



## Optimization of the Process Parameters for Extraction of Millet Milk

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### Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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### ABSTRACT

This study was undertaken to evaluate the effect of different processing methods on extraction of millet milk viz., germinated millet milk extraction, germinated and roasted millet milk extraction; enzyme assisted millet milk extraction and ultrasonicated millet milk extraction. Physical parameters viz., sedimentation rate, separation rate, whiteness index, heat stability and viscosity of extracted millet milk by different processing methods were evaluated. Among the various processing methods, enzyme assisted extraction showed better results as compared to other processing methods in terms of sedimentation rate ( $0.93 \pm 0.07$  to  $1.13 \pm 0.05$  g/40 ml), separation rate ( $45.28 \pm 1.44$  to  $51.97 \pm 0.14$  ml/h), viscosity ( $2.32 \pm 0.02$  to  $2.82 \pm 0.03$  cP) and heat stability ( $24.7 \pm 0.13$  to  $21.2 \pm 0.51$  minutes). The whiteness index was found to be maximum in germinated

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millet milk extraction while the enzyme treated millet milk recorded the lowest value (40.48±0.71 to 62.81±0.64%). Based on heat stability and sedimentation rate, the enzyme assisted millet milk extraction was found to be best for extraction of millet milk and for product development.

**Keywords:** Millets; plant based milk substitute; extraction methods; sedimentation.

## 1. INTRODUCTION

The demand for vegetarian diet is increasing all over the world due to the concern over health and environmental issue, which promotes the development of plant-based milk substitutes [1]. In ancient times, most of the plant-based milk substitutes like soymilk and finger millet based (ragi milk) milk are prepared at home on a very small scale in order to provide it to for the family or on commercial scale for the benefit of the community [2].

Plant-based milk substitutes can be water extracts of dissolved and disintegrated plant materials/edible parts/seeds. These are either soaked or wet milled or the raw material is dry milled and the flour is extracted in water afterwards. Often, the prepared slurry is filtered or decanted, to remove the ground waste and insoluble plant materials. The filtrate thus obtained can be consumed as plant-based milk substitutes [3].

Milk alternatives based on plant homogenates have increased on popularity in recent years because of the perceived nutrient content [4]. The plant-based milk substitutes can be sourced from cereals, pulses, nuts, oilseeds, pseudo cereals etc. The cereal based milk includes oat milk, rice milk and corn milk while pulse based milk includes soymilk, peanut milk and chickpea milk. Nuts based milk substitutes are very common and can be derived from almond, coconut and hazelnut. While seeds such as hemp seeds are used for plant milks, the pseudo cereals such as quinoa are also used as sources for plant milk [5,6,7,8,9].

Millets are considered valuable food crops because they can grow under adverse agro ecological conditions like limited rainfall, poor soil fertility. They can also be stored for more extended periods without any insect and pest damage. They also have the potential to expand the diversity of food baskets among consumers and ensure nutritional and food security [10].

Millets are important food sources since they contain a significant quantity of carbohydrates

(60–70%), protein (7–11%), fats (1.5–5%), dietary fibers (2–7%), B vitamins and minerals especially calcium, iron, zinc and iodine. In addition, millets are low in saturated fats and high in bioactive and antioxidant compounds, which help in maintaining positive health by preventing the onset of non-communicable diseases like obesity, cardiovascular disease, inflammatory disease and diabetes mellitus. [11]. When considering these factors, millets stand to be a good source for development of plant-based milk substitutes due to the presence of numerous water-soluble nutrients [12].

Simultaneously, millet extract is a colloidal system produced by disseminated particles such as proteins, starch granules, and some other solid particles. Due to this reason, it is very hard to produce a stable product without settling particles. Creaming and sedimentation of solid particles can guide the product towards quality loss [13] and heating of extracted millet milk at high temperature leads to gelatinization that results in development of high viscous product. Furthermore, the processing conditions cause changes in the arrangement of components, thus leading to changes in color, particle size, physical stability, and viscosity.

In the view of above mentioned drawbacks, the present study is focused to optimize the process for extraction of millet milk with special reference to reduced sedimentation and viscosity and to develop a product with increased acceptability.

## 2. MATERIALS AND METHODS

Barnyard millet (*Echinochloa esculenta*), little millet (*Panicum miliare*), kodo millet (*Setaria italica*) and finger millet (*Eleusine coracana*) were purchased from local market in Madurai, Tamil Nadu.

