https://doi.org/10.48048/tis.2022.2316

Physicochemical Properties of Rice Bran Hydrolysate Prepared in a Pilot Scale Process and Its Application in Milk Tablets

Chompoonuch Khongla[1,*], Jitpanu Chuaingan[1], Thanachon Siadkhunthod[1], Patiphan Somnam[1], Sumalee Musika[1] and Papungkorn Sangsawad[2]

[1]Department of Applied Biology, Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima 30000, Thailand
[2]School of Animal Technology and Innovation, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand

([*]Corresponding author's e-mail: chompoonuch.2840@gmail.com)

Received: 29 January 2022, Revised: 27 March 2022, Accepted: 5 April 2022, Published: 9 November 2022

Abstract

The objectives of this study were to produce rice bran hydrolysate (RBH) in a pilot-scale process and determine its physicochemical properties including chemical compositions, α-amino contents, molecular weight (MW) distribution and antioxidant activities. The results found that RBH contained a crude protein of 16.14 %, α-amino contents of 51.56 mg Leucine Eq./g RBH, ABTS radical scavenging activity of 20.17 mg Trolox Eq./g RBH, and FRAP value of 5.77 mg Trolox Eq./g RBH. The major peak of MW peptides of RBH was 753 - 6,088 Da (50.81 %). RBH in various levels (0 - 2.0 %) was supplemented in milk tablets. Physicochemical properties, and sensory evaluation (9-point hedonic scale) of all milk tablets were then determined. The result indicated that the milk tablets with 2.0 % RBH (MT_2%RBH) contained higher antioxidant activities than the others, however, the overall acceptability score of all milk tablets was not significantly different ($p \geq 0.05$). MT_2 %RBH was selected for further development by adding 2.0 % cocoa (MT_2%RBHC) and 2.0 % vanilla powder (MT_2%RBHV). The MT_2%RBHC containing higher antioxidant activities and sensory attributes score than the others was further selected for microbiological qualities, consumer and purchase tests (n = 300). It was found that MT_2%RBHC was accepted by 300 people, with an overall acceptability score of 7.16 (Like moderately), and its microbiological qualities, including total bacteria, total yeast and mold and *Escherichia coli* did not exceed the standard limits. These results suggested that RBH produced in the pilot-scale process has a potential as nutritive and antioxidative supplementation in milk tablets.

Keywords: Milk tablets, Pilot scale, Rice bran hydrolysate, Antioxidant activity, Consumer test

Introduction

Milk is a highly nutritive product for humans. The nutrient composition of fresh fluid milk is 88 % water, 3.28 % protein, 3.20 % fat, 4.67 % carbohydrate, 32 µg vitamin A, 0.138 mg riboflavin, 1.1 µg vitamin D and 123 mg calcium [1]. However, its consumption is still low because most children and adults prefer not to drink plain milk [2]. The statistics show that Thai people consume only 18 L of milk person per year, which is much lower than the 25 L recommended by the World Health Organization (WHO) and the world average of 113 L per person per year [3]. In addition, fresh milk has a short shelf life and cannot be stored for a long period of time at room temperature. Therefore, changing the liquid milk form into a chewable milk tablet might be an alternative product for people who prefer not to drink plain milk because milk tablet is delicious, cheap, easily consumed and transported, and can be stored for a long period of time [2]. Furthermore, the milk tablet is abundant in protein, calcium, vitamin A and vitamin D, and has a high nutritious value [4]. The average nutrition facts for the 21 products of the milk tablet of Thailand contained 17.7 % fat, 56.6 % carbohydrate and 15.4 % protein [5]. Therefore, milk tablet is a good choice for consumers of all ages; both children and adults. The ingredients of milk tablets are whole milk powder, whey powder, lactose, sugar or icing sugar, anticaking agent and flavor additives that commercialized in the market. To increase the value of milk tablet, it should be transformed into a functional product by supplementation with functional ingredients.

Rice bran is an underutilized component which is the major by-product of the rough rice milling process. Rice production in Thailand was approximately 18,600 metric tons in 2020/2021 [6]. Rice bran is approximately 5 - 8 % of the whole rice kernel, therefore, about 930 - 1,488 metric tons of rice bran were generated as a by-product in 2020/2021. Rice bran is composed of 15 - 22 % lipids, 34.1 - 52.3 % carbohydrates, 7 - 11.4 % fiber, 6.6 - 9.9 % ash, 8 - 12 % moisture and 10 - 16 % highly nutritional protein [7,8]. However, rice bran is generally used as an animal feed with low value. The proteins in rice bran are of a complex nature mainly including albumin, globulin, glutelin and prolamin with a high digestibility of up to 90 % [9]. In addition, rice bran contains high quality protein with high amounts of essential amino acids, especially aromatic amino acids (9.46 - 11.41 %) that act as strong antioxidants [10]. However, the macroproteins are generally inactive within the sequence of the original structure. Enzymatic hydrolysis is an effective method to expose and release bioactive peptides without affecting nutritional value. It has been reported that rice bran hydrolysate (RBH) has the potential to be used as dietary or nutraceutical agents beneficial for human health [11]. Small peptides in RBH derived from enzymatic hydrolysis can act as antioxidant substances due to being rich hydrogen donors [12,13]. Alcalase was the most effective enzyme that provided RBH with the highest protein yield, protein content and antioxidant activity [11,14]. Previously, our experiments suggested that RBH prepared from Alcalase hydrolysis in a scaled-up process (4 kg of defatted rice bran powder with 40 L of RO water) could be used as an ingredient in functional broken rice beverage with antioxidant activity [15]. Besides peptides in hydrolysate having antioxidant activity, the intermediates, or the final brown polymer in xylose-bovine casein hydrolysate Maillard reaction product (MRPs) also exhibited antioxidant activity [16]. Thus, the addition of RBH in milk tablets might not only increase antioxidant peptides but also produce the MRPs with antioxidant activity.

Therefore, in the present work, we prepared RBH using Alcalase hydrolysis in a pilot-scale process (20 kg of defatted rice bran with 200 L of RO water), determined its physicochemical properties including chemical composition, α-amino content, antioxidant activities and molecular weight distribution. This work also developed a functional milk tablet supplemented with RBH which possesses antioxidant activity.

Materials and methods

Materials

Jasmine rice bran was donated by Phimai Agriculture Cooperative Limited, Nakhon Ratchasima, Thailand. Rice bran was defatted using an oil press machine. 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Biochemika (Buchs, Switzerland). Trinitrobenzenesulfonic acid (TNBS) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals and reagents used were of analytical grade, except for Alcalase and NaOH which were food grade.

