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Minimization of Sodium in Iranian White Brined Cheese

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A chemometric approach was used to minimize NaCl content in Iranian white brine cheese. Influence of different ratios of NaCl:KCl and ripening time on sodium and potassium content, proteolysis, lipolysis, dry matter, TVC, mold count and sensorial quality was explored using a mixture design. Partial substitution of NaCl by KCl did not influence the extent of proteolysis, lipolysis and dry matter. Sodium content of the cheese samples decreased by increasing KCl concentration in brine. Ripening time had positive effect on both Na and K content of the cheese samples. Increasing in KCl concentration in brine had significantly negative effect on TVC and mold. All cheeses were free from Coliform. NaCl and KCl concentrations in brine were effective factors on sensory score. After modelling of Na and K variations and regarding sensory score and dry matter the optimum condition for minimization of Na in Iranian white brine cheese were: concentration of KCl and NaCl in brine were respectively 27.1 and 72.9 % and ripening time of 40 d. Na and K contents in the cheese samples made at optimum condition predicted to be 2.43 and 1.32 %, respectively. KCl at concentrations more than 27.7 % led to significantly low acceptability.

Key Words: NaCl, KCl, Minimization, Mixture design.

INTRODUCTION

Although salt is a flavour enhancer and effective traditional preservative, high sodium intakes can also bring health risks[1-3]. As a result of the association of sodium intake primarily with hypertension[4-7], but also with osteoporosis[8] and the incidence of kidney stones[1], the consumer's concern about sodium in processed foods has increased[9]. The most frequent estimate of the minimum adult daily requirement for sodium is 200 mg (0.5 g of NaCl), while the average total daily sodium intake by most persons in developed countries is 4-5 g (10-12 g of NaCl)[7,10]. These quantities, which are 10-35 times greater than the minimum adult requirement[11,12], are regarded as excessive, even dangerous, by many of those responsible for public health[7]. A sodium intake of 1100-3300 mg (2.8-8.3g of NaCl) per day has been recommended as safe and adequate for adults[13].

Various studies have indicated that an increased intake of potassium *via* the diet can exert a protective effect in individuals with sodium-induced hypertension[14-17], reduces urinary calcium excretion and potentially protects skeletal mass[18].

Some dairy foods such as natural and processed cheeses are high in sodium content and thus means to reduce this have been sought by the dairy industry and scientific community[19]. If the salt content in cheese is simply reduced, proteolysis, acidity, water activity and bitterness all increase, but firmness and saltiness decrease[20]; abnormal fermentations may also occur[21,22]. All of these factors make it difficult to reduce the sodium level in cheese substantially without adversely affecting quality. However, replacing some of the NaCl by KCl helps to address some of the above difficulties[20]. KCl has been the most widely and successfully used partial replacement for NaCl in cheese[19]. Rapacci *et al.*[23] reported that the use of 70 % NaCl + 30 % KCl did not change the intrinsic characteristics of prato cheese.

In Iran white brined cheese is a major item in the diet and the consumption per capita per annum is about 5.4 kg. 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 cheese 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[24].

The present objective in this study was minimization of NaCl in Iran white brined cheese by partial substitution of NaCl by KCl and evaluation of its effects on proteolysis, lipolysis and sensorial quality and dry matter of pickled cheese.

