Cukurova Medical Journal

Araştırma Makalesi / Research Article

Nutritional Composition and Protein Profile of Goat Yogurt PE with Double Culture between Streptococcus Thermophilus and Lactobacilus Species

Streptococcus Thermophilus ve Lactobacilus Bakterileri ile Yapılan İkili Kültürün Keçi Yoğurdu Besin Kompozisyonu ve Protein Profilileri Üzerine Etkisi

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Cukurova Medical Journal 2013; 38 (4): 681-686.

ABSTRACT

Aim: The aim of this study is to characterize the nutrient compositions and protein profiles of Etawah breed (PE) goat yogurt fermented by double cultures .

Material and Methods: To accomplish this, we used goat and bovine milk in five treatment groups: (1) fresh milk bovine, (2) goat,(3) milk fermented by *L. acidophilus* and *S. thermophilus* (LA + ST), (4) *L. bulgaricus* and *S. thermophilus* (LB+ ST),and (5) a commercial mixture. PE goat milk was fermented using 2.5% starting bacterial concentrations at 45^{o}C with a pH ranging from 4.5 to 6.6. Nutrient compositions were measured by proximate analysis.SDS PAGE was conducted using 15% separating and 3% stacking gels. To measure the density of protein bands, we used QuantityOne software.

Results: LA+ST and LB+ST treatments had higher levels of lipids than the control treatment. Conversely, both strain combinations had lower levels of proteins than the control. Organoleptic testing suggests that many attributes (e.g., colour, taste, smell, texture and viscosity) differ significantly from the control. Protein profiles revealed that while the LB + ST and commercial cultures contained proteins with a molecular weight of 36 kDa, the LA + ST cultures did not appear to possess this protein.Based on the molceular weight, we suggest that this protein is in the alpha casein group.

Conclusion: The protein composition of fermented goat and bovine milk is similiar, but the band with molecular weight 36 kDa from goat milk, is absent from the LB+ST and from the mix comercial.

Key Words: Casein Protein, Goat Milk, L. bulgaricus L. acidophillus S. thermophillus

ÖZET

Amaç: Bu çalışmanın amacı: Etawah cinsi (PE) keçiye ait yoğurdun, çift kültür ile fermente edilmesi sonucu besin kompozisyonunun ve protein değerlerinin belirlenmesidir.

Materyal ve Metod: Bu çalışmayı gerçekleştirmek için keçi ve büyükbaş hayvanların sütünü kullanılarak 5 farklı grup oluşturuldu: (1) taze inek sütü (2) keçi sütü (3) *L. acidophilus* and *S. thermophilus* (LA + ST) ile fermente edilmiş süt (4) *L. bulgaricus* and *S. thermophilus* (LB+ ST) ile fermente edilmiş süt (5) ticari süt . Etawah cinsi keçi sütü, 45 ^{o}C'de pH: 4.5-6.6'da %2.5'luk bakteri konsantrasyonu bulunan bakteri kültürü ile fermente edildi. Daha sonra besin konsantrasyonları proximate analizi ile belirlendi. Ayırma gücü %15 ve staking jeli %3'lük olan SDS PAGE ile yürütüldü. Protein bantaların yoğunluğunu ölçmek için QuantityOne yazılımı kullanıldı.

Bulgular: Çalışma LA+ST ve LB+ST ile muamele edilmiş kültürlerin kontrol grubuna gore daha yüksek lipid içerdiğini göstermiştir. Protein seviyeleriyse kontrol grubunda daha yüksek bulundu. Organoleptik test sonucunda da çalışma grubu ile kontrol grubu arasında renk, tat, koku, içerik ve yoğunluk gibi özellikler açısında önemli farklılıklar olduğu tesbit

edildi. LB + ST ve ticari kültürün içerdiği proteinin molaküler ağırlığı 36kDa iken, LA + ST kültürde bu protein tesbit edilemedi. Moleküler ağırlığa dayalı bu karşılaştırmada yeralan bu protein muhtemelen alfa kasein grubundandır.
Sonuç:protein kompozisyonu fermente edilen keçi sütü ve inek sütünde aynı fakat keçi sütünde bulunan 36kDa ağırlındaki protein LB+ST ve ticari sütte bulunmuyor.
Anahtar Kelimeler: Kazein Protein, Keçi sütü, L. bulgaricus ,L. acidophillus ,S. thermophillus

