

Physicochemical and sensory properties of deep fried battered squid containing Brownstripe red snapper (*Lutjanus vitta*) protein hydrolysate

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Abstract

Deep-fried food is a fast and convenient way to prepare food that imparts desirable sensory characteristics of colour, flavour and in particular, a smooth texture, yet has been labelled as not healthy by consumers. Incorporation of other ingredients in the formulation of the batter could reduce the fat absorption in deep-fried foods. This research was aimed to determine the physicochemical and sensory properties of Brownstripe red snapper protein hydrolysate (BRSPH) and its utilisation in reducing the oil intake of deep-fried foods. The BRSPH were extracted using the enzymatic method utilizing Alcalase® as the working enzyme. Batter formulations were prepared by adding 0%, 2%, 4%, 6% and 8% of BRSPH into the sample mixtures. Addition of BRSPH into the batter was found to increase hardness and crispness of deep-fat fried battered squids. The fat content of the deep-fat fried battered squids with 8% BRSPH powder was found to be the lowest compared to those added with 2%, 4% and 6%, while sample without BRSPH powder was the highest (30.15%). Deep-fat fried squids with 4% of BRSPH powder showed the best acceptability scores in terms of crispness, taste and overall acceptability, but no significant differences were determined in the crispness between deep-fat fried squids with and without BRSPH. The findings indicate that enzymatic hydrolysis using Alcalase® has the potential to yield BRSPH with a high degree of hydrolysis (98.19%), low molecular weight (10-15 kDa), and low oil binding capacity (2.38 g oil/ g protein). Enzymatic hydrolysis proved to be a viable technique to produce protein hydrolysate which able to reduce the oil uptake and healthier with high acceptability of the end products. Research on how to optimize the use of BRSPH with a high economic, nutritional and industrial potential of understated resources would allow for the implementation of manufacturing practices to enhance the use of resources and increase the value of the by-product. This approach will offer the potential use of BRSPH for the production of batter formulation which is efficient in the reduction of oil uptake.

1. Introduction

Deep-fried food has become increasingly popular because it is fast and convenient which imparts unique sensory properties of colour, flavour and, in particular, the texture of a smooth, moist interior coupled with a crispy crust outside (Pedreschi and Moyano, 2005). However, customers are becoming more health-

conscious and are increasingly turning to foods that are high in nutritional value and low in fat and caloric content. Moreover, fried foods also constitute high fat content, often exceeding 1/3 of the total food by weight (Mellema, 2003).

In order to reduce the fat in high-fat fried products, surface treatments widely used to reduce oil uptake in

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deep-fried foods (Lumanlan *et al.*, 2019). Surface treatment is the application of coating materials to the surface of food products to limit oil and moisture transfer during frying (Radwan, 2017). Soy protein isolate, whey protein isolate and methylcellulose were very effective in reducing oil uptake in deep-fried foods (Albert and Mittal, 2002). Most of the barriers used in the commercial production of fried foods are protein-based (Hau *et al.*, 2018). Fish protein hydrolysate, such as striped catfish protein, was found to reduce oil binding capacity by increasing the degree of hydrolysis (Tanuja *et al.*, 2014).

Snappers can be found in tropical and subtropical waters of all oceans. Snapper typically feeds on fish, crustaceans and molluscs, particularly squid. In Malaysia, three common types of snappers are available for consumption: red snapper (*Lutjanus argentimaculatus*) and golden snapper (*Lutjanus johnii*) (Rosmilah *et al.*, 2005). Brownstripe red snappers (*Lutjanus vitta*) (Figure 1) can be used as an excellent raw material for the production of hydrolysate due to its low human consumption, which is considered as underutilized marine fish.



