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CHARACTERISTICS OF PROBIOTIC KOUMISS FROM GOAT MILK WITH ADDITION OF ROSELLE EXTRACT (*Hibiscus Sabdariffa* Linn)

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ABSTRAK

Susu kambing dan rosella (*Hibiscus sabdariffa* Linn*)* dikenal masyarakat sebagai bahan makanan yang memiliki sifat fungsional untuk kesehatan. Penelitian ini menggunakan *Lactobacillus acidophilus* RRAM-01 dan *Lactococcus lactis* RRAM-01 sebagai bakteri probiotik, dan *Saccharomyces cereviceae*. Tujuan penelitian ini adalah mempelajari karakteristik koumiss (produk fermentasi) probiotik susu kambing dengan penambahan ekstrak rosella. Hasil analisis menunjukkan bahwa penambahan ekstrak rosella yang berbeda (0%, 0,5%, dan 1%) pada koumiss probiotik susu kambing tidak mempengaruhi karakteristik kimia maupun mikrobiologi koumiss. Pengolahan susu kambing menjadi koumiss dapat menekan pertumbuhan koliform dan memiliki aktivitas penghambatan yang kuat terhadap Escherichia coli ATCC 25922 melalui uji difusi sumur. Analisis mikrobologi menggunakan metode agar tuang. Populasi bakteri asam laktat menunjukkan bahwa koumiss telah memenuhi standar produk probiotik (10^{11} cfu/mL), koliform tidak terdeteksi, sehingga sesuai dengan kriteria Badan Pengawasan Obat dan Makanan (BPOM) sebagai pangan fungsional probiotik. Berdasarkan uji mutu hedonik penambahan ekstrak rosella meningkatkan intensitas warna merah dan kekentalan koumiss, namun tidak berpengaruh terhadap aroma tape dan rasa asam koumiss.

Kata kunci : koumiss, probiotik, rosella (Hibiscus sabdariffa L), susu kambing

ABSTRACT

Goat milk and roselle (*Hibiscus sabdariffa* Linn) are food ingredients with health functional properties. This research used *Lactobacillus acidophilus* RRAM-01 and *Lactococcus lactis* RRAM-01 which had been proven as probiotic, and *Saccharomyces cereviceae*. *Saccharomyces cereviseae* was used to produce specific flavor components in koumiss (bacterial fermentation product). The objective of this research was to study the characteristics of goat milk probiotic koumiss added that with roselle extract. The results showed that the addition of different concentration of roselle extract (0%, 0.5%, and 1%) in the goat milk probiotic koumiss had no effect on chemical and microbiological characteristics of koumiss. Goat milk processed into koumiss had ability to suppress the growth of coliform and had strong inhibition activity against *Escherichia coli* ATCC 25922 as tested by agar diffusion test. Microbiological analysis used in this research was the pour plate method. Population of lactic acid bacteria indicated that the product fulfilled standards as probiotics koumiss (10^{11} cfu/mL), coliform was not detected in the products, therefore the products met the criteria of The National Agency of Drug and Food Control (NA-DFC) as probiotic functional food. Sensory evaluation was conducted by using hedonic quality test. Based on the hedonic quality test, addition of different concentrations of roselle extract increased the intensity of red color and koumiss viscosity, but had no effect to the aroma and sourness of koumiss.

Keywords : goat milk, koumiss, probiotic, roselle (Hibiscus sabdariffa Linn)

INTRODUCTION

Koumis is a bacterial fermentation product, derived from middle Asia and usually made from horse's milk fementation with lactic acid and yeast (Seydim *et al*., 2010). Common bacteria used as culture starter was lactic acid bacteria that made antimicrobial substrates (lactic acid, bacteriosin, alcohol and H_2O_2) and has antagonistic characteristic with pathogen bacteria. In Rusia, koumis had been used as medicine for TBC caused by *Mycobacterium tuberculosis* (Tamime, 2006).

Koumis is traditional beverage that has well known yet to people in the world and the production is still in limited geography (Malacarne *et al*., 2002). It is caused by the limited supply of horse milk production. The solution is to produce koumiss from other animal milk like goat milk.

