Journal of Applied Food Technology

Home page : https://ejournal2.undip.ac.id/index.php/jaft

The Effect of Different Type Drying Methods on Chemical Characteristics and Microbiology of Goat Milk Powder Kefir

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Abstract

The research aims to test and compare the chemical characteristics and total Lactic Acid Bacteria or LAB of goat milk powder kefir that was produced from cabinet drying, freeze drying and spray drying. Kefir was made from goat milk since it was found as a good digestibility, no β-lactoglobulin, and high protein. Data analysis was performed with the test analysis of variance with a significance level of 5% followed by Duncan testing if significant result was detected. Analysis of total acid was conducted using titration. Analysis of proteins was conducted using Kjeldahl method. Water content analysis was performed using oven and ash content was analyzed using furnace. Analysis of fat content was conducted using Soxhlet method. Analysis of total LAB and total yeast were done using Total Plate Count (TPC). As results, drying method significantly affected ($p<0.05$) water content, ash content, and total LAB but there was no significant effect ($p<0.05$) on the total acid, protein content, fat content, and total yeast. As conclusion, the best treatment of drying method in making goat milk kefir powder was spray drying.

Article information:
Received: 10 December 2019
Accepted: 28 May 2020
Available online: 01 June 2020

Keywords:
kefir powder
drying
goat milk

doi:10.17728/jaft.6699

Introduction

Kefir is one of the fermented products, made of several raw ingredients such as cow milk, goat milk, buffalo milk, seeds and fruits, added with kefir grains as a starter which there are LAB and yeast that are bound in the matrix polysaccharides (O'Brien *et al*., 2016). Kefir is also known as a probiotic drink, in general, kefir is made by pasteurizing milk and then inoculated with kefir grains (seeds of kefir) which contain a collection of LAB (Lactobacilli, Lactococci, *Lactobacillus kefir*, *Lactobacillus parakefir*, *Lactobacillus kefiranofirgum*, *Lactobacillus bacteria* and *Lactobacillus kefiranofacien*). Kefir gives health benefits because it acts as inhibitor for the bacteria growth, may causes digestive system disease, in addition, it is able to reduce blood cholesterol levels and increase High Density Lipoprotein (HDL) (Ostadrahimi *et al*., 2015) .

Goat milk has several differences compared to cow milk, the one that make it difference is in fat and protein content. Goat milk has a better digestibility when compared to cow milk due to small fat granules with size less than 1.5 μm and it consists of short and medium chain fatty acids (Schettino *et al*., 2017). Goat milk contains fatty acids which are classified as volatile fatty acids. The total content of volatile fatty acids in goat milk is higher than in cow milk, buffalo milk and horse milk (Erkaya and Sengul, 2011).

Three types of drying methods were used in this study: freeze drying, spray drying, and cabinet drying. Freeze drying is one of the drying methods which has advantage in maintaining drying product quality (Emami *et al*., 2018) besides minimize humidity, no changes in aroma, color and other organoleptic elements, inhibit microbial activity, and prevent chemical reactions which can damage the nutritional content of food (Bourdoux *et al*., 2016). Spray drying converts liquid products into dry powder products using spray system with hot media resulting in evaporation and turning into powder. Spray drying are fast drying cycles, short product retention and the final result is ready to be packaged when the drying process is finished (Wan and Yang, 2016).

Cabinet dryer uses hot air in a closed chamber. The vacuum dryer utilizes a pump for air blowing while the tray dryer utilizes heat flowing on the surface (Aktaş

et al., 2017). The advantage of using cabinet drying is relatively cheap in operating cost (Huddar and Kamoji, 2018). The use of optimum drying should consider the kefir quality such as total acid, protein content, water content, ash content, fat content, total LAB, and total yeast and since less study was found in kefir with variation in dryer, therefore this study was aimed at determining the effect of different drying methods on total acid, protein content, water content, ash content, fat content, total LAB, and total yeast on goat milk kefir powder.

Materials and Methods

This study was conducted in March-November 2019 at the Laboratory of Food Chemistry and Nutrition and the Laboratory of Food Engineering and Post-Harvest, Faculty of Animal Husbandry and Agriculture, Diponegoro University, Semarang.

