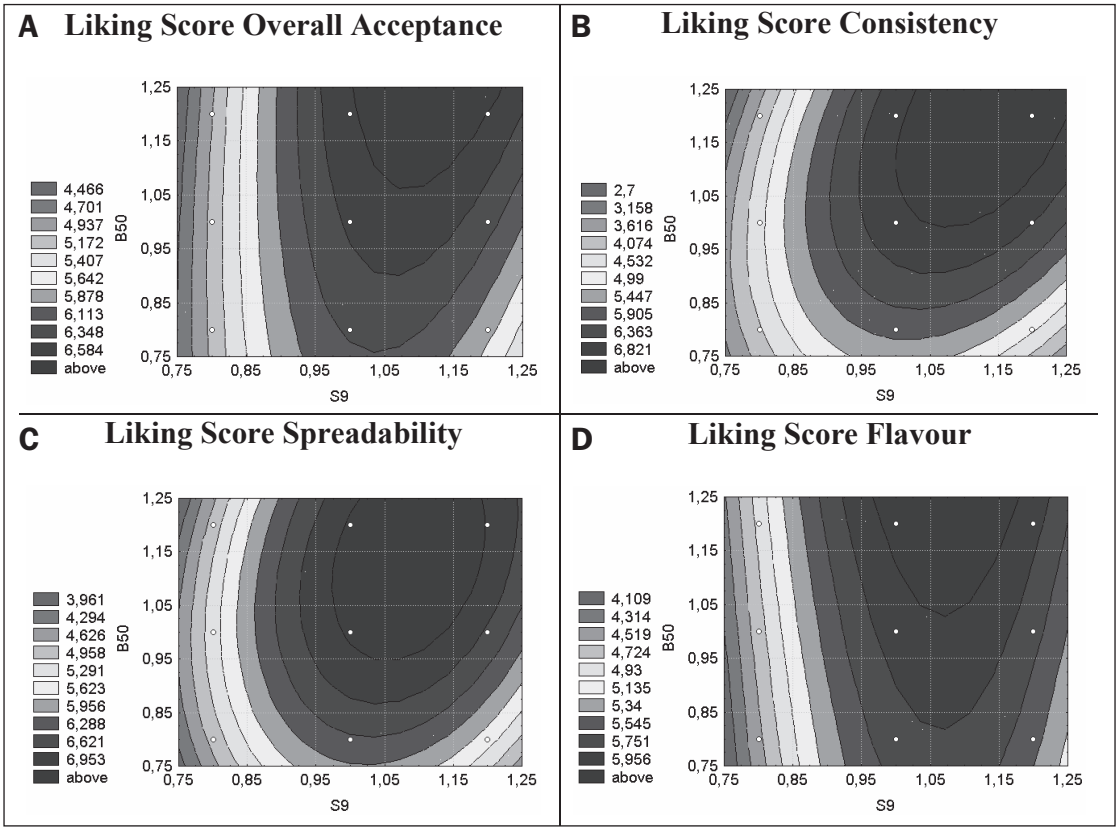

Figure 2: Contour plot of the influence of different combinations of emulsifying salts B50 and S9 used in the manufacture of *requeijão* samples R1 to R11 on the sensory parameters: overall acceptance (A), consistency (B), spreadability (C) and flavour (D).

Table 4: Sensory analysis results for overall acceptance, consistency, spreadability and flavour of the no-fat added reduced-sodium *Requeijão cremoso*-NFARSR (R1 to R11) samples, 1 to 3 days after manufacture, by a panel of 51 consumers.

NFARSR	Overall Acceptance	Consistency (as assessed with a spoon)	Spreadability (spread out with a spatula onto a cracker)	Flavour
R1	5.1 ± 2.0 bcd	4.6 ± 2.2 bc	5.3 ± 2.2 cd	4.5 ± 2.2 b
R2	4.8 ± 2.0 d	3.7 ± 1.8 c	4.5 ± 2.0 d	4.5 ± 2.1 b
R3	5.0 ± 2.2 cd	3.9 ± 2.1 c	4.9 ± 2.0 cd	4.9 ± 2.3 ab
R4	5.9 ±1.5 abc	5.0 ± 2.0 b	5.7 ± 1.9 bc	5.5 ± 2.0 ab
R5	6.5 ± 1.8 a	7.1 ± 1.6 a	7.2 ± 1.4 a	5.7 ± 2.1 a
R6	5.8 ± 1.7 abc	4.6 ± 2.1 bc	5.4 ± 2.1 cd	5.5 ± 2.1 ab
R7	6.0 ± 1.9 ab	6.9 ± 2.0 a	7.0 ± 1.8 a	5.3 ± 2.2 ab
R8	6.6 ± 1.8 a	6.7 ± 2.0 a	6.6 ±1.8 ab	6.0 ± 2.1 a
R9; R10; R11	6.5 ± 1.2 a	6.7 ± 1.0 a	7.1 ± 0.9 a	6.0 ± 1.4 a
L.S.D. (5%)	1.01	1.14	1.06	1.18

L.S.D.: least significant difference of the Tukey test at the 5% probability level. The samples (mean ± standard deviation) followed by the same lower case letter within a column do not differ at the 5% level.

