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Research Article

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Production of Aqueous Extracts from High-Protein Rice and Evaluation of Its Extraction Methods on Quality of Rice Milk

Hemanth Kumar J., V. R. Sinija, S. Shanmugasundaram, S. Bhuvana and Vincent Hema[*]

Indian Institute of Food Processing Technology, Pudukkottai Road, Thanjavur-613 005, Tamil Nadu, India.

*Corresponding Author E-mail: mariagorretti@iifpt.edu.in

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ABSTRACT

Lactose intolerance and cow milk protein allergy after the consumption of dairy milk lead a huge demand in the plant based milk in the present era. Cereals and oilseeds have shown a great concern towards this sector due to its versatile nutritional profile. Therefore, this study was aimed to utilize the high-protein rice for extraction of plant milk. The rice to water in the ratio of 1:5 w/v was prepared for extraction of milk by cold extract, hot extract, mashing, enzymatic method and their nutritional, biochemical properties were determined which was compared to other plant based milk and dairy milk. The aqueous extract from high-protein rice by the enzymatic method showed smaller particle size of 1157±14nm compared to mashing 1356±35nm and cold extract 1945±13nm. The protein retention and the starch breakdown were high in rice milk extracted by enzymatic method. Protein content in rice milk was high 6.83% in enzymatic method compared to mashing 6.3% and conventional 6.49%. The sensory evaluation shows that samples produced using enzyme had a higher preference for taste, flavour and acceptability because this method reduced the raw flavour compound 'octanal' in rice. Proximate analysis showed that high-protein rice will be the good source for plant milk and better substitute to cow milk compared to millet milk and coconut milk.

Key words: *Lactose intolerance, High-protein rice, Aqueous extract, Alpha amylase, Particle Size*

INTRODUCTION

Cow milk or Dairy milk is known to have more nutritional value and is highly perishable in nature. Storhaug *et al.*[1], claimed that lactose malabsorption is around 67% considering almost 81% of the human population in the world. On an average, prevalence of lactose malabsorption is 64% in Asia (except middle east Asian countries which is around 70%) which is the highest followed by 54% in sub-Saharan Africa region and 47% in eastern Europe, Russia and Soviet Republic regions. Among Asian countries, least was recorded in Pakistan of about 58% and the highest lactose malabsorption of about 100% was in South Korea whereas in India it is around 58%.

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Lactose intolerance people is a term used for the people who are facing symptoms such as bloating, abdominal cramps, diarrhea, gas and nausea due to the lactose malabsorption[1]. According to Deora *et al.*[2], 33% of the global population of lactose intolerant and also studies showed that 75% of the adults have decreased lactase, the enzyme activity on ageing[2]. Avoidance of dairy foods leads to defiency of calcium and many other minerals which is the main source in our human diet. Hence, preparation of milk mimics and the development of products which mimic the dairy products is a recent heat in the future. Cereals are in recent trend in this sector nowadays due to its varied nutritional composition. Cereal milk extract will be good for cardiovascular patients because of low fat in cereal based milk extracts. Majorly cereals also have high dietary fibre content which will be beneficial for colon bacteria because of its probiotic nature. Dietary fibre is a non-digestible carbohydrate which has important biological functions in the human body. Dietary fibre has no calorific value, highly resistance to digestible enzymes rather it serves as a prebiotic to beneficial microbes in the gut. Millet which is re-discovered as a healthy cereal has more potential to meet the demands of lactose intolerance people. On the other side, rice which is the worldwide highest produced and consumed cereal due to its ease in digestibility and for as a source of energy also has a potential to meet the lactose intolerance consumers. Mashing of the cereal grains will be helpful as a pre-digestion step in the development of milk mimic beverage. Mitchell *et al.*[12], experimented to know the role on enzymes on the properties of rice starch and concluded that addition of amylases enzyme to the rice starch, reduces the raw rice flavour "octanal" and makes the consumer to be more appeal towards the product[12]. Ozbek *et al.*[20], found that alpha-amylase enzyme breakdown the alpha glyosidic bond present in starch molecule into amylose and amylopectin. The activity of alpha amylase was high at pH 5 and 60°C [20]. As a replacement of dairy milk for cow milk allergens people, soy milk has shown higher demand earlier due to its high protein content but due to its harsh off flavour and high cost of production to reduce flavour has reduced the market. The particle size of any solid particles in the liquid product determines its stability. The lower the particle size of the solid materials, higher will be the stability of liquid product. The range of particle size in the milk will lead to creaming and sedimentation. There was no report on high-protein rice as source of milk extract. This study was therefore aimed to develop a method for the extraction of plant milk from the special variety rice of high-protein jeeraga samba rice and to assess the suitability of its consumption by its physicochemical, nutritional and sensory properties and to compare it to the other established plant milk extracted from coconut, millet and cow milk. The objective of the study is not only to produce the rice milk, simultaneously to prepare low particle size plant based milk especially for lactose intolerant people and to compare with popular non-dairy milk.

