Study on production of Symbiotic Kefir

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Abstract: Kefir is widely known as a probiotic and becoming popular because of its health benefits. It is a fermented milk drink and prepared by inoculating milk kefir grains which is a combination of bacteria and yeasts. Prebiotics impacts on bacterial proliferation and metabolism and consistently demonstrated increases in number of Lactobacillus and Bifidobacterium species which enhance growth of beneficial bacteria in the lower intestine are primarily fibers naturally found in food In addition to feeding your good gut bacteria prebiotics can help you absorb calcium and huge benefits. Addition of flavor, similar like yogurt process which enhance the taste without altering or disturbing the beneficial bacteria present in Kefir. In this article, the production of symbiotic kefir included with the flavors and its physical, nutritional and microbial composition were also discussed.

Index Terms **- Kefir, *Lactobacillus* spp, *Bifidobacterium*, Prebiotic, Natural flavors.**

I. INTRODUCTION

Kefir is a traditional dairy beverage which originated from Caucasian and European regions, obtained by the addition of kefir grains to milk. The polysaccharide called as kefiran contains the bacteria and yeast that encloses the kefir grains. Kefir grains are similar to the shape of cauliflower they are elastic in nature, gelatinous in consistency, irregular in shape with white or an ivory color and ranges from 0.3 to 3.5 cm in diameter. In general kefir grains consist of 45.7% mucopolysaccharide, 4.4% fat, 34.3% total protein, vitamins K and B, Ca, P and Mg, 12.1% ash. Kefir has been recognized to have various health advantages such as antibacterial effects, anti-carcinogenic activity (Gao J, Gu F, Ruan H, et al., 2013) control of glucose in plasma, lactose tolerance, hypo cholesterolaemic effect, antioxidant activity and anti-allergenic activity which resulted in demand for the product in Western countries (Farnworth and Mainville, 2008: Tamime et al., 2011). The milk composition, the sources and the composition of the grains influences the nutritional composition of kefir. The unique smell and flavour of kefir are released from the volatile and non-volatile compounds generated via proteolysis, glycolysis and lipolysis upon fermentation. The acidic pH of 4.6, acidic taste, alcohol of 0.5% to 2% and yeasty flavor are contributed for the physicochemical properties of kefir. In addition to that, the yeast flora's production of carbon dioxide contributes to its yeasty flavor and sharp acid.

The components like lactic acid, acetic acid and aromatic compounds and ethanol are formed after milk fermentation (Güzel-Seydim et al.,2000a; Oner et al., 2010; Megalhaes et al., 2011b; Leite et al., 2012). The kefir grain's most common isolated lactobacilli includes The biotic and abiotic factors influence the relationship between microbial groups and thus, is one of the characteristics of every individual manufacturing process. During fermentation, proteins are digestible more easily, the milk used as substrate is similar to the amino acids profile of the kefir except for tryptophan, serine, alanine, valine, methionine, lysine, phenylalanine and isoleucine in which their quantification are more than unfermented milk (Rosa et al., 2017).

The prebiotic concept was introduced for the first time in 1995 by Glenn Gibson and Marcel Roberfroid. Prebiotics was describes as " a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/ or activity of one or a limited number of bacteria in the colon, and thus improves host health". Based on this definition, only a few compounds of the carbohydrate group, such as short and long chain β-fructans(FOS and inulin), lactulose and GOS, can be classified as prebiotics.

Preparation of flavor mixed drink:

Milk with powdered sugar at 6.5% and high methyl pectin of 0.2% were taken then the carrot juice obtained from the homogenous texture is pasteurized and cooled. Cultures were used were the probiotic

strains is used *Lactobacillus acidophillus*(Mousavi,2006)and prepared with the *lactobacillus* bacteria And the process, were prepared after inoculation with different probiotic bacteria .Each probiotic milk and carrot juice drink was filled in some sterile glass containers all the samples are kept under refrigeration at 4 °C for 20 days samples were taken at 5 days interval for microbiological and chemical analysis (Charanjiv et al.,2006) who showed that carrot flavoured milk remained in good condition for 4 days under refrigeration.