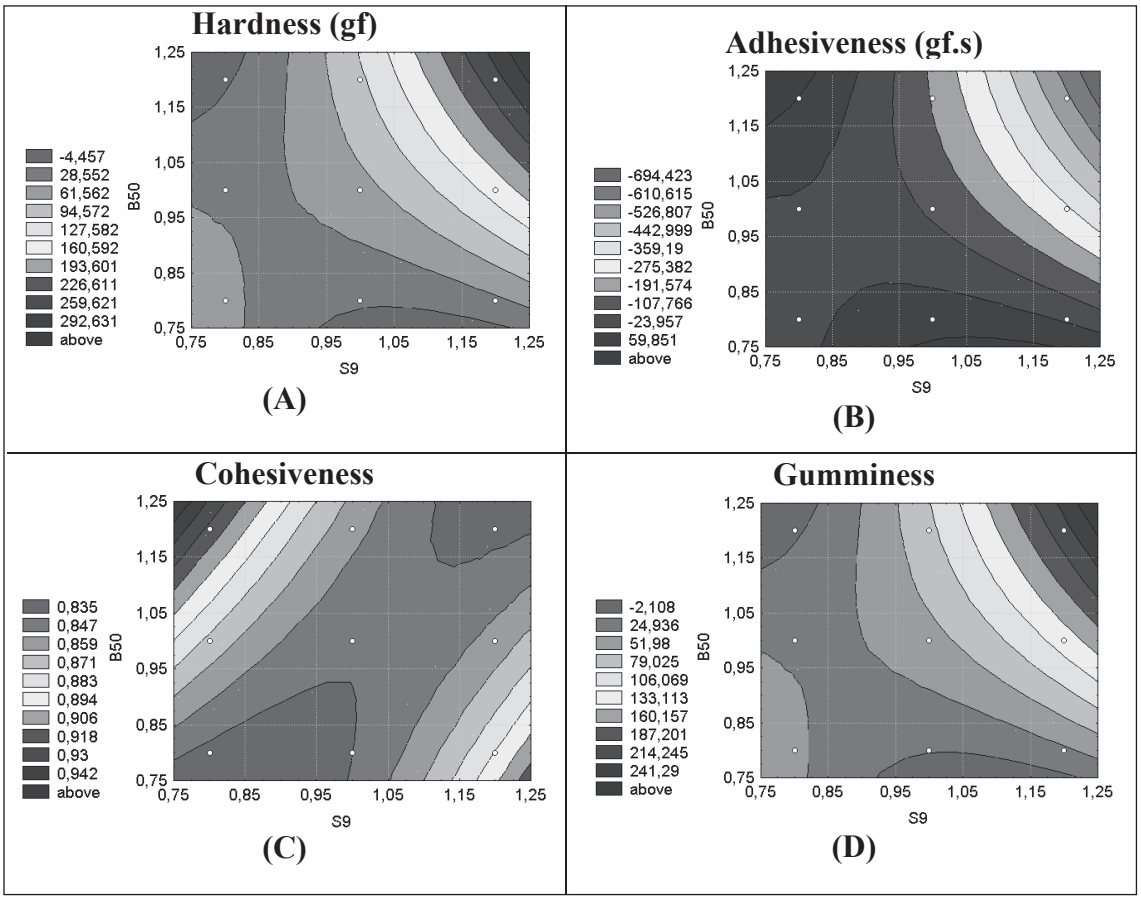

Figure 3: Contour plot of the influence of different combinations of emulsifying salts B50 and S9 used in the manufacture of *requeijão* samples R1 to R11 on the instrumental texture parameters: hardness (A), adhesiveness (B), cohesiveness (C) and gumminess (D).

Table 5: Texture profile analysis results of the samples of no-fat added reduced-sodium requeijão cremoso-NFARSR (R1 to R11), 1-3 days after manufacture.

NFARSR	Hardness (gf)	Adhesiveness (gf.s)	Cohesiveness	Gumminess
R1	20.380 ± 1.310 e	-20.740 ± 4.091 ab	0.830 ± 0.000 b	16.920 ± 1.004 de
R2	15.173 ± 1.502 e	-9.190 ± 0,040 a	0.883 ± 0.015 ab	13.997 ± 0,115 e
R3	14.067 ± 0.719 e	-3.460 ± 0,156 a	0.903 ± 0.006 a	13.090 ± 0.230 e
R4	23.500 ± 3.172 de	-36.913 ±11.330 ab	0.837 ± 0.012 ab	19.670 ± 2.381 de
R5	33.627 ± 0.395 d	- 63,950± 0,400 b	0.873 ± 0.015 ab	27.350 ± 3.954 d
R6	15.980 ± 1.155 e	-10.290 ± 1.340 a	0.883 ± 0.015 ab	14.077 ±0.900 e
R7	115.547 ± 10.032 b	-301.023 ± 31.445 d	0.863 ± 0.006 ab	99.807 ± 8.036 b
R8	280.930 ± 4.074 a	-668.353 ± 44.880 e	0.820 ± 0.020 b	230.717 ± 6.039 a
R9; R10; R11	61.907 ± 1.795 c	-145.077 ± 2.208 c	0.830 ± 0.026 b	50.947 ± 0.579 c
L.S.D.(5%)	11.142	53.565	0.070	10.644

L.S.D.: least significant difference of the Tukey test at the 5% probability level.
The samples (mean ± standard deviation) followed by the same lower case letter within a column do not differ at the 5% level.
** Result expressed as mean média +/- standard deviation.*

Acknowledgments

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