EFFECTS OF VARIOUS STABILIZATION TECHNIQUES ON THE NUTRITIONAL QUALITY AND ANTIOXIDANT POTENTIAL OF BREWER'S RICE

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Abstract

Brewer's rice is a nutritionally rich by-product of rice milling industries but remained unutilized due to its high susceptibility to lipid oxidation. Thus, it is desirable to develop certain strategies to stabilize the brewer's rice for food application. In the present study, the effects of microwave heating, gamma irradiation, and chemical (hydrochloric acid) treatments in stabilizing brewer's rice were investigated. Result showed that microwave-heated brewer's rice contained lower FFA content as compared with control and irradiation. However, FFA content in brewer's rice increased significantly ($p < 0.05$) during storage for all methods, except for hydrochloric acid (HCl) treatment. All the stabilizations methods showed non-significant ($p < 0.05$) effect on protein, fat, and ash content. However, microwave and chemical treatment significantly ($p < 0.05$) reduced moisture content. All methods showed no significant ($p < 0.05$) reduction on phenolic contents but significantly ($p < 0.05$) reduced the γ-oryzanol and α-tocopherol contents. Microwave heating was able to reduce the oxidation of brewer's rice without affecting other bioactive molecules present in brewer's rice. Therefore, microwave heating can be considered as the most suitable technique for stabilizing brewer's rice.

Keywords: Brewer's rice, Oxidation, Microwave heating, γ-Irradiation, Chemical treatment, Antioxidant compounds.

Abbreviations	
FFA	Free fatty acid
GAE	Equivalents of gallic acid
HPLC	High performance liquid chromatography
TPC	Total phenolic compounds

1. Introduction

Rice is a staple food for a large part of the world's human population covering from East and South Asia, the Middle East to Latin America [1, 2]. Rice milling process produces many by-products, which are gaining significant interests among researchers to converts them into value added products addition. The most significant rice-milling by-product is rice bran, which currently showed protection against various maladies, i.e., cancer insurgence, antioxidant activity, and hypocholesterolemic effects [3-5].

Besides rice bran, brewer rice is another rice milling by product, which possesses significant nutritional value. Brewer's rice comprises of mixture of broken kernels with intact rice germ and usually separated during paddy milling process as it passes through the rotary sieves after polishing stage. Intact rice germ consisted of carbohydrate, protein, essential lipids, natural antioxidants such as tocopherols, tocotrienol, γ-oryzanol, vitamin B complex, and minerals [6, 7]. It have been known that dietary intake all of these constituents could result in lowering blood serum cholesterol, reducing the incidence of oxidative-stress related degenerative diseases such as cancer, cardiovascular disorders, inflammation, aging and obesity [7].

Although brewer's rice is rich in high valuable food components, high susceptibility towards lipid oxidation limits its application as a food ingredient. Its limited shelf life and rapid deterioration at ambient temperatures make it unsuitable for human consumption. Storage of brewer's rice at room temperature also leads to development of off-flavour and odours, mainly due to the enzymes, microorganisms, and insects activity, which accelerate its deterioration [8]. One of the methods that could prolong brewer's rice shelf life is by inhibiting the potential sources that accelerate rancidity. Several stabilization methods are able to extend product shelf life up to 6 months when stored at room temperature [9]. Stabilization methods such as extrusion cooking, chemical treatment, gamma irradiation, and microwave treatment are effective in reducing the extent of deterioration of the by-products [10, 11]. Proper stabilization process is able to deactivate several enzymes such as lipase and lipoxygenase that cause rancidity [12].

Since stabilization could inhibit rancidity and prolong brewer's rice shelf life, the selection of appropriate methods that are also able to preserve its nutrient compositions become more challenging. Hence, the present study was conducted to compare the effect of different stabilization methods, i.e., microwave heating, γ-irradiation and hydrochloric acid (HCl) treatment on the free fatty acid (FFA) formation, proximate compositions, total phenolics compounds (free and bound phenolics) and antioxidant contents (γ-oryzanol and α-tocopherols) of brewer's rice. The outcomes of the research are helpful for industries to convert brewer's rice into value added products due to the presence of various bioactive components.

2. Materials and Methods

2.1. Samples preparation

Samples of brewer's rice were freshly collected from local milling factory in Sekinchan, Selangor, Malaysia. The samples collected from receiving bags after milling process of rice were placed immediately into square plastic (polypropylene) containers to protect from light oxidation and stored at 4°C. Prior to analysis, the samples were sieved in order to remove husks, broken pieces of rice, and others unwanted materials.

