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Formulation and Evaluation of Foxtail Millet Composite Mix for Probiotication

VINUTA R. YADAGOUDA AND USHA RAVINDRA
Department of Food Science and Nutrition, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
e-Mail : vinu.y06@gmail.com

ABSTRACT

In the present study composite mix was developed for preparation of non-dairy based probiotic product using foxtail millet and other ingredients. Foxtail millet composite mix was developed by incorporating foxtail millet flour at 40, 50, 60 and 70 per cent level and subjected to sensory evaluation. The best accepted variation was taken and analyzed for its physico-functional, colour, macronutrients and micronutrient composition using standard protocol. The results from the analysis of macronutrients such as moisture, protein, fat, crude fibre, carbohydrate, energy and ash of developed composite mix were 5.75 g, 19.53 g, 5.95 g, 3.45 g, 65.28 g, 392.79 Kcal and 3.53 g/ 100g, respectively. Colour analysis depicted the better lightness (*L**) and slight green colour (*a**) in the acceptable range. Later the best accepted composite mix variation was processed and inoculated with *Lactobacillus acidophilus* and incubated at 37^{0}C for 24-72 hrs. After 72 hrs of fermentation the samples were decimally diluted in prepared dilution series. Dilutions were plated on MRS agar for *Lactobacillus acidophilus* and incubated at 37^{0}C for 24 hrs. Populations in colony forming units (CFU / ml) were counted. *Lactobacillus acidophilus* was found capable of multiplying in foxtail millet composite mix at different inoculum level with CFU / ml ranging from 2.85 x 10^8 to 3.21 x 10^8. Hence, it could be concluded that foxtail millet composite mix can be best suitable substrate for development of non-dairy based probiotic product with good quality protein.

Keywords : Foxtail millet, Functional foods, Composite mix, Probiotics, Protein, Sensory evaluation

IN recent years, there has been an increased interest and change in the food pattern to adapt healthy and balanced diets to prevent life style disorders and diseases. This has led to the development of new functional foods. Probiotics and nutraceuticals foods belong to such diet category. Changes in the eating patterns / habits, living standards and increased health awareness has shifted the consumer acceptance towards nutritious, healthy and disease preventive food with their wider health benefits. Cereal grains are the one of the richest source of nutrition as well as staple food of many parts of the world. Among the cereals, millets are the most unique having the good macro (protein, carbohydrates and fibre) and micro nutrients (minerals and vitamins) with numerous health benefits, highly resistant to drought and can grow in extreme weather conditions (Benerjee *et al.,* 2017). They have a high yielding capacity and can be stored for a longer period. Millets are the nutritious grains and a good substitute to cereals. Therefore, using millets in the preparation of ready-to-eat, ready-to-cook and ready-to-serve products would enhance better nutrition, food and economic value (Fasreen *et al.,* 2017).

As the consumers demand and awareness towards healthy diets have been led to development of more functional foods like probiotics, nutraceutical and protein enriched fermented foods. Use of microbe in the food products to promote health and to prevent diseases has been gradually increasing (Namesha and Usha, 2018). Probiotics are defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (FAO / WHO, 2014). The abundant probiotics in fermented foods also improve digestion and the production of nutrients like vitamins, probiotic lactic acid bacteria that inhibits the growth of food spoilers and can both prevent and treat diarrhea. Many researches proved that addition of probiotics to food leads to several health benefits including the reduction of level of serum cholesterol, improvement of gastrointestinal function, enhancement of immune system, the suppression of diarrhea in young children and lowering the risk of colon cancer (Kavitha and Kiruthika, 2018).

Most of the probiotic products available in the market are dairy based fermented foods. However, consumers' preference has been shifted to plant based functional foods as well as free or minimal cholesterol content foods. Even the increased incidence of lactose intolerance and short life span of dairy based products have led to development of probiotic foods using cereals, millets, fruits and vegetables. This could have better profile of nutrients and therapeutic value which may serve as best alternative to dairy based probiotic products. Therefore, in the present study an attempt was made with an objective to develop and standardize the foxtail millet based composite mix for probiotic food.

METHODOLOGY

Procurement of the Raw Materials

Foxtail millets were procured from the Millet Shop, UAS, GKVK, Bengaluru and other raw materials *viz.,* green gram, groundnut, chia seeds, sugar, drumstick leaves and cardamom required to make composite food mix, were procured from local super market. All samples were segregated, cleaned and stored in air tight containers till further use.

