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FULL PAPER

Effect of Yellowstripe scad (*Selaroides leptolepis*) protein hydrolysate in the reduction of oil uptake in deep-fried squid

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Abstract

Although the fried products are delicious with a tenderizing effect on the crust due to the presence of fat, over-consumption of fried products causes health problems, especially coronary diseases. The tendency of proteins in film formation and thermal gelation to reduce the absorption of oil in fried products is emphasized. The purpose of this study was to determine the reduction of oil in deep-fried squid by the incorporation of protein hydrolysate and to discuss its effect. Yellowstripe scad protein hydrolysate was produced using Alcalase® enzyme. Fat content was determined using the Soxhlet method, subsequently substituted into a formulation for oil uptake calculation. The viscosity of batter was determined using a rheometer. The viscosity of the batter and batter pick-up was found to be directly proportional, showing a significantly reduced pattern from 0 to 20%. Incorporating 10% of fish protein hydrolysate successfully decreased oil absorption by 17.35±0.73% with a good water retention rate of 38.46%. The addition of the Yellowstripe scad fish protein hydrolysate modified the size and shape of the pore. Sensory acceptance portrayed no significant difference among the three samples (0%, 5% and 10% of incorporation), indicating that panellists were able to accept samples incorporated with fish protein hydrolysate. The findings of this study showed that Yellowstripe scad protein hydrolysate can minimize the uptake of oil in fried seafood products and thus could increase the economic value of the Yellowstripe scad fish.

1. Introduction

Yellowstripe scad belongs to a small group of pelagic fish known as low-value species and is one of South China Sea's plentiful marine sources (Bui and Toshiaki, 2014). Although supplies and outputs are relatively high, these fish are usually traded in fresh, frozen or dried snacks as well as in surimi for household consumption by fresh cooking or making fish cakes, generally in low-income families, while the low-quality Yellowstripe scad is used for aquaculture feed or livestock feed (Bui and Toshiaki, 2014). There is also a need to use these fish in a more advantageous way, as fish have a high amount of essential amino acids, namely methionine, lysine, tryptophan, cysteine and threonine, which play a vital role in the control of health and body nutrition (Dezhabadi *et al*., 2012).

Frying is one of the oldest cooking techniques, causing physical and chemical changes in food, but fried food is commonly associated with coronary heart disease because it contains high cholesterol, which can lead to obesity and many other health problems (Rimac-Brncic *et al.,* 2004; Mirzaei *et al.,* 2015). Therefore, fried food is also seen to be at the core of health issues (Falguera *et al.,* 2011). There is also a need to reduce the amount of oil taken up by such goods, as oil has a major effect on wellbeing.

Batters are often used in frying to improve the quality of fried products by improving texture, flavour, weight and volume (Boskou *et al.,* 2006). Apparently, the function of batter as the coating material is meant to reduce water loss during frying, subsequently reduce oil absorption. Besides, coating improves the structural integrity of the product, and retard gas transport so to prevent flavour diffusion to the oil (Kokoszka *et al.,* 2010). Recently, the usage of protein as a coating

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material to reduce oil uptake in fried products has been focused due to its the ability in film-forming and thermal gelation properties in the batter (Brannan *et al.,* 2014). This is because, protein can reduce pores on the batter, subsequently reduce oil uptake on fried products. Hence, many studies on protein to reduce oil uptake have been reported, for example, gelatine reduces oil uptake by 25% (Olson and Zoss, 1985), egg albumin reduces by 27% (Myers and Brannan, 2012) and red snapper protein hydrolysate reduces oil uptake from 30% to 23% with 8% of incorporation (Chew *et al.,* 2020). Fish protein hydrolysate has great potential to reduce oil uptake in fried products, however, the incorporation could be increased to determine the threshold of protein binding with other ingredients to form a good coating material. Thus, various potential protein hydrolysates need to be diversified as a natural oil reducer in fried seafood products. Therefore, this study aimed to determine the oil -uptake reduction by protein hydrolysate incorporated in batter in deep-fried squid.

