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by Samsul Rizal

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Prebiotic activity of *Lactobacillus casei* grown on medium containing of *Hylocereus undatus* extract and its use in the fermentation of goat's milk kefir

MARIA ERNA KUSTYAWATI♥, MUKAROMAH EKA NURLITA, ESA GHANIM FADHALLAH, SAMSUL RIZAL

Department of Agricultural Product Technology, Universitas Lampung. Jl. Prof. Dr. Sumantri Brojonegoro No. 1, Bandar Lampung 34145, Lampung, Indonesia. Tel./fax.: +62-721-782698, ♥email: maria.erna@fp.unila.ac.id

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Abstract. *Kustyawati ME, Nurlita ME, Fadhallah EG, Rizal S. 2022. Prebiotic activity of* Lactobacillus casei *grown on medium containing of* Hylocereus undatus *extract and its use in the fermentation of goat's milk kefir. Biodiversitas 23: 6513-6519.* White dragon fruit extract used as a prebiotic source is thought to be an innovative exploration to enhance its application as a synbiotics in food products. This study aims to quantify the prebiotic index and prebiotic activity score for *Lactobacillus casei* grown on white dragon fruit extract containing media at different concentrations. The study was carried out using a completely randomized design (CRD) experimental method with one factor, concentration of white dragon fruit extract (P1-P5) with 5 levels (2, 4, 6, 8, and 10% respectively), and was repeated 3 times. The data were analyzed statistically by ANOVA, if the treatments were significantly different then the LSD test (5%). The result showed that all tested media had a beneficial effect on the growth of *L. casei* (prebiotic index higher than 1), while they did not support the growth of enteric *Escherichia coli* (prebiotic index less than 1 or negative). The highest score of prebiotic activity for *L. casei* was grown on 10%, while the lowest was 2%. The addition of 10% prebiotic extract of white dragon fruit in goat's milk fermentation produced goat's milk kefir with characteristics that met Codex Stand 243-2003 and had a DPPH radical scavenging activity of 55.13%.

Keywords: *Escherichia coli,* kefir beverage, *Lactobacillus casei*, prebiotic activity score, prebiotic index, white dragon fruit extract

INTRODUCTION

Prebiotics and probiotics are microbiota regulation system in the human digestive tract to improve the health of the host. Prebiotics are non-absorbable oligosaccharides that can be used by probiotics and stimulate their growth and metabolisms and provide health benefits to the host, but these substrates should be non-hydrolysable by enteric bacteria (Huebner et al. 2007). If the substrate can stimulate the enteric growth, it cannot be categorized as a prebiotic. Prebiotics must be resistant to low acids, bile salts, and other hydrolytic enzymes in the intestine; should not be absorbed in the upper gastrointestinal tract, and easily fermented by beneficial gut microflora because they have to be able to pass through the digestive tract to the large intestine so as to be utilized by probiotics for their metabolism. In their metabolism, probiotics provide beneficial effect to the host by producing compounds that have protective functions such as antibacterial, structural functions such as immune system development, and metabolic functions such as SCFA production, vitamins and minerals. Probiotics are live microorganisms that can be found either as supplements or as components in foods, which when foods are consumed the microorganisms are still found alive in the colon in an amount of at least 10^6 CFU/g and provide the health to the host (Dahiya and Nigam 2022). Probiotics must be able to overcome all obstacles when passing through the digestive tract in order to reach the colon and function to nourish the host. Along the digestive tract, each site is inhabited by various microorganisms of different types and numbers, which are influenced by the food matrix that passes through the digestive tract and the length of stay in that site (Roberfroid et al. 2010). Among the sites of the digestive tract, the colon is a suitable part for the proliferation of microorganisms because of its neutral pH conditions, high nutrient availability, and absolutely anaerobic condition, so it is also a source of disease. However, the health benefits of the host will be obtained by a mutualistic symbiosis between prebiotic and probiotics in the colon. Therefore, if the consumption of prebiotic food can survive until it reaches the large intestine and is utilized by probiotics for proliferation, balancing the number of colon microflora by competing for nutrients, inhabiting the lining of the colon, and producing metabolites are occurred to suppress the growth of pathogens and help boost the immune system of the host. Included in the group of probiotics are LAB groups, bifidobacteria, *Bacillus subtilis*, and *Saccharomyces cerevisiae* (Sanders et al. 2019).