Criteria used for Functional Food Formulation (Shilpa, 2013)

- Major ingredients used should possess complex carbohydrates, including rich in protein
- Exploration of small millets
- Combination of materials used to provide good nutritional benefits

Standardization and Evaluation of Millet Based Composite Mix

Specific criteria, preparatory methods, formulations, processing protocols and standardization for food formulations are described below.

Processing of Raw Materials

Germination and roasting were used before development of the composite mix as pretreatments for the foxtail millet. Time and temperature for germination and roasting of foxtail millet was carried out and represented in Fig. 1.

The foxtail millet, green gram and ground nuts were cleaned to remove the impurities and unhealthy grains. Cleaned grains were soaked in water for 12 hours. Later they were milled into a fine powder in a flour mill and were mixed in proportions to develop different variations of millet based composite mix (Fig. 2).

Physical Properties of Foxtail Millet

The physical characteristics like thousand kernels weight (Sangamithra *et al.,* 2016) by counting manually the number of seeds and weight was

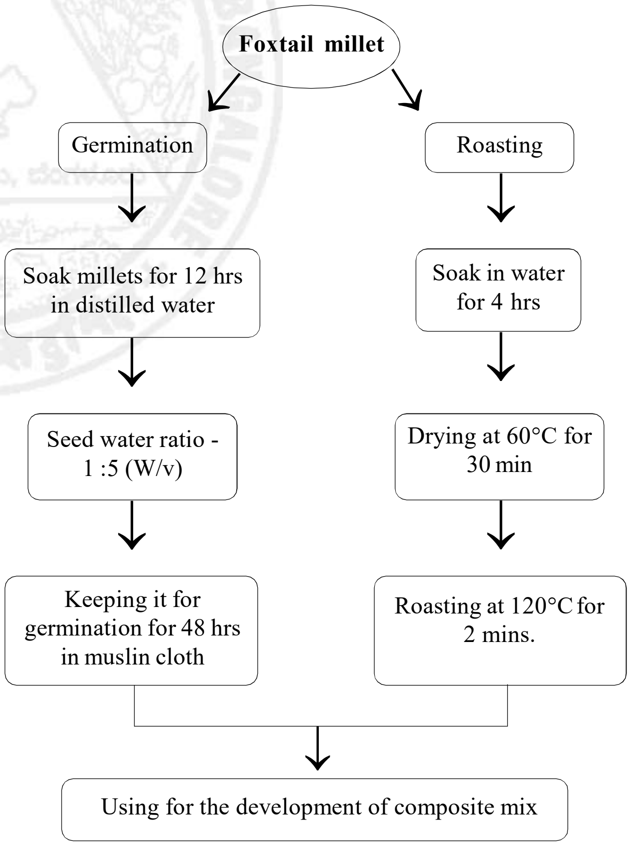


Fig.1 : Standardization of time and temperature for germination and roasting of foxtail millet

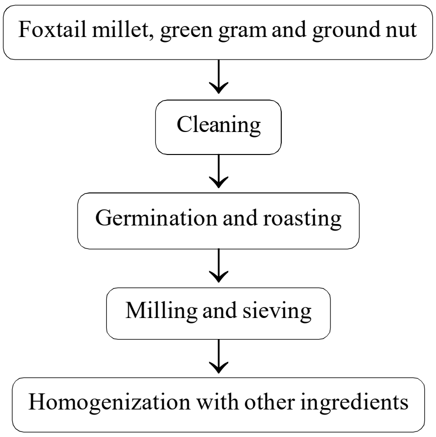


Fig. 2 : Protocol for development of foxtail millet composite mix

recorded using digital electronic balance with 0.01 accuracy. Seed volume (ml / seed) (Yadav *et al.,* 2007) was recorded by water displacement method. Hydration capacity (g / 1000 seeds) determined by soaking 1000 seeds in water and left overnight and excess water were drained off and the seeds were dried and weight was recorded. Hydration index (%) is calculated using formula :

$$\text{Hydration index} = \frac{\text{Hydration capacity (g / 1000seeds)}}{\text{Seed weight (g)}}$$

Swelling capacity (ml / 1000 seeds) was recorded by putting 1000 seeds into a measuring cylinder and initial volume was recorded. These seeds were later soaked in water for overnight, drained and dried. The final volume is measured and swelling capacity was calculated using the formula :

Swelling capacity (ml / 1000 seeds) = Volume after soaking (ml) - Volume before soaking (ml).