Pilot scale preparation of RBH

Twenty kg of defatted rice bran powder was mixed with 200 L of RO water, and the pH was adjusted to 10.0 by the addition of 500 g of NaOH. Subsequently, Alcalase was added into the mixture with the ratio of enzyme to substrate of 2:100 (w/w). Hydrolysis was carried out at 50 ℃ for 2 h in a Solid-liquid extractor. Samples were heated at 100 ℃ for 15 min to terminate the reaction and the extracted liquid was concentrated by using falling film evaporator. The concentrate was mixed with 2 kg of maltodextrin and dried using a Spray dryer. The dried sample, referred to as rice bran hydrolysate (RBH), was kept at −20 ℃ and used for further experiments.

Physicochemical properties of RBH

Chemical composition

The moisture, crude protein, crude fat and ash contents of RBH were determined according to AOAC using sub components 925.09, 979.09, 920.39 and 923.03, respectively [17]. The total carbohydrate content in RBH was calculated by subtracting the sum of moisture, ash, fat and crude protein content from the total matter.

α-Amino content and antioxidant activity assays

RBH (0.1 g) was mixed with 1 mL of deionized water and then centrifuged at 10,000 rpm for 10 min. The supernatant was collected and determined the α-amino content using trinitrobenzenesulfonic acid (TNBS) with L-leucine as a standard [18], and expressed as mg Leucine equivalents (Eq.)/g RBH. The absorbance was monitored at 420 nm using a spectrophotometer. The yield of peptides was calculated from a total weight of RBH powder (g)×α-amino content (mg Leucine Eq./g RBH).

ABTS radical scavenging activity of the supernatant was determined according to the method of Wiriyaphan *et al.* [19]. In brief, the supernatant (20 µL) was mixed with ABTS working solution (1,980 µL) and incubated in the dark at room temperature for 5 min. Absorbance was measured at 734 nm. The result was expressed as mg Trolox equivalents (Eq.)/g RBH.

The ferric reducing antioxidant power (FRAP) was measured according to the method of Benzie and Strain [20]. In brief, the supernatant (100 µL) was mixed with FRAP working solution (1,000 µL) and incubated at 37 °C in a water bath for 15 min. The absorbance was measured at 593 nm. The results were expressed as mg Trolox Eq./g RBH.

Analysis of molecular weight distribution

Peptide molecular weight distributions of RBH were determined using a size exclusion chromatography technique according to the method of our previous study [21] with some modifications. A Superdex Peptide 10/300 GL column (10×300 mm^2; GE Healthcare, Piscataway, NJ, USA) was pre-equilibrated with 30 % ACN + 0.1 % TFA in deionized water. One hundred µL of diluted RBH (1.6 mg leucine eq./mL) were then loaded into the column, and eluted using 1 column volumes of the same solvent at a flow rate of 0.6 mL/min. The absorbance was read at 215 nm. Cytocrom C (12,000 Da), Aprotinin (6,512 Da), peptides (244 - 1480.6 Da) and tyrosine (181 Da) were used as standard. Blue dextran (2,000 kDa) was used as a visible marker for void volume in the gel filtration column.

Development of milk tablets supplemented with various RBH levels

Preparation of milk tablets supplemented with various RBH levels

Ingredients of milk tablets supplemented with various RBH levels (0.0 - 2.0 %w/w) were prepared as described in **Table 1**. All ingredients were mixed in Dry Blender for 10 - 15 min. Subsequently, the mixture was compressed into a tablet with a rotary Tableting Machine (Support Pack Co., Ltd., Samutprakan, Thailand). Milk tablets were packed into an aluminium foil bag and used for further experiments.

Table 1 Ingredients of milk tablets supplemented with various RBH levels.

Ingredients	**Content (%w/w)**				
	T1	**T2**	**T3**	**T4**	**T5**
Milk powder	69.5	69.0	68.5	68.0	67.5
Whey protein concentrate	10	10	10	10	10
Icing sugar	20	20	20	20	20
cab-o-sil	0.5	0.5	0.5	0.5	0.5
RBH	0	0.5	1.0	1.5	2.0

a_w, moisture and color values

a_w of milk tablets was determined by using a water activity meter (AQUA LAB 4TE, dew point, water activity meter, USA). The moisture content of milk tablets was measured according to a method of AOAC [17]. Color values of milk tablets were measured in terms of CIE L*, a*, b* values using a color measurement device (Hunter Lab, Color flex 4510, USA).

Sensory evaluation

Sensory attributes (color, odor, taste, texture and overall acceptability) of milk tablets were evaluated by 30 untrained panelists using a preference test with a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely). The panelists were not informed of the experimental procedure and the samples were blind-coded with 3-digit random numbers. The untrained panel of assessors selected comprised of 15 female and 15 male panelists aged between 17 and 25. The externally recruited panelists had not worked or studied at Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan (Nakhon Ratchsima, Thailand). All panelists declared no food allergies.

α-Amino acid content and antioxidant activity assays

Five g of milk tablets were mixed with 25 mL of DI water. The mixture was homogenized (IKA T25 Digital Ultra Turrax, Staufen, Germany) at 8,000 rpm for 1 min and then filtered using Whatman no.1. The filtrate was used to determine the α-amino acid content, ABTS radical scavenging activity and ferric reducing antioxidant power as mentioned before.

Flavor development of milk tablets supplemented with 2.0 % RBH

Due to high antioxidant activity and low acceptability, a milk tablet supplemented with 2.0 % RBH was selected for further flavor development by the addition of 2.0 % cocoa and vanilla powder. The a_w, moisture content, color values, sensory evaluation, α-amino content and antioxidant activity of the products were determined as mentioned before. The milk tablets supplemented with 2.0 % RBH plus 2.0 % cocoa powder (MT_2%RBHC) exhibited higher antioxidant activities and sensory attributes score than the milk tablets without the addition of cocoa, hence were selected for further experiments.

Microbiological quality test

Microbiological qualities (Total plate count, Yeasts and Molds and *E. coli*) of MT_2%RBHC were determined following the United States Food and Drug Administration (U.S. FDA) Biological Analytical Manual (BAM) [22-24].

