

Improvement of Carbohydrate Quality in Rice Bran Using Microwave Irradiation for Nile Tilapia Feed Production

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Abstract

This study was performed to evaluate the effects of microwave irradiation duration (0, 10, 20 and 30 min) on chemical composition, physicochemical properties and *in vitro* carbohydrate digestibility of rice bran. Moisture content was different among irradiated rice brans and decreased with longer irradiation time ($P < 0.05$). This resulted in an increase in crude protein content on dry matter basis in rice bran irradiated for 10 min ($P < 0.05$). This also caused a decrease in crude fibre content ($P < 0.05$) with a relative increase in available carbohydrate ($P > 0.05$). Lipid and ash contents did not change after irradiation. Microstructure, thermal transition parameters and relative crystallinity were enhanced after irradiation. Changes in these characteristics resulted in the *in vitro* increase in carbohydrate digestibility using crude digestive enzyme extract from Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758). The relationship between microwave irradiation times (t) and carbohydrate digestibility (D) could be explained as a third order polynomial regression [$D = 0.034t^3 - 1.932t^2 + 28.675t + 570.733$ ($r^2 = 0.884$, $P < 0.001$, $n = 24$)]. The results indicate that microwave irradiation for 10 min is appropriate for improving the quality of available carbohydrate in rice bran for tilapia feed production.

Introduction

Rice bran is a by-product from rice milling. In Thailand, 75% of rice bran is used as animal feeds, and 15% is for other foods and edible oil extraction (Wataniyakul et al. 2012). Generally, rice bran contains 11-14% protein, 12-18% lipid and 7-14% ash (Ramezanzadeh et al. 2000; Hien et al. 2010; Thu 2012).

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Moreover, structural constituents including hemicellulose (8.7-11.4%), cellulose (9.0-12.8%) and β -glucan (1%) are also major parts of carbohydrate found in this raw material (Ramezanzadeh et al. 2000). These dietary fibres may affect the digestion due to insufficient production of digestive enzymes from fish or microflora in the gut of fish. Moreover, the structural carbohydrates may impair fish digestibility by encapsulating nutrients or by increasing the viscosity of the intestinal contents. However, rice bran has been partially used for rearing Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758) (Dato-Cajegas and Yakupitiyage 1996), the hybrid of *O. niloticus* $\times$ *O. aureus* (Steindachner 1864) (Lin and Luo 2011), and grass carp, *Ctenopharyngodon idella* (Valenciennes in Cuvier & Valenciennes 1844) (Thu 2012).

Microwave processing is the physical pretreatment that has achieved a remarkable acceptance by the food industry and chemical engineering (Oliveira and Franca 2002). Generation of heat throughout the materials using microwave leads to faster heating rates and shorter processing times, when compared to conventional heating such as boiling and autoclaving (Alajaji and El-Adawy 2006; Arocas et al. 2011; Fan et al. 2013). In feed production, microwave can improve the enzymatic digestibility of feedstuffs by altering some physicochemical properties including microstructure (Kristensen et al. 2008; Thongprajukaew et al. 2013), relative crystallinity (Lewandowicz et al. 2000; Bilbao-Sáinz et al. 2007) and starch gelatinisation (Palav and Seetharaman 2007; Zhong et al. 2013). Therefore, the use of microwave irradiation in rice bran might improve the quality of carbohydrate for digestion.

This study aimed to evaluate whether the effects of microwave irradiation times (0, 10, 20 and 30 min) on the changes in chemical composition and physicochemical properties could improve carbohydrate digestibility of rice bran. *In vitro* carbohydrate digestibility using digestive enzymes extracted from Nile tilapia (*O. niloticus*) was also determined. The findings from the present study may provide the appropriate timing of microwave irradiation for improving rice bran quality for the production of aquafeed.

Materials and Methods

Preparation of rice bran samples

Three hundred grams of rice bran were placed in a plastic box (23 cm diameter $\times$ 10.5 cm height), and mixed with distilled water (1:3 w/v). The plastic box was covered with a lid and was then placed in a microwave oven (SANYO, Model EM-700T, 2,450 MHz). The bran was cooked at 700 W in the oven, under agitation for 10, 20 and 30 min. The temperature range during microwave cooking was 91-94 °C. After irradiation, the irradiated rice bran samples were dried at 60 °C for 48 h to eliminate water, ground and sieved before the chemical composition, physicochemical properties and *in vitro* digestibility of carbohydrate were determined. The control rice bran (0 min) was not irradiated and not dried.