Prebiotics can be used as fortifying agents to improve or maintain the balance of intestinal microflora in an effort to improve human health and well-being. From this point of view, prebiotic substrates have been of interest to the food industry and pharmacies, and several studies have been carried out (Babji and Daud 2021; Akin et al. 2005; Abed et al. 2016). Prebiotics can be mixed or added to

several food products such as dairy products, health drinks, baby food or complementary foods (breast milk), bakery products, sweets, meat products and dietary supplements (Gibson et al. 2004; Patel and Goyal 2012). Exploration of prebiotic products from natural sources such as tubers (Perdinan and Larasat 2019), fibrous fruit (breadfruit, banana) (Zakaria et al. 2018; Budhisatria et al. 2017), soybeans (Zhou et al. 2012), raffinose (Anggraeni 2022), and honey (Aryati et al. 2020) continues to be carried out. The potential of food components as prebiotics can be determined through the prebiotic activity score (PAS), which is a quantitative method to estimate the extent to which prebiotics of such food or food products support the growth of probiotic and not enteric bacteria (Figueroa-González et al. 2019).

Dragon fruit or pitaya (Hylocereus) known to be rich in nutrients, containing bioactive compounds of polyphenols, flavonoids, and vitamin C which are beneficial for health related to its antioxidant activity (Song et al. 2016), besides that, it also contains beta-carotene, anthocyanin, and soluble fiber in the form of pectin (Aji et al. 2012). Extracts of every part of the pitaya plant have biological activity against pathogenic microbes including bacteria, yeasts and molds, and are also useful in controlling degenerative diseases and cancer (Luu et al. 2021). Among the species of dragon fruit, *Hylocereus undatus*, white flesh with pink skin, contains the highest carbohydrates, total sugar, protein, crude fiber, and vitamin C (Ramli and Rahmat 2014; Ruzainah et al. 2009; Jerônimo et al. 2015). Prebiotic properties of white dragon fruit have been studied (Pansai et al. 2020); however, what concentrations of dragon fruit oligosaccharides be added to a food product to function as a prebiotic need to be ascertained.

Kefir is a fermented milk beverage with characteristic of having a viscous carbonated texture, and contains a small amount of alcohol. Microbes that play a role in kefir fermentation are kefir grains (starter kefir), a symbiotic form of a consortium of microorganisms consisting of several lactic acid bacteria and yeast. The aim of this study was to measure the increase growth of *Lactobacillus casei* as probiotic and *Escherichia coli* as enteric bacteria to ferment white dragon fruit extract (*H. undatus*) at various concentrations, and to quantify the prebiotic index and prebiotic activity score for *L. casei* grown on white dragon fruit extract containing media at different concentrations. Furthermore, white dragon fruit extract at a certain concentration was added to fermented goat's milk to make kefir beverage with characteristics that meet Codex Alimentarius description standard 243-2003 for kefir.

MATERIALS AND METHODS

Materials and equipment

The materials used include white dragon fruit (*Hylocereus undatus*) purchased from the Bandar Lampung, Indonesia supermarket, pathogenic bacteria *Escherichia coli* and *Lactobacillus casei* purchased from Microbiology Laboratory of Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia stock culture, MRSB (de Mann Rogosa Sharpe Broth), MRSA (de Mann Rogosa Sharpe Agar) (Difco), NB (Nutrient Broth) (Difco), glucose, filter paper, 80% ethanol, aquadest, inulin (Sigma-Aldrich), saline water, and alcohol. The tools used are scales, mortar, autoclave, incubator, filter cloth, shaker, Erlenmeyer, test tube, petri dish, Rubber bulb, Beaker glass, micropipette, colony counter, thermometer, evaporator, and magnetic stirrer, Bunsen, glass stirrer, hotplate, Ose needle, laminar air flow, test tube rack.