Figure 1. The Brownstripe red snapper (*Lutjanus vitta*)

It is suggested that fish protein hydrolysate with low oil-binding ability and low molecular weight may also minimize the fat intake of deep-fried foods (Zainol *et al.*, 2020). Nevertheless, there is limited information on the use of Brownstripe red snapper hydrolysate protein (BRSPH) in reducing the consumption of deep-fried foods in oil. Successful results may serve as an initiative to enhance or develop a healthy fried food that has contributed commonly to obesity and chronic diseases. Thus, this study aimed to determine the effect of BRSPH on reducing the oil uptake in a fried product and to investigate the potential of BRSPH in the batter formulation as a good source of protein.

2. Materials and methods

2.1 Preparation of Brownstripe red snappers fish protein hydrolysate (FPH)

Brownstripe red snappers were purchased from Jeti Pulau Kambing, Kuala Terengganu, Terengganu,

Malaysia. The fish meat was subsequently homogenized in a food processor (Panasonic, Malaysia) and extracted according to the method of Hau *et al.* (2018). Fish meat of 50 g was deactivated for 10 mins at 90°C. The deactivated fish meat was then mixed with 100 mL pH 8 buffer. A total of 50 g of the homogenized deactivated fish meat was mixed with 500 mL of 90°C water for 10 mins. A further 50 mL of 1 M potassium phosphate buffer (pH 7.5) was added to the fish (ratio of 1:1 between fish and buffer) and the mixture pH was carefully adjusted to 7.5. After 3 hr, the mixture was heated for 5 mins at 90°C to inactivate the enzymes. The mixture was then cooled and centrifuged at 10000 rpm for 20 mins. The resulted hydrolysate was then freeze-dried at -54°C while the vacuum was set at 0.25 mbar.

2.2 Proximate analysis of Brownstripe red snapper

The moisture, ash, fat and crude protein contents of the Brownstripe red snapper were determined based on the standard AOAC (2012) procedures. The samples were analysed in triplicates and calculated according to dry weight basis using AOAC methods (AOAC, 2012).

2.3. Determination of Brownstripe red snapper degree of hydrolysis

Degree of hydrolysis of BRSPH was determined using trichloroacetic acid (TCA) method (Hoyle and Merritt, 1994). One gram of lyophilized FPH powder was determined through Kjeldahl method (AOAC, 2012). As for the 10% TCA soluble nitrogen determination, 1 g of lyophilized BRSPH powder was added into 10 mL distilled water to determine the degree of hydrolysis. Then, 10 mL of 20% TCA was added into the sample and mixed by vortex for 30 s. The sample was then left to stand for 30 mins, to allow protein precipitation and centrifuged at 8000 rpm for 5 mins (High Speed centrifuges 1580R, Cryozen Co, Ltd, Korea). Protein content in the collected supernatant was then measured using Kjeldahl method (AOAC, 2012). The protein content was calculated using the formula:

$$\% \text{ nitrogen} = \frac{\text{mL H}_2\text{SO}_4 (\text{sample} - \text{blank}) \times 1.4}{\text{weight sample (g)} \times 1000} \times 100$$

$$\% \text{ Crude protein} = \% \text{ nitrogen} \times 6.25$$

The degree of hydrolysis was calculated using the formula below:

$$\text{Degree of hydrolysis (\%)} = \frac{10 \% \text{ TCA soluble nitrogen in the sample}}{\text{Total nitrogen in the sample}} \times 100$$

2.4. Determination of Brownstripe red snapper protein hydrolysate (BRSPH) functional groups

The functional groups of BRSPH were determined using FTIR Transmission (Nicolet iS10, Thermo

Scientific, US); in which 5 mg of BRSPH powder was mixed with 250 mg of KBr (1:50). The mixture was homogenized using agate mortar and pestle and then was pressed into pellet (1-2 mm thick films) with a 15-ton hydraulic press. The FTIR spectra were obtained from wavenumber of 600 to 4000 cm^{-1} during 64 scans with 2 cm^{-1} resolution. The resulting spectrum represents the molecular absorption and transmission which then create a molecular fingerprint of the sample (Zainol *et al.*, 2017).

