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Development of kodo millet based functional milk beverage

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Abstract
Kodo millet based functional milk beverage was standardized.100g of kodo millet was cleaned to remove foreign substances and were steeped in excess water for 12 hours and allowed to sprout for 48 hours. To the sprouted grains water was added in the ratio of 1:7 and milk was extracted. Sugar 10% and cardamom 0.1 % were added as a flavouring substance. The Total Soluble Solids were increased to 15° brix. Similarly, milk was extracted from the millet without malting. The millet milk was assessed for their physical characteristics, nutritional characteristics and organoleptic characteristics. The kodo millet-based milk beverage has TSS (15º brix), acidity (0.86), starch (5.73 g%), total sugar (3.26 g%), reducing sugar (1.79g%) and protein (1.75 g%) contents / 100 g. The product cost is Rs. 25/ 200 ml. The shelf life of the kodo millet milk has shelf life of 3 months. Sprouting of millet increased the milk yield with less viscosity and minimum sedimentation. In sensory characteristics, it scored highest values in all aspects like appearance, colour, flavour, consistency, taste and overall acceptability. There was no detectable pesticide residue. In the microbial load, the colony forming unit was1.45± 0.17 (log10^{-2}). The shelf life of the kodo millet beverage was 3 months at refrigerated conditions.

Keywords: Kodo millet, sprouting, milk beverage, storage studies, shelf life

Introduction

Plant-based milk alternatives are fluids that results from breakdown (size reduction) of plant material (cereals, pseudo-cereals, legumes oilseeds, nuts) extracted in water and further homogenisation of such fluids, results in particle size distribution in range of 5–20 µm which imitates cow's milk in appearance and consistency. Recently, demands by consumers for vegetable milk have experienced a noticeable increase due to some problems of milk protein allergenicity and healthy life (Donkor *et al*., 2007) [5]. Considerable attention has been given to soy and almond milk due to their good nutritional value and functionality (Watkins, 2005) [13]. There is no millet milk based functional beverage is available. Kodo millet is a nutritious grain and a good substitute to rice or wheat. millet is an excellent source of fiber (9%), as opposed to rice (0.2%), and wheat (1.2%). Kodo millet contains 66.6g of carbohydrates and 353 kcal per 100g of grain, comparable to other millets. It also contains 1.4% fat and 2.6% minerals. The iron content in kodo millet ranges from 25.86ppm to 39.60ppm (Chandel *et al*., 2014) [4]. Kodo millet flour has a gelatinization temperature range of 13°C (76.6-90°C), which has less resistant to gelatinization (Shinoj *et al*., 2006) [12]

Millets have good grain qualities suitable for Processing of the grain for many end uses involves primary (Wetting, dehulling and Milling) and secondary (fermentation, malting, Extrusion, glaking, popping and roasting) Operations. The emerging principal uses of millets as an industrial raw material include production of biscuits and confectionery, beverages, weaning foods and beer. Germination or malting of cereal grains may result in some biochemical modifications and produce malt with improved nutritional quality that can be used in various traditional recipes. It has been found that germination appreciably improved the *in vitro* protein (14% to 26%) and starch (86% to 112%) digestibility and bio accessibility of minerals. Based on this, the study was carried out. Traditional technologies such as decortication, soaking, germination and fermentation of cereal-based foods reduce the levels of tannins and phytates, increase bioavailability of amino acids and mineral elements and improve protein and starch digestibility. Based on these the study was conducted.

Materials and Methods
The materials like kodo millet, sugar, and cardamom and muslin cloth were purchased from the local departmental store, Coimbatore.
The kodo millets (100g) were cleaned to remove foreign substances and were steeped in excess water for 12 hours to achieve uniform moisture content. At the end of the steeping period, the grains were washed and the excess water was removed. The kodo millet was placed separately in muslin cloth, tied and allowed to sprout for 36-48 respectively with intermittent moistening to prevent dehydration. Malting was carried out at room temperature. To the sprouted grains water was added in the ratio of 1:7 and milk was extracted. Sugar (10%) and cardamom (0.1%) were added as a flavoring substance. The Total Soluble Solids were increased to 15° brix. Then homogenized, heated and bottled in glass as well as PET bottles. This is denoted as T1.The glass bottles were pasteurized, cooled and kept for storage at room (R1)and refrigerated(R2) conditions. Similarly, milk was extracted from the millet without malting (T2). These samples kept for storage. During storage the physico chemical, sensory characteristics, microbial load, pesticide residue analysis were done.

