Buletin Peternakan 43 (1): 52-57, February 2019

ISSTAP
Indonesian Society for Sustainable Tropical Animal Production

Bulletin of Animal Science

ISSN-0126-4400/E-ISSN-2407-876X Accredited: 36a/E/KPT/2016
http://buletinpeternakan.fapet.ugm.ac.id/

Doi: 10.21059/buletinpeternak.v43i1.31496

Physicochemical, Microbiological and Sensory Properties of Fermented Whey using Kombucha Inoculum

Fitri Suciati, Nurliyani*, and Indratiningsih

Department of Animal Product Technology, Faculty of Animal Science, Gadjah Mada University, Yogyakarta, 55281, Indonesia

Article history
Submitted: 20 December 2017
Accepted: 23 January 2019

* Corresponding author:
Telp. +62 816 682 386
E-mail: nurliyani@ugm.ac.id

ABSTRACT

This research aimed to determine physicochemical, microbiological and sensory properties of fermented whey using kombucha inoculum. The material used were kombucha, black tea, green tea, and whey protein concentrate. The research used factorial completely randomized design. The first factor was kombucha inoculum using different mediums (black and green tea) at different levels (5; 10; 15; dan 20%) as a second factor. The whey was fermented at 37°C for 39 hours. Research showed the use of kombucha inoculum using different medium (black and green tea) at different levels (5; 10; 15; dan 20%) did not affect ($p>0.05$) on microbiological properties (the number of Total Plate Count, total lactic acid bacteria, total acetic acid bacteria, and total yeast), dissolved protein content, lactose content, viscosity and acceptability. Black and green tea kombucha inoculum can be used in whey fermentation. Black tea kombucha fermented whey with 20% level addition has the best solid content. Relatively, kombucha fermented whey is quite acceptable.

Keywords: Fermented whey, Kombucha, Sensory, Whey

Introduction

Whey contains nutrients such as lactose, dissolved protein, fat, mineral, vitamin and organic acids. Whey presents 85-95% from the milk volume, it is a byproduct of cheese making. The utilization of whey in fermented beverages is to reduce pollutants potential and increase the value of the product (Assadi *et al.*, 2008). Whey contains 0.7% whey protein, <0.1% casein protein, 0.1% fat, 0.5% ash, 4.9% lactose, with 6.3% total solid (Smithers, 2008). Fermentation process involves microorganism that known as a starter culture, those are bacteria, yeast, mold or combination between the three (Tamime, 2006).

Kombucha utilizes symbiosis between bacteria and yeast with tea and sugar as their substrates with 7-14 days fermentation at room temperature. Tea polyphenols, sugars, organic acids, ethanol, water-soluble vitamin and etc are produced during the fermentation process (Fu *et al.,* 2014). Acetic acid bacteria (AAB) were found in kombucha are *Acetobacter xylinoides, A. pasteurianus, A. xylinum, A. aceti,* and *Bacterium gluconicum,* whereas most of the yeast species were isolated from kombucha including *Brettanomyces bruxellensis, B. lambicus, B. custersii, Kloeckera apiculata, Saccaromycodes ludwigii, Schizosaccaromyces bailii, Candida,* and *Pichia* (Fu *et al.,* 2014) and some of lactic acid bacteria (LAB) such as *Lactobacillus, Lactococcus, Bifidobacterium* and *Leuconostoc* were found in kombucha cellulose and kombucha liquor. The presence of LAB in kombucha was known to assist the growth of AAB and increase cellulose production also in order to assist the growth of *Gluconoacetobacter* in kombucha (Marsh *et al.,* 2014).

