



Analysis of Proximate and Protein Profile of Kefir from Fermented Goat and Cow Milk

Analisis Proksimat dan Profil Protein Kefir Susu Kambing dan Susu Sapi

✉ Erwin Hidayat, Irna Kinayungan W, Muhammad Irhas, Fathurrahman Sidiq, R. Susanti

DOI: 10.15294/biosaintifika.v7i2.3950

Department of Biology, FMIPA, Semarang State University, Indonesia

History Article

Received July 2015
Approved August 2015
Published September 2015

Keywords:

Milk; Proximate analysis; kefir protein

Abstract

This research aims to analyze the characteristics of proximate and protein profile in kefir from fermented goat milk and cow milk with different concentration of kefir grains. The research design was true experimental with Completely Randomized Design (CRD) of 3 repetitions. The research procedures consisted of kefir production, proximate analysis and protein profile characterization. Proximate assay result was analyzed by using LSD, whereas the protein profile was analyzed by descriptive qualitative method. Based on the analysis of kefir proximate levels, the kefir grain (5%) showed the highest proximate level of both kefir from goat milk and cow milk. The analysis of protein profile of cow milk kefir showed 75 kDa of protein ribbon, while the goat milk kefir showed 48 kDa, 60 kDa and 75 kDa. Therefore it can be concluded that the proximate level of goat and cow milk kefir with different concentration of kefir grains showed significant differences in the nutrition content as well as its protein profiles.

Abstrak

Tujuan dari penelitian ini adalah menganalisis karakteristik proksimat dan profil protein pada kefir hasil fermentasi susu kambing dan susu sapi dengan konsentrasi biji kefir yang berbeda-beda. Penelitian ini adalah eksperimen murni, dengan Rancangan Acak Lengkap (RAL) 3 kali ulangan. Prosedur penelitian meliputi pembuatan kefir, analisis proksimat dan profil protein. Data hasil proksimat dianalisis uji BNT, sedangkan profil protein dianalisis deskriptif kualitatif. Berdasarkan analisis kadar proksimat kefir, kefir grains 5% menunjukkan kadar proksimat paling tinggi baik pada kefir susu kambing dan susu sapi. Sedangkan analisis profil protein kefir susu sapi menunjukkan pita protein 75 kDa, pada kefir susu kambing yaitu 48 kDa, 60 kDa dan 75 kDa. Simpulan dari penelitian ini bahwa kadar proksimat kefir susu kambing dan susu sapi dengan konsentrasi kefir grains yang berbeda menunjukkan perbedaan kandungan yang berbeda secara signifikan dengan konsentrasi kefir grains yang paling optimal yaitu 5%. Sedangkan profil protein kefir susu sapi ditemukan pita protein yaitu 75 kDa, dan kefir susu kambing yaitu 48 kDa, 60 kDa dan 75 kDa.

© 2015 Semarang State University

✉ Correspondence Author:

Gedung D6 Lt 1. Kampus Unnes Jl. Raya Sekaran Gunungpati, Semarang 50229
Telp/Fax.8508033 E-mail: erwinhidayat819@yahoo.co.id

p-ISSN 2085-191X

e-ISSN 2338-7610

INTRODUCTION

Milk is essential for human's health, since it consists of many important substances needed by humans such as carbohydrates (lactose), proteins, fats, vitamins, and minerals (Safitri & Swarastuti, 2011). Milk is very sensitive to the effect of physical and microbiological influence so it is easily damaged and has short shelf life. Meanwhile, the development of food technology nowadays provides alternative milk processing as dairy product diversification in order to extend the shelf life of milk. One of the technologies developed is the fermented milk and one of its products is kefir.

Kefir is a fermented milk product that has specific flavor as fermented lactic acid bacteria and yeast that live together and has mutual benefit relationship. Kefir is very beneficial for the body for example to provide good nutrition and inhibit the growth of bacterial pathogens (Zakaria, 2009). The physical changes, nutrient component changes, and production of primary and secondary metabolites happened during the fermentation process. In the fermentation process through the presence of a microbial enzyme activity, components such as starch, fat, protein, toxic substance, and other compounds can be broken. This fermentation technique is widely applied in food, especially milk, because it can increase its nutrients and bring positive influence on health (Khalil, 2006). In addition, in the fermentation process of kefir, the sugar lactose is broken down into glucose and galactose, so lactose intolerance problem can be overcome (Effendi et al, 2009 & Greppi et al, 2008).

