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QUALITY OF DIETARY PROBIOTIC YOGURT PRODUCED BY USING TRANSGLUTAMINASE

KVALITET DIJETALNOG PROBIOTSKOG JOGURTA PROIZVEDENOG UZ PRIMENU TRANSGLUTAMINAZE

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SUMMARY

Different techniques for physico-chemical properties improment of low fat yogurt are used in the dairy industry. Application of transglutaminase (TG) in low fat yogurt manufacture is one of the recent modern techniques. The enzyme transglutaminase is transferase that forms both inter- and intra-molecular isopeptide bonds in and between many proteins by cross-linking of the amino acid residues. The aim of this study was to determine the effect of different quantities of transglutaminase (0.02%, 0.06% i 0.12%) as well as conditions of its application (direct or after enzyme activation) on improvment of physico-chemical properties of yogurt manufactured from two kinds of low fat milk (0.1% w/w fat and 0.5% w/w fat) after production and during of storage. The fermentation in both series was performed with addition of probiotic starter culture ABT-4 (Lactobacilus acidophilus-5, Bifidobacterium-12 i Streptococcus thermophilus, Chr. Hansen A/S, Denmark) to the milk at 43 °C. The results of analyses, after yogurt production, show that the lowest whey separation was determined in yogurt produced with activation of transglutaminase regardless the added amount of the enzyme. Difference of whey separation values between the yogurt samples with 0.1% and 0.5% w/w fat wasn´t significant.

The physical and sensory characteristics of yogurt samples produced with transglutaminase are significantly better compared to yogurt samples produced without enzyme addition.

***Key words**: yogurt, probiotic starter culture, transglutaminase, physico-chemical properties*

REZIME

U industriji mleka primenjuju se različite tehnike za poboljšanje fizičko-hemijiskih karakteristika jogurta sa niskim sadržajem masti. Jedna od savremenih metoda za korekciju navedenih osobina fermentisanih mlečnih proizvoda sa niskim sadržajem masti je primena enzima transglutaminaze (TG) koji utiče na formiranje inter- i intra- molekularnih veza između aminokiselinskih veza unutar kazeinskog matriksa. U radu je ispitan uticaj različitih koncentracija transglutaminaze sa i bez aktivacije enzima (0,02%, 0,06% i 0,12%) i tehnološkog procesa proizvodnje na poboljšanje fizičko-hemjiskih karakteristika jogurta nakon proizvodnje i tokom skladištenja. Za proizvodnju jogurta korišćeno je pasterizovano mleko sa 0,1% i 0,5% mlečne masti. Fermentacija mleka je vršena dodatkom probiotske starter kulture ABT-4 (Lactobacilus acidophilus-5, Bifidobacterium-12 i Streptococcus thermophilus, Chr. Hansen A/S, Denmark) na temperaturi 43°C. Rezultati analiza obavljenih nakon proizvodnje obe vrste jogurta (0,1% i 0,5% mlečne masti) ukazuju da je sinerezis surutke najmanji u uzorcima proizvedenim uz predhodnu aktivaciju transglutaminaze bez obzira na dodatu količinu. Između uzoraka sa 0,1% i 0,5% mlečne masti razlike u promeni sinerezisa surutke tokom skladištenja su neznatne.

Pokazalo se da su uzorci proizvedeni sa transglutaminazom boljih fizičkih osobina kao i senzornih karakteristika u odnosu na uzorke proizvedene bez dodatka enzima.

***Ključne reči**: jogurt, probiotska starter kultura, transglutaminaza, fizičko-hemijiske osobine*

INTRODUCTION

Fermented dairy products include a wide group of products differing regarding the type of milk produced of, the type of fermentation, consistency, milk fat content, the type of additives, etc.. This is a well known group of dairy products of high nutritive and dietetic value. The presence of valuable milk components (proteins, lactose, lactic acid, minerals and vitamins) and their optimal ratio, as well as the application of different starter cultures, especially probiotics, contribute to increased consumer interest for this group of products. Unlike traditional, today, fermented dairy products are produced in strictly controlled conditions defined by the technological process *(Tamime and Robinson, 2004)*.