The component within whey are utilized by bacteria during the fermentation process. Whey contains lactose that can be degraded to glucose and galactose by LAB, whereas yeast utilizes glucose to produce ethanol, furthermore ethanol is utilized by AAB to produce cellulose and acetic acid. The change during fermentation can affect physicochemical, microbiological and sensory properties. Some of the research was conducted such as physicochemical and texture properties of kombucha fermented milk products using black tea and thyme that combine with the use of probiotic. The research result showed that kombucha inoculum that cultivated in different tea significantly affected the texture of fermented milk (Milanović *et al.*, 2012). There was no research conducted on physicochemical, microbiological and sensory properties of fermented whey using black and green tea kombucha, therefore the purpose of this research is to find physicochemical, microbiological, sensory

properties of fermented whey using kombucha inoculum.

Material and Methods

Fermented whey production

The making of kombucha inoculum. Kombucha (tea fungus) culture was cultivated in black and green tea, 0.45% tea and 10% sugar was added to 1,400 ml tap sterile aquadest, then cooled in room temperature for ± 20 minutes. Tea solution then filtered and transferred into a jar, then 2.5% w/v kombucha scoby and 10% v/v of kombucha liquor (from previous fermentation) was added, the jar covered by a cheesecloth and incubated for 7 days in room temperature (Srihari dan Satyanarayana, 2012), the amount of Total Plate Count (TPC), AAB, LAB and *yeast* 8.04; 6.08; 6.32 and 5.30 log CFU/ml respectively for black tea kombucha and 8.28; 6.13; 6.31 and 5.43 log CFU/ml for green tea kombucha respectively.

The making of fermented whey. Reconstituted 7% of whey protein concentrate (containing 6.30% total solid, 93.70% moisture content, 5.27% lactose, and 6.18% protein) in 350 mL aquadest, then pasteurized at 70°C for 30 minutes, then cooled in room temperature. Kombucha inoculum liquor (5; 10; 15; dan 20% v/v) and 2.5% (w/v) kombucha cellulose then was added to the whey. Incubation was conducted at 37°C for 39 hours (Srihari dan Satyanarayana, 2012; Belloso-Morales dan Hernandez-Sanchez, 2003).

Microbiological properties

Microbiological properties method. The determination of microbiological properties consist of TPC test, the total of LAB, total of AAB and total of yeast, the sample was inoculated to Petri dish that containing medium then spread by drigalski spatula and incubated for 24 hours at 37°C (Fardiaz, 1993). PCA (*Plate Count Agar*) medium was used for TPC (Fardiaz, 1993), MRS agar (deMann Rogosa Sharpe agar) medium for total LAB (Fardiaz, 1993), Malt Extract Agar (MEA) medium for total yeast with the addition of 100 ppm chloramphenicol and YPM (*Yeast* Extract, Pepton, Mannitol) medium for total AAB with the addition of 200 U/ml Nystatin (Fu *et al.*, 2014).

Physicochemical properties

Moisture content and total solid. Two grams of sample was placed in a porcelain crucible and introduced in an air oven at 105°C for 12 hours. Porcelain crucible with the sample within cooled in a desiccator and then weighed. The determination of moisture content by weighing the sample before and after drying. The total solid content was obtained from moisture content calculation (AOAC, 1995).

Dissolved protein content. The determination of dissolved protein content using the Lowry method. One ml sample was added with Lowry B solution (50 ml Na_2CO_3 solution added with 1 ml $CuSO_4$.Kna tartat solution), sit in room temperature for 10 minutes, then added 0.5 ml Lowry, A solution (Folin-Ciocalteau reagent solution), let it sit for 30 minutes. Absorbance was read at 750 nm, then the result compared to BSA absorbance as standard (Plummer, 1987).

Lactose content. The determination of lactose content used the colorimetric method. Twenty-five of the sample was placed in 50 ml volumetric flask then added 5 ml of $ZnSO_4$, 5 ml of NaOH 0.75 N solution, then diluted by aquadest until its mark. The suspension then sits for 10 minutes, filtered and the filtrate volume was measured. Five ml of filtrate furthermore placed in covered Erlenmeyer 250 ml. Twenty ml aquadest and KI solution were added then add Chloramine-T solution, shook and let it sit for 90 minutes. Add 10 ml of HCl 2 N solution. The solution then titrated by 0.1 N $Na_2S_2O_3$ until the color of the solution is pale yellow, then added starch solution as an indicator, then titrated until its grey color. Blank solution was made to replaced sample with aquadest, the blank solution then titrated as sample solution (Sudarmadji *et al.*, 1997). Lactose content calculated as follows:

$$\text{Lactose content in 100 ml Sample} = A \times \frac{\text{Filtrate volume}}{100} \times \frac{100 \text{ gram}}{25}$$

Viscosity test. Viscosity test used *Rapid Viscosity Analyzer* (RVA) instrument with TCW (*Thermocline for Windows*) application.