2.5 Determination of BRSPH molecular weight

The BRSPH were analysed for sodium dodecyl sulphate polyacrylamide electrophoresis (SDS-PAGE) (Zainol *et al.*, 2020). SDS-PAGE gel used consisted of 2 layers of acrylamide gels namely resolving gel and stacking gel. The BRSPH sample was diluted in 5% (w/v) and mixed with 1:1 (v/v) ratio loading buffer. The mixture was then heated in 90°C water bath for 10 mins and cooled immediately prior to loading. Sample and protein standard volumes of 25 μL were loaded into individual wells and operated using a discontinuous tris/glycine buffer with 25 mA/gel constant current and 100 V constant voltage for 1 hr. After electrophoresis, proteins were visualized by 0.1% (w/v) Coomassie blue G250 staining and de-staining by soaking in several changes of 40% (v/v) methanol and 10% (v/v) acetic acid until significant clear bands were obtained. Broad protein markers (11 to 245 kDa) were used for molecular weight comparison.

2.6 Oil holding capacity of BRSPH

Palm oil of 10 mL was added to 0.5 g BRSPH and centrifuged in 2000 RCF for 30 mins at 25°C. The unbound oil was decanted off, and oil absorption capacity was determined from the difference in weight of the hydrolysate samples and expressed as gram of oil bound per gram sample (Shahidi *et al.*, 1995).

2.7 Development of battered squid containing BRSPH

Table 1 shows the batter formulation prepared using the modified formulation of Shih *et al.* (2004). Five batter formulations were prepared with the addition of four different amount of BRSPH protein hydrolysate

(2%, 4%, 6%, and 8%) into each formulation and the other one batter formulation was kept constant in which no fish protein hydrolysate was added into the formulation. Then, squid flesh (3 cm x 3 cm) was coated with the 5 batter formulations.

2.8 Deep fat frying

The coated squids were then deep-fried in palm oil for 3 mins at 180°C. The excess oil was then drained the excess oil and cooled to room temperature. Then, the batter crust was peeled off from the squids and the oil was extracted using Soxhlet extraction method Hau *et al.* (2018).

2.9 Determination of oil uptake

Oil-uptake was measured according to a method described by Wasswa *et al.* (2007) with a slight modification. In a 50 mL centrifuge tube, a total of 0.5 g of BRSPH powder sample was added to 9 g of palm oil, mixed with a vortex mixer for 1 min, and then centrifuged at room temperature at 2000 x g for 30 mins. The weight of the extracted oil was determined from the hydrolysate. The difference between the oil weights derived from the hydrolysate and the initial palm oil 9 g was the oil weight consumed by a sample of 1 g. The oil consumption was measured as the quantity of oil consumed by the samples.

2.10 Texture profile analysis

Texture profile analysis was performed using Dogan *et al.* (2005) method with a slight modification. The texture of the samples' crust was analysed in terms of hardness and crispness. Such analyses were measured in the fried samples using texture analyser (TA.XT. plus Stable Micro System Ltd., US). A load cell of 50 N was used. A conical probe was attached to the instrument for the penetration test. The instrument was set to a speed of 55 mm min⁻¹ for 25% penetration of a conical probe into the fried sample.

2.11 Colour profile analysis

The crust colour of fried squid was determined using a colorimeter (Minolta CR 300, Japan), according to the CIE L* a* b* scale. The colour reading included

Table 1. The percentage of ingredients in batter powder formulation

Ingredients	Percentage of ingredients (%)				
Fish protein hydrolysate	0	2	4	6	8
Wheat flour	15.2	13.2	11.2	9.2	7.2
Rice flour	80	80	80	80	80
Sodium chloride	3	3	3	3	3
Sodium bicarbonate	1	1	1	1	1
Disodium pyrophosphate	0.8	0.8	0.8	0.8	0.8

* Batter powder was mixed with water in the ratio of 1:1

lightness (L*), redness (a*), and yellowness (b*). A standard white plate (X = 91.98, Y = 93.97, and Z = 110.41) was used to calibrate the instrument. Each sample was individually measured in triplicate.