New developed concentrated starter cultures (DVS - direct set and an RS - red set) have many advantages in comparison to the conventional forms of starters: simple usage, stable and controlled activity for a long time period, the possibility of infection is reduced to a minimum, require less work and allow uniformed quality and longer shelf life of the product. Yogurt is the most represented dairy product in our country and the world. Yogurt is produced in different variants. Recently, special attention is given to the production of yogurt with reduced energy value – "diet yogurt" (present at the world market as a low fat yogurt with 0.5-2% milk fat or "non-fat yog urt" with 0.1-0.5% milk fat).

Enormous progress in the modern technology of fermented milk beverages is achieved by launching new products for special purposes, which include probiotic microorganisms in starter for fermentation or already fermented milk (Milanović, 1997).

Bacteria of the genus *Lactobacillus*, *Streptococcus* and *Bifidobacterium* are mostly used in the production of fermented dairy drinks with probiotic microorganisms (so-called products of "third generation"). Recently developed fermented milk and dairy drinks show numerous preventive and therapeutic properties, due to multifunctional characteristics of intestinal bacteria (antimicrobial, biochemical, immunodeficiency, physiological). The positive effects of intestinal micro flora taken through fer-

mented milk drinks are: improvment of lactose digestion, stimulation of the immune system, reduction of cholesterol level and triglycerides level, the activation of digestible enzymes, increase of organism resistance to gastrointestinal infections, prevention of diarrhea, production of organic acids (acetic), production of vitamins, increased absorption of mineral substances etc. *(Milanović, 1997, Tamime, Robinson, 2004)*. The primary function of probiotic products is to stimulate the micro flora balance in the digestive tract, *(Topisirović et al., 2001)*. Dairy industry uses different techniques for the improvement of physico-chemical (whey separation) properties of low fat yogurt.

Application of transglutaminase in low fat yogurt manufacture is one of the recent modern techniques. The enzyme transglutaminase is transferase that forms both inter- and intra- molecular isopeptid bonds in and between many proteins by cross-linking of the amino acid residues. Applying transglutaminase is possible to increase significantly the gel strength *(Lauber et al., 2000)*. Also, the sensory analysis shows that the addition transglutaminase can reduce milk fat or protein components and gain yogurt which in respect of texture similar to yogurt produced from fat rich milk and with high protein content *(Lorenzen et al., 2002)*. Also, enzymatic binding significantly reduces the whey separation. The results show that transglutaminase addition to milk is a very useful procedure in the production of yogurt with low milk fat content *(Yokoyama et al., 2004, Milanović et al., 2006, Milanović et al., 2007)*.

MATERIAL AND METHOD

Yogurt samples were produced from skim milk with 0.1 % fat and 0.5% fat. Milk was pasteurized at 72ºC during 15 s and cooled to 8 ºC.

Two series of probiotic yogurt with transglutaminase (ACTIVA Transglutaminase, Ajinomoto Foods EUROPE SAS, Hamburg Branch, Germany) were produced (Table 1).

Table 1. Plan of experiment
Tabela 1. Plan eksperimenta

TG (g/100g)	Yogurt sample Uzorak jogurta	
Without activation Bez aktivacije	Series 1 Serija 1 Milk with 0.1% fat Mleko sa 0.1% masti	Series 2 Serija 2 Milk with 0.5 % fat Mleko sa 0.5% masti
0	Control yoghurt1 Kontrolni jogurt 1	Control yoghurt 2 Kontrolni jogurt 2
0.02	0.02 WAY *	0.02 WA **
0.06	0.06 WAY	0.06 WA
0.12	0.12 WAY	0.12 WA
With activation Sa aktivacijom		
0.02	0.02 AY ***	0.02 A ****
0.06	0.06 AY	0.06 A
0.12	0.12 AY	0.12 A

*WAY- probiotic yogurt with 0.1% milk fat, without activation of TG enzyme

**WA - probiotic yogurt with 0.5% milk fat, without activation of TG enzyme

***AY- probiotic yogurt with 0.1% milk fat, with activation of TG enzyme

****A - probiotic yogurt with 0.5% milk fat, with activation of TG enzyme

In the first series the milk was cooled to 43 ºC and appropriate quantities of TG (0.02%, 0.06% and 0.12%) and probiotic starter culture ABT-4 (*Lactobacillus acidophilus*-5, *Bifidobacterium*-12, *S. thermophilus*, Chr. Hansen A/S, Denmark) were added.

