



## STARTER CULTURES EFFECT ON pH AND SH DYNAMICS OF INOCULUM DURING FERMENTATION PERIOD OF PROBIOTIC YOGURT

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### ABSTRACT

Fermented dairy products are produced by fermentation process of lactose by using microorganisms especially lactic acid bacteria. Probiotics are living microorganisms, which -ingested in sufficient amounts, beneficially influence the health of the host by improving the composition of intestinal microflora. Nowadays, the popularity of these products is growing, not only because of its organoleptic properties, but also because of its nutritional value and health benefits. Probiotic yogurt is a dairy product obtained by milk fermentation process, by adding a probiotic starter culture. The goal of this survey was to follow the pH and SH values of inoculum during fermentation period of probiotic yogurt manufactured with three different starter cultures, which are with the following commercial names: ABT-6, ABT-750 and ABT-10 consisting of *Streptococcus thermophilus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidus*.

**Key words:** probiotic yogurt, inoculum, active acidity, titrable acidity, fermentation period

### INTRODUCTION

Fermented dairy products are products that can be produced by lactose fermentation by microorganisms especially by lactic acid bacteria. Fermented dairy products are usually produced by using lactic acid bacteria and yeasts (Ozer and Kirmaci, 2010). Probiotics are defined as living microorganisms, which when ingested in sufficient amounts, beneficially influence the health of the host by improving the composition of intestinal microflora, (Ejtahed et al., 2011). Probiotic microorganisms that are known to be beneficial to human health can be ingested through fermented dairy products, enrichment of various foods with these bacteria and consumption of pharmaceutical products that are obtained by using viable cells, (Kanmani et al., 2013). Probiotics also are defined as viable microorganisms which can be consumed separately or with different foods, which assist dietary and microbial balance by regulating the mucosal and systemic immunity and beneficially affect the consumer's health,

(Gibson and Fuller, 2000).

The main milk function in the diet of young mammals is providing nutrients that are essential for their normal growth, development and also providing immune protection. Milk is a major source of protein, essential fatty acids, vitamins and minerals that are needed for normal functioning of the human metabolism. To improve the functional, sensory, nutritional, immunologic therapeutic and even technological properties of milk and dairy products substances are added in small amounts. This group of substances includes probiotics that always go together with substances called prebiotics. When combined with probiotics, the two become known as a symbiotic, which is a characteristic of foods that have both good bacteria and the strength to keep them going, (Trajchevski, 2012).

Yogurt is a dairy product with excellent nutritional value, and is a favorite food of all generations (Rašić and Kurmann, 1978). This

product can be consumed by people who are lactose intolerant. Yogurt is characterized by slightly sour taste and pleasant aroma. Specific taste comes from the created lactic acid, which comes from the work of bacteria during the fermentation process of the lactose, while the yogurt flavor comes from the presence of multiple chemical substances produced during fermentation of the lactose in milk.

If we want to produce probiotic yogurt with specific properties, we need to use different probiotic cultures. Recently for yogurt production "ready-set" cultures (dried, deep-frozen or freeze-dried) are used. The effect of this functional food increases by inoculation of

probiotic bacteria and prebiotic that operate in symbiosis. Probiotic product must maintain the vitality of probiotic culture during the production process, and also have to be sustainable and stable during storage time and shelf life of the product.

Probiotics are defined as a single or mixed culture of live microorganisms that have positive effect on human metabolism and also improve the properties of autochthonous microflora.

Titrateable and active acidity are very important parameters, which mostly affect the shelf life and acceptability of fermented dairy products (Mahmoudi et al., 2012).

## MATERIAL AND METHODS

### Milk composition

As a material for this research were used the following: standardized cow milk, (Table 1), 0.1% skimmed milk powder in an amount of 0.7%, three different types of frozen probiotic starter cultures in an amount of 0.2% (Chr Hansen, Copenhagen, Denmark) and addition oligofructose as prebiotic, in an amount of 1.5%.

**Table 1:** Chemical composition of standardized cow milk

Milk components (%)	
Protein	3.46±0.05
Milk fat	1.0 ±0.03
Lactose	4.53±0.01
Dry fat matter	9.0±0.06

### Probiotic starter cultures

The probiotic cultures that were used in this research paper (ABT-6, ABT-750 and ABT-10) were composed with the same types of bacteria, but in different proportion: *Streptococcus thermophilus* St-M5; *Lactobacillus acidophilus*, LA-5 and *Bifidobacterium bifidus*, BB-12; All starter cultures that were used during production process were prepared as operating instructions of the probiotic cultures manufacturer.

### Probiotic yogurt manufacture

Pasteurized cow milk (1.0 % standardized milk fat) was heated at 37°C. Three variants of

probiotic yogurt were produced (A, B and C). Variant A with probiotic culture ABT-6, Variant B – with probiotic starter culture ABT-750 and Variant C with probiotic starter culture ABT-10. All samples were inoculated with the activated starter culture (0.02% v/v) and prebiotic-oligofructose (1.5%) The fermentation was finished at pH 4.6 and the samples stored at 4±1°C for 21 days. First 24 hours the active and titrateable acidity of inoculum was measured.

