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THE EFFECT OF STARTER DOSAGE AND FERMENTATION TIME ON pH AND LACTIC ACID PRODUCTION

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Abstract: The aim of this research is to observed the effect of starter dosage and fermentation time on pH and lactic acid production. This study used Completely Randomized Design (CRD) factorial pattern 5 x 3. The first factor is starter dosage and the second factor is fermentation time. Kefir samples were prepared using of 5, 10, 15, 20 and 25% (v/v) kefir grains, with three fermentation time 8, 16 and 24 hours. Results indicated that kefir with 10% starter and 16 hours fermentation time give the best results in pH (3.95) and lactic acid production (1.476%).

Key words: fermentation time, pH, lactic acid production, starter dosage

Introduction

Kefir, a slightly acidic fermented milk, is produced by adding lactic acid bacteria and yeasts, in the form of grains, to milk. Kefir is an acid, viscous, slightly carbonated dairy beverage *(Garrote et al., 2001)*. Traditionally kefir grains have been used for centuries in many countries, for example, in Tibet, China, as the natural starter in the production of the unique self-carbonated dairy beverage *(Saloff-Coste, 1996)*. Kefir grains looks like a waxy cauliflower substance. It proliferates abundantly when given the right environmental conditions. Kefir is a group of organisms, mostly varieties of lactobacillus and several yeasts, that have evolved to live together in a structure of their own making. Kefir traditionally is used to culture milk which it makes more digestible by consuming the lactose and to some extent breaking down casein and other proteins in some cases making the milk easier for people to digest. The organisms in kefir stop the proliferation of unhealthy organisms in milk, preserving the milk form spoiling. The organisms that grow in kefir are friendly to the human gut ecosystem and highly competitive with organisms that proliferate in unhealthy intestines. In the production of kefir, special kefir grains which consists of yeast, lactobacilli and streptococci are used as

starter. Different types of yeast and lactic acid bacteria have been found in kefir. Kefir generally takes 12 to 48 hours to form. The exact amount of time will vary depending on environmental factors, the most important of which is temperature. Cold retards the fermentation process so kefir will form more slowly in a cold area, and can be stopped by placing the grains in milk in the refrigerator. Heat speeds the process, so kefir will form more quickly in a warm area and will be more likely to over-culture. Standard room temperature were recommended, whenever possible. Allowing the kefir grains to remain in milk, longer than 48 hours risks starving the kefir grains and potentially damaging them. Kefir is an acidic and mildly alcoholic fermented dairy product that is believed to have functional properties *(Farnworth, 1999; Farnworth and Mainville, 2003; Farnworth, 2006)*. The microorganisms contained within the kefir grains typically produce lactic acid and antibiotics, such products inhibit the proliferation of both spoilage and pathogenic microorganisms in kefir milk *(Farnworth, 2006)*. However, a stable and constant starter culture, which is necessary for manufacturing a quality kefir beverage, is difficult to sustain due to complex microbiological composition in kefir grains. Detecting and identifying the bacterial compositions of kefir grains and kefir products with rapid method is often important for quality control of this product. On the other hand, the complete description of kefir microflora gives a clue to specify the several bioactive materials produced and inparticular those involved in grain-forming mechanism. Kefir grains contains a complex microbial symbiotic mixture of lactic acid bacteria (*Lactobacillus, Lactococcus, Leuconostoc,* and *Streptococcus* spp.), and yeasts (*Kluyveromyces, Saccharomyces* and *Torula*) included in a polysaccharide–protein matrix *(Witthuhn et al., 2005; Farnworth, 2006)*. Yeasts and lactic acid bacteria co-exist in a symbiotic association and are responsible for lactic-alcoholic fermentation. Lactic acid bacteria (LAB) in different original kefir grains were first assessed. The bacterial genera that were identified included *Lactobacillus, Leuconostoc* and *Lactococcus* and the yeast genera included *Zygosaccharomyces, Candida* and *Saccharomyces*. The distribution frequencies of the microbes in the different grains were determined and most of the grains were dominated by two microbial species. No pediococci, acetic acid bacteria or propionibacteria were detected *(Witthuhn et al., 2004)*. Full cream milk, low fat milk, can be used for fermentation. Store the culture out of direct sunlight on a bench, for about 24 hours, giving it a gentle stir or shake up to two or three times during that period. According to *(Lengkey, et al, 2013)*, the important conditions for kefir is pH. The yeasts and fungi are able to tolerate more acidic conditions. The mild conditions used in food fermentation produce few of the deleterious changes to nutritional and sensory quality. Using 10% starter, will get the best result for kefir pH (3.95). The kefir produced from cow, goat, sheep and buffalo milk, had the following chemical characteristics such as pH about 4.0, alcohol from 0.55 to 2.0%, fat content depends on the type of milk used, and this fermented milk have an acid, prickly and slightly yeasty taste *(Irigoyen, et al, 2005)*. But *Yaman et*

