Asia-Pacific Journal of Science and Technology: Volume: 23. Issue: 02. Article ID.: APST-23-02-05. Research Article

APST

Asia-Pacific Journal of Science and Technology

https://www.tci-thaijo.org/index.php/APST/index

Published by the Faculty of Engineering, Khon Kaen University, Thailand

Enhanced storage stability of freeze dried rice bran hydrolysates by maltodextrin

Geerada Kaewjumpol[1], Supawan Thawornchinsombut[1, *]

[1] Department of Food Technology, Faculty of Technology and Cardiovascular Research Group (CVRG), Khon Kaen University, Khon Kaen , 40002, Thailand
*Corresponding author: suptha@kku.ac.th

Abstract

In this study, rice bran hydrolysate (RBH) extracted by mild-subcritical alkaline water (mild-SAW) followed by protease hydrolysis was investigated for its storage stability. The effects of the ratio of encapsulating material (maltodextrin, MD with DE10) and storage time on antioxidant activities of RBH were investigated. RBH was freeze dried with and without MD at the ratio of MD to RBH of 0.5:1, 1:1 and 1.5:1, vacuum-packed in an aluminum bag and stored at 40 °C for 4 months. The changes of chemical and physical properties as well as antioxidant capacity were monitored during storage. Total phenolic content (TPC) and ferric reducing antioxidant power (FRAP) of the RBH encapsulated with MD were more stable than those of the control (without MD) during storage. Browning intensity of all samples was constant during storage. The different ratios of MD had no effect on TPC degradation and FRAP, nevertheless MD cannot prevent the loss of protein solubility and ABTS radical scavenging activity throughout storage. The RBH encapsulated with MD at 1:1 and 1.5:1 ratios lowered an increased rate of a_w and the changes of protein secondary structure were determined by ATR-FTIR spectroscopy. Therefore, maltodextrin can prevent deteriorations of antioxidant rice bran hydrolysates to some extent during storage.

Keywords: Rice bran hydrolysates; Maltodextrin; Antioxidant activity; Storage stability; ATR-FTIR spectroscopy

1. Introduction

One of the important crops in the world is rice which was produced around 756.7 million tons in 2017 as paddy [1]. Moreover, it is the staple food of Asian countries, especially Thailand. The co-product after milling the paddy is rice bran, the brown layer existing between the white rice (endosperm) and the outer husk of the rough rice. Whole rice grain comprises around 10 % by weight of rice bran [2] accordingly about 75.7 million tons of rice bran can be estimated from the global production of paddy in 2017. Due to its rich “nutraceutical” oil [2], rice bran is normally used as the raw material in the rice bran oil industry. After oil extraction, defatted rice bran is used as an inexpensive animal feed in Thailand, but still contains high antioxidant compounds such as protein as well as several kinds of phenolic compounds [3].

Previously, an environmental friendly technique with mild-subcritical alkaline water (mild-SAW) treatment followed by proteolysis was used to produce rice bran hydrolysates (RBH) which contained active compounds corresponding to high antioxidant activity from industrially defatted rice bran [4]. Although RBH demonstrated high antioxidant activity, the active ingredients probably deteriorate through storage. Moreover, their degradation reactions can be affected by numerous factors during storage i.e. light, air and temperature. One of the most interesting techniques which can be applied to inhibit the deterioration of active ingredients during storage is microencapsulation. It is an alternative technique that can apply protective encapsulating material, such as biopolymer, to encapsulate small particles of a substance so preventing it from various environments i.e. water, oxygen, or other conditions [5], therefore, it can extend the shelf life of such active ingredients. The encapsulated material or core material or internal phase is the active agent, whereas the wall material (or encapsulating material, coating material, membrane, shell, carrier material, external phase or matrix) is the material that encapsulates the core [5]. The most common biopolymer used as an encapsulating material for freeze and spray drying is

maltodextrin (MD), which is derived from starch (corn, potato or others) by acid hydrolysis, due to its low cost and effectiveness. For freeze drying, after sublimation of the dispersing medium, MD can form amorphous glassy matrices. This glassy matrix is presumed to be a form of solid network connected by hydrogen bonds between carbohydrate chains. A coating of labile food components such as peptides and phenolic compounds within the matrix of MD can increase their stability [6]. There were several researches regarding encapsulation of phenolic compounds by MD. For example, MD was used as the encapsulating material in order to extend the shelf life of phenolic extract from grape juice [7] and anthocyanins from Andes berry [6], but there was no report of its application in RBH which contained both phenolic compounds and peptides.

Therefore, the objectives of this research were to elucidate the effects of the ratio of encapsulating material (MD with DE 10) during 4 months storage under accelerated temperature (40 °C) on their chemical and physical properties as well as the antioxidant activities of the freeze dried RBH. The changes of protein structures during storage were also determined using the FTIR technique.

2. Materials and methods

2.1 Materials

Defatted rice bran from the rice bran oil industry was obtained from Kasisuri Co., Ltd. (Ayudhaya, Thailand). In the rice bran oil factory, a mixture of full fat and parboiled rice bran (~1:1) was stabilized by heat (70 - 80 °C) and defatted with hexane. Then hexane and moisture were evaporated at 140 °C yielding the co-product so called industrially defatted rice bran (IDRB). Protease G6 (PG6) (EC 3.4.21.62) (DuPont™ Genencor® Science, USA) with activity 580,000 DU g^{-1} was purchased from Siam Victory Chemical Co., Ltd. (Bangkok, Thailand). Maltodextrin (or MD) with DE10 was purchased from Abbra Co., Ltd. (Bangkok, Thailand).

2.2 Chemicals and reagents

TPTZ (2,4,6-tripyridyl-s-triazine) was purchased from Sigma (St Louis, MO, USA). Bovine serum albumin (BSA), 2,2'- azinobis-3-ethylzothiazoline-6-sulfonic acid (ABTS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) were purchased from Sigma Aldrich (Steinheim, Germany). Folin-Ciocalteu phenol reagent was purchased from Carlo Erba Reactifs SA (Val de Ruil, France). Other chemicals were obtained from Ajax Finechem Pty Ltd. (Taren Point, NSW, Australia).