2.2. Chemicals and reagents

Hydrochloric acid, isopropanol, sodium hydroxide, sodium sulphate anhydrous, cuprum sulphate, selenium dioxide, sulphuric acid, boric acid, petroleum ether, hexane, ethyl acetate, Follin Ciocalteu's phenol reagent, sodium carbonate, ethanol, acetone, acetic acid, acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from Merck (Germany). Phenolphthalein, methyl red, methylene blue were purchased from Fisher (New Jersey, USA), whilst standard (γ-oryzanol and α-tocopherols) were obtained from Sigma (St. Louis, USA) while gallic acid was from Acros Organic (NJ, USA).

2.3. Stabilization process of brewer's rice

Brewer's rice was being treated with three different stabilization treatments, namely microwave heating, gamma irradiation and HCl treatment. For microwave stabilization, the method proposed by Ramezanzadeh et al. [13] was slightly modified. The moisture content was adjusted to 21% from 12% by adding deionised water prior to microwave stabilization. The microwave chamber of microwave oven (Model R-958A, 2450 MHz Sharp Electronic Corp) was pre-heated at 100 % power for 2 min. The sample (150 g) placed in microwaveable plastic container was heated at medium power for 3 min. Before being packaged using polyethylene zipper top bag, sample was allowed to cool at room temperature.

For gamma irradiation based stabilization, the method of Sung (11) was applied with slight modifications. The samples (150 g) packed in polyethylene bag were subjected to 1kGy γ-irradiation (60Co) doses at the MINTec-SINAGAMA, Agensi Nuklear Malaysia. Meanwhile, the HCl treatment was carried out according to the procedure described by Prabhakar and Venkatesh [14]. Briefly, 40 ml of hydrochloric acid was sprinkled on 1 kg of brewer's rice and mixed well by hand using protective gloves. The samples were then packaged using polyethylene zipper top bag.

2.4. Free fatty acid content

Brewer's rice stabilized with different method were packed and stored into individual polyethylene zipper top bag. The stored samples were analyzed for free fatty acid (FFA) content at 0, 4, 8, 12, and 16 weeks of storage using protocols mentioned by AOCS official methods (AOCS Official Methods Ca 5a-40) [15].

2.5. Proximate analysis

Proximate composition of brewer's rice was determined at 0 and 16 weeks of storage using standard AOAC methods [16] for protein (Method 992.5), fat (Method 945.16A), moisture (Method 985.14), and ash (Method 920.153). The amount of carbohydrates was calculated by subtracting protein, fat, moisture, and ash from 100.

2.6. Determination of total phenolic compounds

The procedures for extraction of free and conjugated phenolic compounds were adapted based on the methods described by de Mira et al. [17] with slight modifications, by varying the sample amount and repeating the extraction steps two times. The brewer's rice was turned into flour by centrifuged mill (Retsch ® ZM 200 Ultra Centrifugal Mill, Retsch GmbH Germany). The flour (10 gram) was slurried with 10 mL of 80% ethanol at room temperature for 10 min. After centrifugation at 5000 x g for 10 min, the supernatant was removed and the residue was re-extracted with fresh 80% ethanol. The supernatants were combined and the final volume was made up to 25 mL with 80% ethanol. The extracts were stored at - 20°C until further analysis.

Non-extractable bound phenolics were solubilized according to the procedure described by de Mira et al. [17] with slight modifications. Brewer's rice flour (10 gram) was extracted twice with 80 % ethanol for 10 min (2×10 mL) and hexane (1×10 mL) to remove soluble phenolic acids and fat, respectively. The extracts were centrifuged at $7000 \times g$ for 10 min and its supernatant was discarded. The residue was hydrolyzed with 60 mL aqueous 4 M NaOH at room temperature and stirred with a magnetic stirrer under nitrogen atmosphere for 1 hours. Later, by gradual addition of ice-cold 6 M HCl, the pH of the mixture was adjusted between 1.5–2.0. It was then subjected to centrifugation at $7000 \times g$ for 30 min and the supernatant was extracted five times with 30 mL ethyl acetate. The ethyl acetate fraction was dried using anhydrous sodium sulphate and evaporated to dryness using a rotary vacuum evaporator at 35°C. The residues were re-dissolved in 80% ethanol to a final volume of 10 mL. All extracts obtained were stored at - 20°C until analysis. TPC of sample extracts were determined using the Folin - Ciocalteu methods according to the procedure described by Singleton et al. [18]. The reaction mixture contained 0.5 ml of ethanol extract and 0.5 ml of Folin-Ciocalteu's phenol reagent was vortexed for 10 s, followed by addition of 10 ml of 7% sodium carbonate. Mixtures were kept in dark at ambient conditions for 60 min to complete the reaction. The absorbance was measured at 715 nm using UV mini 1240 UV-Vis spectrophotometer (Shimadzu, Japan). The analysis was conducted in triplicate and average data was used to calculate TPC content. Gallic acid was used as a standard and TPC was expressed as mg equivalents of gallic acid /g of extract (mg GAE /g).

