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Coconut milk kefir: Nutrient composition and assessment of microbial quality

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Abstract
Kefir is a natural probiotic produced by a mixture of lactic acid bacteria, acetic acid bacteria, and yeast. Kefir was formulated using coconut milk; its nutrient composition and microbial quality were evaluated in this study. The kefir grains were activated and then introduced into coconut milk to produce, coconut milk kefir. The coconut milk kefir was analyzed for major macro and micro nutrients. Microbial quality (qualitative and quantitative) and shelf-life of chocolate, standardized using coconut milk kefir, was also assessed using the Standard Plate Count (SPC) method. Nutrient analysis of the formulated coconut milk kefir revealed that the product was superior in nutrients like biotin, Vitamin B12 and calcium. Qualitative tests showed the presence of microorganisms like *E. coli*, yeast and mold. The quantitative assessment, however, revealed that the number of these microorganisms were within acceptable levels for up to three days.

Keywords: fermented dairy products, kefir, coconut milk kefir, nutrient composition, shelf life, microbial quality

1. Introduction
Fermented dairy products are an important part of the diet in many cultures and can contain a diverse microbiota [1]. They can contain beneficial compounds like vitamins, conjugated linoleic acid, bioactive peptides, and gamma-amino butyric acid, which are produced by the metabolic activity of their microbiota [2]. Fermented milks are produced by the addition of bacterial cultures in raw or heat treated milk. Yeasts, bacteria and fungi are some of the beneficial microorganisms that are used in the fermentation process [3].

Kefir a functional, fermented dairy product that originates from the mountains of the Caucasus [4] is a natural probiotic [5]. It is produced by the action of lactic acid bacteria (LAB), yeasts, and acetic acid bacteria on milk. This complex mixture of microorganisms produces a distinctive fermented milk product with unique properties. Kefir is produced by adding either a starter culture called kefir grains directly or a percolate of the grains to milk [6]. Typical milks used in manufacturing of kefir include cow, goat and sheep milk, each with varying organoleptic and nutritional qualities. Raw milk has been traditionally used. Kefir grains will also ferment milks such as soy milk, rice milk and coconut milk [7].

The National Institute of Nutrition (2002) defines functional food as, "Foods or food components that may have health benefits that reduce the risk of specific diseases or other health concerns" [8]. A subset of functional foods is probiotic foods, from which there are several possible sources of beneficial bioactive ingredients like: exopolysaccharides and bioactive peptides. The microorganisms themselves (dead or alive), and metabolites of the microorganisms formed during fermentation or breakdown of the food matrix, such as peptides, may be responsible for the beneficial effects [9, 10].

Coconut (*Cocos nucifera L.*) is widely used as part of the diet in many cultures. Coconut is mainly known for its nutritional and medicinal values, which are unique and compelling [11]. Coconut is classified as a highly nutritious 'functional food', because it provides many health benefits beyond its nutritional content. Coconut is rich in dietary fiber, carbohydrate, vitamins and minerals; however, notably, evidence is mounting to support the concept that coconut may be beneficial in the treatment of obesity, dyslipidemia, elevated Low Density Lipoprotein (LDL), insulin resistance and hypertension, the risk factors for cardiovascular disease and type 2 diabetes, and also for Alzheimer's Disease [12,13]. Medium Chain Triglyceride, a fraction of coconut has been identified as an important, medically efficacious food [14]. The fatty acid component of coconut consists of lauric acid, a component which is otherwise present only in mother's milk and known to have lot of health benefits. Monolaurin, a disease fighting monoglyceride fatty acid derivative is produced by the body from, lauric acid.

Coconuts are widely used in India and are a regular part of the South Indian diet. Although, kefir is not indigenous to India, fermented dairy products like curd/ dahi are an inherent part of the Indian diet. Therefore kefir, a functional fermented food can be easily incorporated into the Indian diet as coconut milk kefir. Coconut milk and kefir have each been documented to have health benefits. Hence, coconut milk was used in the preparation of kefir. It was used as a replacement for cow's milk. Considering the health benefits of consuming fermented foods on a daily basis, this study was designed to study the synergistic nutritional composition of coconut milk kefir and its shelf life which is key to marketability.