Consumer and purchase tests

A consumer panel of 300 volunteers recruited from the Rajamangala University of Technology Isan evaluated the MT_2%RBHC using a preference test with a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely) to evaluate the acceptability of color, odor, taste, texture and overall acceptability. All panelists declared no food allergies. In addition, the purchase intention test was done by using the question "Would you buy it?" accompanied by a structured scale of 4 points ("No", "Yes", "Maybe buy" and "Maybe not buy"). The consumer panel of volunteers selected comprised of 155 female and 145 male volunteers aged between 15 to 27. The externally recruited volunteers had not worked or studied at Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan (Nakhon Ratchsima, Thailand).

Statistical analyzes

The results were analyzed using a 1-way analysis of variance (ANOVA) at $p < 0.05$. Means comparison was performed using Duncan's multiple-range test (DMRT) using a SPSS package (SPSS 17.0 for Windows, SPSS Inc., Chicago, IL, USA). The α-Amino content, antioxidant activity, a_w, moisture and color values of all samples were analyzed using completely randomized design (CRD). Sensory evaluation was analyzed using a randomized complete block design (RCBD).

Results and discussion

Physicochemical properties of RBH

Chemical compositions, α-amino content and antioxidant activities

The recovery yield of RBH (plus 2 kg of maltodextrin) prepared from hydrolysis of 20 kg defatted rice bran with Alcalase and spray drying was 13.34 kg, which was equivalent to α-amino content of 687.81 g Leucine (α-amino content of 51.56 mg Leucine Eq./g RBH) (**Table 2**). The presence of α-amino content in RBH reflected the presence of oligopeptides and/or amino acids which were released by Alcalase hydrolysis. Moisture, ash, crude fat, crude protein contents and total carbohydrate of RBH were determined to be 9.57, 11.00, 9.64, 16.14 and 53.65 %, respectively (**Table 2**). This indicated that RBH is a rich source of protein and has a potential as nutritive substance supplementation in functional food products. A high content of carbohydrates could be due to the presence of maltodextrin and partial unhydrolyzed rice bran in RBH.

ABTS radical scavenging activity and ferric reducing antioxidant power of RBH were 20.17 mg Trolox Eq./g RBH and 5.77 mg Trolox Eq./g RBH, respectively (**Table 2**). These results indicated that RBH prepared using Alcalase in the pilot-scale process contained peptides with antioxidant properties. Wang *et al*. [10] reported that rice bran contained high quality protein with high amounts of essential amino acids, especially aromatic amino acids (9.46 - 11.41 %) that act as strong antioxidants. Our previous results also suggested that RBH prepared from Alcalase possessed ABTS radical scavenging activity and ferric reducing antioxidant power [15]. Small peptides in RBH can act as antioxidant substances due to being rich hydrogen donors [12,13]. Thamnarathip *et al.* [14] suggested that amino acid with a phenolic hydroxyl group in the peptides of RBH may also be responsible for the ABTS radical scavenging activity [14]. The antioxidative amino acids in the RBH such as histidine, proline, tryptophan, leucine, valine, alanine and methionine could have also contributed to the antioxidant activity [14,25]. The ABTS radical scavenging activity was coincidental with the total phenolic content due to the H-atom-donation of a hydroxyl group in phenolic compounds [14,26]. In addition, ferric reducing antioxidant power of plant extracts was correlated well with phenolic content [27,28]. Therefore, the antioxidant activity of RBH could be due to the presence of antioxidant amino in peptide sequences and phenolic compounds.

Table 2 Chemical properties and antioxidant activity of RBH.

Compositions	Content
Moisture (% d.b.)	9.57 ± 0.19
Ash (% d.b.)	11.00 ± 0.19
Crude fat (% d.b.)	9.64 ± 1.22
Crude protein (% d.b.)	16.14 ± 0.32
Total carbohydrate (% d.b.)	53.65 ± 0.00
α-Amino content (mg Leucine Eq./g RBH)	51.56 ± 1.71
ABTS radical scavenging activity (mg Trolox Eq./g RBH)	20.17 ± 0.66
Ferric reducing antioxidant power (mg Trolox Eq./g RBH)	5.77 ± 0.25
Yields of peptides (g Leucine Eq.)	687.81
Yields of weight (kg)	13.34

Note: Total carbohydrate was calculated from formula; 100 − (moisture + ash + crude fat + crude protein).

Molecular weight (MW) distribution

RBH exhibited the major area peak of MW in the range of 753 - 6,088 Da (50.81 %). In addition, area peaks of peptides at MW of 624, 338 and < 244 Da were also observed in RBH, with area peaks of 19.46, 18.10 and 11.63 %, respectively. This result indicated that RBH is composed of different MW peptide sizes. MW peptides pattern of RBH prepared in the pilot-scale process (20 kg of defatted rice bran powder with 200 L of RO water) were slightly different from the result of our previous work that prepared RBH using Alcalase in the pilot-scale (4 kg of defatted rice bran powder with 40 L of RO water), whereby the MW peptides of RBH in the scale-up process were 837 - 7,462 Da (48.02 %), 621 Da (28.54 %), 414 Da (13.73 %) and < 224 (9.71 %) [15]. These results suggested that the production of RBH in a larger-scale process resulted in different MW distribution of generated peptides. Alcalase is a non-specific endoprotease produced from bacterial source cleaving peptide bonds from the interior of the polypeptide chains and outward which produced rice bran hydrolysate having mainly polypeptides with molecular weight < 16 kDa and small amounts of high- or low molecular weight polypeptides [29,30]. The MW of RBH fractions prepared from Alcalase using tricine-SDS-PAGE clearly showed a band with an MW of 6 kDa [14]. The difference between MW of RBH prepared in our work and previously reported [14,30] could have been due to the difference in hydrolysis conditions and the limitation of the analytical method. The antioxidant activities of peptides were related to the MW distribution [25,31]. It is well known that molecules with a lower molecular weight peptides have a high antioxidant activity because peptides with lower MW could increase solubility, react with free radicals more easily, and enhance easy reduction of free radicals compared to larger, less soluble peptides [32-35]. Therefore, low MW peptides in RBH prepared in a pilot-scale process might have contributed to the antioxidant activity of RBH.