Swelling index (%) (William *et al.,* 1983) is calculated using the formula :

$$\text{Swelling index (\%)} = \frac{\text{Swelling capacity (ml/1000 seeds)}}{\text{Seed volume}}$$

The seed to flour ratio (%) (Usha, 2007) was done by weighing 100 g of foxtail millet and ground into fine powder using mixer grinder. Weight of the foxtail millet flour was recorded and seed to flour ratio was calculated.

Analysis of Functional Properties of the Foxtail Millet Composite Mix

Developed foxtail millet composite mix was analyzed for bulk density (g / ml) (Mandge *et al.,* 2014), water and oil absorption capacity (Rosario and flores, 1981), solubility and swelling power (Iyer and Singh, 1997). Colour analysis was done by using reflecting colorimeter (Chroma meter CR-300) (Ranganna, 2002).

Analysis of Nutritional Composition of Foxtail Millet Composite Mix

Developed foxtail millet composite mix was analyzed for moisture, protein (Kjeldhal method), fat, ash and crude fibre contents (AOAC, 2005). Carbohydrate content was computed by using differential method and energy was calculated using the formula (AOAC, 2005). For mineral estimation, the samples were prepared by dissolving the ash obtained after ashing the samples in a muffle furnace in dilute hydrochloric acid (1:1 v/v). Calcium and magnesium (Lawani *et al.,* 2014), potassium iron and zinc (Ranganna, 2002) contents were estimated according to standard procedures.

Sensory Evaluation

Sensory evaluation was carried out by a panel of twenty-one semi trained judges experienced in quality testing, having good health status and interested in sensory evaluation were selected from Food Science and Nutrition Department, UAS, GKVK, Bengaluru. A nine-point hedonic scale was used to select the best acceptable level of foxtail millet composite mix (Ranganna, 2002). Scores were allotted for appearance, taste, flavor, texture and overall acceptability.

Probiotication of Foxtail Millet Composite Mix

From the sensory analysis best accepted variation of foxtail millet composite mix was used as the substrate media for fermentation at natural pH and 22^0 Brix. The composite mix was cooked with 500 ml of water in a sterilized container. When the solution was boiled at 78^0C, it was retained for further 10 minutes. Later the solution was cooled to 40^0C and inoculated at different concentrations of *Lactobacillus acidophilus* (nu-trish LA-5) and incubated at 37^{0}C for 24-72 hrs. After 72 hrs of fermentation, the samples were decimally diluted in prepared dilution series. Dilutions were plated on MRS agar for *Lactobacillus acidophilus* and incubated at 37^{0}C for 24 hrs. All processes were carried out in laminar air flow cabinet or aseptic condition. Populations in colony forming units (CFU/ml) were recorded.

Statistical Analysis

Analysis of the data was carried out in triplicates. The data was analyzed statistically for the mean, standard deviation and ANOVA was used to test the significance among different levels of incorporation of the product at five per cent significance level.

Results and Discussion

Physico-Functional Properties of Foxtail Millet

Physical properties of foods are necessary to optimize processing operations as well as process design, modeling and optimization. Complete understanding of the physical properties of foods is essential for the development of novel food products and processes in the field of food science. The physico-chemical properties of the foxtail millet are depicted in Table 1. The weight of the 1000 seeds of raw foxtail millet was 4.19 g; thousand kernel volume was 4.26 ml; density recorded was 0.98 g / ml; weight after soaking was 4.68g; hydration capacity was 0.68 g / 1000 kernels; hydration index was 17.31 per cent; volume after soaking was 6.4 ml and the swelling capacity and swelling index were 2.4 ml / 1000 kernels and 60 per cent, respectively. Study conducted by Roopa *et al.* (2013) reported similar result for the functional properties for the little millet which showed that the mean thousand grain weight ranged from 2.15 g to 2.33 g the mean thousand grain volume was 1.60 and 1.65 ml, the bulk density was 1.33 g / m. The mean hydration capacity was 0.15 and 0.17 g per 1000 grains and hydration index was 6.67 to 7.66. Swelling capacity of grains was 0.15 and 0.20 ml per 1000 grains and the swelling index 9.38 to 10.41.