### Measurement of active acidity

The pH (active acidity) was determined by using digital pH MP120 Meter, Mettler Toledo, Switzerland. After each usage the pH-meter was calibrated with buffer solution pH=7 and pH=4. The concentration of hydrogen ions present in the inoculum of all variants probiotic yogurt was measured during fermentation (in a period time of six hours).

### Measurement of Titratable Acidity

The measurement of titrateable acidity (oSH) was according the method described by Caric et al. (2000). In erelenmaer with transfer pipette 20 ml inoculum and 1 ml of 2% w/v solution of phenolphthalein. Content is titrated with 0.1 M NaOH solution till appearance of faint pink color that will not change in a period of 2 minutes. Acidification of inoculum was calculated by the formula:  $K = V \cdot 2$ , where: V-volume of NaOH spent during titration. The titrateable acidity of inoculum of three variants probiotic yogurt was measured during fermentation (in a period time of six hours).

### RESULTS AND DISCUSSION

Dynamic of inoculum titrable acidity during fermentation

Inoculum titrable acidity of three different variants probiotic yogurt, during fermentation process.

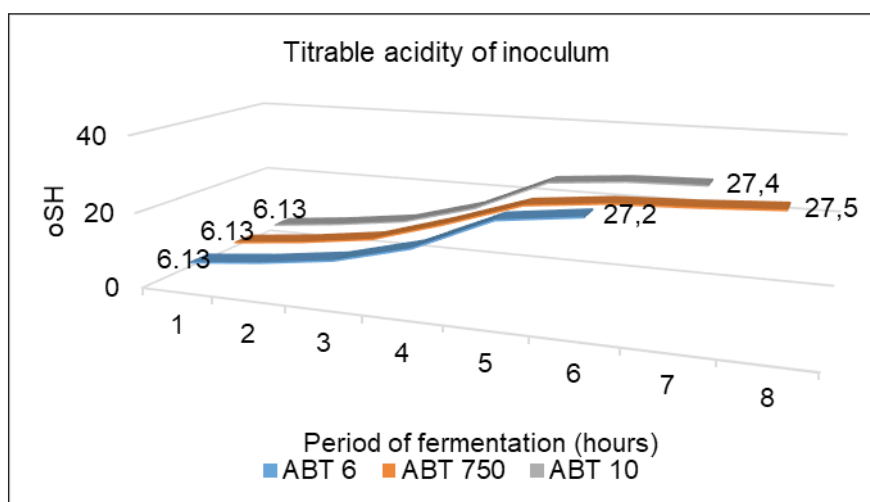
In Table 2 are presented the results of process.

**Table 2:** Dynamic of inoculum titrable acidity during fermentation

Starter culture	Titrable acidity of inoculum (n=5)							
	Period of fermentation (hours)							
	0	1	2	3	4	4.5	5	6
ABT-6	6.13±0.01	8.12±0.02	10.80±0.2	15.90±0.35	24.70±0.3	27.20±0.7	/	/
ABT-750	6.13±0.03	8.15±0.01	10.90±0.05	16.85±0.55	23.53±0.6	25.65±0.9	26.40±0.9	27.50±0.9
ABT-10	6.13±0.03	8.17±0.01	10.80±0.29	16.30±0.4	25.10±0.55	26.85±0.95	27.40±0.9	/

According to the results obtained in this research, constantly increasing of inoculum titrable acidity can be noticed. At the beginning of fermentation process the inoculum titrable acidity was almost equal in all three variants of probiotic yogurt (6.13 oSH). Minimal difference of titrable acidity between inoculums of three different probiotic yogurts was noticed, during the fermentation period. The time for achieving the required inoculum titrable acidity was different at all three examined variants probiotic

yogurt. The probiotic yogurt produced with starter culture ABT-6, has achieved the required titrable acidity of inoculum (27oSH) in shortest time, for four hours and thirty minutes, the probiotic yogurt produced with starter culture ABT-10, have achieved the required titrable acidity of inoculum in five hours and probiotic yogurt produced with starter culture ABT-750 have achieved the required titrable acidity of inoculum in six hours (Figure 1).