MATERIAL AND METHODS

2.1 Raw Materials:

High-protein jeeraga samba rice was identified and purchased from Udhaya Agro Farm, Thanjavur. Millets were procured from Valwill Sudesi Farmers Producers Company, Namakkal. Coconuts were supplied by Thanjavur Consumer Co-operative Warehouse, Tamil Nadu. Raw cow milk was obtained from local farm at Thanjavur.

2.2. High-protein Rice Milk Processing:

Rice milk was prepared using four methods modified from Illinois method of soy milk preparation. The high-protein rice was soaked in water for about 8 hours at $\pm 30^0$C, and the ratio of rice to water was used in the ratio of 1:5 w/v. The soaked rice was washed using clean water and mashed using milk extraction unit (Pristine Plants India Pvt. Limited). The rice was steamed at 100^0C by passing steam through it while mashing with water in cooker cum grinder. After steaming the slurry was mashed again in order to get homogenous aqueous extract at 68^0C for 3 minutes in a pressure of 2 kg/cm^2. The aqueous extract of rice was filtered by filter press and homogenized using colloidal ball mill at the speed of 3000 rpm for 12 minutes (Pilotsmith India Pvt. Limited).

In the other way, the aqueous extract of high-protein rice was treated with different concentrations of alpha-amylase (α-amylase) ranges from 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0 respectively and was incubated for the period of 10, 15 and 20 minutes at 60^{0}C.

The aqueous extract of high-protein rice was also prepared by hot extraction at 72℃ for 15 seconds and raw rice milk was extracted by cold extract at 30^{0}C using colloidal ball mill at the speed of 3000 rpm for 12 minutes. The processed rice milk samples were packed in 300 ml of PET bottles and stored at ±4 ^{o}C for further assessment. Similarly, millet milk was extracted by mashing treatment and coconut milk was extracted by cold extract.

2.3 Quality Analysis:

2.3.1 Physio-chemical analysis

The physio-chemical properties of the rice milk extracted by mashing, enzymatic and conventional techniques were assessed and compared with Millet Milk Extract, Coconut milk and cow milk. pH was measured using LABMAN Scientific pH meter. Total soluble Solids were estimated using ATAGO pocket refractometer, sample was place on the place surface of refractometer and the corresponding TSS was noted down. Water activity was measured using AQUALAB 4TE water activity meter. Colour for all the samples were measured using Commission Internationale de I'Eclairage (CIE) method. Viscosity was measured using Brookfield viscometer at 25^{0}C. Specific gravity was measured using standard AOAC, 932.14 method at 25^{0}C.

2.3.2 Proximate analysis

Moisture was estimated by AOAC 2000 method Protein content was estimated by Kjeldhal apparatus by AOAC 2000 method. Fat was estimated by AOAC 989.05 method. Total carbohydrates and Starch was estimated by anthrone reagent method. Amylose and amylopectin was estimated by Iodine reagent method. Glucose was estimated by glucose oxidase method. Dietary fibre was estimated by AOAC 2009.01 method. Ash content was estimated by AOAC 2000 method.

2.3.3 Particle size analysis

Particle size analysis in the milk extract was done using Malvern Instruments Ltd. zeta sizer. Size of the particles in any liquid determines its quality characteristics.

2.3.4 Sensory analysis

Rice milk extracts were analysed for its acceptability using 9-point hedonic rating scale by 15 semi-trained panellists.

