Effect of microwave heating or simple heat (pan roasted) treatment of rice bran on the rate of release of free fatty acids during storage at room temperature

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ABSTRACT

Development of Free Fatty Acids (FFA) is a major problem which affects the use of rice bran as a feed ingredient. The purpose of this research was to evaluate the effect of microwave heating and pan roasting on FFA development of rice bran during storage, proximate composition and to study the available phosphorous (P) content. Rice bran from raw and parboiled rice obtained from paddy variety BG 358 was treated using microwave heat and pan roasting. An experiment was conducted in a two-factor factorial with 2×3 factorial design. Treated samples were studied for FFA content up to 8 weeks in weekly intervals. Proximate composition was studied immediately after heat treatment and at the eighth week. Availability of P in rice bran was studied after heat treatment. Both the treatments significantly reduced the formation of FFA in rice bran during storage (P<0.05), while microwave heat treatment showed a higher effect on reduction of the formation of FFA content in raw microwave heated rice bran from 2.68 to 12.44 g 100 g⁻¹ and in parboiled microwave heated rice bran from 1.8 to 9.54 g 100 g⁻¹ during eight weeks of storage. There was no significant (P>0.05) difference in proximate composition of rice bran during the period of storage in any of the treatments. Both treatments improved P availability; however, pan-roasting had a high effect on P availability where raw pan roasted rice bran and parboiled pan roasted rice bran showed an increment of 17.67 and 19.43%, respectively. It is concluded that pan roast and microwave heat retard development of rancidity in rice bran, although at different degrees. These findings could be used with suitable modifications to the milling and storage processes to improve the quality of the rice bran available for animal feeding in Sri Lanka.

Keywords: Available phosphorous (P), FFA, microwave heating, pan roasting, rice bran

INTRODUCTION

Rice bran is the cuticle between paddy husk and grain and is produced as a by-product of rice milling. The definition of bran is the “by-product of polishing brown rice, comprising pericarp, aleurone layer, embryo and some endosperm (FAO, 1994). It is also sometimes called as cargo bran, cow bran, polish or white bran (Roy et al., 2011). Rice bran is one of the key ingredients in the animal feed industry in Sri Lanka. High amount of fat in rice bran leads to rancidity with the storage due to hydrolysis and formation FFAs. Development of FFA is one of the core problems of rice bran which affect its use as a feed...
ingredient (Rukmini, 2002). Development of FFA may reduce palatability due to off flavour and cause problems in digestion too.

Rice bran contains fat more than 20% of its weight and thus, it limits its use in animal feed industry. Rancidity occurs soon after production particularly if the grain has not been parboiled (Da Silva et al., 2006). The main factors for deterioration of rice bran during storage are enzymatic activity, microbial enzymatic activity, moulds formation and insect damage. Lipases of rice bran are the major cause of deterioration of oil in rice bran. The hydrolysis is catalysed by endogenous lipases and to some extent by microbial enzymes if the material is of poor quality (Wang et al., 2018).

Rancidity is caused by breaking down of lipids into FFA and production of off flavours is due to lipid hydrolysis and subsequent oxidation during storage. It occurs very rapidly at a tropical temperature (Nayik et al., 2015).

Rancidity in rice bran can be categorized as hydrolytic rancidity and oxidative rancidity (Malekian et al., 2000). Reaction of oxygen with unsaturated lipids involving free radical initiation, propagation and termination processes is called oxidative rancidity (Shahidi and Zhong, 2010). Hydrolytic rancidity occurs due to contact of lipase enzyme with natural fat, causing hydrolysis of fat to FFA and producing glycerol in the bran. Rice bran also contains lipoxygenase and peroxide both having a negative effect on the oxidative state of the bran. The inactivation temperature for lipases and associated enzymes is dependent on the moisture content. At the moisture content of 4%, the inactivation temperatures for lipoxygenase, lipase and peroxidase are 40, 55 and 70 °C, respectively (Juliano, 1985).

