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Effect of rice bran hydrolysates on physicochemical and antioxidative characteristics of fried fish cakes during repeated freeze-thaw cycles

Supattra Supawong[a,b,c], Jae W. Park[b,*], Supawan Thawornchinsombut[a,d]

[a] Dept of Food Technology, Faculty of Technology, Khon Kaen University, Khon Kaen, 40000, Thailand
[b] OSU Seafood Research & Education Center, Oregon State University, Astoria, OR, 97103, USA
[c] Dept of Food Science and Technology, Faculty of Science and Technology, Thammasat University, Prathumthani, 12121, Thailand
[d] Cardiovascular Research Group, Khon Kaen University, Khon Kaen, 40000, Thailand

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ABSTRACT

Rice bran hydrolysates (RBH) produced from hexane defatted rice bran using subcritical alkaline water extraction followed by enzymatic hydrolysis showed high protein and total phenolic contents and showed high antioxidant activity. FTIR results confirmed that RBH consisting of protein (amide I & II), saccharide, phenolic hydroxyl group and Maillard reaction products had antioxidant activity. Adding 1 and 2% RBH significantly reduced fat content in fried fish cakes by 20.9 and 29.3%, respectively, compared to the control. Lipid oxidation was significantly reduced when RBH or BHA/BHT was used. RBH at 2% was equally as effective as 0.02% BHA/BHT. RBH-treated fried fish cakes had higher concentrations of total phenolics (63.9 mg GAE/100 g sample) and showed the highest antioxidant activity (both DPPH$^{\bullet}$ and ABTS$^{\bullet}$ radical scavenging activity). This study showed that RBH can significantly improve the quality of fried cake products as it reduced fat uptake and effectively provided antioxidative protection. Consequently, RBH, as a natural alternative to synthetic antioxidants, might be used for extending the frozen shelf life of fried surimi seafood.

1. Introduction

Meat products are often stored at freezing temperatures to extend storage life. Freezing and frozen storage can cause chemical and structural changes in products, depending on their characteristics (meat source, amount and type of lipids, presence of cryoprotectants, antioxidant status, protective packaging used, etc.) (Serrano, Cofrades, & Jiménez-Colmenero, 2006). Such changes, which occur largely as a result of alterations in the characteristics of proteins (denaturation and aggregation) and lipids (oxidation), lead to undesirable effects on many product characteristics, such as texture, pH, fat, water binding, and color, resulting in reduced quality and shelf life of meat products (Awad, Powrie, & Fennema, 1968). Lipid oxidation is one of the major problems encountered in meat processing, especially in cooking and subsequent refrigerated or frozen storage. Lipid oxidation reduces product quality by contributing to undesirable color, odor and off-flavor, which results in reduced shelf life. The rate of lipid oxidation can be effectively retarded by the use of antioxidants (Coronado, Trout, Dunshea, & Shaha, 2002).

Recently, more attention has been placed on the evaluation of naturally occurring antioxidants. Rice bran hydrolysates (RBH) prepared from rice bran, which is a co-product of rice processing, has potential as a natural antioxidant. After oil extraction of rice bran, considerable protein (approximately 15–20%) remains in defatted rice bran. Rice bran protein is hypoallergenic and has high nutritional value, having a good balance of amino acids (e.g., essential amino acids, net protein utilization and protein efficiency ratio) (Adebiyi, Adebiyi, Ogawa, & Muramoto, 2009). Due to the high quality of the rice bran protein it can be useful in dietary supplements. Moreover, rice bran protein hydrolysates obtained using enzymatic hydrolysis are an excellent source of protein and provide bioactive activities, such as antioxidant, antimicrobial, antithrombotic, antihypertensive, opioid, and immunomodulatory activities (Thamnarathip, Jangchud, Nitisinprasert, & Vardhanabhuti, 2016). According to a previous study (Supawong et al., 2017), RBH produced from hexane defatted rice bran using subcritical alkaline water (SAW) followed by enzymatic hydrolysis showed high antioxidant activity in *in vitro* and *in vivo* tests. In addition, the antioxidant activity of rice bran extract compounds can retard lipid and protein deterioration in food products. A preliminary study showed 2% RBH was effective to minimize lipid oxidation in Pacific whiting surimi gel. Including 2% RBH in Pacific whiting surimi gels reduced TBARS value by 80% compared to untreated control

* Corresponding author. OSU Seafood Research & Education Center, 2001 Marine Dr Ste 253, Astoria, OR, 97103, USA.
E-mail address: jae.park@oregonstate.edu (J.W. Park).

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(Supawong et al., 2017).

Given the observed antioxidative characteristics of RBH, it was hypothesized that incorporation of RBH can improve various quality attributes of frozen fried products (i.e., fried fish cake). In addition, the brown color associated with RBH, when incorporated into a fried product, is not perceived as a negative characteristic (brown color) compared to other food applications where lighter color is preferred. Therefore, the aim of this study was to investigate the physicochemical properties and antioxidant activity of RBH compared to commonly used commercial antioxidants (BHT/BHA and rosemary extract) in fried fish cake during freeze-thaw cycles.

2. Materials and methods

Frozen threadfin bream (*Nemipterus* spp.) surimi (grade A) was obtained from ManA Frozen Foods Co. Ltd. (Muang, Songkhla, Thailand). Rosemary extract produced by steam distillation was purchased from Now Foods (Bloomingdale, IL, USA). Butylated hydroxyanisole (BHA ≥98.5% purity) and butylated hydroxytoluene (BHT ≥99.0%) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and their 50:50 mixture was used. Wheat starch (Midwest Grain Products Inc., Atchison, KS, USA), corn starch (Ingredion Inc., Westchester, IL, USA) and potato starch (KMC, Herningvej, Denmark), pure canola oil (Conagra Brands, Chicago, IL, USA) and plain salt (Signature Kitchens, Boise, ID, USA) were purchased from a local grocery store (Safeway, Astoria, OR, USA). Industrial hexane-defatted rice bran (HDRB) was obtained from Kasisuri Co. Ltd. (Phra Nakhon Si, Ayudhaya, Thailand). Protease G6 (EC 3.4.21.62) (alkaline serine endo-protease) produced by *Bacillus licheniformis* was purchased from Siam Victory Chemical Co. Ltd. (North Klongtoey, Bangkok, Thailand). Citric acid, sodium hydroxide, Folin-Ciocalteu's phenol reagent, sodium carbonate, gallic acid, potassium persulphate, 2,2'-azono-bis(3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8 tetramethyl-chroman-2-carboxylic acid (Trolox), and thiobarbituric acid (TBA) were purchased from Sigma-Aldrich. All other chemicals and reagents used were analytical grade and also purchased from Sigma-Aldrich.

