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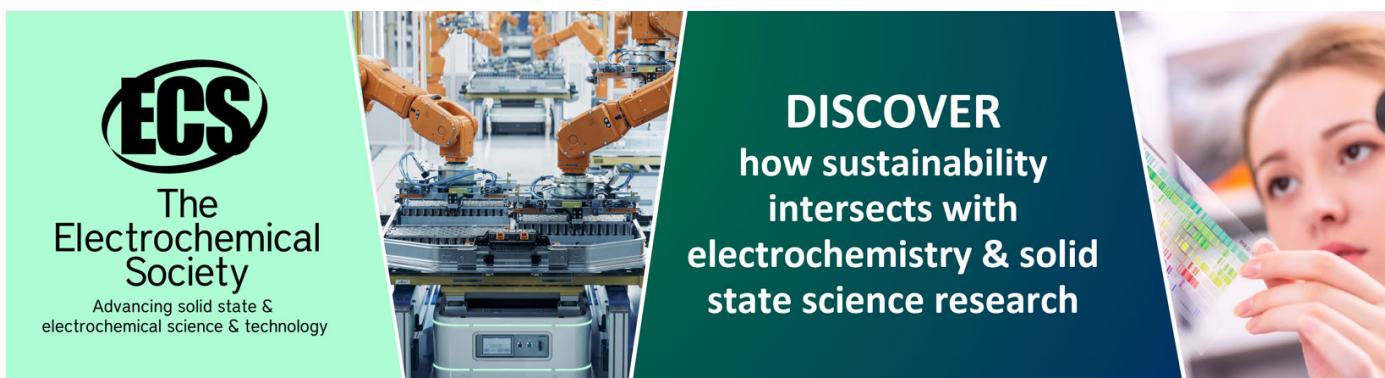
Microbiological properties of preparing facial mask cream from goat milk kefir

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Microbiological properties of preparing facial mask cream from goat milk kefir

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Abstract. Many studies have been published on physicochemical, microbiological and bioactive compound on kefir but the study of microbiological and physicochemical properties to address the preparation of producing facial mask cream from goat milk kefir have never been investigated. The current study provided the optimum incubation time of goat kefir to produce facial mask cream. Total plate count, total lactic acid bacteria, total yeast, titratable acidity and ethanol content were evaluated from goat milk kefir with different incubation time: 18, 24, 30 and 36 h under conditions of controlled incubator temperature ($30\pm1^{\circ}\text{C}$). The lactic acid flora and microbial count between incubation time 18 to 30 h increased by about 1.09 and 1.14 log units, respectively. The sample made using incubation time 24 h had higher total yeast and alcohol content while the sample made using incubation time 36 h had higher titratable acidity.

1. Introduction

Kefir is a fermented milk drink that prepared by inoculating cow, sheep, or goat's milk with kefir grains where lactose hydrolysis during fermentation occurs with the simultaneous action of bacteria and yeasts [1]. Kefir grains consist of kefir, a consortium of several exopolysaccharides as well as various microorganisms [2]. Kefir is a exopolysaccharide (EPS) comprising glucose and galactose in high concentrations, and it is classified as a water-soluble glucogalactan exhibiting very good rheological properties and enhancing gel properties [3]. Kefir also contains significant quantities of CO_2 and variable alcohol quantity due to yeast activity, although lactic acid is a main metabolite. During fermentation, associative growth of various microbial species in kefir is exist, therefore other organic compounds are formed, like bioactive peptides, bacteriocins which are presumed to have a probiotic effect on human health and exopolysaccharides [4, 5].

The increased search for natural polysaccharides has been very significant due to their use in the food, pharmaceutical, and cosmetic industries as additives. Many microorganisms, such as bacteria, fungi, and weeds, have the capacity/ability to synthesize and excrete extracellular polysaccharides, and these polysaccharides can be either soluble or insoluble [6]. Chen et al. [7] proved that kefir whey peptides and lactic acid had skin lightening ability, while only lactic acid inhibited the growth of



Propionibacterium acne. The inhibition of tyrosinase activity was due to the chelation of copper in tyrosinase. Coleman [8] noted that some natural milk products have been used to benefit the skin for topical application to provide healthy ageless skin and specialty cosmetics for centuries in many countries.