INTRODUCTION

Milk is one of healthy food with a lot of essential protein, but the milk production in indonesia are not high. Data from the Ministry of agriculture said that total milk production in the country around 350 thousand tons per year. This amount is still below the number of imported domestic milk that is as much as 1.5 million tons per year[1]. In Indonesia, most of the milk consumption of cow's milk. While cow's milk can cause allergies, allergy posed include: diarrhea, rash, red rash, and indigestion[2]. On the other hand, dairy goat milk Etawah breed that started much farmed in various parts of Indonesia, but has not been utilized[3].Goat Etawah breed is one of the considerable potential as a provider of good animal protein through meat or milk. Compared to cow's milk, goat milk has superior nutritional content, One alternative increasing diversification of Ethawah Breed Goat ‘s milk are extend the shelf life and increase the nutritional value and digested power is making yogurt from Ethawah Breed Goat ‘s milk by using a mixture of lactic acid[4]. The demand for goat's milk and its products has been growing in recent years because of its nutritional and health benefits. These include a higher digestibility and lower allergenic properties than cow's milk[5].

Fermentation technologies are currently developing rapidly, among others, characterized by pure culture of exploiting as single or double culture strains as well as the microbes that are resistant to pathogenic bacteria that can produce high quality milk fermentation and remained on every production. cycle Yogurt made with ferment using specific blends of BAL Streptococcus thermophillus with combination Lactobacillus bulgaricus and/or Lactobacillus acidophilus to produce the consistency of yogurt as a functional food[3]. The combination of several LAB will produce yogurt with active peptides for disease therapy Reumatoid arthithis.

MATERIALS and METHODS

Subject

Lactobacillus. bulgaricus FNCC 0050, S. thermophilus FNCC 0050, and L. acidophilus FNCC 0051 purchased from Gajahmada University (Gajahmada Culture Collection). All the strains were activated from their frozen forms (stored in 40% glycerol at -80 °C)Starter Culture to be used in the production of yogurt is among other things: Streptococcus thermophilus optimum grow temperature 38°C; Lactobacillus acidophilus and Lactobacillus bulgaricus grown at optimum temperature of 45°C[6].

Procedure

This study was approved by ethical review committee of Brawijaya University Research Ethics Committee.

The milk was pasteurised (80°C/5 min), cooled to 45°C -43°C, and inoculated with 2.5% yogurt double culture consisting of Streptococcus thermophilus and Lactobacillus acidophilus and Streptococcus thermophilus and Lactobacillus bulgaricus. The raw milk was divided into four lots are control negatif, control positive and 2 experimental (double cultures). The active acidity (pH) was determined after storage of yogurt. The syneresis of yogurt was studied immediately after storage of milk at 43–45°C for 4-6 hours.

Yogurt was analysis by proximat and organoleptik. Analysis of proximat include: levels of protein, lipid content, and carbohydrate levels. Organoleptic test used was hedonic and test scoring. Each panelist is expected to respond to his perception of color, taste, flavor, texture, and viscosity. The number of panelists being used is 10 people.

Protein Isolation ,Samples of milk 1000 µl, added solution of PBS-Tween-PMSF 5 ml. Mixed solution sonificated for 10 minutes with the amplitude of 20A. Then that solution centrifuged with a speed of 6000 rpm for 15 minutes. The Supernatan that produced is added cold ethanol solution with a 1:1 ratio, then stored in the refrigerator for 12 hours. Samples that had been stored for 12 hours, centrifuged with a speed of 6000 rpm for 15 minutes. The pellets were produced dried until the ethanol is vanishing. Sample added with Tris Cl, pH 6.8 (1: 1) if not used immediately is stored in a temperature of -20 ° C.

