





IMPROVING OF THE MICROBIOLOGICAL AND PROTEOLYTIC PROFILE OF KASHKAVAL CHEESE BY MODIFICATION IN HEAT TREATMENTS OF COW'S MILK AND CHEDDARED CURD

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ABSTRACT

Model samples kashkaval cheese were made by the microwave processing of cow's milk in thermisation mode (62 - 67 °C) and direct microwave treatment of cheddared curd with subsequent dry salting. Control cheese samples were produced by the conventional approach with classical thermisation (62 - 67 °C) of cow's milk and classic hot brining in brine solution (72-74 °C, 14%NaCl). Samples were placed in ripening conditions (8 - 10 °C). The changes of microbiological and proteolytic profile on the 5th, 15th, 30th and 45th day of ripening were studied. Higher survival levels of the starter culture responsible for ripening were established in kashkaval cheese produced by microwave treatment in comparison with classical cheese. It was found that the higher cell concentration of the starter culture leads to active and accelerate process of proteolysis with production of free amino acids in concentrations significantly exceeding the obtained in the classical cheese: 490,10±2,10 mg100g⁻¹ (microwave kashkaval cheese) versus 216,50±2,00 mg100g⁻¹ (classical kashkaval cheese).

Keywords: Proteolysis, microwave treatment, thermisation, amino acids, starter culture

INTRODUCTION

Modern trends in the dairy industry are connected with searching of approaches to enhance the biological potential of dairy products. Much of the studies treating the possibilities for increasing the biological value of dairy products are related to the addition of different ingredients to milk - vegetable oils (Kesenkas et al., 2009), the addition of dietary fibers (Devinder et al., 2012; Vlaseva et al., 2014), the preparation of emulsions fortified with Ω -3 and Ω -6 fatty acids for the purpose of use in dairy products (Ivanova et al., 2012). The approach of ingredient incorporation undoubtedly increase the biological profile of the product but it should use the scheme of mechanical addition with purpose to achieve certain, search functionality. Modern scientific trends related to the search for solutions to improve the functional index of dairy products with complex nature, are based on the acceleration of biotransformation processes by the approaches which retains the nature of milk products and at the same time leading to rapidly increase of the functional characteristics of the product. In this aspect have been reported studies, which have offering alternative approaches to acceleration of ripening process.

Simov et al. (2006) were investigated the contribution of selected starter culture consisting of Lactococcus lactis C11, Streptococcus thermophilus P23 and Lactobacillus casei ssp. casei RP5 for proteolysis of kashkaval cheese by comparing it with a traditional yoghurt culture, consisting of Lactobacillus delbr. ssp. bulgaricus 2-11 and Streptococcus thermophilus 13a. The team reported for reducing in ripening process time of the cheese produced by involvement of the selected starter culture (duration of ripening - 30 days, the level of proteolysis – 17.3%, the depth of proteolysis – 29.9%) in comparison with the control cheese manufactured with addition of yoghurt culture (duration of ripening - 60 days, proteolysis level - 20.2%; depth of proteolysis - 24.9%). Investigators has found three fold higher concentration of free amino acids in the mature kashkaval cheese with the selected starter culture, as compared to that obtained in the mature kashkaval cheese with the traditional yoghurt culture.

Proteolysis is a biochemical process with essential importance for the definition of kashkaval cheese (and cheese at all) as a mature product. It is responsible for the accumulation of valuable biological substances - essential amino acids in the fast absorption form of the body and is relevant for the formation of aroma and taste in a mature product (Rijnen et al., 2000; Yvon and Rijnen, 2001; Kieronczyk et al., 2003). The pattern of proteolysis in many varieties of cheese

can be summarized as follows: initial hydrolysis of casein by residual coagulant activity of plasmin and other present enzymes available to the production of large and medium-size peptides, which in the next step are hydrolyzed by proteinases and peptidases of starter lactobacilli (LAB), non-starter microflora (NSLAB) and secondary microflora to short - chain peptides and free amino acids (Mc Sweeney, 2004).