However, the microorganisms and physicochemical of kefir is change during the incubation time. Lactic acid bacteria decreased between 7 and 14 days in refrigerated storage, while yeast and acetic acid bacterial counts on kefir remained constant.

Concerning physicochemical analysis, the total fat, lactose, dry matter and pH, remained constant until 14 days of storage [9]. During the storage of 1 day, lactobacilli, lactococci, and yeast contents of kefir samples varied between 9.21 and 9.28, 9.23 and 9.29, and 4.71 and 5.53 log cfu/mL, respectively. Contents of *L. Acidophilus* and *Bifidobacterium* spp. were between 5.78 and 6.43 and between 3.19 and 6.14 log cfu/mL, respectively, during 21 day of storage. During the storage period, pH, lactic acid (%), total solids (%), protein (%), acetaldehyde, and ethanol contents of kefir samples ranged from 4.29 to 4.53, from 0.81 to 0.95%, from 7.81 to 8.21%, from 3.09 to 3.48%, from 3.8 to 23.6 mg/L, and from 76.5 to 5,147 mg/L, respectively [3]. The addition of kefir grains to goat milk will determine the ripening duration and acid that is formed. Goat milk is fermented for 12 h using kefir grains having an increased protein and reaching its maximum point at 24 h, ie protein by 2.96% to 3.02% [7].

Kefir can be considered to be a carrier of probiotics and various bioactive compounds, including peptide, polysaccharide and organic acid that may play a functional role for skin care, however, there is limited study to support the time incubation of kefir to preparing facial masker cream. Thus, the aim of the present study was to investigate the effects of different incubation time of kefir on microbiological of facial masker cream such as total plate count, total lactic acid bacteria, total yeast, titratable acidity and ethanol content. The final aim was to preparing a develop new cosmetic product for its possible commercialized and enhance the value of dairy products.

2. Materials and Methods

2.1. Sample preparation

Fresh goat milk were obtained from Agus Farm Bumiaji, Batu, East Java, Indonesia. The kefir grains were from Microbiology Laboratory, Department of Animal Products Technology, Faculty of Animal Science, Universitas Brawijaya.

2.2. Production of facial mask cream

Kefir grains were washed with distilled water and inoculated in fresh milk goat which was obtained from local farms in the village of Bumiaji, Malang. Four batches were made by adding an inoculate consisting 5% (w/w) kefir grains into goat milk under conditions of controlled incubator temperature at $30 \pm 1^\circ\text{C}$ for 18, 24, 30 and 36 h. The grains were separated from the fermented milk by filtering them through a sieve, and then washed for next culture incubation in fresh milk at 4°C . After each elaboration process, kefir was separated between curd and whey at 5°C for 5 days, then curd taken for facial masker cream and analysis were performed.

2.3. Analysis procedure

2.3.1. Microbiological analysis

All the samples were analyzed for microbiological presence using decimal dilutions. Total plate count (TPC), total lactic acid bacteria and yeast count were enumerated by spread plate method using Plate Count Agar (PCA), de Mann Rogosa Sharpe Agar (MRS) and Potato Dextrose Agar (PDA) media, respectively. 5 g of samples were diluted in 45 ml of peptone water solution and 1 ml solution (10^{-1}) from them were diluted in 9 ml of peptone water solution (10^{-2} until 10^{-5}) then spread 0.1 ml of each the last third dilution over media. Incubate inoculated medium in incubator at $35 \pm 2^\circ\text{C}$ for bacteria and $25 \pm 2^\circ\text{C}$ for yeast for 48 hours. The incubation time varied in face of the appearance of Colony

Forming Units (CFU) macroscopically visible on the surface of the media and/or turbidity in the broths. After incubated, the colonies counted are the total number of viable cells and reported as colony-forming units (CFUs). All the procedures were carried out in triplicates

2.3.2. Titratable acidity (TA)

The lactic acid content is analyzed with titratable acidity. The acidity of milk is determined by end point titration using 0.1 eq/l NaOH. The end point value is generally fixed at pH 8.7 and the result is expressed in % of lactic acid. As in this case 1 molecule of lactic acid reacts with 1 molecule of NaOH.

$$R = \frac{V(\text{titr}) \times C(\text{titr}) \times 90 \times 10}{V(\text{smp})} \times 100\% \quad (1)$$

Where:

V (titr) = total volume of titrant to reach the end point in ml

C (titr) = titrant concentration in eq/l (currently 0.1)