Morphology of millet grains
The morphology millet granules were evaluated by scanning electron microscope (SEM) (QUANTA FEG 250 ESEM) before and after sprouting. Samples were mounted on circular aluminum stubs with double-sided sticky tape. The particle granules were evenly distributed on the surface of the tape. Then the samples were coated with 12 nm gold, examined and photographed at an accelerating voltage of 5 kv with a magnification of x5000.

Nutrient analysis
The proximate analysis of the millet based beverage was analysed by AOAC method (2000) [2].

Sensory Analysis
The sensory characteristics were analysed using 9 point hedonic scale as per the method by Watts *et al*., (1989) [14].

Pesticide Residue analysis
Pesticide Residue Analysis **was** done as per the procedure of MPRNL New method – DB5 (STD) 060919, gcm.

Microbial load (TPC)
The total plate count was done as per the method - ISO 4833:2003 (E).

Statistical analysis
Data were analyzed using Data Entry Module for Agres Statistical Software (Version 3.01) developed by Tamil Nadu Agricultural University, Coimbatore. The data obtained from the various experiments were performed in triplicates and were expressed as mean ± standard deviation and to find out the significance between different treatments using Factorial Completely Randomized Design (FCRD) methods described by Gomez and Gomez (1984) [8].

Results and Discussion
Morphology of millet granules
Morphology of the raw and sprouted millet grains given the fig 1 & 2.

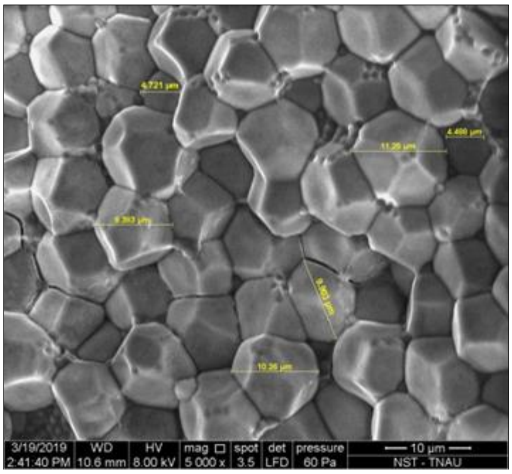

Fig 1: Structure of raw millet grain

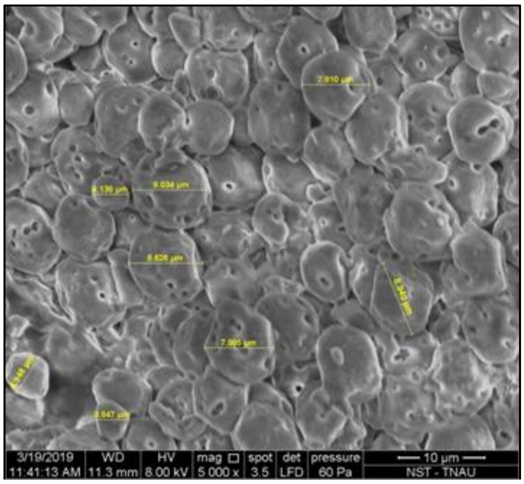

Fig 2: Structure of malted millet grain

The shape of the sprouted kodo millet grain varies like oval, round and hexagon (Fig. 2). The shape of particles size in kodo millet appeared as different geometrical shapes where as shape of the raw millet grain varies like hexagon (Fig. 1). Moreover, the surfaces of the sprouted millet starch granules appeared to be smooth. The mean granule size of raw millet starch molecule (10.47µm), was bigger than the malted millet granule (5.641µm). The pitted granules showed the amylase activity. Similar studies were observed by Pushpa Devi and Narayanaswamy Sangeetha (2013) [7] in maida and composite millet powders.
These structures are also found in broomcorn starch, millet starch, wheat starch, and barley starch. The pores in the starch can be generated during either granule formation or the starch isolation process. The image on the first day of germination was not largely different from the images on day 0 (B). As the germination time progressed, the maize starch granules obviously changed (Xianhong Ma *et al*., 2020) [15]. The present sudy was on par with the results.