2. Materials and Methods
2.1 Preparation of Coconut Milk Kefir and Nutrient Composition
The inactive milk kefir grains were procured from an online store in the United States of America and shipped to India. Fresh coconut milk was extracted using a standardized

procedure. The inactive kefir grains were activated using fresh cold pasteurized cow's milk, by allowing the grains to ferment in the milk for 12 to 24 hours. The grains were then separated by filtering and added to another batch of fresh milk. The milk was changed every time until it reached a maximum of four cups and the kefir grains were then removed by filtering. The kefir grains thus activated were then introduced into the coconut milk and allowed to ferment for 12-24 hours, followed by the straining process to separate the kefir grains. Thus, Coconut milk kefir was prepared.

Nutrient composition of coconut milk kefir was analyzed, in a certified laboratory, for nutrients like energy, carbohydrates, fat, protein, calcium, Vitamin B5, Vitamin B7, Vitamin B1, Vitamin B12 and Vitamin C.

2.2 Microbial Quality

Chocolate incorporating coconut milk kefir at 25 % was standardized and assessed for microbial quality and shelf life at room temperature on the first, third and fifth day after preparation. Both qualitative and quantitative tests (SPC method) for *Escherichia coli (E. coli)*, yeast and mold were done to evaluate the microbial quality and shelf life of coconut milk kefir chocolate.

3. Results and Discussion

3.1 Nutrient Composition

The nutrient composition of 100 g of the formulated coconut milk kefir was analyzed in a reputed food laboratory. The product was analyzed for nutrients like energy, carbohydrates, fat, protein, calcium, biotin (Vitamin B7), pantothenic acid (Vitamin B5), thiamine (Vitamin B1), cobalamin (Vitamin B12), and Vitamin C; the results of which has been presented in table 1.

Table 1: Nutrient composition of coconut milk kefir

Nutrient	Composition (per 100gm)
Energy (kcal)	117.5
Carbohydrates (g)	1.32
Protein (g)	1.27
Fat (g)	11.91
Calcium (mg)	3.89
Vitamin B1 (mg)	0.25
Vitamin B5 (mg)	0.04
Vitamin B7 (µg)	55.0
Vitamin B12(µg)	2.50
Vitamin C (mg)	0.50

Table 1, presents the nutritive value of the formulated coconut milk kefir. The nutritional composition of milk kefir is variable and not well defined [15]. It depends on the source and the fat content of milk, the composition of the grains or cultures and the technological process of kefir [16].The nutrient composition of coconut milk kefir, is likely to vary from that of coconut milk and kefir, which might be due to the process of fermentation [17].

Since, coconut milk kefir is a novel product there are no published, scientific data available for comparison, hence this discussion is based on the comparison between unfermented coconut milk and coconut milk kefir. There exists a complex interactions between yeast and bacteria and their interdependence in kefir grains are not completely understood[18,10,19]; this interaction between yeast and lactic acid bacteria can be stimulated or inhibited by the growth of one or both, in co-cultures. These microorganisms can compete for nutrients for growth, or may produce metabolites that inhibit or stimulate one another [20].

The energy value and carbohydrate content of 100g of the formulated coconut milk kefir were 117.5 kcal and 1.32 g respectively, whereas the energy value and carbohydrate content of coconut milk was found to be 230 kcal / 100g and 5.54g/100g [21], the decrease in the energy and carbohydrate values for the coconut milk kefir may be attributed to the influence of fermentation.

Protein content of 100 g of the formulated product was 1.27 g, whereas the protein content of coconut milk was 2.29 g /100 ml [21]. Some yeast species present in the kefir grains are proteolytic or lipolytic, which contributes to the complete proteins that are partially digested, facilitating digestion by the body [19] this could also be the possible reason behind the reduction in the fat content of coconut milk kefir of 11.91 g/100g from that of the 23.84g/100g of the coconut milk[21].The fat present in the product consists of medium chain fatty acids which are easily assimilated by the body and hence do not contribute to increase in cholesterol levels [22].

Coconut milk kefir had 0.25 mg/ 100 ml of thiamine. The Vitamin B5 content of the formulated coconut milk kefir was found to be 0.04 mg/100 ml. Vitamin B7 content in the product was found to be 55 µg. The coconut milk kefir contained 2.50 µg and 0.5 µg of Cobalamin and Vitamin B12 respectively. There is a significant increase in the B-complex vitamin content of the coconut milk kefir when compared with that of coconut milk [21].

The Vitamin C and Calcium content of the coconut milk kefir was 0.50 mg and 3.89 mg/100g respectively. The pH of the formulated coconut milk kefir was found to be 4. The pH of kefir is found to be influenced by the temperature, and fermentation time. This is similar to a study by Tietze (1996) who reported that kefir had a pH of 3.8 to 4.1 after 72 hours of fermentation [23].