Horse milk production in Indonesia is limited in supply and certain area also the production is still low. Dairy goat is considered a second rank in milk production after cow's milk. Korhonen and Anna (2006) reported that goat milk protein contain bioactive component such as *angiotensin conventing enzyme* (ACE), *inhibitory peptides* and *peptide hypertensive*. The component has effect to give immunity for non-immune disease and to control microorganism infection (Hayes *et al.*, 2007). According to the last research, goat milk fermentation can decrease *Mycobacterium tuberculosis* growth because it had the function as natural antiseptic in human body; this property is similar to horse milk fermentation (Pana, 2004).

Red roselle (*Hibiscus Sabdariffa L*) is a plant with many functions for our health. Roselle contains some nutrition as non hypertension, antioxidant and high content of vitamin C helps digestion process. Roselle has been used at agent therapy in treating cancer, treating hypertension and its lipid-lowering. In addition, roselle can also improve sensory value of the product (Kustyawati and Ramli, 2008). The research about characteristics of goat milk probiotic koumiss added with roselle extract is one way process to know the characteristics of goat milk as raw material to replace horse milk in koumiss production and the effect of roselle to the characteristic of koumiss product. The functional properties of roselle for health is usefull to improve functional characteristic of fermented milk for human's health therapy. The purposes of this research were to evaluate the chemical and microbiology characteristics, antimicrobial activity, and sensory quality of koumiss probiotic goat milk with different treatments of roselle extract (*Hibiscus Sabdariffa L*).

MATERIALS AND METHODS

Roselle Extraction

Dry roselle flower was prepared and smoothed in blender. Twenty gram of roselle powder was put in Erlenmeyer and mixed with 100 mL aquadest. The solvent of roselle was heated in waterbath at temperature of 60ºC for 30 min (Hermawan *et al..,* 2010).

Koumiss Production

Goat milk was produced from Faculty of Animal Science's farm. One liter of goat milk was pasteurized at 65°C for 30 min, then cooled to a temperature of 28°C. Koumiss starter culture was divided into three equal parts, each part of milk was inoculated with *Lc. lactis, Lb. acidophilus* and then incubated at 37°C for 7 h and *Sc. cereviceae* at 25°C for 5 h, respectively. Next, it was mixed *Lc. lactis*, *Lb. acidophilus* and *Sc. cereviceae* as much as 3-5% (v/v) into the goat pasteurized milk. The mixture of milk and starter cultures were incubated at 28°C for 24 h to form the desired starter (modified method of Rahman *et al*., 1992). To produce koumiss, one liter of goat milk was pasteurized at 65°C for 30 min, then inoculated with a starter (5%) after temperature reached 28°C. Then, it was incubated at 25°C for 18 h (modified method of Astawan *et al*., 2012) (Figure1).

Chemical characteristics Analysis

The chemical composition of a processed product was the criteria for assessment the quality of the product. Koumiss characteristic was analyzed by using AOAC method (1995) consisted of pH, total acidity (TA), water, ash, protein, crude fiber, and fat contents.

Microbiology Characteristics Analysis

The method used in koumiss microbiology enumeration was pour plate method (Fardiaz, 1992). Colony microbe was enumerated based on Plate Count Standard (PCS) based on BAM (2011).