2.1. Rice bran hydrolysates preparation

HDRB was sifted through a 50-mesh screen and soaked in 1.5% (w/v) citric acid solution prepared with distilled water at a 1:7 (w/v) ratio for 18 h. The pH of the suspension was adjusted to 8 using 2 M NaOH. Next, the alkaline mixture was heated in an autoclave (Consolidated Stills & Sterilizers, Allston, MA, USA) at 130 °C for 2 h to help with the extraction (Kaewjumpol, 2017). The pressure from a water phase diagram showed that pressure was ~170 kPa. The mixture was kept at room temperature (20 ± 2 °C) for approximately 2 h. The suspension was incubated with 2% Protease G6 mixed with 98% HDRB at 60 °C, pH 8 for 6 h. The degree of hydrolysis was 26.6%, which was investigated using the pH-stat method according to Kaewjumpol (2017). The pH-stat method is based on the number of protons released during hydrolysis. By calculating the volume of alkali used in maintaining a constant pH (pH 8) of the reaction mixture during the reaction, the percentage of peptide bonds cleaved can be determined (Kaewjumpol, 2017). The protease G6, which is an alkaline protein, was used to extract glutelin, the major protein in rice (Shih, 2003). The enzyme reaction was stopped by holding at 95 °C for 2 min. Next, the mixture was left at room temperature for approximately 1 h before centrifuging at 10,000 × *g* for 15 min (Sorvall RC-5B, Newtown, CT, USA). The supernatant, which was mainly rice bran hydrolysates (RBH), was adjusted to pH 7 using 1 M HCl, freeze-dried (Gamma 2–16 LSC Freeze dryer, Sciquip Ltd., Shropshire, UK) and kept at −18 °C for a maximum of 3 wk.

2.2. Determination of physicochemical properties and antioxidant activities of RBH

2.2.1. Attenuated total reflection - fourier transform infrared spectroscopy (ATR-FTIR) measurement of RBH

The conformation of freeze dried RBH was obtained using an ATR-FTIR spectrometer (Bruker BioSpin Corp., Billerica, MA, USA). Diffusive reflectance of the IR was measured with an average of 32 scans at a resolution of 4 cm^{-1}. ATR-FTIR spectra were obtained in the wave number ranging from 400 to 4000 cm^{-1}.

2.2.2. Crude protein content

The concentration of crude protein was measured using the Kjeldahl method (AOAC, 2000) and multiplying the nitrogen content with crude protein conversion factor of rice at 5.95 (FAO, 1986).

2.2.3. Total phenolic content

Total phenolic compound content was determined using the Folin-Ciocalteau method according to Singleton and Rossi (1965) with some modifications. RBH solution (200 μL) was added to 800 μL of the 2 N Folin–Ciocalteu solution and 4 mL deionized water (C2108800, 142 EVOQUA Water Technologies, Pittsburgh, PA, USA). The mixture was Vortexed (MX-S Biobase, Jinan, Shandong, China) for 1 min and left to stand at room temperature for 10 min followed by adding 2 mL of 7.5% sodium carbonate solution. The reaction mixture was Vortexed for 1 min after incubation at room temperature for 2 h. The absorbance of phenolics was measured spectrophotometrically (UV-2401PC Recording Spectrophotometer, Shimadzu, Tokyo, Japan) at 765 nm. The quantification of phenolic compounds was based on the standard curve obtained using gallic acid and was expressed as mg gallic acid equivalent/g of sample (mg GAE/g RBH).

2.2.4. Color

CIE L* (lightness), a* (redness), b* (yellowness) values of RBH powder were determined using a Minolta colorimeter (CR-310; Minolta Camera Co. Ltd., Osaka, Japan). The instrument was calibrated using a Minolta calibration plate and a standard hitching tile by Hunter Lab Inc., (Reston, VA, USA). This hitching tile was used to enhance the calibration for measuring the color of surimi gels (NFI, 1991). In this color space, L* indicates lightness, 0 is black, 100 is light, where +a* (+50) is redness, -a* (−50) greenness, +b* (+50) yellowness, and -b* (−50) blueness.

2.2.5. $ABTS^{\bullet}$ radical scavenging activity

The $ABTS^{\bullet}$ radical scavenging activity was determined using the method of Yin, Wang, Gu, Gu, and Kang (2012) with slight modification. Seven mM ABTS was prepared using 0.1 M sodium phosphate buffer (pH 7.4) containing 0.818% NaCl and 0.0015% KCl. ABTS was mixed with an equal volume of 2.45 mM $K_2S_4O_8$ and incubated at refrigeration temperature (~4 °C) for 12 h in the dark to form the $ABTS^{\bullet}$ radical. To 100 μL RBH solution, 3 mL of $ABTS^{\bullet}$ was added and mixed. The sample was incubated at room temperature for 6 min in the dark and the absorbance at 734 nm was measured. Trolox was used to construct the standard curve and the results were expressed as trolox equivalent antioxidant capacity (mg TEAC/g RBH).

2.2.6. Ferric reducing antioxidant power (FRAP)

A preliminary test showed DPPH radical scavenging activity tests could not use with RBH. Methanol in the DPPH solution caused the precipitation of proteins in RBH. Therefore, the FRAP assay was carried out using the method of Benzie and Strain (1996) with some modification. The method is based on the reduction of a ferric 2,4,6-tripyridyl-*s*-triazine complex (Fe^{3+}-TPTZ) by antioxidants to the ferrous form (Fe^{2+}-TPTZ). RBH was added to 10 mM ferric-TPTZ reagent and the increase in absorbance at 593 nm was measured at 8 min. Deionized water was used as a control. Iron (II) sulfate ($FeSO_4$) was used to

Table 1
Formulations of fried fish cake prepared with various antioxidants.

Ingredients (%)	Control	1-RBH	2-RBH	Rosemary	BHA/BHT
Surimi	40	39	38	39.95	39.98
Wheat starch	9	9	9	9	9
Salt	1.7	1.7	1.7	1.7	1.7
Starch[a]	5.6	5.6	5.6	5.6	5.6
Ice/water	43.7	43.7	43.7	43.7	43.7
RBH	–	1	2	–	–
Rosemary[b]	–	–	–	0.05	–
BHA/BHT[c]	–	–	–	–	0.02
Total	100	100	100	100	100

[a] Starch mixture was made by mixing corn starch, potato starch and wheat starch at 1:2:1.
[b] Rosemary used at 0.05% (Movileanu et al., 2013). This level is a threshold point that does not show any negative odor.
[c] BHA and BHT were mixed at 50:50 and used at 0.02% (Sebranek et al., 2005).

construct the standard curve and the results were expressed as mg $FeSO_4$/g RBH.

2.3. Fried fish cake preparation

Fish cake pastes were prepared according the method of Fowler and Park (2015) with some modifications. Cake paste samples (Table 1) were prepared using various antioxidants: no antioxidant (control), 1% RBH, 2% RBH (Supawong et al., 2017), 0.05% rosemary essential oil (Movileanu, Núñez de González, Hafley, Miller, & Keeton, 2013) and 0.02% BHA/BHT (Sebranek, Sewalt, Robbins, & Houser, 2005). Frozen surimi was thawed to approximately −5 °C (internal) by keeping at room temperature for 1 h. Partially thawed surimi (approximately 3 cm cubes) was chopped at 1800 rpm for 1 min using a silent cutter (UM 5 Universal, Stephan Machinery Corp., Columbus, OH, USA). With the addition of 1.7% salt, surimi was chopped for an additional 1 min at 1800 rpm. Then 9.0% wheat starch, 5.6% mixed starch (corn:potato:-wheat 1:2:1) as well as different antioxidants (Table 1) were added. The moisture content of the ingredients was measured (AOAC, 2000). The moisture content of surimi, RBH, wheat starch, and mixed starch were determined to be 75.6, 10.8, 10.9, and 12.9%, respectively. The final moisture content of the paste was 68.3%. Following the addition of ice and other dry ingredients, chopping resumed at 1800 rpm for 1 min. Chopping then continued at 3600 rpm under vacuum (40–60 kPa) for an additional 3 min, for a total chopping time of 6 min. Final temperature of the surimi paste was maintained below 25 °C. The mixture from each formulation was used to make individual fish cakes using a plastic patty former (5 × 5 × 1 cm) (Warren L. Junes Ltd., Astoria, OR, USA). The fish cakes were deep-fried in canola oil at 177 °C for 6 min (Presto 05466 Dual ProFry Immersion Element Deep Fryer, Presto Industries Inc., Eau Claire, WI, USA) in 4.5 L oil. Frying oil was changed after frying 12 pieces. The fried fish cakes were cooled on a rack at room temperature for 30 sec before freezing at −18 °C for 2 h. The frozen fried cakes were vacuum packed (three cakes in a bag) and frozen at −18 °C for 24 h and used for further analysis after 0, 1, 3, 6 and 9 freeze-thaw (FT) cycles. The packaging film (75 μm nylon/polyethylene) was purchased from Winpak (Winnipeg, MB, Canada). FT cycles were used to approximate long-term commercial cold storage. One FT cycle was defined as 2 days freezing at −18 °C followed by 1 day thawing in a cold room (5 °C).