Determination of chemical composition

Chemical composition of the rice bran samples including crude protein, lipid, ash and fibre were analysed according to standard methods of the AOAC (2000). The nitrogen free extract (NFE) represented carbohydrate was calculated by the difference.

The moisture content was reported. However, the other chemical compositions were reported on dry matter basis because of too high variation in moisture content between the control and the microwave irradiated samples.

Determination of physicochemical properties

Determination of pH

One gram of each rice bran sample was suspended in 25 mL of water at 25 °C (Sokhey and Chinnaswamy 1993). The measurement of pH in suspended samples was conducted after agitating for 10 min using a pH meter.

Thermal transition property

The thermal transition property of the rice bran samples was measured using a differential scanning calorimeter (DSC7, Perkin Elmer, USA), as described by Chung et al. (2010) with some modifications. Three mg of each dried sample was placed in an aluminum pan, sealed, allowed to equilibrate at room temperature for 1 h, and then heated from 40 °C to 400 °C at a rate of 5 °Cmin⁻¹. The thermal parameters including onset (T_o), peak (T_p) and conclusion (T_c) temperatures, and transition enthalpy (ΔH), were recorded automatically. Degree of gelatinisation (DG, %) was calculated from $[1 - (\Delta H \text{ of irradiated sample} / \Delta H \text{ of control sample})] \times 100$.

Microscopic observation

The microscopic observation of the rice bran samples was carried out as described in Thongprajukaew et al. (2013) with some modifications. The rice bran samples were mounted by double-sticky tape on an aluminum stub and coated with gold. Microscopic pictures of the samples were produced using a scanning electron microscope (JSM 5600 LV, Jeol LTD., Tokyo, Japan) at 100× and 2,000× magnifications. The energy potential during micrography was 10 kV.

X-ray diffraction pattern

The diffraction patterns of the rice bran samples were determined with an X-ray diffractometer (X' Pert MPD, Philips, Netherlands) operated at a voltage of 40 kV and a current of 40 mA (Chung et al. 2010). Diffractograms were recorded between 3° and 35° (2 θ) with a scanning rate of 2° min⁻¹. The relative crystallinity (%) was calculated from the ratio of peak area to the total area (sum of peak areas and amorphous areas) of a diffractogram using Microsoft Excel 2007 (Microsoft Corp., Redmond, WA, USA).

***In vitro* carbohydrate digestibility**

Fish sampling and digestive enzymes extraction

Nile tilapia (67.28 ± 2.78 g body weight and 15.47 ± 0.20 cm total length) were randomly collected from a farm in Nakhon Pathom Province, Thailand. The fish were then killed by chilling in ice. The small intestines were carefully collected, kept in ice and then transported to the Department of Zoology, Faculty of Science, Kasetsart University. Subsequently, the samples were pooled and homogenised in 50 mM Tris-HCl buffer pH 8 containing 200 mM NaCl (1:2 w/v) using a micro-homogeniser (THP-220, OMNI International, USA). The homogenate was centrifuged at 15,000 g for 30 min at 4 °C. The supernatant of the crude enzyme extract was collected and then kept at -80 °C until use for *in vitro* digestibility study.

***In vitro* digestibility**

In vitro digestibility of carbohydrate of the rice bran samples was performed according to the method described in Thongprajukaew et al. (2011). The carbohydrate digestion was determined by quantifying the increase in reducing sugar from digestion of carbohydrate after incubation of each rice bran sample with the dialysed crude enzyme extract, by comparing with maltose standard curve. The carbohydrate digestibility values were calculated, standardised with equal amylase activity, and expressed as $\mu\text{mol maltose} \cdot \text{g}^{-1}$. The amylase activity in the dialysed crude enzyme extract was determined as described in Thongprajukaew et al. (2011).

