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RESEARCH ARTICLE

Optimizing the stabilization techniques and enhancing the oil yield of Foxtail Millet Bran (FMB)

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Abstract

Millet bran, a by-product of preliminary processes is often discarded or utilised as animal feed. Despite of the nutritional advantage, the main challenge in using bran as food is due to its shortened shelf life. Stabilization of bran is essential to prevent hydrolytic rancidity and free fatty acid (FFA) formation by inactivating lipase enzymes. FMB was stabilized by three methods *viz.*, microwave (900 W, 2450 MHz), ultrasonication (500 W, 40 KHz) and blanching. Lipase activity and oil yield were studied. FMB was stabilized by microwave technique, maintaining an initial moisture to 21 % for 1 min (MT_1), 2 min (MT_2), 3 min (MT_3) and ultrasonication for 20 min (UT_1), 40 min (UT_2), 60 min (UT_3) and blanching for 20 min (BT_1), 40 min (BT_2), 60 min (BT_3) followed by cabinet drying. Lipase activity was measured in control and stabilized millet bran. Lipase activity of control (untreated bran) was 0.01 and it was reduced to 0.0005, 0.001, 0.0003, 0.00042 and 0.0001 in UT_1, MT_1, MT_2, BT_1 and BT_2 respectively. Lipase activity was found to be nil in UT_2, UT_3, MT_3 and BT_3. Oil yield was found to be increased in stabilized bran from 10.13 % (MT_2) to 10.95 % (MT_3) compared to control (10.01 %). Changes in nutritional characteristics of stabilised FMB were studied. In stabilised FMB increase in protein content a decrease in moisture, ash and fiber was observed in MT3. In conclusion, microwave (MW) treatment for stabilization of FMB significantly improved the stability and oil yield. The emerging debranning and stabilizing technologies may accelerate the utilization of millet bran for industrial application.

Keywords: debranning; lipase activity; microwave technology; stabilization; ultrasonication

Introduction

India cultivates 16.9 Mt of millets on about 12.7 Mha, which accounts for about 6 % of the country's total food grain supply. The two main categories of millets are major and minor millets. It yields 0.35 Mt of minor millet from 0.44 Mha produced in India. One minor type of millet is foxtail millet (1). The Chinese-born foxtail millet (*Setaria italica*) is currently grown all over the world and is one of the most significant food crops in the semiarid tropics (2). The husk and bran content of minor millets vary, with foxtail millet having 13.5 %husk and 1 - 2 % bran (3). Milling, dehulling, dehusking and debranning are primary processes that usually remove 16 - 17 % of the bran from minor millets. For tiny millets, the production figures show that after dehulling, between 0.062 to 0.066 Mt of bran are produced. The percentage of bran recovered from foxtail millet was greater at 14.37 ± 1.10 % (4).

Acquired as a by-product, this bran is either thrown away or used as animal feed. The analysis of FMB revealed the following composition: 9.39 ± 0.17 % crude oil. The oil extracted from FMB contained 64.83 ± 0.83 mg of tocopherol per 100 g, primarily composed of 15.53 ± 0.31 mg/100 g α-tocopherol and 48.79 ± 0.46 mg/100 g γ-tocopherol. Additionally, the foxtail millet bran oil (FMBO) was rich in linolenic acid (66.5 %), oleic acid (13.1 %) and saturated fatty acids, including stearic and palmitic acids (5). The generation of FFA and lipase activity are the main markers of hydrolytic breakdown in rice bran. The more common and uncomplicated measurement of FFAs is still the most extensively used and accurate indication of degradation, even though lipoxygenase and peroxidase activities are also utilized as indicators of rice bran degeneration (6). Bran's inherent instability caused by the breakdown of lipids into FFA through lipase activity leads to its underutilization and relegation as cattle feed, despite its significant nutritional benefits as well as potential for human consumption. Therefore, bran must be promptly stabilized

after extraction to preserve its nutritional integrity and prevent degradation, ensuring its value for potential human consumption (7).

