Processing and Characterization of Coconut Milk Kefir

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Abstract

Kefir is a delicious dairy product and also rich in probiotics. In this research work, coconut milk kefir was prepared by the fermentation of fresh coconut milk with milk kefir grains. Kefir grains are a mixture of beneficial lactic acid bacteria and yeast with a polysaccharide matrix. Mature and fresh coconuts were collected from Mingaladon Township, Northern District, Yangon Region. Firstly, the physico-chemical and nutritional values of raw coconut milk were determined by the AOAC method. Secondly, the most suitable processing conditions were studied. The applied fermentation conditions for the processing of milk kefir were fermentation temperature, 25°C and fermentation time, 24 h. About 10 % (w/w) of milk kefir grains was found to be the most suitable amount of coconut milk (4.5 ± 0.1, % Brix) for coconut milk kefir processing. The physico-chemical characteristics and nutritional values of processed coconut milk kefir were found to be acceptable when compared with the literature values. Furthermore, the organoleptic properties of processed coconut milk kefir were studied, and the microbial examinations were also conducted for the shelf-life. The processed coconut milk kefir (without preservative) under the refrigerated condition (0 – 4 °C), 7 days stored, was found to be safe and nutritious under the guidelines for the microbiological quality of various foods.

Keywords: kefir, coconut milk kefir, fermentation, coconut milk, milk kefir grains, lactic acid bacteria, and yeast.

Introduction

Kefir is a powerful probiotics rich milk beverage. Milk kefir is traditionally made with cow's milk, but milk from other sources such as goat's, sheep's, buffalo's, coconut milk, or soymilk can also be used. It is a creamy type that is a little thinner than normal yogurt, and is also designated as a drinkable yogurt. The cultural condition of milk kefir is mesophilic in nature while that of yogurt is thermophilic. Consumption of milk kefir is recommended because of its probiotic bacteria and mixture of yeast. Kefir grains were usually applied as the starter for the fermentation of dairy products (Gao & Li, 2016). The probiotic properties of kefir are derived from kefir grains or cultures containing various types of Lactobacillus, Lactococcus, Leuconostoc, acetic acid bacteria, yeast, and more. The types of milk have a greater influence on the characteristics of milk kefir than those of starter cultures and grains.

Milk kefir, a fermented milk beverage, is also called kephir and originated in the Caucasus Mountains, cultured with kefir "grains", a yeast/bacterial fermentation starter. Traditional kefir is fermented at ambient temperatures, generally for one night. Fermentation of the milk containing lactose yields a sour, carbonated, slightly alcoholic beverage, with a consistency, flavor, and taste similar to thin yoghurt (Schwan et al., 2015). Kefir grains have a complex composition of microbial species, mainly lactic acid bacteria, acetobacter, yeasts, and fungi. These species fall into four groups: homofermentative and heterofermentable lactic acid bacteria, and lactose and non-lactose anabolic yeasts. In kefir grains, the main polysaccharide, kefiran is a heteropolysaccharide made by equal proportions of glucose and galactose and is mainly obtained by *Lactobacillus kefiranofaciens* (Prado et al., 2015). Kefiran improves the viscosity and viscoelastic properties of milk kefir. It gives a creamy texture and feeling in the mouth. These grains are small, irregularly shaped and semi-hard granules that resemble tiny cauliflower blossoms (Schwan et al., 2015).

The colour range of these grains is from creamy white to pale yellow. The pale yellow colour is the outcome of leaving the grains in the same milk during fermentation. If left longer

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than the optimal 24 h period and continually doing so over many batches, it becomes pale yellow colour (Schwan et al., 2016).

Lactobacillus in kefir can be present in concentrations that vary from about 1 million to 1 billion colony forming units per milliliter of milk kefir. They are the most prominent bacteria involved in the synthesis of the polysaccharide kefiran. Probiotic bacteria found in kefir processing mainly involve *Lactobacillus acidophilus, Bifidobacterium bifidum, Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus helveticus, Lactobacillus kefiranofaciens, Lactococcus lactis and Leuconostoc* species (Ismail, 2007).

The liquid inside a coconut is coconut water, while the fruit's white flesh provides coconut milk. The nature of coconut milk is a thick or thin liquid. When making thick coconut milk, the flesh of mature coconuts is selected, and squeezed through cheesecloth to extract the coconut liquid. Thick coconut milk contains more fat than thin milk (Coconut Kefir, 2017).

Like regular milk kefir, coconut milk kefir is made by using milk kefir grains or starter culture to ferment the milk of coconut, but of course it is a dairy free beverage. Coconut milk kefir also has a much milder and less-tangy taste than other traditional milk kefirs (Schwan et al., 2015).

The objectives of the present study are as follows: to study the most suitable process conditions and amount of milk kefir grain in the processing of coconut milk kefir, to evaluate the characteristics and nutritive values of processed coconut milk kefir and to substitute the processed milk kefir instead of using probiotic supplements for digestive disorders.