Preparation of milk carrot juice mixed drink:

Carrot is a root vegetable and first used for medical purposes. Carrot juices considered as low-acidic nature and has pH of 6 .It contains many nutrients and vitamins and also carrot has unique combination of three flavonoids they are kaempferol, quercetin and luteolin which helps to maintain the pH of the drink. Carrots mildly gives sweetness to the probiotic drink. Most importantly, it does not affect the growth of beneficial bacteria such as *Lactobacillus* and *bifidobacterium* spp.

Banana mixed with probiotic drink:

Bananas are considered to be a good source of prebiotic it has pH of 6.5 and helps to activate probiotic bacteria found in yogurt and kefir. Concentrating on enhancing the flavor, bananas improves flavor in probiotic drink and it also serves many nutrients and bananas are considered as perennial fruit. Commercially, available yogurts are flavored with peach, apricot and mangoes to play a substitute bananas could be a best find and it also cost friendly.

II.MATERIALS AND METHODS

2.1.Traditional method

In traditional method the making of kefir is done by directly adding kefir grains raw milk is boiled at 72°C and cooled to 20-25°C. The temperature for inoculation should be maintain in the range 20-25 °C. After inoculation fermentation for 18-24 hours at 24 -25°C. Kefir grains are separated from the milk with sieve or with mesh cloth can be dried at room temperature (Ozlem caginudi 2003) Fermentation of milk by traditional methods employing kefir grains results in display in product quality due to diverse microflora and uncontrolled fermentation . Two methods have been suggest to overcome the drawbacks of traditional methods of kefir production, Kefir can be produced either by simultaneous (Tamaietal 1996) or consecutive lactic acid and yeast fermentation. Traditional method involve slow souring of milk using kefir grains .

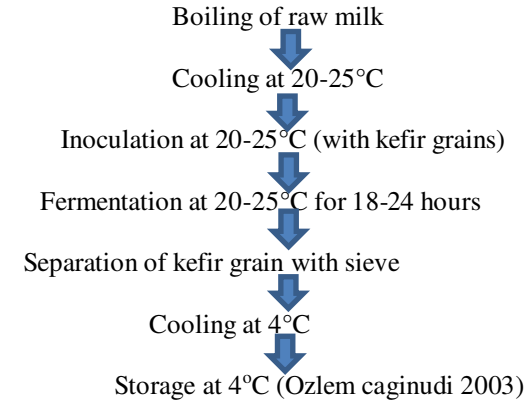

Fig.1. Flowchart for traditional process

2.2.Symbiotic kefir preparation

Initially for preparing the probiotic sample, the milk is selected with the fat content of 4.5% and SNF of 8.5% . Milk is been pasteurized at 63°C(145°F) for 30 minutes and cooled at room temperature. Once the milk has cooled, Kefir culture is measured and added to the room temperatured milk at the ratio of (1:500) (w/v) which is 1gram of kefir sample to 500ml of milk. Incubate the sample for 18- 24 hours at room temperature.Prebiotic (Inulin) is measured and added to the fermented kefir sample at the ratio of (1:100) (w/v) which is 1gram to prebiotic to 100ml of kefir. Trials had done by varying the prebiotic combination but 1:100 blends well and also provides right consistency.

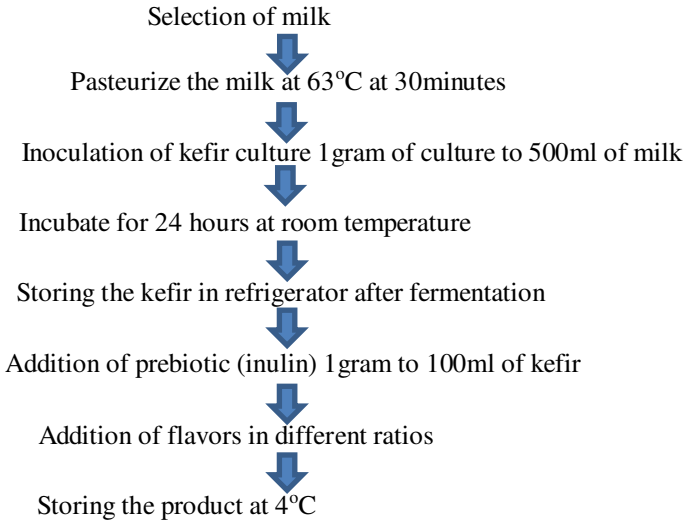

Fig.2.flowchart of symbiotic flavored kefir

Fig3. Kefir after incubation

2.3.Addition of flavors

Adding of natural flavor, banana and carrot were selected and blended using water to prepare juice at similar measurement of 100gram of fruits with 100gram of water. The blended juices were added to the symbiotic kefir at different ratios of 1:1, 1:3, 1:5. In this all the ratios, banana juices were only altered in ml but carrot composition played a standard measurement. We have taken four different samples of A, B, C,D. In this sample A has taken as plain kefir, Sample B has taken as 100ml of kefir mixed with 10ml of flavor, Sample C has taken 30ml of flavor with 100ml of kefir, Sample D has taken 100ml of kefir mixed with 50ml of flavor.