2.3 Sample preparation and encapsulation

Rice bran hydrolysates (RBH) were extracted from IDRB according to Kaewjumpol et al. [4]. Briefly, IDRB was treated with mild-SAW at 130 °C (1.68 atm) for 2 h then hydrolyzed by PG6 at pH 9.5, 60 °C, 2% (v/w) enzyme concentration for 6 h. After that the IDRB mixture was centrifuged at 10,000 ×g (Sorvall Legend Mach 1.6R centrifuge, Thermo Fisher Scientific, Osterode am Harz, Germany) and 4 °C for 15 min. The supernatant (RBH) was collected and filtered through a microfiltration membrane with pore size of 0.45 µm (polyethersulfone material, CFP-4-E-3MA, GE Healthcare Bio-Sciences Crop., MA, USA). RBH supernatant after 0.45 µm microfiltration was mixed with and without maltodextrin (MD; DE10) at different ratios of MD:RBH and freeze dried (Gamma 2-16 LSC plus, Christ, Osterode am Harz, Germany). There were 4 treatments as follows: control (without MD), 0.5 MD, 1 MD, and 1.5 MD, which were MD additions at 0.5:1, 1:1, and 1.5:1 ratios of MD:RBH, respectively. Freeze dried samples were vacuum packed in aluminum bags and stored at 40 °C in a hot air oven (WTC binder oven 7200, WTC Binder, Tuttlingen, Germany) for 4 months. The analyses of total phenolic content (TPC) [3], antioxidant activity including ferric reducing antioxidant power (FRAP) [8] and ABTS radical scavenging activity (ABTS-RSA) [9], protein solubility, water activity (a_w) (Water activity meter; Aqualab Series 3 TE, Decagon Devices Inc., Washington, USA) at 25 °C, pH and browning intensity were conducted monthly except for ATR-FTIR spectroscopy analysis at 0, 2, 4 months. TPC, antioxidant activity and protein solubility were calculated based on the weight of RBH in the sample (excluding weight of MD).

2.4 Protein solubility in water

The protein solubility in water was determined according to Wang et al. [10] with modifications. RBH powder (200 mg) was suspended in 4 mL of deionized (DI) water and shaken for 30 min. Afterwards the suspension was centrifuged at 5000 ×g for 10 min. Next the supernatant was collected and the protein content in the supernatant was analyzed by the Lowry method [11]. Protein solubility was expressed as a soluble protein fraction in supernatant per g RBH powder (mg protein per g RBH).

2.5 Browning intensity and pH

The measurement of the browning intensity was adapted from Dong et al. [12]: 30 mg of RBH powder were dissolved in 10 mL of distilled water (DW). The suspension was measured by pH meter (FiveEasy FE20-I, Mettler Toledo group, Greifensee, Switzerland) before shaking for 20 min then centrifuged at 10,000 ×g for 10 min. After that the supernatant was collected and the browning intensity of the supernatant was measured by monitoring the absorbance at 420 nm in which a suitable dilution of supernatant was prepared to obtain absorbance at less than 1.5.

2.6 Attenuated total reflectance-fourier transform infrared spectroscopy (ATR-FTIR Spectroscopy)

The sample (100 mg) was analyzed by the Fourier transform infrared (Tensor 27, Bruker, Bremen, Germany) using the attenuated total reflectance technique for the infrared spectrum. The spectral analysis was in the range of 600-4000 cm^{-1} using 16 scans. Signal averages were obtained at a resolution of 4 cm^{-1} [13]. Magic plot software (Magic plot software for students, Saint Petersburg, Russia) was used for a Lorentzian curve fitting in the region of the amide I & II bands to separate overlapping bands. The secondary structure content of samples was detected from IR second-derivative amide I & II spectra and estimated from the relative peak areas under the bands assigned to a particular substructure.

2.7 Statistical analysis

The statistics were analyzed by using the SPSS software program version 19 for Windows (SPSS Inc., IBM company, Chicago, IL, USA) with analysis of variance (ANOVA) and the means among samples were compared using Duncan's new multiple range test (DMRT) at an acceptable significance of $P<0.05$. All experiments were performed in triplicate. The correlations among parameters were considered by the Pearson correlation coefficient (r) and probability-value (P). A probability value of $P<0.01$ was considered to be statistically significant.

3. Results and discussion

3.1 Total phenolic contents (TPC)

Three forms: soluble free, soluble conjugates and insoluble bound forms of phenolics exist in rice bran [14]. Its free forms are found in the plant cell vacuoles [14], while soluble conjugates are bound with sugars and other low molecular mass components by ester bonds. Insoluble bound forms are found in rice bran cell walls i.e. cellulose, hemicellulose, lignin, pectin and rod-shaped structural proteins [14]. Previously, mild-SAW followed by proteolytic enzymes was applied to extract RBH and this process helped to release phenolic compounds linked with rice bran cell walls in which the ferulic acid and para-coumaric acid, the soluble bound forms [15], were found as the major phenolic compounds in RBH [4].

TPC in RBH with and without maltodextrin (MD) at different ratios of MD to RBH (w:w) including control (without MD), 0.5MD (0.5:1), 1MD (1:1), and 1.5MD (1.5:1) during 4 months storage were illustrated in Figure 1. The results showed that there was a statistically significant interaction effect between the ratio of encapsulating material and storage time ($P<0.05$) on TPC changes. TPC of the control was decreased significantly, while TPC of 0.5 MD, 1 MD and 1.5MD were relatively stable until the end of storage. However, the fluctuation of TPC in all MD treatments was observed during storage. This fluctuation may be caused by a decrease in insoluble bound phenolics and an increase in free and soluble conjugated phenolics [16] in RBH during storage. Similar fluctuation of TPC levels has been found in other studies. Htwe et al. [16] observed that bound, free, soluble conjugated phenolics and TPC levels were fluctuated during 4months storage of black and red rice milled at 40 °C. Nevertheless, the overall trends of levels of free and bound phenolics decreased, whereas the levels of soluble conjugated phenolics increased during storage. They can be explained by the fact that these changes involve the oxidation of ferulate esters of hemicellulose which cause a decrease in bound phenolics. In addition, protein-phenolic interaction divided into irreversible and reversible forms [17] which might cause the fluctuation of phenolic content during storage as well as protein solubility (Figure 2).