2. Materials and methods

2.1 Preparation of Yellowstripe scad fish protein hydrolysate

About 50 g of fish meat was heated at 90°C for 10 mins and then mixed with 100 mL of sodium phosphate buffer at pH 8. The Alcalase® enzyme (Merck, USA) used were at a concentration of 2.0% and the hydrolysis was performed at 55°C for 2 hrs at a constant (220 rpm). The resulting fish protein hydrolysate was centrifuged at 10000 rpm for 20 mins and filtered using a cellulose filter (Whatman No.1, USA) (Hau, Amiza, Mohd Zin *et al.,* 2018). The liquid hydrolysate was then freeze-dried (Labconco, USA) prior to incorporation in batter for further analysis (Hau, Mohd Zin, Zuraidah *et al.,* 2018).

2.2 Preparation of batter

The quantities of dry ingredients used for the preparation of the batter are shown in Table 1. Water was added to the dry ingredients at a ratio of 1.5:1 (75 mL of water to 50 g of dry ingredients). The batter was then mixed well until a smooth mixture was obtained. Then, the batter was prepared with the incorporation of powdered protein hydrolysate instead of wheat flour. The white squid flesh used was cut to 3.5 cm x 5.0 cm and dipped in the prepared batter (3 secs). The batter-coated squid was then deep-fried for approximately 1 min (160 to 170°C).

2.3 Water binding capacity of batter

A suspension of batter with the incorporation of protein hydrolysate was weighed and placed into a centrifuge tube. The mixture was let to agitate for 1 hr at 25°C and centrifuged at 3000 rpm for 10 mins. Free water was removed and drained for 10 mins. The weight of the pallet left in the centrifuge tube was recorded (Medcalf and Gilles 1965).

2.4 Batter pick up

The weight of the raw sample and the amount of coating picked up by the sample before frying were recorded (g) (Mukprasirt *et al.,* 2000). The value was calculated based on Equation (1):

$$Percentage\ of\ batter\ pick\ up\ (\%) = \frac{Weight\ after\ coating\ (g)}{Weight\ before\ coating\ (g)} \times 100 \quad (1)$$

2.5 Flow behaviour

The flow behaviour (viscosity) and time dependency were investigated using a 40 mm parallel plate rotational rheometer (DHR 2- TA Instrument) at 25±1°C (Dogan *et al.,* 2005b). A drop of batter was placed in the flat plate and equilibrated for 2 mins and tested using 1 mm gap. A linear increasing rate from 0 to 200 s^{-1} in 300 s was set for sample shearing. The time dependency of batter was conducted by measuring the apparent viscosity under constant shear rate of 100 s^{-1} for 300 s.

2.6 Determination of fat content

Approximately 1 g of crust was weighed and wrapped into a filter paper before placing it into a thimble. Extraction was conducted using petroleum ether. The system started with boiling, continued with rinsing, recovery and pre-drying steps. The extraction cup was removed and dried in the oven at 103°C for 2 hrs before weighing (AOAC, 2000). The percentage of fat was determined by Equation (2):

$$Percentage\ of\ fat\ content\ (\%) = \frac{Weight\ of\ fat\ (g)}{Weight\ of\ smple\ (g)} \times 100 \quad (2)$$

Table 1. Formulation of batter used for batter analysis and oil uptake determination

Sample	Percentage for each ingredient (%)				
	A	B	C	D	E
Fish protein hydrolysate (FPH) (%)	0	5	10	15	20
Wheat Flour (%)	30.2	25.2	20.2	15.2	10.2
Rice flour (%)	65	65	65	65	65
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

2.7 Oil uptake

Oil uptake analysis is derived from fat content as described in 2.6, where oil uptake is the amount of fat content in sample against fat content of control sample (0% of incorporation), as shown in Equation (3) (Garmakhany *et al.,* 2008):

$$Percentage\ of\ oil\ uptake\ (\%) = \frac{FC\ (incorporated\ with\ FPH) - FC\ (control)}{FC\ (control)} \times 100 \quad (3)$$

2.8 Determination of moisture content

The oven-drying method was used to determine the moisture content of samples. The samples were placed in the crucible and dried in an oven (Memmert, Germany) at 105°C until a constant weight was recorded (AOAC, 2000).