Antimicrobial activity by Agar Diffusion Test

The inhibition test to *E. coli* bacteria used

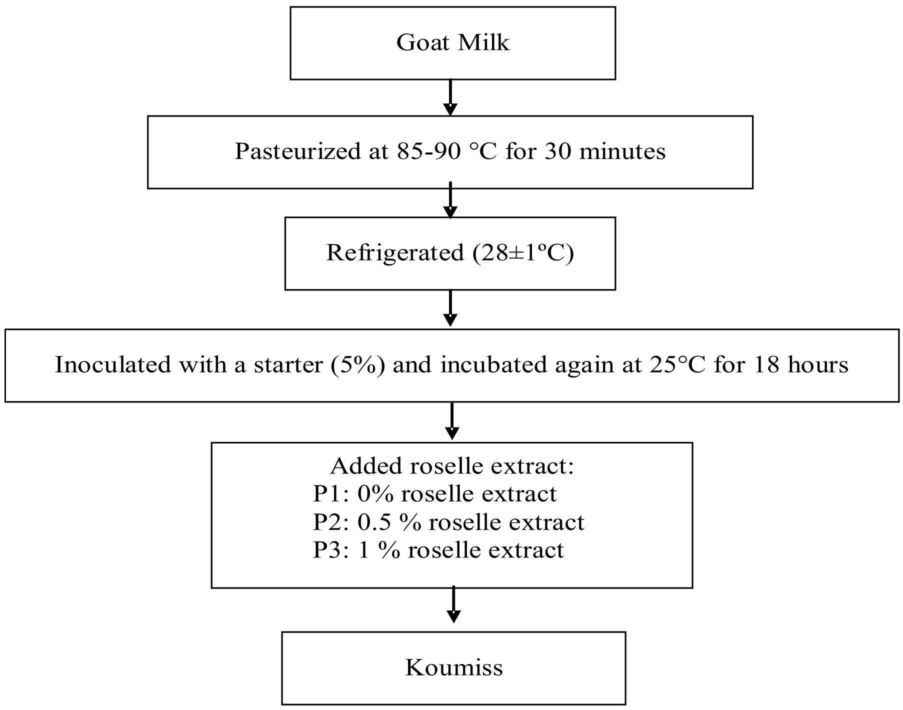

Figure 1. The Process of Producing Koumiss Product

agar diffusion test (Arief *et al*., 2012).

The Test of Sensory Characterictic

The sensory characteristic test used was the hedonic quality test. The test consisted of color, aroma, viscosity, and sourness. Panelists used were trained panelist about 30 people. Three samples of koumiss with different roselle extract concentration had already marked with specific code were given to panelists. The scale of value that used in the test was 1, 2, 3, and 4.

Statistical Analysis

The experimental design used in this study was a completely randomized design (CRD). Treatment being tested was the different percentage addition of roselle extract (0%, 0.5%, and 1%). Each treatment was repeated for three replication and duplo for each replicate. Parametric data were analyzed using analysis of variance (ANOVA) and data were analyzed using the nonparametric kruskal-wallis at the 95% significance level using software statistix 8. Tukey test used for significantly difference (P<0.05) tukey test (Steel and Torrie, 1980). Mathematical model used was according to Matjik and Sumertajaya (2000) as follows:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$$

Where:

Y_{ij} : The response obtained from standard treatment and replicates i-th to j-th

μ : Average value

αi : Effect of treatment to level-i

ε_{ij} : Error

RESULTS AND DISCUSSION

Morphological Examination and Total Bacteria Colonies

Koumiss starter culture examination was aimed to study the characteristics of the cell wall (Gram staining), morphology (form and structure) and to determine the total population from each bacteria. Microbial strains used in this study consisted of *Lactococcus lactis* bacteria RRAM-01, *Lactobacillus acidophilus* RRAM-01 and yeast *Saccharomyces cereviceae*. Test results and characteristics of the total population of each bacteria is presented in Table 1.

Lactic acid bacteria population had met the minimum number required in the manufacture of fermented milk that was equal to 10^8 cfu/mL (Salminen and Wright, 1998). Calculation of population of each type of bacteria, was performed on the starter koumiss and the result

Table 1. Characteristics and Total Microbial Population

Bacteria	Population (cfu/mL)	Gram stain	Morphological
Lactococcus lactis	1.06 x 10^{10}	Gram-positive	Globular, short or long chain.
Lactobacillus acidophilus	6.40 x 10^{11}	Gram-positive	Rod-shaped, short-chain chains.
Saccharomyces cereviceae	2.50 x 10^{8}	-	Oval-shaped, single-cell morphology

was population of total LAB and yeasts were 2.80 x 10^{12} cfu/mL and 5.20 x 10^{11} cfu/mL, respectively.

Test results showed that the starter culture koumiss demonstrated suitability to the morphological characteristics of each bacteria and yeast. Bacterium *Lactococcus lactis* and *Lactobacillus acidophilus* was belongs to the group of Gram-positive lactic acid bacteria, according to Chandan *et al.* (2008)

Chemical Characteristics of Koumiss

The nutritional quality of fresh goat milk was met to SNI (2009). Water content of fresh goat milk was 84.25 %wb, ash content was 0.78 %wb, peotein content was 3.24 %wb and fat content was 6.54 %wb. Coliform was below of SNI (2009).

