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Process optimization for preparation of fruit kefir

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Abstract
The present research work was carried out at the Department of Animal Husbandry and Dairy Science**,** Mahatma Phule Krishi Vidyapeeth, Rahuri- 413722 Dist. Ahmednagar, Maharashtra province of INDIA. The study was conducted to standardize the procedure for production of fruit kefir. For preparation of fruit kefir three different fruit pulp were used *viz.* Mango, Jamun and Strawberry. For fermentation two different steps were followed (Step I 37 ºC for 9 hrs. + 24 ºC for 15 hrs. and Step II 37 ºC for 24 hrs.). Trials were carried out for optimization of level of fruit pulp and fermentation process. Sensory evaluation was carried out for finalising level.

Keywords: Kefir, fruit kefir, fermented products

Introduction
Kefir is a fermented dairy product which has been originated from the Caucasus Mountains (Gronnevik *et al*., 2011) [1]. It is a traditional popular Middle Eastern beverage. The word kefir is said to have originated from the Turkish word 'Keyif' which means 'good feeling' (Leite *et al*., 2013) [9]. It has various names in European countries including kephir, kiapher, knapon, kefer, kepi, kippi snd kippe (Farnworth and Mainville, 2008). The main difference between kefir and other fermented milk product is the use of either a starter culture called "Kefir grains" or a percolate of the grains to ferment milk (Farnworth and Mainville, 2008). Kefir grains are sometimes called "Millet of the prophet" or "Mahomet grains". Kefir grains are a symbiotic association of yeast and bacteria They are dump-shaped, gelatinous granules measuring about 1-2 mm to 3-6 m, sometimes up to 2-15 mm in diameter with irregular, rough and convoluted surfaces. It's reported that traditional kefir grains are obtained from periodic coagulation of cow milk with calf or sheep abomasum (fourth stomach) in a goatskin bag. After a few weeks a water-insoluble, gel-like and spongy layer is formed on the inner surface of the bags where coagulation occurred, this layer is divided into small pieces and dried to obtain kefir grains.

In the present situation, due to busy schedules and changes in the lifestyle of people, the demand for functional food has been increasing. Its popularity is mainly based on its nutritive content and its health benefits. Kefir is a natural probiotic, which contains live active cultures of normal flora which is made of very strong strains of micro-organisms. It has various benefits to human health, such as improving lactose digestion and tolerance in adults as well as antimicrobial, antitumoral, antioxidant, antimutagenic, and antiapoptotic effects (Lopitz-Otsoa *et al.*, 2006) [4].

Recently there has been a trend to add fruit juices and pulp to cultured milk production. As the addition of natural flavor creates a wide variety of sensory choices in texture, color and flavor in fermented milk making it appealing to the consumer. Various fruits also provide nutritional benefits and enhance the product's nutritional value. Considering the above facts, the addition of Mango, Jamun and Strawberry fruit pulp is carried out in kefir.

Mango *(Mangifera indica* L.) is a juicy stone fruit that belongs to the family of Anacardiaceae in the order of Sapindales and is grown in many parts of the world, particularly in tropical countries. It is the national fruit of India and the Philippines and the national tree of Bangladesh. The energy value per 1009 is 250 kJ. Mango contains a variety of phytochemicals and nutrients. The mango pulp composition varies depending on many factors like variety, locality, climate, and stage of maturity. The ripe Mango pulp contains Water 78.9-82.8 g, Protein 0.36-0.40 g, Fat 0.30-0.53 g, Carbohydrates 16.20-17.18 g, Fiber 0.85-1.06 g, Ash 0.34-0.52 g, Calcium 6.1-12.8 mg, Phosphorous 5.5-17.9 mg, Iron 0.20-0.63 mg, Thiamine 0.020–0.073 mg, Riboflavin 0.025–0.068 mg, Niacin 0.025–0.707 mg, Ascorbic Acid

7.8–172.0 mg, Tryptophan 3–6 mg, Lysine 32–37 mg, Methionine 4 mg, 27.7 mg of vitamin C and 3894 IU of vitamin A per 100 g of pulp. Mango peel and pulp contains other compounds, such as carotenoid pigment and polyphenols, omega-3, and omega-6 polyunsaturated fatty acids (Masibo and Quian, 2009) [5].