2.3.5 Statistical analysis

The triplicates results obtained were analysed by Two-way Anova, Mean and Standard Deviation with 5% level of significance using IBM SPSS Statistics 20 software.

RESULTS AND DISCUSSION

3.1. Effect of alpha-amylase (α-amylase) enzyme on Total Soluble Solids

The effect of enzyme concentration and incubation time on total soluble solids in aqueous extract of high-protein rice is presented in Table 1. The enzyme concentration was standardized based on the total soluble solids content of aqueous extract of rice after enzyme treatment. The breakdown of sugars was assessed by total soluble solids as shown in Table 1.

The result was observed that increase the concentration of enzymes ranges from 0.1 to 7.0% in aqueous extract of rice increase the total soluble solids from 0.7 to 3.5 ^{o}brix. It was found that concentration of enzyme at different incubation time at 10, 20 and 30 minutes was more effective in breakdown of starch. Similarly, Zarnkow *et al*.[5], reported that use of alpha-amylase enzyme was increase the breakdown of starch by disrupting the integrity of the cell wall bond and able to increases the extractability of the sugars in cereals[5].

As shown in the result, alpha amylase concentration at the level of 2.5% to 5% seems to be higher total soluble solids 3.3 to 3.5° Brix. Nirmala *et al*.[21], found that breakdown of compound sugars into simpler sugars was in malted grains, which increases the solubility of sugars[21]. Similarly, the results were observed that use of alpha-amylase for aqueous extract of rice increases TSS content, which reduces the particle size and enhance the solubility of particles. The results were in linear and support of Mitchell *et al*[12]. Statistical analysis such as two-way ANOVA was estimated and the observations are as depicted in Table 1.

Table 1: Effect of incubation time and enzyme concentration on TSS (°Brix)

Incubation Time (mins.)	Enzyme Concentration (%)												
	1.0	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**	**4.0**	**4.5**	**5.0**	**5.5**	**6.0**	**6.5**	**7.0**
10	2	2.1	2.9	3.2	3.3	3.3	3.2	3.2	3.3	3.3	3.3	3.4	3.4
20	1.9	2.4	3.2	3.4	3.4	3.4	3.4	3.4	3.5	3.5	3.4	3.4	3.4
30	1.9	2.4	3.2	3.4	3.4	3.4	3.4	3.4	3.5	3.5	3.5	3.5	3.5

Note: Values are mean of three determinations ± standard deviation.

The enzyme concentration of 1.0%, 1.5%, 2% shows significant difference between all the treatments at that respective time. 2.5% enzyme concentration showed significant difference in all the enzyme concentrations except 3%, 3.5%, 4% and 4.5% enzyme concentration. 3% enzyme concentration showed significant difference in all the enzyme concentrations except 2.5%, 3.5%, 4% and 4.5%. 3.5% enzyme concentration shows significant difference in all the enzyme concentrations except 2.5%, 3%, 4% and 4.5% enzyme concentration. 4% enzyme concentration shows significant difference in all the enzyme concentrations except 2.5%, 3%, 3.5% and 4.5%. 4.5% enzyme concentration shows significant difference in all the enzyme concentrations except 2.5%, 3%, 3.5% and 4% enzyme concentration. 5% enzyme concentration shows significant difference in all the enzyme concentrations except 5.5%, 6%, 6.5% and 7% enzyme concentration. 5.5% enzyme concentration shows significant difference in all the enzyme concentrations except 5%, 6% and 6.5% enzyme concentration. 6% enzyme concentration shows significant difference in all the enzyme concentrations except 5%, 5.5%, 6.5% and 7% enzyme concentrations. 6.5% enzyme concentration shows significant difference in all the enzyme concentrations except 5%, 5.5%, 6% and 7% enzyme concentration. 7% enzyme concentration shows significant difference in all the enzyme concentrations except 6% and 6.5% enzyme concentrations.