V (smp) = sample volume in ml

90 = molecular weight of lactic acid

10 = factor for result expression in %

2.3.3. Alcohol content

Gas Chromatographic (Newlett Packard Gas Chromatographer) analysis was applied after isolation of ethanol by a dynamic head space system, sample was purged for 3 hours with nitrogen gas (99.99%) at flow rate of 100 ml/min. Head space extracts were swept into cold traps at -10°C containing diethyl ether: pentane (1:1, v/v). Volatile were obtained by evaporation of solvent under reduced pressure and the compounds were separated on column DB5. Oven temperature was raised initially at 50°C to 180°C at a rate of 3°C/min. Carrier gas contain Helium at a flow rate of 1.0 ml/in. Injection volume was 1.0 µl. Quantification of ethanol from the sample was based on retention time of standard ethanol solution and its standard curve.

2.3.4. Statistical analysis

Each experiment was repeated three times and the obtained results were expressed as mean ± SD. Statistical data processing (one-way ANOVA; p<0.05) was performed by using Microsoft Office Excel 2013 (Microsoft Corporation, Redmond, WA, USA) on each of the physicochemical and microbiological variables to disclose possible differences among the samples. If there were significant differences the analysis was continued using Duncan Multiple Range Test (DMRT).

3. Results and Discussion

Microbiota is important to make the good quality of kefir, such as lactic acid bacteria, acetic acid bacteria and yeast. The analysis result of facial mask cream from goat milk kefir based on microbiology analysis, titratable acidity and ethanol showed at Table 1.

3.3.1 Microbiological analysis

Figure 1 depicts the changes in the microorganism population during incubation time of the facial mask cream from goat milk kefir. Initial levels of total plate counts in facial mask cream for 18 h of incubation time were 6.29 ± 0.60 log cfu/ml and increased until 36 h, the greatest increase gradually to 7.43 ± 0.15 log cf/ml for 30 h. This pattern behaviour was observed of growth phase of bacteria on logarithmic stage during fermentation, although microorganism population decreases on dead stage. In agreement of with the reported by Irigoyen et al. [9], the microbiota population in kefir amount of 10^8 cfu/ml lactobacilli and lactococci, 10^6 acetic acid bacteria and 10^5 yeast in 24 h fermented. Stadie et al. [10] and Guetouache et al. [11] noted that the growth factors such as pH, nutrition, temperature and

water activity affected to total bacteria. Available total bacteria are depended on nutrition content in goat milk. Goat milk is rich of lactose, protein and fat, however, had similar mineral content with cow milk.

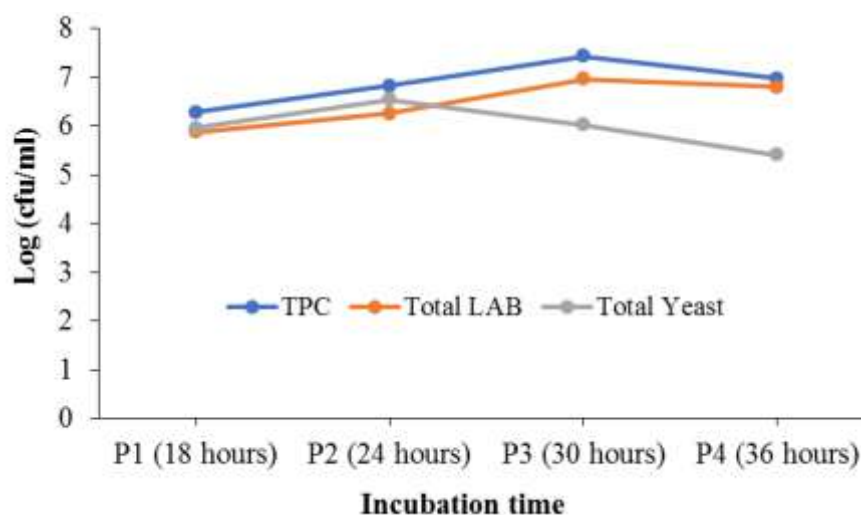


Figure 1. Microorganism population during incubation time of the facial mask cream from goat milk kefir.

