

Ultrasound Treated-Freeze dried white finger millet-based probiotic beverage powder: Effect on proximate, colorimetric, and technological properties

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ABSTRACT: Finger millet is one of the common millets having numerous health benefits. It is rich in calcium and contains various functional compounds with anti-tumorigenic, antimicrobial, anti-diabetic, and antioxidant properties. White finger millet KMR 340 is one of the new varieties of finger millet developed in Mandya's zonal agricultural research station. Probiotic foods are majorly available as milk products. Owing to its high cholesterol level and lactose intolerance, consumer's preference for non-dairybased probiotic beverages is increasing now-a-days and that paved the way for the development of probiotic beverages using white finger millet. Since, heat treatment deteriorates the product quality, the application of non-thermal technology is gaining importance in recent days. The present work investigates the effect of ultrasound treatment and freeze drying on white finger millet-based probiotic beverage powder and analyzes its effect on proximate composition, color values, and technological properties. Technological properties like the Carr index and Hausner ratio did not show any significant difference in the obtained results. Though colorimetric properties showed a significant difference between control and the US-treated samples, it did not impact its visual appearance. The obtained results could help the food processors to find its application in the development of millet-based beverage premix and in designing the storage structures.

Keywords: White finger millet, Ultrasound, Colorimetric and technological properties, Probiotic.

INTRODUCTION

Millets are the major food in developing countries. In general, the production and consumption of millets in developing countries is more than 97%. According to estimates, the area of land used for millets cultivation has decreased worldwide by about 25.71% between 1961 and 2018. Furthermore, from 1961 to 2018, millet productivity increased globally by 36%, from 575 kg/ha to 900 kg/ha. Except for Africa, the average statistics from the previous 58 years showed that millet output decreased globally. In the case of India, millet production peaked in the 1980s and then progressively declined as a result of an increasing reduction in the area that is cultivated. India produces the most millets, accounting for 37.5% of the total global output, followed by Sudan and Nigeria. The year between 2011 and 2017 had maximum import and export values of millets in terms of trade (155.26 and 127.60 million US dollars, respectively). Several factors may contribute to the ongoing decline in the area for millets crop including relocating millets for other crops, alterations in dietary preferences, assurance of irrigation

infrastructure, and guaranteed returns from important commercial crops (Meena *et al.*, 2021).

Millets are often grown in soils that are too deficient in sustaining any other crop. They are different from other cereal crops because of their short growing season and have a high tolerance to drought, poor nitrogen application, and temperature variations. Millets are year-round, all-season crops that provide a variety of security (food, fodder, nutrition, and ecology), making them the crops of agricultural security that are available at reasonable prices (Malathi *et al.*, 2016). Sorghum, Pearl millet, Finger millet, Foxtail millet, little millet, Kodo millet, Proso millet, and Barnyard millet are the major millets grown in India. Among these, finger millet (*Eleusine coracana* L.) is rich in calcium, and it is almost ten times higher than that of rice or wheat (Malathi *et al.*, 2016). It belongs to the Poaceae family. It has some of the functional components like catechin, gallicocatechin, epicatechin, epigallocatechin, vitexin, myricetin, quercetin, apigenin, etc., having anti-tumorigenic, anti-diabetic, antimicrobial, and antioxidant properties (Thakur & Tiwari 2019). White finger millet (WFM) KMR 340 is a new variety

developed in a zonal agricultural research station in Mandya, India. It has a high level of calcium (392 mg/100 g), magnesium (144 mg/100 g), protein (11.98 g/100 g), and fiber (4.2 g/100 g) (Navyashree *et al.*, 2021).

When given to a host in sufficient quantities, live microorganisms that positively affect their health are known as probiotics. To exhibit therapeutic effects, the microorganisms must be alive and present in large quantities, often more than 10^8 to 10^9 cells per gram of products at the moment of ingestion. Probiotics must also be able to live in the challenging environment of the human gastrointestinal tract (Kandylis *et al.*, 2016). Though most probiotics are dairy-based, several concerns like the emergence of vegetarianism and veganism, the allergy risk associated with dairy products, and customer preferences for diverse, unique flavors pave the way for developing non-dairy-based probiotic beverages (Rasika *et al.*, 2021).

