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Modeling of proteolysis and lipolysis in Iranian white brine cheese

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Abstract

The simultaneous effects of processing variables such as ripening time (20–60 days), ripening temperature (6–10 °C), level of rennet added (1–2 g/100 kg milk) and brine concentration (8–14%, w/v) on the proteolysis, lipolysis and sensory score of Iranian white brined cheese (Feta type) were explored by the means of response surface methodology. The most important effect in proteolytical terms was produced by ripening temperature and ripening time in linear form, but level of rennet added and brine concentration were also significant at the 5% level. In terms of lipolysis, ripening time was dominant factor in both linear and quadratic forms; quadratic effect of ripening temperature was greater than its linear effect.

Based on sensory score, optimum conditions were: ripening temperature = 6.7 °C, ripening time = 49.5 days, level of rennet added = 1.4 g/100 kg of milk and brine concentration = 10.9%, w/v.

Keywords: White brined cheese; Modeling; Proteolysis; Lipolysis; Response surface methodology

1. Introduction

In Iran, white brined cheese is a major item in the diet and the consumption per capita per annum is about 5.4 kg. At the industrial level, the ripening time is about 45–90 days (Azarnia, Ehsani, & Mirhadi, 1997). However there are trends to reduce this period due to economical reasons.

White brined cheese, like other types of ripened cheese, requires maturation to develop the required sensory properties. In warm climates it is necessary to preserve cheeses in brine. The specific characteristics of brine cheese develop in the salted water and chemical, physical and sensorial properties of this type of cheese are controlled by processing and environmental conditions (Abd El-salam, 1987; Abou-Donia, 1991; Caric, 1987; Scott, 1986).

White brined cheese manufacturing plants in Iran, work with different levels of processing variables. For example, brine concentration varies from 8% to 16%w/v or ripening temperature varies from 6 to 16 °C and so on. It is evident that in these conditions, the produced cheeses will not have uniform quality.

Response surface methodology (RSM) is an effective tool regularly used for studying the separate and interactive effects of system factors on a desired response variable (Hunter, 1959). RSM is currently the most popular technique in food science for empirical optimization studies, due to its comprehensive theory, reasonably high efficiency and simplicity (Arteaga, Li-Chan, Vazquez, & Nakai, 1994). First or second order polynomials are the most common model functions used to describe the RSM (Khuri & Cornell, 1996).

In this study, we have taken four factors – ripening temperature, ripening time; the level of rennet added and brine concentration – to evaluate their effects on the proteolysis and lipolysis of the Iranian white brined cheese.

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2. Materials and methods

2.1. Cheesemaking

The brine cheese was manufactured for this study according to the method used in Iranian cheese making plants. White brined cheese was prepared from cows' milk. The milk was standardized to a fat content of 2.5%, pasteurized at 72 °C for 15 s and cooled to 32–35 °C. $CaCl_2$ was added at a level of 15 g/100 kg of milk followed by the addition of 1% starter culture (Hansen's Laboratory, Denmark) 30 min before renneting. Cultures of *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* were used as starter. Commercial powdered microbial rennet (Meito, Sangyo Co., Japan) with milk clotting activity of 1 g/100 kg of milk was added at three level of experiment (1, 1.5 and 2 g/100 kg milk) to coagulate milk samples. Following coagulation, the curds were cut and then stirred. The curds were pressed by using weights for 1 h (20 kg weights/30 kg final curd). The curds were then cut to a suitable shape and size and soaked in sterile brine (22%, w/v) for 16 h. The curd pieces were then placed in tins; brines with three different concentrations (8, 11 and 14%, w/v) were added to cover the curds completely and to fill tins. The filled tins were sealed immediately after brining. The sealed tins were stored at three different ripening temperatures (6, 10 and 14 °C) for three different ripening times (20, 40 and 60 days).

2.2. Experimental design

A 3-level-4-factor experimental design with 3 replicates at the center point was used (Box & Behnken, 1960). The 4 factors (processing variables), levels and experimental design in terms of coded and uncoded are given in Table 1.

2.3. Proteolysis and lipolysis evaluation

Water soluble nitrogen (WSN): 20 g of cheese were homogenized with 100 mL distilled water by a Stomacher apparatus for 5 min and left at 40 °C for 1 h, centrifuged ($3000g \times 30$ min, 4 °C) and filtered through Whatman (Whatman International Ltd., Maidstone, England) filter paper No. 42.