Syzygium cumini belongs to the family Myrtaceae, in Hindi, it is commonly called Jamun. The ripened fruit has a combination of sweet, mildly sour and astringent flavors. Jamun is a rich source of Vit A. and Vit C. The pulp of Jamun contains anthocyanin, delphinidin, petunidin, and malvidin glucides. Fruits are rich in raffinose, glucose, fructose, citric acid, malic acid, gallic acid, delphinidin-3-gentiobioside, malvidin-3-laminaribioside, petunidin-3-gentiobioside and cyanidin glycoside. Pulp of Jamun has various nutrients like ascorbic acid, thiamine and niacin, free amino acids like alanine, asparagine, tyrosine, glutamine and cysteine (Ramya *et al.* 2012) [6].

Strawberry (Fragaria × ananassa) is a valuable crop cultivated worldwide. All strawberry species belong to the genus Fragaria and are members of Rosaceae. The fruit of the strawberry plant is packed with beneficial nutrients, particularly Vitamin C and flavonoids. One cup of strawberries weighs approximately 144 grams and contains between 45 and 50 calories. Strawberries are over 90% water, 7% carbohydrates, about 2% fibre, and less than 1% each of protein, fat, and ash. Strawberries are also a dietary source of minerals and vitamins. The strawberries have various minerals *viz*. potassium, phosphorus, calcium, magnesium, sodium, iron, manganese, zinc, copper, and selenium. Strawberries are also a good source of the following vitamins: Vitamin C, thiamine, riboflavin, niacin, pantothenic acid, Vitamin B6, Folate, Vitamin B12, Vitamin A, and Vitamin E. Additionally, strawberries contain 18 different amino acids.

Materials and Method

Composite samples of fresh crossbred cow milk samples were procured from Research- cum-Development Project (RCDP) on Cattle, MPKV, Rahuri, Dist. Ahmednagar (Maharashtra) for preparation of *kefir* samples. Kefir grains were procured from Matru cultures and probiotics, Bangalore. The *Kefir* samples under experimental trials were subjected to sensory evaluation using the method described in IS: 6273, Part –I and II (1971) [7] adopting 9-point Hedonic Scale. A panel of 5 semi trained judges was formulated for this purpose. The samples were coded every time to conceal their identity and were offered to the judges for evaluation of the quality attributes.

Result and Discussion

One of the objectives of this research was to optimum incubation temperature. Kefir was prepared with slight modification to procedure given by the Guzel-Seydim *et al.*, 2010 [8].

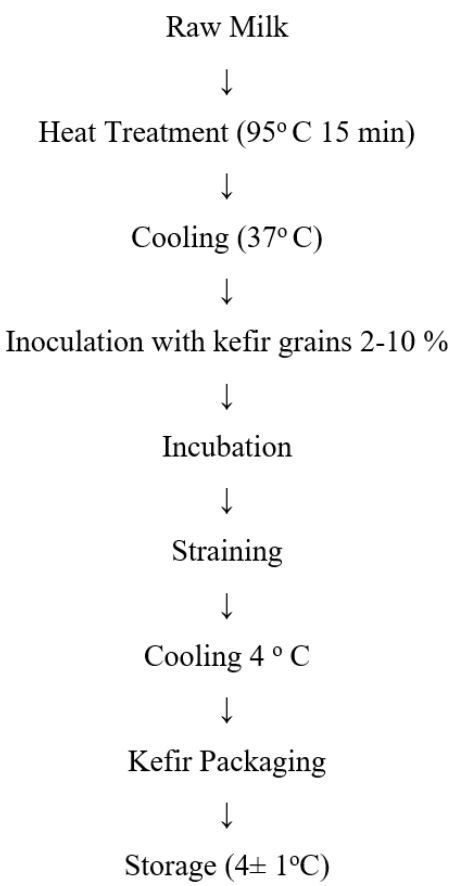

Fig 1: Flow diagram for preparation of kefir

During trials kefir was prepared by a two step fermentation process for optimization of incubation temperature. The time temperature combination was as follows.

Step I: - 37° C for 9 hrs + 24° C for 15 hrs.

Step II: - 37° C for 24 hrs.

On the basis of the result of sensory score of the fermentation process best rated incubation temperature was finalized for further experiment trials (Table 1).