Sensory. Sensory characteristics that were tested including taste, aroma and total acceptability. The test of sensory properties using hedonic test for acceptability and intensity test for taste and aroma, and this experiment used 5 scale assessment respectively, it is showed in Table 1. Sensory characteristics were examined by 15 untrained panelists. Organoleptic procedure test, untrained panelists were given 8 coded samples and questionnaire. Each panelist was given cleanser palate (mineral water and biscuit).

Data analysis

Research Design using factorial completely randomized design with 2 factors treatment (2 x 4) with 3 replications that consist of

Table 1. The scale of taste and aroma intensity test and hedonic test of fermented whey

Scale	Taste	Aroma	Acceptability
1	No sour at all	No typically kombucha at all	Not acceptable at all
2	No sour	No typically kombucha	Not acceptable
3	Slightly sour	Slightly typically kombucha	Fairly acceptable
4	Sour	Typically kombucha	Acceptable
5	Extremely sour	Extremely typically kombucha	Very Acceptable

microbiological (TPC, total LAB, total AAB, and total yeast), physicochemical (viscosity, total solid, moisture content, lactose content, dissolved protein content) and sensory properties. The data that obtained then analyzed by using *two way* ANOVA and significant differences between the mean then tested by DMRT.

Result and Discussion

Microbiological properties

The microbiological properties of fermented whey using different medium and level of inoculum results shown in Table 2. Based on Table 2 different medium and inoculum level did not affect the amount of TPC, total LAB, total AAB, dan total yeast. Bacteria involved in kombucha fermentation such as *Acetobacter* spp., *Gluconobacter,* and *Lactobacillus*. *Yeast* also forms a symbiosis with bacteria in kombucha, some of the species are *Brettanomyces/Dekkera, Candida, Schizosaccharomyces, Torulaspora* and *Zygosaccharomyces* (Teoh *et al*., 2004). Some of the LAB species are *Lactobacillus, Lactococcus, Bifidobacterium* and *Leuconostoc* were found in kombucha, both in cellulose or kombucha liquor (Marsh *et al.,* 2014). Acetic acid bacteria in kombucha is able to convert glucose to gluconic acid, and fructose into acetic acid. The presence of caffeine and xanthines in tea can stimulates kombucha cellulose synthesis by bacteria in kombucha (Balentine, 1997). Acetic acid that produced by acetic acid bacteria is able to stimulate yeast to produce ethanol, and ethanol, in turn, can induce acetic acid bacteria to grow and produce acetic acid. Ethanol and acetic acid, both of them had antimicrobial activity against pathogenic bacteria with the result that can protect kombucha (tea fungus) from contamination (Liu *et al*., 1996).

Total Plate Count showed the entirety of the microbiological condition in fermented whey. The amount of TPC fermented whey at the range of 8.18-8.54 log CFU/ml in this experiment higher than the traditional kombucha with 8 days duration of fermentation reached 6.64 log CFU/ml (Wistiana dan Zubaidah, 2015). The amount of TPC of fermented whey in this experiment still in normal range compared with CODEX standard for fermented milk at the amount of 7 log CFU/g (CODEX Alimentarius Commission, 2010). Lactic acid bacteria is one of the bacteria involved in the kombucha fermentation process. The amount of total lactic acid bacteria (LAB)of kombucha fermented whey at the range of 7.36-7.67 log CFU/ml higher than green tea kombucha that fermented for 90 hours with total 5.45 log CFU/ml (Fu *et al*., 2014). The presence of LAB in kombucha had known able to assist the growth of AAB to increase cellulose production and induce growth of *Gluconoacetobacter* in kombucha (Marsh *et al.,* 2014).