Statistical analysis

Statistical analysis was conducted using SPSS Version 14 (SPSS Inc., Chicago, USA). Data were reported as mean $\pm$ SEM. Significant differences between means were analysed by Duncan's multiple range test at 95% confidence levels. Third order polynomial regression between microwave irradiation time (t) and *in vitro* digestibility of carbohydrate (D) was analysed to predict the suitable microwave irradiation time as well as their relationship.

Results

Chemical compositions of microwave-irradiated rice bran

Chemical compositions of different irradiated rice brans in comparison with the control rice bran are shown in Table 1. After irradiation, the moisture content was significantly different among the rice bran samples, and decreased with longer irradiation time ($P < 0.05$). This had made the level of crude protein on dry matter basis significantly highest in 10 min irradiated rice bran sample ($P < 0.05$) whereas no differences were found between 20 and 30 min irradiation times and control ($P > 0.05$). Crude lipid and ash contents were unchanged after microwave irradiation ($P > 0.05$).

Crude fibre was significantly decreased in microwave-irradiated rice bran ($P < 0.05$), and the levels were similar among all treated groups. Available carbohydrate increased slightly after microwave irradiation, albeit insignificantly ($P > 0.05$).

Table 1. Moisture content (%) and other chemical compositions (% on dry matter) of control and microwave-irradiated rice bran at different cooking times. Data were calculated from duplicate observations.

Chemical composition	Microwave irradiation time (min)			
	0 (control)	10	20	30
Moisture	5.65±0.05 ^d	76.46±0.10 ^a	73.07±0.24 ^b	71.11±0.52 ^c
Crude protein	14.72±0.13 ^b	15.22±0.15 ^a	14.88±0.08 ^{ab}	14.50±0.03 ^b
Crude lipid	19.34±0.58	19.04±0.26	19.98±0.14	20.12±0.24
Crude ash	9.87±0.08	9.59±0.08	9.61±0.15	9.90±0.14
Crude fibre	5.53±0.09 ^a	4.47±0.20 ^b	4.52±0.18 ^b	3.97±0.23 ^b
Carbohydrate	50.54±0.61	51.68±0.40	51.01±0.28	51.51±0.36

Within the same row, the values with different superscripts are significantly different ($P < 0.05$).

Physicochemical properties of microwave-irradiated rice bran

pH

No differences in pH were observed between irradiated and control rice bran ($P > 0.05$). However, a relatively higher pH was found in the microwave-irradiated rice bran for 10 (6.48±0.07), 20 (6.52±0.03) and 30 (6.44±0.07) min when compared with the control (6.34±0.05).

Thermal properties

Thermal transition properties were different between control and microwave-irradiated rice bran (Table 2). The ΔH of rice bran decreased after irradiation. The irradiation processes increased the onset temperature (T_o) values while they decreased the peak (T_p) and conclusion (T_c) temperature values. These changes reduced the melting temperature range ($T_c - T_o$) of the microwave-irradiated rice bran when compared with the control.

Microstructure

The microwave-irradiated and control rice brans were dissimilar in general morphology (Fig. 1). The control rice bran appeared to have spherical and irregular shapes with smooth surface (Figs. 1a and 1b) whereas the dominance in irregular shape with more rough surface was found in the microwave-irradiated rice bran (Figs. 1c–1h), especially for 10 (Figs. 1c and 1d) and 20 (Figs. 1e and 1f) min.