2.12 Sensory evaluation

A fresh batch of fried squid was used for sensory studies. The deep-fried battered squid was subjected to sensory evaluation for colour, odour, oiliness, crispness, and overall acceptability. The sensory evaluation was carried out by thirty panellists using a 7-point hedonic scale from 1 to 7 (1 = dislike extremely, 2 = dislike very much, 3 = like slightly, 4 = neither like nor dislike, 6 = like very much, 7 = like extremely) (Mau *et al.*, 2017). The sample was packed and coded with a 3 digit code. The coded samples were served in a tray to the panellist (Lawless and Heymann, 2010). The mean score for each attribute was reported.

2.13 Statistical analysis

Analysis of oil uptake was conducted triplicate and the statistical analysis was carried out using MINITAB 14 statistical software packaged. The results were expressed as mean±standard deviation. The significant difference at ($p < 0.05$) was performed by one-way analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) test (Mamat *et al.*, 2018).

3. Results and discussion

3.1 Chemical composition of raw material

As shown in Table 2, fresh Brownstripe red snapper mince contained 17.34% crude protein, 77.90% moisture and 5.43% crude fat. Crude protein level was within the range based on a previous study (Benjakul *et al.*, 2009). The fishes of the family Lutjanidae demonstrated that the average moisture content was between 71 and 75%, fresh Brownstripe red snapper mince was out of this range, indicating that chemical composition of fish varies within and between species.

3.2 Protein content and degree of hydrolysis (DH) of Brownstripe red snapper protein hydrolysates (BRSPH)

Table 2 also shows that as predicted protein hydrolysate exhibited higher protein content (38.71%) compared to the fresh Brownstripe red snapper mince (17.34%) and could be an essential protein source. The high protein content could have resulted from the solubilisation of protein during hydrolysis, the removal of insoluble undigested non-protein substances and the partial removal of lipid after hydrolysis (Benjakul and Morrissey, 1997). The percentage of solubilised protein was found to be dependent on the amount of lipid in the raw material. Degree of hydrolysis (DH) is an important

factor highly related to the hydrolytic process yield (Adler-Nissen, 1986). DH value of BRSPH was measured at 98.19%. The data also suggested that the soluble protein also increased as the DH value increased. Nevertheless, it has been mentioned that DH has an impact on FPH's physicochemical properties (Harun *et al.*, 2017), which could result in a low oil uptake.

Table 2. Chemical composition of Brownstripe red snapper fish edible flesh

Moisture content (%)	77.90±0.40
Protein (%)	17.34±0.72
Fat content (%)	5.43±0.03
Ash (%)	2.0±0.12
Degree of hydrolysis (%)	98.19±0.24
Protein in hydrolysate powder (%)	38.71±1.71

3.3 Determination of functional groups of BRSPH

Fourier Transform Infrared (FTIR) spectroscopy is an important and well-established technique to study the secondary structure of proteins and polypeptides. Amide I (1700-1600 cm^{-1}) band is the most sensitive and widely used in studies of protein secondary structure. Amide I band is mainly due to C = O stretching vibration (approximately 80%) of the amide group coupled with in-plane NH bending (less than 20%) (Kong and Yu, 2007). Amide II (1575-1480 cm^{-1}) derives mainly from in-plane NH bending and CN stretching vibration and shows less protein conformational sensitivity compared with amide I while other amide vibrational bands have less practical use in protein conformational studies (Kong and Yu, 2007). The FTIR spectra (Figure 2) of BRSPH demonstrated the absorption band at 1559.46 cm^{-1} and 1647.62 cm^{-1} to 1652.39 cm^{-1} region, indicated the existence of amide II and amide I, respectively. Amide I bands of protein hydrolysate was found at 1630 cm^{-1} (Benjakul *et al.*, 2009). Higher frequencies of amide I bands is attributed to greater loss of molecular order of triple helix due to uncoupling of intermolecular cross-links and disruption of intramolecular bonding when protein hydrolysate was extracted at higher temperature or longer time (Ahmad and Benjakul, 2011).