2.7. Determination of *γ*-oryzanol

Brewer's rice (20 g) was extracted with acetone in the ratio of 1:10 w/v by vortex mixing vigorously for 3 min at room temperature (RT). After extraction, the samples were centrifuged for 20 min at 2500 rpm. The supernatant was collected

and the residue was re-extracted two more times with fresh acetone. The supernatants were pooled, evaporated using rotary evaporator, and filtered using a 0.45 µm nylon membrane filter prior to high performance liquid chromatography (HPLC) analysis.

For HPLC procedures, the procedures outlined by Chen and Bergman [19] were employed with slight modification. The HPLC system for γ-oryzanol detection consisted of a Waters 486 tuneable absorbance detector, Waters 600 Pump and UV/Vis detector. The separation was carried out using Eclipse XDB-C18 (4.6×250, 5 µm) analytical column at room temperature. The mobile phase-A consisted of aceto-nitrile: MeOH: IsOH: aqueous acetic acid (1%) at the ratio of 45:45:5:5. Mobile phase B consist of acetonitrile: MeOH: IsOH at the ratio of 25:70:5. The gradient used was as follows: 100 to 0 % solvent A (0 to 20 min.) and 0 to 100% solvent A (20 to 40 min). The flow rate was kept at 1 mL /min. γ-Oryzanol was detected at 330 nm. Peak identification and quantification was carried out using retention time and area of curve covered by γ-oryzanol.

2.8. Determination of *α*-tocopherol

The extraction procedure for α-tocopherol was similar to γ-oryzanol. The protocols for HPLC analysis was carried out according to the Chen and Bergman [19] with slight modification. The HPLC system for α-tocopherol consisted of Jasco MD-2010 Plus–Multi-wavelength detector, Jasco PU-2080 Plus-Intelligent-HPLC pump and Jasco FP-2020 Plus-Intelligent-fluorescence detector (FLD). The separation was carried out using Eclipse XDB-C18 (4.6x250, 5µm) Analytical column at room temperature. The mobile phase A consisted of acetonitrile:MeOH:IsOH:aqueous acetic acid (1%) at the ratio of 45:45:5:5. Mobile phase B consisted of acetonitrile: MeOH: IsOH at the ratio of 25:70:5. The flow rate was kept at 1 mL /min. The gradient condition for α-tocopherols was as follows: 100 to 0% mobile phase A (0 to 15 min) and 0 to 100% mobile phase A (15 to 30 min). The α-tocopherols were detected at excitation set at 295 nm and emission set at 350 nm. Peak identification and quantification were carried out based on comparison of retention time and area of α-tocopherol.
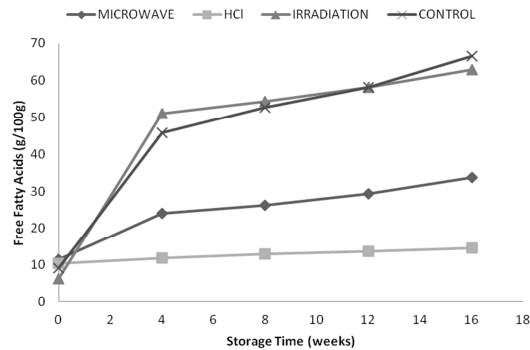
2.9. Statistical analysis

Data were analysed using the SPSS, version 16.0, (SPSS Inc., Chicago, IL, USA). The analysis of variance (ANOVA) using a general linear model was applied and the means comparison was conducted according to Duncan multiple range test procedure at $p < 0.05$.

3. Results and Discussion

3.1. Free fatty acid

In the present study, the effects of different stabilization methods including microwave heating, gamma irradiation and HCl treatments on the free fatty acids content of brewer's rice during storage were investigated. Figure 1 shows the content of FFA in brewer's rice during storage at room temperature up to 16 weeks. FFA content increased in all samples with the passage of time except for

HCl treatment. FFA content for control and γ-irradiation methods tremendously increased from week 0 to week 4, then slowly increased during the storage time until week 16. The FFA content at initial for all methods was around 6.23 – 11.48 %. At week 16, the control and γ-irradiation brewer's rice contained significantly ($p < 0.05$) higher FFA content than others with 66.61% brewer's rice and 62.87% brewer's rice, respectively. Similar trend was showed by microwave brewer's rice. FFA content increased at the early storage period until week 4, then gradually increased up to week 16. However, its' FFA content at week 16 was 33.68%, which is significantly ($p < 0.05$) lower than control and γ-irradiation brewer's rice. It is interesting to note that there was no significant ($p < 0.05$) difference in the FFA content for HCl treatment. The FFA content during week 0 and week 16 for HCl treatment were around 10.28 – 14.58 %, respectively.