EXPERIMENTAL

Cheese manufacture: Fresh raw milk obtained from Animal Husbandry of Urmia University, Iran was bath-pasteurized at 65 for 5 min in a stainless steel container placed in a water bath, cooled to 35 and transported carefully to a cheese vat (FT20-MKII CHEESE VAT, Armfield Ltd. , Ringwood, Hampshire, UK). $CaCl_2$ was added at a level of 15 g/100 kg of milk followed by addition of 1 % starter culture 0.5 h before renneting. A lyophilized direct-to-vat mesophilic mixed culture (R-704, Chr, Hansens Dairy Cultures, Denmark) containing *Lactococcus lactic* subsp.cremoris and *Lactococcus lactic* subsp.lactis was used as starter. As coagulant, chymosin derived by fermentation of *Aspergillus niger* var.awamori was used[25] ([standard rennet] ChyMax, Chr, Hansen Inc., Denmark: 183 International Milk Clotting Units (IMCU)/mL). Rennet was diluted 30-fold with cold water then added to each 7 kg batch of milk. After 55 min, the curd was cut crossways in cubes of 2 cm^3. After being cut, the curd was allowed to settle for 3-5 min and then gently agitated at a gradually increasing rate for 10 min to avoid fusion of freshly cut curd cubes and facilitate whey expulsion. This was followed by whey draining and pressing the transferred curd into molds (25 × 12 × 10) for 2.5 h (under the initial pressure of 0.3 KPa at the first hour and held constant to the end of pressing) to complete draining, 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 containers; brines

with five different mixtures of NaCl/KCl (92.5:7.5, 77.5:22.5, 70:30, 62.5:37.5 and 50:50) were added to cover the curds completely and to fill tins, the concentration of brines was 13 % w/v. Brine was beforehand pasteurized at 80 for 10 min. After brining, the filled tins were sealed immediately and refrigerated at 5-6 for three different ripening times (20, 40 and 60 d).

Compositional analysis: Cheese was analyzed for moisture content by heating to a constant weight by the method of the Association of Official Analytical Chemists[26].

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[27].

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 for 1 h, centrifuged (3000 g @ 0.5 h, 4) and filtered through Whatmann 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 Whatmann no. 42 filter paper.

Determination of the N content: Total nitrogen (TN) and the N content of the nitrogenous fraction were determined by the Kjeldahl method[28].

Sensory evaluation was carried out with a trained panel of 10 judges. Attributes evaluated were bitterness and total score of each sample. Evaluations were made using a 150 mm line scale anchored by the appropriate references.

Determination of Na and K content: 2 g of cheese were homogenized with 500 mL distilled water by a Stomacher apparatus for 15 min, then filtered through Whatmann filter paper No. 42. Na and K content of above solution determined by Flame photometer (Coring Flame photometer 410).

Microbial analysis: Methods used to determine the total viable counts (TVC) of cheese samples were as described by IDF[29] and Coliform bacteria were enumerated in cheeses using the method described by IDF[30]. The yeast and mould counts were determined according to IDF[31].

Experimental design: A D-optimal combined design (Table-1) was selected to determine experimental points and to investigate interactions between mixture components (KCl and NaCl), as well as their interactions with ripening time.

A combined design is a combination of mixture components and process factors. The design consists of 28 experiments including 5 replications for estimation of pure error and 9 experiments for the lack-of-fit test. Second order Scheffe polynomials were fitted to the data allowing response prediction in the experimental region.

Statistical analysis: Experimental data were analyzed by regression analysis (Proc Reg) procedure[32] to fit the following Scheffe quadratic polynomial model:

$$Y = \beta_1X_1 + \beta_2X_2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{123}X_1X_2X_3 + \beta_{133}X_1X_3^2 + \beta_{233}X_2X_3^2 + \beta_{1233}X_1X_2X_3^2$$