### 2.1 Germination of Millet Grains

Millets were cleaned and rinsed in tap water twice and soaked in water. Soaking time was optimized as 18 h, 13 h, 20 h, 16 h respectively for barnyard millet, little millet, kodo millet and

finger millet based on its water absorption capacity. The soaked grains were drained, and tied tightly with muslin cloth and kept in dark for germination. The germination process was carried out at ~25°C for about 24 h for all millets (germination time for all millets were standardized based on shoot length). The germinated millet grains were dried at 45°C for 8 h to obtain the final moisture content of about 7 - 8%.

## 2.2 Millet Milk Extraction Methods

### 2.2.1 Germinated millet milk extraction (GMM)

Germinated millets and water were mixed at different ratios 1:4, 1:5 and 1:6 w/v. The millet to water mixture was ground for 20 min using mixer grinder. Then, the millet milk slurry was filtered through double layered muslin cloth to obtain millet milk. The filtered millet milk from various millets were heated at 85°C for 10 min [14] and stored at refrigerated temperature (4°C) for further analysis.

### 2.2.2 Germinated and roasted millet milk extraction (GRMM)

Germinated millets were roasted at 180°C for 5 min and the roasted grains were ground for 20 min using food processor with water at different ratios 1:4, 1:5 and 1:6 w/v. The resulted millet milk extract was filtered to obtain final product. The obtained millet milks from different millet grains were pasteurized at 85°C for 10 min and stored at refrigerated temperature (4°C) for further analysis.

As the sedimentation rate was lower in 1:6 ratio of millet and water, it was finally taken for enzyme assisted and ultrasonication assisted millet milk extraction.

### 2.2.3 Enzyme assisted millet milk extraction (EMM)

Germinated millets and water were taken at the ratio of 1:6 to make millet milk. The millet and water mixture were ground for 20 min using a food processor. The milk extracts were filtered and filtrates were added with heat stable alpha amylase enzyme at 0.25, 0.5 and 1.0% concentration. Enzyme added millet milks were incubated at 75°C for 60 min [5]. After incubation, the enzyme was inactivated by heating the millet milks at 100°C for 15 min. The obtained product was pasteurized at 85°C for 10

min and stored at refrigerated temperature (4°C) for further analysis.

### 2.2.4 Ultrasonication assisted millet milk extraction (UMM)

The soaked and germinated grains were dried at 60°C for 8 h and the dried grains were pulverized. The pulverized flour was sieved through BS 60 micron sieve. Millet flour to water was taken at 1:6 ratio and the slurry was processed by ultrasonicator fitted with 13 mm probe. Probe was put 2– 3 cm below the surface of millet and water slurry. Then, the millets and water slurry was ultrasonicated at a constant frequency of 20 kHz and power level of 100 watts for the periods of 10, 20 and 30 minutes. The resultant millet milks were stored at refrigerated temperature (4°C) for further analysis.

## 2.3 Determination of Physical Quality Parameters

### 2.3.1 Sedimentation index

Sedimentation rate was determined through phase separation analysis using analytical centrifuge. The samples were centrifuged at 1000 rpm for 30 min. Then, weights of sediment were determined and expressed as g/volume of centrifuge tube [14].

### 2.3.2 Separation rate

Separation tests were performed by filling 100 mL graduated cylinders with 100 mL of each milk type; foam was not included in the volume, so the foam rested above the 100 mL mark. Each sample was tested at ambient temperature (23 to 25°C). The millet milks in the graduated cylinders were observed for 1 h and recorded the separation layer after an hour. Measurements were read from the top of the cylinder [15].

### 2.3.3 Whiteness index

The colour values of developed millet milks were obtained from Hunter colour lab meter. L\*, a\*, and b\* values were recorded and whiteness indices of millet milk samples were calculated by the formula given by Manzoor et al. [16].

### 2.3.4 Heat stability

For the determination of heat stability, 100 mL of samples were placed in beaker and sealed with aluminum foil. Beakers were immersed in a water bath which was thermostatically controlled at

75°C. The heat coagulation time was examined visually and taken as the time in minutes that elapsed between placing the sample in the water bath and the onset of coagulation [2].

### 2.3.5 Viscosity

Viscosity of samples were analysed by Brooke field viscometer using spindle no 62 at 100rpm. 500 ml of millet milks were heated at 75°C were placed in beaker for viscosity analysis. values (cP) were recorded after 30 s for 3 times. The sample holder was cleaned after each reading and values were recorded [17].