Table 1

Physico - functional properties of foxtail millet rice (unpolished)

Millet	Thousand kernel weight (gm)	Thousand kernel volume (ml)	Bulk Density (g/ml)	Weight after soaking (g)	Hydration capacity (g/1000 kernels)	Hydration index (%)	Volume after soaking (ml)	Swelling capacity (ml/1000kernels)	Swelling Index (%)
Foxtail millet	4.19 ± 0.35	4.26 ± 0.28	0.98 ± 0.01	4.68 ± 0.42	0.68 ± 0.09	17.31 ± 0.45	6.4 ± 0.45	2.4 ± 0.54	60 ± 1.15

Note: Values are mean of three replications ± SD

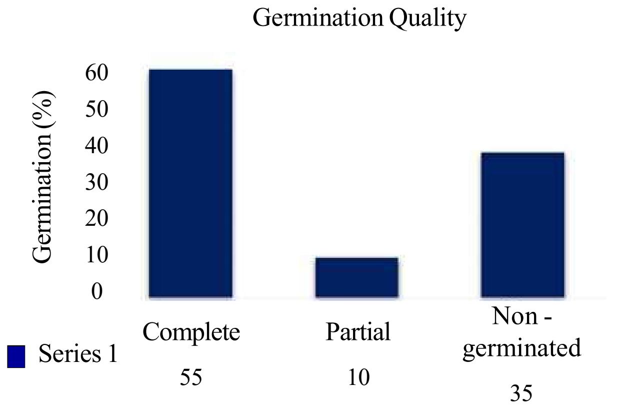
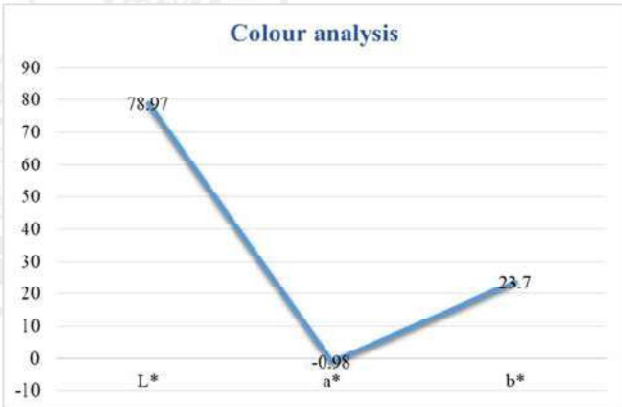


Fig. 3: Germination quality of the foxtail millet rice (unpolished)

The germination quality of the foxtail millet was recorded in Fig. 3. Fifty-five per cent of the foxtail millet rice showed complete germination, ten per cent partial and 35 per cent were non germinated. The results are in line with the soaking time and germination percentage conducted by Nadeem *et al.* (2017) and Pritham (2021). The results revealed that the germination percentage ranged from 10 per cent for 6 hrs of soaking with 12 hrs of germination and 70 per cent of germination for 10 hrs of soaking and 24 hrs of germination.

Table 2 represents the functional properties of the foxtail millet composite mix. The bulk density was 0.90g / ml, solubility percent was 0.85, the swelling power was found to be 4.12 per cent, water and oil absorption capacity was 1.29 and 1.05 g / ml, respectively. Similar results were found for the functional properties for the composite mix developed by Shilpa (2013) and the bulk density ranged from 0.76-0.80g / ml, solubility percentage was in the range of 0.74 to 0.75, swelling power ranged from 3.96 to 4.02 per cent. Water and oil absorption capacity were in the range of 1.10 to 1.18 and 0.10 to 0.91, respectively. Similar results were found from the studies conducted by Itagi & Singh (2012) and Baphiralang (2011) reported that swelling power of these mixes was about 10 per cent; however, solubility varied from 17 to 22 per cent and the swelling power of sugarcane bagasse samples were ranged between 1.00 to 4.03. Twinomuhwezi *et al.* (2020) reported that, the water absorption capacity ranged between 5.79 to 7.17 per cent; the oil absorption capacity ranged between 5.89 to 7.72 per cent; bulk density ranged between 0.99 to 1.21 per cent; and the swelling capacity ranged between 5.0 ml to 23.5 ml for developed composite flour.