Procedures

Inoculum preparation

Lactobacillus casei pure cultures from streak culture stock were activated in 10 mL deMan Rogosa Sharpe broth for 24 h (Kustyawati et al. 2021). A loop of the broth was streak in MRS agar and incubated at 37°C for 48 h. Next, the bacteria were subcultures in MRS broth, by taking single colony. In order to getting same amount of bacteria in the broth within the range $10^6 - 10^7$ CFU mL^{-1}, *L. casei* was incubated at 37°C for 48h. One (1) mL of bacteria in their specific broth was transferred into universal bottles containing different carbon sources, which are glucose, inulin, and growth media with 2%, 4%, 6%, 8%, 10% (w/v) of dragon fruit extract as P1, P2, P3, P4, P5 respectively, prior to fermentation process. Meanwhile, *E. coli* was activated in nutrient broth. A loop of this *E. coli* culture transferred into 10 mL of nutrient broth to reactivate. Then, a loop of the broth was streaked onto nutrient agar and incubated at 37°C for 24 h. Next, the bacteria then subculture in 35 mL of nutrient broth to obtained the amount of bacteria within the range 10^6 - 10^7 CFU mL^{-1}then incubated at 37°C for 8 h. One (1) mL of broth was transferred into universal bottles with addition of different carbon sources which is growth media, inulin, glucose, extract dragon fruit.

Extraction of white dragon fruit

White dragon fruit was extracted by maceration method using ethanol solvent in a ratio of 1:5 (w/v) followed the procedure done by Andrianto et al. (2017) with some modifications. Maceration was carried out for 2x24 hours with stirring every 1x24 hours. The maceration process was carried out by placing the sample in an Erlenmeyer shaker at a speed of 130 rpm. The macerate was then filtered with filter paper and the filtrate was concentrated using a rotary evaporator at a temperature of 50°C. The concentrated extract was stored in the refrigerator until analysis.

Fermentation condition with utilization white dragon fruit extract as a prebiotic activity assay

The fermentation was carried out in 20 mL of media in universal bottles. The assay was performed by adding 1% (vol/vol) of an overnight *L. casei* and *E. coli* to separate tubes containing growth media (MRS broth) with 1% (wt/vol) glucose or 1% (wt/vol) white dragon fruit extract at various concentrations. There were growth media (MRS broth) with glucose, growth media with 2% (w/v) inulin, and growth media with 2%, 4%, 6%, 8%, 10% (w/v) of dragon fruit extract as P1, P2, P3, P4, P5 respectively. The tubes were then incubated at 37°C for 24h in anaerobic

condition in the anaerobic jar. One (1) mL of sample was diluted in serial dilution for growth enumeration. Meanwhile, for *E. coli*, one (1) mL inoculum *E. coli* (3% v/v) was added into fermentation medium which is growth media Nutrient broth and glucose as a negative control without prebiotic, growth media with 2%(w/v) inulin as positive control, growth media with 2%, 4%, 6%, 8%, 10% (w/v) of white dragon fruit extract as prebiotic respectively. The aerobic condition was given for growing *E. coli*.

Enumeration of bacterial growth and pH

Serial dilution was carried out immediately after sampling by using saline water. One (1) mL fermentation media was taken for serial dilution at tent fold dilution (10^{-2} to 10^{-6}) using saline water to obtain countable CFU plate count (30-300 CFU/plate). The viable count of bacterial cultures was enumerated by spread plate method using MRS agar in duplicate analysis, and the counts were reported as colony forming per millimeter suspension (CFU mL^{-1}). The media of MRS and Nutrient were prepared according to the instructions. The measurement of pH was carried out on the samples using *Lovibond* digital pH meter.

Prebiotic index

The prebiotic index (I_{preb}) value was calculated according to the equation of (Palframan et al. 2003), which is the ratio of probiotic growth in the prebiotic to probiotic growth in a control carbohydrate (in the study we used glucose). A prebiotic index higher than 1 means that the carbohydrate positively affects the probiotic growth. If the prebiotic index is near to 1, indicates a low effectiveness of the evaluated carbohydrate. The prebiotic index was calculated according the equation below:

$$I_{preb} = \frac{CFU\ of\ probiotics\ in\ prebiotic\ carbohydrate}{CFU\ of\ probiotics\ in\ control\ carbohydrate}$$

Prebiotic Activity Score

Prebiotic activity score (PAS) of *L. casei* was calculated against *Escherichia coli* strains, as reported by (Figueroa-González et al. 2019). Carbohydrates have a positive activity score if they are metabolized as well as the control by probiotic strains, and are selectively metabolized by probiotics but not by other intestinal bacteria. Changes in cell density were calculated as differences in the differences in log 10 CFU mL^{-1} between viable count at 0 h and the viable count at 24 h.