SDS PAGE using discontinuous system on separating gel 15 % and 3 % stacking gel. Protein sample yogurt 3.16 µl into the Tris-Cl added 20 µl RSB with a 1: 1 comparison. the sample heated for 5 minutes at a temperature of 100 ° c. The sample is inserted into the lane (± 30 µl). Running electrophoresis is carried out at constant current of 200 mA until the tracking dye reaches 0.5 cm above the base of the gel. The distribution of protein bands can be known by using the gel coloring is Coomasie Briliant Blue (CBBR 250). Each band of electrophoresis results calculated molecular weight.

Statistical Analysis

Statistical test used was a Kruskal wallis and Mann-Whitney analysis with degrees of freedom (df) 0.05.

RESULTS

Culture Bacteria and Microbiological Test

The Group of Lactic Acid Bakteria (LAB) had a diverse heterogeneous morphology, or shape and have a *bacil* short or long, and *cocci*, which became physical characteristics. Microbiological character *S. thermophilus* i.e. *cocci*-shaped, purple, including gram positive. The character *of L. acidophilus* and *L. bulgaricus* has *bacil-shaped*, purple and including gram positive. Optimum growth of different starter including *S. thermophilus* optimum growth at a temperature of 38 ° C, while the *L. acidophilus* and *L. bulgaricus* at temperature 45°C. All members of *Lactobacteriaceae* is gram positive and do not form spores generally non-motile and negative catalase.

The Amount Of starter *Sterptococcus thermophilus, Lactobacillus bulgaricus, and Lactobacillus acidophillus* show that the initial population is sufficient for use as starter cultures in production of yogurt that is approximately 10^6 to 10^7.

Table 1. Microbiological character and the starter population number of lactic acid bacteria (cfu/ml)

No	Starter Culture	Microbiological character	Population numbers cfu/ml
1	*Sterptococcus thermophilus*	*Cocci*-shaped, violet, Gram positive, optimum grow at temperature of 38 ° C, negative endospore, and negative catalase	1,51 x 10^6
2	*Lactobacillus bulgaricus*	*Bacil*-shaped, purple, Gram positive, optimum grow at temperature of 45 ° C, negative endospore, and negative catalase	4,53 x 10^6

3	*Lactobacillus acidophillus*	*Bacil*-shaped, purple, Gram positive, optimum grow at temperature of 45 ° C, negative endospore, and negative catalase	5,74 x 10^6

Proximat Analysis and Organoleptic Test

Based on the proximat analysis (Figure 1A) can be seen that the protein and carbohydrate levels of fresh goat's milk have the highest value compared to the double culture yogurt. Double culture yogurt LA+ST, LB+ST and mix using bacterial activity that break down carbohydrates and proteins into energy supply and lactic acid. Lactose is the only carbohydrate that has the enzyme lactate dehydrogenase for the forming of lactic acid and starter energy .Organic acids will reduce the pH of goat's milk yogurt. Furthermore, lactic acid bacteria have a secondary lipolytic activity that is considered important after other microorganisms reduce lipids into simpler components .

The organoleptic testng can be seen figure 1B. The physical appearance of double culture yogurt (LA+ST and LB+ST) with organoleptic analysis based on hedonic and scoring test can be seen in Figure 1B. Mix commercial yoghurt has the highest average score. The best flavor is produced by culture with combination of *L .bulgaricus.* The main component in yogurt is acid from lactic acid and flavor produced by Lactobacilli .In the process of fermenting *S. thermophilus* will form lactic acid, the compound of diasetil and asetoin which gives the taste and flavor of yogurt, while *L. bulgaricus* only form lactic acid, so the use of this starter affect to acid taste and flavor yoghurt that produced.