Koumiss is a type of fermented milk generally made from fermented horse milk using lactic acid bacteria and yeasts. Lactic acid bacteria (LAB) plays important role in producing lactic acid and yeast which is responsible for producing ethanol and carbon dioxide (Surono, 2004). Koumiss has similarity with kefir, because koumiss also produces lactic acid and alcohol as well as kefir. The difference lies in the structure of starter cultures used in the manufacture of both types of milk fermentation, where the starter koumiss is not granular (Wang *et al*., 2008). Based on the pH value, koumiss is grouped into three types: strong koumiss (pH 3.3-3.9), medium (pH 3.9-4.5), and mild (pH 4.5-5) (Danova *et al*., 2005). The research used application of goat's milk as a raw material for making probiotic koumiss and the addtion of roselle flower extract (*Hibicuss sabdarifa L*) as the treatment. Goat milk is milk from a healthy mother goat udder and obtained with the good way (BSN, 1992). Goat milk composition per 100 g that used in this research was 87.34% moisture, 0.8% ash, 5.22% fat, 3.9% protein, and 0.19% crude fiber based on wet weight.

Chemical analysis include total pH, total acidity (TA), moisture content, ash content, protein content, fat content, and crude fiber content. The results presented in Table 2. The main results of the metabolism of lactic acid bacteria fermentation were the lactic acid that reduced pH of the final product. The decrease in pH and increase in TA was occurred due to accumulation of organic acid metabolism. Koumiss pH values ranged from 3.6 to 3.8. Based on the pH value, the resulting koumiss was included in the group of strong koumiss (pH 3.3-3.9) (Danova *et al*., 2005). Low pH value was due to the production of organic acids by *Lactococcus lactis* and *Lactobacillus achidopillus*. Total acidity value of koumiss with different treatments fulfilled Indonesian standards for flavour fermented milk which were 0.5-2% (BSN, 2009). The addition of roselle extract had no significant different (P>0.05) on pH value and TA.

Addition of roselle extract had no significant effect to the moisture, ash, protein, fat and crude fiber content. This was caused by addition of small percentage of extract (0%, 0.5%, and 1%). Levels of protein and fat in goat milk koumiss were higher than horse milk koumiss. Koumiss mare's milk has a protein content of 21% and a fat content of 12% (Seydim, 2010). This was caused by differences in the nutritional composition of mare's milk with goat milk (per 100 g). Protein and fat content of goat milk is higher than horse milk (Chandan *et al*., 2008).

Microbiological Characteristics Koumiss

Microbiological characteristics observed were total lactic acid bacteria (LAB), total yeast and total coliform (Table 3). Koumiss microbiological characteristics of goat's milk with

Table 2. Chemical Characteristics of Koumiss Probiotic Goat Milk

Variable	Koumiss with roselle extract		
	0%	0.5%	1%
pH	3.72 ± 0.06	3.78 ± 0.01	3.76 ± 0.06
Total Acidity (TA)	1.52 ± 0.05	1.56 ± 0.05	1.58 ± 0.04
Water (% wb)	85.72 ± 0.14	85.82 ± 0.39	85.67 ± 0.32
Ash (% wb)	0.78 ± 0.056	0.76 ± 0.046	0.78 ± 0.040
Protein (% wb)	4.04 ± 0.171	4.09 ± 0.387	3.91 ± 0.257
Fat (% wb)	6.56 ± 0.137	6.38 ± 0.533	6.60 ± 0.457
Crude fiber (% wb)	0.11 ± 0.038	0.16 ± 0.053	0.30 ± 0.164