3.2 Effect of extraction methods on biochemical and nutritional components

The aqueous extraction of high-protein rice by cold extract, hot extract, mashing and enzyme methods and its effect on biochemical constituents are presented in Table 2. The carbohydrate content of rice milk was decreased in all extraction condition than raw rice milk extracted by cold method which contained carbohydrate of 15.68%. When compared between raw rice milk to other extraction condition such as conventional heat treatment, mashing and enzymatic method found that carbohydrate content in raw rice milk reduced to 15.51%, 15.49% and 14.75% by hot extract, mashing and enzymatic method respectively. The high-protein rice selected for this study showed high amount of amylopectin than amylose and the similar results were obtained in rice milk contains amylose 5.42%, 5.53%, 5.28% and 2.82% and amylopectin 6.62%, 5.93%, 7.8% and 4.89% by cold, hot, mashing and enzyme method. It was indicated that the amylose and amylopectin structural chain breakdown was high in enzyme treatment Ozbek *et al*.[20], The higher carbohydrate in raw rice milk was influenced by the breakdown of sugars and starch matrix in various extraction condition[20]. The breakdown of oligosaccharides into simpler sugar molecule glucose was high 3.4% in enzyme method. In case of hot extract and mashing showed lesser glucose 0.43% and 0.78% respectively than enzyme treatment but higher than the cold extract 0.29% of glucose. Dietary fibre which has important biological functions in the human body. Mudgil *et al.*[15], Dietary fibre with no calorific value have higher resistance to digestive enzymes rather it serves as a prebiotic to beneficial microbes in the gut.

Table 2: Various extraction methods on biochemical constituents of rice milk

Parameter	Cold Extract	Hot Extract	Mashing	Enzyme
Moisture (%)	78.0±1.3	74.5±1.0	73.66±1.2	74.43±0.51
Total Solids (%)	22.0±1.3	25.5±1.0	26.33±1.2	25.56±0.51
Total CHO (%)	15.68±0.04	15.51±0.01	15.49±0.08	14.75±0.07
Dietary fibre (%)	3.63±0.005	3.586±0.05	3.63±0.01	3.64
Starch (%)	12.04±0.06	11.46±0.22	11.08±0.06	7.71±0.01
Amylose (%)	5.42±0.02	5.53±0.02	5.28±0.07	2.82±0.03
Amylopectin (%)	6.62±0.02	5.93±0.02	7.80±0.07	4.89±0.03
Glucose (%)	0.29±0.02	0.43±0.007	0.78±0.009	3.40±0.07
Protein (%)	7.28±0.11	6.49±0.18	6.306±0.005	6.83±0.01
Fat (%)	0.73±0.04	0.71±0.01	0.696±0.015	0.52±0.01
Ash (%)	3.82±0.01	3.92±0.002	3.86±0.05	3.90±0.01

