OPEN ACCESS International Journal of Applied Biology

ISSN : 2580-2410
eISSN : 2580-2119

KEFIR OF KEJI BELING TEA *(Strobilanthes crispus)* AS FUNCTIONAL BEVERAGE FOR GLUCOSE INTOLERANCE

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Abstract

Kefir is beverage fermented product, it is usually made from milk. However, there is an arising concern about lactose intolerant. Therefore, taking benefit of herbal tea solution as raw material of kefir will give multiple benefits. The used tea solution was made from keji beling leaves (*Strobilanthes crispus L.)* which has many benefits. Kefir beverage is probiotic beverage which is beneficial for digestion health and can keep immune system, as well as keji beling tea which is rich in antioxidants. The used design in this research was Complete Randomized Design (Rancangan Acak Lengkap) with 4 levels of treatment namely: concentration of keji beling 0%, 10% and 15% with fermentation duration of 12 hours and 24 hours. Based on the research result pointed out the obvious effect between treatment toward pH, total polyphenols, total acetic acid, and total glucose with p score = 0,00. The result of lactic acid bacteria (BAL) total is 1,914 x10^7 CFU/mL and yeast total is 1,532 x 10^7 CFU/mL on concentration of 15% kefir of keji beling tea with fermentation duration of 24 hours. Result for organoleptic test shows the obvious effect (p=0,00) between the treatment toward parameters of taste, aroma, color and power. Panelist takes pleasure on the treatment P1 with concentration of horsewhip tea 150% in fermentation duration of 24 hours.

Article History
Received 12 October 2020
Accepted 31 December 2020

Keyword
Kefir,
Keji beling tea,
Strobilanthes crispus L,
Fermented Beverage.

Introduction

Kefir is fermented milk product which can be made from raw materials of cow milk, goat milk or sheep milk by adding kefir grains which consists of lactic acid bacteria and yeast. According to Gulitz *et al,* (2011), kefir grains formed from cultures of various strains of healthy bacteria and yeast, which existed together on polysaccharide matrix which was made by bacteria. Some yeasts which contained in kefir that rich in probiotics namely; *Kluyveromyces, Saccharomyces, Acetobacter spp., Lactobacillus acidophilus, brevis, casei, fermentum, helveticus, kefiri, kefiranofaciens parakefiri, lactis* and *Leuconostoc mesenteroides* (Cai, Sounderrajan and Serventi, 2020). The relationship of microbial symbiosis produced a stable growth culture. Microbes change glucose to be lactic acid, alcohol (ethanol) and carbon dioxide which will produce carbonated fermented beverage. The alcohol content of water kefir ranges between 0,5-1% (Gulitz *et al*., 2011). All this time, kefir is usually made from milk. However, there is an arising concern about lactose intolerant and also the growing number

of vegetarians which initiates the increasing in availability of non-dairy beverages (Mubin, 2016). Therefore, the suggested raw materials are from fruits or vegetables as the medium of probiotics growth. Water kefir or also known as kefir grains are able to produce alcohol (ethanol) relatively low, carbon dioxide, organic acids (lactic and acetate) and fat content are few in number. The use of water kefir can be used from the compound of herbal tea water, such as keji beling tea.

Keji Beling (*Strobilanthes crispus L)* is one of plants from familia Acanthaceae which has spread in Indonesia that is used empirically as anti-diabetes and as the ingredients of other traditional medicine that has multiple benefits. From various researches was known that keji beling contains chemical substance such as: potassium, sodium, calcium, silicate acid, alkaloid, saponins, flavonoid and polylenoid. One of plants which is used as traditional medicine is keji beling (Setyaningsih, 2008). Some contents of keji beling *(Strobilanthes crispus L.)* are phenol compound which has benefit as anti-bacteria, the content of potassium and silica helps to overcome haemorrhoids and dysentery. The content of vitamin C, B1, B2 and catechins makes keji beling potentially as antioxidants (Amalia *et al*, 2015). Keji beling leaves have a rich antioxidant activities compared with verbate (herbal tea) and vitamin C. Concerning the previous research result of kefir fermentation of black tea with adding carrot juice pointed out the increasing number of lactic acid bacteria (BAL) with the low glucose level (Subardjo, 2017).

The aim of making process of herbal tea kefir on this keji beling is to know effectiveness making process of kefir with raw material of herbal tea which is expected will produce simultaneous benefit for health effect of herbal tea and functional effect of herbal tea kefir.

Methodology

This research was conducted on May until July 2019 at Biology Laboratory, FBS Wijaya Kusuma University of Surabaya and laboratory of research association and industrial consultation (BPKI).