al (2010), conclude that the type of milk has an influence on pH than the starter culture at 21 hours fermentation time for kefir that made from cows milk. According to *Simova et. al (2002),* the pH of kefir between 4.35 – 4.5 and the lactic acid are between 8.18 – 8.20.

Material and Methods

The objectives of this study were to determine the pH of the kefir and the lactic acid production from some dosage of kefir starter and the fermentation time. The pasteurized cow milk samples contain fat 3.43%, protein 4.72%, lactose 4.30%, titratable acidity number expressed as pH value of 6.70, and lactic acid content 0.13%. 2.50 L milk was pasteurized at 72^{o}C for 15 minutes, and then was cooled until 23^{o}C. The milk then divided into 20 beaker glass (125 ml in each beaker glass). The kefir grain as the starter, collected from homemade kefir, were evaluated in this study. In the laboratory, 10% (w/w) of each grain was propagated at 20^{0}C for 20 h with two to three weekly transfers in sterilized milk, and kept at 4 ^{0} C for short- and long-term storage. The grains were cultured in sterile 10% reconstituted skim milk at 20^{o}C for 20 h. The kefir grains were then filtered and stored at 4 ^{o}C. Kefir grains were recovered from the mother culture having reached the fermentative end-point. Ten grams of each kefir grains were suspended in 90 g of sterile saline buffer (0.85% NaCl) and homogenized with a Stomacher for 20 min. The pasteurized cow milk samples contain fat 3.43%, protein 4.72%, lactose 4.30%, titratable acidity number expressed as lactic acid content 0.13% and pH value of 6.70. The starter were used 5, 10, 15, 20 and 25% (v/v) was added into the milk. Each treatment was four times repeated. The starter was mixed carefully, so the starter was mixed into the milk; and fermented for 24 hours at 27^{o}C (room temperature). And then the kefir was washed with 1 L cooked cold water each. Acidity are denoted by the term pH. The pH may be measured electrometrically or by means of dyes that changes color at different pH values. After 24 hours fermentation, the starter was washed with cool cooked water, and then straining the liquid through a stainless-steel sieve, and then weighed. The lactic acid production, was done according to titration method Manns Acid Test: lactic acid production (%) = ml NaOH x 0.009/sample (g) x 100%.

Results and Discussions

The effect of kefir starter on Kefir pH

Table 1. Kefir pH from some dosage of kefir starter and fermentation time

Fermentation time	Treatments					Average
	D-1 (5%)	D-2(10%)	D-3(15%)	D-4(20%)	D-5(25%)	
F-1 (8 hours)	4.6	4.05	3.89	3.70	3.50	3.958
F-2 (16 hours)	4.5	3.94	3.75	3.60	3.40	3.838
F-3 (24 hours)	4.3	3.86	3.64	3.50	3.30	3.740
Total	13.4	11.85	11.28	10.80	10.30	
Average	4.46	3.95	3.76	3.60	3.43	