Nitrogen soluble in 12% trichloroacetic acid (TCA-SN): 4 mL of 60% TCA solution was added to 16 mL of WSN filtrate. After 1 h the mixture was filtered through Whatman No. 42 filter paper.

Table 1
Box–Behnken design used to evaluate the effects of process variables on sensory score of cheese

Run	Temperature (°C) (X1)		Time (day) (X2)		Rennet added (g/100 kg milk) (X3)		Brine concentration (%w/v) (X4)	
	Uncoded value	Coded value	Uncoded value	Coded value	Uncoded value	Coded Value	UncodedValue	Coded Value
1	6	−1	20	−1	1.5	0	11	0
2	6	−1	40	0	1.0	−1	11	0
3	6	−1	40	0	1.5	0	8	−1
4	6	−1	40	0	1.5	0	14	1
5	6	−1	40	0	2.0	1	11	0
6	6	−1	60	1	1.5	0	11	0
7	10	0	20	−1	1.0	−1	11	0
8	10	0	20	−1	1.5	0	8	−1
9	10	0	20	−1	1.5	0	14	1
10	10	0	20	−1	2.0	1	11	0
11	10	0	40	0	1.0	−1	8	−1
12	10	0	40	0	1.0	−1	14	1
13	10	0	40	0	1.5	0	11	0
14	10	0	40	0	1.5	0	11	0
15	10	0	40	0	1.5	0	11	0
16	10	0	40	0	2.0	1	8	−1
17	10	0	40	0	2.0	1	14	1
18	10	0	60	1	1.0	−1	11	0
19	10	0	60	1	1.5	0	8	−1
20	10	0	60	1	1.5	0	14	1
21	10	0	60	1	2.0	1	11	0
22	14	1	20	−1	1.5	0	11	0
23	14	1	40	0	1.0	−1	11	0
24	14	1	40	0	1.5	0	8	−1
25	14	1	40	0	1.5	0	14	1
26	14	1	40	0	2.0	1	11	0
27	14	1	60	1	1.5	0	11	0

Determination of the N content: total nitrogen (TN) and the N content of the nitrogenous fraction were determined by the Kjeldahl method (IDF, 1993).

Fat extraction from cheese samples was carried out using diethyl ether and the acidity index of the fat (meq/100 g of fat) was calculated from ethanolic titration (Nunez, Garcia-Aser, Rodriguez-Martin, Medina, & Gaya, 1986).

Sensory evaluations of cheese samples were obtained for flavor, body and texture, odor and appearance by Iran's standard-2344-1 for white white brined cheese. In this sensory system, the product is graded on a 25 point scale as follows: 10 points maximum for flavor, 5 points maximum for body and texture, 5 points maximum for odor and 5 points maximum for appearance.

Sensory evaluation of cheese samples was performed by five trained panelists according to a scoring card (Bodyfelt, Tobias, & Trout, 1988).

In this study, sensory score refers to the total score of each sample and it was used in statistical analysis and modeling.

2.4. Data analysis

The statistical analysis of the data was performed using the MINITAB Statistical Software, Release 13.1. Initially, the full term second order polynomial response surface models were fitted to each of the response variables, according to the following equation:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \beta_{ii} x_{ii}^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} x_i x_j,$$

where Y is response; $\beta_0, \beta_i, \beta_{ii}$ and β_{ij} are constant coefficients and x_i the coded independent variables.

The analysis was performed using coded units. Where possible, stepwise deletion of terms was applied to remove the statistically non-significant terms, so simplifying the model. However, when the exclusion of such terms from the model decreases R^2 (adjusted) and increases the estimator of the variance S, the term was included in the model. The statistically non-significant linear terms also remained in the model when the respective quadratic or interactive effects were statistically significant. Based on such considerations, besides the significant terms (at 0.05 level), statistically non-significant terms may also be included in the final models. The results are means of two replicates. All generated models adequately explain the variation of the responses with satisfactory R^2 values ($R^2 > 0.80$) and non-significant lack-of-fit. The models for WSN, TCA-SN, FFA and sensory score explained 98%, 92.4%, 80.1% and 84.2% of the variability, respectively, and can be considered adequate, since the probability level of F was $P < 0.001$ (Thompson, 1982).

SAS software (Version 6.12, SAS Institute.Inc.) was used to compute the estimated ridges of maximum and minimum response for increasing radii from the center of the original design.

Pareto charts were created using Statistica version 6.0 (Statsoft, Tulsa, OK, USA).