2.9 Water retention

The percentage of water retention was calculated using water content (WC) (as described in 2.8) of fried sample incorporated with fish protein hydrolysate (FPH) relative to the control (moisture content of fried sample with 0% incorporation), according to the formula shown in Equation (4) (Garmakhany *et al.,* 2008).

$$\%\ Water\ retention = \frac{WC\ (incorporated\ with\ FPH) - WC\ (control)}{WC\ (control)} \times 100 \quad (4)$$

2.10 Microstructure of fried crust

The samples were coated with 99% pure gold using JFC 1600 Auto fine coater, before being analysed using Scanning Electron Microscope (SEM) (Jeol-6360, USA). The specimen was viewed using 40X magnification (Hau, Mohd Zin, Zuraidah *et al.,* 2018).

2.11 Sensory acceptance

Sensory analysis was conducted to identify the sensory acceptance of 40 untrained panellists using 7-point Hedonic scale (7-like very much, 4-neither like nor dislike and 1-dislike very much) on attributes namely colour, smell, taste, texture, oiliness and overall acceptance (Zainol *et al.,* 2020). The attributes were evaluated based on personal preference among the panellists on 3 samples presented to panellists, including 1 control (0% incorporation) were presented to panellists. The data were analysed using one-way ANOVA.

2.12 Statistical analysis

The statistical analysis for fried samples analysis was conducted by using SPSS software at the confidence level at $\alpha \leq 0.05$. The data obtained were analysed using one-way ANOVA to compare samples in different percentages of incorporation in the batter. Comparisons of means were carried out using Fisher's Least Significant Difference (LSD) test and the data were presented in the form of mean ± standard deviation.

3. Results and discussion

3.1 Water holding capacity

There was no major difference in the water holding capacity of sample A (control - 0% incorporation) to 15% incorporation of fish protein hydrolysate. Batter with 20% incorporation, however, showed substantial difference ($p<0.05$) with to 0% incorporation, as shown in Table 2. High water binding ability of protein hydrolysate has been able to regulate the loss of moisture, reducing oil absorption during frying (Dogan *et al.,* 2005b). Sample E exhibited the lowest value of all, possibly due to the inclusion of too much protein hydrolysate that reduced the efficiency of the batter ingredients (protein hydrolysate, flours, sodium chloride, sodium bicarbonate and disodium pyrophosphate) to bind together. Increasing the batter's water holding ability has shown to reduce oil absorption, as it prevents oil replacement of water. The finding was in line with the study by Holownia *et al.* (2000), who stated that the use of edible coating with high water holding ability could minimize fat absorption and increase the preservation of moisture. Batters with higher amounts of protein had a higher water holding capacity (Senthil *et al.,* 2002). In addition to the surface properties, the water retained in batter plays a significant role in influencing texture and appearance (Chen *et al*., 2008).

3.2 Viscosity

Figure 1 indicates that increasing the amount of fish protein hydrolysate incorporation makes the batter

Table 2. Means of physicochemical analysis for different percentages of protein hydrolysate incorporation on batter, crust and flesh.

Sample	Water holding capacity	Batter pick up	Fat content	Oil uptake	Moisture content	Water retention
A (0%)	66.14±2.66^{a}	50.41±2.95^{a}	60.40±2.21^{a}		41.21±0.59^{b}	
B (5%)	66.64±0.74^{a}	44.89±1.11^{ab}	55.50±2.33^{ab}	-9.87±3.32^{c}	52.85±5.53^{a}	29.67±3.59^{a}
C (10%)	66.56±2.15^{a}	34.29±1.81^{abc}	49.92±0.44^{bc}	-17.35±0.73^{b}	53.21±1.53^{a}	38.46±6.08^{a}
D (15%)	65.13±1.81^{a}	30.68±0.13^{bc}	46.11±2.60^{cd}	-23.66±4.31^{ab}	43.21±1.35^{b}	16.42±3.11^{b}
E (20%)	59.43±1.74^{b}	23.66±0.27^{c}	41.03±2.11^{d}	-32.07±3.50^{a}	41.47±2.60^{b}	-5.15±1.11^{c}

Values are expressed as mean±SD. Means with same letter superscript are insignificantly different ($p>0.05$) in each column.