Colour attributes includes L^*, a^* and b^* where L^* value represents lightness and darkness, a^* gives indication of greenness and redness while b^* shows blueness and yellowness. Results revealed from the Fig. 4 that the lightness value was 78.97, a^* was -0.98 indicating slight green colour of the mix and b^* was 23.7. Study carried out by Bembem and



L^* - Lightness a^*- green to red b^*- blue to yellow

Fig. 4: Colour analysis of the foxtail millet composite mix

TABLE 2

Functional properties of foxtail millet composite mix

Treatments	Bulk density (g/ml)	Solubility (%)	Swelling power (%)	Water absorption capacity (g/ml)	Oil absorption capacity (g/ml)
Foxtail millet composite mix	0.90	0.85	4.12	1.29	1.05

Note: Values are mean of three replications

TABLE 3

Sensory scores for concentration variations of foxtail millet composite mix

Treatments	Appearance	Colour	Texture	Aroma	Taste	Overall acceptability
FT1 (40%)	8.00	8.12	7.00	7.15	7.23	7.58
FT2 (50%)	8.12	7.98	7.45	8.18	8.31	8.17
FT3 (60%)	7.55	6.87	7.13	7.18	6.81	6.95
FT4 (70%)	6.12	5.78	6.72	6.15	5.45	6.00
Mean	7.44	7.18	7.07	7.16	6.95	7.15
F – value	67.944*	74.437*	NS	24.794*	47.709*	51.386*
S.Em±	0.556	0.523	0.516	0.494	0.474	0.486
CD (p<0.05)	1.814	1.705	-	1.609	1.544	1.586

*-Significant; NS - Non Significant @ p≤0.05 %

FT1 - Foxtail Millet Treatment 1 (40%), FT2 - Foxtail Millet Treatment 2 (50%), FT3- Foxtail Millet Treatment 3 (60%), FT4- Foxtail Millet Treatment 4 (70%) to specify composition of other ingredients in treatments

Murugkar, (2020) showed similar values for *L** for optimized beverages from millet flour ranged from 45.35 to 60.39 and *b** values ranged from 22.55 to 28.91 based on the variations.

The mean sensory scores for concentration variations of foxtail millet composite mix which indicated that FT2 variation scored very good in overall acceptability (8.17), taste (8.31), aroma (8.18) and appearance (8.12) followed by FT1 variation. The parameters differed significantly for appearance, colour, aroma, taste and overall acceptability except for the texture when compared between the variations (Table 3). The FT2 variation had good scores for acceptability compared to other variations. Similar study conducted by Ananthan *et al.* (2013) and Shobha *et al.* (2019) indicated that the sensory attributes of flax oat nutty bar by incorporating multipurpose mix exhibited significant differences with respect to colour, aroma, taste, texture and overall acceptability of bars with variation in ingredient levels.

Proximate principles *i.e.,* moisture, crude protein, crude fat, crude fibre, carbohydrate, total mineral matter (ash), energy and micronutrient (minerals) composition of mixes were analyzed and results of are represented in Table 4. The results for the analysis of macronutrients such as moisture, protein, fat, crude fibre, carbohydrate, energy and ash of developed foxtail millet composite mix were 5.75 g, 19.53 g, 5.95 g, 3.45 g, 65.28 g, 392.79 Kcal and 3.53g / 100g, respectively. The micronutrient composition of composite mix, was 58.95 mg calcium, 70.57 mg magnesium, 2.56 mg iron, zinc

TABLE 4

Nutrient composition of the foxtail composite mix per 100g on dry weight basis

Nutrient	Composition
Proximate (g)	
Moisture	5.71
Protein	19.53
Fat	5.95
Ash	3.53
Crude Fibre	3.45
Carbohydrates	65.28
Energy (K cal)	392.79
Minerals (mg)	
Calcium	58.95
Magnesium	70.57
Potassium	278.27
Iron	2.56
Zinc	1.59