Table 1: Mean sensory score for incubation temperature

Treatment	Colour and Appearance	flavor	Consistency	Overall Acceptability
Step I	8.51	8.50	8.58	8.53
Step II	8.10	7.75	7.55	7.80

On the basis of the sensory score of fermentation process the best rated incubation temperature was finalized for further experiment trials.

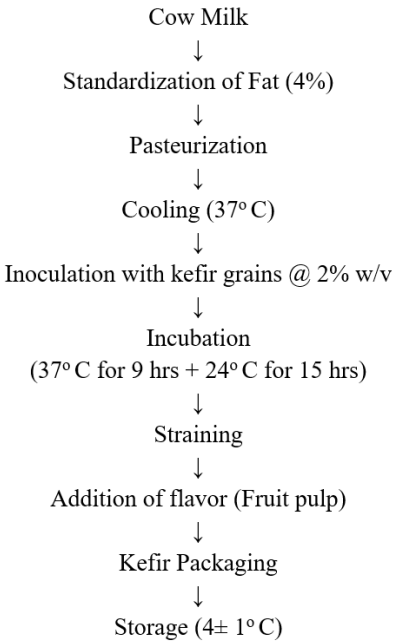

Fig 2: Flow diagram for preparation of fruit kefir

Kefir produced by Step I gives mild sour and alcoholic flavor as compared to Step II which had strong sour and acidic flavor. The Step II method kefir showed low consistency as compared to Step I where the consistency of kefir was sour-cream like. There was not much difference in colour as both showed white colour but the kefir from Step II method was less viscous and lacks creaminess as compared to kefir produced from Step I method. This may be the reason that panellists gave more score to L_1. Based on Sensory analysis the fermentation temperature L_1 i.e 37° C for 9 hrs + 24° C for 15 hrs was carried forward in experimental trials for production of kefir**.**

For optimization of level of Mango pulp fruit kefir was prepared by addition of different levels of pulp. Kefir samples were prepared with six levels of mango pulp – 10% (M_1), 12% (M_2), 14% (M_3), 16% (M_4), 18% (M_5) and 20% (M_6) (Table 2). Mango pulp contains beta carotene, which lends their interiors a beautiful yellow hue. Mango pulp is packed with carotenoids, which are produced using phytoene. The phytoene leads to an integration of orange-yellow carotenoids, thereby turning it yellow. The pulp contains myrcene, a kind of terpenoid, a naturally occurring chemical in plants responsible for flavor and aroma. Mango pulp gives juicy, sweet and tropical fruit flavor and yellow colour. Addition of mango pulp gives kefir a typical sweet and juicy flavor. The table illustrates that the highest mean score was obtained by kefir having 16% mango pulp (M_4) as depicted in Table 2. The score for treatment M_5 and M_6 is lower than M_4 as higher concentration of pulp masks the sourness and typical acidic flavour of kefir. The higher concentration of mango pulp also increases the consistency of kefir making it more viscous. As the table suggests, treatment M_4 tends to be a perfect blend of Mango pulp to kefir without compromising the novelty of the product. Therefore, a concentration of 16% mango pulp was selected for trials.

Table 2: Mean sensory score for different levels of Mango pulp

Treatment	Colour and Appearance	Flavour	Consistency	Overall Acceptability
M_0 (Control)	7.12	7.12	6.71	6.98
M_1	7.46	7.47	7.32	7.42
M_2	7.72	7.74	7.62	7.69
M_3	8.03	8.24	8.05	8.11
M_4	8.59	8.61	8.60	8.60
M_5	8.44	8.31	8.20	8.32
M_6	7.92	7.76	7.81	7.83
SE±	0.096	0.142	0.134	0.074
CD at 5%	0.280	0.413	0.389	0.216

For optimization of the level of Jamun pulp, fruit kefir was prepared by adding different levels of pulp. Kefir samples were prepared with six levels of Jamun pulp – 12% (J_1), 14% (J_2), 16% (J_3), 18% (J_4), 20% (J_5) and 22% (J_6) (Table 3). Jamun has a subtly sweet, tart, and sour flavor followed by an astringent aftertaste. Anthocyanins naturally occurring pigments that give characteristic colour to jamun the sensory score reveals that's the lower concentration of pulp than 18% has not as much of effect on the sensory attributes of kefir, whereas higher concentration seems to give more sourness and low consistency to the kefir. The 18% jamun pulp has optimum effect on the kefir and was selected for experimental trials. On the basis of sensory evaluation, the highest mean score was obtained by kefir having 18% jamun pulp (J_4).