TABLE-1
EXPERIMENTAL DESIGN MATRIX USED FOR MINIMIZATION OF NaCl IN IRANIAN WHITE BRINE CHEESE

Run	Block	KCl (%)	NaCl (%)	Time (d)	Run	Block	KCl (%)	NaCl (%)	Time (d)
1	Block 1	30.0	70.0	40	15	Block 4	15.0	85.0	60
2	Block 1	30.0	70.0	60	16	Block 4	22.5	77.5	20
3	Block 1	7.5	92.5	60	17	Block 5	22.5	77.5	60
4	Block 1	7.5	92.5	40	18	Block 5	22.5	77.5	60
5	Block 2	15.0	85.0	40	19	Block 5	0.0	100.0	20
6	Block 2	15.0	85.0	40	20	Block 5	0.0	100.0	60
7	Block 2	0.0	100.0	20	21	Block 6	50.0	50.0	40
8	Block 2	15.0	85.0	20	22	Block 6	37.5	62.5	20
9	Block 3	0.0	100.0	60	23	Block 6	50.0	50.0	40
10	Block 3	7.5	92.5	20	24	Block 6	37.5	62.5	60
11	Block 3	30.0	70.0	20	25	Block 7	22.5	77.5	40
12	Block 3	7.5	92.5	20	26	Block 7	37.5	62.5	20
13	Block 4	15.0	85.0	60	27	Block 7	37.5	62.5	40
14	Block 4	0.0	100.0	20	28	Block 7	50.0	50.0	60

where Y = a dependent variable, β_1, β_2, β_{12}, β_{13}, β_{23}, β_{1233} = the corresponding parameter estimates for each linear and crossproduct term produced for the prediction models, X_1 = KCl, X_2 = NaCl, X_3 = ripening time. The intercept and quadratic terms of mixture components were removed from the models[33]. The intercept was not included in the analysis because the mixture components should equal 100 % of the mixture. Selection of variables was systematically performed based on the p-values to provide reduced models which were used to generate constrained contour plots[32].

RESULTS AND DISCUSSION

Regression analysis was performed to fit the different response variables to the Scheffe quadratic model. Table-2 summarizes the estimated regression coefficients of the models for the response variables, along with the corresponding R^2 and F values. The response surfaces (Figs. 1-8) are presented to aid in visualizing the effects of variables.

Dry matter: The dry matter content increased during ripening time about 41.17 %. NaCl: KCl different ratios did not influence on dry matter. When cheese is placed in brine, a dynamic mutual diffusion process is established as NaCl molecules move from the brine into the cheese; while, water diffuses out through the cheese matrix[33]. It decreases the moisture content of cheese and increases the salt content of cheese as it is ripened[34-36]. Ripening time was a major factor in the dry matter changing. NaCl:KCl ratio in brine did not influence the DM levels of cheese throughout the ripening time (20-60 days) (Fig. 1).

TABLE-2
REGRESSION EQUATION COEFFICIENTS FOR THE RESPONSE FUNCTIONS IN THE ACTUAL LEVEL OF VARIABLES

Factor	DM (%)	Sqrt(FFA/Tfat)	log (NPN)	1/Sensory score
Constant	37.48369	1.88467	0.83278	–
T	0.092160	0.012403	4.35518E-003	–
K_c	–	–	–	5.48678E-003
N	–	–	–	1.81050E-004
K_c*N	–	–	–	-9.42204E-005
K_c*T	–	–	–	–
K_c*N*(K_c-N)	–	–	–	-5.13256E-007
N*T	–	–	–	–
K_c*N*T	–	–	–	–
K_c*T^2	–	–	–	–
N*T^2	–	–	–	–
K_c*N*T^2	–	–	–	–
K_c*N*T^2*(K_c-N)	–	–	–	–
K_c*N*T*(K_c-N)				
Factor	Na^+ (%)	K^+ (%)	$\log_{10}$ (TC)	$\log_{10}$ (MC)
Constant	–	–	–	–
T	0.15912	–	–	–
K_c	-1.36127	0.24309	-0.053659	0.22734
N	0.073505	1.28036E-003	-0.025708	2.04526E-003
K_c*N	0.025526	-3.79652E-003	–	-4.92943E-003
K_c*T	0.096153	-8.78743E-004	2.99011E-003	-9.12047E-003
K_c*N*(K_c-N)	1.76807E-006	-1.7379E-005	–	–
N*T	-3.18249E-003	2.91365E-005	2.45203E-003	5.95759E-004
K_c*N*T	-1.87563E-003	1.79320E-003	–	2.06771E-004
K_c*T^2	-1.27553E-003	–	-2.80141E-005	9.11693E-005
N*T^2	2.40946E-005	–	-2.43868E-005	-5.06634E-006
K_c*N*T^2	2.46346E-005	–	–	-2.03939E-006
K_c*N*T^2*(K_c-N)	1.64231E-009		–	–
K_c*N*T*(K_c-N)	-1.24099E-007			

Aly[37] and Katsiari *et al.*[38] have reported that moisture content of UF white-brined cheese and traditional feta cheese decreased during the storage time, respectively, although in this case this would be primarily due to loss into the brine.