Extraction of milk from the raw and sprouted millet
The milk yield was better in sprouted kodo millet (475 ml) than the raw millet which is only 400 ml, which was shown in Table 1.

Table 1: Extraction of millet milk

S. No	Particulars	Grain wt (g)	Grain: water	Germinated yield (g)	Milk Yield (ml)
1	Raw Kodo millet	100	100:700	-	400
2	Sprouted Kodo millet	100	100:700	160	475

Shelf life

The control sample had a shelf life of one day. The pasteurised millet milk had a shelf life up to 4 days without any spoilage at room temperature. The refrigerated samples had a shelf life of three months.

Table 2: Physico-chemical parameters of Kodo Millet milk beverage

S. No.	Nutrients	Refrigerated Kodo Millet milk(raw) beverage (R2 T1)	Refrigerated Kodo Millet milk (sprouted) Beverage (R2 T2)
1.	Protein (g%)	1.71^a ±0.144	1.75^a ±0.149
2.	Starch (g%)	6.01^a ±0.15	5.73^a ±0.13
3.	Reducing sugar (g%)	1.36^a ±0.07	1.79^a ±0.12
4.	Total Sugar (g%)	3.82^a ±0.16	3.26^a ±0.17
5.	Fat (g%)	1.22^a ±0.078	1.21^a ±0.074
6.	Calcium (mg%)	1.05^a ±0.05	1.63^a ±0.26
7.	T.S.S °brix	15^a ±0.10	15^a ±0.10
8.	Acidity	0.74^a ±0.07	0.86^a ±0.09

All means are based on triplicate value. Means with different letters in each column differ highly significantly $P< 0.05$*: wet weight basis.

Physical Characteristics of the millet milk

The physico - chemical parameters of kodo milletis projected in Table.2.

During storage there was no significant difference during the storage days. In the case of protein, there was slight increase in protein content of sprouted millet milk beverage (1.75%). This is because of sprouting. Malting is a traditional processing technology is used to improve the nutritional quality of protein. This could be attributed to a synthesis of enzymatic proteins by germinating seed. Similar results were observed in the Aminat O. Adelekan *et al*., (2013) [1] in malted traditional beverage (Soy – Kunnu zagi)

Malting also had significant effect ($p < 0.05$) on the carbohydrate and fats content of the samples. The carbohydrate and fats content of the unsprouted samples was higher than that of the sprouted samples, this was as a result of fats and carbohydrate used as energy for respiration or metabolic activities during malting and this is in accordance with Sade, (2009) [11]. Ocheme and Chinma (2008) [10] reported that malting decrease fat contents of cereals. Due to pasteurization of millet milk all the enzymes are inactivated no off flavour was found in the milk. Similarly the total sugar content is reduced in sprouted sample (3.26%) compared to raw millet milk samples. This due to malting process the amylase acted upon the sugars and converted in to simpler forms.

During germination, vital kernels activate and synthesize endogenous enzymes after water absorption and a requisite period of respiration. These enzymes subsequently promote the exit of the kernel capsules and the exposition of the kernel roots from the kernel embryos. Following the activity of the endogenous enzymes during germination, macromolecules (e.g., proteins, fats, carbohydrates) are decomposed and nutrients (e.g., vitamins, minerals) are released, thereby causing changes to kernel composition. Moreover, the effects of germination on starch digestion decreased the resistant starch. The results of the aforementioned studies imply that germination moderately affects grain starch digestion. The beverage derived from the malted millets had higher protein digestability. pH of the raw millet milk sample is 0.74 while the pH of the sprouted millet milk sample is 0.86 making it slightly acidic. The results were on par with the studies conducted by Ashiru *et al*., 2003 [3]. The acidity of the beverage has been noted to be a result of lactic acid production by some bacteria during fermentation, which is not affecting the sensory qualities of the beverage.