2.4. Physicochemical properties of fried fish cake

2.4.1. pH, moisture and crude fat content

Fried samples were prepared for pH analysis in quadruplicate by blending 10 g sample in 90 mL distilled water using a pH meter (Fisher Scientific, Atlanta, GA, USA). Moisture content was determined using the AOAC Official Method 950.46 (AOAC, 2000) in quadruplicate. Crude fat content was measured in triplicate using an acid hydrolysis method according to the AOAC Official Method 948.15 (AOAC, 2000).

2.4.2. Surface color

The color of fried samples was determined using the Minolta colorimeter. Lightness (L∗), redness (a∗) and yellowness (b∗) values were measured (Park, 1994). The results were expressed as an average of at least 10 measurements of 5 different spots on the surface of each fried samples. The samples were kept at room temperature during the color measurements.

2.4.3. Texture analyses

Breaking force and distance of fried samples were measured using a wire cutter (TA-26) attached to a TA-XT plus Texture Analyzer (Texture Technologies Corp., Hamilton, MA, USA), provided with the Texture Expert software. The evaluation was carried out with the following conditions: test speed, 1 mm/s; trigger force set, 30 g; and temperature, 25 °C. The samples were kept at room temperature for 2 h before measurements. The average of 8 samples was reported.

2.5. Lipid oxidation of fried fish cakes

Lipid oxidation was measured as Thiobarbituric Acid Reactive Substances (TBARS) using the method of Buege and Aust (1978). Each fried sample (0.5 g) was homogenized in 10 mL thiobarbituric acid (TBA) solution containing 0.375% TBA, 15% trichloroacetic acid (TCA) and 0.25 N HCl for 1 min at high speed using a homogenizer (985,370-07, BioSpec, Bartlesville, OK, USA). The solution was heated (95–100 °C) for 10 min then the solution was cooled with running tap water and centrifuged at 3600 × *g* for 20 min at 25 °C. The absorbance of the supernatant was measured at 532 nm using a UV-2401PC Recording Spectrophotometer. The TBARS results were reported as mg of malonaldehyde/kg of sample.

2.6. Determination of antioxidant properties of fried fish cakes

Ten g of each fried sample was homogenized with 90 mL of two different extracting solutions: (1) hydrophilic extract: 0.05 M phosphate buffer (pH 7) and (2) lipophilic extract: methanol. The homogenates were then centrifuged at 12,000 × *g* for 1 h at 4 °C. The supernatant obtained was used to estimate total phenolic content and antioxidant activity, respectively. In measuring antioxidant activity, this study used two different assay methods, namely ABTS and DPPH.

2.6.1. DPPH• radical scavenging activity

DPPH• radical scavenging activity was estimated using the method of Qwele et al. (2013) with slight modifications. One mL of 0.2 mM DPPH• prepared in methanol was added to 1 mL supernatant. The mixture was Vortexed and left to stand at room temperature for 30 min. The control was DPPH• solution and the blank was methanol. The Abs was measured at 517 nm. Percent inhibition was calculated as:

$$\% \text{ Inhibition of DPPH}^{\bullet} = [1 - (\text{Abs of sample}/\text{Abs of control})] \times 100$$

2.6.2. ABTS• radical scavenging activity

The scavenging potential against ABTS• radical was determined using the method described by Qwele et al. (2013) with slight modifications. The radical was obtained by mixing equal volumes two stock solutions of 7 mM ABTS and 2.4 mM potassium persulphate and incubating in the dark for 12 h at room temperature. This solution was diluted with 0.1 M phosphate buffer, pH 7.4 to adjust its absorbance to 0.70 ± 0.02 at 734 nm. The diluted ABTS• solution (3 mL) was reacted with 500 μL of the supernatant followed by measuring 734 nm using

buffer solution as the blank. Percent inhibition was calculated as:

% Inhibition of ABTS$^{\bullet}$ = [1 - (Abs of sample/Abs of control)] × 100

2.7. Experimental design and statistical analysis

The experimental design was a split plot design. The FT cycle was allocated to the main plot while types of antioxidants were allocated to a sub-plot. Statistical analysis was done using the one-way analysis of variance (ANOVA) with the Statistical Package for the Social Science for Windows version 19.0 (SPSS Inc., Chicago, IL, USA). Statistical significance ($p^{<}0.05$) among samples and between treatments was analyzed using Duncan's New Multiple Range Test.

3. Results and discussion

3.1. Physicochemical properties and antioxidant activities of RBH

3.1.1. FTIR spectra analysis of RBH

FTIR spectroscopy is a useful technique for studying protein–carbohydrate systems. There are several identifiable regions of the mid-infrared spectrum where the chemical fingerprints of carbohydrates and proteins do not overlap significantly (Wen-qiong, Yi-hong, & Ying, 2013). According to the FTIR spectrum of RBH (Fig. 1A), a broad band at 3246 cm^{-1} belongs to stretching vibration of phenolic hydroxyl group (-OH) representing hydrogen bonding (Srivastava et al., 2011).

The series of overlapping peaks located in the region of 1140–1021 cm^{-1} results from vibration modes such as the stretching of C–C and C–O and the bending mode of C–H bonds (Cael, Koenig, & Blackwell, 1974). These are often referred to as the "saccharide" bands. The absorptions in the region of 1146–1021 cm^{-1} were strong in RBH, suggesting that there were saccharides attached to the RBH, which is in agreement with results reported by Wen-qiong et al. (2013). In their study, the whey protein isolate-sugar conjugate showed increased intensity in the region of the saccharide bands (1180-953 cm^{-1}). It might be expected that chemical changes accompanying the Maillard reaction (MR) in RBH would lead to several changes in the mid-infrared spectrum as a result of the consumption of some functional groups and the appearance of others. Functional groups, including NH_2, especially from lysine, may be lost, while the amount of those associated with Maillard reaction products (MRP), such as the Amadori compound (C═O), Schiff base (C═N) and pyrazines (C–N) may be increased by MR (S'miechowski & Stangret, 2008; Srivastava et al., 2011).