Lipolysis: For evaluation of lipolysis in the cheese samples, acidity index of the fat, expressed as meq/100 g of fat was used.

As shown in Fig. 2, ripening time was a major factor in the total amount of FFAs. FFAs at the end of ripening time increased to 6.75 meq/100 g of fat. Other investigations have confirmed the strong positive effect of ripening time on lipolysis[39,40].

The ratio of NaCl:KCl didn't have any significance effect on lipolysis during ripening time.

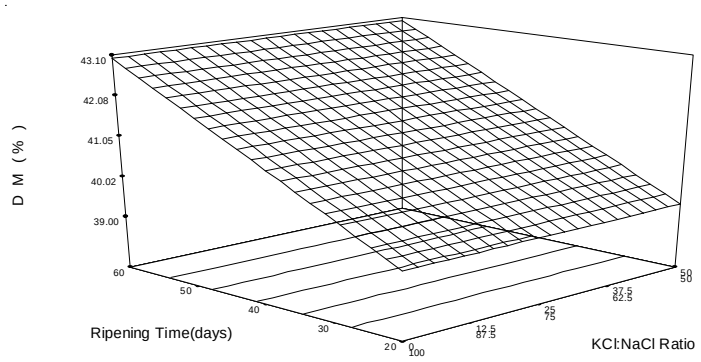

Fig. 1. Influence of ripening time and NaCl:KCl ratio in brine on dry matter

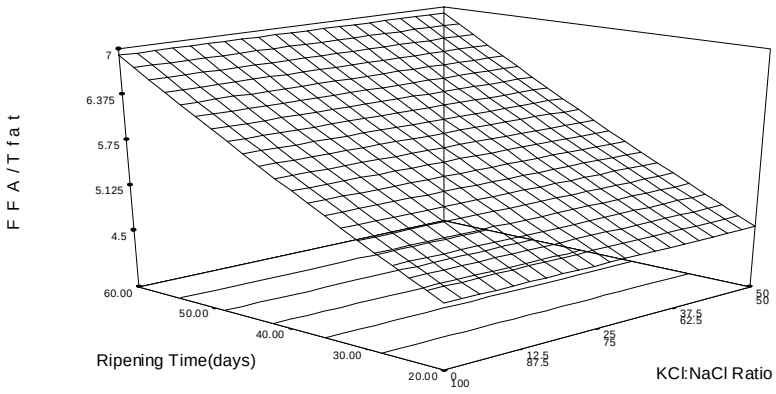