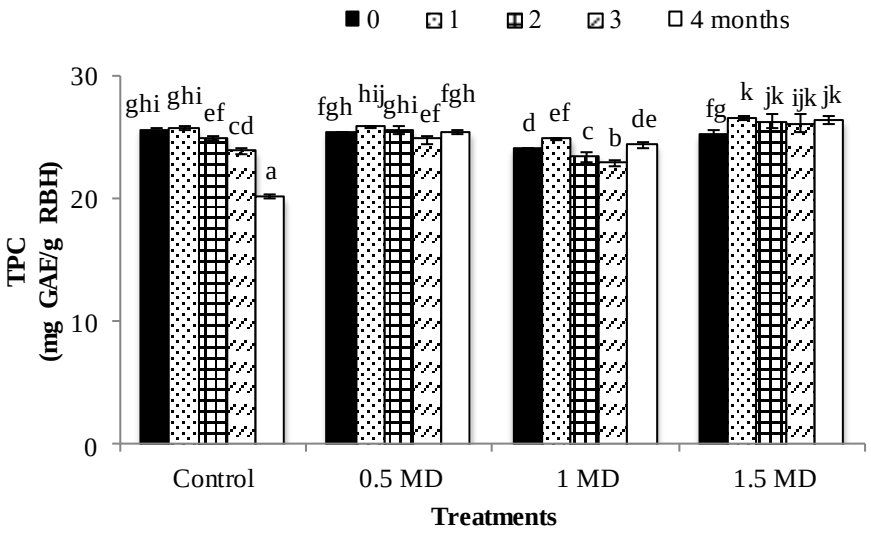

Figure 1 Total phenolic content (TPC) of RBH with and without maltodextrin (MD) at different ratios during 4 months storage at 40 °C. The different letters on the columns indicate significant differences (P<0.05). Treatments-Control: RBH without maltodextrin (MD); 0.5 MD, 1 MD or 1.5 MD: with the ratios of MD to RBH at 0.5:1, 1:1 or 1.5:1, respectively. GAE: gallic acid equivalent.

Our results demonstrate that the addition of MD (DE 10) at different ratios of MD to RBH slow down a decrease in TPC during storage. Similarly, Laine et al. [7] freeze dried cloudberry phenolics extract with 9% w/w MD (DE 5-8 and DE 18.5) and investigated their stability during storage for 64 days at 25 °C. An overall decrease in TPC was detected and was proposed to be caused by hydrolysis, oxidation, and polymerization reactions. The storage stability of phenolics was enhanced when MD was added with DE 5-8 (higher molecular weight), whereas MD with DE 18.5 did not preserve TPC during storage. They explained that MD with a larger molecular weight revealed a higher efficiency to encapsulate phenolics than a lower molecular weight containing more hydroxyl groups. Consequently, it perhaps formed tighter complexes with phenolic compounds [7]. Negrão-Muragami et al. [18] studied the influence of different DE values of MD including 10.2, 15.2 and 18.6 (30% w/v) on the storage stability (for 90 days at 45 °C) of spray dried concentrated mate (*Ilex paraguariensis* A. St. Hil.*)* which is a rich source of phenolic compounds. They found that all microcapsules with different DE values of MD were more stable in TPC and antioxidant activity (FRAP and DPPH) than non-encapsulated one. Among microcapsules, MD with DE 10.2 showed a higher stability for phenolic compounds than the other two MD treatments.

3.2 Protein solubility in water

The ratio of encapsulating material and storage time significantly affected protein solubility of RBH. Generally, protein solubility in water of all treatments decreased with the same trends while in storage (Figure 2). These results indicate that MD addition did not slow down protein aggregation during storage. Moreover, this reduction is possibly related to the secondary structure changes (β-sheet, α-helix and β-turn) of proteins which will be described later.

Protein aggregation typically occurs during storage due to protein conformation changes leading to inter- and intra-molecular interactions among proteins and/or with other compounds i.e. phenolic compounds, oligosaccharide and reducing sugar. Physical aggregation can also be formed such as moisture-induced aggregation of lyophilized insulin during storage [19]. The opposite trend between changes of protein solubility and water activity (a_w) of our samples was observed. During extended storage, protein solubility decreased, while the a_w increased (Figures 2 and 3). This probably involves an increase in chemical reaction activities with a greater mobility of reactants at higher a_w. This demonstrates that moisture content or humidity is one important factor of protein aggregation formation which affects its solubility during storage.

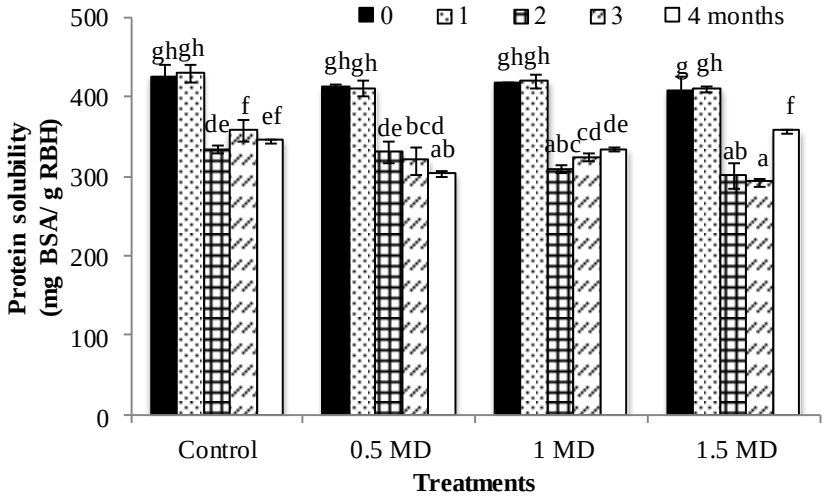

Figure 2 Protein solubility of RBH with and without maltodextrin (MD) at different ratios during 4 months storage at 40 °C. The different letters on the column indicate significant differences (P<0.05). Treatments are as in Figure 1.