In the second series transglutaminase was activated in milk for 2 h at 40 ºC, after that the milk was heat-treated at 80 ºC for 1 min, cooled to 43 ºC and inoculated with the same probiotic starter at 43 ºC. In both trials the fermentation lasted to reach pH 4.5. The yogurt samples were cooled and gently homogenized. The samples were packed in plastic containers and stored at 4 ºC. The control sample was produced without TG *(Milanović et al., 2007)*.

The quality of probiotic yogurt was analyzed after the production and after 10 days storage.

- TOTAL ACIDITY was determined by the Soxhlet-Henkel (ºSH) titration method *(Carić and Milanović, 2000);*
- pH. Active acidity is determined by using pH-meter (pH Spear, Eutech Instruments Oakton).
- WHEY SEPARATION is expressed in mL of whey separated during filtration of a 50g sample for 3 hours, at room temperature *(Atamer et al., 1996)*;
- SENSORY ANALYSIS of yogurt was performed by the expert commission to set points system. Evaluation included appearance (maximum number of points 1), colour (maximum number of points 2), consistency (maximum number of points 4), odour (maximum number of points 3) and taste (maximum score 10). The maximum number of points is 20.

RESULTS AND DISCUSSION

Fermentation time

Change of pH during milk fermentation is presented in Figs. 1 and 2. The pH of the samples produced from milk of 0.1% and 0.5% fat decreased during fermentation. Fermentation time of yogurt produced without TG enzyme activation prior to fermentation, was shorter than in yogurt samples produced with enzyme activation. The obtained results are in accordance with the literature *(Lorenzen et al., 2002; Ozer et al., 2007)*.

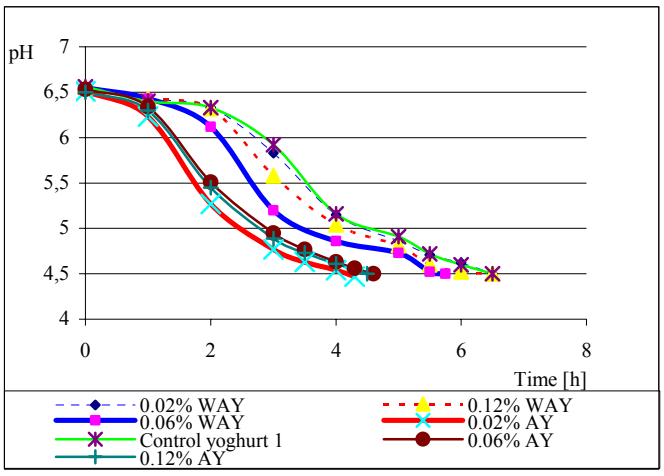

Fig. 1. Fermentation time of yogurt samples with TG produced from milk of 0.1% w/w fat
Sl. 1. Fermentaciono vreme uzoraka jogurta proizvedenog uz primenu TG iz mleka sa 0,1% m.m.

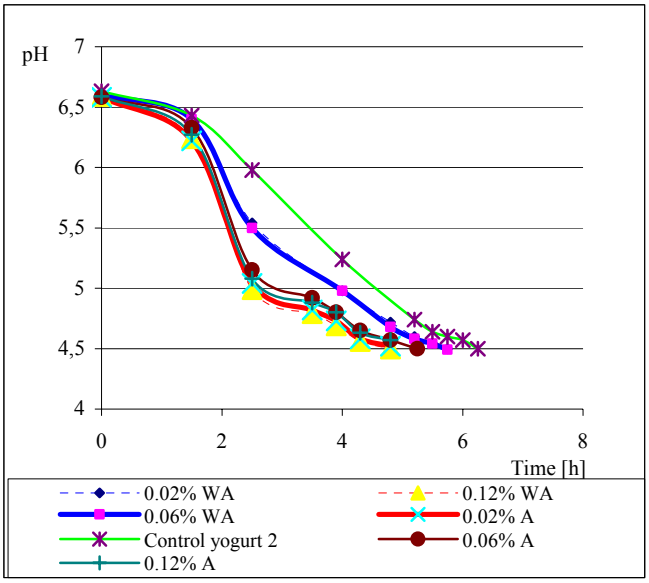

Fig. 2. Fermentation time of yogurt samples with TG produced from milk of 0.5% w/w fat
Sl. 2. Fermentaciono vreme uzoraka jogurta proizvedenog uz primenu TG iz mleka sa 0,5% m.m.