Ultrasound (US) is an emerging technology that works on the cavitation phenomenon, which causes the formation, expansion, and collapse of gas bubbles leading to various chemical and mechanical effects. In food fermentation, low-frequency ultrasound (20–50 kHz) enhances mass transfer and cell permeability, resulting in increased process efficiency and output rates (Ojha *et al.*, 2017). Therefore, US is applied to analyze its effect on fermentation of the developed beverage.

Knowledge of the technological properties like bulk density, tapped density, Carr index and Hausner ratio help to determine the flow characteristics of the powder (Shishir *et al.*, 2014). At the same time, the colorimetric properties of freeze-dried white finger millet-based probiotic beverage (WFMPB) powder gives information about the level of perception. Thus, the present work is performed to determine the effect of ultrasound treatment and freeze drying on the WFMPB powder in terms of its proximate, technological and colorimetric properties. The results obtained in this study help the food processors to find its application in the development of millet-based beverage premix and in designing the storage structures.

MATERIALS AND METHODS

A. Materials

WFM (KMR 340) was obtained from V.C Farm, Mandya, Karnataka, India. *Lactobacillus rhamnosus* GG NCDC 347 was acquired from the National Dairy Research Institute (NDRI), Karnal, India for inoculation.

B. Sample preparation

Preparation of WFMPB. Cleaned millets were soaked in water and then germinated at 30 °C for 48 h. The sprouted millets were dried and milled using a hammer mill (Almech Enterprise, Coimbatore, India) to obtain germinated millet flour (GMF). It was then sieved with a sieve having a mesh size of 250 µm, and flour was collected and stored in high-density polyethylene (HDPE) bags at the refrigerated condition for further

analysis. The probiotic culture was revived as per the protocol given with the ampoules.

A suspension of GMF (14% w/v), and sugar (5% w/v) were mixed in 100 mL water and sterilized. It was then inoculated with *L. rhamnosus* having a cell density of 6 log CFU/mL. Then the mixture was fermented at 37 °C in a shaker incubator (Scientech, New Delhi, India) till it attained the pH value of 4.6 (Medve & Lipt 2008). The pH was measured using a pH meter (Horriba scientific, Japan). A sample without US treatment was taken as a control.

The mixture was treated with the US at two different conditions. In the first condition, the mixture was treated with the US after the inoculation of probiotic culture (after inoculation). In the second case, the mixture was treated with US and then inoculated with probiotic culture (before inoculation). For after inoculation, the amplitude and treatment time of 41.42% and 2.63 min were used, respectively. On the other hand, before inoculation treatment, the amplitude and treatment time of 40.11% and 11.09 min were used, respectively. After treating the samples with US, the mixture was inoculated with *L. rhamnosus* and fermented at 37 °C. After fermentation, WFMPB was freeze-dried and the powder was collected. Freeze dryer (Lyophilizer 80 °C, Lark innovative fine teknowledge, Chennai) was used to dry the samples. The freeze-dried WFMPB powder was stored in HDPE bags at 4 °C. Freeze-dried WFMPB powder was used for analyzing color and technological properties.

Proximate analysis. WFMPB powder was analyzed for moisture, fat, fiber, ash, protein and carbohydrate content according to AOAC 2019 methods.

Moisture content. The sample's moisture content was determined according to AOAC 2019, 931.04 method. 2 g of sample was taken in a petriplate and kept in a hot air oven for 3 h at 105°C. Moisture content was calculated using the following formula.

$$\text{Moisture (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Weight of sample}} \times 100$$

Ash content. The ash content was determined according to AOAC 2019, 923.03 methods. 2 g of sample was taken in the pre-weighed crucible and then placed in a muffle furnace for 5 h at 600 °C. After 5 h, the crucible was taken and allowed to cool in a desiccator. The crucible and its content were weighed.

$$\text{Ash (\%)} = \frac{\text{Final weight of flask} - \text{Empty weight of flask}}{\text{Weight of sample}} \times 100$$