Fig. 1. The Free Fatty Acids Content of Treated Brewer's Rice and the Control during Storage at Room Temperature (27±2°C).

The increment of FFA content in the brewer's rice stabilized by γ-irradiation was higher than microwave heat treatment. Initial FFA content in the brewer's rice stabilized by gamma irradiation was 6.23% and increased almost ten-fold up to 62.87% at week 16. While for microwave stabilization method, FFA content in the brewer's rice slowly increased during storage period.

Milling process of the rice caused the removal of bran layer from endosperm and disrupted the individual cells. The lipids present in bran usually react with highly reactive lipase enzymes resulting in the rapid hydrolysis of the triglycerides into glycerol and FFA. Further oxidation which can naturally occur due to the present of oxygen molecules and lipoxygenase resulted in the enzymatic rancidity of the food [10, 20]. Previously, several stabilization methods such as heat treatment including microwave heating, blanching, and chemical stabilization method inactivated the lipase and lipoxygenase, prolonged the shelf life of the product [10, 12, 13].

In the present study, FFA content in control increased during storage indicating the activities of lipases and lipoxygenase. The FFA content in week 0 was 9.16 % and increased to 66.61 % at week 16. Some other scientists reported the similar observations over the globe. In one such research study, Malekian et al. [10] found that FFA content in untreated rice bran increased from an initial value of 2.5% to 48.0% for sample packed in zipper-top bags and 54.3% for

sample packed in vacuum packs over 16 weeks of storage at room temperature. The rapid development of hydrolytic rancidity in un-stabilized rice bran makes the product unsuitable for human consumption [21, 22]. The amount of FFA for human consumption should not exceed more than 10 % FFA and 5 % FFA in bran oil and bran, respectively [21]. Thus, un-stabilized brewer's rice can be considered as unsuitable for human consumption owing to higher susceptibility for lipid oxidation.

It is interesting to note that HCl stabilization method controlled the formation of FFA in brewer's rice. The FFA content in the brewer's rice as stabilized through HCl treatment showed an increment only from 10.28 ± 0.09 % during initial stage of storage up to 14.58 ± 0.14 % within 16 weeks storage period. The present study showed that HCl treatment effectively inhibited the formation of FFA in the brewer's rice as compared to the others stabilization methods. Addition of hydrochloric acid to the brewer's rice decreased the pH of the environment being created and may be unsuitable for lipase to react with its substrate. Similar observation was reported by Prabhakar and Venkatesh [14] when HCl treatment stabilized rice bran with FFA contents in the range of 3.0–9.3%, during initial stages and after 51 days storage at room temperature which were significantly ($p < 0.05$) lower than un-stabilized rice bran. The optimum pH condition for lipase in rice bran is around 7.5-8.0 and addition of HCl decreased the pH and completely inhibited the lipase activity (Fig. 1). However, there is still less information on how low pH inhibited lipases activity and prevent rancidity.

Microwave stabilized brewer's rice contained significantly ($p < 0.05$) lower FFA content as compared with γ-irradiation and control. The low FFA indicate that the lower rate of enzymatic oxidation in brewer's rice. Microwave heating inactivates the enzymes that cause rancidity such as lipases and lipoxygenases through the internal heating of particles within the microwave cavity. The cavity makes the dipolar water molecules in the samples excited by the electromagnetic waves, resulting in enhancement kinetic energy along with the friction and produces an even distributed of heat through the samples [10]. In the present study, microwave treatment partially inhibited the activity of lipases and thus halting the process of rancidity. This indicated that longer heating time or heating at higher power is required for complete inactivation of the lipases by exciting more electromagnetic waves.

