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IMPACT OF THE MODIFIED METHOD OF USING KNO_3 ON TRANSFORMATIONS OF NITRATES V AND III DURING RIPENING OF DUTCH TYPE CHEESE

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On a commercial scale Dutch cheese was produced with the addition of KNO_3 to brine but not to cheese-making milk. Three variants of experiment depended on salting the cheese into brine with KNO_3 the level of which ranged from 0.05% to 0.15%. Cheeses without saltpetre and with traditional addition of KNO_3 into milk were produced for comparison. In ripening cheese the level of nitrates V and III was low and did not exceed permissible values by national and European regulations. In ripe experimental cheeses contents of nitrates V were on average from 5.06 to 11.13 mg NO_3^-/kg whereas contents of nitrates III were on average from 0.73 to 0.81 mg NO_2^-/kg. Control cheeses were characterised by similar contents of nitrates V and III, but cheeses produced without the addition of saltpetre had the lowest levels of nitrates V and III. Cheeses produced by means of this modified method of using KNO_3 were of good quality.

INTRODUCTION

Nitrates V and III in the form of KNO_3, $NaNO_3$ and $NaNO_2$ are used as food additives in the food industry. The above compounds are also chemical food contaminants introduced into plants and animal materials and water.

As contaminants they appear mainly in water and plants which are consumed by people or are fodder for animals. In plants nitrates V and III accumulate as a result of excessive mineral soil fertilization or as a result of the fall of nitrogen compounds emitted by industrial works. Plants which are not able to transform excessive amounts of nitrates into amides, amino acids or proteins accumulate nitrates in their various parts. Nitrates V and III are ingested by animals with fodder and water [Gapper *et al*., 2004].

Nitrates V are acknowledged as substances noxious for humans and animals because of their suppleness to reducing to nitrates III. The reduction of nitrates V into nitrates III may take place in dairy products and in water before consumption, in the digestive tract before consumption or in some animal tissues after absorbing them from the digestive tract. The process of reducing nitrates V and III always takes place with the help of reducing bacteria, mainly such strains as: *E. coli, A. aerogenes, P. vulgaris, S. aureus* or some strains from *Pseudomonas* and *Bacillus* genera [Matijasic *et al*., 2007; Su & Ingham, 2000].

Mostly babies and elderly people are subject to the toxic activity of nitrates V and III. In those age groups methemoglobinemia is reported to occur most often, which is caused by oxidizing, under the influence of nitrates, the bivalent iron hemoglobin to trivalent form, which does not have the ability of a reversible oxygen bond. The above causes cyanosis, stomach aches, dizziness, fall of blood pressure, acceleration of heart action and in more serious cases death [Lima *et al*., 2006].

Nitrates III, except methemoglobinemia, cause also the blocking of other biologically-active substances, such as carotenes, vitamin A, B-group vitamins or proteins by oxidizing the –SH group. Nitrates III may also exert a diuretic influence, thus causing dispensation of excessive chlorides, which leads to alkalosis, hypochloremia and digestion disorders. Also nitrates V, after reducing to nitrates III, may lead to the formation of carcinogenic N-nitrosamines as a result of their reaction with amines of secondary and third rate importance [Nabrzyski & Ganowiak, 1992; Bouchikhi *et al*., 1999].

The presence of nitrates V and III in milk as well as in its products has already been researched for a long time. Domestic research showed in fresh milk on average from 0.0 to 3.3 mg of KNO_3/dm^3 and from 0.3 mg of $NANO_2/dm^3$ [Śmiechowska & Przybyłowski, 1994].

In nutritive milk the presence of nitrates V may range from 3.7 to 19.6 mg of $NaNO_3$ /kg and that of nitrates III may range from 0.0 to 0.6 mg of $NaNO_2$ /kg [Borawska *et al*., 1996].

Cheese is also a dairy product which contains nitrates V and III. In Poland dairy plants produce cheese by adding KNO_3 to cheese-making milk during the technological process. Technological addition of KNO_3 to cheese-making milk (0.01-0.02%) is used to stop the development of Coli-group bacteria and butter fermentation bacteria during cheese bulk ripening [Beresford *et al*., 2001; Luukkonen *et al*., 2005].

In Poland, the contents of nitrates V and III in food are regulated by the Regulation of the Ministry of Health of the 23rd April 2004 [Regulation..., 2004]. In the case of cheese des-

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ignated for consumption the level of nitrates cannot exceed 50 mg of $NaNO_3$ /kg (36.5 mg of NO_3^-/kg) and the level of nitrites cannot exceed -2 mg of $NaNO_2$/kg (1.33 of NO_2^-/kg) [Polish Standard Dz. U. 94, poz. 933,2004].