3.4 Molecular characterization of the BRSPH by SDS-PAGE

Figure 3 shows the characterisation by SDS-PAGE of BRSPH's molecular weights (MW). SDS-PAGE is the most widely used for analysing protein mixture, monitoring protein purity and determining their molecular weights. Characterization of the molecular weights of BRSPH by SDS-PAGE showed the presence of strong bands ranging between 10-15 kDa and 75-250 kDa, which indicated that Alcalase® enzyme was able to produce small-sized peptides in 180 mins. Several

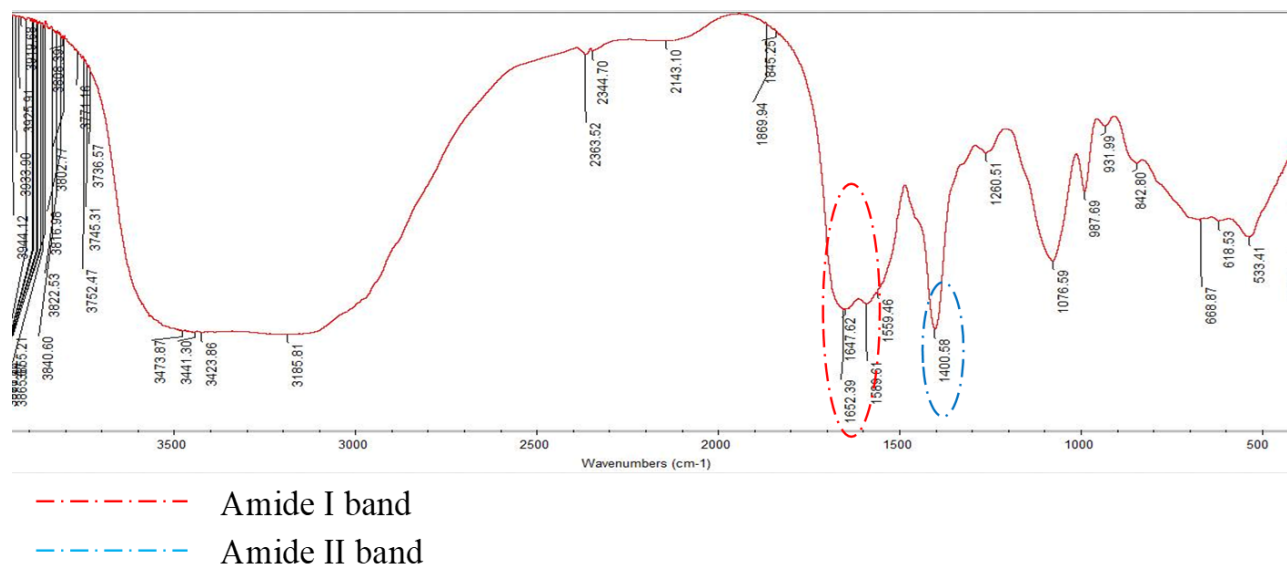


Figure 2. The FTIR spectra of Brownstripe red snapper hydrolysate (BRSPH) powder

studies have shown Alcalase®'s ability to produce low molecular weight peptides via a high degree of hydrolysis (DH) (Liaset *et al.*, 2000). This is in coincident with high DH of Brownstripe red snapper (98.18%). According to Bhaskar *et al.* (2008), fish protein hydrolysate with high nutritional values should be rich in low molecular weight peptides, and the successful production of such desired peptides indicated its potential application in functional food products.