Tab1. *Table for flavor preparation*

SI.NO	SAMPLE	KEFIR(ml)	FLAVOR(ml)
1.	Sample A	100	-
2.	Sample B	100	10
3.	Sample C	100	30

4.	Sample D	100	50

III. QUALITY ANALYSIS

3.1. PHYSICAL ANALYSIS

A) Analyzing Titratable Acidity and pH

Titratable Acidity and pH acts a major important role for determining the shelf life of the kefir. pH should maintain in between 4.0 and 7.0 and measured either in pH paper but could not get an accurate value. According to AOAC, pH was measured using a digital pH-meter. Titratable Acidity were titrated against NaOH and determined according to AOAC official method no 947.05 (AOAC,1999). The Titratable Acidity increases progressively during fermentation.

B) Analyzing moisture content

The percentage of moisture content was determined by oven method from the literature(Igbabul B et al., 2014). Briefly, 2g of yoghurt samples was dried in the oven for 24 hours at 100°C. The percentage moisture content was calculated by the following formula.

$$\% \text{ moisture} = W_1 - \frac{W_2 \times 100}{W_1}$$

where, W1=initial weight of sample; W2=weight of the dried sample.

3.2.NUTRITIONAL ANALYSIS

A) Analyzing protein content

The crude proteins were determined by the macro Kjeldahl method as described in literature (Oladipo et al.,). Briefly, 2g of the sample was introduced into a Kjeldahl digestion flask together with 10g of copper sulphate and sodium sulphate in the ratio of 5:1. 25 mL of concentrated sulphuric acid was added to the digestion flask and the digestion was carried at about 1500°C in the fume cupboard until frothing ceased. A clear and light blue coloration was observed. The digest was cooled and diluted up to the mark with distilled water in 100 mL volumetric flask. 10 mL of the diluted mixture was poured into the distillation apparatus and 18 mL of 40% of sodium hydroxide was added. 25 mL of 2% boric acid was added into the receiving conical flask and two drops of bromocresol green and methyl red mixed indicator was added. The distillation was continued until boric acid solution turned from pink to yellowish green. After the distillation, the solution in the conical flask was titrated against 0.1N hydrochloric acid until the end point was reached. A blank was taken using the same procedure using only with distilled water.

The protein was calculated as:

$$\% \text{ crude protein} = \% \text{ nitrogen} \times 6.38$$

$$\% \text{ nitrogen} = \frac{(ml\ standard\ acid - ml\ blank) \times N\ of\ acid \times 1.4007}{sample\ in\ grams}$$

B) Analyzing fiber content

The crude fibre was determined according to the procedure reported in literature (Mbaeyi-Nwaoha et al., 2017). It was determined as the fraction remaining after digestion with standard sulphuric acid and sodium hydroxide. Briefly, 2g of the sample was hydrolysed in a beaker containing 299 mL of 1.25% of sulphuric acid and then boiled for 30 minutes. The mixture was filtered under vacuum and the residue was washed with hot distilled water for 3 times and then boiled again for 30 minutes with 200 mL of 1.25% of sodium hydroxide and filtered again. The digested sample was washed with hydrochloric acid to neutralize

sodium hydroxide and then with hot distilled water for 3 times. The residue was taken into a crucible, dried at 100°C for 2 hours in an oven, the sample was cooled in a desiccator and then weighed. The sample in the crucible was incinerated at 500°C for 5 hours until all carbonaceous matter were burnt. Finally, the crucible containing the ash was cooled in the desiccator and weighed.

The percentage crude fiber was calculated by the following formula:

$$\% \text{ crude fibre} = \frac{\text{loss in weighed (g) after ignition}}{\text{weight of the original sample (g)}} \times 100$$

$$= \frac{W_1 - W_2}{W} \times 100$$

where: W1= weight of digested sample and crucible before ash; W2=weight of crucible and ash; W=weight of sample used.