A small shoulder at around 1650 cm^{-1} was formed. These bands are associated with typical MRP, i.e., Schiff's base imine group (stretching) and enaminol group (stretching) (Yang et al., 2015). As Lund and Ray (2017) mentioned, the first step of the MR leads to the formation of an imine known as a Schiff's base. The C═N stretching band appears at 1630–1650 cm^{-1} in the infrared spectra of imine containing compounds. Wnorowski and Yaylayan (2003) monitored carbonyl-amine reaction between pyruvic acid and α-amino alcohols by FTIR spectroscopy and reported that the resultant Schiff's base absorbs infrared light at about 1647 cm^{-1}. These observations confirmed the formation of covalent bonds between proteins and polysaccharides through the MR in RBH, which was consistent with the brown color (Table 2). The brown color in RBH was contributed by the MR, which is in accordance with FTIR results.

The regions of 1574 cm^{-1} referred to as C═O from amide I, 1389 cm^{-1} referred to C–N stretching from amide II and 1266 cm^{-1} referred to as C–N stretching and N–H deformation from amide III, similar to those reported earlier (Yang et al., 2015). The most distinctive spectral features for proteins are the strong amide I and II bands centered approximately between 1600 and 1400 cm^{-1} (Oliver, Kher, McNaughton, & Augustin, 2009).

Between the spectra of RBH of defatted rice bran after alkaline water extraction (Fig. 1A) and that of unhydrolyzed sample (Fig. 1B), some shifts were observed in the spectra of amide I (1581 cm^{-1}) and II (1404 cm^{-1}) in RBH. Band position generally shifts as the number of hydrogen bonds increased (Zho & Wang, 2016). The imine and phenolic groups in RBH might be involved in the formation of intramolecular hydrogen bonds in RBH and their corresponding complex. It is known that infrared spectra of a complex containing hydrogen bonds can show a band change, which may indicate the presence of imine and phenolic groups, and this is related to the high content of total phenolic and browning index in RBH. The spectra of imine (small shoulder at around 1650 cm^{-1}) was not seen in unhydrolyzed sample spectra (Fig. 1B) but was seen in RBH spectra (Fig. 1A), indicating imine groups of MRP formed.

FTIR results confirmed that RBH consisting of protein (amide I & II), saccharide, phenolic hydroxyl group, and MRP is responsible for antioxidant activity.

3.1.2. Color, production yield, protein content and protein recovery of RBH

Color, production yield, protein content and protein recovery of freeze-dried RBH are shown in Table 2. Color values indicated RBH was brown or golden-yellow (Pantone 609 XGC) according to the Pantone color (Pantone, 2017). Protein recovery was higher than Rahim, Morad, and Long (2015), which were (51–64%). Production yield (48 ± 2%) and total phenolic content (29 ± 1 mg GAE/g RBH) were higher than the values (32% and 22 mg GAE/g RBH) reported by Thamnarathip et al. (2016). The higher values might have resulted from the use of subcritical alkaline water (SAW) extraction before enzymatic hydrolysis (Sereewatthanawut et al., 2008). Subcritical water (SW) is water under pressurized condition in the temperature range of 100–374 °C thus retaining its liquid state (Sereewatthanawut et al., 2008). Solvation behavior of SW alters from a polar, highly hydrogen bonded solvent to behavior more typical of a non-polar solvent such as hexane (Peterson et al., 2008). A preliminary study showed that using SAW extraction with alkaline condition followed by enzymatic hydrolysis could extract more proteins from defatted rice bran than individually using alkaline treatment or enzymatic hydrolysis. This combined technique resulted in higher yield, protein, and total phenolic content of hydrolysates. The results confirmed that RBH obtained from SAW extraction followed by enzymatic hydrolysis showed high production yield and physicochemical properties.

3.1.3. Total phenolic content and antioxidant activities of RBH

For antioxidant activities of RBH, total phenolic content, ABTS$^{\bullet}$ radical scavenging activity and FRAP are shown in Table 2. ABTS$^{\bullet}$ radical scavenging of this RBH showed higher activity than that reported by Thamnarathip et al. (2016). The antioxidant activity of the hydrolysates is inherent to the phenolic compounds, protein content and MRP (Adebiyi et al., 2009).

RBH is known to contain various chemical compounds including phenolic compounds that contain strong antioxidation effects (Thamnarathip et al., 2016). Adebiyi et al. (2009) assured that the antioxidation activity of RBH was linked to protein concentration, phenolic compounds, and MRP.

Kaewjumpol (2017) recently showed the Tricin SDS-PAGE profile of RBH which was produced from defatted rice bran under the same condition (SAW at 130 °C for 2 h followed by 2% Protease G6 hydrolysis) used in the current study, and filtered by MF (0.45 μm) or UF (10 kDa) membranes. They observed that most peptides in the $^{<}$0.45 μm and $^{<}$10 kDa samples were in the range of 3.7 to > 10.0 kDa. In addition, high contents of lysine (60 mg/g protein), glutamate (70 mg/g protein), leucine (59 mg/g protein), phenylalanine (59 mg/g protein), isoleucine (45 mg/g protein), and proline (44 mg/g protein) were observed in $^{<}$10 kDa RBH. These amino acids may account for bioactive activities of peptides. As some amino acids (e.g., arginine, glycine and histidine), small peptides and nitrogenous metabolites directly