Physico-chemical characteristics of low fat yoghurt after production

The physico-chemical characteristics (acidity, whey separation) of yogurt with 0.1% and 0.5% milk fat are presented in Figs. 3 and 4, respectively.

The acidity of produced yogurt samples ranges from a minimum of 36 °SH (control yogurt) to the maximum 40.4 °SH (sample 0.06% AY) (Fig. 3).

Whey separation value decreased from 34 ml in the control sample, to 30 mL in samples where enzyme was activated (0.02%, 0.06% and 0.12% TG). The lowest whey separation was determined in samples with transglutaminase activation, regardless of the added amount of enzyme.

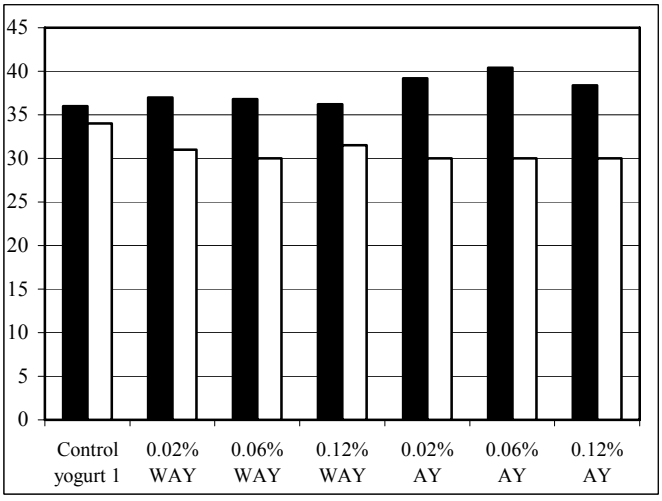

Fig. 3. Physico-chemical properties (●milk acidity(°SH) and ○whey separation(mL/50g)) of low fat probiotic yogurt (0.1% w/w fat) after production
Sl. 3. Fizičko-hemijske karakteristike (●mlečna kiselos(°SH) t i ○sinerezis surutke(ml/50g)) probitskog jogurta sa niskim sadržajem masti (0,1% m.m.), nakon proizvodnje

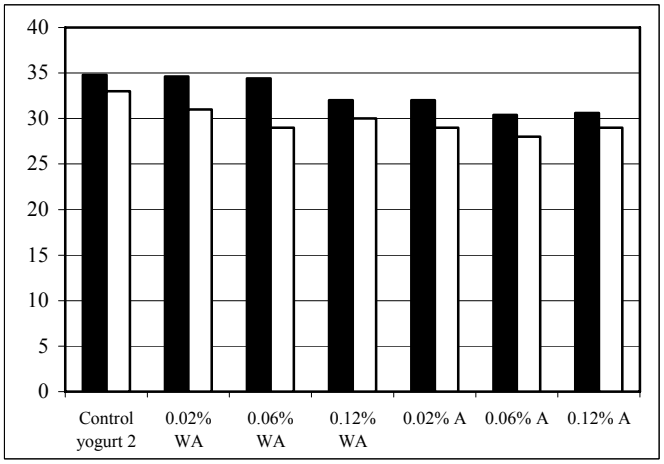

Fig. 4. Physico-chemical properties (●milk acidity(°SH) and ○whey separation(mL/50g)) of low fat probiotic yogurt (0.5% w/w fat) after production
Sl. 4. Fizičko-hemijske karakteristike(●mlečna kiselost(°SH) i ○sinerezis surutke(ml/50g)) probitskog jogurta sa niskim sadržajem masti (0,5% m.m.), nakon proizvodnje

Acidity of produced yogurt samples ranges from a minimum of 30.4 °SH (sample 0.06% A) to a maximum of 34.8 °SH (control yogurt) (Fig. 4).