Fig. 2. Influence of ripening time and NaCl:KCl ratio in brine on FFA/Tfat

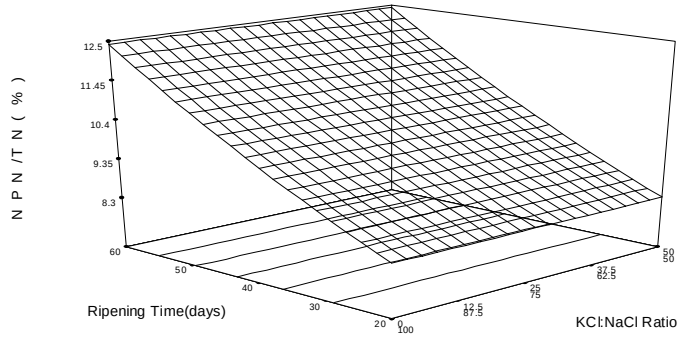

Fig. 3. Influence of ripening time and NaCl:KCl ratio in brine on NPN/TN

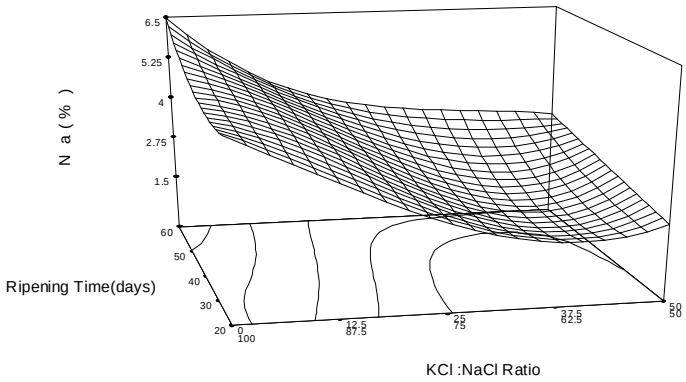

Fig. 4. Influence of ripening time and NaCl:KCl ratio in brine on Na content

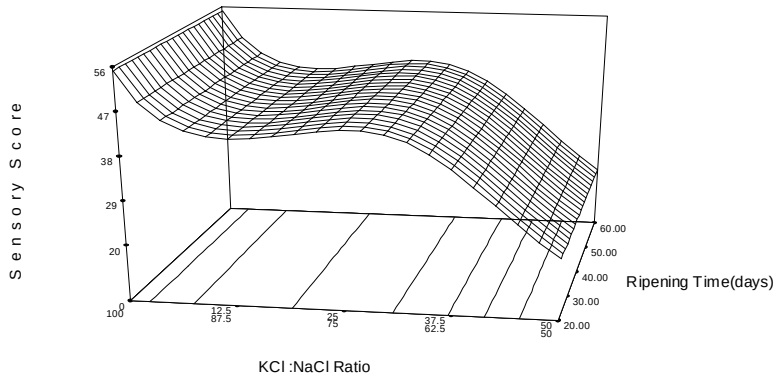

Fig. 5. Influence of ripening time and NaCl:KCl ratio in brine on K content

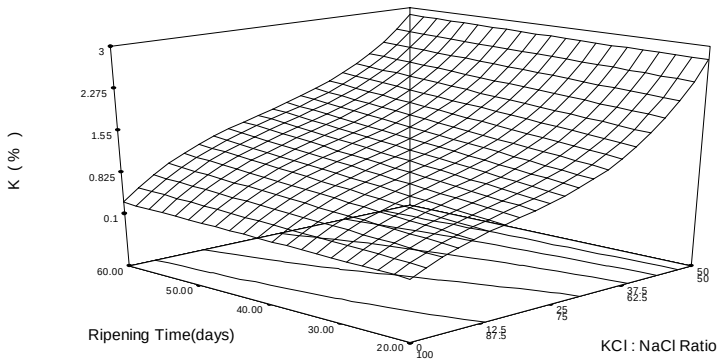

Fig. 6. Influence of ripening time and NaCl:KCl ratio in brine on sensory score

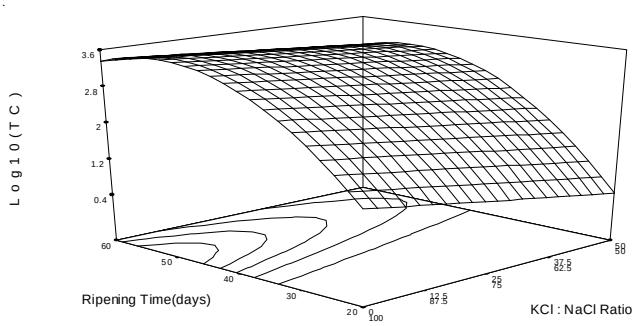

Fig. 7. Influence of ripening time and NaCl:KCl ratio in brine on total viable count (TVC)