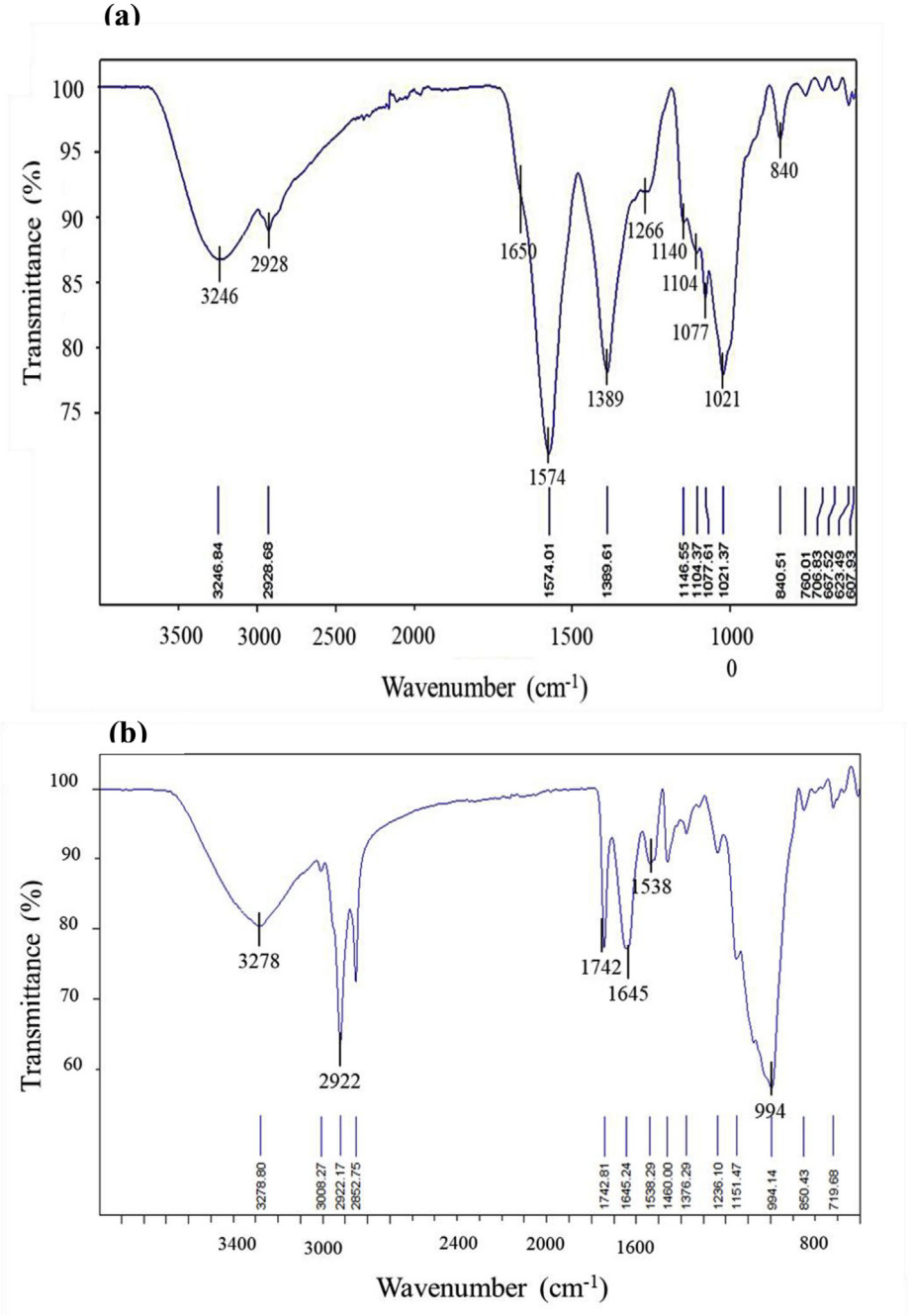

Fig. 1. FTIR spectra of rice bran hydrolysates (a) and defatted rice bran (b).

scavenge oxygen free radicals (Sarojnalini & Devi, 2014), amino acids with a phenolic hydroxyl group in the peptides may also be responsible for ABTS$^{\bullet}$ radical scavenging activity.

The top 4 phenolic compounds in RBH are *p*-coumaric acid (3.0 mg/g RBH), ferulic acid (1.1 mg/g RBH), sinapic acid (0.44 mg/g RBH), and vanillin (0.43 mg/g RBH) (Kaewjumpol, 2017). This confirms that RBH obtained from SAW extraction followed by enzymatic hydrolysis had phenolic compounds (vanillic acid, syringic acid, vanillin, *p*-coumaric acid, ferulic acid, and sinapic acid). Polyphenol and proteins (peptides and amino acids) have both antioxidant activity and chelating capabilities (Cagdas & Kumcuoglu, 2015). The radical scavenging activity can be achieved via H-atom donation of the hydroxyl group in phenolic compounds. A statistically significant relationship between total phenolic content and antioxidant activity was reported with $R^2 = 0.96$ ($p<0.05$) for flaxseed and $R^2 = 0.91$ ($p<0.05$) in cereals and in fruits, vegetables, and grains (Velioglu, Nazza, Gao, & Oomah, 1998).

Another compound responsible for antioxidant activity in RBH is MRP formed during the heating process of RBH. The antioxidative effect of MRP was shown when a glucose-glycine model system was served as an electron donor (Yoshimura, Iijima, Watanabe, & Nakazawa, 1997).

Thus, the antioxidant effect of RBH is presumably linked to its proteins (peptide and amino acid), phenolic compounds, and MRP constituents in RBH. These chemical compositions strongly suggest RBH has high antioxidant potential in fried fish cake.

3.2. Physicochemical properties of fried fish cakes

3.2.1. Change in pH values of fried fish cakes

The pH, a dependable indicator of food stability, is associated with

Table 2
Physicochemical properties of RBH.

Measurement	Value	Unit
Color, crude protein content, yield and protein recovery of freeze dried RBH		
% Yield RBH	48 ± 2	% (based on the quantity of rice bran)
*Protein content	27 ± 1	% (using Kjeldahl analysis)
*Protein recovery	66 ± 4	% (based on protein in rice bran)
Color (L*/a*/b*)	64/2.3/17	Golden-yellow color (Pantone 609 XGC, Pantone, 2017)
Total phenolic content and antioxidant activities of freeze dried RBH		
Total phenolic content	29 ± 1	mg GAE/g RBH
ABTS	13 ± 0.5	mg TEAC/g RBH
FRAP	8.3 ± 0.3	mg $FeSO_4$/g RBH

*Crude protein content and protein recovery were determined using Kjeldahl analysis.
**Crude protein content of defatted rice bran = 19.8%.

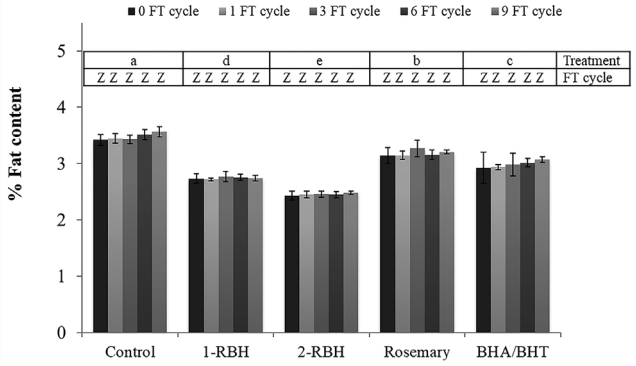

Fig. 2. Fat content of fried fish cakes prepared with various antioxidants during FT cycles. Different letters (Y, Z) indicate significant differences ($p<0.05$) among FT cycles within the same treatments. Different letters (a, b, c, d, e) indicate significant differences ($p<0.05$) among treatments.

microbial and chemical reactions that lead to food deterioration (Hwang et al., 2013). The pH values of fried samples treated with various antioxidants during FT cycles ranged from 6.90 to 7.01 (Table 3). However, samples with 2% RBH showed higher pH than the other treatments. During FT cycles, pH values decreased significantly ($p<0.05$), except for RBH treatments.

The pH of 1% RBH slightly dropped at 6 cycles while the pH of 2% RBH was maintained throughout 9 cycles. As RBH was produced under alkaline digestion as a neutral compound, more alkaline compounds, such as various alkaline phenolic compounds and alkaline proteins, were likely extracted, the change in pH values during FT cycles was, therefore minimized. Based on pH results during 9 F T cycles and the

Table 3
pH, moisture content, texture properties and surface color of fried fish cakes.