The purpose of this research is to present the transformations' dynamics of NO_3^- and NO_2^- in Gouda cheese with a modified method of using potassium saltpetre, *i.e.* means depending on KNO_3 addition not to milk but to brine.

MATERIAL AND METHODS

The research has been carried out on a commercial scale in the "Pasłęk ICC Sery" dairy plant where Dutch Gouda-type ripening rennet cheese has been produced.

For the needs of this research, Gouda cheese has been produced according to the following experimental model:

A – cheese produced from milk without the addition of KNO_3 was pressed and immersed in 0.05% KNO_3 brine,

B – cheese produced from milk without the addition of KNO_3 was pressed and immersed in 0.10% KNO_3 brine,

C – cheese produced from milk without the addition of KNO_3 was pressed and immersed in 0.15% KNO_3 brine,

D – cheese produced from milk without the addition of KNO_3 was pressed and immersed in brine without the KNO_3 addition,

K – control cheese produced from milk to which KNO_3 has been added in the amount of 0.02% and after being pressed the cheese was immersed in KNO_3 free brine.

Each of the above experimental models has been repeated 3 times, respectively.

In general, 15 experimental cheese variants have been produced. Samples of Gouda cheese were taken from 1/4 cheese block, ground and an average was taken of the sample after salting and after 4 and 6 weeks of ripening. In every experimental variant, the samples were taken from 3 cheese blocks.

Determinations of nitrates V and III were made according to method given in Polish Standard including further modifications [PN-EN ISO 14673-1:2004].

Determination of nitrates III depends on inducing a colour reaction among nitrates III and sulfanilamide and dichloride hydride N-1 naphthylene – diamine. The absorbance of the colour compound was measured on a UNICAM UV/VIS spectrophotometer UV2 Spectrometer at a wavelength of λ =538nm. Nitrates V were reduced on a cadmium column to nitrates III and the experiment was continued as in the case of nitrates III determination.

Results of the experiment were subjected to a statistical analysis and statistical differences were determined by using analysis of variance (at a significance level of α=0.05). The statistical analysis was carried out with STATISTICA.PL software produced by Statsoft Polska.

RESULTS AND DISCUSSION

Changes in contents of nitrates V and III in Gouda cheese were presented in Tables 1 and 2.

Cheese of experimental variants A, B and C after salting included, respectively: 27.09 mg of NO_3^-/kg, 46.86 mg of NO_3^-/kg and 75.23 mg of NO_3^-/kg. It was observed that the increasing of KNO_3 concentration in brines of variants A, B and C from 0.05% to 0.15% influenced the variations in the content of nitrates V in cheese of respective variants, reaching the highest level for the experimental variant C. Whereas, variations in the content of nitrates V in cheese among respective

TABLE 1. Changes in contents of nitrates V in Gouda cheese during 6-week ripening.

EV	CB	NO_3^- (mg/kg)									Average NO_3^- (mg/kg)		
		after salting			after 4 weeks			after 6 weeks					
		series											
		I	II	III	I	II	III	I	II	III	after salting	after 4 weeks	after 6 weeks
A	1	28.85	29.39	28.73	6.27	11.59	8.19	3.29	4.66	4.00	27.09	8.79	5.06
	2	28.67	29.87	29.03	7.23	11.11	7.83	3.65	5.74	5.08			
	3	15.47	24.79	28.97	9.26	9.86	7.77	4.66	9.68	4.78			
B	1	44.86	45.82	46.89	14.28	18.22	10.46	6.81	10.16	8.78	46.86	15.62	8.34
	2	46.00	47.49	44.98	14.52	17.21	13.68	7.59	8.78	8.01			
	3	53.58	53.40	38.71	13.92	15.95	22.34	6.57	9.02	9.32			
C	1	56.03	57.77	80.82	21.27	19.89	18.82	10.52	11.05	12.67	75.23	19.67	11.13
	2	79.87	80.46	81.72	21.51	18.16	19.12	11.47	10.40	11.17			
	3	80.70	79.21	80.46	21.63	17.62	19.00	10.75	11.11	11.05			
D	1	10.16	9.14	6.33	2.75	1.73	1.50	1.26	1.26	1.14	9.13	2.05	1.12
	2	13.86	9.56	6.87	2.57	2.03	1.97	1.08	1.08	0.90			
	3	12.20	10.10	6.04	2.57	2.09	1.50	1.38	1.14	0.90			
K	1	43.25	31.66	37.87	14.52	6.57	11.11	6.93	4.66	6.99	38.52	11.24	6.19
	2	41.16	35.78	40.50	13.56	8.78	10.69	6.69	5.20	6.51			
	3	37.63	37.40	41.46	14.04	10.99	10.87	7.11	4.42	7.23			

EV – experimental variant, CB – cheese block number.