3.3 Water activity (a_w)

The ratio of encapsulating material and storage time had a significant interaction effect on a_w of RBH. Overall, a_w of all treatments increased markedly after storage (Figure 3). However, a_w of the control sharply increased and reached the highest a_w after 1 month. Its a_w was relatively stable until the end of storage. Among MD treatments, 1 MD and 1.5 MD showed a lower rate of increased a_w than 0.5 MD treatments during storage. Negrão-Muragami et al. [18] found that spray dried concentrated mate (*Ilex paraguariensis* A. St. Hil.) with MD at different DE of 10.2, 15.2 and 18.6 (30% w/v) had a_w of 0.148, 0.157,0.126, respectively, which was lower than control without MD (a_w of 0.237). Our study, which used an MD (DE10) addition in RBH could not improve the initial a_w of freeze dried RBH, but at the 1:1 ratio or higher it preserved a_w better than control during storage. Rocha-Parra et al. [20] performed drying of red wine using a freeze dryer with MD (DE10): gum arabic (65:35) at final concentration of 9% (total weight basis) and stored at 38 °C for 145 days. At initial a_w (0.11), the main phenolics in red wine including Malvidin 3-G and total anthocyanins were stable during storage. For our samples, 0.5 MD, 1 MD and 1.5 MD had very low a_w (between 0.08-0.22) and also demonstrated fairly constant TPC values during storage (Figure 1). Nonetheless, in their study, the phenolic compounds showed a higher loss during storage when a_w increased from 0.11 to 0.58. This indicates that a_w (or moisture content) is a key factor influencing the stability of active compounds during storage.

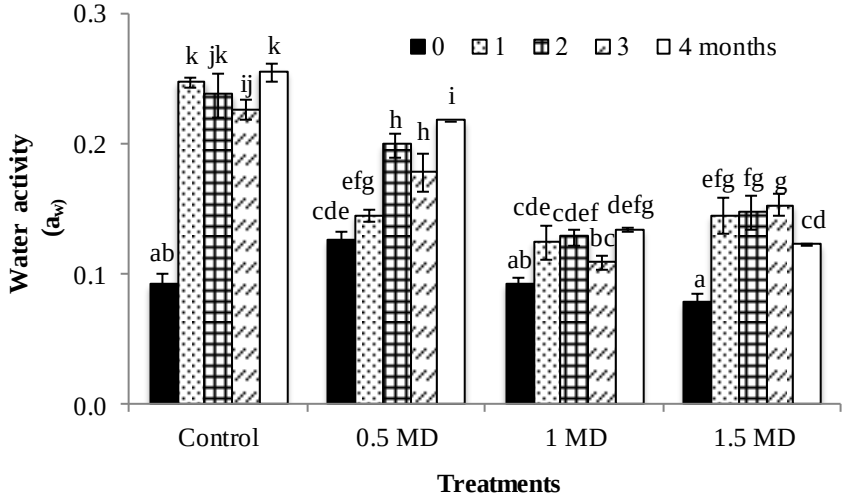

Figure 3 Water activity (a_w) of RBH with and without maltodextrin (MD) at different ratios during 4 months storage at 40 °C. The different letters on the column indicate significant differences (P<0.05). Treatments are as in Figure 1.

3.4 Protein secondary structure

There was an interaction effect between the ratio of encapsulating material and storage time on the protein secondary structure changes in RBH. The secondary structure changes during storage were monitored using ATR-FTIR spectroscopy between 600 and 4000 cm^{-1} (Figure 4). The protein IR spectrum showed two characteristics including the amide I (C=O stretching; 1700 to 1600 cm^{-1}) and amide II (N-H bending; 1600 to 1500 cm^{-1}) bands [21]. Previously, the secondary structures of the protein were commonly based on the amide I band analysis [22]. In our study, the overlapping between amide I and amide II peaks was observed so these two peaks were analyzed between 1480 and 1730 cm^{-1} (Figure 5) by Lorentzian curve-fitting [23]. However, the percentage of protein secondary structures of β-sheet, random coil, α-helix and β-turn were calculated in amide I regions which overlapped with amide II between 1560 and 1700 cm^{-1} (Figure 6). The characteristic peaks at 1611 to 1639 cm^{-1} were attributed to β-sheets. Other peaks corresponded to the following structures: random coils, 1640 to 1649 cm^{-1}; α-helixes, 1650 to 1658 cm^{-1}; and β-turns, 1660 to 1700 cm^{-1} [24].

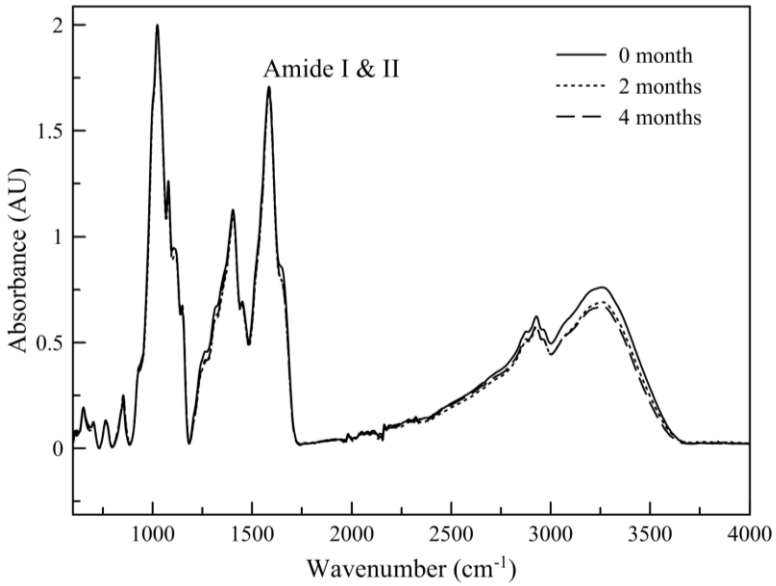

Figure 4 Fourier transform infrared (FTIR) spectra (600-4000 cm^{-1}) of RBH powders (the control without MD) at 0, 2 and 4 months.