Measurement	FT cycle	Treatment				
		Control	1-RBH	2-RBH	Rosemary	BHA/BHT
pH	0	6.98 ± 0.01^{d}	6.97 ± 0.01^{d}	7.00 ± 0.01^{ab}	6.95 ± 0.01^{ef}	6.95 ± 0.01^{ef}
	1	6.98 ± 0.01^{cd}	6.97 ± 0.01^{d}	7.01 ± 0.01^{a}	6.93 ± 0.01^{gh}	6.95 ± 0.01^{ef}
	3	6.95 ± 0.01^{e}	6.97 ± 0.01^{d}	7.00 ± 0.01^{a}	6.92 ± 0.01^{i}	6.94 ± 0.01^{fg}
	6	6.93 ± 0.01^{h}	6.95 ± 0.01^{e}	6.99 ± 0.01^{bc}	6.90 ± 0.01^{j}	6.92 ± 0.01^{hi}
	9	6.90 ± 0.01^{j}	6.95 ± 0.01^{ef}	6.99 ± 0.01^{ab}	6.89 ± 0.01^{j}	6.91 ± 0.01^{j}
Moisture content (%)	0	57 ± 1^{c}	58.2 ± 0.5^{b}	61 ± 1^{a}	55 ± 1^{d}	57 ± 2^{c}
	1	57.2 ± 0.3^{c}	59.1 ± 0.3^{b}	61.4 ± 0.5^{a}	55 ± 1^{d}	57 ± 1^{c}
	3	57 ± 1^{c}	59.2 ± 0.4^{b}	62 ± 1^{a}	55 ± 1^{d}	57.4 ± 0.3^{c}
	6	57 ± 1^{c}	57.9 ± 0.4^{b}	61.3 ± 0.3^{a}	55 ± 1^{d}	56 ± 2^{c}
	9	57 ± 1^{c}	57 ± 1^{b}	62 ± 1^{a}	56 ± 1^{d}	57 ± 1^{c}
Texture	0	4.4 ± 0.4^{a}	4.3 ± 0.6^{ab}	3.5 ± 0.2^{h}	4.5 ± 0.2^{a}	4.4 ± 0.4^{a}
Breaking force (kg)	1	3.3 ± 0.3^{de}	3.9 ± 0.1^{def}	3.4 ± 0.3^{hi}	4.2 ± 0.1^{bc}	4.2 ± 0.1^{bc}
	3	3.9 ± 0.1^{d-g}	3.9 ± 0.1^{d-g}	3.3 ± 0.1^{i}	4.0 ± 0.1^{cd}	4.0 ± 0.2^{cd}
	6	3.8 ± 0.2^{efg}	3.8 ± 0.1^{d-g}	3.3 ± 0.1^{i}	3.9 ± 0.1^{de}	3.9 ± 0.1^{d-g}
	9	3.5 ± 0.3^{h}	3.7 ± 0.2^{fg}	3.3 ± 0.2^{i}	3.9 ± 0.2^{d-g}	3.7 ± 0.2^{g}
Penetration distance (mm.)	0	14 ± 1^{Ac}	14 ± 1^{Ac}	12 ± 1^{Ad}	14 ± 1^{Ab}	14 ± 1^{Aa}
	1	13 ± 1^{Ac}	14 ± 1^{Ac}	12.3 ± 0.5^{Ad}	14.1 ± 0.5^{Ab}	14 ± 1^{Aa}
	3	13 ± 1^{Bc}	13 ± 1^{Bc}	12.1 ± 0.5^{Bd}	14 ± 1^{Bb}	14 ± 1^{Ba}
	6	13 ± 1^{BCc}	13 ± 1^{BCc}	12 ± 1^{BCd}	13 ± 1^{BCb}	14 ± 1^{BCa}
	9	12.5 ± 0.5^{Cc}	13 ± 1^{Cc}	12.3 ± 0.5^{Cd}	13.1 ± 0.4^{Cb}	13.9 ± 0.5^{Ca}
Surface color	0	71 ± 2^{hi}	69 ± 2^{jk}	66 ± 2^{m}	72 ± 3^{h}	71 ± 1^{hi}
L*	1	75 ± 2^{ef}	70 ± 2^{ij}	68 ± 2^{l}	74 ± 3^{fg}	72 ± 3^{h}
	3	77 ± 2^{cde}	72 ± 2^{h}	69 ± 1^{k}	78 ± 2^{bcd}	76 ± 1de
	6	79 ± 1^{ab}	74 ± 2^{g}	69 ± 1^{k}	78 ± 1^{abc}	77 ± 2^{cde}
	9	79 ± 1^{a}	74 ± 1^{g}	69 ± 1^{k}	78 ± 1^{abc}	77 ± 1de
a*	0	-3.3 ± 0.8^{d-f}	-0.6 ± 0.5^{b}	0.6 ± 0.1^{a}	-3.1 ± 0.5^{d}	-3.5 ± 0.5^{e-g}
	1	-3.3 ± 0.5^{de}	-0.7 ± 0.7^{bc}	0.6 ± 0.5^{a}	-3.1 ± 0.6^{d}	-3.4 ± 0.2^{d-g}
	3	-3.7 ± 0.2^{f-h}	-1.1 ± 0.5^{c}	0.4 ± 0.2^{a}	-4.0 ± 0.5^{h}	-4.4 ± 0.3^{i}
	6	-4.0 ± 0.3^{h}	-1.0 ± 0.7^{c}	0.5 ± 0.1^{a}	-3.2 ± 0.7^{de}	-3.5 ± 0.5^{e-g}
	9	-3.8 ± 0.5^{gh}	-1.1 ± 0.3^{c}	0.5 ± 0.1^{a}	-3.3 ± 0.4^{de}	-3.5 ± 0.4^{e-g}
b*	0	19 ± 0.4^{ghi}	22 ± 2^{bcd}	24 ± 2^{a}	18 ± 3^{hi}	18 ± 3^{hi}
	1	21 ± 2^{cde}	23 ± 2^{abc}	24 ± 3^{a}	22 ± 3^{bcd}	21 ± 2^{def}
	3	22 ± 2^{bcd}	21 ± 2^{def}	23 ± 1^{ab}	21 ± 3^{def}	18 ± 1^{i}
	6	20 ± 2^{d-g}	22 ± 2^{bcd}	24 ± 1^{a}	22 ± 3^{bcd}	20 ± 2^{efg}
	9	21 ± 1^{def}	21 ± 1^{cde}	24 ± 2^{a}	22 ± 3^{bcd}	20 ± 2^{fgh}

biochemical properties of RBH, it can be speculated that RBH likely controls pH during frozen storage.

3.2.2. Moisture and fat content of fried fish cakes

The fat content of fried fish cakes prepared with various antioxidants during FT cycles ranged between 2.5 and 3.5% (Fig. 2). Samples with RBH showed the lowest fat content, which was significantly ($p<0.05$) lower than the control and other antioxidant treatments. FT cycles did not affect significant ($p \geq 0.05$) changes in moisture and fat content of samples as the samples were kept in vacuum packs. The moisture content of fried fish cakes treated with 1% RBH and 2% RBH were significantly ($p<0.05$) higher than other treatments, respectively; control, rosemary and BHA/BHT (Table 3).

The fat content of fried fish cakes treated with RBH were significantly ($p<0.05$) lower than other treatments (control, rosemary and BHA/BHT) (Fig. 2). This indicates RBH may function to expel fat globules during frying of fish cake. These results were supported by He, Franco, and Zhang (2015), who used Yellow Kingfish protein hydrolysates in the formulation of deep-fried fish cakes and obtained significantly higher moisture and significantly lower fat content than the control ($p<0.05$). The thermogelling ability of myofibrillar proteins (surimi) might have had an additional role in reducing the fat content of fried surimi seafood (Supawong, Park, & Thawornchinsombut, 2018). In our preliminary dynamic rheology results, with increasing temperature, surimi paste completed gelation, resulting in a three-dimensional network structure. The surimi paste, through thermogelling by frying, became a firmer gel with a smooth surface, thereby reducing evaporation. Surimi proteins form a gel layer (film) that prevents moisture migration from inside to outside and oil migration from outside to inside (Athaillah & Park, 2016). Additionally, phenolic compounds in RBH possibly had cross-linking ability. According to Prodpran, Benjakul, and Phetcharat (2012), phenolic compounds (caffeic acid, catechin, ferullic acid and tannic acid) can react with some amino acids in proteins (tyrosine, lysine, cysteine), resulting in cross-linked protein molecules. Thus, RBH could have acted as a cross-linking agent and further improved the properties of protein films on the surface of fried fish cake.