thinner. Sample A (control - 0% incorporation of FPH) showed the batter was most viscous followed by 5%, 10%, 15%, and 20%, respectively. The viscosity of batter was directly related to the water binding capacity of dry ingredients (Dogan *et al.,* 2005b). Batter with 0% of protein hydrolysate incorporation had the highest viscosity, probably due to the strongest binding strength among the batter ingredients. Substituting flour with fish protein hydrolysate causes the viscosity of batter to reduce as the binding strength of other batter ingredients, especially flour as the thickening agent, could be reduced. This is in agreement with the previous study which reported that developing viscosity in batter depends on structural association, the solubility of other ingredients and molecular weight of components (Seyhan *et al.,* 2005; Meyer, 1989). Altunakar *et al.* (2006) deduced that viscosity of batter developed was dependent on water binding capacity of the ingredients. Ingredients that bind higher quantities of water will form a higher consistency batter, but the most appropriate batter should be in moderate consistency because the viscous batter may result in excessive oil absorption while the too diluted batter produces unsatisfactory coating.

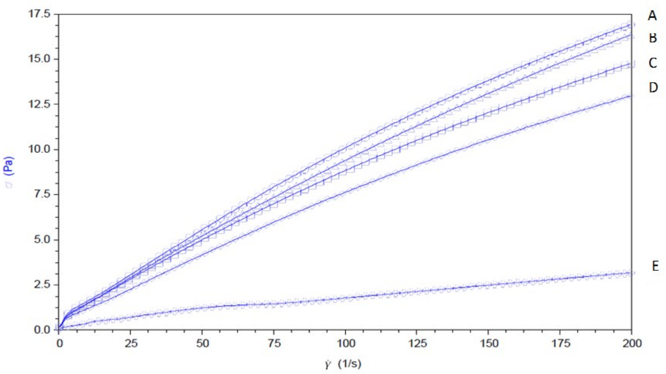


Figure 1. Flow behaviour of batter incorporated with different percentage of protein hydrolysate. A-0%, B-5%, C-10%, D-15% and E-20% incorporation of FPH

3.3 Batter pick up

Table 2 portrays the highest batter pick-up was 0% of protein hydrolysate incorporation. The result showed a reducing trend starting with 50.4% (A), 44.9% (B), 34.3% (C), 30.7% (D) and 23.7% (E), significantly different ($p < 0.05$) from each other, as the incorporation of fish protein hydrolysate varied from 0% to 20%. This could be due to reducing the viscosity of the batter as the incorporation of fish protein hydrolysate increased. Batter pick-up was found to be directly proportional to batter viscosity, which was in accordance with the study by Dogan *et al.* (2005b). Altunakar *et al.* (2004) and Dogan *et al.* (2005a) reported that correlation between batter viscosity and batter pick up, at which increasing batter pick up is due to increased viscosity of batter. The higher consistency batters give higher batter pick up, with the correlation up to r = 0.98, whereas the consistency of the batter is largely dependent on the ingredients and other parameters (Gamonpilas *et al.,* 2013). A good film-forming and thermal gelling ability is a crucial property in the batter to reduce oil uptake because uniform coating formation and stronger coating with smaller pore size are essential to minimise mass transfer during frying (Balasubramaniam *et al.,* 1997; Huse *et al.,* 1998; García *et al.,* 2002; Garmakhany *et al.,* 2008).

3.4 Fat determination (oil uptake) on fried crust

Table 2 also shows that as the incorporation of protein hydrolysate increased from 0% to 20%, fat content reduced from 60% to 55%, 50%, 46% and 41%. A significant difference ($p<0.05$) was observed between protein hydrolysate incorporation and fat content on fried crust. Oil uptake showed a reducing trend as the percentage of incorporation increased, where the samples had significant interaction ($p<0.05$) among the percentage of incorporation. As the incorporation increased from 5% to 20%, the oil reduction increased from 10% to 32%, suggesting that the addition of protein hydrolysate could reduce oil uptake. The result was in agreement with Zainol *et al.* (2020) who reported that Toothpony fish protein hydrolysate reduced fat content in fried seafood products. Proteins were being emphasized in several previous studies to reduce oil uptake due to the ability in film-forming and thermal gelation properties (Brennan *et al.,* 2014). Cross-linking of protein could also reduce the porosity in the sample, which will reduce oil absorption (up to 30%). Mohamed *et al.* (1998) reported that batter coating reduces moisture loss during frying, subsequently reducing oil absorption. Oil uptake showed a reducing trend as the amount of protein hydrolysate increased because of the functional properties of protein to reduce moisture loss during frying. Even though the oil-uptake reduction in samples C and D was higher than the other samples, it is not preferable, as the reduction could be attributable to the lower crust formed during frying, so that less oil is contained in the crust. Angor (2016) and Dogan *et al.* (2005b) reported that different amounts of hydrocolloid, especially protein hydrolysate, may reduce the oil uptake of fried products to varying degrees.