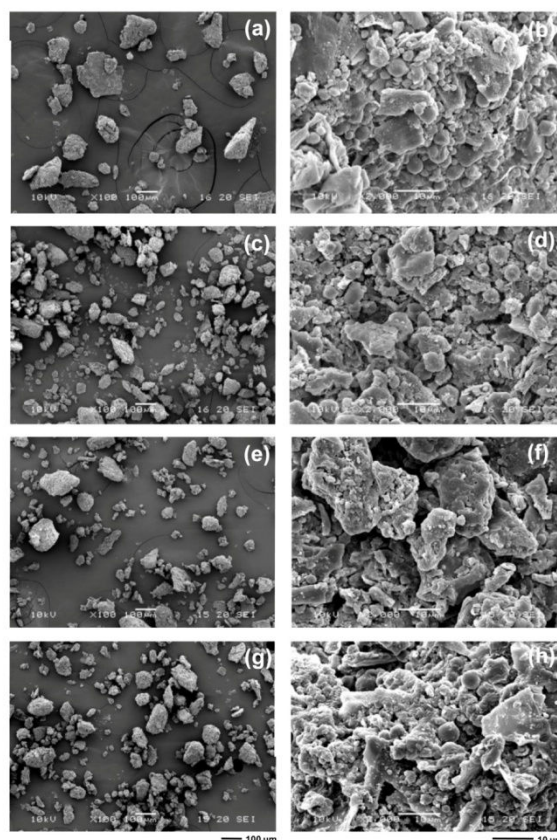


Fig. 1. Microstructures of control rice bran (a and b), and after microwave irradiation for 10 (c and d), 20 (e and f) and 30 (g and h) min. Magnifications of photographs were recorded at 100 $\times$ (left panel) and 2,000 $\times$ (right panel).

Diffraction pattern

No differences in diffraction pattern and diffraction peak were observed among the samples (Fig. 2). The relative crystallinity was highest in control rice bran (33.16%) and gradually decreasing when heated for 10 (31.58%), 20 (29.39%) and 30 (27.84%) min.

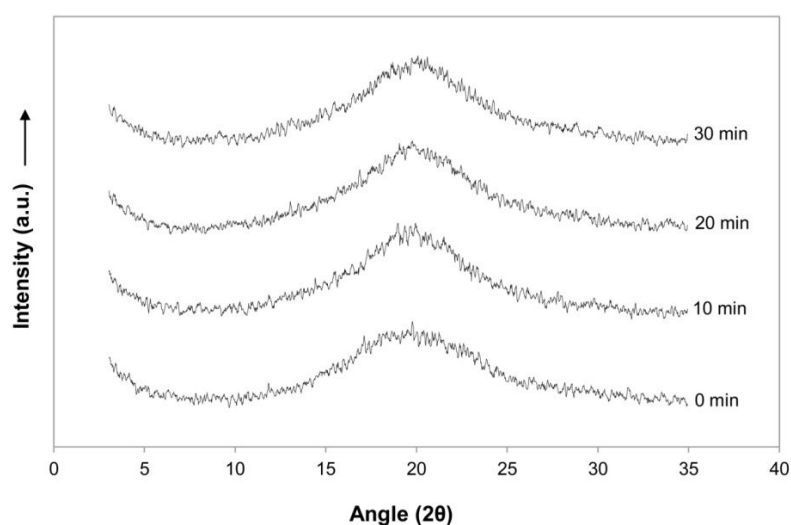


Fig. 2. Diffraction patterns of control and microwave-irradiated rice bran at different cooking times. Diffraction patterns were detected between 4° and 35° (2 θ).

In vitro digestibility of microwave-irradiated rice bran

Significant increase in carbohydrate digestibility was found in the irradiated rice bran when compared with the control material (Fig. 3, $P < 0.05$). The value was highest after irradiation for 10 min and then decreased with longer irradiation times (Fig. 3). Third order polynomial regression analysis between irradiation times (t) and carbohydrate digestibility (D) was $D = 0.034t^3 - 1.932t^2 + 28.675t + 570.733$ ($r^2 = 0.884$, $P < 0.001$, $n = 24$). This equation predicts that 10 min is appropriate for improving carbohydrate quality in this raw material.

Table 2. Thermal transition properties of control and microwave-irradiated rice bran at different cooking times.

Thermal parameter	Microwave irradiation time (min)			
	0 (control)	10	20	30
T_o (°C)	77.94	82.45	81.03	82.67
T_p (°C)	117.50	117.00	116.00	114.33
T_c (°C)	161.56	157.11	154.11	150.17
$T_c - T_o$ (°C)	83.62	74.66	73.08	67.50
ΔH (J g ⁻¹)	98.21	54.70	40.88	33.85
DG (%)	0.00	44.31	58.38	65.91

T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperature, $T_c - T_o$ = melting temperature range, ΔH = enthalpy, DG = degree of gelatinisation.