C)Analyzing the ash content

The ash content was determined by direct heating method as described in literature (Igbabul B et al., 2014).Briefly, 2g of each one of the yoghurt samples was weighed in dried glass crucibles separately. The samples were then incinerated to ash in a muffle furnace for 3 hours at 550°C. The crucibles were then removed, cooled in desiccator and the weight of the ash was determined. The percentage ash content was calculated by the following formula.

$$\% \text{ ash} = (Z\text{-}X/Y\text{-}X) \times 100$$

where; X=weight of empty crucible; Y=weight of crucible + sample; Z=weight of crucible + ash

Fig.4. Ash content obtained after muffle furnace

3.3.Microbial analysis

The kefir includes numerous bacterial species from lactic acid and acetic acid groups, yeasts and filamentous fungi. In this relationship, yeasts produce vitamins ,amino acids and other essential growth of energy source. Additionally, the culture added to the milk, agitation and incubation temperature can influence the microbial composition. Lactic acid bacteria are mesophiles grows at temperature range of 10-45°C(Fontan et al.,2006) were frequently reported that kefir sample, ranging from 10^8 to 10^9 cfu/ml (Paramita et al., 2008). According to Kandler and Weiss (1986) the growth of lactic acid bacteria were reported better in neutral pH, optimally at the pH of 5 to 9.

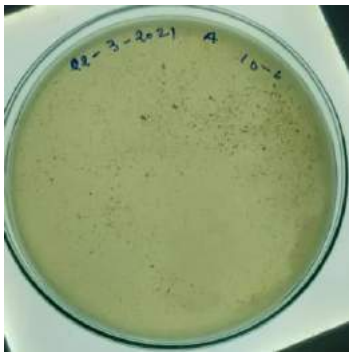

Fig.5. Microbial plating of kefir

3.4 Sensory Analysis

Sensory Analysis were done over 20 panelists with four samples to analyze which sample would be the desired one according to the community. The sensory chart includes appearance, sourness, consistency, texture, flavor, aroma, taste, acidity and finally to conclude with overall acceptability.

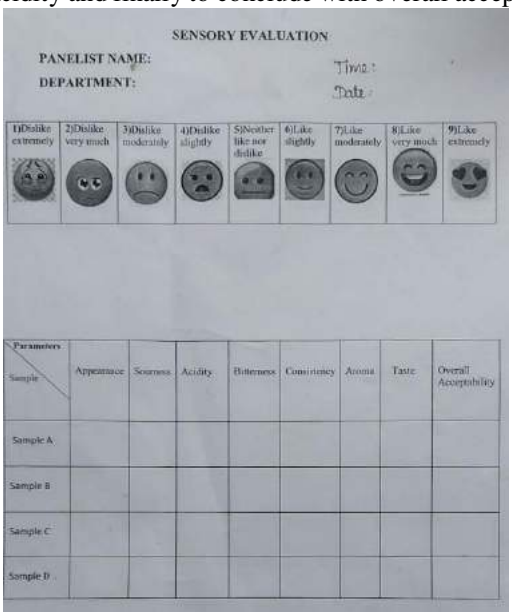

Fig.6.Sensory Evaluation Chart

IV. RESULT AND DISCUSSIONS

4.1. Physical Analysis

In the present work, tested in pH meter 24 hours fermented kefir after incubation reports at pH of 4.3-4.6 after 15 days of storage at 4^{o}C pH ranged from 4.5-4.8. (O.A. Adebo et al.,2018) suggested that pH decreases at longer fermentation time. Values of the pH and titratable acidity are taken throughout the storage period. The noticeable pH that obtained during processing are which are decreases during storage so the pH has been maintained throughout the processing. It helps us to adding prebiotic in when pH decrease the stability of the prebiotic in the drink is more stable. And when comparing the samples of sample A,B,C and D obviously sample D shown high acidic content than other samples.

4.2. Nutritional Analysis

Proteins are become easily digestible due to the action of the coagulation and proteolysis it occurs during the fermentation process (Ferreira C, 2010). Protein analysis were done for the yogurt and resulted that it has high protein content(Kemelo et al.,) while we are analyzing and sample D shows high in protein content.