Fat uptake is dramatically affected by the water content in deep-fried food; higher water reduction is generally associated with higher fat uptake. Oil can only penetrate where water has evaporated. Upon addition of the food into the hot oil, the surface temperature of the food rises rapidly. The water at the surface immediately starts boiling and the resultant vapor generates surface porosity, which leaves voids for fat to enter. In addition, free water is easier to evaporate than bound water (He et al., 2015). Hydrophilic RBH likely binds more water inside the fish cakes due to its strong hydrophilicity. Therefore, water molecules inside the fish cakes with added RBH are bound and unable to be released, which subsequently minimizes fat uptake (Supawong et al., 2018).

3.2.3. Changes in surface color of fried fish cakes

RBH-treated samples showed the lowest lightness (L*) and highest redness (a*) (Table 3). Lightness decreased, whereas redness increased with increasing RBH concentration ($p<0.05$) (Table 3). These were probably due to the natural color of RBH, which is relatively brown or golden-yellow in color (Pantone 609 XGC) according to the Pantone color (Pantone, 2017). There was no significant ($p \geq 0.05$) difference in yellowness in samples treated with various antioxidants. Lightness significantly ($p<0.05$) increased as FT cycles extended, while redness decreased after 6 F T cycles.

Control fried fish cake samples showed a rapid increase in lightness values during FT cycles, whereas RBH-treated samples showed a slight increasing trend. A possible explanation could be the protective effect of RBH against discoloration due to the high antioxidant activity of protein (peptide and amino acid) and phenolic compounds, which are present in RBH (Table 2 and FTIR spectra). This is consistent with the preliminary study, in which much lower TBARS values of fried fish cake were noted for samples with RBH compared to others.

3.2.4. Texture properties of fried fish cakes

Changes in texture properties (breaking force and penetration distance) of fried fish cakes during FT cycles were measured (Table 3). A significant interaction ($p<0.05$) between various antioxidants and FT cycles on breaking force, indicating gel hardness, was observed, but there was a non-significant ($p \geq 0.05$) interaction between various antioxidants and FT cycles on penetration distance, denoting gel cohesiveness. RBH-treated (2%) samples showed the lowest breaking force and distance. A similar influence of eel head protein hydrolysates on textural properties of surimi gel was also reported (Yanan, Li, Hua, Wei, & Meilan, 2012). This effect was attributed to possible interferences of RBH in the surimi protein gelation processes, which limit gel network formation. Gel formation is a complex process. As RBH contains long and short chains of peptides and amino acids, respectively, they might inhibit dissociation and/or aggregation, thus decreasing gel strength (Yanan et al., 2012). However, the negative role of RBH seems to be minimal. RBH-treated sample (2%) reduced breaking force by only 6.32% compared to the control after 9 F T cycles.

Breaking force and distance of all treatments significantly ($p<0.05$) decreased as FT cycles repeated, probably because FT led to structural damage to the proteins. Freezing and frozen storage have long been recognized to cause chemical and structural changes in muscle foods. This damage essentially resulted from changes in protein characteristics (Serrano et al., 2006).

3.3. Lipid oxidation of fried fish cakes

TBARS values represent secondary lipid oxidation products (aldehydes and carbonyls of hydrocarbons) affecting off-flavors in meat (Hwang et al., 2013). A significant ($p<0.05$) interaction on TBARS (mg malondialdehyde/kg sample) of fried fish cake samples was observed between various antioxidants and FT cycles (Fig. 3). The control samples (no antioxidant) showed significantly higher ($p<0.05$) TBARS values compared to treated samples. The samples formulated with RBH and BHA/BHT showed little changes in TBARS after 1 F T cycle and maintained much lower values (below 2.2) throughout 9 F T cycles.

Lipid oxidation was significantly reduced with RBH as TBARS values were significantly lower compared to the control at all FT cycles ($p<0.05$). The addition of 2% RBH was slightly better than or as effective as BHA/BHT in maintaining low TBARS values, indicating RBH effectively inhibited lipid oxidation. This is probably due to antioxidant compounds in RBH, such as proteins (peptide and amino acid), phenolic compounds and MRP (Adebiyi et al., 2009; Supawong et al., 2017;

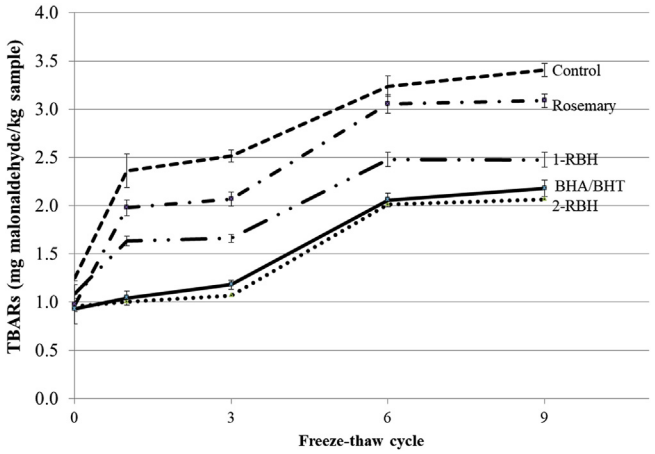

Fig. 3. Lipid oxidation of fried fish cakes formulated with various antioxidants during FT cycles.

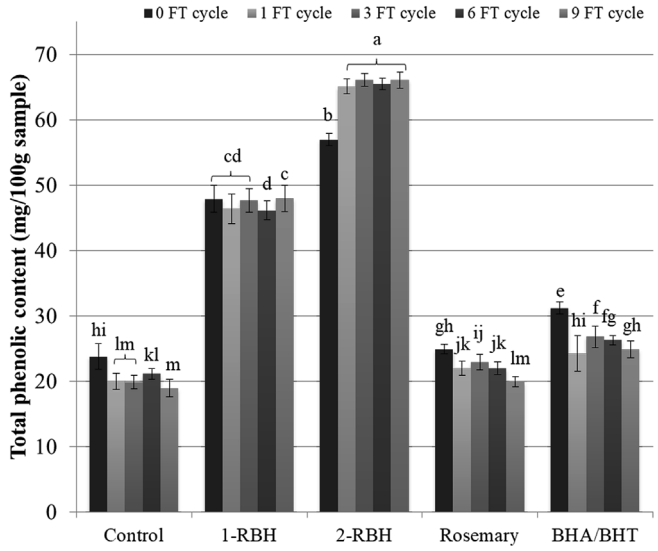

Fig. 4. Total phenolic content of fried fish cakes prepared with various antioxidants during FT cycles.

Thamnarathip et al., 2016). Supawong, Park, et al. (2017) and Supawong, Thawornchinsombut, et al. (2017) showed that 2% RBH was effective to minimize lipid oxidation in Pacific whiting surimi gel: TBARS value was reduced by 79.8%. Wongthahan and Thawornchinsombut (2015) showed that fish cakes made from tilapia byproducts, when prepared with added rice bran protein hydrolysates, also showed lower lipid oxidation during freezing and thawing.