There are different ways of applying heat treatment to inactivate activity of the enzymes. Pan roasting and microwave heat treatment are easy to apply. Microwave heating is becoming increasingly popular and important in cooking and food processing. Microwave heating is considered to be one of the most energy-efficient types and a rapid method for heating food items (Yoshida et al., 1991). This method of cooking or processing saves time and energy. In Sri Lanka, microwave heating is becoming popular as a source of cooking and food processing. It is possible to use microwave heating and pan roasting to destroy lipase activity and to control FFA development in animal feed ingredients. Therefore, the objective of this research was to evaluate the effect of microwave heating and pan roasting on free fatty acid development in rice bran during storage and the available phosphorous (P) content.

**MATERIALS AND METHODS**

**Sampling and treatment**

Samples of paddy were taken from BG358 variety which was grown during Maha season 2009 in North Western Province. One sample was parboiled using
“domestic parboiling method” practiced in Sri Lanka by boiling at 92 °C for 105 min.

Both raw and parboiled paddy samples were milled in a rubber roller two stage mill. Samples of rice bran were exposed to stabilization treatments. Both raw and parboiled rice bran were treated with application of microwave and simple heat using hot pan. For microwave heat treatment, an oven which is operated at 860 W for 5 min was used. Rice bran was roasted in open aluminium pan at 80 °C for 10 min for pan roasting. Untreated rice bran samples from raw and parboiled paddy were used as controls. Treated and untreated rice bran was packed in polyethylene bags and placed in an incubator at 28 °C. Samples were taken for FFA analysis at one-week intervals.

**Determination of FFA content**

Fat extraction was done by using semi-auto fat analyser and petroleum ether (40 - 60 °C) was used as the non-polar solvent. Petroleum ether (25 mL) was taken into receiving flask and they were placed in apparatus. Samples were put into thimbles and they were placed in semi auto fat analyser. After two hours of extraction the receiving flask was kept in oven at 100 °C for drying until it gets a constant weight. Weight of the fat extracted from rice bran was taken.

Diethyl ether (25 mL), alcohol (25 mL) and phenolphthalein (1 mL) were mixed and carefully neutralized with 0.1 M sodium hydroxide. Extracted fat was dissolved with prepared solution and titrated with 0.1 M sodium hydroxide shaking constantly until pink colour was appeared. The volume of used sodium hydroxide (NaOH) was recorded and acid value was calculated using following (ISO 660; IUPAC 2.201 method) equation.

\[
\text{Acid value} = \frac{\text{Titration (mL)} \times 5.61}{\text{Weight of sample used}}
\]

The FFA figure is usually calculated as oleic acid (1 mL of 0.1 M NaOH = 0.0282 g of oleic acid) in which, the acid value is equal to 2 × FFA.

**Proximate analysis**

Crude protein, crude fat, crude fibre, moisture and ash were determined by standard AOAC method (AOAC, 2000). Carbohydrate content was determined by using the formula below;

\[
\% \text{ Nitrogen Free Extract (NFE)} = 100 - (\% \text{ crude protein} + \% \text{ crude fat} + \% \text{ crude fibre} + \% \text{ moisture} + \% \text{ ash})
\]
Determination of total phosphorous and available phosphorous

Total phosphorous and available phosphorous contents were determined using following procedure. Standard solution series was prepared to get the absorbance curve for the spectrophotometer using K$_2$HPO$_4$. Rice bran samples from different treatments were ash at 550 °C. Then, 0.5 g of ash was weighed and 10 mL of nitric and perchloric (4:2 ratio) and solution mixture was kept in block digester at 220 °C for one hour until clear mixture appears. It was kept for cooling and boiled for 15 min. with 10 mL distilled water in the block digester. Each volume was measured in to 50 mL volumetric flasks using pipette. Solutions was shaken well and kept for 15 minutes to develop the blue colour complex. From each sample, 1.0 mL was taken and volume up to 10 mL in volumetric flasks. Ammonium molybdate solution (2 mL) and N.S.A (Nitrosyl sulfuric acid) reagent (0.8 mL) is also added to each sample. Linear direct standard curve was used to measure the absorbance in SP-3000 + uv/vis spectrophotometer at 650 nm wave length.