Materials on this research are solution of keji beling tea brand “Herba Tazakka” and kefir bacteria from Bioteknologi Mikroorganisme Laboratory, TG2 Ubaya. This experimental research used complete randomized design (RAL) with 5 times of repetition namely; variation on concentration of horsewhip tea solution 0%, 10% and 15% with fermentation duration of 12 hours and 24 hours. The used chemical ingredients are sucrose buffer pH 4 and buffer pH 7, PP indicator, aquadest, NaOH 0,1 N, alcohol 70%. Whereas, the used tools are glass bottle, iron sieve, beaker glass, autoclave, measuring cup, pH meter, water thermometer, digital scale, drop pipette, measuring pipe, petri cup, reaction tube, wooden stirrer, plastic spoon, measuring flask 100 mL, ependorf tube, cuvette, big jar, micropipette, spectrophotometer UV-VIS, and centrifuge.

Performing of research was at the first stage as for procedural stage on this research that was as follows: 1. Making process of keji beling tea solution (*Stachytarpheta jamaicensis*) with concentration 10% and 15%. 2. Adding 150 gram kefir grains bacteria in 1 L. 3. Adding 7-9 raisins as kefir grains’ nutrition on kefir of keji beling tea that has been made. Then it stored at room temperature in accordance with the treatment of fermentation duration 12 hours and 24 hours. After that it was continued with measuring acid total by using titration method, polyphenol total by using spectrophotometer UV-VIS at the wavelength 760 nm, glucose total by using *Luff Schoorl* method, and for measuring pH by using pH meter. The stage of total lactic acid bacteria (BAL) analysis that was the sample was diluted into 0,1 % 9 mL peptone

solution (this solution is 10^{-1} dilution), then it was continued until 10^{-8} dilution. On the last three dilution series, each sample was taken1 mL and poured into petri cup which was filled with MRSA media. Colony growth was counted by using Total Plate Count (TPC) number into 1 g by multiplying the average number of colonies with dilution factor which was used with colony forming unit, whereas for the stage of yeast total that was kefir sample was diluted into 0,1 % 9 ml peptone solution (this solution is 10^{-1} dilution), then it was continued until 10^{-5} dilution. Each of them was taken 1 mL from the last three dilutions and poured into sterile petri cup, then poured with sterile PGYA media (warm) until the bottom of cup covered with media. After the media has solidified, it was incubated at 30^{o}C temperature during 48 hours. Then, the growth colony was noted (Mubin and Elok, 2016).

Parameter data on this research is pH, acetate acid total, glucose total, polyphenol total, lactic acid bacteria (BAL) total and yeast total.

Result and discussion

Based on the research result of keji beling tea kefir (*Strobilanthes crispus L.)* toward pH, polyphenol total, acetate acid total and glucose total pointed out the obvious effect with p score = 0,00. The result of pH score of keji beling tea on concentration 10% in duration 12 hours is 4,4 while in 24 hours fermentation pH decreased to be 3,78. For concentration 15% in 12 hours pH score is 4,18 and decreased in fermentation duration 24 hours with pH 3,375 (Table 1). The thicker of tea concentration and the longer fermentation duration then pH score is more acid. On polyphenol total of kefir of keji beling tea indicated the increasing score by the longer of fermentation process from 0,144% up to 3,47%. Whereas, for acetate acid total is around 1,014%-3,26%. However, the longer fermentation proses for glucose total occurred the decreasing from 6,486% until 4,054 on concentration of keji beling tea 10% and for 15% of tea concentration is from 5,08 % until 4 % (Table 1).

Table 1. The average of pH, polyphenol, acetate acid, glucose of kefir keji beling tea

Treatment	pH	Polyphenol (%)	Acetate acid (%)	Glucose (%)
K1	4,4^{d}	0,144^{a}	1,014^{a}	6,486^{c}
K2	4,18^{c}	2,25^{b}	2,84^{b}	5,08^{b}
K3	3,78^{b}	3,30^{c}	3,11^{c}	4,054^{a}
K4	3,375^{a}	3,47^{c}	3,26^{d}	4,0^{a}

Note :

K1= Concentration of keji beling tea 10% and duration of fermentation 12 hours,
K2= Concentration of keji beling tea 15% and duration of fermentation 12 hours,
K3= Concentration of keji beling tea 10% and duration of fermentation 24 hours,
K4= Concentration of keji beling tea 15% and duration of fermentation 24 hours.