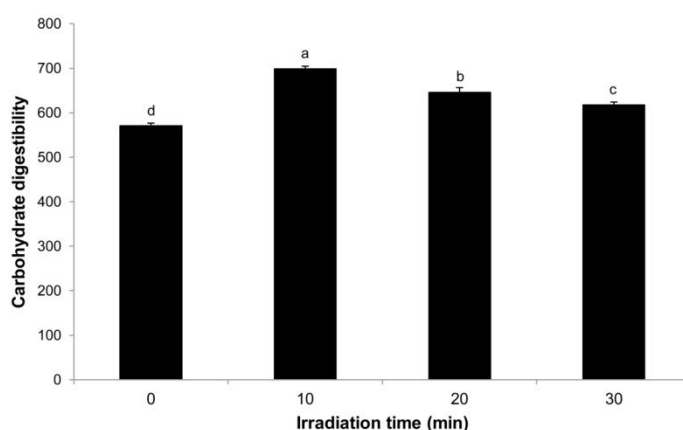


Fig. 3. *In vitro* carbohydrate digestibility ($\mu\text{mol maltose g}^{-1}$) of control and microwave-irradiated rice bran at different cooking times, using dialysed crude digestive enzymes extracted from Nile tilapia. The bars with different superscripts are significantly different ($P < 0.05$).

Discussion

Chemical compositions of microwave-irradiated rice bran

No significant differences in crude protein after irradiation have been reported in microwave-irradiated (8 min) fish feed (Thongprajukaew et al. 2011), microwave-irradiated (3 min) rice bran (Ramezanzadeh et al. 2000), microwave-irradiated green gram (30 min) and Bengal gram (40 min) (Khatoon and Prakash 2006). However, an increase in protein content was reported after microwave irradiation of wheat flour for 30 sec at 45 °C (El-Naggar and Mikhael 2011). Moreover, microwave irradiation could modify the molecular properties of protein by

covalent cross-linkages formed or converted to higher molecular weight aggregates, resulting in a decrease in protein solubility (Sadeghi and Shawrang 2007).

Crude lipid and ash contents were unchanged after microwave irradiation, similar to the observations in microwave-irradiated fish feed (Thongprajukaew et al. 2011) and microwave-irradiated green gram seed (Pande et al. 2012). However, a significant loss of ash constituent in raw materials after boiling, autoclaving or microwave irradiation could be observed (Alajaji and El-Adawy 2006). Loss of lipid during cooking due to the oxidation process of unsaturated fatty acids could occur, depending on time and temperature of processing (Stewart et al. 2003; Malheiro et al. 2009).

Crude fibre was significantly decreased in microwave-irradiated rice bran, and available carbohydrate tended to increase slightly after microwave irradiation. These findings suggest the reduction of main cell wall constituents (cellulose, hemicelluloses and lignin) which causes an increase in nitrogen free extract after pretreatment (Thongprajukaew et al. 2013). Similar observed trends have been found in microwave-irradiated palm kernel meal (Thongprajukaew et al. 2013), microwave-irradiated coconut meal (Chumwaengwapee et al. 2013) and also in gamma-irradiated wheat straw, cotton seed shell, peanut shell, soybean shell, extracted olive cake and extracted unpeeled sunflower seeds (Al-Masri and Guenther 1999). Changes in some chemical compositions after microwave irradiation differed from some earlier reports because of differences in various factors that affect cooking capacity, namely raw material type, quantity of the water used in the preparation of feedstuff, wave intensity and irradiation time.

Physicochemical properties of microwave-irradiated rice bran

pH

A relatively increased pH after modification was postulated due to the higher release of hydroxyl group from lignocellulosic degradation (Chumwaengwapee et al. 2013). Moreover, removal of NH_2 -group under microwave activation might increase the alkaline condition as observed by Izquierdo et al. (2005). The pH increment in rice bran is in agreement with the fluctuation observed in palm kernel meal (Thongprajukaew et al. 2013) and coconut meal (Chumwaengwapee et al. 2013).

Thermal properties

The occurrence of T_c – T_o wideness was hypothesised due to the heterogeneity of crystalline starch (Bao and Corke 2002). Therefore, the narrower T_c – T_o in the irradiated rice bran probably indicates the similar chain length of cleaved amylase and amylopectin, or homogeneity of crystallinity after activation by microwave irradiation. The ΔH of rice bran decreased after irradiation. This indicates that the irradiated rice bran requires lower amount of enthalpy for transformation due to higher portions of partially gelatinised starch content. This presumption is well associated with the increasing degree of gelatinisation in a time dependent manner (Table 2), similar to the data observed in γ -irradiated RS₄ waxy maize starches (Chung et al. 2010). This

characteristic has been reported to play an important role for improving carbohydrate digestibility (Kaur et al. 2010; Thongprajukaew et al. 2011).