## 3. RESULTS AND DISCUSSION

The effect of processing methods on physical parameters like sedimentation rate, separation rate, whiteness index, heat stability and viscosity of barnyard millet milk, little millet milk, kodo millet milk and finger millet milk was analysed and presented in Tables 1 to 4. Among the various physical parameters, sedimentation rate and heat stability was considered for optimizing the best method for extraction of millet milk.

Sedimentation rate of barnyard millet milk varied from 0.98 to 12.26 g/40 ml. The lowest sedimentation rate was observed in 1.0% enzyme treated sample. Significant difference ( $P < 0.01$ ) was found among the different processing methods whereas no significant difference was found between 0.5 and 1.0% enzyme concentration. Rosenthal et al. [18] evaluated the effect of enzymatic treatment and filtration on physical stability of soymilk. Cellulase enzyme treated soymilk had good physical stability index of 0.99% than filtered soymilk (1.00%) As like soymilk, enzyme treated millet milk showed less sedimentation rate than other methods.

A beverage emulsion may contain a number of constituents that partition into different phases within the product [19]. A separation layer on the millet milk is undesirable because it would lead to consumer rejection. The highest separation value of  $72.61 \pm 1.72$  ml/h of barnyard millet milk was recorded in ultrasonicated millet milk and lowest separation of  $46.85 \pm 1.14$  ml/h was found in enzyme treated barnyard millet milk.

Whiteness index of the developed barnyard millet milks were in the range of  $64.93 \pm 0.68$  to

$52.73 \pm 0.93\%$ . The highest whiteness index was observed in germinated millet milk extraction ( $64.93 \pm 0.68$  to  $64.29 \pm 1.60\%$ ) followed by germinated and roasted millet milk extraction ( $62.23 \pm 0.88$  to  $61.97 \pm 2.02\%$ ), ultrasonication assisted millet milk extraction ( $58.75 \pm 0.11$  to  $57.96 \pm 0.63\%$ ) and enzyme assisted millet milk extraction ( $56.34 \pm 0.69$  to  $52.73 \pm 0.93\%$ ). Whiteness index of lentil based milk substitute processed by aqueous extraction method was  $46.27 \pm 0.40\%$  which is lower compared to whiteness index of barnyard millet milk [2].

Heat stability of millet milk is an essential parameter which defines time taken for coagulation of millet milk. The millet milk will get gelatinized during pasteurization at 75°C if the heat stability of millet milk is low. Hence, heat stability of millet milk should be high in order to ensure heating and thereby pasteurization. In the present study, heat stability of millet milks processed by various methods was in the range of  $7.1 \pm 0.06$  to  $24.3 \pm 0.63$  minutes. Enzyme treated millet milk (EMM) had highest heat stability (24.1 to 24.3 minutes) due to the addition of alpha amylase enzyme. Added enzyme converts the starch to sugars thereby preventing the onset of gelatinization and increasing the heat stability [5]. In case of GMM, GRMM, UMM methods, heat stability of millet milks was 6.3 to 14.1 minutes.

Viscosity of barnyard millet milks developed by various processing methods was in the range of  $2.67 \pm 0.50$  to  $21.60 \pm 0.58$  centipoise. The highest viscosity (21.60cp) was found in germinated millet milk which is due to gelatinization of starch at 75°C. The lowest viscosity was observed in enzyme treated millet milk (2.67cP). Jiang et al. [17] evaluated the viscosity of short time germinated soy milk and that was found that 2.55cP. The result was similar to the results of enzyme treated millet milk. The higher moisture content and action of enzyme making the millet milk diluted could be a reason contributing to the reduced viscosity.

Sedimentation rate of little millet milk processed by various extraction methods were in the range of  $1.02 \pm 0.19$  to  $12.26 \pm 0.15$  g/40 ml. Among the various methods, enzyme treated milk had good stability in terms of sedimentation rate followed by germinated and roasted millet milk extraction, germinated millet milk extraction and ultrasonication assisted millet milk extraction.