Brewer's rice stabilized by γ-irradiation showed similar trend as control indicating that lipases activity in brewer's rice was not affected by γ-irradiation. The rise in FFA with storage period may be due to the radiolysis of glycerides by γ-irradiation itself and enzymatic hydrolysis of glycerides by lipases. Similar observation was reported by Shin and Godber [23] whereby the increase of γ-irradiation from 5 to 15 kGy resulted in a greater loss of phospholipids and formation FFA of rice bran. This explained the significant ($p < 0.05$) increased of FFA levels in irradiated brewer's rice with storage time. In the present study, the γ-irradiation applied to brewer's rice was 1 kGy irradiation (60Co). It can be stated that the application of γ-irradiation in certain commodity even at lower doses also could accelerate lipid oxidation.
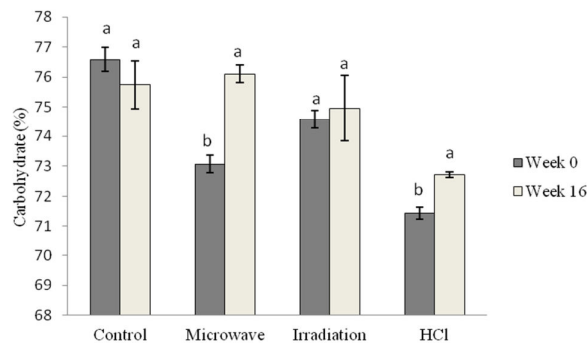
Microwave method also showed considerable efficiency in inhibiting lipid oxidation of brewer's rice since the FFA content at week 16 was significantly ($p < 0.05$) lower than control. In contrast, γ-irradiation showed similar lipid oxidation

pattern as control brewer's rice. Although, HCl treatment effectively inhibited lipase activity in brewer's rice and retarded the lipid oxidation and insignificant ($p > 0.05$) changes of FFA was observed during the storage up to 16 weeks. Even though HCl treatment could effectively inhibit lipid oxidation, microwave method is more applicable to be used in food application due to its energy efficiency.

3.2. Proximate composition

Proximate composition is one of the important nutritional values for food to be considered as raw material for food products. It represents the content of major macro components of food. Generally, rice contained high percentage of carbohydrate followed by protein and fat. The effect of different stabilization methods on the proximate composition of brewer's rice at week 0 and week 16 were compared with control (Figs. 2-6). The result of the study showed that certain stabilization methods caused significant ($p < 0.05$) increased in carbohydrate and significant ($p < 0.05$) reduction in moisture content of brewer's rice at week 16 as compared with initial (Figs. 2 and 3). It is interesting to note that all studied stabilization methods did not significantly ($p > 0.05$) changed the ash, protein and fat content of brewer's rice (Figs. 4-6).

The carbohydrate content for all stabilized brewer's rice was significantly ($p < 0.05$) higher on week 16 as compared with week 0 except for γ-irradiation and control when stored at room temperature (Fig. 2). The carbohydrate content in microwave and chemical stabilized brewer's rice increased significantly ($p < 0.05$) from an initial value of 73.07% to 76.11% and 71.45% to 72.72%, respectively. In the present study, the carbohydrate content of microwave and HCl treatment was significantly ($p < 0.05$) reduced prior to stabilization process at week 0. In contrast, the carbohydrate content in microwave stabilized brewer's rice was significantly ($p < 0.05$) increased along with storage time and resulted in similar carbohydrate content with that of control at week 16.
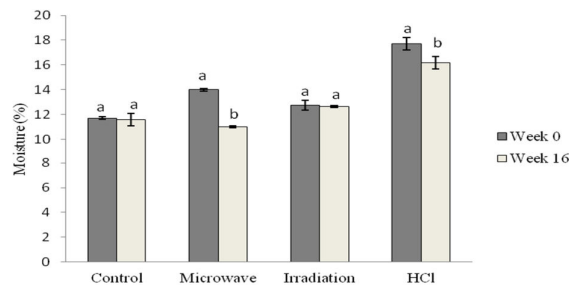

Fig. 2. The Changes of Carbohydrate Content in Treated and Untreated Brewer's Rice during 16 Weeks of Storage at Room Temperature (27±2°C).

Data represents mean ± standard deviation. Means followed by different letters in the same stabilization methods (alb) are significantly different at ($p < 0.05$) according to Duncan's multiple range test. n = 3.
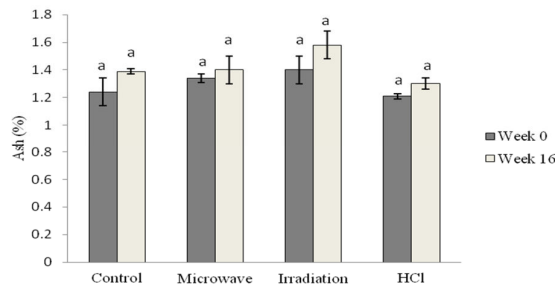
Figure 3 shows the moisture content of microwave stabilized (14%) and chemical stabilized brewer's rice (17.69%) was higher compared to control (11.71%) in week 0, but significantly ($p < 0.05$) decreased to 11% and 16.19%, respectively after storage for 16 weeks. A higher moisture content during week 0 in microwave stabilized samples was due to the addition of water to the sample to make the moisture content become 21% before microwave heating. Similarly with chemical stabilized sample, the addition of hydrochloric acid at the beginning of experiment increased the moisture content. Reduction of moisture content in microwave stabilized sample was due to the microwave process itself. This could be explained that during microwave process, water molecules undergo rotation and absorb microwave energy, resulting in an increase in temperature and thereby reduction in moisture [10, 24].
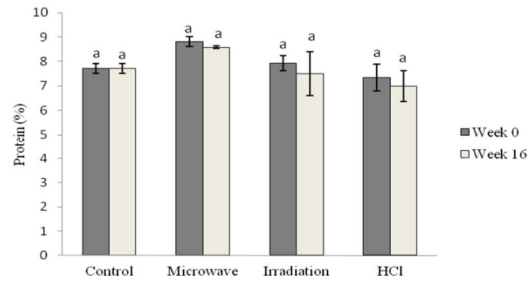