The highest separation is recorded in the control yogurt (33 ml) (Fig. 4). Minimum whey separation values were in samples produced with a previous activation of enzymes ranging from 28 mL/50g (0.06% TG) to 29 mL/50g (0.02% and 0.12% TG).

Physico-chemical changes of low fat yogurt during storage

The physico-chemical changes of yogurt properties in the samples with 0.1% and 0.5% fat, during 10 days of storage, are shown in Figs. 5 and 6.

Comparing the results of diet probiotic yogurt analyses, presented in Figs. 3 and 5, it can be concluded that the acidity increased in all yogurt samples, as expected.

After ten days storage of diet probiotic yogurt samples (0.1% w/w fat) varying whey separation was noticed. Increase of TG concentration with previous activation of enzymes at 40°C during the 2 hours results in tendency of whey separation decrease.

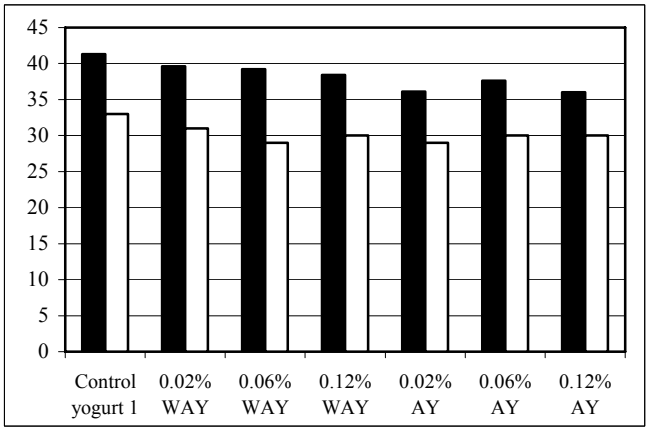

Fig 5. Physico-chemical properties (●milk acidity(°SH) and ○whey separation(mL/50g)) of low fat probiotic yogurt (0.1% w/w fat) during storage
Sl. 5. Fizičko-hemijske karakteristike(●mlečna kiselos(°SH) t i ○sinerezis surutke(ml/50g)) probitskog jogurta sa niskim sadržajem masti (0,1% m.m.), tokom skladištenja

During 10 days of storage the adicity of all probiotic yogurt samples (0.5 w/w fat) increased.

Whey syneresis was below average values by 3.36% in all yogurt samples. Minimum whey syneresis, 28 mL/50g sample, was found in probiotic yogurt samples with transglutaminase activation at 40ºC for 2 hours, regardless of the added enzyme amount.

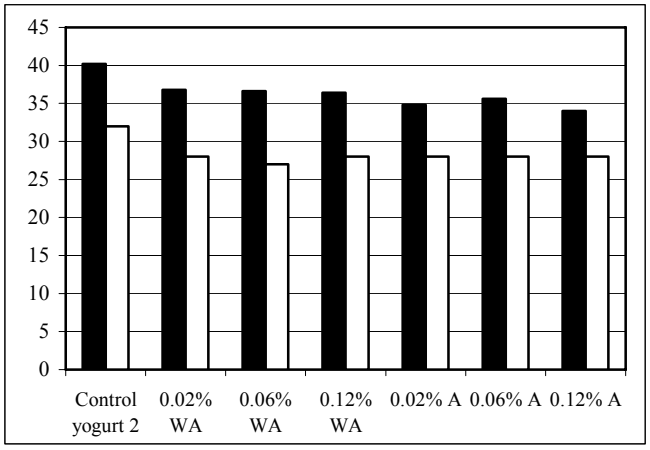

Fig. 6. Physico-chemical properties (●milk acidity (°SH) and ○whey separation (mL/50g)) of low fat probiotic yogurt (0.5% w/w fat), during storage

Sl. 6. Fizičko-hemijske karakteristike (●mlečna kiselost(°SH) i ○sinerezis surutke(ml/50g)) probitskog jogurta sa niskim sadržajem masti (0,5% m.m.), tokom skladištenja

Sensory analysis of low fat yogurt

The sensory analysis of probiotic yogurt samples produced with the application of TG with and without activation is shown in Figs. 7 and 8.