Ash samples (0.5 g) obtained from the different treatments was shaken with 50 ml 2% citric acid for 1 h. Solution was filtered and residues were used to measure phosphorous content using the same procedure mentioned above.

\[
Concentration \text{ calculation} = \frac{Concentration \ (ppm) \times 50 \ mL \times 100 \ mg/g}{1000 \times 10 \ mL \times sample \ weight}
\]

Statistical analysis

Factorial experimental design was used as research design. ANOVA and t-test were performed to analyse FFA development under various treatments and to analyse the proximate composition respectively using MINTAB software.

RESULTS AND DISCUSSION

Effect of microwave heating and pan roasting on free fatty acid development

There was a significant ($P<0.05$) difference of FFA level in raw rice bran (RRB) with all the other treatment combinations one hour after milling. There was no significant ($P>0.05$) difference between parboiled rice bran (PRB) and raw pan-roasted rice bran (RPR). Though there were differences of FFA level in parboiled pan-roasted rice bran (PPR), raw microwave treated rice bran (RMW) and parboiled microwave treated rice bran (PMW), those differences were not significant ($P>0.05$).

PMW sample had the lowest initial FFA value and RRB has the highest initial FFA value. Difference between initial FFA value of RRB and PRB was significant ($P<0.05$). However, the difference between initial FFA of PRB and RPR was not significant ($P>0.05$) and also the difference between FFA values of PPR, RMW was not significant ($P>0.05$) at the initial stage (Table 1).
Malekian et al. (2000) reported that raw rice bran packed in vacuum packed bags and zipper top bags had 3.7% initial FFA level and microwave heated rice bran packed in vacuum packed bags and zipper top bags give 3.2% initial FFA level when stored at 25 °C. Similar results were recorded in the present study when raw and microwave treated rice bran value was obtained at the storage temperature of 25 °C whereas FFA an acid value in this research has been obtained at the storage temperature of 28 °C, at 78% RH.

Rice bran oil normally contains 1.5 - 2% FFA right after milling (Thanonkaew et al., 2012). According to Orthoefer (2005), rice bran oil contains 2 - 4% FFA at the time of milling. Roy et al. (2011) reported that the FFA increasing rate can be high as 5 - 7 % per day. FFAs accumulate to unacceptable levels (>5%) within a few hours after milling (Malekian et al., 2000). Up to 70% of FFA has been reported for a single month of bran storage (Roy et al., 2011). Rice bran is unfitted for human consumption when FFA concentration increases above 5%, which may happen within 12 h of milling. Once the FFA concentration exceeds 12%, it becomes unsuitable even for cattle feed, the lowest economic use of most crop by-products (Rukmini, 2002). Rice bran oil with an excess of 10% FFA is unfit for human consumption and due to high refining loss, bran with more than 5% FFA will be unprofitable for edible oil extraction (Sharif et al., 2014).

Malekian et al. (2000) reported 26.7% FFA in vacuum packed bags and 22.2% in zipper-top bags in raw rice bran during eight weeks of storage. According to Malekian et al. (2000), FFA values of microwave heat-treated rice bran were 3.2% at vacuum-packed bags and 3.3% in zipper-top bags after eight weeks of storage. According to Dubey et al. (2019), after 60 d under normal warehouse storage, the mean FFA of rice bran is 55.37%. The rate of FFA formation is highly dependent on environmental conditions (Orthoefer, 2005).

Pan roasting extends the shelf life of RRB and PRB up to 4 weeks of storage time with FFA level below 12% (Table 1). Microwave heat treatment extends the shelf life of RRB and PRB up to 8 weeks of storage time with FFA level below 12% (Table 1).

During the eighth week, there were differences in FFA% between all the treatments and those differences were significant ($P<0.05$). It shows that there was a significant effect ($P<0.05$) of pan roasting and microwave treatment on FFA development in rice bran, during storage.
Table 1: Free fatty acid content (g 100 g^-1 ether extract) in raw and parboiled rice bran during storage (8 weeks) after application of either microwave heating or simple heat treatment. All values are presented as mean+standard deviation.