Materials and Methods

Sample Collection

Fresh and mature coconuts, Figure (1), were purchased from the local vegetable market of Mingaladon Township, Northern District, Yangon Region. Milk kefir grains were purchased from Bushwick Kombucha Pte. Ltd., Singapore. The required chemicals for analysis were purchased from Super Shell Store, 27th Street, Pabedan Township, Yangon Region.

Processing of Coconut Milk Kefir

Fresh and mature coconuts, Figure (1), were used to produce a high yield and good quality coconut milk. After husking and cracking the coconuts, an electronic grater was used to remove the coconut meat from the shell. To extract the coconut milk, the grated coconut meat, shown in Figure (2), was placed in a blender and added hot water (90-95°C) over the meat with a ratio of 1.5 : 1 (hot water : coconut meat). The mixture was blended for 5 minutes. After blending, the mixture was squeezed to extract the coconut milk and the meat residue was separated as a by-product which can be used as a food ingredient for bakery products. The physico-chemical characteristics and nutritional values of processed coconut milk were analyzed by the AOAC method.

The extracted coconut milk was heated to pasteurize in a pan for a few minutes. After pasteurization, the temperature of the coconut milk was reduced to room temperature. Activated milk kefir grains were required for the fermentation of kefir in an incubator. Milk kefir grains were activated to reach their fermentation potential within 3-7 days. Various amounts (4 %, 6 %, 8 %, 10 % and 12 %) of activated milk kefir grains were placed in a container containing 100 g (4.5 ± 0.1, % Brix) of pasteurized coconut milk. The container containing the mixture was covered with a filter, secured by a rubber band, and incubated at 25°C for 24 h until the pH fell to ~ 4.2 (Elgarhy et al., 2018). The most suitable amount of milk kefir grains was found to be 10 % according to the organoleptic properties.

During incubation, shaking the container two or three times was found to enhance the fermentation. After incubation, the milk was thickened and strained to remove the grains. The processed coconut milk kefir, shown in Figure (3) after the first incubation, was found to have good flavour and taste. The first time, strained grains were reactivated with the same amount of fresh milk until the milk was thickened and fermented. Fully activated grains, that could consistently thicken milk within 24 h, would be used for fermenting coconut milk again. The physico-chemical characteristics and the nutritional values of processed coconut milk kefir were analyzed by the AOAC method.

Figure (1) Raw Coconuts

Figure (2) Grated Coconut Meat

Figure (3) Coconut Milk Kefir

Assessment on Characteristics of Coconut Milk and Processed Coconut Milk Kefir

pH

pH of the coconut milk and kefir samples was measured using a pH meter (HANNA Waterproof pH tester). Before measurement of the liquid sample, the glass electrode was calibrated with pH 4 and pH 7 buffer solutions.

Acidity

Acidity of the processed coconut kefir sample as lactic acid was determined by the titration method. 10 mL of filtered processed kefir and transferred into a 250 mL conical flask and 1-2 drops of phenolphthalein indicator was added. It was titrated with standardized NaOH solution until a colourless to pink colour appeared. The titrant volume was recorded and calculated as follows:

$$\text{Total Acidity, \% (w/w) as Lactic acid} = \frac{\text{mL of 0.1 N NaOH} \times \text{Equilvalent factor} \times 100}{\text{mL of Sample taken}}$$

Soluble Solids Content

The soluble solids content of the raw milks and processed kefirs was measured with a HI-96801 Digital Refractometer (0 – 85 % Brix) Hanna Instruments. The HI-96801 measures the refractive index to determine the percent Brix of sugar in aqueous solutions. The refractive index of the sample is then converted to Brix concentration units. A sample was dipped onto the prism surface until the well was full using a plastic pipette. The result was obtained in the selected unit from the display.

Viscosity Content

The viscosity of the sample was measured using a ATAGO 6800 Digital viscometer VISCO™ made in Japan.

Assessment on the Nutritional Values of Processed Coconut Milk Kefir

The nutritional values such as moisture content, ash content, protein content, crude fiber content, crude fat content, carbohydrate content, and energy value were determined according to the Association of Official Analytical Chemists (AOAC) methods.

Microbial Examination of Processed Coconut Milk Kefir

Microbiological Examination of Total Plate Count

Total Plate Count was examined according to [AOAC (1990)].

$$\text{Total Plate Count (TPC)} = \frac{\text{Total number of colonies/plate}}{\text{Reciprocal of dilution used}}$$

Estimated counts can be made on plates with > 300 colonies and should be reported as estimated counts. In making such counts, the circular growth area can be considered to contain twenty-one cm squares. To isolate colonies for further identification, the top film was lifted and the colonies picked from the gel.