The best appearance, consistency and taste were found in samples of probiotic yogurt produced from milk of 0.1% milk fat with addition of 0.06% and 0.12% TG with previous activation (Fig. 7).

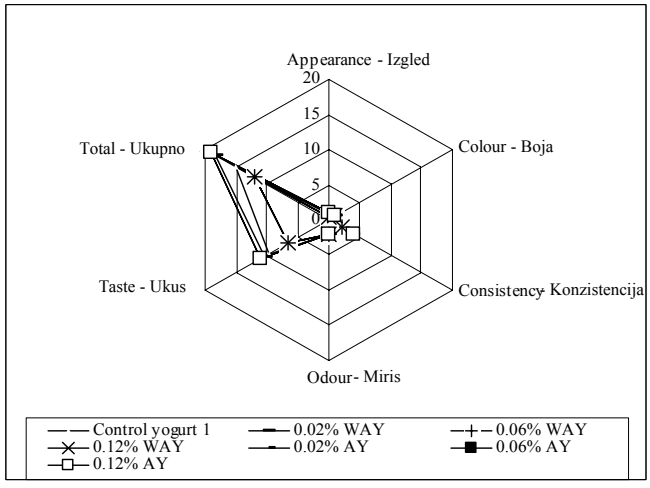

Fig. 7. Sensory evaluation of low fat probiotic yogurt produced from milk of 0.1% fat with TG

Sl. 7. Senzorna ocena probiotskog jogurta sa niskim sadržajem masti, proizvedenog od mleka sa 0.1% m.m. uz dodatak TG

Adition of 0.02% TG, with or without activation, resulted in better sensory characteristics of yogurt produced from milk of 0.5% milk fat compared to control sample (Fig. 8).

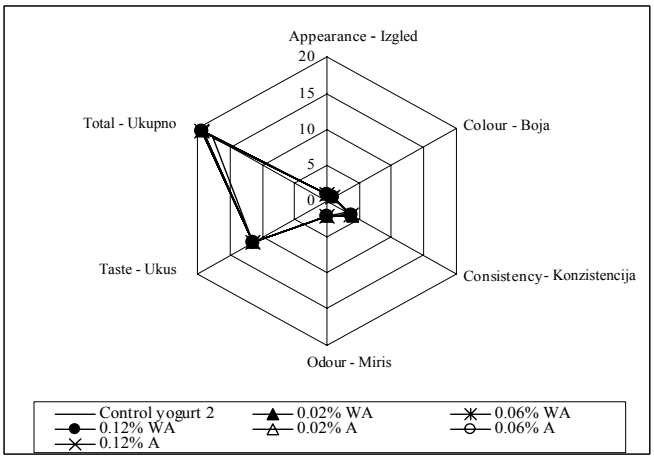

Fig. 8. Sensory evaluation of low fat probiotic yogurt produced from milk of 0.5% fat with TG addition

Sl. 8. Senzorna ocena probiotskog jogurta sa niskim sadržajem masti, proizvedenog od mleka sa 0.5% m.m. uz dodatak TG

Probiotic yogurt samples produced with application of 0.06% and 0.12% TG and with activation of enzyme had higher consistency than the yoghurt samples produced with 0.02% TG with previous activation of enzymes. Also, they had the best appearance, consistency and taste.

CONCLUSION

Different variaties of yogurt samples were produced applying appropriate technological processes from milk of 0.1% and 0.5% milk fat and with addition of chosen probiotic starter culture and different transglutaminase concentration: 0.02%, 0.06% and 0.12% with and without activation of enzyme at 40ºC for 2h.

The application of transglutaminase contributed to improvement of probiotic yogurt properties and reduced the tendency of whey separation during ten days storage.

In order to achieve high nutritive value of probiotic yogurt from milk with 0.1% milk fat, somewhat higher concentration (0.06% or 0.12%) of transglutaminase is necessary.

On the other hand, desirable physico-chemical and sensory characteristics of probiotic yogurt from milk of 0.5% milk fat were obtained by application of TG in concentrations of as low as 0.02%.

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