| Week | RRB   | PBRB  | RSH   | PBSH  | RMW   | PBMW  |
|------|-------|-------|-------|-------|-------|-------|
| 0    | 9.11±0.87 | 4.39±0.29 | 5.54±0.44 | 3.11±0.23 | 2.68±0.38 | 1.80±0.14 |
| 1    | 11.85±0.27 | 10.15±0.01 | 10.75±0.18 | 7.66±0.1 | 3.45±0.02 | 2.46±0.14 |
| 2    | 13.14±0.02 | 12.36±0.22 | 11.33±0.16 | 9.25±0.23 | 4.31±0.19 | 3.52±0.06 |
| 3    | 14.62±0.33 | 13.84±0.16 | 12.65±0.43 | 10.11±0.1 | 6.02±0.05 | 4.94±0.1 |
| 4    | 18.24±0.05 | 15.30±0.03 | 13.50±0.37 | 12.23±0.1 | 6.74±0.01 | 5.88±0.11 |
| 5    | 20.35±0.08 | 16.60±0.4 | 15.51±0.23 | 13.03±0.15 | 7.90±0.05 | 6.76±0.3 |
| 6    | 21.67±0.26 | 18.00±0.07 | 16.80±0.43 | 14.14±0.1 | 9.45±0.01 | 6.97±0.05 |
| 7    | 24.01±0.24 | 19.83±0.19 | 17.76±0.12 | 16.59±0.14 | 10.43±0.14 | 7.63±0.05 |
| 8    | 25.62±0.16 | 21.92±0.06 | 19.48±0.13 | 17.53±0.08 | 12.44±0.13 | 9.54±0.37 |

n=3, Values bearing similar superscripts in each week (a-f) are significantly not different at α = 0.05

Values bearing similar superscripts in each treatment (k-s) are significantly not different at α = 0.05

R RB: Raw rice bran; PB RB: Parboiled rice bran; R-PR: Raw pan roasted rice bran; PB- PR: Parboiled pan roasted rice bran; R MW: Raw microwave heated rice bran; PB MW: Parboiled microwave heated rice bran

Pan roasting extends the shelf life of RRB and PRB up to 4 weeks of storage time with FFA level below 12% (Table 1). Microwave heat treatment extends the shelf life of RRB and PRB up to 8 weeks of storage time with FFA level below 12% (Table 1).

During the eighth week, there were differences in FFA% between all the treatments and those differences were significant. It shows that there is a significant effect (P<0.05) of pan roasting and microwave treatment on FFA development in rice bran during storage.

The rate of development of FFA was high in the first week and it was rapidly declined in the second week according to the Figure 1. Rate of FFA
development was below at 1% FFA/day throughout the storage period (Figure 1). Juliano, 1994; Lacerda et al., 2013 and Da Silva et al., 2006 agree with the present study results, who have observed rapid lipid deterioration, a progressive increase of free fatty acids, reduction of triglycerides in raw rice bran at environmental temperature, achieving acidity levels above 6% after some week of storage.

![Figure 1: Rate of FFA development during 8 weeks.](image)

**Effect of microwave heating and pan roasting on proximate composition of raw rice bran and parboiled rice bran**

Increase of crude protein content was observed in RRB, PRB, RMW and PMW after 8 weeks of storage time. Declining of crude protein content was observed in, RPR and PPR after 8 weeks of storage time (Table 2). Crude protein content in rice bran varies 9.8 to 15.4% according to Roy et al. (2011). Luh and Barber (1991) found that crude protein content in rice bran was 12.0 to 15.6%. In Sri Lanka according to Siriwardana (1969), crude protein content in raw rice bran was 12.5% and crude protein content in parboiled rice bran was 12.3%. Samarasinghe (2007) mentioned that the crude protein percentage in raw rice bran is 12.69% and parboiled rice bran is 10.41%. According to Industrial standards, the percentage of crude protein in rice bran should be a minimum of 13% (Gul et al., 2015). Rice bran used in the present research has indicated a minimum of 14% crude protein level, which is within the industrial standard. Microwave heat treatment and pan-roasting change crude protein contents slightly but changes do not exceed the standards or findings of many authors.
Crude fat content was increased in all treatments except PPR after 8 weeks of storage time (Table 2). Ether extract content in rice bran varies from 7.7 to 22.4% according to Roy et al. (2011). Ether extract content in rice bran varies with 15.0 - 19.7% according to Juliano, (1985). In Sri Lanka, according to findings of Siriwardana (1969), ether extract content in raw rice bran and parboiled rice bran were 14.6 and 20.3%, respectively. According to Samarasinghe (2007), ether extract in raw rice bran and parboiled rice bran was 18.64 and 16.06%, respectively. According to the findings of this experiment, ether extract content ranges from 20.77±0.39 to 24.67±1.07%. In raw rice bran ether extract value varied from 20.77±0.39 to 24.31±0.48%. Ether extract content in parboiled rice bran varied from 22.24±0.11 to 24.67±1.07%. Similar results have been recorded by Roy et al., (2011) parboiled rice bran has higher ether extract content than raw rice bran. According to industrial standards for the percentage of crude fat in rice bran, it should be minimum of 16% (Gul et al., 2015). Rice bran used in the present research has indicated a crude fat level minimum of 20%, which is with in the industrial standard.