3. Results and discussion

3.1. General

Table 2 summarizes the estimated regression coefficients of the quadratic polynomial models for the response variables, along with the corresponding R^2 and F values.

Linear, quadratic and interaction effects can be represented graphically on a standardized pareto chart. The length of each bar on a standardized pareto chart is proportional to the absolute value of its associated regression coefficient or estimated effect. In this chart, the effects are standardized (each effect is divided by its standard error). The order in which the bars are displayed corresponds to the order of the size of the effects, with the strongest effect on the top, which allows the most important effects to be identified. The chart includes a vertical line, which corresponds to the 95% confidence limit indicating statistical significance. An effect is therefore significant if it crosses this vertical line.

3.2. Proteolysis

WSN/TN% and TCA-SN/TN% were used in this study as indices of proteolysis. As shown in Fig. 1, linear effects of ripening temperature and time on the production of WSN and TCA-SN were more significant and these two factors had positive effect on proteolysis but, quadratic effects of these factors were not significant ($P < 0.05$). On the other hand, brine concentration had the most negative effect on WSN and TCA-SN formation. It is well known that high concentration of NaCl in cheese inhibits proteolysis (Guinee & Fox, 1993).

While rennet content linearly affected production of WSN and TCA-SN, but its quadratic effect and interactive effect with ripening temperature were not significant ($P < 0.05$) on the production of TCA-SN (Fig. 3). It may be concluded that the effect of rennet content on the formation of small size peptides was relatively weak. In accordance with this conclusion, various studies have indicated that unlike WSN, the formation of small size peptides did not depend greatly on the level of rennet added. (Fox, Law, & McSweeney, 1993; Martin G. Wilkinson, 1993).

The levels of independent variables that maximize and minimize formation of WSN and TCA-SN were determined using ridge analysis. The method of ridge

Table 2
Regression coefficients of the second-order polynomial model for the response variables (analysis has been performed using coded units)

Factors[a]	WSN/TN%	TCA-SN/TN%	FFA (meq/100 g fat)	Sensory score
Constant	16.469	10.298	9.897	19.663
Te	7.261 *	3.717 *	0.492 **	−3.197
Ti	6.561 *	3.455 *	1.116 *	−2.352
Re	3.875 *	2.080 *	−0.540 **	−0.338
Br	−4.53 *	−2.336 *	−0.059	−0.463
Te^2	–	–	−1.071*	−1.126
Ti^2	–	–	−0.914 **	−1.095
Re^2	2.359 *	1.091 ***	−0.745 **	−2.134
Br^2	3.661 *	1.760 **	−0.498	−2.333
Te*Ti	5.069 *	2.595 *	0.601	−5.509
Te*Re	2.257 **	1.201	−0.598	–
Te*Br	−4.138 *	−2.086 **	−0.401	–
Ti*Re	1.187	–	−0.751 ***	−3.962
Ti*Br	1.285	–	0.383	–
Re*Br	1.186	–	−0.301	–
R^2	0.98	0.924	0.801	0.842
F	104.73	36.35	8.46	14.81
Probability of F	$P \leqslant 0.0001$	$P \leqslant 0.0001$	$P \leqslant 0.0001$	$P \leqslant 0.0001$

[a] Factors: Te, ripening temperature (°C); Ti, ripening time (days); Re, rennet added (g/100 kg milk); Br, brine concentration (%W/V).
* $P \leqslant 0.001$.
** $P \leqslant 0.01$.
*** $P \leqslant 0.05$.