**Table 1. Extraction of barnyard millet milk by various methods**

Extraction methods		Sedimentation rate (g/40 ml)	Separation rate (ml/h)	Whiteness index (%)	Heat stability (min)	Viscosity (cP)
GMM	1:4	7.1±0.20 <sup>f</sup>	71.37±0.19 <sup>c</sup>	64.93±0.68 <sup>a</sup>	7.1±0.06 <sup>g</sup>	21.60±0.58 <sup>h</sup>
(Millets to water ratio)	1:5	7.0±0.21 <sup>ef</sup>	71.83±2.34 <sup>c</sup>	64.78±1.41 <sup>a</sup>	7.1±0.01 <sup>g</sup>	21.53±0.24 <sup>h</sup>
	1:6	6.6±0.21 <sup>d</sup>	72.25±0.49 <sup>c</sup>	64.29±1.60 <sup>a</sup>	7.3±0.18 <sup>g</sup>	21.32±0.04 <sup>h</sup>
GRMM	1:4	6.8±0.16 <sup>de</sup>	63.15±2.10 <sup>b</sup>	62.23±0.88 <sup>b</sup>	8.2±0.20 <sup>f</sup>	20.43±0.68 <sup>g</sup>
(Millets to water ratio)	1:5	6.7±0.04 <sup>d</sup>	62.78±1.79 <sup>b</sup>	62.14±0.23 <sup>b</sup>	8.4±0.03 <sup>ef</sup>	20.17±0.59 <sup>g</sup>
	1:6	6.2±0.10 <sup>c</sup>	62.62±1.74 <sup>b</sup>	61.97±2.02 <sup>b</sup>	8.6±0.18 <sup>e</sup>	20.02±0.10 <sup>g</sup>
GEMM	0.25 %	3.14±0.06 <sup>b</sup>	47.21±0.54 <sup>a</sup>	56.34±0.69 <sup>d</sup>	24.1±0.66 <sup>a</sup>	4.80±0.01 <sup>b</sup>
(Enzyme concentration)	0.50 %	1.07±0.03 <sup>a</sup>	46.92±0.47 <sup>a</sup>	55.80±0.26 <sup>d</sup>	24.3±0.63 <sup>a</sup>	2.75±0.02 <sup>a</sup>
	1.0 %	0.98±0.04 <sup>a</sup>	46.85±1.14 <sup>a</sup>	52.73±0.93 <sup>e</sup>	24.2±0.19 <sup>a</sup>	2.67±0.50 <sup>a</sup>
GSMM	10 min	12.26±0.40 <sup>h</sup>	72.14±1.78 <sup>c</sup>	58.75±0.11 <sup>c</sup>	14.1±0.29 <sup>b</sup>	15.60±0.42 <sup>d</sup>
(Sonication time)	20 min	12.13±0.23 <sup>h</sup>	72.43±0.59 <sup>c</sup>	58.32±1.23 <sup>c</sup>	13.5±0.19 <sup>c</sup>	17.76±0.14 <sup>f</sup>
	30 min	11.54±0.03 <sup>g</sup>	72.61±1.72 <sup>c</sup>	57.96±0.63 <sup>c</sup>	13.0±0.03 <sup>d</sup>	18.92±0.77 <sup>e</sup>

GMM - Germinated millet milk, GRMM - Germinated and roasted millet milk, EMM - Enzyme treated millet milk and UMM - Ultrasonicated millet milk. Values are means of 3 replicates. Means in the same column followed by different superscripts are significantly different at  $P < 0.01$