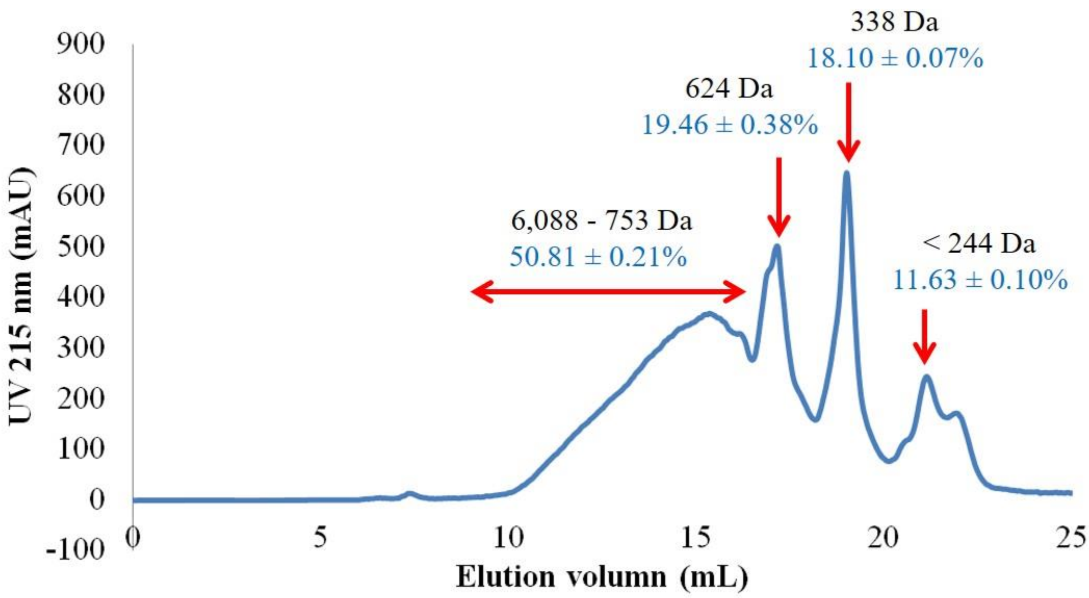

Figure 1 The molecular weight distribution of RBH produced in a pilot-scale process using a size exclusion chromatography.

Development of milk tablet supplemented with various RBH levels (0 - 2.0 %)

a_w, moisture and color value

a_w and moisture content are considered to be the most critical factor for microbial growth, determining the quality, preservation, and shelf life of foodstuffs. The a_w of milk tablets was 0.31 - 0.37, which is classified as dried foods with a_w below 0.6 (**Table 3**). The moisture content of milk tablets was 2.26 - 3.01 % which did not exceed the standard limits of flavored milk (moisture content not more than 5 %).

Color plays an essential role in food appearance and acceptability. L* (lightness) and b* (yellowness) values of milk tablets tended to decrease with an increase in RBH levels while a* (redness) value of T5 was the highest (**Table 3**). These results indicated that the addition of RBH had a direct effect on the darker color of the milk tablet due to the brown color of RBH powder.

Table 3 a_w, moisture content (%) and color values of milk tablet supplemented with 0.0 - 2.0 % (w/w) of RBH.

Treatments	a_w	Moisture content (%)	Color values		
			L*	a*	b*
T1	0.35 ± 0.01^b	2.75 ± 0.19^{ab}	94.89 ± 0.07^a	3.08 ± 0.04^{bc}	10.17 ± 0.06^a
T2	0.37 ± 0.00^a	2.54 ± 0.43^{ab}	95.07 ± 0.11^a	2.36 ± 0.11^d	9.95 ± 0.22^a
T3	0.37 ± 0.01^a	2.71 ± 0.56^{ab}	93.29 ± 0.05^b	2.91 ± 0.08^c	9.28 ± 0.10^b
T4	0.34 ± 0.01^c	2.26 ± 0.34^b	92.38 ± 0.36^c	3.20 ± 0.16^b	8.23 ± 0.68^c
T5	0.31 ± 0.01^d	3.02 ± 0.15^a	89.73 ± 0.07^d	4.43 ± 0.20^a	7.42 ± 0.28^d

Different superscripts within a column indicate significant differences ($p < 0.05$); T1 was milk tablet without the addition of RBH; T2 was milk tablet with the addition of 0.5 % RBH; T3 was milk tablet with the addition of 1.0 % RBH; T4 was milk tablet with the addition of 1.5 % RBH; T5 was milk tablet with the addition of 2.0 % RBH.

Sensory evaluation

Color, taste, texture and overall acceptability attributes score were not significantly different among milk tablets (**Table 4**). These results implied that the addition of RBH at 0.5 - 2.0 % did not affect the color, taste, texture and overall acceptability attributes score of milk tablets. However, the odor attribute score of T3, T4 and T5 was lower than T1 (milk tablet without the addition of RBH). These suggested that the addition of RBH at the level of 1.0 - 2.0 % had a negative effect on the odor attribute score of milk tablets due to an unfavorable flavor of rice bran. Therefore, milk tablet supplemented with RBH should be further developed to increase the acceptability of consumers.

Table 4 Sensory evaluation of milk tablets supplemented with 0.0-2.0% (w/w) of RBH**.**

Treatments	Color ^{ns}	Odor	Taste ^{ns}	Texture ^{ns}	Overall acceptability ^{ns}
T1	6.80 ± 1.28	6.50 ± 1.59^{a}	6.73 ± 1.12	6.37 ± 1.38	6.70 ± 1.16
T2	6.70 ± 1.27	6.27 ± 1.44^{ab}	6.43 ± 1.58	6.23 ± 1.65	6.20 ± 1.49
T3	6.50 ± 1.31	5.97 ± 1.47^{b}	6.23 ± 1.56	6.00 ± 1.61	6.27 ± 1.48
T4	6.50 ± 1.34	5.80 ± 1.90^{b}	6.17 ± 1.75	6.03 ± 1.99	6.13 ± 1.94
T5	6.57 ± 1.15	5.93 ± 1.50^{b}	6.30 ± 1.37	6.13 ± 1.54	6.23 ± 1.31

n = 30 Different superscripts within a column indicate significant differences ($p < 0.05$); T1 was milk tablet without the addition of RBH; T2 was milk tablet with the addition of 0.5 % RBH; T3 was milk tablet with the addition of 1.0 % RBH; T4 was milk tablet with the addition of 1.5 % RBH; T5 was milk tablet with the addition of 2.0 % RBH.