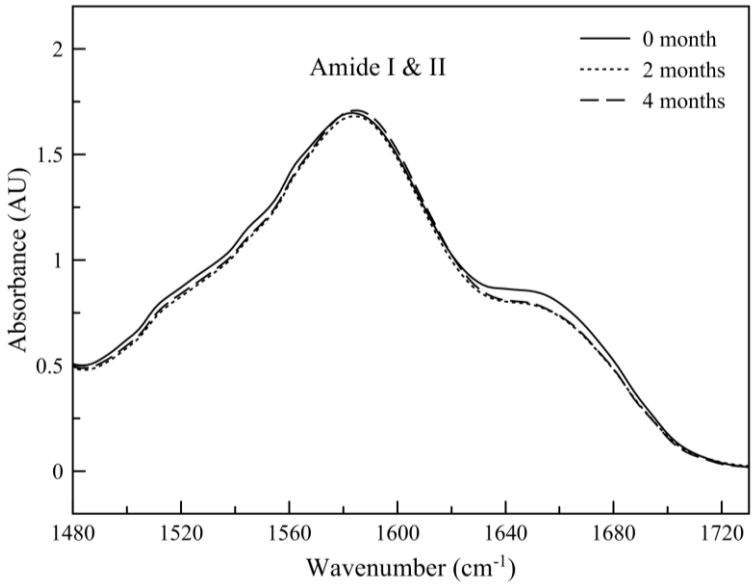

Figure 5 FTIR spectra between amide I and II regions (1480-1730 cm^{-1}) of RBH (the control without MD) at 0, 2, 4 months.

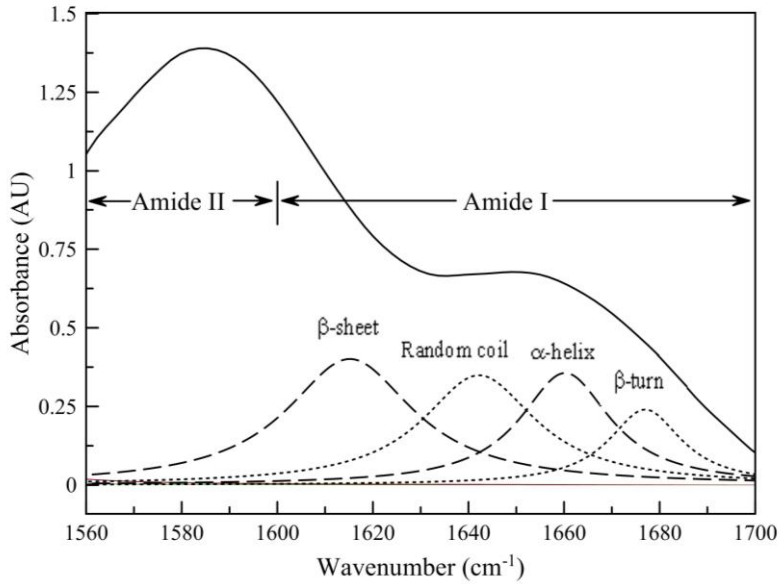

Figure 6 Curve-fitted spectra of β-sheet, random coil, α-helix and β-turn bands of amide I overlapped with amide II region in FTIR spectrum (1560 to 1700 cm^{-1}) of RBH (the control without MD) at 0 month.

Table 1 Peak region (wave number) of α-helix with the enaminol group of RBH stored at 0, 2 and 4 months.

Treatments*	Storage times		
	0 month	2 months	4 months
	α-helix with the enaminol group (cm^{-1})		
Control	1660.52±0.37^{d}	1661.39±0.03^{ef}	1662.31±0.08^{g}
0.5 MD	1658.90±0.08^{a}	1660.06±0.25^{cd}	1661.83±0.16^{fg}
1 MD	1659.88±0.91^{bc}	1660.20±0.12^{cd}	1661.15±0.01^{e}
1.5 MD	1659.32±0.01^{ab}	1659.18±0.33^{a}	1660.38±0.49^{d}

* Treatments: Control - RBH without maltodextrin (MD); 0.5MD, 1 MD or 1.5 MD - ratios of MD to RBH at 0.5:1, 1:1 or 1.5:1, respectively.

$^{a-g}$ the different letters indicate significant differences (P<0.05) among treatments and storage times.

Bands of the Maillard Reaction Products (MRPs), i.e. Schiff's base imine group (stretching) and enaminol group (stretching) are typically found in the amide I region at which the band intensity at 1630-1650 cm^{-1} increased and a new band was formed at around 1660 cm^{-1} [21] (Table 1). The peak region of α-helix overlapped with the enaminol group and increased slightly during storage. Yang et al. [21] found that a new band was formed in the amide I region at around 1660 cm^{-1} of soy protein-soy polysaccharide MRPs. The Maillard reaction (MR) can occur during RBH extraction due to the release of reducing sugar and amino acids or peptides. However, the reaction might still continue during storage. In the present study (Table 1), the peak regions for α-helix and the enaminol group (corresponding to MRPs) were found especially in control which showed the highest peak region (P<0.05). All treatments revealed a shifted peak to a higher wave number after storage (P<0.05).