Table 3. Microbiological Characteristics of Goat Milk Probiotic

Variable	Koumiss with increasing roselle extract ($\log_{10}$ cfu/mL)		
	0%	0.5%	1%
LAB	11.42 ± 0.73	12.49 ± 1.33	13.61 ± 0.31
Khamir	11.37 ± 1.39	12.55 ± 0.37	12.88 ± 0.51
Coliform	0	0	0

the addition of probiotic and roselle extract had no significant different (P>0.05) in relation with the amount of LAB, yeasts, and, coliforms. Lactic acid bacteria used in this study was *Lactococcus lactis* and *Lactobacillus achidopillus*. Each LAB had a high population so that the total population of the final product was relatively high. This result was accomplied with yoghurt probiotic added *Lactobacillus achidopillus* could increase population of lactic acid bacteria of the product (Astawan *et al*., 2012). *Lactobacillus achidopillus* RRAM-01 and *Lactococcus lactis* RRAM-01 was a probiotic bacterium. *Lactobacillus acidophillus* have been proven as probiotic because it has benebicial fuction such as it could give antimicrobial activities againts pathogenic bacteria (Arief *et al*., 2010), and could prevent hematology health status of rat (Astawan *et al*., 2011) According to the result, LAB populations had met with international standards for koumiss probiotic drink that was a minimum of 10^7 cfu/mL (Davidson *et al*., 2000). Probiotics are non pathogenic microbes which when it ingested, produced a positive impact on the health and physiology of the host (Commane *et al*., 2005). Probiotic bacteria can affect the physiology directly or indirectly by stimulating the immune system (Dewater, 2003). Total populations of LAB and yeasts koumiss were higher than the total population of the initial starters. These were due to the synergistic relationship between the yeast (*Saccharomyces cereviceae*) with LAB (*Lactococcus lactis* and *Lactobacillus achidopillus*). Lactic acid bacteria had the ability to break down complex component into simpler components, one of which was lactose. Lactose in milk was broken down into simple sugar monomer called glucose and galactose. The sugars could be utilized by yeast as a source of energy, because the yeasts prefer simple sugars rather than complex components, for its growth (Seydim *et al*., 2010).

In addition, fermentation by *Lactococcus lactis* and *Lactobacillus achidopillus* produced lactic acid, thereby creating acidic conditions (lower pH) in the fermentation medium. This condition was useful for *Saccharomyces cereviceae*, because *Saccharomyces cereviceae* prefered acidic conditions than alkaline conditions (Bennett *et al*., 1999). The existence of *Saccharomyces cereviceae* was profitable for *Lactococcus lactis* and *Lactobacillus achidopillus* due to metabolites produced by *Saccharomyces cereviceae*, namely organic compounds (ethanol) and carbon dioxide. Carbon dioxide balanced oxygen level in the medium and ethanol was able to break down toxic H_2O_2 into non-toxic compounds with the aid of peroxidase. Therefore, the yeast will produce CO_2 which can balance the levels of oxygen in the medium. The statement was supported by Gilliland (1986) which states that excessive oxygen in the growth of bacteria can cause a decrease in cell viability, due to excess oxygen to form hydrogen peroxide which are toxic to cells.

Microorganisms often used as an indicator of sanitation in food were coliform bacteria. Coliform in fresh goat milk was about 2.5 x 10^2 cfu/mL, while coliform in koumiss was not present at all treatments due to the possibility that colliform would not survive at a low pH. Coliform decrease in koumiss was caused by acid production from LAB and roselle extract. Acid would decrease pH to below pH range of bacteria growth and these acids were in the form of not dissolated and could rapidly diffuse into the microbial cell, causing the cell to be damaged. Furthermore acids dissolated would break down into anions and protons, where the proton (H^+) will be entered into the cell that causes the metabolic function is compromised, such as the acidification of the cytoplasm, inhibition transfer substrate, synthesis of macromolecules and overall growth bacteria is inhibited (Dewater, 2003).

Inhibitory activity against *Escherichia coli* by roselle extract and antimicrobial koumiss was seen by observing inhibition zone against pathogenic bacteria *E. coli* with the well diffusion agar method. The amount of inhibition zones was shown in Table 4.

Wide minimum inhibitory zone was one mm, one positive (+) when the clear area between 2-5 mm and strong inhibitory activity (+ +) when more than five mm (Jacobsen, 1999). Based on the inhibition zone diameter, koumiss had strong inhibitory activity against *E.coli. Lactobacillus acidophillus* could inhibit pathogenic bacteria with strong activities such enteropathogenic E. coli by *in vivo* analysis (Arief *et al*., 2010).