**Figure 1.** Dynamics of inoculum titrable acidity of probiotic yogurt variants.

#### Dynamic of inoculum active acidity during fermentation

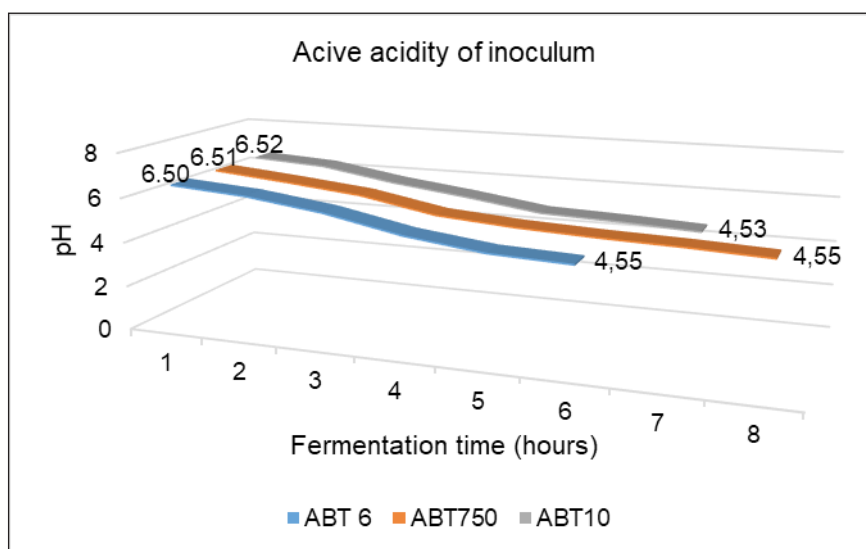
In Table 3 are presented the results of inoculum active acidity during fermentation process.

**Table 3.** The results of inoculum active acidity during fermentation process

Starter culture	Active acidity of inoculum (n=5)							
	Period of fermentation (hours)							
	0	1	2	3	4	4.5	5	6
ABT-6	6.50±0.02	6.26±0.01	5.81±0.07	5.12±0.08	4.69±0.02	4.55±0.02	/	/
ABT-750	6.51±0.06	6.20±0.05	5.85±0.05	5.21±0.05	4.95±0.01	4.80±0.03	4.70±0.03	4.55±0.03
ABT-10	6.52±0.02	6.31±0.03	5.75±0.03	5.32±0.07	4.82±0.03	4.70±0.02	4.53±0.03	/

According to the results obtained in this research, constantly decreasing of inoculum active acidity can be noticed. At the beginning of fermentation process the inoculum active acidity was almost equal in all three variants of probiotic yogurt (pH=6.50). Minimal difference of active acidity between inoculums of three different probiotic yogurts was noticed, during the fermentation period. The time for achieving the required inoculum active acidity was different at all three examined variants probiotic

yogurt. The probiotic yogurt produced with starter culture ABT-6, has achieved the required active acidity of inoculum (pH=4.55) in shortest time, for four hours and thirty minutes, the probiotic yogurt produced with starter culture ABT-10, have achieved the required active acidity of inoculum in five hours and probiotic yogurt produced with starter culture ABT-750 have achieved the required active acidity of inoculum in six hours (Figure 2).



**Figure 2.** Dynamics of inoculum active acidity of probiotic yogurt variants.

### CONCLUSION

The probiotic starter cultures that were used in the production process of three variants probiotic yogurt have a different time for achieving the required titrable and active acidity of yogurt inoculum. The probiotic starter culture ABT-6 has reached the required values for titrable acidity (27oSH) and active acidity (pH=4.55) of yogurt inoculum in shortest time, 4 hours and 30 minutes. The probiotic starter culture ABT-10 has reached the required values

for titrable acidity and active acidity in 5 hours and probiotic starter culture ABT-750 has reached the required values for titrable acidity and active acidity of yogurt inoculum in 6 hours. The probiotic starter cultures have significant impact on the time that is needed for achieving the necessary titrable and active acidity value for yogurt inoculum during fermentation process.

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## ВЛИЈАНИЕ НА СТАРТЕР КУЛТУРИТЕ ВРЗ ДИНАМИКАТА НА pH И SH НА ИНОКУЛУМОТ ЗА ВРЕМЕ НА ФЕРМЕНТАЦИЈА НА ПРОБИОТИЧКИ ЈОГУРТ

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### Резиме

Ферментираниите млечни производи се произведуваат преку процесот на ферментација на лактозата, со помош на микроорганизми, особено млечно-киселинските бактерии. Пробиотиците се живи микроорганизми, кои доколку се внесени во доволна количина во организмот позитивно влијаат на здравјето на домаќинот, преку подобрувањето на составот на интестиналната микрофлора. Денес популарно стана овие производи сè повеќе и повеќе да се зголемуваат, не само поради органолептичките карактеристики, туку и заради нивната нутритивна вредност и здравствени бенифити. Пробиотичкиот јогурт е течен млечен производ добиен преку процес на ферментација на млекото, со додавање на пробиотички стартер култури. Целта на истражувањето е да се следи активната (pH) и титрационата (SH) киселост на инокулумот за време на ферментациониот период кај три различни варијанти на пробиотички јогурт, кои се произведени со следните стартер култури: ABT-6, ABT-750 и ABT-10, кои во својот состав се состојат од *Streptococcus thermophilus*, *Lactobacillus acidophilus* и *Bifidobacterium bifidus*.

**Клучни зборови:** пробиотички јогурт, инокулум, активна киселост, титрациона киселост, ферментационен период.