As much as 21 liters of fresh goat milk and 1 kg kefir grain were purchased at Omah Kefir Ungaran, 0.1 N NaOH solution, H_2SO_4, selenium, NaOH, phenolphthalein, MRSA media, PDA (Potato Dextrose Agar), MRMB, physiological NaCl, ether, aquadest, and aluminum foil. Cabinet dryers (NCD-5, Dongguan Naser Machinery CO., LTD., China), freeze dryers (2018.11.16, ShunDi food., Ltd, Shanghai, China), and spray dryers (OLT-SD8000B, Ollital Technology, Fujian, China), kjeldahl flask, destruction equipment, distillation apparatus, soxhlet tool, furnace, autoclave (Hirayama, Japan), and incubator (Memmert, Germany).

Kefir Manufacture

Previous method (Ot'es and Cagindi, 2003) was used to produce kefir with several modifications. Fresh goat milk was pasteurized at 70° C for 15 seconds and allowed to stand until 27°C, then inoculated with a 5% kefir grains starter. Stirring was then applied until well mixed prior to incubation at room temperature (±27°C) for 24 hours. The kefir was then filtered to separate the kefir grains from the kefir and then proceed with the drying step.

Drying Process

Freeze drying method referred to the method of Zhao and Zhang (2005) with modification. Spray drying process was carried out in a 0.06 mbar T vacuum freeze dryer T ice condenser, frozen in -45°C for 36 hours with temperature inlet was set in 120°C (Aponte *et al.,* 2016). Drying treatment by the cabinet drying method refers to the Pratama *et al.* (2017) with modification by kefir was poured on a baking sheet and put in a cabinet drying at a temperature of 50°C for 24 hours. Then the dried kefir was milled using miller machine (Beater bar-FCT-Z300, Maksindo, Indonesia) for 45 seconds up to 1 minute until the kefir powder was produced.

Total Titratable Acid Analysis

The total titratable acid analysis was carried out using a method that refers to Campbell-Platt (2009) using the titration method which is expressed as a percentage of lactic acid. Goat milk kefir powder samples were prepared and it was rehydrated until 10 ml was obtained and taken into an erlenmeyer, then added with 2-3 drops of phenolphtalein then titrated using 0.1 N NaOH. Titration was stopped until it constantly obtained pink color. Total acid was determined by calculating the molar mass of the acid in the solution as the sum of mass of all atoms in the molecule. Multiply the volume of the standard solution of NaOH by its concentration to determine the number of moles of the titrant was used for the titration. Number of moles is volume (in L) x applied concentration (mol/L).

Total Protein Content Analysis

Total protein content analysis was measured by the Kjeldal method (Sudarmadji *et al.,* 1997). A sample of 0.5 gram was put into a kjeldal flask then it was added with 10 ml of concentrated H_2SO_4 and 0.5 gram selenium then it was destructed until the color turned into clear green then followed by distillation. The distillation results were then titrated using 0.1 N HCl until the color changes into purple and determined the blank form. Protein levels was calculated using the formula as described in Chang and Zhang (2017).

Water Content Analysis

Water content was measured by the drying-oven method (AOAC, 2005). As much as 2 grams of kefir powder samples were prepared and put into a porcelain cup, which had measured its empty weight with an analytical balance then each sample and cup were put into the oven and dried at 105° C for 4 hours. After it reached 4 hours drying oven, the sample was removed from the oven, next it was put in a desiccator for 15 minutes and weighed with an analytical balance. The water content was determined by calculating the weight subtraction between the samples before and after drying, and then was divided by the sample's weight, and finally multiplying it with 100%.

Total Ash Content Analysis

Total ash content analysis refers to Sudarmadji *et al.* (1997). The empty porcelain cup was weighed first, then the kefir powder was weighed as much as 2 g. The kefir was dried with a furnace at 500-600° C for 3 to 5 hours. Next, the furnace was turned off and awaited until reached the lower temperature and weighed the final result with analytical balance. Determination of ash content was carried out by the final weight be diminished with the porcelain weight, and then it was divided by the sample's weight, and finally multiplying it with 100%.

Total Fat Content Analysis

Total fat content analysis refers to AOAC (2005). Samples were weighed 1.5 g in filter paper (as weight A) and wrapped. The wrapped sample was dried in an oven at 105°C for 4 hours then weighed with analytical balance (as weight B). The sample was put into a fat flask and extracted by using ether solvent for 6 hours. After that, the sample was dried in an oven at 105 ° C for 1 hour and it was weighed with analytical balance (as weight C). Percentage of total fat content was determined by calculating the difference weight between dried and extracted dried sample. Then it was divided by the sample weight.