Fiber content were analyzed from yogurt (Mbaeyi-Nwaoha IE,2017) explained on the process and analyzing the samples and while studying usually kefir has low in fiber content because of the addition of prebiotic analyzing the fiber may help to find out the presence of fiber in the samples. Sample A shows less fiber content because of the absence of prebiotic and even without the flavors.

Ash content and moisture content study is done to analyze the minerals present and also the moisture content present in the products. (Kemelo et al.,) studied with the yogurt samples and we analyzed both the contents. Ash and moisture content were high in sample D just to high in flavor since they flavor were added in the form of liquid consistency improved the moisture content in sample D

Tab2.Physical and nutritional analysis of kefir

Sample	Titratable Acidity	pH	Ash content (%)	Protein(%)	Fiber (%)	Moisture content (%)
Sample A	7	4.2	20.7	2.38	0.05	12.10
Sample B	8	4.4	21.8	2.33	1.22	12.21
Sample C	16	4.4	21.2	2.13	1.53	12.24

Sample D	27.2	4.5	23.4	2.35	1.19	12.28

4.3 Microbial Analysis

Additionally, the culture added to the milk, agitation and incubation temperature can influence the microbial composition. Observation of the population microbial composition of bacteria vary from 6.3 x 10^4 to 8.3 x 10^8 CFU/g. After 18hours of fermentation, the *Lactobacillus* species presented in kefir 10^8 CFU/g and yeasts present in 10^6 CFU/g(Irigoyen et al.,2005). When we observing the bacteria growth microbial composition of bacteria varied 6.4 × 10^5 to 8.1 × 10^5 CFU/ g. *Lactobacillus* spp. Present in kefir samples ranged from 10^7 CFU/g. Appearance of Gram positive Lactobacillus rod in microscopic examination.

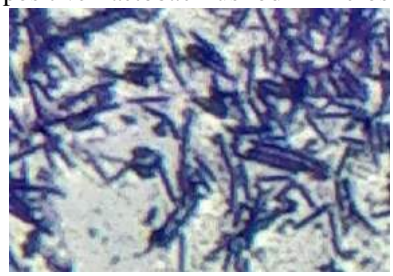

Fig.7.Microscopic structure of Lactobacillus rod present in kefir

4.4. Sensory Analysis

Sensory Analysis were done for all the samples i.e., sample A,B,C and D. Optimization of samples were also done to improve the overall acceptability and plotting the radar chart shows Sample C i.e., 100ml of kefir mixed with 30ml of flavor is more scored the overall acceptability.

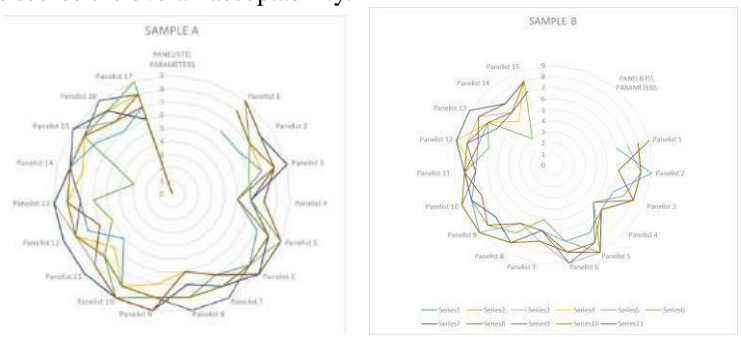

Fig.8. Radar chart for Sample A and B

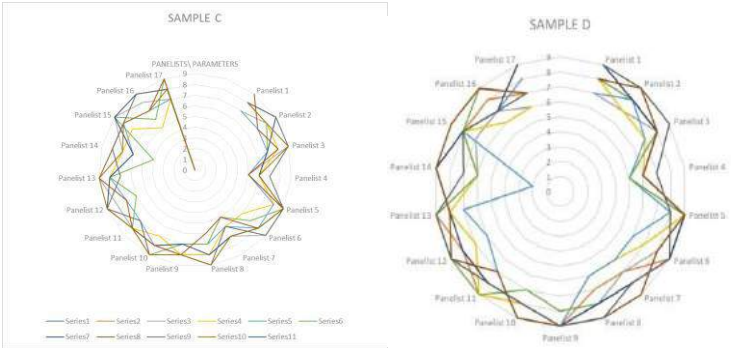

Fig.9. Radar chart for Sample C and D

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