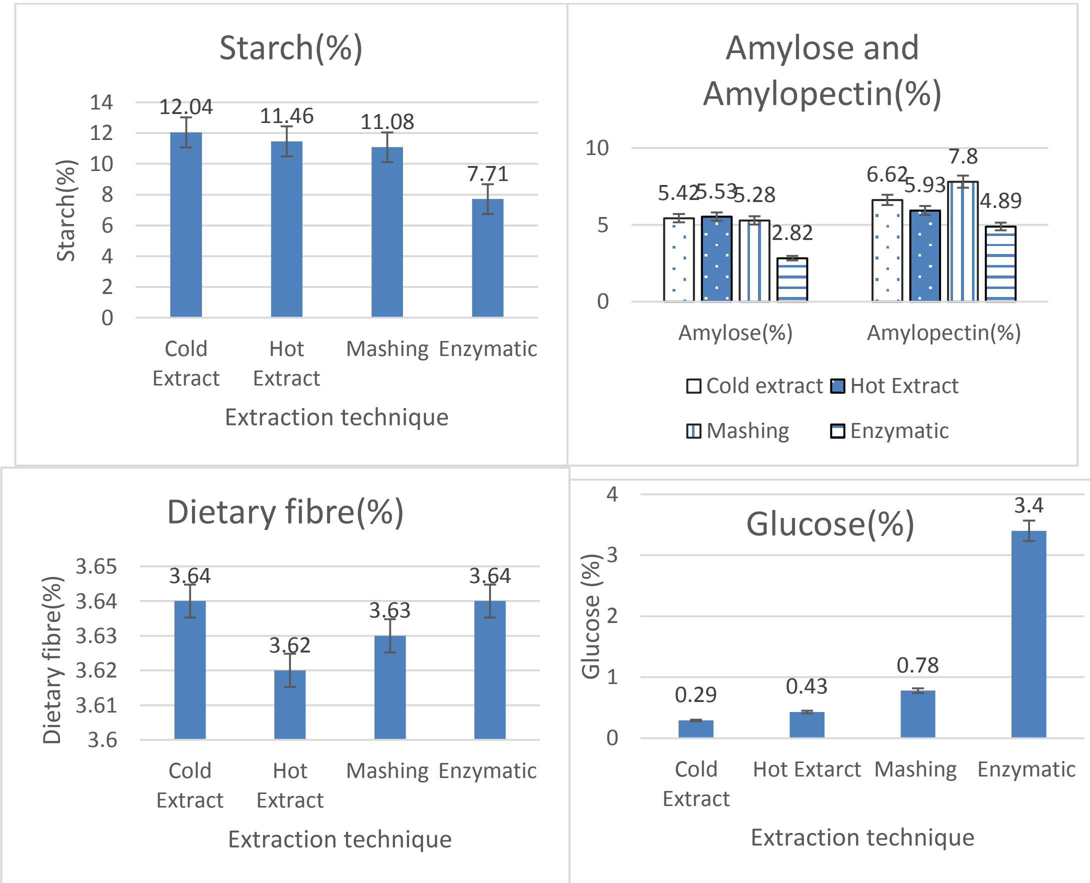

Fig. 1: a, 1 b, 1 c and 1 d. Biochemical constitutes of rice milk extracted by cold, hot, mashing and enzymatic techniques

The protein content was higher in cold extract 7.28% it was reduced to 6.49% in hot extract, 6.31% in mashing and 6.83% in enzymatic rice milk. The retention of protein is high in enzymatic rice milk, since the enzyme might have separated protein from the complex carbohydrate structure. The reduction in hot extract might be due to the denaturation of thermal sensitive amino acids in the protein matrix as the findings of Ju *et al*, [17]. There was a reduction in the fat content in extracted rice milk (0.73% to 0.68%) compared with fat in high-protein rice 1.2%. In this synergetic effect might be hydrophobic nature of water. The fat content was found to be in the range of 0.73 to 0.68% in rice milk extracted by cold, hot extract, mashing and enzymatic method.

The results found for ash showed that a mineral constituent was improved by all extraction methods ranged from 3.91 to 3.92% compared with the ash content of raw rice milk (3.82%) extracted by cold method. However, there was no much changes in the ash content in the rice milk was extracted by hot extract, mashing and enzymatic method. This result was revealed that higher ash content was in rice milk in all extraction other than cold extraction.