However, the manufacture of kefir from goat or cow milk is rarely explored even though society considers that kefir fermentation from goat and cow milk is very beneficial, because it is allegedly expected that there is an active bio peptide that can be used to treat disease. So this research was conducted to analyze the proximate compound and protein profile of kefir from goat milk and cow milk fermented by kefir grains that have the potential to increase the nutritional value as a functional food containing of active bio peptide compound.

METHODS

This research design was true experimental research with Factorial Design of A x B (4x1) with basic design of Completely Randomized Design (CRD) of 3 repetitions. Factor of treatment given was: (A) The distribution of kefir grains (2.5%, 5%, 7.5% and 10%) and (B) duration

of incubation (24 hours).

Kefir Production

One L of goat milk and one L of cow milk was boiled and stirred at temperature of 80 ° C then waited until it was cold. Then milk was divided into 4 containers and added the kefir grain with concentration of 2.5%, 5%, 7.5% and 10% and they were incubated for 24 hours. After that it was filtered process and the appropriate filtered results were used as material of proximate test (fat content, kh and proteins) and protein profile.

Fat Level Test

The method used was Gerber method. The step was putting 10.75 ml of sample into the butyrometer and adding 10 ml of H₂SO₄ 91-92% and 1 ml of amyl alcohol. Then, butyrometer was closed with rubber stopper and shake gently until it was homogeneous and heated at 65-70°C for 10 minutes. After that the centrifugation was done for 5 minutes, and reheated for 5 minutes. Fat content was read on scale in the butyrometer by inserting or removing the rubber stopper gradually to get scale of zero on the boundary between fat and other substances (Selundik et al, 2011).

Protein Level Test

The method used was Formal Titration method consisting of putting 10 ml of sample Erlenmeyer flask, then adding few drops of phenolphthalein 1% and saturated potassium oxalate. Then it was titrated with NaOH 0.1 N solution until resulting re-formed pink color. The amount of used NaOH was recorded as *p* ml. Blank titration and used NaOH was recorded as *q* ml. The protein content was calculated by using the formula of (*pq*) x 1.70 ml (formal factor) (Selundik et al, 2011).

Carbohydrate Level Test

10 ml of kefir added by 50 ml of distilled water and lead acetate were diluted up to 100 ml and filtered. Its filtrate added by Na₂CO₃ was diluted up to 250 ml, then shaken and filtered. Next, 25 ml of filtrate was put into Erlenmeyer by using pipette and added by Luff Schorl solution, then boiled for 10 minutes, cooled and added by 15 ml of KI 20%, 25 ml of H₂SO₄ 25% and titrated with Na₂S₂O₃ 0.1 N until turning pale yellow, and added by starch indicator and finally titrated until turning into white milk, and noted the volume of Na₂S₂O₃ (Barus, 2005).

Protein Isolation

1 ml of sample was added by 4mm PMSF + PBS-T of 5 times of the volume. The solution

mixture was sonicated with 20% amplitude for 10 minutes, then centrifuged at speed of 6000 rpm at temperature of 4 ° C for 15 minutes. Supernatant was added by cold ethanol solution (1: 1), then stored at temperature of 4 ° C for 12 hours. The sample then was centrifuged at a speed of 6000 rpm at temperature of 4 ° C for 15 minutes. Pellet was dried to release ethanol then added by Tris-HCl pH 6.8 (1: 1), and stored at temperature of -20 ° C (Khoiriyah & Fatchiyah, 2013).

SDS PAGE Analysis

SDS-PAGE was used with the discontinuous system in 15% separating gel. Protein sample was measured its levels of protein by using Nanospectro added by Tris-Cl pH 6.8 and Reducing Sample Buffer (1: 1). The sample was heated at temperature of 100 ° C for 5 minutes, Running electrophoresis was conducted at constant current of 200 mA for 95 minutes. Ribbon distribution was determined by gel staining of Coomassie Brilliant Blue (CBBR-250) (Khoiriyah & Fatchiyah, 2013).

Data Analysis

Proximate assay result was analyzed by using LSD, whereas the protein profile was analyzed by descriptive qualitative method.

RESULTS AND DISCUSSION

Proximate Level of Goat and Cow Milk

Kefir is fermented milk fermenting by the number of microbes of bacteria producing lactic acid (BAL), acetic acid-producer bacteria, and yeasts. Kefir is made through microbial fermentation process by using bacteria and yeast (Winarno & Inone, 2007). Based on the analysis of proximate level by using Anova and LSD it showed significant differences. The analysis result is presented in Table 1.