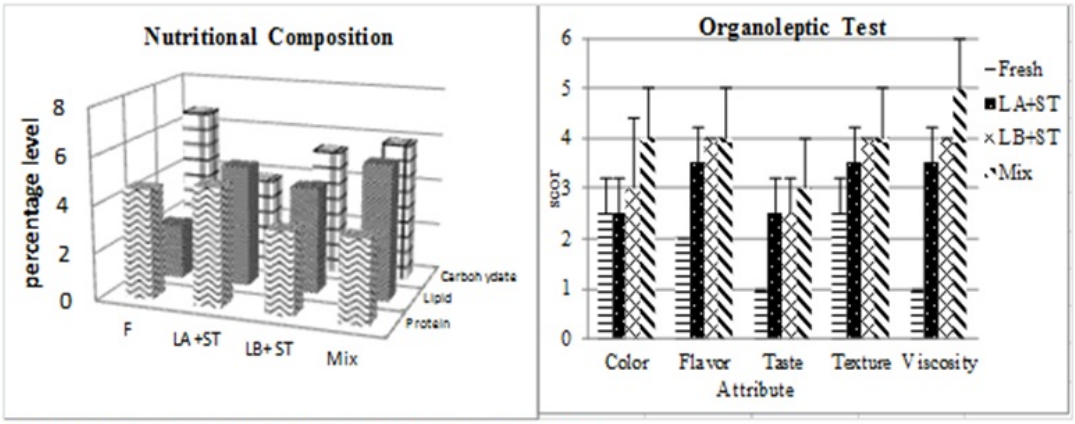

Figure 1. Yoghurt Analysis A : Proximat B : Organoleptic Test

*(*Ismi *et al., 2013)*

The higest score of yoghurt viscosity appearance can be found in mix commercial yogurt. The fresh goat milk has low viscosity because there is no addition of starter bacteria.

The addition of double culture bacteria increase the value of viscosity, it might be due to the effect of proteolytic enzymes from two symbiotic bacteria that can break down polypeptide bonds shorten and the protein was denatured until become solids.The difference in level of preference panelists probably from the combination of some starter from the mix commercial yogurt starter which the bacteria is still unknown.

The data obtained processed by categories attribute of yogurt and analyzed using Microsoft

Excel and SPSS for Windows 15:00 software, Statistical test used was a Kruskal wallis and Mann-Whitney analysis with degrees of freedom (df) 0.05. Kruskal wallis test count was found that the value sig 0,113 > 0.05, then there are not differ significantly between organoleptic tests including color, flavor, taste, texture, and viscosity. Analysis of Mann Whitley with with degrees of freedom (df) 0.05 and variable appearance of yogurt (character) count was found that the value sig 0,004 < 0.05. Thus, there is a differ significantly between the combination starter: fresh goat *L.acidophilus* + *S.thermophilus* , *L.bulgaricus* + *S.thermophilus*, and Mix.

The results of electrophoresis double culture yogurt *L. bulgaricus* and *S. thermophilus* (Figure 2A) by SDS PAGE have migration patterns with equation y= -0.098 x + 1.6348.The protein bands betwen fresh goat and fresh bovine are differnly, in fresh goat have protein band with molecular weigh 36 kDa and fresh bovine did not appear to possess this protein. Based on molecular weight belongs to the goat milk, LB + ST_1, LB + ST_2 and mix commercial. The density protein band was measured quantitatively the 562.88 INT/mm^2 . While the yogurt culture *L. acidophilus* and *S. thermophilus* can see in figure 2B has a pattern of migration with the equation y = x +-0.0336 2.0496. Yogurt with cultures LA + ST_1, LA + ST_2 ,LA + ST_3 shows the existence of protein band with molecular weight 34 kDa with density protein band 692.47 INT/imm^2.Subunits of proteins with molecular weights of the emerge of the be running gels, whereas subunits with a molecular weight of will appear at the bottom.