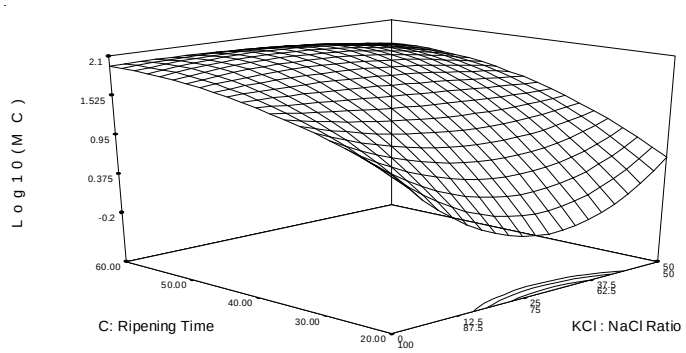

Fig. 8. Influence of ripening time and NaCl:KCl ratio in brine on mold count

Proteolysis: In this study, TCA-SN/TN % was used as index of proteolysis. As shown in Fig. 3 linear effect of ripening time on the production of TCA-SN was more significant. NaCl and KCl concentration in brine had no significant effect on the production of TCA-SN. Throughout this study, the levels of TCA-SN in all cheeses at a identical ripening times were similar ($p < 0.05$). These results are in agreement with the results of Reddy and Marth[9], who reported no significant ($p < 0.05$) difference in the TCA-SN values at a given sampling time among Cheddar cheeses made with NaCl, KCl or mixture of the two salts. Iwanczak *et al.*[41] also found that partial substitution (1:1) of NaCl by KCl in the salting of Camembert, Camping, Tilsit and Gouda-type cheeses did not affect their proteolysis and specifically the levels of pH 4.6 soluble nitrogen, non-protein nitrogen and peptide nitrogen.

Na and K content of cheese samples: As shown in Figs. 4 and 5, NaCl and KCl concentrations in brine had significant effect on Na and K content in cheese. Increasing in NaCl concentration in brine had positive effect on Na content in cheese and increasing in KCl concentration in brine had the same effect on K content of cheese. Ripening time had also positive effect on Na and K content in cheese.

Sensory evaluation: As shown in Fig. 6, NaCl and KCl concentration in brine are effective factors on sensory score. When KCl was at low level, the sensory score of cheese samples was at high level, when KCl concentration increases to 10 %, the sensory score decreased, after that from 10 to 27.7 % of KCl, sensory score was constant and there wasn't significant difference between cheeses with 22.5-27.7 % KCl in brine and control cheese. KCl concentration in brine more than 27.7 % had significantly low acceptability.

Total bacterial count and molds: As shown in Figs. 7 and 8, increasing in KCl concentration in brine had significantly negative effect on TVC and molds. It is possible that the K^+ was partly responsible for the inhibitory effect of the NaCl/KCl mixture as compared to NaCl.

Coliforms: All cheeses were free from coliform. The absence of these organisms may be attributed to a combination of adequate pasteurization and hygienic during the production and storage of the cheese.

Conclusion

This study indicated that partial substitution of NaCl by KCl in manufacture of Iranian white brined cheese, using mixture of NaCl/KCl (100:0, 92.5:7.5, 77.5:22.5, 70:30, 67.5:32.5, 50:50) did not influence the extent of proteolysis (TCA-SN), lipolysis) and dry matter. Increasing in NaCl concentration in brine had positive effect on Na content in cheese and increasing in KCl concentration in brine had the same effect on K content of cheese. Ripening time had also positive effect of Na and K content in cheese. Increasing in KCl concentration in brine had negative effect on TVC and molds count. All cheeses were free from Coliforms. NaCl and KCl concentration in brine were effective factors on sensory score. Acceptability of cheese samples significantly decreased by increasing KCl concentration in brine more than 27.7 %.

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