PERUBAHAN SIFAT KIMIA KEFIR-AIR YANG DIFERMENTASI PADA BERBAGAI SUHU

CHEMICAL PROPERTIS CHANGES OF WATER-KEFIR FERMENTED AT VARIOUS TEMPERATURES

SRR Pertiwi[1a], N Novidahlia[1], dan I Juliana[1]

[1]Program Studi Teknologi Pangan, Fakultas Ilmu Pangan Halal, Universitas Djuanda Bogor
Jl. Tol Ciawi No.1 Kotak Pos 35 Bogor 16720
[a] Koresponsdensi: Sri Rejeki Ratna Pertiwi, Email: sri.rejeki.pertiwi@unida.ac.id
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ABSTRACT

Water-kefir is a probiotic fermented water drink made by inoculating water with water-kefir grains. One of the factors influencing the growth of microbes is temperature. Chemical properties changes of water-kefir fermented at various temperatures were studied. Water-kefir grains (crystal algae) were fermented in water containing 2% of sugar for 3 days at 5^{o}C, 15^{o}C, and 26^{o}C. Every 12 hours of fermentation, total soluble solid, total sugar, total lactic acid, alcohol, and pH value were determined. Temperature affected significantly ($p<0,05$) on total sugar, total lactic acid, pH, and alcohol, but did not affect significantly ($p>0,05$) and total soluble solid. During fermentation, all treatments showed that soluble solid did not change, whereas total sugar and pH value decreased, total lactic acid and alcohol increased. Water-kefir containing sugar 2% fermented at 5^{o}C and 15^{o}C could be fit for consumption on longer than 3 days fermentation, and no longer than 1 day when fermented at 26^{o}C

Key words: water-kefir, crystal algae, lactic acid bacteria, probiotik.

ABSTRAK

Kefir-air adalah minuman fermentasi probiotik yang dibuat dengan menginokulasi air dengan biji kefir-air. Salah satu faktor yang memengaruhi pertumbuhan M suhu ikroba adalah suhu. Pada penelitian ini dipelajari perubahan sifat kimia kefir-air yang difermentasi pada berbagai suhu. Biji kefir-air (alga kristal) difermentasikan di dalam larutan gula 2% selama 3 hari pada 5^{o}C, 15^{o}C, dan 26^{o}C. Setiap 12 fermentasi dianalisis kadar total padatan larutan, gula, asam laktat, alkohol, dan pH. Suhu berpengaruh ($p<0,05$) terhadap kadar gula, alkohol, dan pH, tetapi tidak berpengaruh ($p>0,05$) terhadap total padatan terlarut. Selama fermentasi, semua perlakuan menunjukkan bahwa total padatan terlarut tidak berubah, sedangkan kadar gula dan pH menurun, kadar asam laktat dan alkohol meningkat. Kefir-air berkadar 2% yang difermentasi pada suhu 5^{o}C dan 15^{o}C dapat dikonsumsi hingga fermentasi selama 2 hari, dan hanya 1 hari jika difermentasi pada suhu 26^{o}C.

Kata kunci: kefir-air, alga kristal, bakteri asam laktat, probiotik.

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PENDAHULUAN

Water-kefir is a probiotic fermented water drink made by inoculating water with water-kefir grains. Water-kefir grains are also known as sugary kefir grains, Gingerbeer plants, California bees, tibicos, tibis grains, tibi complex, of Japanese water crystals (Pidoux 1989; Zoe 2010; Boeitus 2011).

The grains make up a culture of various strains of healthy bacteria (lactic acid bacteria) and yeast, held together in a polysaccharide matrix created by the bacteria. The symbiotic relationship of the microbes produces a stable growing culture. The microbes feed on sugar and produce lactic acid, alcohol (ethanol), and carbon dioxide, yielding a fermented carbonated beverage (Pidoux 1989; Zoe 2010).

Microorganism found in water-kefir grains are lactobacilli, lactococci, and Yeasts (Bottazzi *et al*. 1994). Genus lactobacillus brevis, Lactobacillus viridescens, Lactobacillus casei, Lactobacillus kefiri, Lactobacillus kefiranofaciens, Lactobacillus kefirgranum, Lactobacillus parakefir. Genus lactococci include Leuconostoc spp. and lactococcus lactis (Marshall *et al*. 1984; Takizawa *et al*. 1994; Garrote *et al*. 1997). Yeasts inclede Candida kefir, Saccaromyces cerevisiae, Candida holmii, Saccarom unisporus, and Saccaromyces lopolytica (Angulo *et al*. 1993; Garotte *et al*. 1997).