3.5 Moisture content (water retention) on flesh of squid

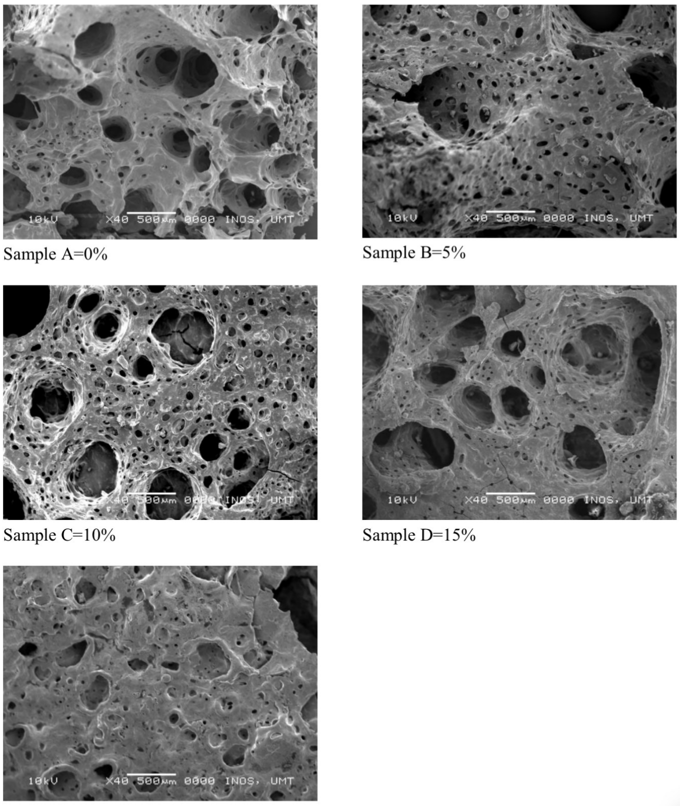
Table 2 shows that sample C (10% incorporation) was significantly higher than sample A, D and E in moisture content and water retention. This data strongly suggests that 10% incorporation of fish protein hydrolysate was the best formula to retain moisture in fried products, subsequently reducing oil uptake. Table 2 also shows the percentage of water retained in the fried sample as the incorporation of protein hydrolysate increased. As the percentage of incorporation increased to 10%, water retention increased to 38% but dropped to 16% then to -5%. This showed that crusts with 0 to 10% of incorporation retained water positively and successfully reduced oil uptake. This is because water retained is inversely proportional to oil absorption (Mellema, 2003).

The increase in water retention and reduction in oil absorption could be due to incorporation of ingredients as coating film was also agreed by Freitas *et al.* (2009). Protein can control moisture loss, subsequently oil uptake due to its high water binding capacity (Dogan *et al.,* 2005b). Sakhale and Pahade (2012) reported that oil absorption could be prevented by the hydrocolloids through the ability of film-forming, hence increasing moisture retention in the product. Thus, incorporation of protein hydrolysate to 10% showed somewhat a positive effect in reducing oil uptake but 15% and 20% gave a somewhat negative effect. Lower water retention could be caused by lesser crust formed. Water in the sample could not be retained by the thin crust which acts as a barrier that prevents moisture loss. This probably due to the properties of protein hydrolysate as the batter formed was less viscous as the percentage of incorporation increases. Crust formation is required to limit mass transfer during frying. Thus, lesser crust formation could not absorb oil and retain water in the sample, causing a negative result in water retention as shown in Table 2.