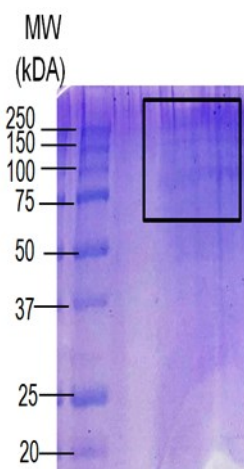


Figure 3. SDS-PAGE patterns of Brownstripe red snapper hydrolysate (BRSPH) powder

3.5 Oil binding capacity

Oil binding capacity expresses the quantity of oil which directly bound by the protein and is of great interest as it is an important functional characteristic, especially expected by meat and confectionery industries (Gbogouri *et al.*, 2004). It is an important attribute that will influence the taste of a product, hence increasing the acceptance of the product. In this study, BRSPH powder exhibited oil binding capacity of 2.38 g oil/g protein, which may be considered as low. The hydrolysates can be potentially used as functional ingredients for the meat and confectionery industry. The decrease in oil binding

capacity as DH increased might have been due to the hydrolytic degradation of the protein structure itself. Oil binding capacity of proteins also correlates with surface hydrophobicity (Kristinsson and Rasco, 2000). Generally, fish protein hydrolysates may be expected to have significant changes in oil absorption, compared to those of native proteins or common food protein ingredients (Sathivel *et al.*, 2003; Gbogouri *et al.*, 2004).

3.6 Analytical methods of batter crusts of fried squids

3.6.1 Colour profile

Table 3 shows the effect of different amount of BRSPH on colour development of deep-fat-fried squid that was examined in terms of Hunter L* a* b* values. Deep-fat-fried foods develop a golden colour, between yellow and brown, when they are properly fried. This is a characteristic that consumers normally consider very attractive. A light golden tone is the benchmark colour for determining the end of the final frying process. A brownish yellow colour is the benchmark colour for the deep-fat-fried squids in this study. There was no significant trend in the variation of the lightness (L*) with an increased amount of BRSPH in the batter. When the frying time was extended longer, the L* value decreased at all the frying temperatures. At the same frying time, the higher frying temperature provided the lower L* value. The decrease in L* value has been linked with non-enzymatic browning reactions which accelerate at high temperatures (Dueik *et al.*, 2010). Dogan *et al.* (2005) also reported that the colour of deep-fat fried chicken nuggets was studied by they found that as frying time increased, L* values decreased. Therefore, the insignificant differences in L* values can be explained by the consistent frying time (3 mins) carried out in the frying process. Redness (a*) is not a desirable colour in fried food products (Ansarifar *et al.*, 2012). The changes in a* values were mainly related to myoglobin oxidation

Table 3. Colour profile (L*, a*, b*) of deep-fat-fried squids incorporated with different amount of BRSPH

Percentage of hydrolysate added	0%	2%	4%	6%	8%
L* (Lightness)	66.62±0.36 ^a	66.79±0.47 ^a	66.69±0.08 ^a	67.16±0.58 ^a	66.34±0.01 ^a
a* (Redness)	1.71±0.17 ^a	2.71±0.08 ^a	4.69±0.07 ^b	4.62±0.19 ^b	5.09±0.05 ^b
b* (Yellowness)	20.68±0.41 ^a	20.79±0.26 ^a	21.09±0.52 ^a	21.14±0.03 ^a	21.30±0.73 ^a

Values are mean±standard deviation of three replications. Values with same superscript letters within the same row are not significantly different ($p < 0.05$).