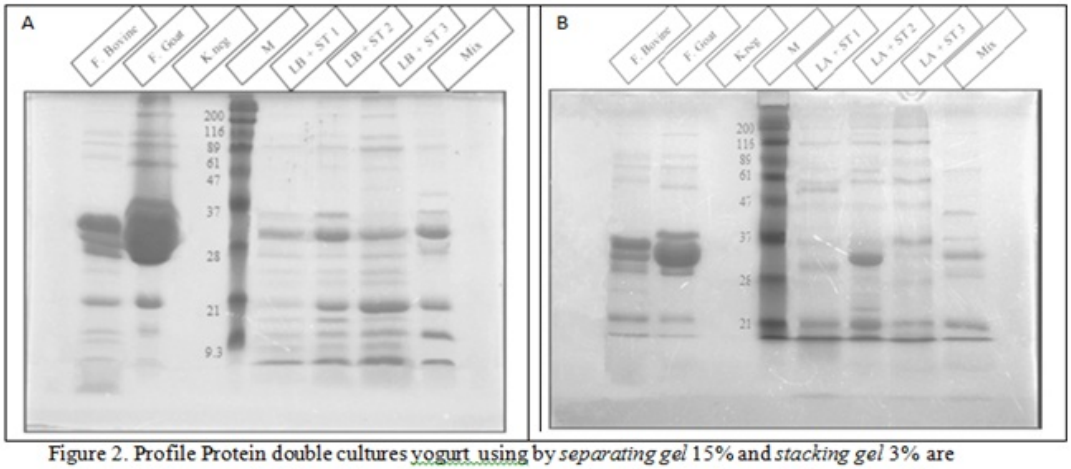

Figure 2. Profile Protein double cultures yogurt using by *separating gel* 15% and *stacking gel* 3% are stained with *Commassie Briliant Blue* A: double culture yogurt *L. bulgaricus* and *S. thermophillus* concentration of 1.64 mg/ml B: double culture yogurt *L. acidophillus* and *S. thermophillus* concentration of 1.1 mg/ml

(Ismi et al.,2013)

DISCUSSION

The number of total population of microorganisms that live and active frozen yogurt to reach the number of 10^8 cfu / g, while the manufacture of frozen yogurt at least the number of active bacteria must reach 10^7 cfu / g^8. In order to obtain the desired therapeutic effect, the number of active probiotic organisms in food minimum 10^6 cfu / ml. This figure is recommended to compensate for the possible reduction in the amount of probiotic organisms during the trip to the intestine[7]. According to Chilliard said that levels of lipid from fermented lactic acid bacteria have a secondary lipolitic activities are considered important as other microorganisms reduced fat milk into simpler components. Symbiosis double

cultured *S. thermophilus* will form lactic acid, the compound that gives asetoin diasetil and smell and typical yogurt flavor, while *L. bulgaricus* only form lactic acid, so that the use of a starter this affect the taste and flavor[9]. BM from casein protein will separated properly when using SDS-PAGE or urea-PAGE, with a range of BM 25 - 38kDa. Both protein bands 36 kDa was suspected as the protein α-casein: goat CSN1S1 or CSN1S2 protein[10].

In summary, Nutritional yogurt double culture between *S. thermophilus* and *L. acidophilus* or *L. bulgaricus* treatments had higher levels of lipids than the control treatment. Conversely, both strain combinations had lower levels of proteins than the control. Profil protein by SDS PAGE showed band protein α casein in LA + ST : 34 kDa and LB+ ST 36 kDa with density 692.47 INT/mm^2 and 562.88INT/mm^2.

Acknowledgements

This research is supported in part by grant of the RUPT-BOPTN UB-Decentralization of Directorate of General Higher Education, Ministry of Education and Culture of Republic Indonesia. We thank to Biosains Laboratory UB for providing the laboratory equipment for research analysis.We acknowledged to Jeffrey W. Streicher (University of Texas, Arlington) and Bambang Setiawan M.Biomed (Lambung Mangkurat University, Banjarmasin) for manuscript correction.

Conflict of Interest Statement

Theres no conflict of interest statement

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geliş tarihi/received :05.03.2013
kabul tarihi/accepted:18.04.2013