Microstructure

The microwave-irradiated rice bran appeared to have more rough surface than the control rice bran. Thongprajukaew et al. (2013) reported an increase in surface roughness enhancing the *in vitro* enzymatic hydrolysis of raw material. A similar finding has been reported in wheat straw after modification (Kristensen et al. 2008). This characteristic probably promotes an efficiency of digestion by providing a large surface-to-volume ratio as well as loading with high enzyme volume (Ji et al. 2008). In addition, the absence of spherical structure might occur due to the destruction of starch granules after irradiation for 10-20 min. This result is well associated with granule rupture, followed by formation of film coating the surface, observed by Palav and Seetharaman (2007).

Diffraction pattern

No differences in diffraction pattern and diffraction peak were observed among the samples. This indicates no or minor changes of crystallinity which is mainly generated by association between macromolecules, especially for cell wall constituents and amylose and amylopectin in irradiated rice bran. The relative crystallinity was highest in control rice bran which gradually decreased when heated. This finding indicates an increase of amorphous region after irradiation. Similar results supported the significant role of microwave irradiation for destruction of crystalline structure in raw material (Chumwaengwapee et al. 2013). With regards to crystallinity, a negative relationship between *in vitro* digestibility of rapidly and slowly digestible starches has been reported (Kaur et al. 2010). Therefore, microwave irradiation of rice bran may provide the proper carbohydrate architecture which contributes to enzymatic hydrolysis along the alimentary tract of animals.

In vitro digestibility of microwave-irradiated rice bran

Microwave irradiation had a potential to increase digestible undegraded protein in cotton seed meal (Sadeghi and Shawrang 2007). However, the *in vitro* digestibility of the experimental rice bran was studied only for carbohydrate, because microwave irradiation for 8 min could significantly increase carbohydrate digestibility in fish feed but not protein digestibility, compared to 4 min irradiation (Thongprajukaew et al. 2011).

The carbohydrate digestibility was highest after irradiation for 10 min, similar to the suitable irradiation time of 8 min for fish feed (Thongprajukaew et al. 2011). Improvement in carbohydrate digestibility associated with the observed physicochemical changes (as described above in Table 2 and Figs. 1 and 2), showed the best improved properties at 10 min irradiation.

The *in vitro* digestibility study using fish crude enzyme extract is a very sensitive technique, as significant differences in carbohydrate digestibility could be observed (Fig. 3) although the levels of available carbohydrate were not significantly different (Table 1). The

characteristics, namely water solubility, starch gelatinisation, crystallinity, amylose content, starch diameter and starch degradation, were reported to play an important role in contributing to enzymatic digestion of carbohydrate (Sadeghi and Shawrang 2008; Chung et al. 2010; Kaur et al. 2010). Improvement of carbohydrate quality in different raw materials using microwave irradiation associated with *in vitro* digestibility has been reported (Negi et al. 2001; Khatoon and Prakash 2006; Thongprajukaew et al. 2013). Longer irradiation time could cause a significant reduction in nutritional quality and digestibility of carbohydrate, such as changing pasting property (Palav and Seetharaman 2007) and starch granule aggregation (Anderson and Guraya 2006). Therefore, microwave irradiation of rice bran for 10 min before its use in the processing of Nile tilapia feed should be of interest.

Conclusion

Microwave irradiation could improve carbohydrate quality of rice bran. This technique decreased the crude fibre content and provided a relatively higher amount of available carbohydrate. Physicochemical properties (microstructure, relative crystallinity and thermal transition parameters) were changed after irradiation and enzymatic hydrolysis enhanced. These changes contributed to the increase in the *in vitro* digestibility of carbohydrate when screening was done using dialysed crude digestive enzymes extracted from Nile tilapia. The results indicated that the appropriate irradiation time is 10 min which exhibited significant potential improvement for preparation of rice bran for use in Nile tilapia feed production.

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