As related to the physicochemical properties of RBH, its protein content, total phenolic content, ABTS• radical scavenging activity and FRAP of RBH were 27.6%, 29.4 mg GAE/g RBH, 13.4 mg TEAC/g RBH and 8.35 mg $FeSO_4$/g RBH, respectively (Table 2). The RBH-treated fried fish cakes had higher concentrations of total phenolic content (63.9 mg GAE/100 g sample) and showed the highest antioxidant activity (both DPPH• and ABTS• radical scavenging activity) (Figs. 4–6). Polyphenol and proteins (peptides and amino acids) have both antioxidant activity and capabilities to eliminate the radicals and chelate metals (Cagdas & Kumcuoglu, 2015). Thus, the capability of RBH to prevent lipid oxidation is presumably contributed by its good polyphenol and protein constituents.

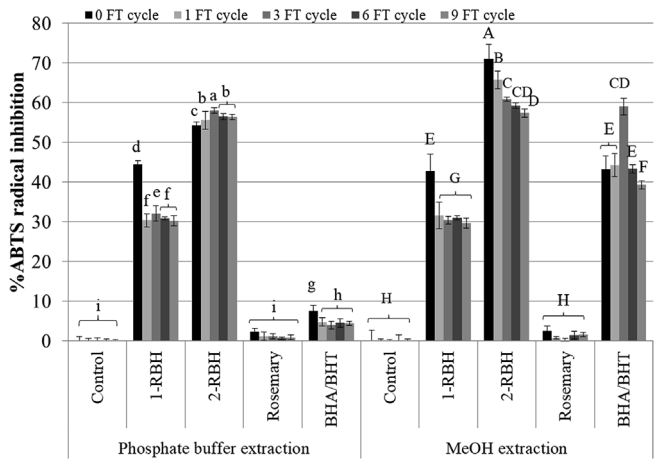

Fig. 5. ABTS• radical scavenging activity of fried fish cakes extracts during FT cycles. a-i letters listed in columns indicate statistically significant difference of phosphate buffer extraction group. A-H letters listed in columns indicate statistically significant difference of MeOH extraction group.

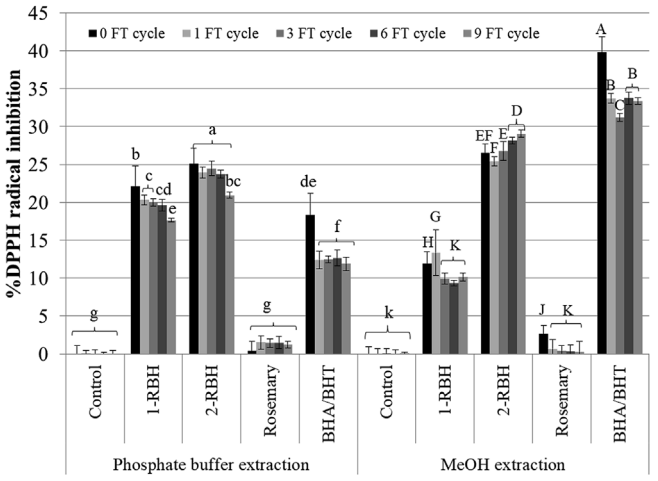

Fig. 6. DPPH• radical scavenging activity of fried fish cakes extracts during FT cycles. a-i letters listed in columns indicate statistically significant difference of phosphate buffer extraction group. A-H letters listed in columns indicate statistically significant difference of MeOH extraction group.

3.4. Antioxidant properties (total phenolic content, ABTS• and DPPH• radical scavenging activity) of fried fish cakes

The total phenolic content (mg GAE/100 g sample) of fried fish cakes treated with various antioxidants is shown in Fig. 4. The samples treated with 2% RBH had a significantly higher ($p^<0.05$) total phenolic content (63.9 mg GAE/100 g) than other treatments: 1% RBH (47.2 mg GAE/100 g); BHA/BHT (26.7 mg GAE/100 g); rosemary (22.4 mg GAE/100 g) and control (20.7 mg GAE/100 g). The higher total phenolic content of 2% RBH strongly suggests this treatment has the highest antioxidant potential in fried samples.

The samples treated with 2% RBH showed the highest ABTS• radical scavenging activity for both hydrophilic and lipophilic extracts with 56.2 and 62.8% inhibition of ABTS• radical compared to the control, respectively (Fig. 5). For hydrophilic extracts, samples with RBH also showed the highest DPPH• radical scavenging activity (Fig. 6). Meanwhile, samples with BHA/BHT had the highest percentage of DPPH• radical inhibition for lipophilic extracts (37.4%), followed by 2% RBH (27.2%). Lipophilic (hydrophobic) BHA/BHT, unlike hydrophilic RBH, would have better interaction with relatively hydrophobic DPPH radical. This could explain the higher DPPH• radical scavenging capacity of BHA/BHT capacity compared to RBH in lipophilic extracts.

However, overall antioxidant activity of samples with RBH were significantly higher ($p^<0.05$) than the other treatments (Figs. 5 and 6). This is probably due to antioxidant compounds in RBH, such as proteins (peptides and amino acids), phenolic compounds and MRP (Supawong et al., 2017; Thamnarathip et al., 2016; Adebiyi et al., 2009). As for the physicochemical properties of RBH (Table 2), protein content, total phenolic content, ABTS• radical scavenging activity and FRAP were 27.6%, 29.4 mg GAE/g RBH, 13.4 mg TEAC/g RBH and 8.35 mg $FeSO_4$/g RBH, respectively. Rice bran protein hydrolysates also have a high nutritional value consisting of amino acid composition and protein qualities (e.g., essential amino acids, net protein utilization and protein efficiency ratio) (Adebiyi et al., 2009).

Small peptides and nitrogenous metabolites directly scavenge oxygen free radicals (Sarojnalini & Devi, 2014). In addition, phenolic compounds in RBH are responsible for antioxidant activity. Another compound responsible for antioxidant activity in RBH was MRP, which could be formed during the thermolysis process of RBH. Yoshimura et al. (1997) investigated the antioxidative effect of MRP using a glucose-glycine model system and showed that MRP could function as electron donors. Thus, the antioxidant effect of RBH on fried fish cakes may result from the proteins (peptide and amino acid) (Kaewjumpol,

2017), phenolic compounds, MRP and tannin constituents in RBH.

4. Conclusion

RBH, when compared with rosemary or BHA/BHT showed the highest antioxidant activity (both $DPPH^{\bullet}$ and $ABTS^{\bullet}$ radical scavenging activity) in fried fish cakes through repeated FT cycles. This study showed the potential of RBH as a healthy ingredient for fried foods, in which its brown color is not viewed by consumers as negative. Comparing RBH with synthetic antioxidants for the safety and toxicity, RBH appears to be natural and more consumer-friendly for the food industry. The fat content of fried fish cake was significantly ($p<0.05$) reduced with the addition of 1–2% RBH likely because hydrophilic RBH dispelled fat globules during frying and surimi protein concomitantly formed a gel that prevented the migration of fat. The role of RBH in reducing fat uptake in fried fish cake requires further investigation.

Practical application

Rice bran hydrolysates (RBH) have shown their effectiveness in reducing fat uptake during frying and controlling lipid oxidation of fried fish cake during frozen storage. Unlike artificial antioxidants, RBH can be used as a health-friendly ingredient for fried food products.

Author's contribution

S. Supawong collected test data, interpreted results, and drafted the manuscript. J. Park designed the study with S. Thawornchinsombut and S. Supawong. J. Park and S. Thawornchinsombut interpreted the results and reviewed the draft.

Declaration of competing interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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