Microwave processing is the process of heat treatment with a heating mechanism, radically different from the classical heat transfer. Basically microwave processing is a treatment of food with electromagnetic energy with frequencies in the microwave region (usually 2450MHz), causing rotation of the dipole water molecules in the product and conducting migration of dissolved ions in the electromagnetic field - two phenomena that generate heat in the product on the basis of so-called molecular friction (Mudgett, 1988; Ramaswamy and Tang, 2008). It was found that the rapid nature of heating by microwave treatment reduced destructive effects of heat on the food composition as compared to longer conventional approaches of heat treatment (Dumuta et al., 2010; Dehghan et al., 2012).

Literature discussing the application of microwave processing in dairy industry indicating data only for its application as a heat treatment process for the purpose of sterilization and pasteurization of milk (Kovacs et al., 2006; Korzensky et al., 2013; Villamiel et al., 1996). There are no data in the literature relating to the study of kinetics of proteolysis (essential process in the cheese ripening) in cheeses produced by replacing of the classical heat treatment with microwave treatment.

The alternative solution, which is based this study is the application of microwave processing for the production of traditional Bulgarian hot brined cheese - kashkaval by modification of heat treatments in the technology of cheese manufacturing in three aspects:

» Replacement of classical heat treatment of milk in regime - thermisation (62 - 67 °C) with microwave thermal treatment in the same thermal regime;

» Replacement of classical hot brining (72 - 74 °C, 14% solution of NaCl) with direct microwave treatment of cheddared curd (with processing parameters f = 2450 MHz, p = 800 W, generate temperature of 72 - 74 °C);

»Dry salting of microwave treated cheese curd with NaCl.

The objective of our study was to investigate the microbiological and proteolytic profile of cheese produced by applying of microwave heat treatment.

MATERIAL AND METHODS

For conduct of the study raw cow's milk was used; Microwave oven LG (model № MS2389 BS / 00 power 800W, frequency 2450 MHz); Electric heater Alaska (Model KP 180, SIG, GmbH); starter cultures for kashkaval cheese (*Lb. delbr. ssp. bulgaricus, Streptococcus thermophilus, Lactococcus lactis ssp. lactis, Lactobacillus helveticus*); rennet (Biokom Trendafilov - Ltd., Sliven, Bulgaria); vacuum packaging film; vacuum - packaging machine.

Production of kashkaval cheese samples

Kashkaval cheese samples were produced in the laboratory conditions in the Department of Technology of milk and dairy products at University of Food Technologies - Plovdiv, Bulgaria. Classical technology for the production of cheese and modified technology with application of microwave treatment were applied. 20 dm3 cow's milk was used for production of classical kashkaval cheese and 20 dm³ cow's milk was used for production of kashkaval cheese by applying of microwave treatment. Cow's milk was appraised by physicochemical parameters and adopted pursuant to statutory regulations. At the next stage the milk was subjected to heat treatment. Cow's milk used for production of the classical cheese was heat treated in a conventional mode thermisation (62 - 67 °C), and milk intended for the manufacture of microwave cheese was treated in the same temperature regime, but with application of microwave thermisation (62 - 67 °C). At the next stage, samples of cows' milk was cooled to a temperature of coagulation (34 - 35 °C), and then inoculate with the starter culture for kashkaval cheese in an amount 1 g.100g⁻¹ consisting of Lactobacillus delbr. ssp. bulgaricus, Streptococcus thermophilus, Lactococcus lactis ssp. lactis and Lactobacillus helveticus. Biological ripening of the inoculated milk for a period of 15 - 20 min was conducted with finalizing stage at the moment in which the milk increased its titratable acidity (parameter for rate of lactic acid process in Thorner degrees -°T) with 1 - 2 °T. To biologically ripened milk was added 50% solution of CaCl₂ in the amount of 3 cm³.10dm³ milk. Coagulation was carried out at maintained temperature of 34 ± 1 °C throughout the whole period of coagulation with fixation occurs of initial coagulation in 10 - 12 min, and finalizing of coagulation at 35 -40 min. After finishing of coagulation cheese curd is cut initially to the prism, and then to grains with size 6-8 mm, which was stirred for 15-20 min. In next stage cheese grains were subjected to cooking, which was performed with gradually increasing at the temperature of the whey to 40±1 °C. In each 5 min temperature was increased by 1 °C with constant stirring for 40 – 60 min. End of cooking was set at achieving sufficient elasticity of the cheese grains and titratable acidity (parameter for rate of lactic acid process in Thorner degrees -°T) of the whey - 18 °T. After finishing of the cooking process greater amount of whey was removed and cheese grains were placed in filter material for squeeze. Then, the cheese grains were subjected to pressing, which is achieved by a gradual increase in pressure over a period of 20 – 30 min. After pressing cheese curd is cutting into pieces with a size 10/25cm and left for chedderization. The chedderization process completed with reaching pH 5.3 for 2h with maintaining a constant temperature of 36±1 °C throughout the process. After chedderization cheese curd of classical cheese was hot brining (salt concentration - 14%, t = 72 -74 °C), while chedderized cheese curd for production of microwave cheese was shredded, portioning and subjected to direct microwave treatment with parameters: frequency of waves f = 2450 MHz, power p = 800 W, generated temperature in cheese curd – 72 - 74 °C. After that microwave treated cheese curd was dry salted with NaCl at 2%. The two types of kashkaval cheese were formed into cylindrical forms (200 g). After stabilization cheeses are removed of forms and left in a refrigerated conditions at a temperature of 10 - 12 °C to drying for 3 days. After drying the cheeses were packaged in PVC packaging under vacuum and placed in conditions of ripening (8 - 10 °C and humidity of 75-80%) over 45 days. Cheese samples were analyzed in the dynamics of ripening of the 5th, 15th, 30th, 45th day.