Microbiological Examination of Yeasts and Molds

Yeasts and Molds Count was examined according to [AOAC (1997)].

$$\text{Yeasts and Molds Count} = \frac{\text{Total number of colonies / plate}}{\text{Reciprocal of dilution used}}$$

Microbiological Examination of Escherichia coli (E. coli)

The presence of *Escherichia coli* of samples was examined according to [AOAC (1990)] method.

E. coli, cfu/g = No. of Positive Colonies x Dilution Factor (DF)

Results and Discussion

Processing and Characterization of Coconut Milk Kefir

In the processing of coconut milk kefir, physico-chemical characteristics of coconut milk were determined, and the results are shown in Table (1). The quality of coconut milk was found to be good and conditions were found for the processing of coconut milk kefir according to the results of pH, acidity, soluble solids, and viscosity.

Table (1) Physico-chemical Characteristics of Coconut Milk

Hot water : Coconut meat = 1.5 : 1 (w/w)

Sr. No.	Characteristics	Coconut Milk	Literature Value*
1	pH	6.4 ± 0.2	6.3 – 6.8
2	Acidity, % (w/w)	0.28 ± 0.02	-
3	Soluble Solids, % (Brix)	4.5 ± 0.2	-
4	Viscosity, cP	7.46 ± 0.2	12.8 – 33.6

* (CODEX STAN 240-2003)

From the results of Table (2), the nutritional values of coconut milk were analysed and compared with the literature value. Among these values, the water content 78.35 ± 0.2, % (w/w) and the fat content 13.14 ± 0.05, % (w/w) were considerably higher than the other non-fat solids values but in the range of the literature values. The other values, such as carbohydrate

content and protein content, were slightly higher than the literature values. The energy value of processed coconut milk was found to be 150.62 kcal per 100 g.

Table (2) Nutritional Values of Coconut Milk

Sr. No.	Characteristics	Coconut Milk	Literature Value*
1	Water Content, % (w/w)	78.35 ± 0.2	75.6 – 87.3
2	Crude Fat Content, % (w/w)	13.14 ± 0.05	10 – 15
3	Carbohydrate Content, % (w/w)	5.64 ± 0.2	5.5
4	Protein Content, % (w/w)	2.45 ± 0.03	2.3
5	Ash Content, % (w/w)	0.27 ± 0.005	-
6	Crude Fiber Content, % (w/w)	0.15 ± 0.005	-
7	Energy Value (kcal/100 g)	150.62	-

* (CODEX STAN 240-2003) (Codex Alimentarius)

Table (3) shows organoleptic properties of coconut milk kefir in various formulations. 100 g (4.5 ± 0.1, % Brix) of coconut milk was fermented at 25°C and 24 h. In this formulation, various weights of kefir grains of 4 %, 6 %, 8 %, 10 % and 12 % were used to produce coconut milk kefir. According to the organoleptic results, 10 % of the milk kefir grains were found to be the most suitable amount for the processing of coconut milk kefir. In this condition, complete fermentation, a creamy colour, good taste and good flavor were obtained. When an excess amount of grains, 12 %, was used in the processing of milk kefir, the quality of coconut milk kefir was found to be pale yellow in colour with a sour taste, and a sour flavor.

Table (3) Organoleptic Properties of Coconut Milk Kefir by Various Formulations

Coconut Milk = 100 g (4.5 ± 0.1, % Brix)

Fermentation Temperature = 25 °C

Fermentation Time = 24 h

Sample No.	Milk Kefir Grains (%)	Organoleptic Properties
I	4	Poor Fermentation, Light Taste, Milky Colour and Light Flavour
Ii	6	Poor Fermentation, Light Taste, Milky Colour and Light Flavour
Iii	8	Incomplete Fermentation, Milky Colour, Light Taste but Good Flavour
Iv	10*	Complete Fermentation, Creamy Colour, Good Taste and Good Flavour
V	12	Complete Fermentation, Pale Yellow Colour, Sour Taste and Flavour

* The most suitable amount

Figure (4) demonstrates the comparison of the effect of fermentation time on the acidity of coconut milk kefir. When the longer fermentation time was studied, the higher amount of acidity content as lactic acid was found during the incubation of coconut milk.