α-amino content and antioxidant activity

The α-Amino content of milk tablets tended to increase with an increase in RBH levels up to 1 % RBH (T3) and gradually increased after that but was not significantly different with T3 ($p \geq 0.05$) (**Figure 2(a)**). This could have been due to the limitation of peptides solubility. In addition, Maillard's browning reaction might have occurred during compressing milk tablets. Pizzoferrato *et al.* [36] reported that the percentage of blocked lysine, which indicated the occurrence of Maillard reaction in milk tablets, was approximately 13 %. Phisut and Jiraporn [37] suggested that the increase in browning intensity and formation of intermediate products were concomitant with the decrease in a free amino group. Therefore, amino acids and/or small peptides in RBH might have reacted with reducing sugars (lactose in milk powder and whey protein) leading to Maillard browning products, resulting in a non-significant difference in α-amino acid content between T3, T4 and T5.

ABTS radical scavenging activity of milk tablets tended to increase with an increase in RBH levels (**Figure 2(b)**). T3 - T5 had higher ABTS radical scavenging activity than T1 ($p < 0.05$) while only the FRAP value of T5 was significantly higher than T1 (**Figure 2(c)**). Our results agreed with Thamnarathip *et al.* [14] who reported that FRAP of RBH did not correlate well with ABTS radical scavenging activity. The authors suggested that the type of radical, the reaction mechanism, and condition (pH) between FRAP and the ABTS radical scavenging activity were different. These could be reasons for the non-correlation between ABTS radical scavenging activity and the FRAP of RBH.

FRAP and ABTS radical scavenging activity were not correlated well with α-amino acid content (**Figure 2(a)**). During heat treatments of food containing both carbohydrate and amino acid, peptide or proteins, the Maillard reaction occurs generally leading to brown-colored products with antioxidant activities [36-38]. The Maillard reaction occurs in all milk-based products during heat treatment of milk [38]. It has been reported that the intermediates, or the final brown polymer in xylose-bovine casein hydrolysate MRPs derived from Maillard reaction possessed antioxidant activity [16]. Therefore, Maillard browning products in milk tablets supplemented with RBH might have also contributed to the antioxidant activity of milk tablets. This implied that not only peptide content but also Maillard browning products affect antioxidant activity. Based on both FRAP and the ABTS radical scavenging activity assays, a milk tablet supplemented with 2.0 % RBH was selected for further flavor development by adding cocoa and vanilla powder.

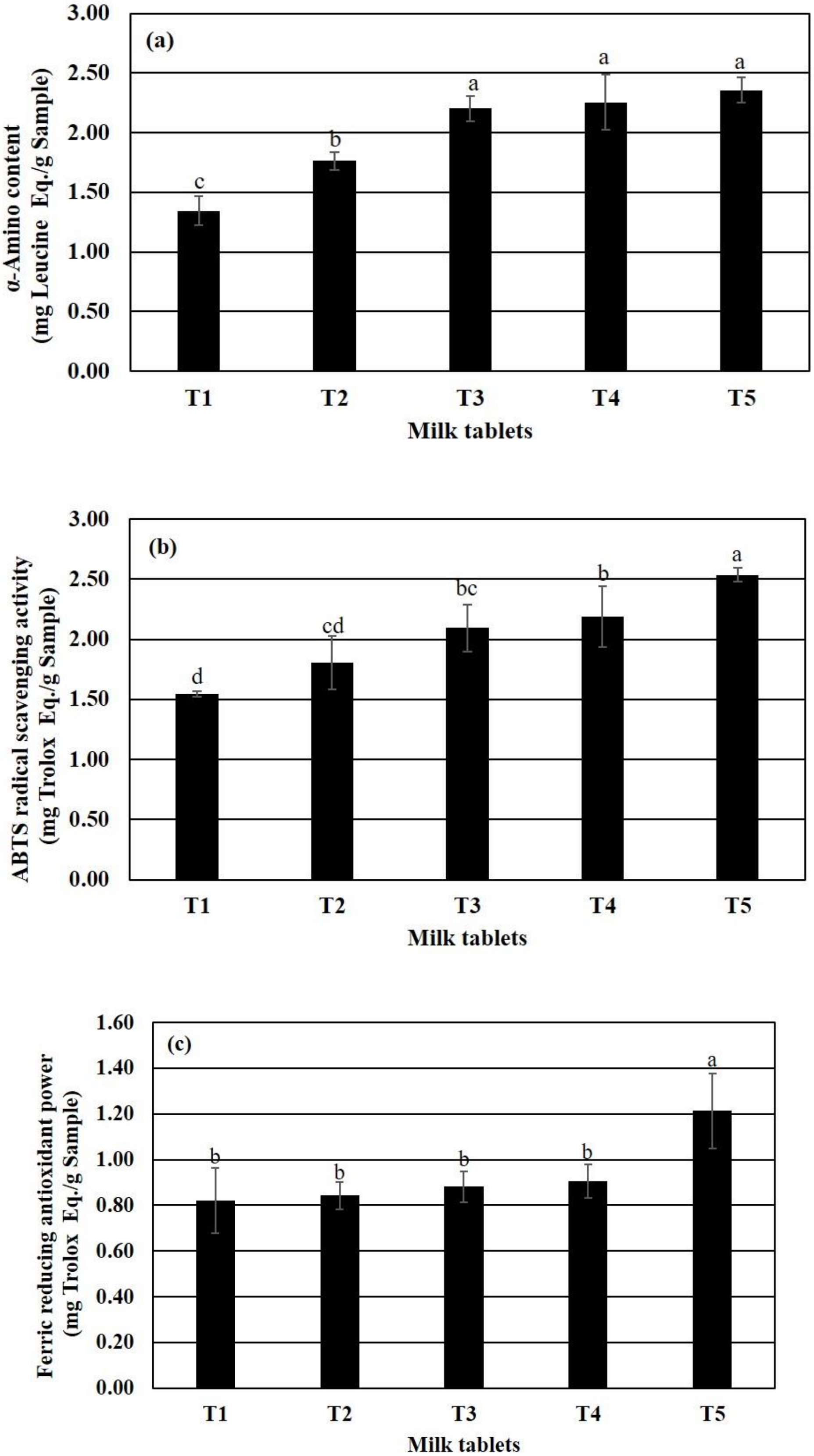

Figure 2 α-Amino content (a), ABTS radical scavenging activity (b), and ferric reducing antioxidant power (c) of milk tablets supplemented with rice bran hydrolysate at levels of 0.0, 0.5, 1.0, 1.5 and 2.0 % (T1 - T5, respectively). Bars with different letters indicate means with significant differences ($p < 0.05$).