Pan roasting and microwave heating had a positive effect on reducing crude fibre content during the storage (Table 2). The high fibre content in bran makes it largely unsuitable for inclusion in unlimited proportions in rations for monogastric animals. According to Roy et al. (2011), crude fibre content was varied with 5.7 to 20.9%. Luh and Barber (1991) found that crude fibre content in rice bran was 7.0 - 11.4%. In Sri Lanka, according to Siriwardana (1969), the crude fibre content in parboiled rice bran was 10.7%. Samarasinghe (2007) mentioned that crude fibre content in raw rice bran and in parboiled rice bran was 9.38 and 19.67%, respectively. Industrial standards for rice bran according to Gul et al. (2015) specifies that good quality rice bran should have crude fibre less than 9%. The crude fibre content reported in the experiment is within this limit.

Nitrogen-free extract (NFE) percentage was decreased in all treatments except pan-roasting after 8 weeks of storage (Table 2). According to Roy et al. (2011), Nitrogen-free extract contents in rice bran were between 34.1 to 46.1%. Luh and Barber (1991) found that nitrogen-free extract content is between 31.1 to 52.3% in rice bran. In Sri Lanka, Siriwardana (1969) found that Nitrogen free extract contents in raw rice bran were 47.9% and nitrogen-free extracts contents in parboiled rice bran were 36.1%. According to Samarasinghe (2007), Nitrogen free extract contents in raw rice bran and in parboiled rice bran from Sri Lanka were 49.04 and 39.92%, respectively.
Table 2: Effect of pan roasting and microwave heating on proximate composition during the 1st to the 8th week.

| Treatment | Week | Ash (%) | Crude protein (%) | Crude fat (%) | Crude fiber (%) | Nitrogen free extract (%) |
|-----------|------|---------|-------------------|---------------|-----------------|---------------------------|
|           |      | Mean±SD | Mean±SD           | Mean±SD       | Mean±SD         | Mean±SD                   |
| R RB      | 0    | 8.24±0.02 | 14.32±0.02       | 21.92±0.07    | 7.83±0.05       | 47.69±0.10                |
|           | 8    | 8.37±0.03 | 14.47±0.08       | 24.31±0.48    | 8.13±0.08       | 44.22±0.05                |
| P RB      | 0    | 8.18±0.06 | 14.26±0.06       | 23.13±0.07    | 7.97±0.04       | 46.46±0.06                |
|           | 8    | 8.36±0.08 | 14.43±0.05       | 23.74±0.64    | 8.14±0.08       | 45.32±0.71                |
| R MW      | 0    | 7.65±0.15 | 14.97±0.06       | 22.89±0.08    | 7.93±0.02       | 46.56±0.10                |
|           | 8    | 8.53±0.05 | 15.14±0.09       | 24.27±0.61    | 7.92±0.02       | 44.14±0.74                |
| P MW      | 0    | 7.47±0.09 | 15.11±0.09       | 23.68±0.02    | 7.82±0.02       | 45.92±0.05                |
|           | 8    | 8.76±0.06 | 15.21±0.13       | 24.67±1.07    | 7.81±0.04       | 43.55±1.08                |
| R PR      | 0    | 7.61±0.08 | 15.57±0.10       | 20.77±0.39    | 8.33±0.09       | 47.71±0.38                |
|           | 8    | 7.47±0.06 | 15.54±0.06       | 20.78±0.46    | 8.28±0.05       | 47.93±0.58                |
| P PR      | 0    | 8.82±0.09 | 15.54±0.08       | 22.35±0.42    | 8.61±0.24       | 44.68±0.46                |
|           | 8    | 8.63±0.19 | 14.96±0.25       | 22.24±0.11    | 8.07±0.04       | 46.10±0.27                |