From the result of Lactic Acid Bacteria (BAL) and yeast total in kefir of keji beling tea on concentration of keji beling tea 10% with duration 12 hours pointed out the BAL total is 0,662 x10^{7} cfu/mL and yeast total is 0,4460 x10^{7} cfu/mL,while for duration of fermentation 24 hours

pointed out BAL total is 1,694 x10^7 cfu/mL and yeast total is 0,798 x10^7 cfu/mL. The result of concentration of keji beling tea 15% within 12 hours of fermentation is BAL total 0,792 x10^7 cfu/mL and yeast total 0,582 x10^7 cfu/mL, while within 24 hours of fermentation produces BAL total 1,914 x10^7 cfu/mL and yeast total 1,018 x10^7 cfu/mL (Table 2). From the result of statistical test, kefir of keji beling tea toward BAL and yeast total indicates the obvious effect with p score =0,000.

The average of BAL total above pointed out that the longer of fermentation duration will increase the growth of lactic acid bacteria. Where environmental factor will also affect the growth of BAL namely salinity, temperature, pH, availability of carbohydrates as its source of nutrition (Pelczar *et al.*,2005). The average of pH condition approaches 4. From the previous research, the fermentation process produced pH 3,5 - 4,5 and alcohol content under 1% and was conducted at temperature 25 - 30 ° C in anaerobic condition. As for yeast strains which included within it are *Kluyveromyces* and *Saccharomyces* and rich in probiotic bacteria, such as lactic acid bacteria *Acetobacter spp., Lactobacillus acidophilus, brevis, casei, fermentum, helveticus, kefiri, kefiranofaciens parakefiri, lactis* and *Leuconostoc mesenteroides*. Furthermore, yeast strains was from *Kluyveromyces lactis* and *Saccharomyces cerevisiae* (Cai, Sounderrajan and Serventi, 2020). This indicates that in pH 4 on this research, lactic acid bacteria can change sucrose to be lactic acid on kefir of tea media then the growth of BAL will be obstructed and this condition is used by yeast for growing to do metabolism (Manik, 2005 as cited by Nadhiroh, 2018).

Some of good microorganisms which contained in kefir of tea make it as functional health beverage for glucose intolerant. This kefir of tea is included on water kefir as probiotic beverage which is health and low glucose (Laureys, 2014). The extract of keji beling can produce antibacterial because the existence of some chemical compound on this leaves extract, such as; polyphenol, cathechins, caffeine, alkaloid, tannin, β-citosterol and stigmaste (Setyawan, A. Budi, Winarto, 2016). Whereas, antibacterial compound which contained in kefir is lactic acid and alcohol. Therefore, during the fermentation process between kefir grains bacteria and tea solution also occur the high activities. On picture 1, it is seen the difference of color on concentration 15% which looks slightly brownish while on concentration 10% looks clearer.

Generally, this taste of kefir is sour and slightly sweet. This is caused by the activities of yeast, bifidobacteria and lactic acid bacteria which involved in kefir fermentation (Laureys, 2014). The success of fermentation process in kefir of tea needs to consider the duration of fermentation. If the length of fermentation duration is excessive then it produces the excessive lactic acid bacteria and occurs the reducing lactic acid bacteria is caused by reducing need of nutrition that will cause the failure in fermentation. On fermentation process will occur fission of nutrients that exist in the ingredients (Talattof, 2019).

Table 2. Result of BAL Total of Kefir Keji beling tea

Treatment	BAL Total (X^7) CFU/mL	Yeast Total (X^7) CFU/mL
K1	0,662^a	0,4460^a
K2	0,792^b	0,582^b
K3	1,694^c	0,798^c

K4	1,914[d]	1,018[d]

Note:

K1= Concentration of keji beling tea 10% and duration of fermentation 12 hours,
K2= Concentration of keji beling tea 15% and duration of fermentation 12 hours,
K3= Concentration of keji beling tea 10% and duration of fermentation 24 hours,
K4= Concentration of keji beling tea 15% and duration of fermentation 24 hours.

Picture 1. Kefir of Keji beling tea (a. Concentration 10%, b. Concentration 15%)

Conclusion

Kefir of keji beling tea (*Stachytarpheta jamaicensis*) with concentration 10% and 15% in duration of fermentation 12 hours and 24 hours gives the obvious effect with p score = 0,00 toward pH total, acetate acid total, glucose total, polyphenol total, BAL total and yeast total. The best treatment of keji beling kefir pointed out on concentration 15% within duration 24 hours. For pH of keji beling kefir shows score 3,375, polyphenol total 3,47%, acetate total 3,26% and glucose total 4. Whereas, the result of BAL (lactic acid bacteria) total is 1,914 x 10^7 cfu/mL and for yeast total is 1,018 x10^7 cfu/mL.

Acknowledgment

The researcher acknowledges the great gratitude due to team of LPPM Wijaya Kusuma University, Surabaya for financial support that has been given and to Dean of Languange and Science Faculty for the assistance of laboratory facilities during the research process.

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