It was observed that all treatments showed an increased trend of β-sheet content while α-helix and β-turn content decreased after four months storage (Table 2). However, random coil content of all treatments did not change during storage (data not shown). The increased β-sheet and decreased α-helix and β-turn might imply that hydrophobic peptides were more exposed during storage [22]. MD addition especially 1.5 MD treatments can delay an increase of β-sheet and a decrease of α-helix and β-turn compared to control (Table 2). These results reveal that MD addition helps to retain the secondary structure (β-sheet, α-helix and β-turn) of peptides in RBH. Wang et al. [22] found that the surface hydrophobicity of soy bean protein isolates increased with the decrease of α-helix and random coil content and decreased with the increase of β-sheet content. In the case of β-turn, a relationship with surface hydrophobicity was not found. Besides, it has been reported that an increase in β-sheet content during freeze drying is often an indication of protein aggregation and/or increased intermolecular interaction [25]. Protein aggregation is one of the main instabilities for freeze dried protein pharmaceuticals during storage [26]. Thus protein aggregation may be one of the reactions leading to protein solubility change during storage. In this study, although MD addition at 1.5:1 ratio slowed down the changes of β-sheet, α-helix and β-turn structures, protein solubility of all treatments dramatically decreased at 2 months storage. Changes of

secondary structures resulting in protein aggregation were not the only reactions leading to the reduction of protein solubility as the phenolic-protein interaction might also affect the decrease of protein solubility. It has been reported that binding between protein and phenolic resulted in a decrease of protein solubility [27]. Labuckas et al. [27] reported that the hull of walnut kernels contained a substantial content of phenolic compounds. When the oil is extracted from the ground whole kernels, most phenolic compounds in the flour can bind with proteins via different bonding such as hydrophobic and ionic interactions, and hydrogen and covalent bonds. As a result, both protein aggregation and phenolic-protein interaction could reduce protein solubility.

Table 2 Changes of β-sheet, α-helix and β-turn contents in RBH after 4 months storage.

Treatments [1]	Change (%)		
	β-sheet	α-helix	β-turn
Control	+13.0	-15.2	-20.36
0.5MD	+11.1	-12.4	-17.09
1MD	+12.1	-13.2	-14.33
1.5MD	+11.6	-7.2	-11.93

Note: Protein secondary structure contents obtained by curve-fitting of FTIR spectra of amide I region
[1] Treatments are as in Table 1.

3.5 Antioxidant activity

It was found that the ratio of encapsulating material and storage time had an interaction effect on FRAP and ABTS radical scavenging activity (P<0.05). Overall, FRAP of the control decreased, while FRAP of the MD treatments slightly increased at the end of the storage (P<0.05) (Figure 7). However, fluctuations of FRAP levels were found during storage. The major change in FRAP may be explained by the alteration of phenolic compounds. Some phenolic compounds might be dissociated and some might be formed during processing and/or storage, while their antioxidant activities are perhaps still maintained or promoted [6]. Phenol-protein interactions can also improve antioxidant property [28]. It should be noted that there was a relatively similar trend between TPC and FRAP (r: 0.524 and P<0.01).

Generally, ABTS radical scavenging activity (ABTS-RSA) in the control and MD treatments significantly decreased after 1 month and then slightly increased at the end of storage (P<0.05) (Figure 8). These results indicate that MD addition cannot prevent loss of ABTS-RSA, which is the opposite of FRAP because they have a different mechanism to measure antioxidant activity. There were relatively similar trends between protein solubility and ABTS-RSA (r: 0.408 and P<0.01). This might indicate that the reduction of ABTS-RSA is related to a decrease in protein solubility.

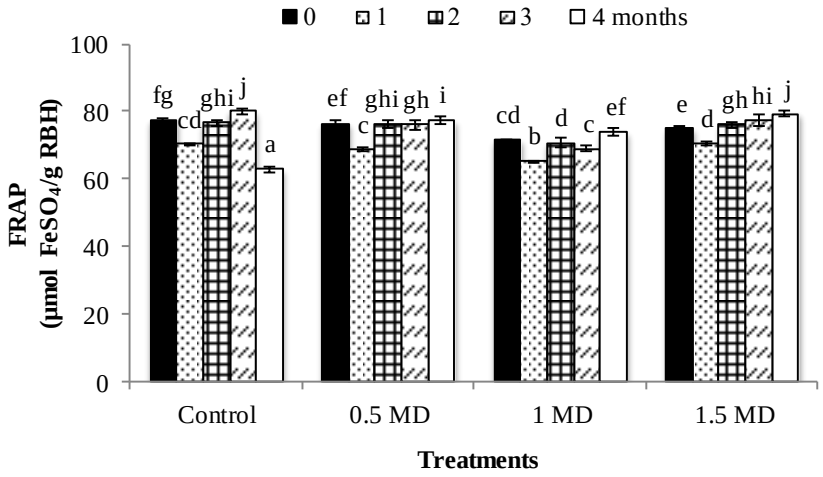

Figure 7 Ferric reducing antioxidant power (FRAP) of RBH with and without maltodextrin (MD) at different ratios during 4 months storage at 40 °C. The different letters on the column indicate significant differences (P<0.05). Treatments are as in Table 1.

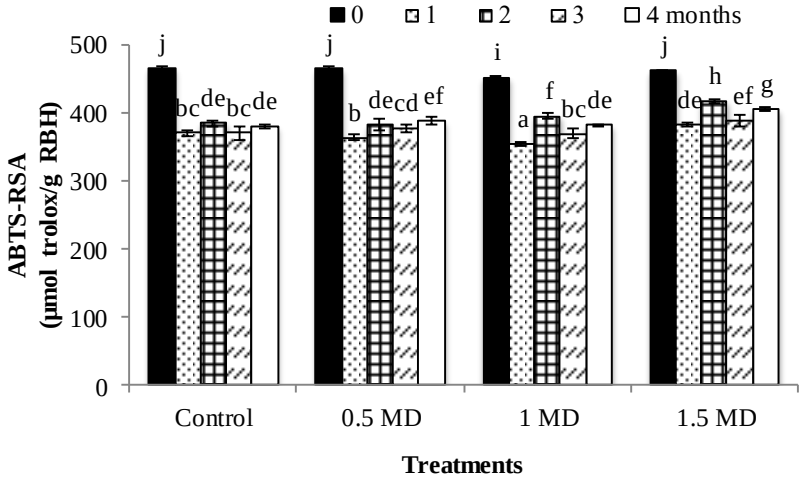

Figure 8 ABTS radical scavenging activity (ABTS-RSA) of RBH with and without maltodextrin (MD) at different ratios during 4 months storage at 40 °C. The different letters on the column indicate significant differences (P<0.05). Treatments are as in Table 1.