Ash percentage has increased in all treatments except pan-roasting after 8 weeks of storage time (Table 2). In both raw and parboiled rice bran ash contents were slightly change due to the application of simple heat or microwave heat treatment. According to Luh and Barber (1991) crude ash content was 6.6 – 9.9% in rice barn. The change reported did not exceed the findings of Luh and Barber (1991) and findings of Roy et al. (2011). Moreover, Roy et al. (2011) founds that ash content in rice bran was 7.1 to 20.6%. In Sri Lanka, according to Siriwardana (1969), ash contents of raw rice bran and parboiled rice bran were 10.7 and 10.7%, respectively. Industrial standards for rice bran according to Gul et al. (2015) mention maximum ash content for raw rice bran and parboiled rice bran were 10 and 15%, respectively.

In parboiled rice bran phosphorous content was varied from 12.1 - 15.4 mg g⁻¹ in Sri Lanka (Samarasinghe, 2007). According to Ibrahim (1988), phosphorous content in rice bran varies from 8.6 to 10.4 mg g⁻¹. Findings of this experiment within the range of the findings of Samarasinghe (2007) close to the range of findings of Ibrahim (1988).
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Figure 2: Total phosphorous contents in rice bran after different treatments (R RB - Raw rice bran, P RB - Parboiled rice bran, R PR - Raw pan roasted rice bran, P PR - Parboiled pan roasted rice bran, R MW - Raw microwave heated rice bran P MW - Parboiled microwave heated rice bran)

Effect of microwave heating and pan roasting on available phosphorous content in rice bran

The current study indicated that there was a significant effect ($P<0.05$) in available phosphorus content due to six different treatments (R RB, P RB, R MW, P MW, R PR, P PR). In RRB, available phosphorous content was 0.0198±0.0003 mg g$^{-1}$ and PRB contained 0.0211±0.002 mg g$^{-1}$ of available phosphorous. RMW has 0.0201±0.0006 mg g$^{-1}$ of available phosphorous (Figure 3). PMW has available phosphorous content of 0.0220±0.0002 mg g$^{-1}$. RPR has available phosphorous content of 0.0233±0.002 mg g$^{-1}$. PPR has 0.0252±0.002 mg g$^{-1}$ of available phosphorous.

In RRB available phosphorous content has increased by 0.003 mg g$^{-1}$ after microwave treatment 0.003 mg g$^{-1}$ and percentage of increase was 1.51%. Increment of available phosphorous in RRB after pan-roasting was 0.0035mg g$^{-1}$ and percentage increase was 17.67%. In parboiled rice bran increment of available phosphorous after microwave treatment was 0.0009 mg/g and percentage of increase was 4.26%. Increment of available phosphorous in parboiled rice bran after pan-roasting was 0.0041 mg g$^{-1}$ and percentage increase was 19.43%.
Figure 3: Available phosphorous contents in rice bran after different treatments (R RB - Raw rice bran, P RB - Parboiled rice bran, R PR – Raw pan roasted rice bran, P PR – Parboiled pan roasted rice bran, R MW - Raw microwave heated rice bran, P MW - Parboiled microwave heated rice bran).

RRB contains 0.03% of available phosphorous. Available phosphorous percentage in PRB was 0.35%. RMW consists of 0.31% of available phosphorous. Available phosphorous content in PMW sample was 0.36%. RPR contains 0.35% of available phosphorous content. The available phosphorous content in PPR sample was 0.40%. This shows the effect of pan roasting over microwave treatment in increasing the available phosphorous content. Parboiling also increases the available phosphorous content.

CONCLUSIONS

Both pan-roasting and microwave heating are having a significant effect on controlling free fatty acid development in rice bran under Sri Lankan condition. Microwave heating is more efficient in controlling FFA development. Microwave heating should be applied as early as possible to reduce FFA development. There is no significant effect of domestic parboiling, pan roasting and microwave heating on proximate composition of rice bran during storage of the period of 8 weeks. Both pan roasting and microwave heating treatments improves phosphorous availability.

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