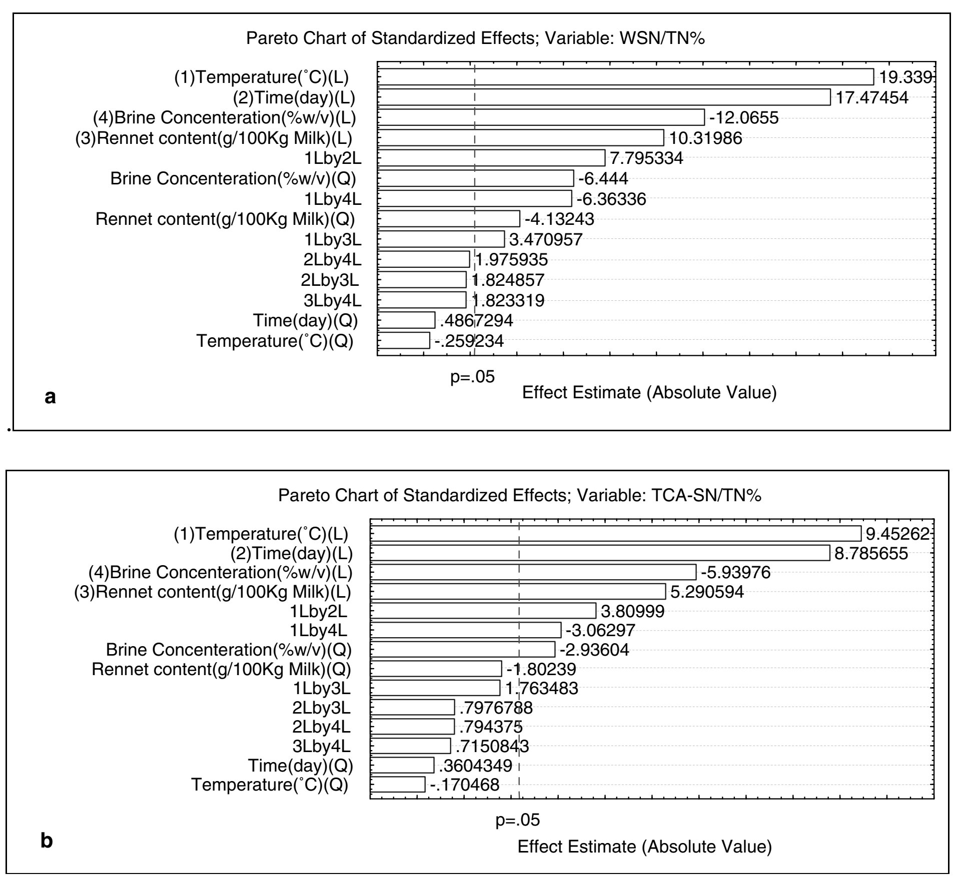

Fig. 1. Pareto chart showing the significance of processing variables on: (a) WSN/TN% and (b) TCA-SN/TN%. L and Q represent linear and quadratic effect of variable, respectively.

analysis computes the estimated ridge of optimum response for increasing radii from the center of the original design (Chwen-Jen, Koehler, & Akoh, 1996).

The ridge analysis (Table 3) indicated that maximum WSN/TN% (32.109 ± 0.68) would be at ripening temperature = 12.6 °C, ripening time = 49.4 days, level of rennet added = 1.7 g/100 kg of milk and brine concentration = 9.5%, w/v at the distance of coded radius 1.0.

As shown in Table 3, minimum of proteolysis would occur at ripening temperature = 9.72 °C, ripening time = 22.2 days, level of rennet added = 1.4 g/100 kg of milk and brine concentration = 12.1%, w/v at the distance of coded radius 1.0.

3.3. Lipolysis

Acidity index of the fat, expressed as meq/100 g of fat, was used to evaluate lipolysis in the cheese samples. Pareto chart of standardized effects of various factors on the total amount of FFAs is shown in Fig. 2.

As shown in Fig. 2, ripening time was a major factor in the total amount of FFAs and affected lipolysis both linearly and quadratically. The strong positive effect of ripening time on lipolysis is well known and has been confirmed by other investigations (Cha'varri et al., 1999; Virto et al., 2003).

Quadratic effect of the ripening temperature was more than its linear effect. Brine concentration had no significant effect on lipolysis ($P < 0.05$).

The effect of rennet content on lipolysis was rather complex as it exerts curvilinear and interactive effects on lipolysis and the direction of its linear effect was in contrast with its quadratic effect (Fig. 2).

The ridge analysis (Table 3) indicated that maximum FFA (10.9 ± 0.29 meq/100 g of fat) would be at ripening temperature = 11.6 °C, ripening time = 53.4 days, level of rennet added = 1.2 g/100 kg of milk and brine concentration = 11.4%, w/v at the distance of coded radius 1.0.

3.4. Sensory evaluation

Significance of processing variables on sensory score is shown in a Pareto chart (Fig. 3). This chart shows that temperature and interaction of time and temperature are the most effective factors on sensory score.

The linear effect of brine concentration and rennet added is not significant ($P < 0.05$), but their quadratic effect is significant (Fig. 3). Also, Fig. 3 shows that compared to other factors, brine concentration in studied range (8–14%, w/v) had the least significant effect on sensory score.

The relationship between factors and response can best be understood by examining the series of contour plots generated by holding constant two factors and plotting response as a function of two other factors.