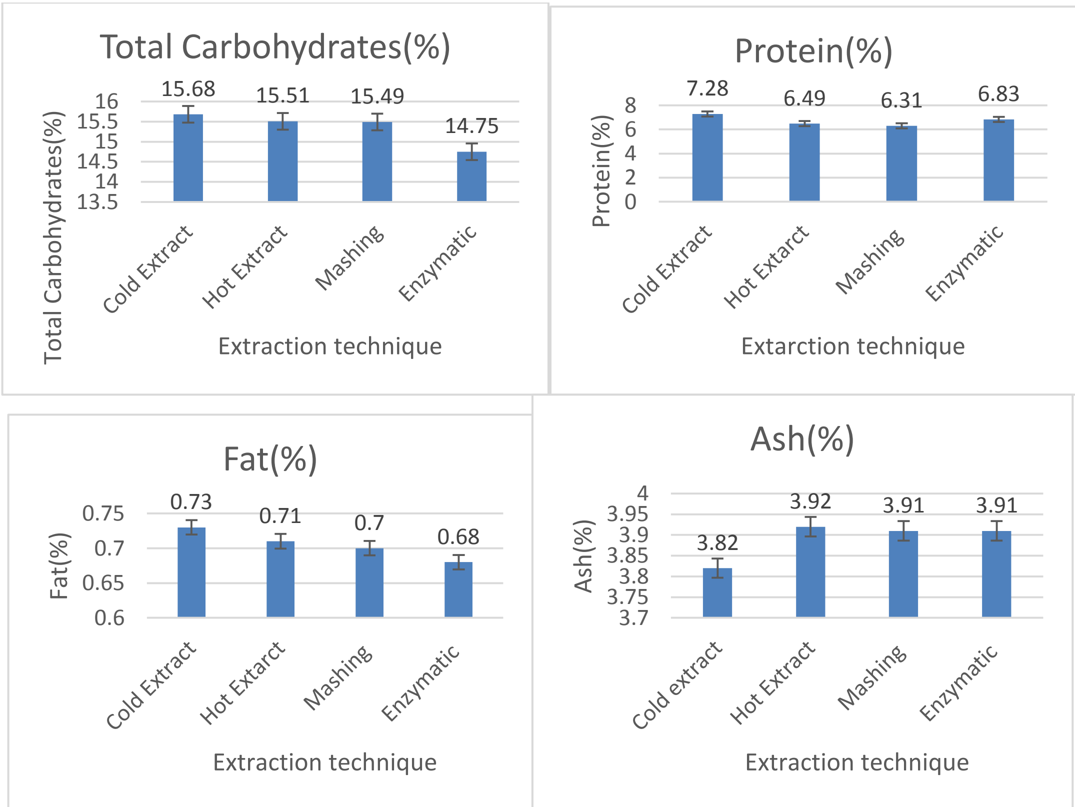

Fig. 2: a, 2 b, 2 c, 2 d. Proximate value of rice milk extracted by cold, hot, mashing and enzymatic methods

3.3 The impact of extraction method of rice milk compared to dairy and non-dairy milk

3.3.1. Physicochemical and nutritional composition:

The physio-chemical properties of rice milk extract were compared to non-dairy milk such as millet milk, coconut milk and dairy milk as shown in Table 3. The developed rice milk had no significant differences in pH, viscosity and colour when compared to cow milk. The specific gravity was high in rice milk, millet milk and coconut milk and which might be particle size of plant milk as per the similar findings by Hooper *et al.*[19]. The physio-chemical parameters of the results indicated that it enhances the process parameters of plant milk at pilot scale and also which determines the palatability of the milk.

Table 3: Physio-chemical properties of rice milk, dairy milk and non-dairy milk

Properties	RMH	RMM	RME	MM	CM	DM
pH	6.62±0.01	6.656±0.006	6.71±0.01	6.753±0.005	6.826±0.005	6.616±0.005
Viscosity	1.045±0.002	1.032±0.05	1.01±0.001	0.757±0.012	5.193±0.011	1.21±0.02
Specific Gravity	3.819±0.01	3.862±0.001	3.942±0.005	3.8416±0.002	3.62±0.001	1.028±0.001
Colour	0.12±0.012	0.115±0.007	0.145±0.021	24.55±0.21	6.26±0.07	-
Water activity	0.9992	0.9991±0.0001	0.9991	0.9995±0.0001	0.9989	0.9993
TSS	0.86±0.05	1.13±0.05	3.4±0.1	1.13±0.06	9.32±0.05	3.46±0.06

Note: RMM: Rice milk extracts Mashing, RME: Rice milk extracts Enzyme, RMH: Rice milk extracts Hot method, MM: Millet milk, CM: Coconut milk, DM: Cow milk

Nutritional composition of rice milk was compared to millet milk, coconut milk and dairy milk as depicted in Table 4. Protein content in the rice milk extracted by mashing method was 6.306±0.005% and in enzymatic method was 6.83±0.01 respectively which is high as compared to dairy milk with 3.50±0.05 %. Rice protein is easily digestible, non-allergic than other plant protein and dairy protein sources. Rice milk extract can be suitable to cardiovascular, lactose intolerance people and gastrointestinal disorders. The result was found that low fat content in mashing and enzymatic method of rice milk ranges from 0.696±0.015% to 0.52±0.01% than the dairy and non-dairy plant milk which contain high amount of fat content. The highest energy value was observed in rice milk extract 93.5kcal/100g than dairy milk 62.5kcal/100g whereas lower than coconut milk 277.3kcal/100g. These results are in linear with Belewu *et al.* [8].