Table 3: Mean sensory score for different levels of Jamun pulp.

Treatment	Colour and Appearance	Flavour	Consistency	Overall Acceptability
J_0(Control)	6.75	6.85	8.34	7.31
J_1	7.64	7.75	8.22	7.87
J_2	8.00	8.07	8.10	8.06
J_3	8.35	8.28	7.98	8.20
J_4	8.58	8.64	7.87	8.36
J_5	8.12	8.02	7.64	7.93
J_6	7.74	7.61	7.45	7.60
SE±	0.12	0.12	0.03	0.06
CD at 5%	0.37	0.36	0.10	0.19

For optimization of the level of Strawberry pulp fruit kefir was prepared by adding different levels of pulp. Kefir samples were prepared with six levels of strawberry pulp – 14% (S_1), 16% (S_2), 18% (S_3), 20% (S_4), 22% (S_5) and 24% (S_6) (Table 4).

On the basis of sensory evaluation, the highest mean score was obtained by kefir having 20% strawberry pulp (S_4) as depicted in Table 4. Strawberry pulp is valued for its characteristic red colour, juicy texture, and distinct aroma. Flavor of pulp is sweet, fruity, caramel, spice and green notes. The 20% strawberry pulp seems to be optimum concentration as in lower concentration flavor and colour of strawberry is not well distinguished whereas, in higher concentration the flavor of kefir gets masked by sweet fruity flavor of

strawberry. Red tinge obtained to kefir at 20% concentration was more appealing to the panellist compared to sharp colour at high concentration which has resembled in the score for colour. The table reveals that with highest sensory score treatment S_4 (20% strawberry pulp) was a proper blend of kefir and strawberry pulp, which was selected for experimental trials.

Table 4: Mean sensory score for different levels of Strawberry pulp.

Treatment	Colour and Appearance	Flavour	Consistency	Overall Acceptability
S_0(Control)	6.69	6.85	8.48	7.34
S_1	7.25	7.23	8.28	7.59
S_2	7.63	7.72	8.06	7.81
S_3	8.24	8.13	7.90	8.09
S_4	8.56	8.66	7.80	8.34
S_5	7.99	8.21	7.62	7.94
S_6	7.72	7.79	7.42	7.64
SE±	0.10	0.11	0.06	0.06
CD at 5%	0.31	0.34	0.19	0.18

Conclusion

The kefir was produced by Step I (37 ^{0}C for 9 hrs + 24 ^{0}C for 15 hrs) and Step II (37 ^{0}C for 24 hrs). Kefir produced by Step I gives mild sour and alcoholic flavour as compared to Step II. There was not much difference with colour. Both produced whitish kefir but the consistency was creamy of kefir produced with Step I. Kefir produced with Step II gave watery consistency. This was the reason Step I was preferred by panellists over Step II during sensory evaluation for further study. The kefir produced with 16% mango pulp seems to be preferred by panellists over other treatments. As lower concentration of pulp does not give sweetness whereas, higher concentration masks the typical sourness and alcoholic flavor of kefir. Kefir produced with 16% mango pulp seems to have optimum consistency. As the score by the panellist suggests, the kefir produced with 16% mango pulp is a perfect blend without compromising the originality of the product. Therefore 16% concentration of mango pulp was selected for further experimental trials.

Kefir produced with 18% jamun pulp gives a purple tinge to kefir which seems to attract the panellist gaining its highest score for colour and appearance. As the score by the panellist suggests, the kefir produced with 18% jamun pulp is the perfect blend for preparation of jamun flavor kefir. Therefore 18% concentration of jamun pulp was selected for further experimental trials.

The kefir produced with 20% strawberry pulp seems to be preferred by panellists over other treatments. As in lower concentration of pulp typical sweet fruity, caramel, spice flavor of strawberry is not realized whereas, higher concentration mask seems to increase sourness of kefir. Kefir produced with 20% strawberry pulp gives a red tinge to kefir which seems to be more appealing to panellists gaining its highest score for colour and appearance. As the score by panellists suggest, the kefir produced with 20% strawberry pulp is the perfect blend for preparation of strawberry flavor kefir. Therefore 20% concentration of jamun pulp was selected for further experimental trials.

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