Based on proximate analysis of goat milk kefir it showed difference of different proximate content to the use of different kefir. The use of

5% kefir grains showed the best proximate level compared to the use of 2.5%, 7.5% and 10% kefir grains. Then fermented kefir grains were proved containing 5% of carbohydrate 21.3%, 8.65% of fat and 5.26% of protein. Meanwhile the proximate analysis results of cow milk kefir showed difference of different proximate content to the use of different kefir. The use of 5% kefir grains showed the best proximate level compared to the use of 2.5%, 7.5% and 10% kefir grains. Fermented 5% kefir grains were proved containing 7.28% of carbohydrate, 10.6% of fat and 3.75% of protein.

These results indicated that the proximate level of goat and cow milk kefir was higher than pure milk's. According to Buckle et al. (1987), during fermentation, carbohydrate, protein, fat and nucleic acid can be broken down into simpler components and affected to the flavor and texture of food. Component first attacked by the bacteria is carbohydrates then the protein and fat. These components will be degraded to simple compounds, lactose component will be broken down into glucose and galactose, protein into amino acids and fat into fatty acids.

Protein Profile of Goat and Cow Milk Kefir

Results of protein analysis of goat and cow milk kefir showed the presence of different protein ribbon. Only one protein ribbon of 75 kDa was found cow milk kefir, whereas three protein ribbons of 48 kDa, 60 kDa and 75 kDa were found in goat milk kefir. The giving of concentrations of different kefir grains did not result difference on the isolated protein ribbon. Each visible protein ribbon showed different thickness. The thicker the protein ribbon, the higher its protein concentration, and the thinner protein ribbon the lower its protein concentration (Aristya et al. 2013).

Analysis of 75 kDa protein ribbon of cow milk kefir was lactoperoxidase protein, while goat milk kefir showed more varied results of 48 kDa was α casein and 60 kDa was k-casein and 75 kDa was lactoperoxidase protein (Tay & Gam

Table 1. ANOVA and LSD Test Result

Kefir Grains (%)	Goat Milk			Cow Milk		
	Carbohydrate (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Fat (%)	Protein (%)
2.5	7.3967 ^a	5.6167 ^a	5.3167 ^a	6.54 ^a	5.8 ^a	3.6167 ^a
5	21.3033 ^b	8.65 ^b	5.26 ^{ab}	7.2867 ^b	10.6 ^c	3.75 ^b
7.5	14.6067 ^c	7.4 ^c	3.15 ^c	10.1767 ^d	5.3 ^{ab}	3.74 ^{bd}
10	15.7 ^d	6.3767 ^d	3.19 ^{cd}	7.4067 ^{bc}	4.5333 ^d	3.68 ^{ac}

Note: different letters in each section show significant difference.

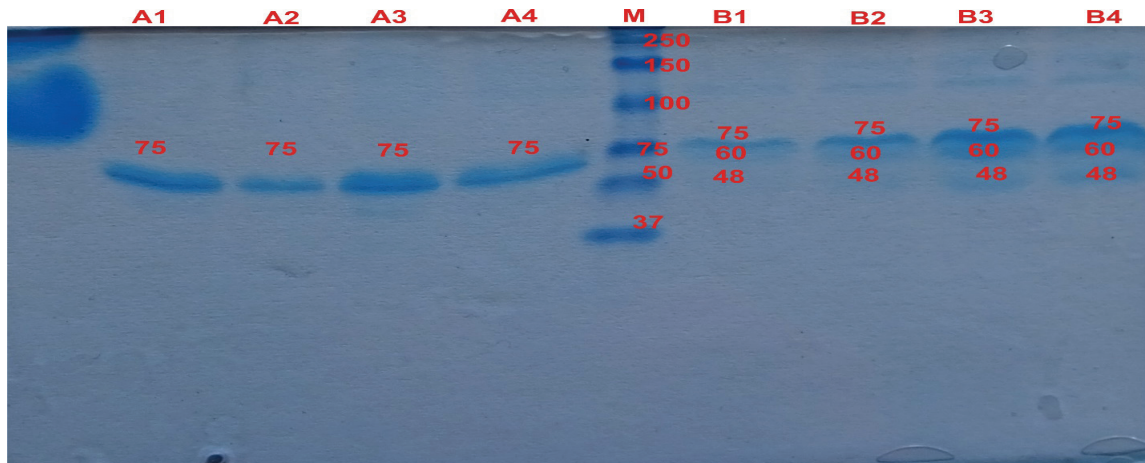


Figure 1. A. Cow Milk Kefir, A1. Kefir Grains 2.5%, A2. . Kefir Grains 5%, A3. Kefir Grains 7.5%, A4. . Kefir Grains 10%. B. Cow Milk Kefir, B1. Kefir Grains 2.5%, B2. Kefir Grains 5%, B3. . Kefir Grains 7.5%, B4. Kefir Grains 10%. M. Marker.