$$\text{PAS} = \frac{(Log\,P_{24} - Log\,P_0)_{prebiotic}}{(Log\,P_{24} - Log\,P_0)_{glucose}} - \frac{(Log\,E_{24} - Log\,E_0)_{prebiotic}}{(Log\,E_{24} - Log\,E_0)_{glucose}}$$

Where Log P is the log of growth (CFU mL^{-1}) of the probiotic bacteria at 24 h (P_{24}) and 0 h (P_0) of culture on prebiotic and glucose; Log E is the log of growth (CFU mL^{-1}) of *E. coli* at 24 h (E_{24}) and 0 h (E_0) of culture on prebiotic and glucose. By definition, substrate with high PAS support good growth of probiotic bacteria, with cell count (CFU mL^{-1}) comparable with that when grown on glucose. On the other hand, the growth of *E. coli* grown on the prebiotics should be very low (according to the theory) compared to that on glucose. Therefore, using this equation the prebiotic activity score of an oligosaccharide can be determined relative to any given strain.

Making kefir with the addition of white dragon fruit extract

Goat's milk and kefir grains were purchased from a private household in Lampung (Indonesia). Kefir grains were washed with distilled water prior to use, and the goat's milk was pasteurized at 70°C for 30 seconds. Pasteurized goat's milk was allowed to cool down to room temperature. Next, the milk was mixed with 10% (w/v) dragon fruit extract and stirred, and then inoculated with 3% (w/v) kefir starter. The mixture was stirred until smooth and then poured into sterile glass jars with lids. Then, it was incubated at room temperature for 24 hours so that it was viscous into kefir beverage. After incubation, kefir grains were separated from the kefir beverage by filtration through plastic sieve and washed before the next incubation. The kefir beverage was bottled in sterile glass bottles with lids. Characterization of kefir was carried out according to the standard Codex Stan 243-2003. Measurement of antioxidant activity was carried out to determine the health function of kefir added with white dragon fruit extract.

The chemical analysis of kefir with the addition of white dragon fruit extract included titratable acidity using the acid-base titration method (Sudarmadji et al. 1997), protein content using Micro-Kjeldahl method, alcohol content using the Pygnometer method followed Setyawardani et al. (2015). Analysis of microbiology included total yeast, LAB and aerobic bacteria using spread plate method followed by Kustyawati et al. (2021). The assay of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was followed by Biadala and Adzahan (2021).

Data analysis

Data obtained from the observations were performed statistically using a one-way ANOVA (IBM SPSS Statistics Data Editor, Edition 20) for comparing the mean values of the data of bacterial growth, prebiotic index and probiotic score. All significant differences between means will assessed at significant level of $\alpha = 0.05$ (95% confident level). Analysis of variance (ANOVA) and Duncan Multiple Range Test was used to compare any significant different between samples, where $p<0.005$ was considered statistically significant. Prebiotic index and probiotic activity score were presented in Histogram figure.

RESULTS AND DISCUSSION

The pH and increase in cell densities of probiotic and enteric bacteria in media containing prebiotics (white dragon fruit extract)

The increase in cell densities for *L. casei* and enteric bacteria (*E. coli*) following growth for 24h on 1% (wt/vol) glucose or specified amount (wt/vol) of prebiotic (white

dragon fruit extract) are shown in Table 1. For a specific given sugar to have prebiotic activity, that sugar should be metabolized by test bacteria as well or nearly as well, as glucose is metabolized. Growth (CFU mL^{-1}) of *L. casei* on the prebiotic (white dragon fruit extract) was high than on glucose, with all combinations having a significantly higher ($p<0.005$) increase in cell densities on the prebiotic compared to glucose. Furthermore, there was a gradual increase in cell densities of *L. casei* as the concentration of prebiotic contained in the growth medium increased. In contrast, the increase in cell densities of *E. coli* grown on all media tested was less ($p<0.005$) than for glucose.