3.2 Microbial Quality

The microbial quality of, chocolate, prepared using coconut milk kefir, was assessed using both qualitative and quantitative analysis. Microorganisms like *E.coli*, yeast and mold were identified by qualitative analysis. Quantitative analysis helped enumerate the identified microorganisms, the results of which have been presented in table 2. Standard Plate Count of the sample, stored at room temperature, was assessed on the first, third and the fifth day after preparation.

Table 2: Microbial quality of coconut milk kefir chocolate

Microorganism	Medium	Day 1	Day 3	Day 5
		Number of CFU/100µl		
E. coli and Coliform count	MacConkey Agar	$2x10^4$	$2.2 x10^5$	$18.05 x10^5$
Total Plate Count	Nutrient Agar	$3.019x10^5$	$3.9x10^5$	$5.23 x10^5$
Yeast and Mold count	Yeast extract peptone dextrose agar	1.6 x103	7.4 x105	13.4 x105

Based on, "Microbiological Guidelines for ready to eat foods", (2007) [24] assessment of microbial quality of the chocolate indicated that the microbial quality was acceptable ($<10^5$) on day 1 and day 3 but not on day 5 (figures 1 to 9). The microbial quality of the coconut milk kefir, chocolate, was examined when stored at room temperature. This might be one of the reasons why the product was considered safe for consumption only until the third day. Refrigerating the product could help prolong the shelf life. Physical examination revealed that there were no visible signs of spoilage observed on the chocolate stored at room temperature even after 5 days from preparation.

Microbial growth (*E.coli* and Coliform) in chocolate

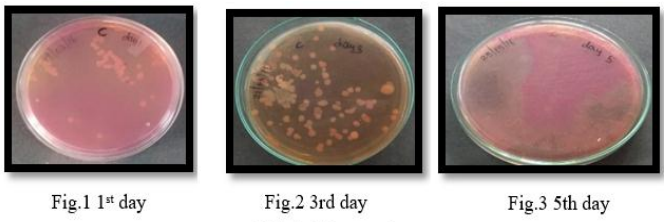

Fig.1 1st day Fig.2 3rd day Fig.3 5th day

Total plate count

Fig.4 1st day Fig.5 3rd day Fig.6 5th day

Yeast and mold count

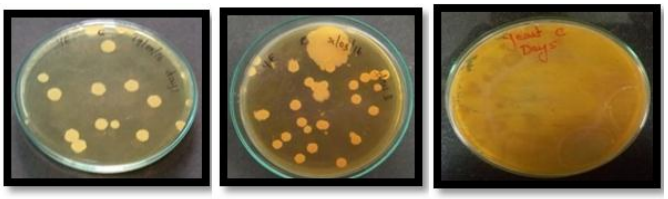

Fig.7 1st day Fig.8 3rd day Fig.9 5th day

The use of starter cultures or grains can help standardize the production of kefir, however if the selection of species and strains of yeasts and bacteria is carried out accurately and carefully [25, 26]. Kefir when manufactured on a commercial scale, was found to have a life period of up to 28 days. It is recommended that kefir produced with kefir grains be consumed between 3 to 12 days [19], hence, this can also be applicable to the product standardized using coconut milk kefir. According to Puniya, 2016, the fermentation of milk preserves or increases the shelf life of the perishable milk, in addition to improving the taste and digestibility of the milk [27]. The coconut milk kefir was prepared under sterile conditions, using fresh cold pressed coconut milk instead of using commercial brands of processed coconut milk, in order to avoid use of preservatives. This may have contributed to the limited shelf life of the coconut milk kefir chocolate.

4. Conclusion

This study aimed to ascertain the nutrient composition, shelf life and microbial quality of coconut milk kefir, a novel product; in an attempt to promote its use as a simple yet ideal probiotic food that can be included in our daily diet. The results of the nutrient analysis of coconut milk kefir, indicate that it may have varied nutrient benefits on human health owing to the co-existence of bacteria and yeast in the kefir grains as well as the nutrients present in it. The microbial quality studies reveal that the coconut milk kefir incorporated recipe, the chocolate, was found to be ideal for consumption for three days, from the date of manufacture when stored at room temperature. Due to the nutritional properties of kefir it is likely to confer health benefits on humans when consumed on a regular basis.

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