2011). Differences of type and weight of protein molecule in goat milk kefir were caused by the process of glycation between carbon groups of reduction sugar and free amino acid of milk protein in Maillard reaction to form heavier protein molecule weight. This is related with Diftis & Kiosseoglou (2006) opinion, that explained the Maillard reaction between protein and polysaccharide can result heavier protein molecule weight. According to Van Boekel (2001) factors affecting results of Maillard reaction are the heating time, pH, water activity, intrinsic properties of protein and sugar, and ratio of amino acid group with reduction sugar.

CONCLUSION

The Proximate level of goat and cow milk kefir with different concentration of kefir grains showed significantly different content to the most optimal kefir grains concentration of 5%. While 75 kDa protein ribbon was found in protein profile of cow milk kefir, and 48 kDa, 60 kDa and 75 kDa were found in goat milk kefir.

ACKNOWLEDGEMENT

We would like to say thank you to Directorate General of Higher Education which has provided grant funding of research 2014.

REFERENCES

- Aristya AL, Anang M, Legowo & Baarri AN. (2013). Total Acid, Total Yeast, Protein And Profile Kefir Goat Milk, With Addition Concentration Of Sugar In Different Level. *Jurnal Pangan dan Gizi* 4 (7): 39-47.
- Barus, P. (2005). Studi Penentuan Kandungan Karbohidrat, Protein Dan Mineral Dalam Air Rebusan Beras Sebagai Minuman Pengganti Susu. Medan: Universitas Sumatera Utara. *Jurnal Sains Kimia (Suplemen)* Vol. 9 No. 3, 2005: 15-16
- Buckle KA, Edwards RA, Fleet GH & Wootton M. (1987). *Ilmu Pangan*. Jakarta: UI-Press.
- Diftis, N & Kiosseoglou, V. (2006). Stability against heat-induced aggregation of Emulsions prepared with a dry-heated soy protein isolate-dextran mixture. *J. Food Hydrocolloids* 20(6): 787-792.
- Effendi, MH Hartini, S & Lusiastuti, AM. (2009). Peningkatan Kualitas Yogurt Dari Susu Kambing Dengan Penambahan Bubuk Susu Skim Dan Pengaturan Suhu Pemeraman. *J. Penelit. Med. Eksakta*. pp. 185-192.
- Greppi, GF, Roncada, P & FortiN, R. (2008). *Protein Components of Goat's Milk*. Dairy Goats Feeding and Nutrition. Italy. pp.71-94.
- Khalil, AA. (2006). Nutritional Improvement Of An Egyptian Breed Of Mung Bean By Probiotic Lactobacilli. *J. Biotechnology. Egypt*. pp.206-212.
- Khoiriyah, LK & Fatchiyah. (2013). Karakter Biokimia dan Profil Protein Yogurt Kambing PE Difermentasi Bakteri Asam Laktat (BAL): Malang: Universitas Brawijaya. *J.Exp. Life Sci*. Vol. 3 No.3
- Safitri, MF & Swarastuti, A. (2011). Kualitas Kefir Berdasarkan Konsentrasi Kefir Grain. Semarang: Universitas Diponegoro. *Jurnal Aplikasi Teknologi Pangan* Vol. 2 No. 2
- Salundik, Suryadi, Mansjoer, SS, Soepandi, D & Ridwan, W. (2011). Analisis Kualitas Fisik dan Kimia Susu Sapi Perah dengan Pakan Klobot Jagung dari Limbah Organik Pasar. Bogor: ITB. *Agrista* Vol.15 No.3
- Tay, Eek-Poei & Gam, LH. (2011). Proteomics of human and the domestic bovine and caprine milk. *J. Mol. Biol. Biotechnol.*, 19, 45-53. Van Boekel,

- M. A. J. S. 2001
- Van Boekel, M. A. J. S. (2001). Kinetic aspects of the Maillardreaction: A critical review. *J. Nahrung.* 45 : 150-159
- Winarno FG. & Fernandez IE. (2007). Susu dan Produk Fermentasinya. Bogor: M-BRIO press.
- Zakaria, Y. (2009). Pengaruh Jenis Susu dan Persentase Starter yang Berbeda terhadap Kualitas Kefir. Banda Aceh: Fakultas Pertanian Universitas Syiah Kuala. *Agripet* Vol. 9 No. 1:26-30