The health benefits of consuming water-kefir are endless. The are a natural supplier of probiotics to our digestive track. Another health advantage of water-kefir is that people who do not wish to consume dairy or have a vegan type diet may find that water-kefir provides the living probiotics without the need for dairy (Zoe 2010).

There are two types of kefir grains: milk-kefir and water-kefir grains. Though similar in function, milk-kefir grains are quite different than water-kefir grains. Milk-kefir grains are white, cottage cheese-looking with slimy feel to them. Water-kefir grains, however, are translucent, cauliflower-like and easily break apart under pressure. Also, milk-kefir grains feed on the lactose in milk, whereas water-kefir grains feed on sugar (Pidoux 1989; Schroeder 2011).

Several factors influence the growth of microbes. Those factors are factors categorized as physical factors including temperature, pH, oxygen, hydrostatic pressure, osmotic pressure, (moisture, aw, RH), light, and factors categorized as chemical or nutritional factors including carbon, nitrogen, sulfur, phosphorus, trace elements, vitamins. Concentration of sucrose as a source of carbon for water-kefir has been investigated on the previous study. In that study, water-kefir grains were fermented in water containing sugar at 2%, 5%, 8% and 11%, and all treatments produced water-kefir drink judged suitable for human consumption after 72 hours or 3 days fermentation, with the least pH and highest alcohol content were 4,67 and 1,321% respectively (Pertiwi *et al*. 2012).

Another factors influencing the growth of microbes that will be a focus of our research as temperature. Every microbe has a specific growth temperature range. Typical growth rate and temperature can be divided into three: minimum, optimum, and maximum growth temperature. Minimum growth temperature is the lowest temperature which microbe can grow, optimum growth temperature is the temperature at which grow is possible. Zoe (2010) suggests that water-kefir should be fermented at room temperature away from direct sunlight. The temperature during fermentation of water-kefir is not critical as long as it is not above one that will kill the culture (about 40^{o}C), or much below 4oC where the process will cease.

This study was aimed to carry out water-kefir fermentation for 27 hours to evaluate the affects of temperature on chemical properties changes of water-kefir including total soluble solid, total sugar, pH, production of lactic acid and alcohol.

MATERI DAN METODE

Water-kefir grains (crystal algae) were fermented in water containing 2% of sugar for 3 days at 5, 15 and 26^{o}C. fermentation was done in a plastic container having 500 mL capacity. Each container consisted 0f 400 mL sugar solution, 5% water-kefir grains, and 2% raisin. Every 12 hours of fermentation, one plastic container from each sugar concentration was taken out from the refrigerator and total soluble solid, total sugar, total lactic acid, alcohol and pH value were determined. Total sugar and alcohol were determined every 36 hours, instead of 12 hours. Total soluble solid was determined using hand refractometer, total sugar was determind by Sutadi *et al*. (1997) procedure, total lactic acid and pH were determined by Apriyantono *et al.* (1985) procedure, alcohol was measured using PHLC. The data were then analyzed using Analysis or Variance at a 5% for completely Randomized Design and linear regression.

HASIL DAN PEMBAHASAN

For the development of water-kefir for home scale, the evaluation of temperatures which could produce water-kefir drink with pH and alcohol content suitable for human consumption was required. Our previous work had shown that water-kefir drink grains fermented in water containing sugar at 2%, 5%, 8% and 11% could produce water-kefir drink suitable for human consumption after 72 hours or 3 days

fermentation, with pH ranging from 4,93 to 4,67 and alcohol content ranging from 0,835% to 1.321% (Pertiwi *et al.* 2012). According to Gilliland and Kim (1984) in Pudjianti (1987), lactic acid bacteria do not use carbohydrate more than work and fermentation was done for 3 days three different temperature, 5, 15, and 26°C.