**Table 2. Extraction of little millet milk by various methods**

Extraction methods		Sedimentation rate g/40 ml)	Separation rate (ml/h)	Whiteness index (%)	Heat stability (min)	Viscosity (cP)
GMM	1:4	10.8±0.11 <sup>f</sup>	74.80±2.13 <sup>c</sup>	69.81±1.58 <sup>a</sup>	6.3±0.01 <sup>g</sup>	25.20±0.05 <sup>h</sup>
(Millets to water ratio)	1:5	10.5±0.14 <sup>de</sup>	75.13±1.73 <sup>c</sup>	69.73±0.42 <sup>a</sup>	6.5±0.02 <sup>fg</sup>	24.91±0.13 <sup>g</sup>
	1:6	9.7±0.13 <sup>c</sup>	75.50±2.04 <sup>c</sup>	69.13±0.62 <sup>ab</sup>	6.7±0.14 <sup>f</sup>	24.74±0.14 <sup>g</sup>
GRMM	1:4	10.7±0.14 <sup>ef</sup>	66.92±1.00 <sup>b</sup>	68.32±0.92 <sup>ab</sup>	8.3±0.26 <sup>e</sup>	22.00±0.63 <sup>f</sup>
(Millets to water ratio)	1:5	10.4±0.07 <sup>d</sup>	66.43±0.63 <sup>b</sup>	67.85±2.21 <sup>ab</sup>	8.4±0.04 <sup>d</sup>	21.89±0.46 <sup>f</sup>
	1:6	9.5±0.30 <sup>c</sup>	66.14±0.49 <sup>b</sup>	67.71±0.36 <sup>b</sup>	8.7±0.07 <sup>d</sup>	21.78±0.48 <sup>f</sup>
GEMM	0.25%	3.25±0.04 <sup>b</sup>	52.13±0.07 <sup>a</sup>	61.77±1.93 <sup>d</sup>	26.2±0.35 <sup>a</sup>	3.47±0.05 <sup>b</sup>
(Enzyme concentration)	0.50%	1.13±0.05 <sup>a</sup>	51.97±0.14 <sup>a</sup>	61.38±1.50 <sup>d</sup>	26.1±0.33 <sup>a</sup>	2.32±0.06 <sup>a</sup>
	1.0%	1.02±0.19 <sup>a</sup>	51.92±1.09 <sup>a</sup>	60.79±0.04 <sup>d</sup>	26.2±0.10 <sup>a</sup>	2.01±0.04 <sup>a</sup>
GSMM	10 min	12.26±0.15 <sup>h</sup>	75.64±0.71 <sup>c</sup>	65.75±1.23 <sup>c</sup>	14.2±0.40 <sup>b</sup>	11.60±0.18 <sup>c</sup>
(Sonication time)	20 min	12.13±0.14 <sup>h</sup>	75.83±1.13 <sup>c</sup>	65.32±0.95 <sup>c</sup>	13.1±0.03 <sup>c</sup>	13.76±0.25 <sup>d</sup>
	30 min	11.54±0.31 <sup>g</sup>	76.02±2.06 <sup>c</sup>	64.96±1.89 <sup>c</sup>	13.2±0.12 <sup>c</sup>	16.59±0.43 <sup>e</sup>

GMM - Germinated millet milk, GRMM - Germinated and roasted millet milk, EMM - Enzyme treated millet milk and UMM - Ultrasonicated millet milk. Values are means of three replicates. Means in the same column followed by different superscripts are significantly different at  $P < 0.01$

**Table 3. Extraction of kodo millet milk by various methods**

Extraction methods		Sedimentation rate (g/40 ml)	Separation rate (ml/h)	Whiteness index (%)	Heat stability (min)	Viscosity (cP)
GMM	1:4	9.96±0.18 <sup>f</sup>	70.63±2.16 <sup>c</sup>	52.85±1.08 <sup>a</sup>	6.0±0.25 <sup>f</sup>	20.40±0.52 <sup>g</sup>
(Millets to water ratio)	1:5	9.52±0.17 <sup>de</sup>	70.92±2.36 <sup>c</sup>	52.43±1.60 <sup>a</sup>	6.2±0.18 <sup>f</sup>	20.31±0.67 <sup>g</sup>
	1:6	9.34±0.05 <sup>cd</sup>	71.28±0.63 <sup>c</sup>	52.41±0.28 <sup>a</sup>	6.2±0.11 <sup>f</sup>	20.25±0.55 <sup>g</sup>
GRMM	1:4	9.73±0.19 <sup>ef</sup>	62.75±0.85 <sup>b</sup>	52.13±0.38 <sup>a</sup>	7.1±0.09 <sup>e</sup>	19.20±0.43 <sup>f</sup>
(Millets to water ratio)	1:5	9.45±0.11 <sup>d</sup>	62.34±1.90 <sup>b</sup>	52.06±0.74 <sup>a</sup>	7.1±0.13 <sup>e</sup>	19.06±0.32 <sup>f</sup>
	1:6	9.18±0.21 <sup>c</sup>	62.06±1.68 <sup>b</sup>	51.86±0.42 <sup>a</sup>	7.2±0.47 <sup>f</sup>	18.82±0.34 <sup>f</sup>
GEMM	0.25%	4.17±0.05 <sup>b</sup>	46.69±1.27 <sup>a</sup>	41.16±0.47 <sup>d</sup>	21.2±0.51 <sup>b</sup>	2.61±0.01 <sup>b</sup>
(Enzyme concentration)	0.50%	1.02±0.02 <sup>a</sup>	45.52±1.08 <sup>a</sup>	40.48±0.71 <sup>d</sup>	22.1±0.05 <sup>a</sup>	2.44±0.13 <sup>ab</sup>
	1.0%	1.01±0.17 <sup>a</sup>	45.41±1.26 <sup>a</sup>	40.12±0.04 <sup>d</sup>	22.2±0.38 <sup>a</sup>	1.97±0.24 <sup>a</sup>
GSMM	10 min	14.64±0.40 <sup>h</sup>	71.87±0.59 <sup>c</sup>	47.74±0.34 <sup>b</sup>	12.4±0.02 <sup>c</sup>	11.73±0.15 <sup>c</sup>
(Sonication time)	20 min	14.45±0.03 <sup>gh</sup>	71.93±1.51 <sup>c</sup>	45.63±0.22 <sup>c</sup>	11.5±0.07 <sup>d</sup>	14.81±0.20 <sup>d</sup>
	30 min	14.31±0.23 <sup>g</sup>	72.05±0.93 <sup>c</sup>	45.52±0.37 <sup>c</sup>	11.4±0.21 <sup>d</sup>	16.25±0.22 <sup>e</sup>