Similar pattern of microorganism population, the initial lactic acid bacteria (LAB) level in facial mask cream for 18 h on incubation time was increasing until 36 h which the greatest value was 30 h of incubation time ($6.97^{\circ} \pm 0.35$ log cfu/ml). It was established that the present of greater bacteria contained is lactic acid bacteria. Arora et al. [12] stated that lactose contains in goat milk around 4.4 g/100 g milk that used as lactic acid bacteria feed. Yüksekdağ et al. [13] and Tamime [14] noted that hydrogen peroxide and bacteriocin, which available metabolites substrate from fermentation by LAB, as an antimicrobial compound produced by Lactococci can inhibited of *S. aureus*, *E. coli* and *Pseudomonas aeruginosa* and other gram positive and gram negative bacteria that had closely related affected acne on face skin. Market study by Zhang [15] established that skin care product contain Lactobacilli had claimed mechanism competitive with pathogenic bacteria and stimulate the natural immune system in skin.

The yeast population in facial mask cream was increasing from 18 h to 24 h of incubation time, however decrease of gradually 0.52 and 1.14 log units that took place from 30 h to 36 h of incubation time being statistically significant ($p < 0.05$). The decreasing of yeast population is because yeast and LAB had symbiosis mutualism for support their growth condition during the kefir production. Adriana and Socaciu [16] and Du et al. [17] reported that β -glucan exist in the cells wall of yeast is most important compound for cosmetics product as possess skin immune revitalizing, regeneration of collagen, anti-aging, anti-wrinkles and skin health promotion activities.

3.3.2. Titratable acidity

The lactic acid content in facial mask cream was increased during incubation time (Table 1). Such data are probably related to the lactic acid bacteria that fermented lactose of goat milk became lactic acid compound. The obtained values of TA range from $1.052\% \pm 0.33$ to $2.40\% \pm 0.63$ of lactic acid, in agreement with the findings reported by Coleman [8], the maximum lactic acid used in cosmetics is 2.5% as may damage skin sensitivity on sunscreen. Mukul et al. [18] noted that one of ingredients used for skin care product is lactic acid compounds. Sharma et al. [19] stated that cosmeceuticals are

product with active biology ingredients with medicinal properties and beneficial topical action. Lactic acid is kind of Alpha-Hydroxy Acids (AHA's) that important for making cosmeceuticals for skin. Their action onto skin claimed to increase collagen forming and decrease pigmentation.

Table 1. Changes in titratable acidity and ethanol content of facial mask cream from goat milk kefir

Incubation time	Titratable acidity (%)	Ethanol content (%)
18 h	1.05 ^a ±0.33	0.46±0.44
24 h	1.21 ^a ±0.70	0.56±0.05
30 h	2.14 ^b ±0.49	0.54±0.03
36 h	2.40 ^b ±0.63	0.51±0.02

^{a,b} Means in the same column with different superscript letters are significantly different ($p < 0.05$).

Kaptan et al. [20] and Guetouache et al. [11] reported that lactose in kefir turned into lactic acid during fermentation period which could lower the pH of milk as casein coagulation mechanism. Thus, casein micelle polymerize and coagulate to form a curd by separation from the whey, in the presence of Ca^{2+} ion. The curd form was used to produce facial mask cream.

3.3.3. Ethanol content

Considering ethanol content, average values of approximately 0.52%±0.14 detected in all samples. The highest ethanol contents were detected in samples incubation time of 24 h (Table 1), which corresponded well to previously reported by Bensmira and Jiang [21] that ethanol content in kefir increase significantly by 18 h of incubation time. However, Lachenmeier [22] noted that ethanol in skin care used as skin penetration enhancer system that ethanol removed lipids from stratum corneum when enters into skin barrier. And Kramer et al. [23] stated that human skin is frequently exposure with ethanol by cosmetics application and there is little or no risk developing their habit. Ethanol concentration of cosmetics may carry the risk of sensitization of the skin by topical applications. Topical application of 10% ethanol had positive influence for wound healing stimulation of wound healing.

4. Conclusion

Facial mask cream from milk goat kefir with different incubation time has ability to produce face care product. The present findings indicated that levels of yeasts and lactic acid bacteria were directly proportional to the quantity of 30 h incubation time to produce facial mask cream. Although the present study proved that certain kefir components had skin care properties, further studies are necessary for developing a new commercialized cosmetic product. Additionally, studies intending to optimize desaturase activity during fermentation conditions may contribute to obtaining a fermented product with a higher nutritional lipid quality.

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