During fermentation of water-kefir, the microbes feed on sugar and produce lactic acid, alcohol, and carbon dioxide. So, during fermentation process, sugar content will decrease, pH will also decrease due to the formation of lactic acid, and alcohol content will increase. Total sugar and pH value of water kefir during fermentation for 3 days decreased, on the other hand total lactic acid and alcohol increased. Both the decrease and increase every hour of fermentation were showed by the value of the gradients.

Chemical properties changes of water-kefir fermented for 3 days in various temperatures ranging from 5 to 26°C were compared (Table 1, 2, 3, 4, 5). In this study temperature affected significantly ($p<0,05$) on sugar, total lactic acid, pH, and alcohol, but did not affect significantly ($p>0,05$) on total soluble solid. During fermentation, all treatments showed that total soluble solid did not change, whereas total sugar and pH value decreased, total lactic acid and alcohol increased.

Table 1. Total soluble solid content of water-kefir fermented at various temperature for 3 days

emp.of Fermentation	Fermentation time (hours)							Linear Regression
	0	12	24	36	48	60	72	
A1 (5°C)	2,2[a]	2,2[a]	2,2[a]	2,2[a]	2,2[a]	2,2[a]	2,2[a]	-
A2 (15°C)	2,2[a]	2,2[a]	2,2[a]	2,2[a]	2,2[a]	2,2[a]	2,2[a]	-
A3 (26°C)	2,2[a]	2,2[a]	2,2[a]	2,2[a]	2,2[a]	2,2[a]	2,2[a]	-

A value within a column with letters are not significantly different ($p<0,05$)

Table 2. Total sugar content of water-kefir fermented at various temperatures for 3 days

Temp. of fermentation	Fermentation time (hours)			Linear regression
	0	36	72	
A1 (5°C)	2,140[a]	1,967[a]	1,560[a]	Y=-0,008 X+2.179, r=-0,97
A2 (15°C)	2,148[a]	1,841[a]	1,325[a]	Y=-0,011 X+2.183, r=-0,99
A3 (26°C)	2,148[a]	1,716[a]	0,747[a]	Y=-0,020 X+2.238, r=-0,98

a-c values within a column with different letters are significantly different ($p<0,05$)

At all temperature treatments, there was no change in total soluble solid in water-kefir fermented for 3 days (Table 1). This result did not mean that the microbes in water-kefir fed on nothing. During fermentation, the microbes feed on sugar and produce lactic acid, alcohol, and carbon dioxide. This was confirmed by the observation in our study, the decrease in total sugar during fermentation for 3 days (Table 2). Total soluble solid content was measured using a hand-refractometer. In this analysis, not only sugar but also acid and alcohol were included in the determination of total soluble solid, and this caused the total soluble solid in water-kefir remained the same during fermentation for 3 days. This result was similar to the previous research with factor affecting microbe growth of sugar concentration (Pertiwi *et al.,* 2012).

Table 3. Total lactic acid content of water-kefir fermented at various temperatures for 3 days

Temp. of fermentation	Fermentation time (hours)							Linear regression
	0	12	24	36	48	60	72	
A1 (5oC)	0,009a	0,013a	0,017a	0,022a	0,024a	0,031a	0,039a	Y=0,0004X=0,008r=0,97
A2 (15oC)	0,012a	0,020a	0,032b	0,040a	0,045b	0,048ab	0,053a	Y=0,0006X+0,015r=0,98
A3 (26oC)	0,010a	0,027a	0,042a	0,058a	0,072c	0,083b	0,115b	Y=0,0014X+0,009r=0,99

a-c values within a column with different letters are significanty different ($p<0,05$)

Levels of total sugar (Table 2) and total lactic acid (Table 3) produced at three temperature during 3 days fermentation were compared. The total sugar levels off all three temperature treatments decreased with fermentation time. The decrease of total sugar was showed by the negative value of gradient from linear regression equation. Fermentation at 26°C had the least total sugar (0,747%) after 3 days fermentation with the rate of decreased 0,020% per hour. In contrast to the results on the sugar, lactic acid levels increased with fermentation

time. During three days fermentation, the tendency of total sugar was opposite that of lactic acid in all fermentation temperatures. Fermentation at 26°C produced the most lactic acid (0,115%) during 3 days fermentation with the rate of production 0,0014% per hour. This opposite tendency pointed out that a certain amount of sugar was taken up by the microbes for their growth and production of lactic acid. The biggest value of gradient regardless the sign occurred at temperature fermentation 0f 26°C indicated that the microbes in water-kefir were most active at 26°C.