(Pilar and Reyes, 2007). Deep-fat-fried squid without BRSPH has significantly lower a* value compared to deep-fat-fried squid incorporated with 2%, 4%, 6% and 8% of BRSPH. Addition of 8 % BRSPH provided the highest a* value (highest redness) indicated that the development of golden brown to dark brown colour in deep-fat-fried squids due to non-enzymatic browning reactions (Garayo and Moreira, 2002). Mohamed *et al.* (1988) also observed that the addition of ovalbumin to a batter improved crispness and colour owing to the amine groups present in proteins participating in the Maillard reaction. For yellowness, no significant difference was observed between control and deep-fat-fried squids incorporated BRSPH. This could be due to the consistent frying time (3 mins) carried out in this study. Ansarifar *et al.* (2012) reported that b* value of deep-fat-fried crust decreased with frying time which implied that frying time has a significant effect on the yellowness of the fried product.

3.6.2 Texture profile analysis

The texture profiles of the deep-fat-fried squids which include hardness and crispness were assessed in this study. The addition of BRSPH produces higher hardness compared to the control and 6% added BRSPH to the batter showed the highest value compared to all other treatments. This result was identical to those reported by Mohamed *et al.* (1988), indicating that the addition of protein, such as egg yolk to the batter has reported improving the hardness of fried batter. Crispness was described as the most versatile single texture parameter of a product because it was universally liked, it enhanced or contrasted texture, and was the prominent texture attribute related to top-quality cooking. Deep-fat-fried squid without BRSPH showed the least crispness (3.52 kg.s) among all samples. Incorporation of BRSPH could be the cause of increased

crispness in deep-fat-fried squid batter crust and this is in the agreement with Mohamed *et al.* (1988), denoting that the addition of protein, ovalbumin to a batter improved crispness.

3.6.3 Oil uptake analysis

Oil uptake of deep-fat-fried squid batter crust incorporated with 0%, 2%, 4%, 6%, 8% BRSPH is shown in Table 4. The oil uptake of deep-fat-fried squid batter crust incorporated with 8% BRSPH was significantly the lowest (23.30 %) among the samples. These results indicated that the higher the BRSPH, the lower the oil absorption. Oil uptake of deep-fat-fried squid batter crust incorporated with 2%, 4%, 6% was significantly lower than the control sample, which also demonstrated that the oil uptake of the control sample was the highest among samples. This result suggested that FPH was able to reduce fat uptake when applied to the batter system of deep-fried battered food products as a functional food ingredient. Oil uptake reduction of deep-fried food using other protein based ingredients in batter coating has also been reported before. Albert and Mittal (2002), found that batter coating systems of soy protein “isolate”/methyl cellulose could reduce the fat uptake of deep-fried cereal products by up to 99.8%.

3.6.4 Sensory evaluation of deep-fat-fried squids

The sensory panels were convened to assess the effects on the colour, crispness, oiliness, taste and overall acceptability of deep-fat-fried squid incorporated with 0%, 2%, 4%, 6%, and 8%. Garcia *et al.* (2002) compared the effects of various cellulose derivatives and additionally the sorbitol effect on selected cellulose derivatives in French fries and found that a fat uptake reduction of 41% could be achieved (Table 5). The deep-fat-fried squids with added 4%, 6% and 8% of BRSPH produced higher colour scores than the control ($p < 0.05$).

Table 4. The oil uptake, hardness and crispness values of deep-fat-fried squid batter crust incorporated with different amount of BRSPH

Amount of Tooth-pony FPH in Batter	0%	2%	4%	6%	8%
Oil uptake (%)	30.15±1.35 ^a	28.20±0.13 ^b	27.67±0.31 ^b	27.39±0.52 ^b	23.30±0.61 ^c
Hardness (kg)	0.02±0.01 ^b	0.03±0.01 ^{ab}	0.04±0.01 ^a	0.04±0.01 ^a	0.03±0.01 ^{ab}
Crispness (kg.sec)	3.52±0.01 ^b	3.55±0.01 ^{ab}	3.57±0.02 ^a	3.57±0.01 ^a	3.59±0.01 ^a

Values with same superscript letters within the same row are not significantly different ($p < 0.05$).