Temperature and level of rennet added had significant and determinant effect on sensorial quality of brine cheese (Fig. 4). As Shown in Fig. 4(a), when temperature and rennet added were at low level, the sensory score of cheese samples increased by increasing ripening time. By contrast when temperature and rennet added were at their high level, sensory score of cheese samples decreased by increasing ripening time (Fig. 4(c)) and most of the cheese samples ripened in this condition have bitter taste. It is known that high level of rennet added

Table 3
Estimated ridge of maximum and minimum value for the different responses

Response	Te	Ti	Ti	Re	Br	Minimum	Maximum
WSN/TN%	9.72	22.2	22.2	1.4	12.1	8.166±0.68	
	12.6	49.4	49.4	1.7	9.5		32.109±0.68
TCA-SN/TN%	9.7	22.2	22.2	1.4	12.1	5.91±0.71	
	12.6	49.5	49.5	1.7	9.5		18.41±0.71
FFA(meq/100g of fat)	10.6	20.8	20.8	1.5	11.7	7.8±0.29	
	11.6	53.4	53.4	1.2	11.4		10.9±0.29
Sensory score	12.6	53.6	53.6	1.7	11.5	11.3±0.94	
	6.7	49.5	49.5	1.4	10.9		23±0.94

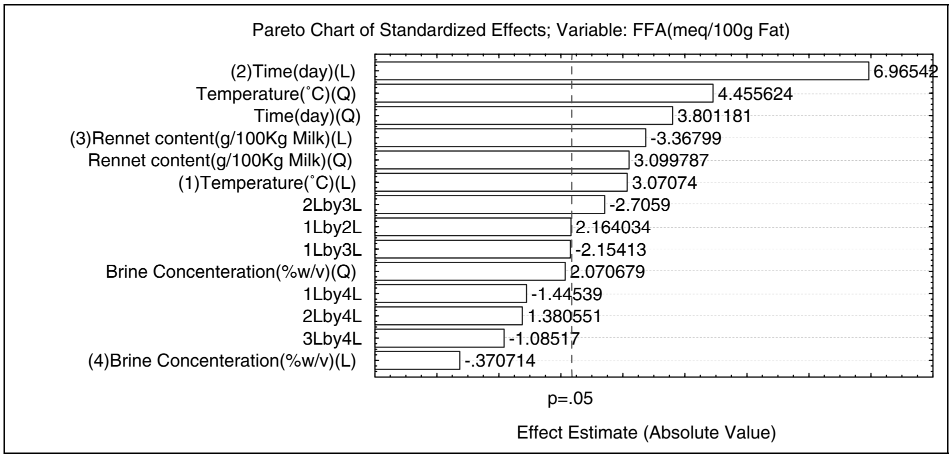

Fig. 2. Pareto chart showing the significance of processing variables on FFA (meq/100 kg of fat).

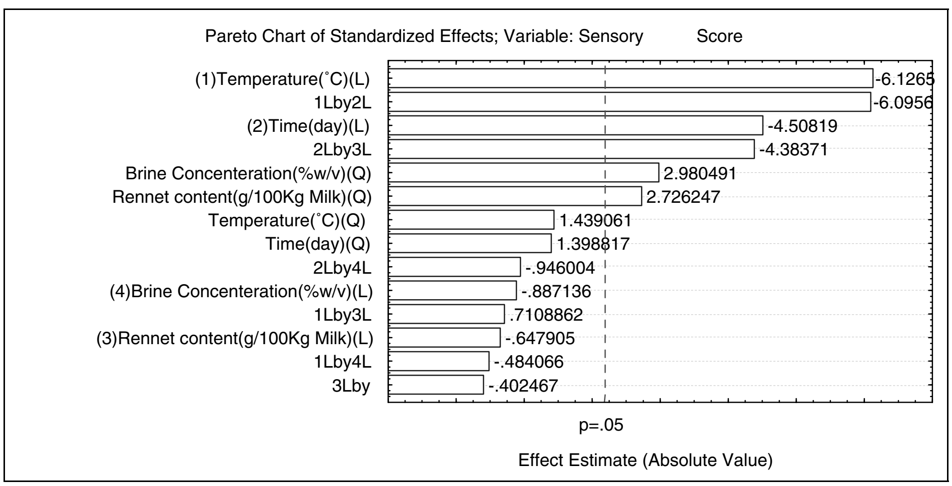

Fig. 3. Pareto chart showing the significance of processing variables on sensory score.