The literature has explored a wide range of methods for stabilizing rice bran, including advanced techniques such as extrusion, MW heating, hot air heating, autoclaving, infrared (IR) heating, ohmic heating and radio frequency (RF) heating. Additionally, treatments like ultraviolet (UV) exposure, ultrasound treatment and gamma irradiation have been investigated for their potential to preserve rice bran. Beyond thermal and irradiation methods, the incorporation of antioxidants, enzymes and phenolic compounds has also been studied to enhance stability. Furthermore, traditional methods such as parboiling, toasting, roasting and steaming have been examined for their effectiveness in improving the shelf life and nutritional quality of rice bran. This present study explains about the impact of microwave, ultrasonication and blanching techniques on inactivating the lipase and other nutrition composition.

Materials and Methods

Freshly harvested FMB was procured from Amutham foods, Pudukkottai, Tamil Nadu. The procured bran was stored at low temperature until the analysis of proximate composition.

Optimization process for stabilization of FMB

Microwave oven

In the experiment, three samples of 100 g each with 21 % moisture content were subjected to MW heating at 100 % power (IFB model 900W, 2450MHz), with varying exposure times. The moisture content was adjusted to 21 % by adding the required amount of water, as calculated and mixing continuously at medium speed in a blender to ensure the even distribution of water (8). The first sample (MT1) was heated for 1 min, while the second sample (MT2) received 2 min of heating and the third sample (MT3) was exposed to 3 min of heating.

Ultrasonication

A 100 g sample was placed in a beaker, to which 500 mL of distilled water was added. The beaker was then submerged in an ultrasonic cleaner (LMU C-25 model 500W, 40KHz) for treatment. After the ultrasonic treatment, the sample was drained and transferred to a cabinet dryer set to 50 °C for further drying. Three distinct drying conditions were tested: UT1, where the sample was treated for 20 min at 40 °C; UT2, where the treatment was extended to 40 min at 47 °C and UT3, where the sample was dried for 60 min at 51 °C.

Blanching

A 100 g sample was placed in a domestic steam cooker and subjected to different treatment durations. BT1 was treated for 20 min, BT2 for 40 min and BT3 for 60 min.

Proximate analysis

Moisture estimation

Moisture content was determined by AACC, 2000 method (9). 2 g of the sample weighed in a pre-weighed Petri dish, which was then dried at 130 ± 3 °C for 3 hr. After drying, the Petri dish was cooled in a desiccator and weighed. This drying, cooling and weighing process was repeated every 30 min until the weight difference between two consecutive measurements was less than 1 mg.

Protein estimation

The protein estimation was done according to AOAC 954.01 - 2010 (10). The nitrogen content of the sample was measured using the Kjeldahl method with Pelican Kel plus equipment. The crude protein was subsequently calculated by multiplying the nitrogen content by a factor of 6.25.

Fat estimation

Fat content in the bran samples was determined by AOAC 922.06 - 2007 method (11). Fat content in the FMB was estimated in Soxhlet apparatus as crude ether extracted method using moisture-free samples. The solvent was evaporated and the remaining fat residue was weighed.

Fiber estimation

The crude fiber content was estimated according to the method given by AOAC 962.09 - 2007 (12). Crude fiber in the sample was measured by using samples that were free of moisture and fat. Crude fiber is estimated by boiling a 2 g of bran sample with dilute acid and alkali to replicate the digestive processes. The fiber, which remains undissolved, is collected by filtration. The residue is then dried, weighed and ashed to remove any mineral contamination.