Acetic acid bacteria play role in kombucha fermentation. Acetic acid bacteria utilize glucose to produce gluconic acid, and ethanol to produce acetic acid (Dufresne dan Farnworth, 2000), besides that, the acetic acid bacteria also convert glucose into glucuronic acid through pentose phosphate pathway and fructose is metabolized into acetic acid with the lack of acetic acid or without gluconic acid (Alvarez dan Martı´nez-Drets, 1995). Total acetic acid bacteria in fermented whey with black and green tea kombucha inoculum at various level turn between 6.15-6.64 log CFU/ml. Kombucha with 2 days fermentation duration with 7% lactose medium showed the amount of AAB at the rate 6.79 log CFU/ml (Markov *et al*., 2012) slightly higher than black and green tea kombucha fermented whey. Total AAB of fermented *sweet whey* reached 6.95 CFU/ml at the end of fermentation, the fermentation process taking place 95 hours fermentation (Belloso-Morales dan Hernandez-Sanchez, 2003) higher than fermented whey in this experiment and kombucha fermentation using 7% lactose as its medium. Fermentation of traditional kombucha for 90 hours had total AAB at 7 log CFU/ml (Fu *et al*., 2014) higher than fermented milk product using kombucha inoculum in this experiment. Comparing product in this experiment with the product of previous experiments, the amount of total AAB still at normal range. Different medium and duration of fermentation can affect the amount of total AAB, for fermented milk product and its derivatives had total AAB lower than traditional kombucha.

Yeast is one of the microorganisms that are present in kombucha. *Yeast* in the kombucha fermentation process converts sucrose into fructose and glucose also produce ethanol (Reiss, 1994). Total *yeast* in fermented kombucha with

Table 2. Microbiological properties of fermented whey using different medium and level of kombucha inoculum (CFU/ml)

Inoculum medium	Inoculum Level (%)	TPC	Total LAB	Total AAB	Total yeast
Black tea	5	8.31±0.24	7.40±0.36	6.54±0.28	5.31±0.13
	10	8.37±0.14	7.36±0.16	6.35±0.28	5.27±0.15
	15	8.43±0.40	7.35±0.28	6.58±0.35	5.35±0.14
	20	8.18±0.02	7.33±0.34	6.15±0.03	5.39±0.11
Green tea	5	8.15±0.44	7.37±0.33	6.25±0.19	5.61±0.33
	10	8.40±0.06	7.46±0.37	6.15±0.06	5.36±0.37
	15	8.40±0.10	7.67±0.27	6.64±0.06	5.31±0.27
	20	8.54±0.07	7.31±0.18	6.47±0.25	5.59±0.18
M		NS	NS	NS	NS
L		NS	NS	NS	NS
M*L		NS	NS	NS	NS

M = Inoculum medium; L= Inoculum level; NS= non significant (p>0.05)

7% lactose as its substrate with 2 days fermentation had total *yeast* 6.68 log CFU/ml (Markov *et al.,* 2012) higher total *yeast* of kombucha fermented whey in this experiment. The different of the amount of total yeast because of using a different medium as its substrates.

Physicochemical properties of fermented whey

Table 3 showed the physicochemical properties of fermented whey using different medium and level of kombucha inoculum. Based on Table 3, total solid of fermented whey using kombucha inoculum significantly ($p<0.05$) affected by medium and level of inoculum, but there is no interaction between the factors, whereas the moisture content only affected by the level of inoculum. Lactose content, dissolved protein, and viscosity did not affect by medium and inoculum level of kombucha. The moisture content of kombucha fermented whey at the range of 93.59-94,27%. Fermented whey using 5% black tea kombucha inoculum treatment had the highest moisture content 94.35% and the lowest is fermented whey using 20% black tea kombucha inoculum has 93.58% moisture content. The moisture content of whey is 93.70%, comparing to kombucha fermented whey. Whey generally contains 93% moisture content (Kumar and Joshi, 2015).