Determination the number of viable lactic acid bacteria in cheeses

With a sterile spatula was removed and weighed 5±0.1 g cheese. The prepared sample was homogenized whit peptone water, to obtain a dilution of 10⁻¹. The sample is diluted to a certain degree of dilution. The 1ml of so prepared samples are transferred into a sterile petri dishes. For the determination of lactobacilli to this sample was added 15 ml MRS agar (Merck, Germany), cooled to 45 °C. For the determination of cocci to the sample was added 15 ml M17 agar (Merck, Germany), cooled to 45 - 47 °C. Immediately after pouring the agar inoculum gently mix by shaking the Petri dishes, after which the plates were placed on a flat surface to harden the agar.

The plates to establish the viability of lactic acid bacteria were incubated at 37 °C for 48 hours. After finishing of the incubation process, colonies were counted and the number of lactic acid bacteria was determined according to the formula (Simova and Spasov, 2007):

$$N = \frac{\sum c}{V.(n1+0,1.n2).d}$$
, $CFUg^{-l}$, where:

 $\sum C$ - amount of these colonies in two successive dilutions selected for enumeration;

V - volume of seed material;

- n1 number of dishes from the first dilution, which count the colonies;
- n2 number of dishes in second dilution, which count the colonies;
- d the dilution factor corresponding to the first dilution, which count the colonies

Amino acid composition

Free amino acids were determined after precolumn derivatization with phenylisothiocyanate using Pico. Taq method (Cohen et al., 1984). One gram cheese was mixed with 2 ml 30% methanol in 0.3N hydrochloric acid containing 50 nmol norvaline as internal standard. The mixture was homogenized, kept in refrigerator for thirty minutes, and after that centrifuged at 10000 g for 5 minutes. Five hundred microliters from the supernatant were transferred to ultrafiltration cartridge with cut-off 5000 Daltons (Microcon YM-5, Milipore). The cartridges were centrifuged for 30 minutes at 12000 g. Twenty five microliters from the filtrate were transferred to small Pyrex tubes (6x50 mm) and the samples were evaporated under vacuum. After that the derivatization was conducted according to the Pico. Taq method (WARERS) using specially designed reagents and HPLC column for free amino acids. Ten microliters were injected into the column and the analysis was performed using HPLC equipment SHIMADZU 10A. The UV detection was at 254 nm.

The method is suitable for determination of all free amino acids including tryptophan, arginine, asparagine, and glutamine.