Flavor development of milk tablet supplemented with 2.0 % RBH (MT_2%RBH), cocoa (MT_2%RBHC) and vanilla powder (MT_2%RBHV)

a_w*, *moisture content and color value

a_w and moisture content of MT_2%RBH, MT_2%RBHC, and MT_2%RBHV were 0.30 - 0.32 and 2.86 - 3.29 %, respectively (**Table 5**). The addition of 2 % cocoa powder decreased L* (lightness) and b* (yellowness) values, and increased a* value (redness) of milk tablet when compared to MT_2%RBH due to the dark color of cocoa powder. While the addition of 2 % vanilla powder increased L* (lightness) and b* (yellowness) values, it decreased a* value (redness) of milk tablet when compared to MT_2%RBH due to the white color of vanilla powder.

Table 5 a_w, moisture content (%), and color values of milk tablet supplemented with 2 % RBH (MT_2%RBH), cocoa (MT_2%RBHC) and vanilla powder (MT_2%RBHV).

Treatments	a_w	Moisture content (%) ^{ns}	Color value		
			L*	a*	b*
MT_2%RBH	0.32 ± 0.01^a	3.02 ± 0.15	89.73 ± 0.07^b	4.43 ± 0.20^a	7.42 ± 0.28^b
MT_2%RBHC	0.31 ± 0.01^a	3.29 ± 0.30	79.25 ± 0.10^c	8.45 ± 0.05^b	6.08 ± 0.07^c
MT_2%RBHV	0.30 ± 0.01^b	2.86 ± 0.30	91.41 ± 0.09^a	3.53 ± 0.01^c	8.49 ± 0.10^a

Different superscripts within a column indicate significant differences ($p < 0.05$); ns means not significantly different in the same column ($p \geq 0.05$)

Sensory evaluation

All sensory attributes scores of MT_2%RBHC and MT_2%RBHV were higher than those of MT_2%RBH, except for color and texture of MT_2%RBHV which were not significantly different from MT_2%RBH (**Table 6**). Overall acceptability of MT_2%RBHC and MT_2%RBHV was 7.43 and 7.13 (Like Moderately), respectively. These results indicated that the addition of 2 % cocoa and vanilla powder could improve the sensory attributes score of the milk tablet.

Table 6 Sensory attributes score of milk tablet supplemented with 2.0 % RBH (MT_2%RBH), cocoa (MT_2%RBHC) and vanilla powder (MT_2%RBHV).

Treatments	Color	Odor	Taste	Texture	Overall acceptability
MT_2%RBH	6.57 ± 1.15^b	5.93 ± 1.50^b	6.30 ± 1.37^b	6.13 ± 1.54^b	6.23 ± 1.31^b
MT_2%RBHC	7.07 ± 1.09^a	7.17 ± 1.27^a	7.17 ± 1.42^a	6.90 ± 1.45^a	7.43 ± 1.36^a
MT_2%RBHV	6.70 ± 1.22^{ab}	7.03 ± 1.30^a	7.00 ± 1.06^a	6.47 ± 1.36^{ab}	7.13 ± 1.02^a

N = 30 Different superscripts within a column indicate significant differences ($p < 0.05$)

Antioxidant activity

ABTS radical scavenging activity of MT_2%RBHC was higher than MT_2%RBHV ($p < 0.05$) but was not significantly different from MT_2%RBH ($p \geq 0.05$) (**Figure 3(a)**). While the FRAP value of MT_2%RBHC was higher than MT_2%RBH, it was not significantly different from MT_2%RBHV (**Figure 3(b)**). This indicated that the addition of 2 % cocoa into MT_2%RBH could improve the antioxidant activity of milk tablets due to the presence of phenolic compounds in cocoa powder. Therefore, the milk tablet with the addition of 2.0 % RBH plus 2.0 % cocoa (MT_2%RBHC) was selected for further experiments.

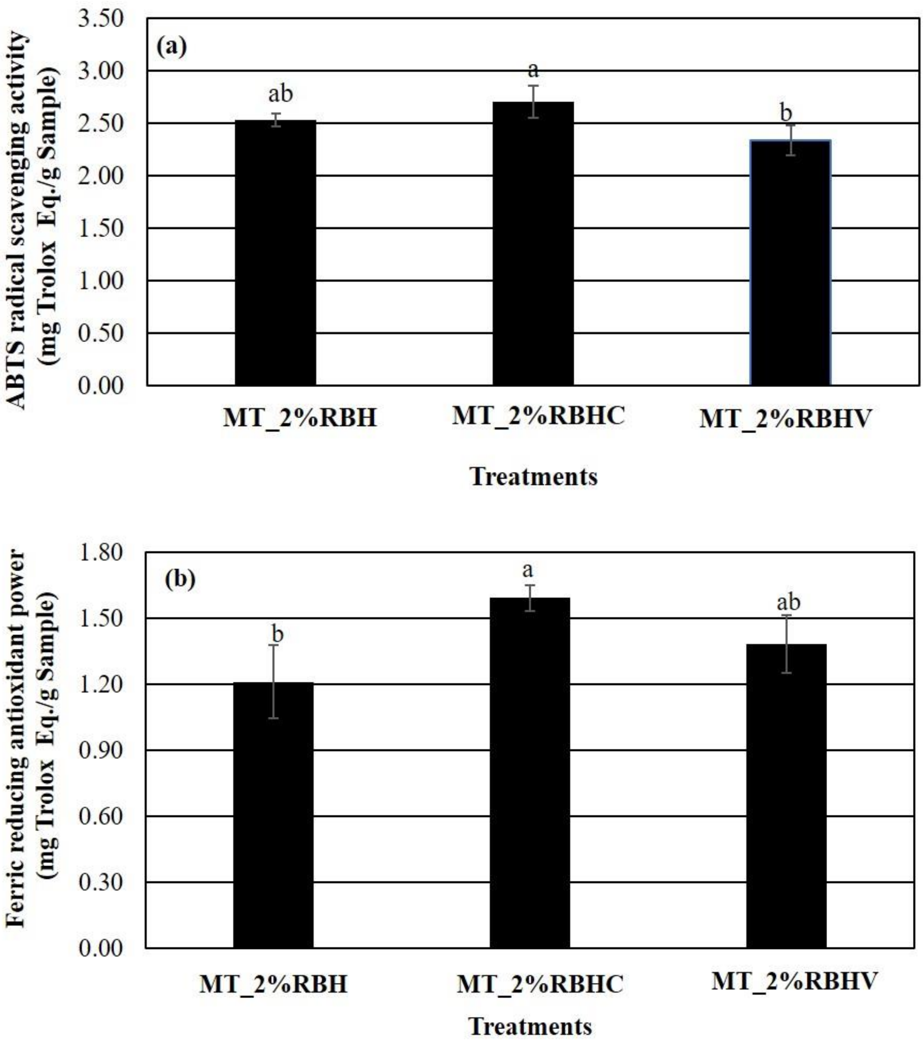

Figure 3 ABTS radical scavenging activity (a) and ferric reducing antioxidant power (b) of milk tablet supplemented with 2 % RBH (MT_2%RBH), MT_2%RBHC was the milk tablet with the addition of 2.0 % RBH plus 2.0 % cocoa; MT_2%RBHV was the milk tablet with the addition of 2.0 % RBH plus 2.0 % vanilla. Bars with different letters indicate means with significant differences ($p < 0.05$).