Total LAB Analysis

LAB analysis used TPC method with MRSA media (Yunivia *et al*., 2019). Kefir powder sample was weighed as much as 1 gram and then dissolved in 10 ml of distilled water. Next, 1 ml of sample was put into a test tube containing 9 ml of physiological NaCl 0.85% as a dilution of 10^1. Dilution was then carried out to 10^4. At the last 3 dilution, carried out a search in which 1 ml of sample was put into a petri dish and added with 15 ml of sterile MRSA medium and mixed well. After solidifying, the plates were incubated at 37°C for 24 hours.

Total Yeast Analysis

Total yeast testing referred to Yunivia *et al.* (2019) using TPC method. The kefir powder sample was weighed as much as 1 g and dissolved in 10 ml of distilled water. As much as 1 ml of sample was put into a test tube containing 9 ml of physiological NaCl 0.85% as a dilution of 10^{-1}. The dilution was carried out up to 10^{-4} and carried out the duplication of the last 3 dilutions. After that, the PDA media were added with a temperature of 50°C and mixed well then incubated at 37°C for 48 hours.

Data Analysis

The data obtained were processed using the SPSS application for Windows 22.0 with a significance level of 5%. The results of total acid, water content, ash content, fat content, total LAB, and total yeast were analyzed by analysis of variance and if there was a significant effect, then continued with Duncan test.

Results and Discussion

Total Titratable Acid

Based on Table 1, it can be seen that the differences in the drying method did not show any significant effect ($p<0.05$) on the total acid of goat milk kefir powder. The average total acid score on cabinet drying treatment was 0.785%, on freeze drying treatment was 0.785% and on spray drying treatment was 0.78%. Based on Table 1, it was found that the average total acid in each drying treatment had a bit range from 0.78% to 0.785%. This is in accordance with the standards set by Codex (2003) which states that the total standard of lactic acid kefir milk is at least 0.6%. This shows that the total acid obtained in goat milk kefir powder with the different drying method has reached the set standard for total kefir acid. The total lactic acid in goat milk kefir powder was produced from the fermentation process by lactic acid bacteria and yeast, in the fermentation process there was lactose degradation into single sugars, named glucose and galactose which became lactic acid. This is consistent with the opinion of Nursiwi *et al.* (2015) which stated that the levels of lactic acid in goat milk kefir powder depended on the amount of lactose and the amount of kefir grain, and the length of fermentation process.

Total Protein Content

Based on Table 1, it can be seen that differences in the type of drying method had no significant effect ($p> 0.05$) on the total protein content of goat milk kefir powder. The average of total protein content in goat milk kefir powder produced was quite high. The higher of total microbes contained in goat milk kefir, the higher protein content in kefir due to the microbial constituent (Hanum 2016). The raw material influenced the total protein content of goat milk kefir (Haenlein, 2014). According to Adriani *et al.* (2014) goat milk protein was 4.57%. A research by Widiantara et al. (2018) stated that milk processing with high temperature and long time might caused denaturation of protein compounds and reduce their protein content.

Water Content

Based on Table 1, it is known that the drying method had a significant effect ($p<0.05$) on the moisture content of goat milk powder kefir. The average score of water content at cabinet drying treatment was 17.86%, freeze drying treatment was 8.37%, and spray drying treatment was 7.75%. This might influenced by the use of the temperature used when drying with an inlet temperature of 125°C in the spray drying causing decrease in water content highly, as stated by Quek et al. (2007) that the higher of drying temperature, the greater temperature difference between the heating media and the material, the faster heat transfer occurs so the more water would be evaporated resulting in the faster drying speed. Based on all applied methods, the water content produced was not in accordance with SNI 01-2970-2006 regarding the requirements for the quality of fatty milk powder that was equal to 5.00% due to originally protein content in goat milk which was less than 5%. It is known that during fermentation, protein in goat milk might stimulated the growth of starter by hydrolyzing into dissolved components generating the specific formation of textures. This is consistent with the opinion of Pereira *et al.* (2015), that the protein denaturation might appeared due to acid formation during fermentation.