The other characteristic property of a prebiotic substrate is that it should be selective and not fermented by enteric bacteria. For this reason, growth on each white dragon fruit extract containing medium was also evaluated for an enteric bacterium (we used *E. coli* in this experiment). Growth of the *E. coli* on all of the prebiotics (white dragon fruit extract) containing medium was significantly less ($p<0.005$) compared with growth on glucose. This finding was in line with Huebner et al. (2007) found that a mixture of three enteric bacteria *E. coli* ECOR 1, ECOR 2, and ECOR 22 had less ($p<0.005$) growth in prebiotic medium compared with growth on glucose.

The study showed that the *L. casei* were able to grow using carbohydrates and oligosaccharides contained in the extract white dragon fruit as a carbon source, although its ability to grow was different in each substrate. *Lactobacillus casei* is a probiotic extensively used as a fermentation starter culture in milk, vegetables and fruit based food products. Growth of *L. casei* is greatly influenced by substrate and pH of the medium. The pH and nutrient of white dragon fruit containing considerably high vitamin C, 31.05 mg $100g^{-1}$ (Luu et al. 2021), and fatty acids (Jerônimo and Costa Orsine 2015) may also contribute to the growth of *L. casei*. Bandiera et al. (2013) reported that *L. casei* added to the yogurt fermentation remained viable in populations of more than 10^8 CFU/g during 21 days of storage, in which the pH ranged from 4.93-4.32. The study by de Melo et al. (2014) reported that pitaya pulp has a pH of 4.82-6.1. On the other hand, the viability of *E. coli* 15.44 - 22.84% was due to favorable conditions such as temperature and growth medium.

Prebiotic index

Prebiotic index obtained with the different concentration of white dragon extract is shown in Figure 1. The highest value was for *L. casei* in P5 (1.82), significantly different from other prebiotics (white dragon extract). Furthermore, all prebiotic indexes obtained in this study indicated a beneficial effect of the tested carbohydrates (white dragon extract containing media) over the growth of probiotic *L. casei*, with a prebiotic index of 1, except for inulin. On the other hand, the prebiotic indexes of negative or less than 1 showed that tested carbohydrates did not support the growth of enteric bacteria. This study was in accordance with the prebiotic index for *L. casei 1* on commercial prebiotic lactulose, Frutafit, and Oligomate (Figueroa-González et al. 2019). Similar to our finding, Pansai et al. (2020) reported the I_{preb} score of selected fecal bacterial population on dragon fruit oligosaccharides (DFO) were less than 1 (in the range of 0.068, 0.024, and 0.073 respectively, in the proximal, transverse and distal colon). In contrast to our findings, LHEPS (*L. helveticus* LZ-R-5- EPS) and LPEPS (*L. pentosus* LZ-R-1-EPS) had very high prebiotic indexes of 13.88 and 11.78 for the growth of *Lactobacillus* and *Bifidobacterium*, respectively (Xu et al. 2022). This can be expected because LHEPS and LPEPS are heteropolysaccharides composed of galactose and glucose fractions that are easily metabolized by probiotics. LHEPS and LPEPS are exopolysaccharides EPS isolated from Tibetan kefir grains fermented by *L. helveticus* and *L. pentosus*.

Prebiotic activity score

Probiotic activity scores presented in Figure 2 were derived from the cell density value from Table 1. The highest prebiotic activity score (1.84) was for *L. casei* on medium P5, and the lowest (0.97) was for growth media P2. Furthermore, *L. casei* showed prebiotic activity score of higher than 1 means that the growth of *L. casei* is better in the entire evaluated growth media (P) than *E. coli*. Rubel et al. (2014) stated that the relative growth rate (RGR) value >1 means that tested samples (in this research were white dragon fruit extract), prebiotics/oligosaccharides addition as growth media could support the growth of probiotic (in this research was *L. casei*) than using glucose media. Prebiotic activity score of *L. casei* grown on white dragon fruit extract in our study was higher than prebiotic activity score found by Budhisatria et al. (2017). It was reported that low-value prebiotic activity score (less than 1) for *L. paracasei* grown on purified oligosaccharides (POS) from banana Uli, Raja Sere, Tanduk, and Cavendish with the score of 0.33; 0.15; 0.15; and 0.77 respectively. The cause could be due to differences in oligosaccharide compounds in prebiotic sources and species or probiotic strains. Figueroa-González et al. (2019) explained that variation in prebiotic activity scores for the different prebiotic used by a single probiotic strain was due to the metabolic diversity of the lactobacilli. For example, *L. rhamnosus* had significantly higher scores than *L. casei* on Oligomate55 compared to Fruitafit and lactulose. Another study on Breadfruit resistant starch for the growth of *L. plantarum* and *B.bifidum* (Zakaria et al. 2018) showed that PAS score of *B.bifidum* growth on Breadfruit resistant starch was 0.65, which was lower than our findings. The low water holding capacity of the starch in Breadfruit was very likely to be one of the causes of the low PAS value of probiotics in Breadfruit starch.