Statistical analysis

The statistical analysis of the data is carried out by determining the standard deviation (SD), with triple repetition of the analyses. It is performed with the Excel 2007 software application of the Microsoft Office 2007 suite (Microsoft Corporation, USA).

RESULTS AND DISCUSSION

Starter bacteria for the production of kashkaval cheese (Lactobacillus delbr. ssp. bulgaricus, Streptococcus thermophilus, Lactococcus lactis ssp. lactis, Lactobacillus helveticus) are the basis of microbiological and proteolytic processes occurring during the ripening of cheeses. The growth dynamics of the starter culture in the ripening process of kashkaval cheese are presented in Figure

Bacteria from the starter culture are reproduced intensively during cheddarization and form a high cell concentration, respectively 2.7*10⁶ CFU.ml⁻¹ for lactobacilli and 2.2*10⁸ CFU.ml⁻¹ for the streptococci in the kashkaval cheese produced by the application of microwave treatment versus 5.2*10⁴ CFU.ml⁻¹ for lactobacilli and 9.8*10⁴ CFU.ml⁻¹ for streptococci in classical cheese. The direct microwave treatment of chedderized cheese curd reduce the levels of cheese microflora and kashkaval cheese produced by the application of microwaves is started ripening process with a concentration of lactobacilli – 2.4*10⁵ CFU.ml⁻¹ and streptococci – 2.9*10⁷ CFU. ml⁻¹. Reduction of microflora after brining in hot brine solution (72 - 74 °C, 14% NaCl) lead to a concentration of lactobacilli – 4.3*10³ CFU.ml⁻¹ and streptococci – 8.6*10³ CFU.ml⁻¹ in which concentrations started ripening process in classical kashkaval cheese.

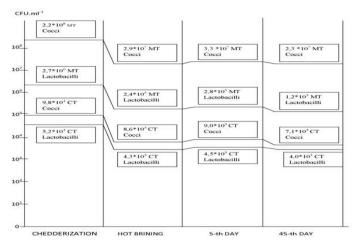


Figure 1 Dynamics of active microflora in the ripening process of kashkaval cheese

*MT - Kashkaval cheese produced with application of microwave treatment

*CT - Kashkaval cheese produced with application of conventional heat treatment

Higher levels of lactic acid bacteria in cheese produced with application of microwave processing were established. On the 5th day of ripening in cheese produced by microwave treatment of milk and direct microwave treatment of chedderized cheese curd was observed a slight increase in the concentrations of lactobacilli and streptococci; $2.8*10^5$ CFU.ml⁻¹ and $3.3*10^7$ CFU.ml⁻¹.

The same trend of a slight increase in the levels of lactobacilli and streptococci was observed in the classical cheese too $-4.5^{*}10^{3}\,\text{CFU.ml}^{-1}$ and $9.0^{*}10^{3}\,\text{CFU.ml}^{-1}$. Trend of more active reproduction of the starter culture was observed in cheese produced by the application of microwave treatment and levels of starter culture overtaking the concentration levels reported in the classical cheese.

Table 1 Concentration of amino acids in the kashkaval cheese during the ripening period (5,15 days)