Table 4: Nutritional composition of rice milk, dairy milk and non-dairy milk

Properties	RMM	RME	MM	CM	DM
Moisture (%)	73.66±1.2	74.43±0.51	78.9±0.55	54.26±0.98	85.16±1.04
Protein (%)	6.306±0.005	6.83±0.01	2.173±0.11	0.74±0.01	3.50±0.05
Fat (%)	0.696±0.015	0.52±0.01	2.17±0.02	18.5±0.1	3.736±0.005
Total CHO (%)	15.493±0.08	14.75±0.07	12.5±0.03	26.99±0.09	4.45
Ash (%)	3.86±0.05	3.9±0.01	4.2±0.03	1.2±0.03	0.796±0.005
Total Solids (%)	26.33±1.2	25.56±0.51	21.03±0.55	46.73±2.44	14.83±1.04
Energy (kcal/100 g)	93.5	91.2	78.05	277.3	65.5

Note: RMM: Rice milk extracts Mashing, RME: Rice milk extracts Enzyme, RMH: Rice milk extracts Hot method, MM: Millet milk, CM: Coconut milk, DM: Cow milk

3.4 Particle size:

In the present study the enzyme treatment was carried out to reduce the particle size of total solids in the rice milk extracts to avoid sedimentation. A standard deviation of different particle size was obtained in each of the rice extraction methods and dairy and non-dairy milk were shown in the table 5.

Table 5. Particle size of rice milk, dairy milk and non-dairy milk:

Milk Samples	Particle size (nm)
RMH	1945±13
RMM	1356±35
RME	1157±14
MM	2744±64
CM	2568±45
DM	560.8±29

Note: RMH: Rice milk extracts Hot method, RMM: Rice milk extracts Mashing, RME: Rice milk extracts Enzyme, MM: Millet milk, CM: Coconut milk, DM: Cow milk

The result observed that the particle size with 560.8± 29nm had stability for more than 24 hrs at refrigeration condition without cream separation. Coconut milk with particle size of 2568±45nm had stability for 2 hrs without cream separation. The raw rice milk extracted by hot method with particle size of 1945±13nm was stable for 120 minutes without sedimentation. The reduced particle size was observed in mashing and enzymatic method of rice milk extraction ranges from 1356±35nm to 1157±14nm and was stable for 150 minutes and 175 minutes respectively. The stability of rice milk extract was enhances by hydrocolloids such as xanthan gum was added at concentrations of 0.5% to rice milk extract showed positive effect to increase the stability time and reduced the sedimentation from 2 to 7 hours. The results were in support of Binks *et al.*[13], as particle size reduction brings longer stability of product[14].

3.5 Sensory evaluation

Breakdown of oryzanol, which is raw rice flavour reduction in the enzymatic method, was noticed during the flavour analysis of rice milk extracts. Mean values in the sensory evaluation by 9-point hedonic rating scale is as shown in the Table 6.

Table 6: Particle size of rice milk, dairy milk and non-dairy milk:

Milk Samples	Colour	Flavour	Consistency	Taste	Overall acceptability
RMM	8.8	6.13	7	6.46	6.4
RME	8.86	8.26	6.93	6.8	7.26
MM	6.53	7.06	7.46	6.26	6.6
CM	7.66	7.66	7	8	7.33
DM	9	8.33	9	8.26	8.6

Note: RMM: Rice milk extracts Mashing, RME: Rice milk extracts Enzyme, MM: Millet milk, CM: Coconut milk, DM: Cow milk

CONCLUSION

The developed rice milk with enzymatic treatment will be best suitable for vegan and lactose intolerance population. The high-protein jeeraga samba rice packed with high amount of protein and minerals such as calcium and iron helps to overcome the prevalence of hidden hunger deficiency and protein-energy malnutrition, since the developed rice milk is good in protein content and other functionalities. The use of alpha amylase showed breakdown of starch to maximum extent and also it reduced the octanal flavour which increased the palatability and acceptability to the consumer. The acceptability of rice milk showed high preference compared to dairy milk and non-dairy milk. The developed rice milk extract was stable than non-dairy milk which can be commercialized for lactose intolerance people as it has high energy value with no lactose sugar.

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