Microbial qualities

Total plate count and yeasts and molds of MT_2%RBHC were $< 2\times10^3$ CFU/g. *E. coli* was not detected in 1 g of milk tablet (**Table 7**). Microbial qualities (TPC, yeasts and molds and *E. coli*) did not exceed the standard limits of flavored milk [39].

Table 7 Microbiological qualities of milk tablet supplemented with 2.0 % RBH pus 2.0 % cocoa powder (MT_2%RBHC).

Microbiological test	**Microbiological qualities**	
	MT_2%RBHC	**Standard**
Total plate count	$< 2\times0^3$ CFU/g	$< 1\times10^5$ CFU/g
Yeasts and molds	$< 2\times10^3$ CFU/g	-
E .coli	Not detected in 1 g	Not detected in 1 g

Consumer and purchase tests

MT_2%RBHC was evaluated for consumer and purchase tests by using the consumer´s panel consisting of 300 people, mostly aged between 17 - 25 years (95 %). The panelists were recruited among the staff and student population of the Rajamangala University of Technology Isan. Odor and texture attribute scores of MT_2%RBHC were 6.63 and 6.89, respectively, which were described as like a little bit to like moderately (**Table 8**). Color, taste and overall acceptability of MT_2%RBHC were 7.08, 7.18 and 7.16, respectively, which were described as like moderately (**Table 8**). The purchase test indicated that 29.0 % of the panelists responded "I would buy it"; 62.7 % of the panelists responded “maybe buy it”; while only 6.3 and 2.0 % of the panelists responded “maybe not buy it” and "I would not buy it", respectively, to the poll (**Table 9**). These results suggested that the milk tablet supplemented with 2.0 % RBH plus 2.0 % cocoa powder was accepted by consumers.

Table 8 Consumer test of milk tablet supplemented with 2 % RBH plus 2 % cocoa powder (MT_2%RBHC).

Sensory attributes	Score (Means ± SD)	Description
Color	7.08 ± 1.20	Like moderately
Odor	6.63 ± 1.51	Like a little bit to like moderately
Taste	7.18 ± 1.20	Like moderately
Texture	6.89 ± 1.37	Like a little bit to like moderately
Overall acceptability	7.16 ± 1.18	Like moderately

n = 300

Table 9 Purchase test of milk tablet supplemented with 2% RBH pus 2 % cocoa powder (MT_2%RBHC).

Purchase test	Number of people	Percentage
“I would buy it”	87	29.0
“maybe buy it”	188	62.7
“maybe not buy it”	19	6.3
"I would not buy it"	6	2.0

n = 300

Conclusions

Rice bran hydrolysate (RBH) prepared in a pilot-scale process contained substances that possessed antioxidant activity. The major MW of RBH was 753 - 6,088 Da (50.81 %). The addition of 2 % RBH plus 2 % cocoa could improve the antioxidant activity and sensory attribute score of milk tablets. The developed MT_2%RBHC was accepted by consumers, with an overall acceptability score of 7.16, and the microbial qualities (TPC, yeasts and molds and *E. coli*) were within the standard of flavored milk. The obtained results from this work will provide potential information for further RBH production on an industrial scale and the application of RBH in functional milk tablets. However, the milk tablet still has a little bit an unfavorable flavor of rice bran which should be developed in the future by adding other flavor enhancers.

Acknowledgements

This research project is supported by the Thailand Research Fund (Grant No. RDG59A0014.1-2). The authors would like to thank the Rajamangala University of Technology, Isan, Thailand for its facilities support. We would also like to thank Ms. Martha Maloi Eromine for her support in editing the English language in this manuscript.

References

[1] United States Department of Agriculture (USDA), Available at: https://fdc.nal.usda.gov/fdc-app.html#/food-details/1097512/nutrients, accessed March 2022.

[2] A Kamlangdee, S Saiut, S Upasen and P Wattanachai. Milk tablet processing techniques for novel milk product. *In*: Proceedings of the Academicsera 17th International Conference, Istanbul, Turkey. 2018, p. 25-9.

[3] The Nation Thailand, Available at: https://www.nationthailand.com/in-focus/30378894, accessed March 2022.

[4] DB Kapadiya, DB Prajapati, AK Jain, BM Mehta, VB Darji and KD Aparnathi. Comparison of Surti goat milk with cow and buffalo milk for gross composition, nitrogen distribution, and selected minerals content. *Vet. World* 2016; **9**, 710-6.

[5] Open Food Facts, Available at: https://world.openfoodfacts.org/category/milk-tablet, accessed March 2022.

[6] United States Department of Agriculture (USDA), Available at: http://www.aseanthai.net/english/mobile_detail.php?cid=14&nid=4030, accessed December 2021.

[7] SASC Faria, PZ Bassinello and MVC Penteado. Nutritional composition of rice bran submitted to different stabilization procedures. *Braz. J. Pharm. Sci.* 2012; **48**, 651-7.

[8] BS Luh. *Rice oil*. *In*: SB Luh (Ed.). Rice Production. 2nd ed. Springer Publishing, New York, 1991, p. 714-30.

[9] M Wang, NS Hettiarachchy, M Qi, W Burks and T Siebenmorgen. Preparation and functional properties of rice bran protein isolate. *J. Agr. Food Chem*. 1999; **47**, 411-6.

[10] X Wang, H Chen, X Fu, S Li and J Wei. A novel antioxidant and ACE inhibitory peptide from rice bran protein: Biochemical characterization and molecular docking study. *LWT Food Sci. Tech.* 2017; **75**, 93-9.