	Content of amino acids (mg.100g ⁻¹)				
Amino acids	Type of the sample kashkaval cheese				
	Microwave kashkaval		Classical kashkaval		
	5-th day	15-th day	5-th day	15-th day	
Alanin	3.50±0.12	3.60±0.12	2.50±0.04	3.30±0.05	
Glycine	1.10±0.11	1.20±0.07	1.10±0.02	1.30±0.04	
Valine	7.20±0.02	6.50±0.11	3.90±0.06	5.00±0.12	
Leucine	10.60±0.08	11.30±0.21	5.20±0.12	6.90±0.13	
Isoleucine	2.60±0.09	2.80±0.23	1.40±0.09	1.80±0.09	
Threonine	1.80±0.13	1.70±0.09	0.90±0.07	1.20±0.02	
Serine	8.20±0.10	7.40±0.11	4.10±0.01	4.70±0.12	
Proline	14.90±0.11	13.60±0.03	9.80±0.03	11.10±0.10	
Asparagine	1.70±0.09	1.90±0.04	0.90±0.04	1.00±0.03	
Aspartic acid	1.60±0.01	1.50±0.12	2.20±0.01	2.70±0.07	
Methionine	1.60±0.03	1.60±0.19	0.80±0.02	0.90±0.02	
Glutamic acid	12.00±0.06	16.30±0.23	13.40±0.06	16.60±0.19	
Phenylalanine	5.80±0.10	5.60±0.12	2.40±0.09	3.00±0.04	
Glutamine	2.60±0.12	2.80±0.04	4.40±0.01	2.80±0.02	
Lysine	18.20±0.17	21.00±0.15	13.80±0.04	18.60±0.05	
Histidine	0.80±0.20	2.70±0.19	2.70±0.03	2.40±0.02	
Tyrosine	5.40±0.07	5.60±0.09	2.40±0.08	3.00±0.03	
Tryptophan	1.70±0.08	1.50±0.03	0.80±0.02	0.90±0.03	
Cysteine	ND	ND	ND	ND	
Arginine	0.30±0.01	0.30±0.01	0.20±0.01	0.20±0.01	
TOTAL CONTENT	101.60±1.70	109.00±2.18	73.10±0.85	87.50±1.18	

Table 2 Concentration of amino acids in the kashkaval cheese during the ripening period (30, 45days)

	Content of amino acids (mg.100g ⁻¹)				
Amino acids	Type of the sample kashkaval cheese				
	Microwave kashkaval		Classical kashkaval		
	30-th day	45-th day	30-th day	45-th day	
Alanin	11.70±0.03	12.60±0.11	9.20±0.05	10.40±0.07	
Glycine	4.20±0.05	5.00±0.07	2.80±0.07	3.90±0.03	
Valine	34.00±0.04	39.80±0.17	23.40±0.12	27.30±0.21	
Leucine	58.30±0.06	68.30±0.23	35.60±0.14	37.20±0.20	
Isoleucine	11.40±0.08	13.50±0.12	9.40±0.11	10.90±0.17	
Threonine	9.10±0.03	8.00±0.08	6.60±0.06	7.70±0.12	
Serine	35.70±0.10	38.00±0.11	4.90±0.09	5.60±0.09	
Proline	39.30±0.14	41.50±0.15	25.20±0.13	26.80±0.16	
Asparagine	13.90±0.12	16.00±0.09	19.80±0.12	20.70±0.18	
Aspartic acid	3.90±0.08	4.30±0.06	3.90±0.04	4.30±0.05	
Methionine	8.10±0.09	9.40±0.01	1.70±0.04	2.20±0.10	
Glutamic acid	29.50±0.05	42.10±0.02	17.20±0.11	19.50±0.21	
Phenylalanine	44.00±0.04	53.30±0.12	4.80±0.06	5.60±0.12	
Glutamine	33.00±0.09	35.40±0.13	3.40±0.05	3.60±0.09	
Lysine	49.30±0.19	67.70±0.21	19.60±0.09	21.60±0.03	
Histidine	4.10±0.05	6.10±0.09	2.90±0.10	3.80±0.01	
Tyrosine	17.80±0.07	20.40±0.11	3.50±0.12	3.70±0.04	
Tryptophan	6.60±0.08	7.60±0.03	1.00±0.04	1.50±0.10	
Cysteine	ND	ND	ND	ND	
Arginine	1.00±0.02	1.10±0.01	0.20±0.01	0.30±0.02	
TOTAL CONTENT	415.00±1.41	490.10±2.10	195.20±1.55	216.50±2.00	

On the 45th day of ripening in cheese produced by microwave treatment was observed a slight decrease in the number of lactobacilli and streptococci, compared to 5-th day $-1.2*10^5 \ {\rm CFU.ml^{-1}}$ and $2.3*10^7 \ {\rm CFU.ml^{-1}}$. Thus possibly due to a depletion in the levels of energy substrate (lactose) for metabolism of starter culture. However, the obtained results shows that in the microwave cheese, even after stages of ripening (45 day) were observed large concentrations of viable lactic acid microflora. In the control sample (classical cheese) on day 45th compared to day 5th decrease in the number of lactobacilli and streptococci was observed too $-4.0*10^3 \ {\rm CFU.ml^{-1}}$ and $7.1*10^3 \ {\rm CFU.ml^{-1}}$.