3.6 Browning intensity and pH

Maillard reaction products (MRPs) are formed between reducing sugars and amino acids, peptides or proteins in several food products. The formation of final MRPs or browning compounds can be estimated by an indirect method, which is a measurement of browning intensity by monitoring the absorbance at 420 nm. Only the ratios of encapsulating material to RBH significantly affected the browning intensity of RBH solution. The more the MD ratio increased, the lower the browning intensity of the sample solution (P<0.05) (data not shown). The lower browning intensity is most likely a reflection of the dilution of the brown RBH solution with the white powder of MD. Normally, MRPs formation is induced by heat and pH during processing and storage. The Maillard reactions (MRs) affect protein aggregation and protein solubility loss during storage [29]. The a_w or moisture content is also an important factor affecting MRPs formation during storage, notably with a high moisture content [29]. It was found that the browning intensity typical of MRPs, was quite stable during storage (P>0.05) (data not shown). This might be because a_w of all treatments during storage was very low (<0.26), when non-enzymatic reactions are unlikely to occur. In addition, RBH powder tightly packed under vacuum conditions with light protection might help to slow down MRs. Similarly, Rao et al. [30] stored egg white hydrolysate powder at 45 °C for 2 months with controlled a_w between 0.05-0.79. They found that a sample with a_w between 0.05-0.31 had a low rate of MRs after 1 month storage.

Another factor which relates to non-enzymatic reaction is pH. There was a positive correlation between pH and browning intensity (r: 0.748 and P<0.01). Both factors, MD content and storage time excluding their interaction significantly influenced the pH values of RBH. The control had higher pH than MD treatments. When MD content increased, pH decreased (P<0.05) (Table 3). This could be due to the dilution of the original alkaline condition of the RBH with MD addition. However, pH in all treatments was slightly reduced in the same trends within 4 months storage (P<0.05). It significantly decreased after one month of storage then was stable until 4 months which is a similar process to browning intensity. Some other researchers have reported varied results of non-enzymatic browning with pH values. To improve protein solubility and functionality, the MRs between rice protein and glucose (weight ratio of 1:1) were conducted at pH 10 and 11 at 100 °C [31]. Rice protein solubility and rate of MR increased to a higher pH. In this research, however, RBH powder might have been subjected to certain conditions (very low a_w, vacuum packed in an aluminum foil bag) that can impede browning reactions. Likewise, Cao et al. [32] observed that storage of dehydrated *Brassica parachinensis* under vacuum packaging or in darkness at 37 °C could delay the browning process.

Table 3 pH values of RBH with and without maltodextrin (MD) at different ratios during 4 months storage at 40 °C.

Treatments*	pH value[1]
Control	9.28 ± 0.17^{d}
0.5 MD	9.08 ± 0.17^{c}
1 MD	8.89 ± 0.18^{b}
1.5 MD	8.65 ± 0.17^{a}
Storage times (months)	pH value[1]
0	9.21 ± 0.28^{B}
1	8.82 ± 0.28^{A}
2	9.02 ± 0.21^{AB}
3	8.96 ± 0.25^{A}
4	8.93 ± 0.25^{A}

* Treatments are as in Table 1.
[1] Values give means ± standard deviation from triplicate observations.
[a-d] The different letters indicate significant differences (P<0.05) among treatments.
[A-B] The different letters indicate significant differences (P<0.05) among storage times.

4. Conclusions

MD addition with different ratios of MD to RBH (0.5:1, 1:1 and 1.5:1) can preserve total phenolic content and FRAP values during storage, but cannot impede the reduction of protein solubility and ABTS-RSA during 4 months storage at 40 °C. The changes of the conformational structures (increasing of β-sheet and decreasing of α-helix and β-turn) of protein in RBH were possibly caused by protein aggregation leading to loss of protein solubility. These changes were hindered by encapsulating RBH with MD especially at 1:1 and 1:1.5 ratios. In addition, MD impeded an increase of a_w and maintained the browning intensity characteristic of MRPs of all samples during storage compared to the control. Consequently, maltodextrin can prevent deteriorations of antioxidant rice bran hydrolysates to some extent during storage.

5. Acknowledgements

The authors greatly appreciate the assistance of the Thailand Research Fund through the Royal Golden Jubilee Ph.D. Program (RGJ: Grant no. PHD/0128/2553 to GK and ST) for their financial support. This research was also funded by Khon Kaen University through the National Research Council of Thailand (Grant no. 581101/2015). Special thanks are due to Mr. Varapong Supachok, Kasisuri Co., Ltd. for supplying partial funds and raw materials (industrially defatted rice bran, IDRB).

6. References

[1] Food and Agriculture Organization of the United Nations., 2017. FAO Rice Market Monitor [WWW Document]. URL http://www.fao.org/economic/est/publications/rice-publications/rice-market-monitor-rmm/en/ (accessed 01. 02. 18).
[2] Shih, F.F., 2003. An update on the processing of high-protein rice products: A review. Nahrung/Food 47, 420–424.
[3] Iqbal, S., Bhanger, M.I., Anwar, F., 2007. Antioxidant properties and components of bran extracts from selected wheat varieties commercially available in Pakistan. LWT-Food Science and Technology 40,361–367.
[4] Kaewjumpol, G., Oruna-Concha, M.J., Niranjan, K., Thawornchinsombut, S., 2018. The production of hydrolysates from industrially defatted rice bran and its surface image changes during extraction. Journal of the Science of Food and Agriculture.
[5] Jeyakumari, A., Zynudheen, A.A., Parvathy, U., 2016. Microencapsulation of bioactive food ingredients and controlled release - A review. MOJ Food Processing and Technology 2, 1–9.
[6] Estupiñan, D.C., Schwartz, S.J., Garźon, G.A., 2011. Antioxidant activity, total phenolics content, anthocyanin, and color stability of isotonic model beverages colored with Andes berry (Rubus glaucus Benth) anthocyanin powder. Journal of Food Science 76, S26–S34.
[7] Laine, P., Kylli, P., Heinonen, M., Kirsi, J., 2008. Storage stability of microencapsulated cloud berry (Rubus chamaemorus) phenolics. Journal of Agricultural and Food Chemistry 56, 11251–11261.
[8] Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Byrne, D.H., 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 19, 669–675.