Lipase activity

Lipase activity was measured according to the method given in Biochemical Methods book with some modifications (13). Lipase (Triacylglycerol lipase (EC 3.1. 1.3)) hydrolyses triglycerides to release FFA and glycerol. The principle behind determining lipase activity is based on measuring the amount of fatty acid released over time, which is indicated by the quantity of NaOH needed to keep the pH constant. The enzyme activity is then quantified by the mEq of alkali consumed. The sample was defatted prior to enzyme estimation using n-hexane. It was mixed with hexane at a 1: 3 ratio and stirred for 30 min in three separate batches. After each stirring period, the hexane layer was decanted and added fresh hexane for the next batch (14). A 3 g defatted sample was ground and homogenized with twice its volume of ice-cold acetone. The mixture was then filtered; the resulting powder was washed sequentially with acetone, a 1:1 acetone-ether mixture and ether, before being left to air dry. For enzyme extraction 1 g of the dried powder was dissolved in 20 mL of ice-cold water, followed by centrifugation at 15000 rpm for 10 min. The supernatant was collected as the enzyme source. To prepare the substrate, 2 mL of coconut oil was neutralized to pH 7.0, mixed with 25 mL of distilled water, 100 mg of bile salts and then stirred to form an emulsion. To enhance emulsification, 2 g of gum arabic was added. In a 500 mL beaker, 20 mL of the substrate and 5 mL of phosphate buffer (pH 7.0) were combined, stirred at 35 °C and the pH was adjusted to 7.0. Then, 0.5 ml of enzyme extract was added, the pH was monitored and maintained at 7.0; by adding 0.1 N NaOH as required. This process continued for 30 to 60 min and the volume of alkali consumed was recorded. Enzyme activity was expressed as the amount of enzyme that produces one mEq of FFA per min per g of sample. Specific activity was given in mEq per min per g (mEq/min/g) (eq. 1).

$$\text{Activity/meq/min/g sample} = \frac{\text{Volume of akali consumed x strength of alkali}}{\text{Weight of the sample in gram x time in minute}} \quad \text{Eq. 1}$$

Oil yield

Oil yield was determined by Soxhlet apparatus using petroleum ether as solvent. 2 g of the sample was weighed and placed in a filter paper thimble in the Soxhlet apparatus. The thimble was attached to a weighed receiver containing 150 mL of petroleum ether (B.P. 60 - 80 °C). The condenser was set up, with water circulating in the condenser. As the petroleum ether evaporated and condensed, it dissolved the fat from the sample, which was then siphoned back into the bottom flask after filling the extraction chamber. This siphoning process was repeated 6 - 8 times for complete extraction. After evaporating the petroleum ether, the flask was cooled in a desiccator, the residue was weighed and the fat content was calculated as a percentage using eq. 2.

$$\text{Percentage of crude fat} = \frac{(W2-W1)}{X} \times 100 \quad \text{Eq. 2}$$

Where,

W2= Weight of the flask + ether extract (g)

W1=Weight of the receiver/bottom flask (g)

X= Weight of the bran sample taken

Statistical analysis

The experiments were carried out in triplicate and the results are expressed as mean values. A completely randomized design, one-way analysis of variance (ANOVA) with Duncan's multiple test was conducted using OPSTAT software to assess significant differences among the various treatments i.e., control, MT1, MT2, MT3, UT1, UT2, UT3, BT1, BT2, BT3.

Results and Discussion

Effect of different stabilization methods on oil yield and lipase activity

Lipase activity

Effect of different stabilization treatment (microwave, blanching and ultrasonication) on lipase activity was studied. The results of lipase activity are depicted in Fig. 1. Inactivating lipase is crucial for preventing rancidity and preserving the quality of the oil. Lipase activity is expressed as the amount of enzyme that produces one mEq of FFA per min for each g of sample (meq/g/min). The lipase activity in the control sample was measured at 0.01. However, in the MW-treated samples, there was a significant reduction in enzyme activity, dropping from 0.001 (MT1 = 900 W, 1 min) to 0.00 (MT3 = 900 W, 3 min). This decline in activity can be attributed to the denaturation of the enzyme's protein structure, induced by the high power and frequency of the MW treatment. Several studies have emphasized that lipase is unstable and easily inactivated at high temperatures (15). Lipase activity in the blanched treatments was measured at 0.00042 (BT1 = 20 min), 0.0001 (BT2 = 40 min) and was undetectable in BT3 (60 min). By using the domestic kitchen equipment for steaming at 130 °C for 2 min, the FFA, acid value and peroxide value of rice bran has been significantly reduced while comparing to its raw form (16). MW and high-pressure steam treatments were effective in slowing the rise in FFA and inhibiting lipase activity, whereas steaming had the least impact (17). Steaming will increase free fatty acid content from 2.8 % to 6.1 % (Amarasinghe). This demonstrated that steaming had a more effective stabilization impact than dry heating. The ultrasonication treatment demonstrated a significant inactivation effect, effectively stabilizing the FMB. The resulting lipase activity was 0.0008 (UT1 = 500 W, 40 kHz, 20 min), whereas it was undetectable in both UT2 (500 W, 40 kHz, 40 min) and UT3 (500 W, 40 kHz, 60 min). High-frequency sound waves that create mechanical energy which leads to the denaturation of FMB lipase, causing