3.6 Microstructure of fried crust

Figure 2 illustrates the influence of different percentages of protein incorporation on the crust of battered squid after deep frying. Sample A with 0% incorporation illustrated that the crust had many large and deep pores on the crust, while sample B had large and many small pores. Sample C with 10% incorporation had many pores but the pores were shallow with layering at the bottom, whereas sample D had many large pores. Sample E had no notable pores, probably because a layer of skin from the sample was stuck to the crust since a very thin layer of batter-coated on the sample. Porosity on fried crust showed consensus with oil uptake analysis, showing that lesser oil absorbed as fish protein hydrolysate incorporation elevated. Smaller pores seen as the percentage of incorporation increased because of thermal gelation and film-forming properties shown by fish protein hydrolysate (Angor, 2014). Dragich and Krochta (2010) suggested that protein altered the surface structure of fried samples by filling the pores, in order to reduce empty space for oil absorption.

Protein hydrolysate in batter altered the size and shape of the pores supported the oil uptake analysis, at which smaller and shallower pores absorbed lesser oil. There was consensus with the previous study where larger pores on crust drawn more oil adhere to the surface of the crust when a sudden drop in internal pressure due to water vapour condensation creates a vacuum-effect (Ufheil and Escher, 1996; Brennan *et al.,* 2014). The oil absorbed in this study relied largely on the microstructure of the crust, suggesting that fried crust plays a vital role in oil absorption.



Sample A=0% Sample B=5% Sample C=10% Sample D=15% Sample E=20%

Figure 2. The effect of protein hydrolysate incorporation on the microstructure of crust

3.7 Sensory acceptance on fried products

Table 3 shows that there was no significant difference (p>0.05) between samples incorporated with different percentages of fish protein hydrolysate in each attribute. This showed that the panellists were able to accept samples incorporated with protein hydrolysate. The previous study reported that consumers prefer fried food despite health diseases due to better taste (Garmakhany *et al.,* 2008). Thus, this study proved that incorporating protein hydrolysate in batter could be accepted (taste, appearance and texture) with lower oil absorbed in fried samples, based on the personal preferences of panellists. Kilincceker *et al.* (2009) suggested that the smell, taste and flavour of fried products were improved using coating material. Chew *et al.* (2020) reported that incorporation of Brownstripe red snapper (*Lutjanus vitta*) protein hydrolysate increases the overall acceptability of fried squid. Generally, panellists were able to identify samples with lower oiliness and

Table 3. Sensory acceptance scores for different percentage of incorporation in batter

	Appearance	Smell	Taste	Oiliness	Texture	Overall preference
A (0%)	5.55±1.26[a]	5.30±1.51[a]	5.38±1.25[a]	4.93±1.40[a]	5.40±1.61 [a]	5.40±1.36[a]
B (5%)	5.07±1.54[a]	5.50±1.41[a]	5.65±1.29 [a]	4.53±1.84[a]	5.48±1.38[a]	5.58±1.22[a]
C (10%)	5.33±1.27[a]	5.28±1.20[a]	4.93±1.49 [a]	5.05±1.60[a]	5.35±1.46[a]	5.05±1.52[a]

Values are expressed as mean ± SD. Means with same letter superscript are insignificantly different (p>0.05) in each column.

these panellists were able to accept the taste and smell of the fried sample incorporated with protein hydrolysate.

4. Conclusion

The incorporation of Yellowstripe scad fish's protein hydrolysate in batter clearly expressed its ability in reducing oil uptake. The most optimum percentage of incorporation was shown to be at 10% of incorporation, at which the moisture retention was the highest with optimum oil reduction. Besides, a clear SEM image indicating protein hydrolysate was able to form film that reduces pore size which accommodates oil compounds. Hence, incorporating 10% of protein hydrolysate has successfully reduced oil uptake, with high potential to be applied to the food industry. The outcomes of this study are beneficial in further exploring the usage of these functional proteins, increasing the value of Yellowstripe scad fish by utilizing them as value-added components in food. The world, where a healthy driven society focused on natural products to boost better living, exploring the amino acid compositions, sequencing and other bioactivities in the future study can widen the application in nutraceutical and food sciences.

Conflict of interest

The authors declare that there is no conflict of interest.

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