Notes : D-1 = 5% starter dosages
D-2 = 10% starter dosages
D-3 = 15% starter dosages
D-4 = 20% starter dosages
D-5 = 25% starter dosages
F-1 = 8 hours fermentation time
F-2 = 16 hours fermentation time
F-3 = 24 hours fermentation time

From Table 1, the pH of the kefir, will more acid when the percentage of the dosage of starter used more higher. The pH of the kefir with 5% starter is 4.46 and the kefir with 25% starter is 3.43. When the dosage of starter is higher, the acidity of the kefir will more acid. *Irigoyen et al. (2005)* in their study reported that kefir produced from cow, goat, sheep and buffalo milk had the following chemical characteristics such as pH about 4.0, and this fermented milk have an acid, prickly and slightly yeasty taste. Proper treatment of the kefir is an important step in producing cultured milk. A prolonged shelf life of cultured milk products, be achieved by subjecting the finished product to heat treatment. Due to the low pH existing in cultured milk, it will have a shelf life of several weeks at room temperature.

Table 2. Analysis of variance for Kefir pH

Treatments	Kefir pH	Significancy 0.05
Starter dosage		
D-5	3.43	a
D-4	3.60	b
D-3	3.76	c
D-2	3.95	d
D-1	4.46	e
Fermentation time		
F-1	3.958	a
F-2	3.838	b
F-3	3.740	c

Notes : D-1 = 5% starter dosages
D-2 = 10% starter dosages
D-3 = 15% starter dosages
D-4 = 20% starter dosages
D-5 = 25% starter dosages

F-1 = 8 hours fermentation time
F-2 = 16 hours fermentation time
F-3 = 24 hours fermentation time

From Table 2, the pH of kefir are between 3.43 to 4.46 for 25% starter dosage to 5% starter dosage; and for fermentation time between 8 – 24 hours, the pH are 3.958 to 3.740. The important conditions are pH, the yeasts and fungi are able to tolerate more acidic conditions. The mild conditions used in food fermentation produce few of the deleterious changes to nutritional and sensory quality *(Lengkey et al, 2013)*. The taste and texture of kefir depends on several factors including fermentation time of the kefir cultures, the room temperature and the ratio of kefir grains to milk. If the room temperature has changed, may need to adjust the fermentation time to allow the kefir to culture. If the kefir grains have multiplied, then may find the taste and texture of the kefir change. To remedy this problem, simply remove a portion of the kefir grains and either start a second batch of kefir or find them a good home. Using 10% starter, will get the best result for kefir pH. Kefir generally takes 12 to 48 hours to form. The exact amount of time will vary depending on environmental factors, the most important of which is temperature. Heat speeds the process so kefir will form more quickly in a warm area and will be more likely to over-culture. We recommend standard room temperature whenever possible. The room temperature when this experiment was done is 27^0 C and the fermentation time are between 8 – 24 hours. Allowing the kefir grains to remain in milk longer than 48 hours risks starving the kefir grains and potentially damaging them. According to *Yaman, et al (2010)*, the kefir made from cows milk with fermentation time for 21 h, has pH 4.41 and the type of milk has an influence on pH than the starter culture.

The effect on lactic acid production

From Table 3, the lactic acid production will increase as the starter dosages rise. As the starter dosages 5% (D-1), the lactic acid production 0.933 and when the starter dosages 25% (D-5), the lactic acid production is 2.00. According to the fermentation time, when the fermentation time 8 hours (L-1) the lactic acid production is 0.774 and the highest is from 24 hours fermentation time (L-3) 1.910.