Total Ash Content

Table 1 shows significant effect on the total ash content in goat milk kefir powder from all drying method has a. The results of the average levels of total ash content of goat milk kefir powder on this three drying methods did not meet the standards. Due to the absence of SNI for kefir, SNI 2981: 2009 on yogurt is stated that the maximum total ash content is 1.0%. Drying treatment

Table 1. Results of chemical characteristics analysis of goat milk kefir powder

Treatments	Total Acid (%)	Total Protein (%)	Water Content (%)	Total Ash Content (%)	Total Fat Content (%)
Cabinet drying	0.785±0.071^a	27.56±1.51^a	27.56±1.51^a	30.37±5.96^{ab}	37.17±7.99^b
Freeze drying	0.785±0.071^a	25.97±4.95^a	8.37±1.009^a	5.59±0.785^a	37.17±7.99^b
Spray drying	0.78±0.0067^a	1.66±4.73^a	7.75±3.671^a	58.98±61.90^b	1.026±0.09^a

Notes : Data shown as mean value from 7 replications. The number followed by the same superscript shows no significant difference at the α level of 0.05

with the freeze drying method produces total ash content with the lowest mean value. This is consistent with the opinion of Riansyah et al. (2013) states that total ash content formed during the drying process will increase with increasing temperature and length of drying time. Drying with low temperatures will produce less ash content. According to Sundari et al. (2015), ash content in food shows the inorganic mineral content in it and ash content is influenced by the method of ashes and the raw materials thereof. The minerals contained in goat's milk are calcium, magnesium, and phosphorus.

Total Fat Content

Based on Table 1 shows that the drying method does not significantly affect the fat content in goat milk. The results of fat content in goat milk kefir powder did not meet the standards, because according to SNI 01-2970-2006 the fat content in milk powder is between 1.5 and 26%. Drying treatment using the cabinet drying method resulted high or exceeding standard fat content that might influenced by the drying temperature and length of drying time (Purbasari, 2019; Riansyah *et al.,* 2013). Drying using the spray drying method resulted in low or below standard of fat content due to longer time was required to finalize the process that allowed lipase enzyme to break down the more fat resulting in the decrease in fat content (Bayu *et al*., 2017). Kefir fat content also depends on the total fat content of raw material (Utomo *et al*., 2017).

Total Lactic Acid Bacteria

Table 2 shows significant effect ($p<0.05$) on the total LAB of goat milk powder kefir among drying methods but did not meet the requirements of SNI 2891-2009 (at least 10^7 CFU/ml). The lowest amount of bacteria was found in kefir powder by the method of drying cabinet drying. This is due to high drying temperatures as high as 50°C for 24 hours causing the decrease in the number of living lactic acid bacteria (Al-Baarri *et al*., 2016). The highest total LAB yield was found in kefir powder with the freeze drying method, which was 7.14 x 10^5 CFU/ml because this method provided suitable condition for sensitive products and able to maintain the quality of the final product to be more durable, especially heat-sensitive products (Dewi, 2009).

Table 2. Results of microbiological analysis of goat milk kefir powder

Treatments	Total LAB (CFU/ml)	Total Yeast (CFU/ml)
Cabinet drying	-	$4.87x10^6$
Freeze drying	$7.14x10^5$	$2.88x10^5$
Spray drying	$2.25x10^2$	$1.33x10^4$

Notes : Data shown as mean value from 7 replications. The number followed by the same superscript shows no significant difference at the α level of 0.05

Total Yeast

Table 2 shows no significant effect of total yeast in goat milk powder kefir among drying methods. The highest total yield of yeast to the lowest was found in the cabinet drying, freeze drying and spray drying methods. Yeast growth is influenced by several factors such as temperature, humidity, pH, oxygen, and the nature of the microbes themselves (Anggraini *et al*., 2017; Hardianto *et al*., 2018). The total standard of yeast according to Codex (2011) is at least 10^4 CFU/ml representing all the average total yield of yeast has met the standard. According to Ningsih *et al*. (2018), yeast works by degrading various types of sugar, especially simple sugar as an energy source to produce alcohol and CO_2 as the final product of its metabolism. This process occurs because of the enzymes produced by yeast that fermenting sugar into the simplest form such as glucose to produce ethanol and carbon dioxide (Hasanah *et al.* 2012).

Conclusion

The different type of drying method specifically affected the water content, total ash content, total LAB and yeast of goat milk powdered kefir. The best treatment of drying method was spray drying. This may reach maximum shelf life product.

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