Fig. 3. The Changes of Moisture Content in Treated and Untreated Brewer's Rice during 16 weeks of Storage at Room Temperature (27±2°C).

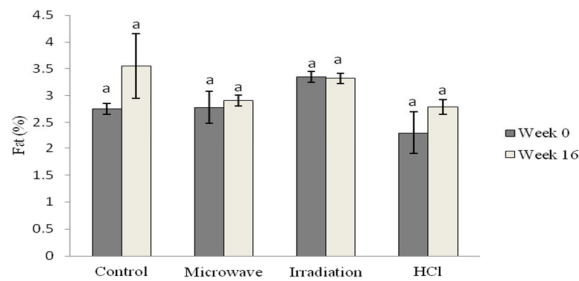*Data represents mean ± standard deviation. Means followed by different letters in the same stabilization methods (a,b) are significantly different at ($p < 0.05$) according to Duncan's multiple range test. n = 3.*

Fig. 4. The Changes of Ash Content in Treated and Untreated Brewer's Rice during 16 weeks of Storage at Room Temperature (27±2°C).

Data represents mean ± standard deviation. Means followed by different letters in the same stabilization methods (a,b) are significantly different at ($p < 0.05$) according to Duncan's multiple range test. n = 3.

Fig. 5. The Changes of Protein Content in Treated and Untreated Brewer's Rice during 16 weeks of Storage at Room Temperature (27±2°C).

Data represents mean ± standard deviation. Means followed by different letters in the same stabilization methods (a,b) are significantly different at (p < 0.05) according to Duncan's multiple range test. n = 3.

In the present study, there were no significant ($p > 0.05$) changes in protein, fat and ash content as compared with control as illustrated in Figs. 4-6 respectively. This data agreed with the earlier findings of Malekian et al. [10] which reported that microwave stabilization did not significantly change the contents of protein and fat in rice bran, even after storage for 16 weeks at room temperature. It can be stated that stabilization of brewer's rice through microwave heating, γ-irradiation and chemical treatment had little effect on the nutritional composition of the brewer's rice.
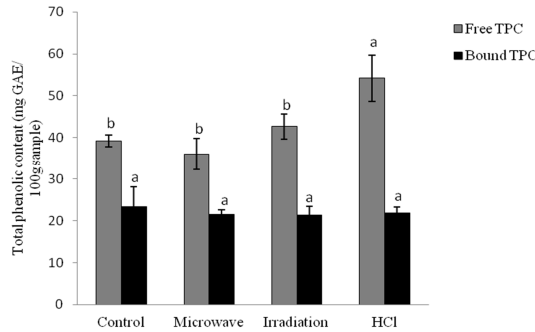

Fig. 6. The Changes of Fat Content in Treated and Untreated Brewer's Rice during 16 weeks of Storage at Room Temperature (27±2°C).

Data represents mean ± standard deviation. Means followed by different letters in the same stabilization methods (a,b) are significantly different at (p < 0.05) according to Duncan's multiple range test. n = 3.

3.3. Total phenolics compounds

The contents of free and bound phenolic compounds in control and stabilized samples at week 0 were shown in Fig. 7. The free TPC of chemical treatment (54.22 mg GAE/100 g sample) was significantly ($p < 0.05$) higher than other treatment and control at week 0. There was non-significance ($p < 0.05$) difference for free TPC

between control (39.22 mg GAE/100 g sample), microwave (36.04 mg GAE/100 g sample) and γ-irradiation (42.66 mg GAE/100 g sample) stabilized brewer's rice. Similar observation was found for bound phenolic compounds, where there was no significant ($p < 0.05$) difference between all stabilized brewer's rice and control. The content of bound TPC in the control sample was 23.34 mg GAE/100g sample, while the bound TPC in the microwave, γ-irradiation and chemical stabilized samples were 21.5 mg GAE/100g, 21.3 mg GAE/100g and 21.92 mg GAE/100g sample, respectively.