The production of lactic acid during fermentation brings about the change of pH in water-kefir. At the start of fermentation, the pH of all water-kefir fermented at three different temperature was neutral, ranging 6,92 to 6,98 (Table 4). There was a decrease in pH and inversely an increase in lactic acid content during 72 hours of fermentation (Table 4 and 3). In the three temperature of fermentation, significant differences were found in pH and lactic acid content after 24 hours until 72 hours fermentation. The changes of pH and lactic acid content during 72 hours fermentation increased with the fermentation temperature. At the end of fermentation water-kefir (72 hours), fermentation at 5°C resulted in highest pH (5,26) with the rate of decrease 0,02 per hour due to the lowest production of lactic acid, on the contrary, fermentation at 26°C resulted in lowest pH (3,79) with the rate of decrease 0,044 per hour due to the highest production of lactic acid.

Table 4. pH water-kefir fermented at various temperatures for 3 days

Temp. of fermentation	Fermentation time (hours)							Linear regression
	0	12	24	36	48	60	72	
A1 (5°C)	6,95[a]	6,12[a]	5,84[b]	5,75[b]	5,56[b]	5,36[c]	5,26[c]	Y=-0,0205X+6,570, r=-0,93
A2 (15°C)	6,98[a]	6,02[a]	5,98[b]	5,87[b]	5,64[b]	5,06[b]	4,84[b]	Y=-0,0258X+6,700, r=-0,95
A3 (26°C)	6,92[a]	6,00[a]	5,24[a]	4,48[a]	4,10[a]	3,92[a]	3,79[a]	Y=-0,0437X+6,495, r=-0,96

a-c values within a column with different letters are significantly different (p<0,05).

Table 5. Alcohol content of water-kefir fermented at various temperature for 3 days

Temp. of fermentation	Fermentation time (hours)			Linear regression
	0	36	72	
A1 (5°C)	0,040[a]	0,215[s]	0,611[a]	Y= 0,0079 X+0,003, r=0,98
A2 (15°C)	0,035[a]	0,297[a]	0,817[b]	Y= 0,0109X+0,008, r=0,98
A3 (26°C)	0,042[a]	0,421[b]	1,378[c]	Y= 0,0186X+0,054, r=0,97

a-c values within a column with different letter are significantly different (p<0,05).

Water-kefir grains contain lactic acid bacteria and yeast, so, there are two types of fermentation, lactic acid and alcohol fermentation, occure in water-kefir. According to Foster *et al.* (1961), glucose was converted intolactic acid by lactic acid bacteria, and into alcohol by yeast. D'amore and Stewart (1987) cited by Chen *et al*. (1998), demonstrated that *S. cerevisiae* had the ability to take up and ferment a wide range of sugars, e.g. sucrose, glucose, fructose, galactose, maltose, and maltotriose. The tendency of alcohol production during 72 hours fermentation was similar to that of lactic acid, but opposite to that of sugar content. These opposite tendency to sugar and similar tendency to lactic acid of alcohol production showed that a certain amount of sugar was used by the microbes for their growth and production of lactic acid and alcohol. There were found significant differences in alcohol production 3 days fermentation in all temperature treatments and the changes increased with the fermentation temperature. Fermentation at 26°C produced the most alcohol (1,378%) during 3 days fermentation with the rate of production 0,019% per hour.

KESIMPULAN DAN IMPLIKASI

Water-kefir containing 2% of sugar and fermented at 5°C, 15°C, and 26°C showed that total soluble solid did not change, whereas total sugar and pH value decreased at rate of 0,008-0,02% and 0,02-0,04% per hour respectively. The biggest value of gradients regardless the sign occurred and 0,008-0,02% per hour respectively. The biggest value of gradients regardless the sign occurred at temperature fermentation of 26°C indicated that the

microbes in water-kefir were most active at 26°C. During fermentation at 5°C and 15°C, microbes in water-kefir grain took up only a small amount of sugar, but higher amount of sugar was consumed when fermentation was taken place at 26°C. water-kefir containing sugar 2% fermented at 5°C and 15°C could de fit for consumption no longer that 3 days fermentation, and no longer than 1 day when fermented at 26°C.

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