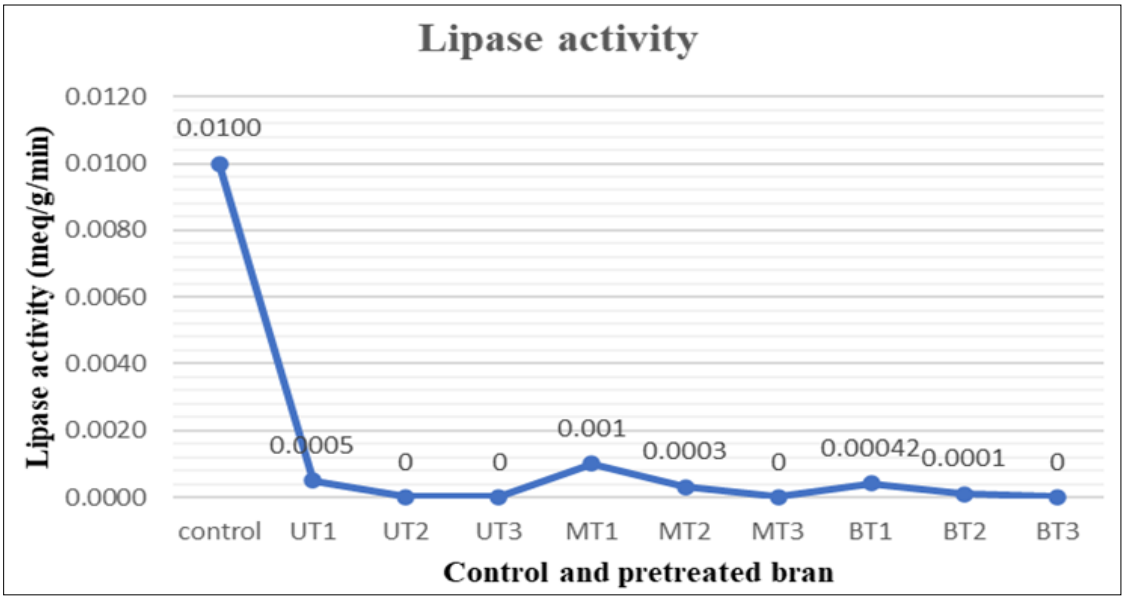

Fig. 1. Effect of different stabilization treatment (microwave, blanching and ultrasonication) on lipase activity.

UT1 = 500 W, 40 khz, 20 min, UT2 = 500 W, 40 khz, 40 min, UT3 = 500 W, 40 khz, 60 min

MT1 = 900 W, 1 min, MT2 = 900 W, 2 min, MT3 = 900 W, 3 min

BT1 = 20 min, BT2 = 40 min, BT3 = 60 min

changes in its tertiary and quaternary structures and resulting in the loss of catalytic activity. However, there is limited research on the use of non-ionizing radiation for stabilizing rice bran and further studies are required. If successful, non-ionizing radiation could provide a scalable, cost-effective and convenient method for stabilizing rice bran (18).