Fig. 7. Total Phenolic Compounds (TPC) of Brewer's Rice Treated with Different Types of Treatments.

Data represents mean ± standard deviation. Means followed by different letters in the same stabilization methods (a,b) are significantly different at ($p < 0.05$) according to Duncan's multiple range test. n = 3.

Cereals and grains have been reported to contain various classes of phenolic compounds such as derivatives of benzoic and cinnamic acids, anthocyanidins, quinines, ferulic acid and diferulates whereby sometimes its content complement with those in fruits and vegetables [25, 26]. These phenolic compounds in grains and grain products have been reported to possess significant antioxidant properties and showed a positive correlation with health benefit effects [25]. In natural, phenolics exist in the grain in different forms such as free, soluble conjugate, and insoluble bound, which bound to the cell wall materials [25, 27]. Most of the total phenolic present in rice (74%) and corn (69%) are in the forms of insoluble bound with major phenolic compound present is ferulic acid [25].

In the present study, all stabilization method used did not change TPC content except for HCl. However, the finding for γ-irradiation in present study showed different result as reported by Zhu et al. [28], where the authors reported that γ-irradiation significantly ($p < 0.05$) decreased total phenolic contents in rice sample. The reduction of the TPC during γ-irradiation may be due to disruption of phenolic acids during γ-irradiation ray. In contrast, Oufedjikh et al., [29] reported that γ-irradiation process also could enhance the synthesis of phenolic contents by activating some enzyme that induces the synthesis of the phenolic acids. However, in the present study, the dose of γ- ray used was 1kGy γ-irradiation

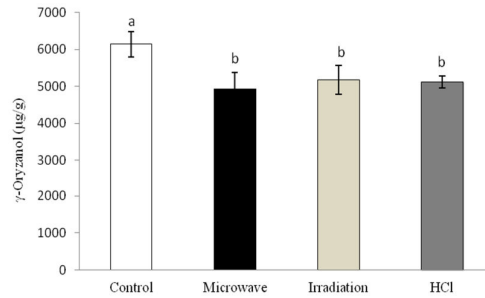(^{60}Co), which might be quite low in order to possess the same detrimental effect as others.

Phenolic compounds particularly in cereals exist in both free and bound forms in cereals. In the present study, the bound TPC in brewer's rice was in range of 23.34 mg GAE/100 g for control. The application of all stabilization methods did not significantly ($p > 0.05$) changed the brewer's rice bound TPC. This is interesting to note since most of the bound TPC possesses functional properties. Recently, Okarter [3] reported the TPC of non-soluble-bound fraction of whole and refined wheat (Barretta and Magnolia) were in range of 97.5-95.8 mg gallic acid equivalent/ 100 g and 2.8 to 13.8 mg gallic acid equivalents / 100 g, respectively were able to possess anti-proliferative activity and cytotoxic effects towards Caco-2 human colon cancer cells. Similarly, high percentage of bound phytochemicals could contribute to the total antioxidant activity can be found in wheat (90%), corn (87%), rice (71%) and oats (58%). Since bound phytochemicals could survive during digestion in stomach and intestine prior to reaching the colon may partly explained the prevention of grain consumption against colon cancer and other digestive cancers, breast cancer, and prostate cancer [25].

It can be concluded that all stabilization methods did not significantly ($p > 0.05$) reduced the both free and bound TPCs of brewer's rice. High TPC could be one of the significant properties that should be preserved in order to further extend brewer's rice into other food products.
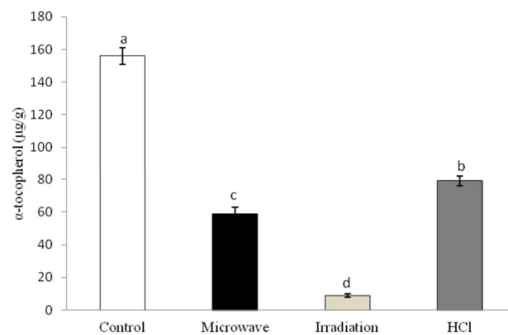
3.4. γ-Oryzanol and α-tocopherol

In the present study, the γ-oryzanol and α-tocopherol were analysed in brewer's rice stabilized by different methods. Figure 8 showed that the extract obtained from control (6134±348 µg/g) had the highest γ-oryzanol followed by that of others, microwave (4934±432 µg/g), γ-irradiation (5167±387 µg/g) and HCl treatment (5115±158 µg/g). The highest α-tocopherol content was also seen in control brewer's rice (155.8±5µg/g), followed by HCl treatment (78.99±3 µg/g), microwave (58.96±4 µg/g) and γ-irradiation (9.16±1 µg/g) as illustrated in Fig. 9.