In regard to our finding, white dragon fruit extract at concentration less than or equal to 10% may serve as good growth for probiotic and have the potential to be added to the diet. Dahiya and Nigam (2022) stated that incorporation of oligosaccharide prebiotic into a diet in modest quantities of about 5 to 20 g per day stimulate the growth of bifidobacteria and lactobacilli in the intestine of adult.

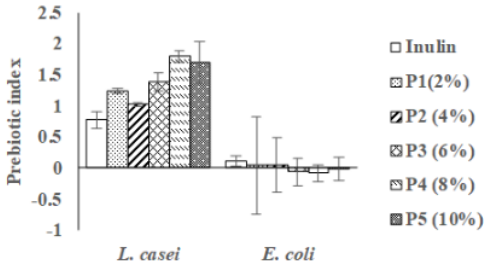

Figure 1. Prebiotic index values of *L. casei* (probiotic) and *E. coli* (enteric bacteria) grown on white dragon fruit extract at different Concentrations

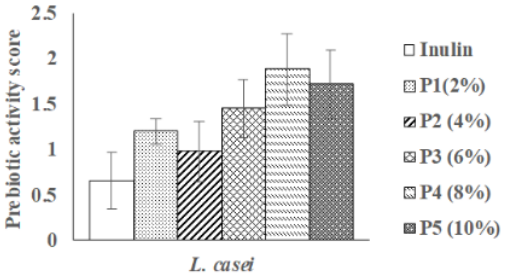

Figure 2. Prebiotic activity score of *L. casei* grown on white dragon fruit extract at different concentrations

Table 1. pH and increase cell densities between time 0 and time 24h (Log CFU mL^{-1}) ± standard deviation for *L. casei* and *E. coli* grown in the white dragon extract containing medium at various concentrations

Tested medium	pH	*L. casei* (0h)	*L. casei* (24h)	*E. coli* (0h)	*E. coli* (24h)	*ΔL. casei*	*ΔE. coli*
Glucose	6.5	6.40	8.06	4.38	6.54	0.85±0.74 [a]	1.94±0.21 [a]
Inulin	6.2	6.88	8.10	4.52	5.78	0.65±0.49 [a]	0.23±0.14 [b]
P1 (2%)	6.0	6.93	8.04	4.60	5.65	1.05±0.26 [a]	0.08±0.04 [ab]
P2 (4%)	6.0	6.98	8.35	4.81	5.56	0.86±0.64 [a]	0.09±0.03 [ab]
P3 (6%)	5.7	6.65	8.29	4.85	5.61	1.17±0.51 [a]	-0.13±0.14 [c]
P4 (8%)	5.5	6.18	8.46	4.58	5.49	1.41±0.50 [a]	-0.17±0.10 [c]
P5(10%)	5.3	6.23	8.15	4.59	5.54	1.54±0.60 [a]	-0.03±0.33 [ab]

Note: Mean value (± standard deviation) with different letter in the same column were significantly different ($p<0.005$). Δ Log CFU $mL^{-1}$is T_{24}-T_0.

Table 2. Chemical analysis value of kefir added with 10% of white dragon fruit extract and the Codex Stan 243-2003

Chemical parameters	Result of analysis	Codex Stan 243-2003
Milk Protein (% w/w)	2,9	Min 2,7
Milk fat (%)	Not measured	<10
Titratable acidity (%)	0.18	Min 0,6
Ethanol (% vol/w)	0.21	Not stated
Total bacteria count (cfu/mL)	2.51 x 10^{10}	Min 10^7
Total LAB (cfu/mL)	3.5 x 10^8	
Yeast (cfu/mL)	4.5 x 10^5	Min 10^4
Antioxidant (%)	55.13	

Fermentation of goat's milk with the addition of 10% white dragon fruit extract for kefir beverage