Fat content. The fat content of the samples was estimated using Soxhlet extraction methods (AOAC 945.38). 2 g of sample was weighed into an oil flask. About 100 mL of n-hexane was added into oil flask and kept in Soxhlet apparatus for extraction. After the extraction period, contents in the flask were evaporated to dryness in a hot air oven. The total fat content of the samples was measured using the following equation.

$$\text{Fat (\%)} = \frac{\text{Final weight of flask} - \text{Empty weight of flask}}{\text{Weight of sample}} \times 100$$

Protein estimation. The protein content was determined by kjeldahl method. The samples were

digested with H₂SO₄ in the presence of a digestion mixture for 3-4 h until the solution turns colorless. Upon the addition of 40% NaOH, ammonia was released from the samples during distillation, and it was then collected in a flask containing 4% boric acid. The nitrogen content of the samples was estimated by titrating it against 0.1 N HCl and methyl red was used as an indicator.

$$\text{Protein (\%)} = \frac{(\text{Sample} - \text{Blank}) \times \text{Normality of acid} \times 14.01 \times 6.25}{\text{Weight of sample}}$$

Crude fiber. The crude fiber was estimated according to the method proposed by Shendage *et al.* (2020). A 2 g of the sample that was free of moisture and fat was put into a 1000 mL beaker. In the beaker, 200 mL of a 1.25 % H₂SO₄ solution were added and boiled for 30 minutes. After that, it was filtered and the residue was cleaned with hot water until it became acid-free. Later, the residue was again kept in a 1000 mL beaker and heated for 30 minutes with a 200 mL solution of 1.25 % H₂SO₄. It was again filtered, and the residue was then put into a crucible that had already been weighed. It was dried for 24 h at 100°C until a constant weight was attained. The crude fiber was calculated using the following formula.

$$\text{Crude fiber (\%)} = \frac{\text{Weight of residue} - \text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Total carbohydrate. The total carbohydrate content of the samples was estimated using difference method and its formula is given below.

$$\text{Carbohydrate} = 100 - \% (\text{Moisture} + \text{Protein} + \text{Fat} + \text{Ash} + \text{Crude fiber})$$

Colorimetric properties. The color values of freeze-dried WFMPB powder was determined using Hunter Lab Colorimeter (Hunter Associates Laboratory, Reston, VA). L* indicates lightness to darkness (0 to 100), a* indicates redness to greenness (+ to -), and b* indicates yellowness to blueness (+ to -) and these values were noted. Total color difference (ΔE), chroma (ΔC), hue angle, and whiteness index (WI) were determined using the following equations (Navyashree *et al.*, 2021).

$$\Delta E = \sqrt{(L - L^*)^2 + (a - a^*)^2 + (b - b^*)^2}$$

$$\Delta C = \sqrt{(a^*)^2 + (b^*)^2}$$

$$\text{Hue angle} = \tan^{-1} \frac{b}{a}$$

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$

C. Technological properties

Bulk and tapped density. Bulk density and tapped density of freeze-dried WFMPB powder were determined as per the method described by Shishir *et al.* (2014). 2 g of powder were placed into a 10 mL graduated cylinder and the volume changes were noted. The mass-to-volume ratio of the sample served as a measure of the bulk density of the powder. Whereas 2 g of powder was taken in a 10 mL graduated cylinder and

counting the volume after the sample was smoothly dropped 120 times, the tapped density of the samples was ascertained.

$$\text{Tapped density} = \frac{\text{Weight of powder}}{\text{Volume of powder after tapping}}$$

Flowability. The Carr index (CI) and Hausner ratio (HR) were used to assessing the powders' flowability (Shishir *et al.*, 2014). Bulk and tapped densities of the freeze-dried WFMPB powder were used to compute both the CI and HR. They were calculated using the following formula.

$$CI = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

$$HR = \frac{\text{Tapped density}}{\text{Bulk density}} \times 100$$

D. Statistical analysis

Statistical analysis was performed using SPSS Statistics 20 (IBM, USA). Paired t-test was used to determine the probability level of $p \leq 0.05$ for significant difference. All the analyses were performed in duplicates and values were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