GMM - Germinated millet milk, GRMM - Germinated and roasted millet milk, EMM - Enzyme treated millet milk and UMM - Ultrasonicated millet milk. Values are means of 3 replicates. Means in the same column followed by different superscripts are significantly different at  $P<0.01$

**Table 4. Extraction of finger millet milk by various methods**

Extraction methods		Sedimentation rate (g/40 ml)	Separation rate (ml/h)	Whiteness index (%)	Heat stability (min)	Viscosity (cP)
GMM	1:4	12.7±0.22 <sup>f</sup>	70.26±2.24 <sup>c</sup>	70.24±0.91 <sup>a</sup>	5.1±0.05 <sup>g</sup>	26.40±0.37 <sup>d</sup>
(Millets to water ratio)	1:5	12.6±0.11 <sup>f</sup>	70.83±1.20 <sup>c</sup>	70.17±0.52 <sup>a</sup>	5.3±0.03 <sup>g</sup>	26.31±0.80 <sup>d</sup>
	1:6	12.1±0.25 <sup>de</sup>	71.58±1.12 <sup>c</sup>	70.04±0.33 <sup>a</sup>	5.3±0.02 <sup>g</sup>	26.18±0.65 <sup>d</sup>
GRMM	1:4	12.4±0.13 <sup>f</sup>	64.79±0.52 <sup>b</sup>	70.21±0.90 <sup>a</sup>	6.2±0.13 <sup>f</sup>	25.39±0.57 <sup>c</sup>
(Millets to water ratio)	1:5	12.0±0.04 <sup>ce</sup>	64.52±1.09 <sup>b</sup>	70.02±2.04 <sup>a</sup>	6.4±0.15 <sup>f</sup>	25.18±0.17 <sup>c</sup>
	1:6	11.7±0.01 <sup>cd</sup>	64.19±0.17 <sup>b</sup>	69.93±0.71 <sup>a</sup>	6.5±0.12 <sup>f</sup>	25.03±0.30 <sup>c</sup>
GEMM	0.25%	4.03±0.06 <sup>b</sup>	45.61±0.18 <sup>a</sup>	63.46±1.69 <sup>b</sup>	23.6±0.18 <sup>c</sup>	2.99±0.04 <sup>a</sup>
(Enzyme concentration)	0.50%	0.93±0.07 <sup>a</sup>	45.28±1.44 <sup>a</sup>	62.81±0.64 <sup>b</sup>	24.5±0.33 <sup>a</sup>	2.82±0.70 <sup>a</sup>
	1.0%	0.92±0.22 <sup>a</sup>	45.03±0.18 <sup>a</sup>	62.53±0.18 <sup>b</sup>	24.1±0.13 <sup>b</sup>	2.43±0.32 <sup>a</sup>
GSMM	10 min	16.83±0.46 <sup>h</sup>	70.73±1.04 <sup>c</sup>	68.87±1.55 <sup>a</sup>	9.2±0.24 <sup>d</sup>	23.35±0.15 <sup>b</sup>
(Sonication time)	20 min	16.45±0.48 <sup>g</sup>	71.24±2.29 <sup>c</sup>	68.74±1.68 <sup>a</sup>	8.1±0.19 <sup>e</sup>	24.94±0.78 <sup>c</sup>
	30 min	16.24±0.19 <sup>g</sup>	71.60±0.43 <sup>c</sup>	68.68±0.83 <sup>a</sup>	8.4±0.23 <sup>e</sup>	24.72±0.39 <sup>e</sup>