Table 3. Lactic acid production from some dosage of kefir starter

Fermentation Time	Treatments					Average
	D -1 (5%)	D-2 (10%)	D-3 (15%)	D-4 (20%)	D-5 (25%)	
F-1 (8 hours)	0.37	0.52	0.82	1.00	1.26	0.774
F-2 (16 hours)	1.10	1.18	1.40	1.58	2.12	1.476
F-3 (24 hours)	1.24	1.53	1.80	2.36	2.62	1.910
Total	2.71	3.23	4.02	4.94	6.00	
Average	0.933	1.073	1.34	1.646	2.00	

Notes : D-1 = 5% starter dosages
D-2 = 10% starter dosages
D-3 = 15% starter dosages
D-4 = 20% starter dosages
D-5 = 25% starter dosages
F-1 = 8 hours fermentation time
F-2 = 16 hours fermentation time
F-3 = 24 hours fermentation time

During the fermentation time, the kefir with 25% starter dosage has the highest lactic acid level, and the lowest is 5% starter dosage and based on fermentation time, the highest lactic acid percentage are from 24 hr fermentation time and the lowest is 8 hr fermentation time. To find out the distinction of the treatment in lactic acid, we used Duncan multiple range test in Table 4.

Tabel 4. Analysis of Variance for Kefir Lactic Acid (%)

Treatments	Lactic Acid average .....%.....	Significancy 0.05
Starter dosage		
D-5	2.00	a
D-4	1.646	b
D-3	1.34	c
D-2	1.073	d
D-1	0.933	e
Fermentation time		
F-3	1.910	a
F-2	1.476	b
F-1	0.774	c

Notes : D-1 = 5% starter dosages
D-2 = 10% starter dosages
D-3 = 15% starter dosages
D-4 = 20% starter dosages
D-5 = 25% starter dosages
F-1 = 8 hours fermentation time
F-2 = 16 hours fermentation time
F-3 = 24 hours fermentation time

As the starter dosage increase, it also increased the lactic acid, because the microorganism in kefir starter will increase the lactic acid content in kefir. This result is in accordance with the opinion, that during the fermentation process, the starter dosage will influence the lactic acid production. The dosage starter indicated that the effect of bacteria on lactic acid production, the ability of the bacteria to break the lactose, that is why when the starter dosage increased, will produce lactic acid more faster and then the lactic acid in kefir more higher. Lactic acid in the kefir was expected for 4 %, and it will recommended in kefir production, with 18 hr fermentation time with 10% starter dosage.

The kefir that produced from kefir grain, will produced kefir with pH between 4.35 – 4.5 and the lactic acid were between 8.18 – 8.20 were determined by enzymatic methods as described by Boehringer Mannheim (*Simova et al, 2002*). Kefir is an acidic and mildly alcoholic fermented dairy product that is believed to have functional properties *(Farnworth, 1999; Farnworth and Mainville, 2003; Farnworth, 2006)*. The microorganisms contained within the kefir grains typically produce lactic acid and antibiotics, such products inhibit the proliferation of both spoilage and pathogenic microorganisms in kefir milk *(Farnworth, 2006)*.

Conclusion

In conclusion, this study showed that the starter dosage and fermentation time has an influence on the pH and lactic acid of the kefir. Although the longer the fermentation time and the more starter dosage will result lower pH and also higher percentage of lactic acid, and it may influence the quality of kefir, but the best results are for 10% dosage starter and 16 hours fermentation time, because of the result of kefir pH 3.95 and lactic acid 1.476%.

Uticaj doze startera i vremena fermentacije na pH i proizvodnju mlečne kiseline

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Rezime

Cilj ovog istraživanja je bio da se utvrdi uticaj doze startera i vremena fermentacije na pH i produkciju mlečne kiseline. U ovom istraživanju korišćen je Completely Randomized Design (CRD) obrazac 5 x 3. Prvi faktor je doza startera i drugi faktor je vreme fermentacije. Kefir uzorci su pripremljeni korišćenjem 5, 10, 15, 20 i 25% (v/v) kefir zrna, sa tri vremena fermentacije 8, 16 i 24 časa. Rezultati

pokazuju da kefir sa 10% startera i 16 sati fermentacije daje najbolje rezultate što se tiče pH (3.95) i proizvodnje mlečne kiseline (1,476%).

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