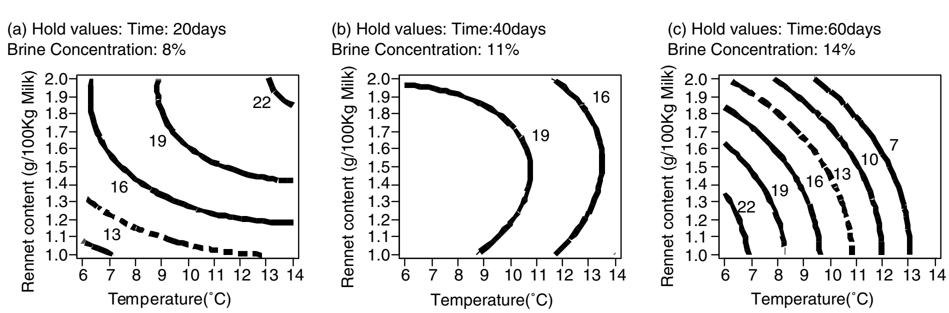

Fig. 4. Contour plots showing the effect of ripening temperature and rennet content on sensory score under constant brine concentration and ripening time. The numbers inside the contours represent sensory scores of cheese samples from maximum score of 25.

leads to accumulation of bitter peptides in cheese (Frister, Michaelis, Schwerdtfeger, Folkenberg, & Sorensen, 2000; Visser, 1977; Lemieux & Simard, 1991).

The level of rennet added and brine concentration at their high level had negative effect on sensory score of cheese (Fig. 5). This can be attributed to intensive proteolysis in cheese samples that originates from high levels of rennet added and NaCl. It is shown that NaCl content of cheese (up to 6%) has a positive effect on activity of proteolytic enzymes in cheese (Guinee & Fox, 1993; Ustnol & Zeckler, 1996; Visser, 1977).

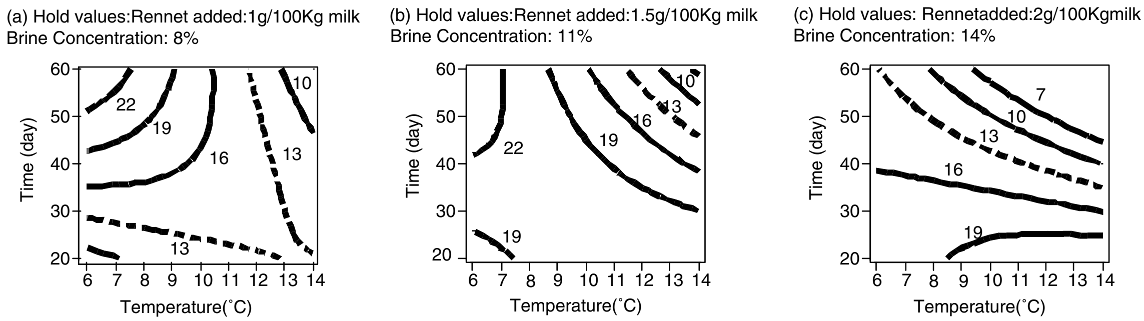

Fig. 5. Contour plots showing the effect of ripening time and temperature on sensory score under constant rennet content and brine concentration.

Maximum sensory score in the experimental region was determined by ridge analysis (Draper, 1963; Myers, 1971). As shown in Table 3, the maximum sensory score (23 ± 0.94) would be at ripening temperature = 6.7 °C, ripening time = 49.5 days, level of rennet added = 1.4 g/100 kg of milk and brine concentration = 10.9%, w/v at the distance of coded radius 1.0.

4. Conclusion

Results showed that four processing variables (i.e., ripening time, ripening temperature, level of rennet added and brine concentration) affected proteolysis, lipolysis and sensory score quit differently. In all studied responses, the ripening temperature and ripening time were pronounced more significant than other two factors (i.e., level of added rennet and brine concentration).

Since maximum sensory score was final goal of our study, the processing variables (i.e., ripening temperature = 6.7 °C, ripening time = 49.5, level of added rennet = 1.4 g/100 kg of milk brine concentration = 10.9%) that brought about the cheese samples with maximum sensory score were considered as optimum. By replacing the above factor values (in the coded form) in the mathematical models of the three other responses (Table 2), the values of WSN/TN%, TCA-SN/TN% and FFA (meq/100 g of fat) in the cheese samples with high sensory scores were predicted to be 10.71%, 7.39% and 8.86 (meq/100 g of fat), respectively.

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