GMM - Germinated millet milk, GRMM - Germinated and roasted millet milk, EMM - Enzyme treated millet milk and UMM - Ultrasonicated millet milk. Values are means of 3 replicates. Means in the same column followed by different superscripts are significantly different at  $P<0.01$

Separation rate of little millet milk prepared by various methods were significantly different. However, no significant difference was observed among each processing methods. Enzyme treated sample had lowest separation rate ( $52.13 \pm 0.07$  to  $51.92 \pm 1.09$  ml/h) compared to other treatments. Based on the statistical results, GMM and UMM were on par with one another ( $75.50 \pm 2.04$  to  $76.02 \pm 2.06$  ml/h). The findings in present study were comparable to that reported by Issara and Rawdkuen [20] for rice bran beverage which had separation rate of 80 ml/h when kept at  $4^\circ\text{C}$  for 7 days.

Germinated little millet milk had highest whiteness index which varied from  $69.81 \pm 1.58$  to  $69.13 \pm 0.62\%$ . When water to millet ratio is increased, whiteness index of millet milk gets decreased. A significant difference was observed ( $P < 0.01$ ) among the different processing methods. Enzyme treated little millet milks had lowest whiteness index ( $61.77 \pm 1.93$  to  $60.79 \pm 0.04\%$ ) which may be due to action of amylase enzyme and also heat treatment.

Heat stability of little millet milks produced by different processing methods significantly differs from one another. Germinated millet milks coagulated easier ( $6.3 \pm 0.01$  min) than enzyme treated millet milks ( $26.2 \pm 0.10$  min). The increasing order of heat stability in various processing methods was germinated millet milk < germinated and roasted millet milk < ultrasonicated millet milk < enzyme treated millet milk.

Viscosity of little millet milks showed significant difference among the various processing methods. When increasing the millet to water ratio in GMM and GRMM and enzyme concentration in EMM, the viscosity of respective products get decreased ( $2.01 \pm 0.04$  to  $25.20 \pm 0.05\text{cP}$ ). But, in case of, ultrasonicated milk, the viscosity of millet milks get increased with increasing sonication time ( $11.60 \pm 0.18$  to  $16.59 \pm 0.43\text{cP}$ ). This may probably due to internal heat production with increasing sonication time. The viscosity of pulsed electric field treated almond milk was reported by Manzoor et al., [16] and the values were in the range of  $3.86 \pm 0.05$  to  $2.31 \pm 0.06\text{cP}$ . The viscosity of almond milk increased with increased pulsed electric field treatment which was similar to present finding of ultrasonicated milk.

Sedimentation rate of kodo millet ranged from  $1.01 \pm 0.17$  in EMM to  $14.64 \pm 0.40\text{g}/40$  ml in UMM. Jeske et al. [21] analysed the sedimentation of

enzyme treated quinoa milk substitutes which is  $4.41 \pm 0.52\text{mm}$ , whereas quinoa milk which is not treated by enzyme showed higher sedimentation of  $6.65 \pm 0.08\text{mm}$ . In the present research work, all enzyme treated millet milks showed lower sedimentation rate than enzyme treated quinoa milk.

Separation rate of kodo millet milk ranged from  $72.05 \pm 0.93$  in GMM to  $45.41 \pm 1.26$  ml/h in EMM. The higher separation rate of kodo millet milk may probably due to lack of naturally occurring emulsifying or stabilizing agent in millet milk. Results of present study are contrary to results of peanut beverages. Lower separation rate ( $0.13$  to  $0.31$  ml/h) in peanut beverage was probably due to presence of casein protein that can act as an emulsifier to bind the water and lipid-containing components into the solution more effectively which emulsify the beverage which resulted in lower separation rate [15].

Kodo millet milk had lowest whiteness index among the millet milk which is mainly due to colour of kodo millet grain. Whiteness index of kodo millet milk prepared by various processing methods were in the increasing order of enzyme treated millet milk ( $41.16 \pm 0.47$  to  $40.12 \pm 0.04\%$ ), ultrasonicated millet milk ( $47.74 \pm 0.34$  to  $45.52 \pm 0.37\%$ ), germinated and roasted millet milk ( $52.13 \pm 0.38$  to  $51.86 \pm 0.42\%$ ) and germinated millet milk ( $52.85 \pm 1.08$  to  $52.41 \pm 0.28\%$ )

Heat stability of kodo millet milks varied from  $6.0 \pm 0.25$  to  $22.2 \pm 0.38$  minutes for germinated kodo millet milk and enzyme treated kodo millet milk respectively. Jeske et al. [2] reported that in lentil based milk substitute, untreated lentil protein beverage had lower heat stability of  $8.28 \pm 0.25$  minutes whereas the heat stability was increased to  $17.41 \pm 0.80$  minutes in homogenized lentil protein beverage.