Table 5. Sensory scores of deep-fat-fried squid incorporated with 0%, 2%, 4%, 6% and 8% of BRSPH

	Colour	Crispness	Oiliness	Taste	Overall acceptability
0%	4.33±1.92 ^b	4.00±1.68 ^a	4.33±1.83 ^{ab}	4.47±1.93 ^b	5.07±1.20 ^{ab}
2%	4.63±1.56 ^{ab}	3.50±1.68 ^a	3.57±1.57 ^b	4.50±1.48 ^b	4.70±1.37 ^b
4%	5.20±1.16 ^a	4.23±1.65 ^a	4.70±1.51 ^a	5.43±1.19 ^a	5.43±0.93 ^a
6%	5.30±1.42 ^a	4.20±1.73 ^a	3.93±1.68 ^{ab}	5.17±1.53 ^{ab}	4.97±1.25 ^{ab}
8%	5.10±1.24 ^a	4.17±1.95 ^a	3.93±1.76 ^{ab}	5.13±1.38 ^{ab}	4.93±1.14 ^{ab}

Values are mean±standard deviation of three replications. Values with same superscript letters within the same row are not significantly different ($p < 0.05$).

Taste scores were increased by the addition of BRSPH. The samples with 4% BRSPH showed the highest taste scores than that of other samples. Maillard is a non-enzymatic browning reaction, which causes the decomposition of certain amino acids (the building blocks of proteins), in the presence of reducing sugars. This reaction leads to the formation of brown pigments, or melanoidins, which are not well defined and may result in numerous flavour and odour compounds (deMan, 1999).

Textural quality is an important attribute for the acceptability of fries and it is influenced by both material and process conditions (Troncoso *et al.*, 2009). A longer frying time benefits for getting a crisper fried food. In the present study, crispiness values were not significant differences between the five samples. This was probably due to the consistent frying time (3 mins) carried out in this study. Addition of different proteins at different concentrations to the batter decreased the oil content of the final product. Less oil absorption may be related to the formation of covalent links within films during heating. The reduced oil uptake may also be related to thermal gelation and the film-forming ability of proteins. Ovalbumin, the main protein in egg albumen, was also reported to reduce the oil uptake of the fried product, probably owing to its lipophobic nature (Kato and Nakai, 1980). However, panels perceived that the deep-fat-fried squid batter with 4% BRSPH added had undesirable oiliness.

Overall, with regard to the flavour and crispness characteristics, the samples with 4% BRSPH exhibited the highest acceptability scores than that of other samples. Enzymatic hydrolysis of protein develops formation of short chain peptides, thus causing development of bitter taste in the product (Kristinsson and Rasco, 2000). However, the lowest molecular weight of BRSPH was in the range of 10-15 kDa, indicating that the presence of higher amount (4%) of BRSPH did improve the taste and overall acceptability of deep-fat-fried squid instead of generating bitterness. These findings are in concert with the study by Hoyle and Merritt (1994), who reported that Alcalase®-hydrolyzed herring with a higher DH generated less bitter taste

hence giving the higher sensory score. The high acceptability scores of deep-fat-fried squid incorporated with 4% of BRSPH could be explained by umami taste existed in FPH. Umami was one of the dominant tastes of threadfin bream hydrolysates produced by Alcalase® (Normah *et al.*, 2004).

4. Conclusion

This study shows that oil uptake of batter crust incorporated with 2%, 4% and 6% of BRSPH exhibited significant differences ($p < 0.05$) compared with the control sample (30.15%). In sensory evaluation, with regard to the flavour and crispness characteristics, the samples with 4% of BRSPH had the highest overall acceptability scores than other samples. In general, sensory evaluations indicated that deep-fat-fried squids with BRSPH were accepted. Thus, the inclusion of BRSPH may offer better colour and flavour of the deep-fat-fried squids, thereby improving the overall acceptability of deep-fat-fried squids.

Conflict of interest

The authors declare that there is no conflict of interest in conducting this study.

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