Note: Values are mean of three replications

1.59 mg and 278.27 mg potassium per 100 g, respectively. Study conducted by Shilpa (2013) reported higher moisture (11.00 g), slightly lower protein (18.10 g), fat (5.70 g), crude fibre (3.47 g), carbohydrate (59.00 g), energy (359 Kcal) and ash (3.19 g) and high calcium (107.10 mg), iron (5.66 mg) and zinc (2.03 mg) per 100g. Study conducted by Shobha *et al.* (2019) have reported the similar results for the macronutrients and micronutrients. Nutritional characteristics of grain based composite mixes developed by Teradal (2013) also showed 15.2g moisture, 18.3g protein, 5.2g fat, 5.9g crude fibre, 48.3g carbohydrate, 313 Kcal energy and 6.8g ash per 100g of the sample. The results on proximate composition from the study conducted by Twinomuhwezi *et al.* (2020) showed that the moisture content ranged from 5.99 to 9.82 per cent. The ash content ranged from 1.01 to 2.83 per cent. the fat content ranged between 0.77 to 8.96 per cent followed by theprotein content ranged between 6.91 to 18.17 per cent, carbohydrate content ranged between 67.1 to 81.58 per cent; the total energy ranged between 363.95 kcal to 413.94 kcal and fiber content ranged between 0.99 per cent to 1.12. The differences in value of the foxtail millet composite mix is mainly due to processing techniques, drying temperature and genetic variation of the grains. However, all the mixes reviewed in the present investigation for protein showed very good percentage. Therefore, composite mixes could be used as nutridense products and consumed any time of the day.

Storage of any product determines its wholesomeness during the definite period of time (Shobha *et al.,* 2011). It is evident from the Table 5, that the microbial quality of the mix is good as there were no fungal and mould colonies throughout the mentioned storage period. Similar results were found from the study carried out by Shobha (2019) where very few bacterial colonies were found after four months of storage.

TABLE 5

Microbial load of foxtail millet composite mix

Period of microbial analysis	Fungi Cfu/ml of sample (10^{-5})	Bacteria Cfu/ml of sample (10^{-3})	Moulds Cfu/ml of sample(10^{-5})
Initial (0)	Nil	Nil	Nil
15 days	Nil	Nil	Nil
1 month	Nil	Nil	Nil

Changes in microbiological parameters of probiotication of foxtail millet composite mix by *L. acidophilus* influenced by different inoculums level (%) are given in Fig. 5. Inoculum concentration of 8 per cent gave the good LAB population of 3.21 × 10^8 CFU / ml. Similar observations were found in study

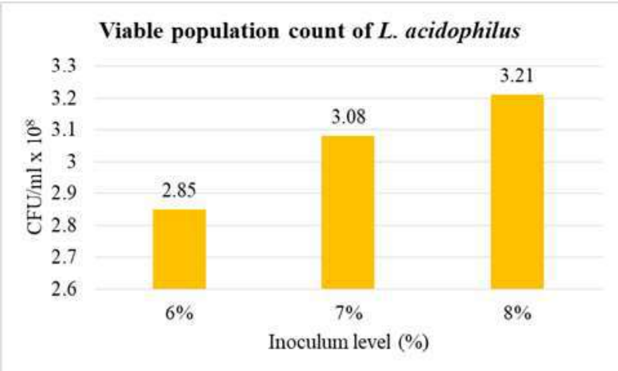


Fig. 5 : Viable population count of *L. acidophilus* in the probioticated foxtail millet composite mix

conducted by Fasreen *et al.* (2017) and reported 2.21 x 10^8 to 7.0 x 10^7 CFU / ml from 4 hrs to 6 weeks of storage study. Study conducted by Shilpa (2013), recorded the viability count for *Lactic acid bacteria i.e.,* 7.33 x 10^7 to 0.61 x 10^7 CFU / ml from initial day to 60 days of storage period in the probiotic composite mix. Therefore, developed composite could serve as a better substrate for development of *L. acidophilus* population without dairy ingredients.

From the present study it could be concluded that developed foxtail millet composite mix has got good source of protein, crude fibre and minerals. It has got good storage quality without any microbial spoilage. Thus, considering from its physical, functional, nutrient composition and microbial storage, the composite mix can be used for the probiotication by using suitable probiotic microbial strains. This mix would serve as a best substrate without dairy ingredients to produce economically viable probiotic mix. This could be an alternative product for consumers with lactose intolerance problem.

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