A. Proximate analysis

Sonicated samples' moisture and carbohydrate content did not show significant difference ($p > 0.05$). Whereas protein, crude fiber, fat and ash content showed significant differences ($p < 0.05$). The proximate composition of WFMPB and US-treated WFMPB were presented in Table 1. It shows that moisture content of control (85.27±1.50) was higher followed by before inoculation (83.28±1.38) and after inoculation (82.72±1.37) sample. US treatment slightly increased the fat content of WFMPB from 0.08% in control to 0.1% in before inoculation and 0.12% in after inoculation sample. The lower fat content values could be due to the utilization of fat as the source of energy for the germination process (Hejazi & Orsat 2016). The protein content of WFMPB decreased after US treatment when compared to control. This could be due to the variation in the proteolytic activity of *L. rhamnosus* during the fermentation process (Apaliya *et al.*, 2017). The crude fiber of control was found to be 0.59±0.02, which was higher than the fiber content of before inoculation (0.52±0.01) and after inoculation (0.49±0.00) samples. The ash content of control was 2.94±1.38 which was higher than the sonication after inoculation (2.17±1.01) and before inoculation (1.47±0.70) samples. Sonication increased the carbohydrate content of after inoculation (13.60±0.30) and before inoculation sample (13.65±1.96) when compared to control (9.82±0.11). The carbohydrate content of the samples might be due to the amyolytic activity of lactic acid bacteria during the fermentation process. The obtained results were found to be inconsistent with the results reported by Shendage *et al.* (2020).

Table 1: Proximate composition of US-treated WFMPB.

Parameters	Control	After inoculation	Before inoculation
Moisture (%)	85.27±1.50 ^a	82.72±1.37 ^b	83.28±1.38 ^b
Fat (%)	0.08±0.00 ^c	0.12±0.00 ^a	0.10±0.01 ^b
Protein (%)	1.28±0.02 ^a	0.87±0.04 ^c	0.97±0.11 ^b
Crude fiber (%)	0.59±0.02 ^a	0.49±0.00 ^c	0.52±0.01 ^b
Ash (%)	2.94±1.38 ^a	2.17±1.01 ^b	1.47±0.70 ^c
Carbohydrate (%)	9.82±0.11 ^b	13.60±0.30 ^a	13.65±1.96 ^a

The means in the row with different superscripts were significantly different at $p < 0.05$; a, b represents the significant effect of ultrasound on millet beverage in control, before, and after inoculation samples.

B. Effect of US treatment on color properties

Color is one of the most significant characteristics of a product that influences its acceptability in markets and among consumers. Color values exhibited a significant difference between US treated and control samples ($p < 0.05$). Colorimetric properties of control and US-treated samples were presented in Table 2. The L* value of US treatment after inoculation (86.03±0.14) and before inoculation (84.78±0.02) was higher than control (81.13±0.09). This might be due to the control sample's high-temperature treatment that resulted in the WFMPB powder browning. The redness (a*) and yellowness (b*) values of control was higher than US treatment after and before inoculation samples. High-temperature control treatment caused gelatinization of starch present in WFM flour which contributed to higher a* and b* values for control than US treated samples (Sharma *et al.*, 2018).

Hue angle of all the samples was less than 90°. The hue angle of control (83.50±0.10) sample was higher

followed by US treatment after inoculation (81.98±0.10) and before inoculation samples (80.37±0.04). Colors red, yellow, green, and blue are represented by hue angles of 0, 90, 180, and 270 degrees, respectively. For perception and acceptance, the hue angle is the most important factor for people with normal color vision (Ramashia *et al.*, 2018). Since hue angle of all samples is in the range of 80, it represents that WFMPB flour is slightly yellow. Chroma indicates the intensity of the color. Chroma of control sample is higher than the US treated samples. Though the hue angle for all samples was less than 90° indicating the yellow color of WFMPB flour, the intensity of yellowness is found to be less, which could be seen from the chroma values of the samples. WI values of control and US treatment after and before inoculation samples were 76.16±0.009, 84.49±0.20, and 84.22±0.01, respectively. The results for color analysis were in range with those obtained by Mohite *et al.* (2020).

Table 2: Color analysis of freeze-dried US treated WFMPB.