Oil yield

The results of oil yield of different stabilized FMB are given in Table 1. The oil yield results from the various treatments were analysed for statistical significance at 5 % level of significance. The control treatment yielded 10.010 ± 0.207 %, which was significantly ($p < 0.05$) higher than the oil yield from UT2 (8.090 ± 0.361 %), which was the lowest among the ultrasonication treatments. Ultrasonication treatments, specifically UT1 (9.003 ± 0.340 %) and UT3 (9.050 ± 0.298 %), exhibited moderate yields, with UT1 and UT3 not showing significant differences from each other. MW treatments yielded higher amounts of oil, with MT3 (10.950 ± 0.190 %) achieving the highest yield among all treatments, followed by MT1 (10.237 ± 0.093 %) and MT2 (10.130 ± 0.262 %). The oil yields for MT1, MT2 and MT3 were statistically similar, indicating no significant difference between them. Blanching treatments showed competitive results, with BT2 (10.447 ± 0.057 %) and BT3 (10.480 ± 0.406 %) yielding significantly higher amounts of oil compared to BT1 (9.313 ± 0.304 %). BT2 and BT3 exhibited similar oil yields, while BT1 had a significantly lower yield. In conclusion, MW treatments, particularly MT3 yielded the highest oil amounts, followed by blanching treatments (BT2 and BT3) which also provided competitive results. Ultrasonication treatments, on the other hand were less effective, with UT2 yielding the lowest oil amount. MW treatment of rice bran leads to an increase in both oil and protein content, enhancing its nutritional and commercial value (8). In bran that had been MW-heated and had a higher first moisture content (21 %), bran pellets were created due to the aggregation of bran particles because of microwave heating and this bran particle aggregation is thought to be advantageous when it comes to oil extraction (19).

Table 1. Effect of different stabilization technique on oil yield

Sl. No	Treatments	Oil yield
1	Control	$10.010^{a} \pm 0.207$
2	UT1	$9.003^{b} \pm 0.340$
3	UT2	$8.090^{c} \pm 0.361$
4	UT3	$9.050^{b} \pm 0.298$
5	MT1	$10.237^{a} \pm 0.093$
6	MT2	$10.130^{a} \pm 0.262$
7	MT3	10.950 ± 0.190
8	BT1	$9.313^{b} \pm 0.304$
9	BT2	$10.447^{a} \pm 0.057$
10	BT3	$10.480^{a} \pm 0.406$

all values are noted as mean ± standard deviation (sd)

Note: the means with different letters as superscripts are significant ($p < 0.05$). the means with same letters or having common letter (s) are not significantly different

UT1 = 500 W, 40 khz, 20 min, UT 2 = 500 W, 40 khz, 40 min, UT 3 = 500 W, 40 khz, 60 min

MT1 = 900 W, 1 min, MT2 = 900 W, 2 min, MT3 = 900 W, 3 min

BT1 = 20 min, BT2= 40 min, BT3 = 60 min

The oil yield and lipase activity of various stabilization treatments were evaluated, with MT3 treatment (MW treatment at 900 W for 3 min) showing the highest oil yield and the lowest lipase activity. As a result, MT3 was selected for further proximate analysis, because it demonstrated superior performance compared to the other treatments.

Effect of MW treatment on moisture, protein, ash and fiber

Moisture, fat, protein, ash and fiber (Proximate) were analysed for control and MW treated (MT3) bran to understand the effect of MW treatment on macronutrients. For bran to remain stable throughout storage, its moisture content is essential. The rate at which lipids decompose into FFA decreases when the moisture content drops (20). Following the MW treatment (MT3), the moisture content dropped from 7.86 ± 0.21 % to 6.8 ± 0.3 % (Fig. 2). The microwave procedure alone was responsible for the samples' decreased moisture content. As a bipolar molecule, moisture absorbs the microwave power, heats up and then evaporates from the bran samples, therefore this is to be expected (21). After being MW-treated, the protein level rose from 11.18 ± 0.14 % to 12.42 ± 0.21 %. This might be because the protein was denatured by exposing bran to a suitable temperature and accessible microwave power. Similarly, ash content was increased from 11.05 ± 0.05 % to 11.19 ± 0.01 % after the treatment. Fiber is the core nutrient in the bran for which nutritionally recognised. Upon the treatment, fiber content was degraded to 48.02 ± 1.56 % from 58.03 ± 1.31 %. A study on microwave (MW) treatment of Lemont rice bran reported minor changes in nutrient composition. Protein content increased slightly from 16.07% to 16.33%, while fat and fiber content decreased marginally from 19.20% to 19.00% and