[9] Yin, L.J., Tong, Y.L., Jiang, S.T., 2005. Improvement of the functionality of minced mackerel by hydrolysis and subsequent lactic acid bacterial fermentation. Journal of Food Science 70, 172–178.
[10] Wang, C., Li, D., Xu, F., Hao, T., Zhang, M., 2014. Comparison of two methods for the extraction of fractionated rice bran protein. Journal of Chemistry 2014, 1–10.
[11] Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry 193, 265–275.
[12] Dong, S., Panya, A., Zeng, M., Chen, B., McClements, D.J., Decker, E.A., 2012. Characteristics and antioxidant activity of hydrolyzed β-lactoglobulin-glucose Maillard reaction products. Food Research International 46, 55–61.
[13] Schmidt, C.G., Cerqueira, M.A., Vicente, A.A., Teixeira, J.A., Furlong, E.B., 2014. Rice bran protein-based films enriched by phenolic extract of fermented rice bran and montmorillonite clay. CyTA-Journal of Food 13, 204–212.
[14] Wang, W., Guo, J., Zhang, J., Peng, J., Liu, T., Xin, Z., 2015. Isolation, identification and antioxidant activity of bound phenolic compounds present in rice bran. Food Chemistry 171, 40–49.
[15] Pourali, O., Feridoun, S.A., Yoshida, H., 2010. Production of phenolic compounds from rice bran biomass under subcritical water conditions. Chemical Engineering Journal 160, 259–266.
[16] Htwe, N.N., Srilaong, V., Tanprasert, K., Tongchitpakdee, S., Kanlayanarat, S., Uthairatanakij, A., 2010. Effect of storage time and temperature on radical scavenging activities and bioactive compounds in colored rice varieties. Journal of Food, Agriculture and Environment 8, 26–31.
[17] Haslam, E., Williamson, M.P., Baxter, N.J., Charlton, A.J., 1999. Astringency and polyphenol protein interactions. Recent Advances in Phytochemistry 33, 289–318.
[18] Negrão-Muragami, A.N., Nunes, G.L., Pinto, S.S., Murakami, F.S., Amante, E.R., Petrus, J.C.C., Prudêncio, E.S., Amboni, R.D.M.C., 2017. Influence of DE-value of maltodextrin on the physicochemical properties, antioxidant activity, and storage stability of spray dried concentrated mate (Ilex paraguariensis A. St. Hil.). LWT-Food Science and Technology 79, 561–567.
[19] Costantino, H.R., Langer, R., Klibanov, A.M., 1994. Moisture- induced aggregation of lyophilized insulin. Pharmaceutical Research 11, 21–29.
[20] Rocha-Parra, D.F.R., Lanari, M.C., Zamora, Chirife, J., 2016. Influence of storage conditions on phenolic compounds stability, antioxidant capacity and colour of freeze-dried encapsulated red wine. LWT-Food Science and Technology 70, 162–170.
[21] Yang, Y., Cui, S.W., Gong, J., Guo, J., Guo, Q., Wang, Qi., Hua, Y., 2015. A soy protein-polysaccharide Maillard reaction product enhanced the physical stability of oil-in-water emulsions containing citral. Food Hydrocolloids 48, 155–164.
[22] Wang, C., Jiang, Wei, Li, Siu, X., Wang, Z., Li., 2011. Effect of secondary structure determined by FTIR spectra on surface hydrophobicity of soybean protein isolate. Procedia Engineering 15, 4819–4827.
[23] Goormaghtigh, E., Cabiaux, V., Ruysschaert, J.M., 1990. Secondary structure and dosage of soluble and membrane proteins by attenuated total reflection Fourier-transform infrared spectroscopy on hydrated films. The FEBS Journal 193, 409–420.
[24] Meng-Xia, X., Yuan, L., 2002. Studies on the hydrogen bonding of aniline's derivatives by FT-IR. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 58, 2817–2826.
[25] Wang. W., 2000. Lyophilization and development of solid protein pharmaceuticals: A review. International Journal of Pharmaceutics 203, 1–60.
[26] Costantino, H.R., Schwendeman, S.P., Langer, R., Klibanov, A.M., 1998. Deterioration of lyophilized pharmaceutical proteins. Biochemistry (Moscow) 63, 357–363.
[27] Labuckas, D.O., Maestri, D.M., Perelló, M., Martínez, M.L., Lamarque, A.L., 2008. Phenolics from walnut (Juglans regia L.) kernels: Antioxidant activity and interactions with proteins. Food Chemistry 107, 607–612.
[28] Almajano, M.P., Delgado, M.E., Gordon, M.H., 2007. Changes in the antioxidant properties of protein solutions in the presence of epigallocatechin gallate. Food Chemistry 101, 126–130.
[29] Rao, Q., Kamdar, A.K., Labuza, T.P., 2016. Storage stability of food protein hydrolysates: a review. Critical Reviews in Food Science and Nutrition 56, 1169–1192.
[30] Rao, Q., Rocca-Smith, J.R., Schoenfuss, T.C., Labuza, T.P., 2012. Accelerated shelf-life testing of quality loss for a commercial hydrolysed hen egg white powder. Food Chemistry 135, 464–472.
[31] Li, Y., Lu, F., Luo, C., Chen, Z., Mao, J., Shoemaker, C., Zhang, F., 2009. Functional properties of the Maillard reaction products of rice protein with sugar. Food Chemistry 117, 69–74.
[32] Cao, H., Zhang, M., Mujumdar, A.S., Xiao, G., Sun, J., 2007. Study on reduction of water activity and storage stability for dehydrated Brassica parachinensis with intermediate moisture. Drying Technology 25, 669–674.