Parameters	Control	After inoculation	Before inoculation
L*	81.13±0.09 ^c	86.03±0.14 ^a	84.78±0.02 ^b
a*	1.50±0.007 ^a	0.96±0.01 ^c	1.17±0.02 ^b
b*	14.49±0.13 ^a	11.34±0.14 ^c	12.34±0.01 ^b
ΔE	-	5.85±0.20 ^a	4.24±0.02 ^b
ΔC	14.57±0.13 ^a	11.38±0.14 ^c	12.39±0.01 ^b
Hue angle	83.50±0.10 ^a	81.98±0.10 ^b	80.37±0.04 ^c
Whiteness index	76.16±0.009 ^c	84.49±0.20 ^a	84.22±0.01 ^b

The means in the row with different superscripts were significantly different at $p < 0.05$; a, b represents the significant effect of ultrasound on millet beverage in control, before, and after inoculation samples.

C. Effect of US treatment on technological properties

Bulk density and tapped density. The results found that the tapped density was higher than the bulk density in all the samples (Table 3). This could be due to the insignificant volume of voids which resulted as a consequence of external force. The tapped density of US treatment after inoculation (0.39±0.00) and before

inoculation (0.35±0.01) samples showed higher values than control (0.31±0.003). Bulk density also showed a similar trend as in the case of tapped density. Rao *et al.* (2021) observed slightly higher values for bulk density (0.61-0.64) and tapped density (0.70-0.73) in case of microwave-treated foxtail millet flour.

Table 3: Technological properties of freeze-dried US treated WFMPB.

Parameters	Control	After inoculation	Before inoculation
Bulk density (g/mL)	0.24±0.008 ^c	0.30±0.01 ^a	0.25±0.01 ^b
Tapped density (g/mL)	0.31±0.003 ^c	0.39±0.00 ^a	0.35±0.01 ^b
Carr index (%)	22.50±3.53 ^b	23.29±4.48 ^b	29.16±1.35 ^a
Hausner ratio	1.29±0.05 ^b	1.30±0.07 ^b	1.41±0.02 ^a
Flowability	Passable	Passable	Poor

The means in the row with different superscripts were significantly different at $p < 0.05$; a, b represents the significant effect of ultrasound on millet beverage in control, before, and after inoculation samples.

Carr index and Hausner ratio. CI and HR of WFMPB flour were evaluated to determine the difference between their flow ability. CI and HR of US treatment after inoculation and control samples showed no significant difference ($p > 0.05$). The CI value for control and US treatment after inoculation were found to be 22.5% and 23.29%, respectively. Similarly, HR values for control (1.29) and treatment after inoculation (1.30) were found to be comparable. The CI in the range of 0-25% indicates good quality powder, and the range 26-40% is of lower quality, whereas HR in the range of 1.00-1.34 and 1.35-1.6 indicates good quality and lower quality, respectively. Therefore, control and after inoculation samples fall under passable flow ability. However, before inoculation, samples showed poor flow ability, as indicated by their CI (29.16%) and HR (1.41) values. This demonstrates strong particle cohesion, which causes the particles to withstand the tapping force. This could be due to the grinding of dried WFMPB, which created fine particles and increased the specific surface area. Through the creation of active binding sites, particle interactions and interparticle bonding may be enhanced. Industrial silos used to store cohesive powders are driven with mechanical and pneumatic assistance for continuous discharge to prevent arching and ratholing. However, free-flowing powders can be passed without using any external means (Sengar *et al.*, 2022).

CONCLUSION

In the present study, US treated-Freeze dried WFMPB powder significantly changed color values. No significant difference was observed for CI and HR values. The proximate composition of control and US treated samples had slight variations. Flow characteristics of CI and HR for control and treatment after inoculation was found to have passable flowability. In comparison, CI and HR for treatment before inoculation resulted in poor flowability. In future, the flowability of treatment before inoculation could be improved by using suitable processing techniques, so that, the developed product could exploit its application in designing storage structures.

FUTURE SCOPE

In this fast-running world, people are preferring ready to eat and ready to drink food products. Therefore, US treated freeze-dried WFMPB powder could be used as a premix in the development of ready to drink products.

In order to be used as premix, the stability and solubility of US treated freeze dried WFMPB powder should be improved using suitable processing techniques in further studies.

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Conflict of Interest. None.

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