Viscosity of ultrasonicated kodo millet milk ( $11.73 \pm 0.15$  to  $16.25 \pm 0.22\text{cP}$ ) was higher than other processing methods. The dilution ratios in GMM and GRMM didn't significantly influenced the viscosity values of kodo millet milk. The higher viscosity in GMM and GRMM was due to gelatinization of kodo millet milk at  $75^\circ\text{C}$ . Enzyme treated milk had lowest viscosity in the range of  $2.61 \pm 0.01$  to  $1.97 \pm 0.24\text{cP}$ . Jeske et al. [14] evaluated viscosity of cereal based milk substitutes which was in the range of 2.21 to 2.77cP. The similar results were observed in the present study.

Finger millet milk had lowest sedimentation rate at 1.0% enzyme concentration among the millets ( $0.92 \pm 0.22$  mg/40 ml). Significant difference was found among processing methods. In all the samples, germinated millet milks had highest sedimentation ( $12.7 \pm 0.22$  to  $12.1 \pm 0.25$  g/40 ml) which may be due to deposition of starch and protein molecules.

Separation rate of finger millet milk varied from  $71.58 \pm 1.12$  to  $45.03 \pm 0.18$  ml/h. Enzyme treated finger millet milk showed lower separation rate compared to germinated finger millet milk. According to the results of Jeske et al. [21] amount of quinoa milk separated in an hour is  $52.29 \pm 0.29$  ml/h for control quinoa milk and  $61.86 \pm 0.60$  ml/h for enzyme treated quinoa milk.

The nature of processing influenced the whiteness index of millet milks. Among the millets, finger millet milk had highest whiteness index which is  $70.24 \pm 0.91$  to  $70.04 \pm 0.33$  for GMM,  $70.21 \pm 0.90$  to  $69.93 \pm 0.71$  for GRMM,  $63.46 \pm 1.69$  to  $62.53 \pm 0.18$  for EMM and  $68.87 \pm 1.55$  to  $68.68 \pm 0.83$  for UMM. No significant difference were found among GMM, GRMM, UMM prepared by various millet to water ratios and time combinations. Whiteness index of millet milk prepared with various concentration of enzyme was on par to one another. According to the results of Jeske et al. [22], glucose isomerase enzyme treated quinoa-based milk substitutes had lower whiteness index of  $51.18 \pm 1.23$  and to quinoa milk fermented with *Lactobacillus brevis* TR055 had higher whiteness index of  $54.32 \pm 1.50\%$ .

Heat stability of finger millet milks were in the range  $5.1 \pm 0.05$  to  $24.5 \pm 0.33$  minutes. The higher heat stability in enzyme treated sample is considered as a favorable parameter because the product will be stable under pasteurization temperature of  $75^\circ\text{C}$  for 15 minutes which ensures the microbial stability of the final product.

Viscosity of finger millet milks were in the range of  $2.43 \pm 0.32$  to  $26.40 \pm 0.37$  cP. Faccin et al. [23] concluded that the increase in viscosity of rice bran beverage is due to starch retrogradation. Similarly, in the present study, the higher viscosity of GMM, GRMM and UMM is due to starch gelatinization at high temperature.

#### 4. CONCLUSION

There has been an increased demand for the plant based alternative milks around the world. In such aspects, millet milk stands to be a better

choice. Millets such as barnyard millet, little millet, kodo millet and finger millet was processed into millet milk by various processing methods. All the processing methods significantly affect the physical parameters of developed millet milks. Among the four processing methods, enzyme ( $\alpha$  amylase) assisted extraction showed better results at 0.5 and 1.0% concentration. In order to minimize the use of enzyme, addition of 0.5% enzyme was considered as best for the development of millet milk. Though the whiteness index of all the enzyme treated millet milks were lower, the major parameters viz., sedimentation rate, separation rate, heat stability and viscosity of millet milk showed better results which decides the product quality and consistency of plant based milk alternatives. The developed millet milk can be served with sweeteners and flavouring agents to maximize the consumer acceptability.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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