TABLE 2. Changes in contents of nitrates III in Gouda cheese during 6 week ripening.

EV	CB	NO_2^- (mg/kg)									Average NO_2^- (mg/kg)		
		after salting			after 4 weeks			after 6 weeks					
		series											
		I	II	III	I	II	III	I	II	III	after salting	after 4 weeks	after 6 weeks
A	1	1.03	0.63	0.68	1.60	0.54	0.85	1.16	0.37	0.63			
	2	0.94	0.72	0.68	1.56	0.68	0.68	1.34	0.23	0.72	0.69	1.07	0.73
	3	0.54	0.28	0.72	1.60	1.38	0.72	1.25	0.19	0.72			
B	1	0.68	0.63	0.72	1.43	0.72	1.16	1.21	0.28	0.85			
	2	0.72	1.16	0.81	1.47	0.59	1.87	1.29	0.41	0.98	0.79	1.20	0.81
	3	0.85	1.43	0.14	1.69	0.63	1.21	1.16	0.23	0.90			
C	1	0.59	0.41	0.81	1.56	0.59	1.16	1.12	0.28	0.85			
	2	0.63	0.63	1.47	1.60	0.76	1.16	1.07	0.32	0.85	0.70	1.20	0.76
	3	0.68	0.45	0.63	1.52	1.16	1.25	1.16	0.23	0.94			
D	1	0.72	0.37	0.23	0.68	0.32	0.28	0.45	0.23	0.32			
	2	0.63	0.50	0.28	0.81	0.32	0.37	0.59	0.14	0.28	0.45	0.46	0.38
	3	0.59	0.54	0.23	0.81	0.28	0.28	1.03	0.10	0.28			
K	1	0.76	0.94	0.32	1.25	0.94	1.12	1.12	0.59	0.85			
	2	0.76	0.98	0.37	1.07	0.63	0.98	1.07	0.63	0.76	0.72	0.98	0.83
	3	0.72	1.12	0.54	0.94	0.76	1.07	0.98	0.54	0.90			

EV- experimental variant, CB – cheese block number.

cheese blocks within the same experimental variant confirm the Jakubowski's thesis about individual "material constant" for respective cheese blocks [Jakubowski, 1967]. Namely, for example in variant A the content of nitrates V in cheese ranged from 15.47 to 29.87 mg of NO_3^-/kg. However in variant B it ranged from 38.71 to 53.58 mg of NO_3^-/kg and in variant C – from 56.03 to 81.72 mg of NO_3^-/kg.

Content of nitrates V in cheese from variant D, produced from milk without the addition of KNO_3, salted in industrial brine without the addition of KNO_3, after the salting process was on average 9.13 mg of NO_3^-/kg, ranging from 6.04 to 13.86 mg of NO_3^-/kg.

In control cheese from the control variant K after salting in brine without the addition of KNO_3 the average content of NO_3^- was 38.52 mg of NO_3^-/kg, ranging from 31.66 to 43.25 mg of NO_3^-/kg.

Contents of nitrates III in cheeses from variants A, B, C and K after salting were low and reach on average, respectively: 0.69, 0.79, 0.70 and 0.72 mg of NO_2^-/kg. The cheese from variant D showed a lower average content of nitrates III, *i.e.* 0.45 mg NO_2^- /kg.

After 4-week ripening the level of nitrates V was reduced by about 70-80%. After this period of ripening, the average content of nitrates V in cheeses from variants A, B and C accounted for 8.79, 15.62 and 19.67 mg of NO_3^-/kg, respectively. The variation of levels of nitrates V among cheese blocks in every experimental variant, within the same research series was observed as well (Table 1). After 4-week ripening, cheese from variant D was characterised by a low level of nitrates – 2.05 mg of NO_3^-/kg, as compared to the control cheese (11.24 mg of NO_3^-/kg).

In Gouda cheese a significant reduction of nitrates V in cheese did not cause large accumulation of nitrates III in cheese (Table 2). After 4-week ripening, in cheeses from variants A, B, C and K the level of nitrates III was similar and accounted for 1.07, 1.20, 1.20 and 0.98 mg of NO_2^-/kg, respectively. In turn, in cheese of variant D the level of nitrates appeared to be low and reached 0.46 mg of NO_2^-/kg.

Concluding, after 4 weeks of cheese ripening the increase in the content of nitrates III was small and did not depend on the rate of reducing nitrates V in this period. This may be due to a significant activity of bacterial reductases and xanthioxidases [Harrison, 2006].