Table 3: Sensory evaluation of kodo millet milk beverage

Quality attributes	Kodo Millet milk beverage (raw)	Kodo Millet milk beverage (sprouted)
Appearance	7.02^a ±0.22	8.07^a±0.26
Colour	6.50^a±0.21	6.75^a±0.16
Flavour	7.02^a±0.27	8.13^a±0.32
Texture	7.29^b±0.26	8.00^a±0.22
Taste	7.20^a±0.21	8.00^a±0.33
Over all acceptability	7.12^a±0.20	7.87^a±0.35

All means are based on triplicate value. Means with different letters in each column differ highly significantly $P< 0.05$*: wet weight basis.

Sensory evaluation of kodo millet beverages

Table 3 showed the sensory evaluation results. The sensory evaluation showed that sprouted kodo millet milk beverage was good in all characteristics (7.87) even though the color was slightly dull, appreciated like chocolate drink. Modha and Pal (2014) [9] reported that fermented pearl millet based milk beverage has sensorily acceptable quality and shelf-life of 7 days without any preservative at refrigerated storage (5–7°C) when packed in glass bottles. The results were on par with the present study.

Table 4: Microbial Load in the kodo millet milk beverage

S. No	Particulars	Kodo Millet milk beverage (raw)	Kodo Millet milk beverage (sprouted)
1.	Total Plate Count (log 10^2cfu/g)	1.27± 0.10	1.45± 0.17

Microbial load

The table 4 showed that the microbial load of the raw millet drink had lesser (1.27 cfu/g) than the and sprouted millet milk beverage (1.45 cfu/g). This may be due to the fermentation total plate count is increased. A study conducted by Nor' Aishah and binti Hasan (2012) showed that Total counts and Micro coccacceae were reduced by 2.21 and 3.64 log cycles at pasteurized almond milk while no viable Enter obacteriaceae nor yeast and mould remained in any pasteurized milk samples. In this study also no yeast and mould were present in the millet milk.

Pesticide Residue Analysis (MPRNL New method – DB5 (STD) 060919, gcm) showed (Fig.3) that there were no detectable pesticide residues in the sample.

Studies on the Magnitude of Residues in Processed Commodities provide data on the transfer of residues to different processed commodities from the raw agricultural commodity. Studies on the magnitude of residues are conducted, typically using industrial or simulated industrial processes, in order to quantify levels of residues in processed commodities and to provide the distribution of residues (active ingredient, and/or metabolites, degradation products) in various processed products resulting from the processing of a commodity. This information about dilution and concentration of residues and the estimation of processing factors (the ratio of residue levels in processed commodities to those in the raw agricultural commodity (RAC)) is used to conduct refined dietary exposure assessments with primary processed products to assess consumer safety; provide results on residues in commodities that may be used as animal feedstuffs and thus to allow a more realistic calculation of the dietary burden of livestock; establish MRLs for processed commodities; and monitor compliance with the RAC MRL. Processing guidance applies to raw agricultural commodities of plant origin. It also applies to RACs of animal origin in cases of direct animal treatment or veterinary use. Applicability of studies on the magnitude of residues in processed commodities depends upon the importance of a processed product in the human and/or animal diet; the possibility of residue levels in processed foods/feeds exceeding the level in a RAC; the level of residue in the plant or plant product to be processed (RAC); the physical-chemical properties of the active ingredient or relevant metabolites; and the possibility that degradation products of toxicological significance may be found after processing of the plant or plant product. (ENV/JM/MONO, 2009)

Conclusion

The sprouted millet milk beverages contained 1.25% protein, 5.73 % starch, 1.79% reducing sugar, 3.26 % Total sugar, 1.21 % fat, 1.63 % calcium, 15° brix TSS and 0.86 acidity. The average overall acceptability of the beverage was 7.86. The present study revealed the feasibility of the sprouted millet milk beverage based on the nutrient analysis and sensory evaluation. The method of processing is very simple and this can be taken up for industrial production

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