There is similarity in the trend for both γ-oryzanol and α-tocopherol degradation in brewer's rice where all stabilization methods had significantly ($p > 0.05$) lower of these components as compared with that of control. γ-Oryzanol composed of a mixture of at least 10 phytosteryl ferulates. The three major components of γ-oryzanol, cycloartenyl ferulate, 24-methylenecycloartanyl ferulate and campesteryl ferulate have been identified, accounting for 80% of **γ-**oryzanol in rice bran oil [30]. The level of γ-oryzanol in rice bran is 13 to 20 times (w/w) greater than total tocopherols and tocotrienols [31]. It is one of the potent antioxidant compounds that has been studied widely. According to Cicero and Gaddi [32], γ-oryzanol is able to improve plasma lipid pattern, reduce the total plasma cholesterol, increase HDL cholesterol levels and inhibit the platelet aggregation. In food application, γ-oryzanol improves the storage stability of foods [33, 34]. α-Tocopherol is one form of tocopherol isomers that is present abundantly in nature. It has been considered as valuable vitamin-E homolog due to its high level of physiological activity [19, 35].

Fig. 8. The Content of γ-Oryzanol in Different Treatments of Brewer's Rice.

Data represents mean ± standard deviation. Means followed by different letters in the same stabilization methods (a,b) are significantly different at ($p < 0.05$) according to Duncan's multiple range test. n = 3.

Fig. 9. The Content of Alpha Tocopherol in Different Treatment of Brewer's Rice.

Data represents mean ± standard deviation. Means followed by different letters in the same stabilization methods (a,b) are significantly different at ($p < 0.05$) according to Duncan's multiple range test. n = 3.

In the present study, stabilization process affected the amounts of bioactive compounds, i.e., γ-oryzanol and α-tocopherol (Figs. 8 and 9). Both compounds were lower in all stabilized brewer's rice as compared with control. However, γ-oryzanol was more stable than α-tocopherol possibly due to its molecular structure. Kim et al. [34] also reported that the different roasting time and temperature didn't affect the levels of γ-oryzanol in rice germ oil significantly. However, Yoshida et al. [36] reported that α-tocopherol content in sesame oil decreased with microwave oven heating time.

In the present study, γ-irradiation stabilized brewer's rice contained lowest γ-oryzanol and α-tocopherol as compared with control. The α-tocopherol of γ-irradiation stabilized brewer's rice was also significantly ($p < 0.05$) lower than

microwave and HCl treatment. This also may be due to different stability of these two bioactive compounds against γ-irradiation ray.

A research carried out by Shin and Godber, [23] found that the α-tocopherol in irradiated rice bran was more sensitive to γ-irradiation as compared to the γ-oryzanol. Similar observation has been reported by others when α-tocopherol content decreased markedly in irradiated rice seed [37]. The possible explanations proposed were the loss of α-tocopherol during γ-irradiation process was due to the free radicals formation and the secondary reaction product produced during the reaction may attack α-tocopherol [23]. These also could explain the significantly ($p < 0.05$) lowest of α-tocopherol content in *γ*-irradiation brewer's rice as compared to other treatments.

However, the content of γ-oryzanol in all stabilized brewer's rice were not significantly ($p < 0.05$) different between each other. The content of γ-oryzanol in all stabilized brewer's rice was in range of 4934±432 µg/g to 5167±387 µg/, which can be considered higher as compared with other cereals. The γ-irradiated rice seed contained around 96 to 246 µg/g γ-oryzanol [37], which is lower than brewer's rice. It can be stated that all stabilization methods reduced the γ-oryzanol and α-tocopherol in brewer's rice. However, the final concentration for these two bioactive compounds can be considered as still in adequate level.

4. Conclusions

This study focused on the effects of various stabilization techniques on the nutritional quality and antioxidant potential of brewer's rice. Based on the observations it can be concluded that:

- The different stabilization methods affected brewer's rice properties differently.
- All the stabilization methods showed non-significant ($p < 0.05$) effect on protein, fat, and ash content. However, microwave and chemical treatment significantly ($p < 0.05$) reduced moisture content.
- Microwave and HCl treatment significantly ($p < 0.05$) inhibited lipases activity in brewer's rice. These stabilization methods significantly ($p < 0.05$) increased free TPC and reduced bioactive compounds including α-tocopherol and γ-oryzanol in brewer's rice.
- Chemical stabilization method could prevent the production of FFA. However, due to restriction of HCl application in food industry, microwave stabilization method is the most suitable method to be used in stabilizing brewer's rice.
- Microwave was able to reduce FFA formation significantly ($p < 0.05$) along with retaining its bioactive components. More work is needed to determine the optimum time and condition for microwave stabilization of brewer's rice.

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