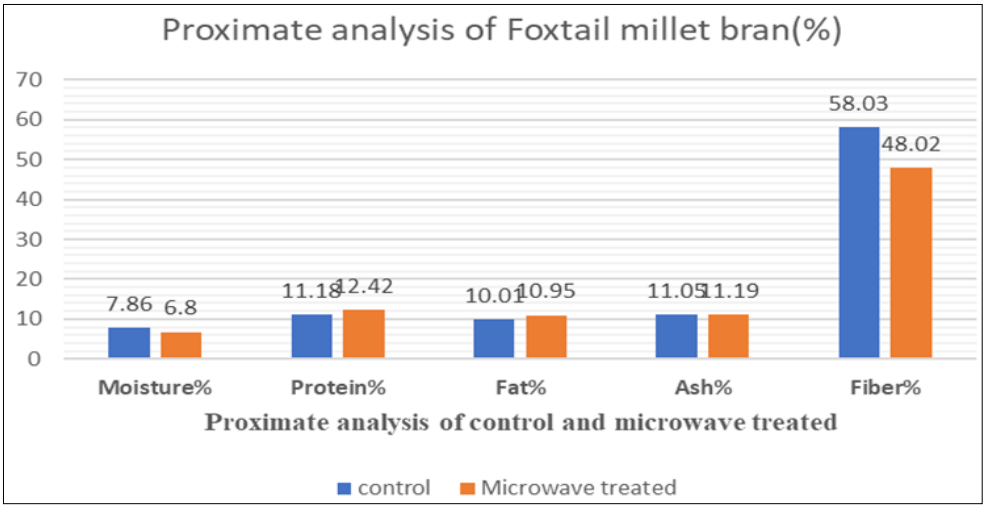

Fig. 2. Proximate analysis of control and MW-treated (MT3) FMB.

8.49% to 8.07%, respectively. Ash content showed a minimal reduction from 9.22% to 9.17%. Nitrogen-Free Extract (NFE) increased modestly from 47.02% to 47.42%, indicating a slight improvement in carbohydrate content. The most significant change was a substantial reduction in moisture content from 11.20% to 7.07% following MW treatment (19).

Conclusion

Stabilizing FMB is crucial for preserving its nutritional and therapeutic benefits. This process reduces lipase activity, preventing rancidity and maintaining the integrity of beneficial fats, antioxidants and bioactive compounds; thereby enhancing its health-promoting properties. Based on the above study, it can be concluded that MW treatment for 3 min at 900 W effectively reduced lipase activity while maximizing oil yield, making it an optimal method for enhancing the stability and nutritional quality of FMB. This treatment demonstrated the best results in enhancing the nutritional components of FMB, increasing protein (12.42 %), fat (10.95 %) and ash (11.19 %) compared to the control sample. However, it also led to a reduction in fiber content (48.02 %) and moisture (6.8 %), highlighting a trade-off in composition that may contribute to improved stability and oil yield. The conditions for stabilization should be optimized and modified for continuous, large-scale operations to ensure efficiency, consistency and scalability in commercial production.

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Authors' contributions

KP carried out experimental analysis and writing of article. VM carried out conceptualization, literature review, correction and proofreading of article. RV has done proof reading and put design for the article. MLM has helped to collect literature and has given technical help. AK contributed for literature collection and STS contributed in prefinal correction.

Compliance with ethical standards

Conflict of interest: All authors do not have any conflict of interest to declare.

Ethical issues: None

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