[11] C Uraipong and J Zhao. *In vitro* digestion of rice bran proteins produces peptides with potent inhibitory effects on alpha-glucosidase and angiotensin I converting enzyme. *J. Sci. Food Agr.* 2018; **98**, 758-66.

[12] JJ Fan, X Luo and Z Dong. Extraction isolation and purification of rice bran peptides. *Food Sci. Tech.* 2008; **33**, 169-72.

[13] X Zhang, Q Chen and G Pang. The preparation of the oligopeptides and the investigations of the antioxidative activity of oligopeptides from the corn gluten meal. *J. Food Sci*. 2005; **26**, 33-5.

[14] P Thamnarathip, K Jangchud, S Nitisinprasert and B Vardhanabhuti. Identification of peptide molecular weight from rice bran protein hydrolysate with high antioxidant activity. *J. Cereal Sci*. 2016; **69**, 329-35.

[15] C Khongla, S Musika, P Sangsawad, L Srithamma, P Kiatbenjakul and V Tanamool. Antioxidant activity of Jasmine rice bran hydrolysate and its application in broken rice beverages. *Suranaree J. Sci. Tech.* 2022; **29**, 1-10.

[16] K Chen, J Zhao, X Shi, Q Abdul and Z Jiang. Characterization and antioxidant activity of products derived from xylose - bovine casein hydrolysate Maillard reaction: Impact of reaction time. *Foods* 2019; **8**, 242.

[17] AOAC. *Official methods of analysis*. 17th ed. Association of Official Chemists, Gaithersberg, Maryland, United States, 2000.

[18] J Adler-Nissen. Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *J. Agr. Food Chem.* 1979; **27**, 1256-62.

[19] C Wiriyaphan, B Chitsomboon and J Yongsawadigul. Antioxidant activity of protein hydrolysates derived from threadfin bream surimi byproducts. *Food Chem.* 2012; **132**, 104-11.

[20] IFF Benzie and JJ Strain. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power the FRAP assay. *Anal. Biochem.* 1996; **239**, 70-6.

[21] C Khongla, S Musika and P Sangsawad. Changes in antioxidant activity of fermented fish sauce (Nam Pla-ra) from *Osteochilus Hasseltii* during fermentation. *Suranaree J. Sci. Tech.* 2020; **27**, 1-8.

[22] L Maturin and JT Peeler. BAM chapter 3: Aerobic plate count, Available at: https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm063346.htm, accessed August 2021.

[23] V Tournas, EM Stack, BP Mislivec, AH Koch and R Bandler. BAM: Yeasts, molds and mycotoxins, Available at: https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071435.htm, accessed August 2021.

[24] P Feng, SD Weagant, MA Grant, W Burkhardt, M Shellfish and B Water. BAM: Enumeration of *Escherichia coli* and the Coliform Bacteria, Available at: http://www.fda.gov/Food/FoodScience Research/LaboratoryMethods/ucm064948.htm, accessed August 2021.
[25] AP Adebiyi, AO Adebiyi, J Yamashita, T Ogawa and K Muramoto. Purification and characterization of antioxidative peptides derived from rice bran protein hydrolysates. *Eur. Food Res. Tech.* 2009; **228**, 553-63.
[26] P Goupy, C Dufour, M Loonis and O Dangles. Quantitative kinetic analysis of hydrogen transfer reactions from dietary polyphenols to the DPPH radical. *J. Agr. Food Chem*. 2003; **51**, 615-22.
[27] S Nanasombat, K Yansodthee and I Jongjaited. Evaluation of antidiabetic, antioxidant and other phytochemical properties of Thai fruits, vegetables and some local food plants. *Walailak J. Sci. Tech.* 2019; **16,** 851-66.
[28] P Yongpradoem and N Weerapreeyakul. Evaluation of antioxidant activity and inhibition of tyrosinase activity of *Raphanus sativusvar. caudatus* Alef extract. *Walailak J. Sci. Tech.* 2020; **17**, 838-50.
[29] J Adler-Nissen. *Enzymic hydrolysis of food proteins*. Elsevier Applied Science Publishers, New York, 1986, p. 110-85.
[30] P Thamnarathip, K Jangchud, S Nitisinprasert and B Vardhanabhuti. Functional properties of protein hydrolysates from Riceberry rice bran. *Int. J. Food Sci*. 2016; **51**, 1110-9.
[31] X Zhang, YL Xiong, J Chen and L Zhou. Synergy of licorice extract and pea protein hydrolysate for oxidative stability of soybean oil-in-water emulsions. *J. Agr. Food Chem*. 2014; **62**, 8204-13.
[32] MBK Foh, I Amadou, BM Foh, MT Kamara and WS Xia. Functionality and antioxidant properties of Tilapia (*Oreochromis niloticus*) as influenced by the degree of hydrolysis. *Int. J. Mol. Sci.* 2010; **11**, 1851-69.
[33] S Phongthai, S D'Amico, R Schoenlechner, W Homthawornchoo and S Rawdkuen. Fractionation and antioxidant properties of rice bran protein hydrolysates stimulated by *in vitro* gastrointestinal digestion. *Food Chem.* 2018; **240**, 156-64.
[34] E Nguyen, O Jones, YHB Kim, F Martin-Gonzalez and AM Liceaga. Impact of microwave-assisted enzymatic hydrolysis on functional and antioxidant properties of rainbow trout *Oncorhynchus mykiss* by-products. *Fish Sci.* 2017; **83**, 317-31.
[35] JM Kim, AM Liceaga and KY Yoon. Purification and identification of an antioxidant peptide from perilla seed (*Perilla frutescens*) meal protein hydrolysate. *Food Sci. Nutr.* 2019; **7**, 1645-55.
[36] L Pizzoferrato, P Manzi, V Vivanti, I Nicoletti, C Corradini and E Cogliandro. Maillard reaction in milk-based foods: Nutritional consequences. *J. Food Protect.* 1998; **61**, 235-9.
[37] N Phisut and B Jiraporn. Characteristics and antioxidant activity of Maillard reaction products derived from chitosan-sugar solution. *Food Res. Int.* 2013; **20**, 1077-85.
[38] S Stojanovska, N Gruevska, J Tomovska, J Tasevska, A Krstanovski and M Menkovska. Maillard reaction and lactose structural changes during milk processing. *J. Chem. Res.* 2017; **2**, 139-45.
[39] Notification of the Ministry of Public Health (No.351), Available at: food.fda.moph.go.th/law/data/ announ_ moph /V.English/P351_E.pdf, accessed August 2021.