Kefir is fermented milk produced by the activity of bacteria and yeast contained in the kefir grain starter culture, and reported as having unique taste of sour, creamy, and alcoholic scent (Farnworth 2005). Starter kefir is a symbiotic form of a consortium of microorganisms consisting of several homofermentative and hetero-fermentative lactic acid bacteria (Streptococcus, *Lactobacillus kefiri*, Lactococcus, and Leuconostoc), Acetobacter, and lactose fermenting yeast (*Kluyveromyces marxianus*) and non-lactose fermenting yeast (*Saccharomyces cerevisiae*), Candida and Pichia (Nikolaou et al. 2016). Fermentation of milk using kefir is traditionally done by the Russians.

In this research, the use of prebiotic white dragon fruit extract by adding it to fermented milk for kefir beverage aims to determine whether these prebiotics can be utilized by kefir probiotic and provide health benefits by measuring antioxidant activity. Kefir microflora known as probiotic that utilize dragon fruit prebiotics for their growth and reproduction so as to provide health benefits to the host attributed to the production of metabolites and colonization of enteric bacteria. Table 2 shows the chemical analysis of kefir made from fermentation of goat's milk with the addition of 10% prebiotic white dragon fruit extract. The titratable acidity did not fulfill Codex stand 243-2003. The fermentation conditions such as concentration of kefir grain starter and nutrition (substrate fermentation) may influence the final product. The ratio of kefir and substrate (milk) in the fermentation is very important as reported by Farnworth (2005), the optimum ratio is 1:30 to 1:50. During the fermentation, homofermentative LAB, Lactobacillus, Streptococcus, and Leuconostoc, grow rapidly and produce lactic acid causing a drop in pH. The low pH is causing the decline number of streptococci, but favors the growth of lactobacilli. Yeasts encourage the growth of streptococci and Acetobacter to produce aroma. Lactose-fermenting yeasts hydrolyze milk's lactose to produce glucose and galactose, then glucose was utilized by non-lactose fermenting yeasts to produce alcohol and CO_2. However, as the fermentation proceeds, growth of LAB is favored over the growth of yeasts and acetic bacteria. Therefore, only a slight alcohol was present. The

addition of glucose may increase the *S. cerevisiae* numbers, lactic acid, and ethanol production (Liu and Lin 2000). Milk fat from Etawa crossbreed goat's milk was not measured in this research, but it was about 5%, as reported by Yusa et al. (2016). The growth of yeasts was fulfill Codex Stan 243-2003, and the growth of LAB was quite high of about >10^6 cfu/mL which was an indication that the prebiotic can be hydrolyzed by probiotic kefir.

Antioxidants are chemical compounds that prevent the oxidation process from producing free radicals by providing electrons to neutralize them. Kefir has higher antioxidant capacity than milk. Milk itself has the antioxidants capacity, primarily attributed to the presence of sulfur-containing amino acids in milk such as cysteine (Biadala and Adzahan 2021); however, antioxidant capacity in Goat's milk kefir can be associated with metabolite products and hydrolysis products by kefir microflora during fermentation. The addition of prebiotic white dragon fruit extract increased the antioxidant capacity in kefir beverage (Table 2) may be due to the present of polyphenols, flavonoids and vitamin C containing in white dragon fruit flesh. Umam et al. (2021) reported the antioxidant capacity (DPPH radical capturing activity) (%) of goat's milk kefir was 41.33± 1.51, while that of kefir added with prebiotic of white dragon fruit extract in our study was 55.13.

In conclusion, quantitative prebiotic index and prebiotic activity scores that describe the extent to which 5 different concentration of white dragon fruit extract containing media support selective growth of probiotic *L. casei* were determined. All of white dragon fruit extract at different concentration have the beneficial effect on the growth of *L. casei* (prebiotic index higher than 1) except inulin, while the medium tested did not support the growth of enteric *E. coli* (the prebiotic index less than 1 or negative). The highest score of probiotic activity was obtained for *L. casei* grown on 10% (P5) of white dragon fruit extract, while the lowest was on 2% (P1) of white dragon fruit extract. This study can be used as a basis for evaluating the combinations of probiotic and prebiotic white dragon fruit extract at concentration less than or equal to 10% for applications as synbiotics in the product of kefir beverage.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7