After 6-week ripening, a decrease in the contents of nitrate ions V in cheese in comparison with contents of these ions after salting was 80-90%. After 6-week ripening, average residues of nitrates V in cheese from variant A accounted for 5.06 mg of NO_3^-/kg, in that from variant B – for 8.34 mg of NO_3^-/kg, and in that from variant C – for 11.13 mg of NO_3^-/kg. In control cheese, the content of nitrates V was on average 6.19 mg of NO_3^-/kg, whereas cheese from variant D showed the lowest remains of nitrates V – 1.12 mg of NO_3^-/kg on average.

In addition, after 6 weeks of ripening cheeses of all experimental variants did not show accumulation of nitrates III and their average contents in the cheeses examined did not exceed 0.83 mg of NO_2^-/kg. For cheeses from variants A, B, C and K the level of nitrates III residues was similar. However, for cheese of variant D the average content of NO_2^- ions was twice lower and reached 0.38 mg of NO_2^-/kg.

The analysis of results obtained from experimental variants of Gouda cheese ripening is presented in Table 3. It dem-

TABLE 3. Results of variance analysis of the content of nitrates V in 6-week Gouda cheese.

Cheese	Source of variation	Value of test F	
		nitrate V	nitrate III
Gouda	Experimental variant	88.23978*	2.20088
	Cheese block	0.45602	0.018581
	Interaction: variant x block	0.72770	0.063289
Gouda	Experimental series	2.3750	202.3929*
	Experimental variant	177.4751*	29.3038*
	Interaction: series x variant	5.0755*	5.6305*

*- statistically significant correlation at a significance level of α= 0.05.

onstrated to what extent particular factors influenced changes in the contents of nitrates V and III in cheese.

Analysing the influence of experiment variant and cheese block (material constant) it was proved that the experimental variant (F=88.23978*) influenced significantly the level of nitrates V. The second system of sources of changes, taking into consideration independent constants, series and experimental variant, also showed a significant influence of the experimental variant (F=177.4751*) on the content of nitrates V.

Dependencies in variants' analysis for nitrates III are different. In the independent variables system: variant-cheese block, the two-factor variance analysis did not show any significant influence of these factors on the content of NO_2^- ions in ripening Gouda cheese. In turn, in the independent variables system: series- experimental variant, it showed that both factors were a source of nitrates III variation (F=202.3929* and F=29.3038*, respectively). A significant correlation of the above factors was proved: of series and experimental variant (F=5.6305*).

Śmiechowska & Przybyłowski [1994] examined changes in the content of nitrates V in Żuław, Edam and Gouda cheese in relation to the addition of KNO_3 to cheese-making milk. In ripening cheese they proved the variety of remains of the content of nitrates V in relation to the level of addition of saltpetre to milk. The highest nitrates remains were shown for cheese with 0.02% addition of KNO_3 of saltpetre to milk (2.3 mg/kg for Żuław cheese, 4.4 mg/kg for Gouda cheese and 4.3 mg/kg for Edam cheese). In turn, the lowest nitrates remains were demonstrated for cheese without addition of saltpetre to milk. Results obtained in the study of Śmiechowska & Przybyłowski [1994] are consistent with findings received in this work for Gouda cheese produced with the addition of saltpetre to cheese-making milk. Cheese produced without the addition of saltpetre showed the lowest remains of nitrates V after the ripening period [Śmiechowska & Przybyłowski, 1994].

Comparing content of nitrates V and III in ripening cheese produced in this experiment (addition of KNO_3 to brine) with those of domestic cheeses available on the market and foreign cheeses it can be proved that their quantities are comparable [Koréneková *et al*., 2000].

Receiving whey of low nitrogen contents of ions V and III is an additional advantage of the modified method of using of KNO_3, which enables its further industrial usage [Glibowski, 2004].

The modified method of using KNO_3 in Gouda cheese production, presented in this work, enables producing cheese of good quality [Stasiuk &Przybyłowski, 2005].

CONCLUSIONS

1. The addition of KNO_3 to brine allows producing Dutch cheese of good quality.
2. Dynamics of transformations of nitrates V and III during ripening of cheese produced with the modified method of KNO_3 addition did not lead to accumulation of nitrates V and III. In ripening cheese the level of these ions was low and did not exceed permissible values stipulated by national and European regulations.
3. Statistical analysis of results showed that an essential source of variations in nitrates V and III in cheese during ripening were: " material constant" of cheese block, concentration of KNO_3 in brine, and ripening time. The most advantageous turned out to be the application of KNO_3 to brine in the amount from 0.05% to 0.10%.
4. Applying KNO_3 to brine and not to cheese-making milk allows obtaining nitrate-free whey, which enables its further application in the dairy industry.

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