The reported high levels of cell concentration affect the amino acid profile with demonstration the process of active proteolysis of casein throughout the ripening period. The data of the amino acid profile in kashkaval cheese are presented in Table 1 and Table 2.

Higher cell concentration of microorganisms in cheese produced by the application of a microwave treatment, established on 5th day of ripening

compared with classical cheese leads to more active biodegradation of casein still in the early stages of ripening (5 days) with established higher concentration levels of amino acids in the microwave cheese $-101.60\pm1.70~\text{mg}.100\text{g}^{-1}$, as compared with $73.10\pm0.85~\text{mg}.100\text{g}^{-1}$ for the classical cheese.

In the process of ripening (15th, 30th, 45th day) this dynamic has remained and cheese produced by microwave treatment demonstrated higher concentration levels of total amino acids in compared to classical cheese, respectively: $109.00\pm2.18~mg.100g^{-1}$ versus $87.50\pm1.18~mg.100g^{-1}(15~days);~415.00\pm1.41~mg.100g^{-1}$ versus $195.20\pm1.55~mg.100g^{-1}$ (30 days); $490.10\pm2.10~mg.100g^{-1}$ versus $216.50\pm2.00~mg.100g^{-1}$ (45 days). The ripening process finalize with a double higher concentration of free amino acids in the cheese produced by the microwave treatment in comparison to the classical cheese, respectively: $490.10\pm2.10~mg.100g^{-1}$ (microwave cheese) versus $216.50\pm2.00~mg.100g^{-1}$ (classical cheese).

After ripening in cheese produced by microwave treatment is established all essentials amino acids in high concentrations, significantly exceeding those of the control (classical cheese), respectively: valine $-39.80\pm0.17~mg.100g^{-1}$ versus $27.30\pm0.21~mg.100~g^{-1}$; leucine $-68.30\pm0.23~mg.100g^{-1}$ versus $37.20\pm0.20~mg.100g^{-1}$; isoleucine $-13.50\pm0.12~mg.100g^{-1}$ versus $10.90\pm0.17~mg.100g^{-1}$, threonine $-8.00\pm0.08~mg.100g^{-1}$ versus $7.70\pm0.12~mg.100g^{-1}$; methionine $-9.40\pm0.01~mg.100g^{-1}$ versus $2.20\pm0.10~mg.100g^{-1}$; lysine $-67.70\pm0.21~mg.100g^{-1}$ versus $21.60\pm0.03~mg.100g^{-1}$; and tryptophan $-7.60\pm0.03~mg.100g^{-1}$ versus $1.50\pm0.10~mg.100g^{-1}$.

The established result for the amino acid profile focuses on significantly increasing of biological potential and functional index of kashkaval cheese produced by applying of microwave treatment. Generated better survival rate of microflora added as a starter cultures for cheese production recorded after microwave processing of chedderised cheese curd contributes to active metabolic activity of lactic acid bacteria, leading to increased proteolysis with accumulation of significantly higher concentration levels of free amino acids in the mature (45 days) cheese produced by the application of microwave treatment.

CONCLUSION

The application of microwave treatment as an alternative approach for cheese production significantly improve the microbiological and proteolytic profile after cheese ripening. In the cheese produced by the application of microwave treatment were identified higher survival levels of the starter culture responsible for ripening. After direct microwave treatment of chedderized cheese curd was found that the microwave cheese ripening starts with levels of lactic acid microflora, significantly exceeding those of the classical cheese in which applied classical hot brining (14% NaCl, 72 - 74 °C). Even after finishing of the ripening process was established higher levels of lactic acid microorganisms in the mature microwave cheese as compared to conventional. Higher cellular concentration of starter culture in microwave cheese lead to active and rapid process of proteolysis, leading to the accumulation of amino acids in concentrations significantly exceeding that obtained in classical cheese – 490.10 \pm 2.10 mg.100g $^{-1}$ (microwave cheese) versus 216.50 ± 2.00 mg.100g $^{-1}$ (classical cheese).

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