Total solid of fermented whey in this experiment is at the range of 5.65-6.42% and the highest at fermented whey using 20% black tea kombucha is 6.42% and the lowest at the using 5% black tea kombucha inoculum is 5.65%. The total solid of whey before fermentation is 6.30%, the addition of black and green tea kombucha and various level of inoculum showed the changes during fermentation. Total solid of fermented whey in this experiment lower than in the experiment of Fatma *et al*. (2012) in fermented whey dangke using *Lactobacillus acidophilus* FNCC 0051 with 6.95% total solid. The differences between these experiments are caused by the addition of tapioca flour in Fatma *et al*. (2012) experiment, therefore the total solid of fermented whey dangke higher than in this experiment.

Lactose is a disaccharide that consists of glucose and galactose. Lactose is carbohydrate that can be found in milk and its derivatives. Lactose becomes a source of energy for the microorganism in kombucha symbiosis in whey fermentation. Whey contains 5.27% lactose after fermentation the trend showed decreasing lactose content, kombucha fermented whey in this experiment contains 4.36-4.67% lactose, It is defined that lactose is hydrolyzed during the fermentation process. Kombucha is able to utilize 20-30% of lactose in its fermentation process (Ilicic *et al.*, 2012). Lactose becomes the main carbon source for bacteria and yeast in kombucha, degradation rate occurred fast at the beginning of fermentation then become low during the fermentation, kombucha fermented whey has low lactose content, it is safe to be consumed by lactose intolerance sufferer (Belloso-Morales dan Hernandez-Sanchez, 2003).

The amount of inoculum level that added does not affect the time of fermentation, therefore the addition of 10% level of inoculum does not mean a low rate of fermentation and higher level does not mean a faster rate of fermentation (Malbaša *et al*., 2009). The use of kombucha inoculum with different medium and level of inoculum in this experiment did not affect lactose content, however Milanović *et al.* (2012) reported that the used of kombucha inoculum with different medium (black and *thyme* tea) with addition of probiotic and combined with incubation temperature affected the lactose content, whereas in this experiment there is no addition of probiotic or combination between treatments and various temperature of incubation. The lowest dissolved protein content is fermented whey using 5% black tea kombucha inoculum is 6.71% and the highest fermented whey using 15% green tea kombucha is 7.14%. Milanović *et al.* (2008) reported that kombucha inoculum level did not affect the rate of fermentation, therefore inoculum level did not affect the dissolved protein content of fermented whey in this experiment.

The viscosity of fermented whey using kombucha inoculum at the range of 2.70-2.97 cP. Viscosity defines as an internal movement in liquid or tends to against the flow. Viscosity includes a rheological component that describes as physical properties of food (Bourne, 2002). Measurement of viscosity can be used to observe product stability, including fermented milk products (Tamime, 2006). Fermented whey using black and green tea kombucha inoculum in this experiment showed no difference with the experiment that conducted by Milanović *et al.* (2012) reported that fermented milk using kombucha inoculum with different medium black and *thyme* tea affected viscosity, the viscosity of black tea kombucha fermented milk is higher than thyme tea kombucha fermented milk. Milk fermentation in the experiment of Milanović *et al.* (2012) using milk as the main material that fermented by black and thyme tea kombucha and combined with the addition of probiotic, gel formation during fermentation with different medium of kombucha inoculum allegedly affected viscosity, whereas in this experiment the main material is reconstituted whey protein concentrate, the fermentation product that produced slightly aqueous, therefore fermented whey using black and green tea kombucha inoculum at various level did not affect its viscosity.