Table 4. Inhibition Zone of Koumiss and Roselle Extract against *E. coli*

Sample	Inhibition Zone (mm)
Roselle extract	19.37 ± 2.03
Koumiss	8.46 ± 1.10
Koumiss 0.5 % Roselle extract	9.48 ± 1.95
Koumiss 1% Roselle extract	8.08 ± 0.77

Roselle and koumiss extract could inhibit pathogenic bacteria *E.coli* in a population of 10^6 cfu/mL, this also supported by Rostinawati (2009) which showed that the roselle flower had antibacterial activity. Inhibition zone formed on roselle extract was greater than the inhibition zone formed by koumiss which reached zone diameter of 19.37 ± 2.03 mm. This was due to high concentration of flavonoids and organic acids that occured in roselle extract like inhibiscus acid, ascorbic acid, malic acid and protokatekin acids (Arelano *et al*., 2004). Inhibition occured because the content of antimicrobials in koumiss were in the form of organic acids, bacteriocins and alcohol (Surono, 2004).

Organoleptic Characteristics of Koumiss

Organoleptic test performed in this study was hedonic quality test. The parameters used were color, aroma alcoholic aroma, consistency and flavor. Koumiss organoleptic test results is presented in Table 5.

Hedonic quality test results showed that the addition of roselle extract treatment was significant (P<0.05) on the color of koumiss. Koumiss with the addition of roselle extract was intended from white to pink. The color difference was caused by the differences concentration in the addition of roselle extract. The red color on koumiss was formed due to the red pigment in the roselle anthocyanin (Astawan and Andreas, 2008).

Hedonic quality test results showed that the addition of Roselle extract treatment had no significant effect (P > 0.05) to the koumiss aroma. Aroma on koumiss was included into weak aroma. Aroma was due to a number of volatile components derived from the products that can be detected by the sense of smell (Rachmawati,

Table 5. Organoleptic Characteristics of Probiotic Goat Milk Koumiss

Variable	Koumiss with addition roselle extract		
	0%	0.5%	1%
Colour	1.00 ± 0.00[a]	1.50 ± 0.51[b]	1.93 ± 0.52[c]
Tapay aroma	2.17 ± 0.75	1.97 ± 0.72	2.10 ± 0.76
Viscosity	2.17 ± 0.65[a]	2.00 ± 1.02[ab]	2.63 ± 0.81[b]
Sour taste	2.40 ± 0.77	2.37 ± 0.76	2.77 ± 0.77

Color: 1 = white; 2 = pink; 3 = red; 4 = very red. Tapay aroma: 1 = very weak; 2 = weak; 3 = strong; 4 = very strong . Viscosity: 1 = very dilute; 2 = rather thick; 3 = thick; 4 = highly viscous. Sour taste: 1 = not sour; 2 = slightly acid; 3 = acid; 4 = highly acidic.

Different small superscript in the same row indicate significantly different results (P <0.05).

2001). Koumiss aroma associated with total acid and alcohol content. Ester component koumiss aroma was affected by the reshuffle resulting from alcohol. Ester produced from the reaction between organic acids with alcohols (Hesseltine, 1979).

Hedonic quality test results showed that the addition of Roselle extract treatment had significant (P<0.05) effect on the viscosity koumiss which was not added roselle extracts and with added 1% koumiss roselle extract. Differences in viscosity at koumiss was influenced by the addition of acid formation due to differences in roselle extract, which caused protein denaturation process at the isoelectric pH so that proteins precipitated to form a gel (Sodini *et al*., 2002).

Hedonic quality test results showed that the addition of Roselle extract treatment had no significant effect (P > 0.05) to the sour taste in koumiss. These results were consistent with the results of testing the acidity (pH) koumiss which also showed that the addition of roselle treatment had no significant effect to the acidity.

CONCLUSION

The addition of roselle extract different had no affect to the chemical and microbiological characteristics goat milk koumiss probiotic. Koumiss goat milk could suppress the growth of coliform and had a strong inhibitory activity against *E. coli*. Population koumiss lactic acid bacteria in probiotic products had met the standards (10^7 cfu/mL). Based on the pH value, the resulting koumiss was strong koumiss group. Hedonic quality tests showed that the addition of roselle extract was capable in increasing red color and viscosity of koumiss.

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