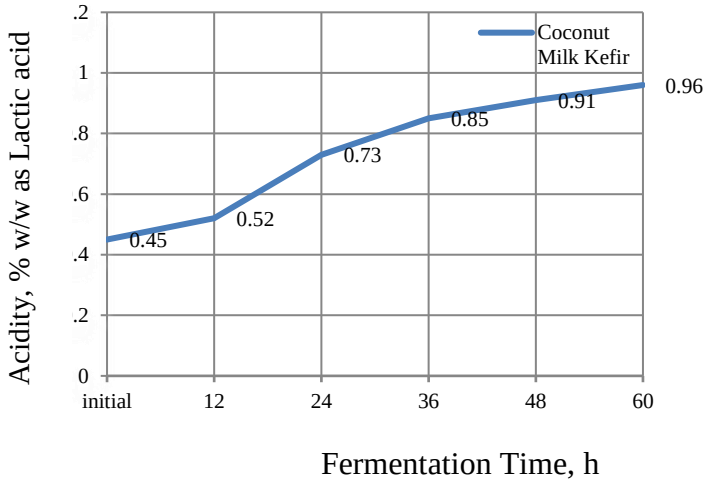

Figure (4) Comparison of Effect of Fermentation Time on Acidity of Coconut Milk Kefir

A comparison of the physico-chemical characteristics of coconut milk kefir with literature values is shown in Table (4). From these results, the pH of processed coconut milk kefir was considerably lower than that of the raw coconut milk. But the amount of acidity and soluble solids, and viscosity content of processed coconut milk kefir were considerably higher than there of raw coconut milk.

Table (4) Physico-chemical Characteristics of Processed Coconut Milk Kefir

Sr. No.	Characteristics	Coconut Milk Kefir	Coconut Milk
1	pH	4.5 ± 0.1	6.4 ± 0.2
2	Acidity, % (w/w)	0.73 ± 0.02	0.28 ± 0.02
3	Soluble Solids, % Brix	6.5 ± 0.2	4.5 ± 0.2
4	Viscosity, cP at 20 °C	24.7 ± 0.5	22.1 ± 0.5

Table (5) shows the comparison of the nutrient composition of coconut milk kefir with literature value. Among these values, fat content and protein content were considerably higher than the literature values; on the other hand, moisture content, carbohydrate content and ash content were considerably lower than the literature values. The energy value of processed coconut milk kefir was found to be 67.50 kcal per 100 g.

Table (5) Comparative Analysis of Nutrient Composition of Coconut Milk Kefir

Sr. No.	Characteristics	Coconut Milk Kefir	Literature Value*	Literature Value**
1	Moisture Content, % (w/w)	75.34 ± 0.2	90.1	87.5
2	Fat, % (w/w)	3.74 ± 0.2	0.9	3.5
3	Carbohydrate, % (w/w)	3.94 ± 0.02	4.5	4.0
4	Protein, % (w/w)	4.52 ± 0.02	3.8	3.3
5	Ash, % (w/w)	0.5 ± 0.02	0.7	-
6	Crude Fiber, % (w/w)	0.2 ± 0.02	-	-
7	Energy Value, kcal/100 g	67.50	41	65

* (Kefir — Nutrition Facts) ** (Otles & Çağındı, 2003)

Table (6) indicates the microbial examination of processed coconut milk kefir. The shelf-life, 7 days, of processed coconut milk kefir (without preservatives) under refrigerated conditions (0 – 4°C) was found to be safe and nutritious under the guidelines for the microbiological quality of various foods. Both the Total Plate Count (TPC) and the yeasts & molds of processed coconut milk kefir were under the legal limit of guideline levels. No growth of *E. coli* was found in processed coconut milk kefir.

Table (6) Microbial Examination of Processed Coconut Milk Kefir

Storage Temperature = Refrigerated Temperature (0 - 4 °C)

Shelf-life = 7 Days

Sr. No.	Microorganisms	Processed Coconut Milk Kefir	Guideline Levels*
1	Yeasts & Molds, cfu/g	5×10^3	1×10^5
2	Total Plate Count (TPC), cfu/g	$< 1 \times 10^3$	1×10^5
3	*Escherichia coli* (*E. coli*), cfu/g	ND	ND

*Guidelines for the microbiological quality of various ready-to-eat foods, Commercial disease and public health, Vol.3, No.3, September 2000, London.

Conclusion

Milk kefir could serve as a probiotic for the digestive system. In the processing of coconut milk kefir, 10 % of milk kefir grains was found to be the most suitable amount of pasteurized coconut milk (4.5 ± 0.1, %, Brix). The processed coconut milk kefir was found to be a substitute instead of using probiotics supplements for digestive disorders.

Fresh coconut milk kefir gave a tart and effervescent yogurt-like flavour with a taste of coconut. The coconut milk kefir was found to be thicker than regular coconut milk. In

microbial examination, both Total Plate Count (TPC) and yeasts & molds were under the legal limit of guideline levels. No growth of *E. coli* was found in processed coconut milk kefir. The shelf-life of processed coconut milk kefir (without preservatives) was found to be 7 days under the refrigerated conditions (0 – 4 °C). According to the results, with the consumption of milk kefirs, the positive health benefits would be obtained.

Kefir with enhanced functional properties from adding probiotic bacteria will attract the attention of many consumers. In addition, to resolve the issues in the production of this kefir, new studies and projects should be conducted that provide detailed information on future expectations.

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