Sensory

Characteristics of kombucha fermented whey that was examined are taste, aroma, and acceptability. The data in Table 4 showed medium and level of kombucha inoculum significantly ($p>0.05$) effect to the taste of kombucha fermented whey, however, there is no

Table 3. Physicochemical properties of fermented whey using different medium and level of kombucha inoculum

Inoculum medium	Inoculum level (%)	Moisture content (%)	Total solid (%)	Lactose content (%)	Dissolved protein content (%)	Viscosity (cP)
Black tea	5	94.35±0.53	5.65±0.53	4.67±0.27	6.71±0.12	2.77±0.21
	10	93.97±0.07	6.03±0.07	4.56±0.42	6.81±0.17	2.80±0.20
	15	93.94±0.25	6.06±0.25	4.50±0.09	6.89±0.33	2.83±0.25
	20	93.58±0.09	6.42±0.09	4.36±0.27	6.91±0.73	2.83±0.23
Green tea	5	93.84±0.22	6.16±0.22	4.56±0.08	6.75±0.53	2.77±0.21
	10	93.78±0.18	6.22±0.18	4.46±0.03	6.98±0.44	2.80±0.20
	15	93.63±0.22	6.37±0.22	4.36±0.17	7.14±0.23	2.83±0.25
	20	93.60±0.20	6.40±0.20	4.40±0.13	7.06±0.43	2.83±0.23
M		NS	*	NS	NS	NS
L		*	*	NS	NS	NS
M*L		NS	NS	NS	NS	NS

M = Inoculum medium; L= Inoculum level; NS= non significant (p>0.05); *= the significant different (p<0.05).

Table 4. The sensory average of fermented whey using different medium and level of kombucha inoculum

Inoculum medium	Inoculum level (%)	Taste	Flavor	Acceptability
Black tea	5	2.13±0.64	2.13±0.64	2.67±0.90
	10	2.33±0.62	2.27±0.80	2.67±0.82
	15	2.73±0.59	2.20±0.77	2.60±0.98
	20	3.20±0.68	1.80±0.68	3.00±0.84
Green tea	5	2.86±0.64	2.53±0.83	2.67±0.72
	10	3.20±0.94	2.40±0.74	2.80±0.94
	15	3.27±0.80	2.47±0.74	3.00±1.07
	20	3.13±0.74	2.53±0.74	3.00±1.07
M		*	*	NS
L		*	NS	NS
M*L		NS	NS	NS

M = Inoculum medium; L= Inoculum level; NS= non significant (p>0.05); *= the significant different (p<0.05).

interaction between the factors, higher level of inoculum, sour taste of kombucha fermented whey becomes stronger.

Taste score of kombucha fermented whey using inoculum level at 5, 10, 15 and 20% were 2.50 (not sour), 2.77 (slightly sour), 3.00 (slightly sour) dan 3.17 (slightly sour) respectively. The sour taste appears because of the presence of organic acids in fermented whey. Kombucha culture metabolites kultur kombucha mainly are acetic and lactic acid and it caused a low value of pH then occurs sour taste in kombucha fermented whey.

The flavor of kombucha fermented whey was produced during fermentation. The flavor of kombucha fermented whey was specific, it is different compared to traditional kombucha. It still has a flavor of whey. The used of black and green tea kombucha has given a different flavor of kombucha fermented whey. Panelists reported that kombucha fermented whey had different flavor compared to traditional kombucha.

Acceptability was examined by panelists using the hedonic scale, fermented whey using kombucha inoculum is not a common product, because it is a new product. The mean score of inoculum level treatment at the range 2,.67-3.00 (fairly acceptable) and the mean score of the use of different teas at the range of 2.73-2.87 (fairly acceptable). Panelists reported that kombucha fermented whey had a unique taste, it still had whey flavor, however, it also did not have juga traditional kombucha taste. Based on hedonic taste and report of panelists it can be concluded that kombucha fermented can be accepted to consume.

Conclusion

Black and green tea kombucha inoculum can be used in whey fermentation. Kombucha fermented whey using 20% black tea kombucha showed the highest total solid. Black and green tea kombucha fermented whey were acceptable for panelists.

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