MALAYSIAN JOURNAL OF BIOCHEMISTRY & MOLECULAR BIOLOGY

The Official Publication of The Malaysian Society For Biochemistry & Molecular Biology (MSBMB)
http://mjbmb.org

EFFECT OF CRUDE AND PURIFIED FISH PROTEIN HYDROLYSATE FROM YELLOWSTRIPE SCAD (*Selaroides leptolepis)* IN REDUCING OIL UPTAKE IN DEEP FRIED BATTERED SQUID

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History
Received: 19th December 2019
Accepted: 4th May 2020

Keywords:

Reducing oil uptake, Yellowstripe scad, protein hydrolysate, fried seafood product

Abstract

Frying is one of the oldest cooking methods to prepare desirable food. Fat is a naturally palatable *par excellence* when hot frying fat replaces water loss, exerting a tenderizing effect on the crust for flavour, crispness and pleasant taste. However, fried products are commonly related to chronic coronary health diseases. Thus, the desire to find a way to produce healthy, yet delicious food is crucial. This study aimed to determine the effect of crude and purified protein hydrolysate incorporated in batter in deep fried seafood products. The purified protein hydrolysate A and B were obtained at different retention time. The viscosity of batter and batter pick up were found to be insignificantly different among the protein hydrolysate. Purified protein hydrolysate A and B had significantly lower oil uptake than crude protein hydrolysate. In contrast, water retention was seen highest in purified protein hydrolysate A followed by purified protein hydrolysate B and crude protein, (59%, 57% and 35%) respectively. The crust formed by incorporating crude protein had a combination of few large pores and many small pores. There were a few large pores but a very small number of small pores in the crust incorporated purified protein hydrolysate A, while purified protein hydrolysate B incorporation resulted in a higher number of small pores.

INTRODUCTION

Obesity is defined as a condition of abnormal and excessive fat accumulation in adipose tissue to the extent where health may be adversely affected; like heart attack, high blood pressure and many more [1]. Studies have shown an alarming rate of childhood overweight and obesity in Malaysia [2]. It is a serious epidemic health problem which ranks as the fifth mortality caused disease worldwide [3]. Despite these alarming health diseases, fried food has unique taste and attraction that makes it a favourite food among consumers [4]. Fried seafood products such as fried squid, fishes, prawns and others are popular local cuisine, loved by many consumers. These products are mainly deep fried with batter or flour. Batters are often used in frying to improve the quality of fried products by improving texture, flavour, weight and volume [5, 6]. Major features of fried foods are texture, moisture, oil content, porosity, nutrition, taste and colour [7]. Ingredients in batter act as a defence material that

increase water holding capacity to reduce water lost in the form of steam, subsequently reducing oil uptake [8].

Fish protein is an essential nutrient to many consumers. Functional proteins derived from fishes are found to have many contributions pharmaceutically. These active proteins have great roles as antioxidative compounds, antithrombotic, mineral binders, antimicrobial, antihypertensive, anti-appetizing, immunomodulatory and cytomodulatory [9]. However, only plant proteins, such as wheat gluten, soy protein and whey protein are widely investigated for their film forming properties [10]. These film forming proteins are seen to be added as an ingredient in coating to reduce oil uptake in fried food. Fish protein hydrolysate obtained from extraction has many kinds of protein with different properties and functions, so it has to be purified before proceeding to specific usage [11]. Yellowstripe scad belongs to the small pelagic group which is categorised as low value fishes. They are abundantly found in the Vietnam sea area. However, up to date, lack of study reported on utilizing protein-fat interaction properties to reduce the amount of oil uptake in fried seafood products. Thus, this study aimed to determine the effect of protein-fat interaction in order to reduce oil uptake in fried seafood products using crude and purified protein hydrolysate.

MATERIALS AND METHODS

Powdered protein hydrolysate was obtained from enzymatic hydrolysis of Yellowstripe scad using 2.0% of Alcalase enzyme with 2 h of hydrolysis [12]. The liquid hydrolysate was freeze dried prior to incorporation in batter for further analysis. Freeze dried protein hydrolysate was subjected to purification. This purified protein hydrolysate was dried and incorporated into batter, along with crude protein hydrolysate. Freeze dried protein hydrolysate powder obtained from 2 h of enzymatic hydrolysis contained 57.59±2.08% protein with 47.72±4.80% degree of hydrolysis [12].

Frying was conducted using a 16 cm stainless steel pot, filled with 500 ml of palm oil. The oil was heated 10 min prior to frying. Squid coated with batter that was incorporated with 10% of fish protein hydrolysate was fried for approximately 1 min (160 to 170^{o}C). The white squid was obtained from Gong Badak, Terengganu where the squid was cut according to 3.5 cm X 5.0 cm. Batter was then prepared with the incorporation of powdered protein hydrolysate to replace wheat flour, shown in Table 1.

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

	%
Fish protein hydrolysate (FPH)	10
Wheat Flour	20.2
Rice flour	65
Sodium chloride	3
Sodium bicarbonate	1
Disodium pyrophosphate	0.8

Protein Purification

Protein purification was performed using ion exchange chromatography. AKTA Explorer (Unicorn 5.11 system, GE Healthcare, USA) had been used for ion exchange chromatography. Anion exchange with XK 16/20 column attached to the system was used to purify the protein hydrolysate. The buffers used were A-20 mM sodium acetate buffer and B-2 M sodium chloride buffer with a linear gradient of 100% B and wavelength of 280 nm. Two millilitres of sample were injected into the sample loop and the flow rate was set at 2.00 ml/min throughout the elution. Different peaks at different retention times indicated protein with different net charge.

Properties of Protein Hydrolysate

Properties of freeze-dried powdered protein hydrolysate were determined using FTIR (Thermo Scientific Nicolet iS10 – Transmission) where samples were held using potassium bromide (KBr) [13]. When potassium bromide is pressed at high pressure where it melts and becomes transparent. The ratio of samples to KBr was 1:100. The data was analysed and recorded in percentage of transmission.

Microsturcture of Protein Hydrolysate

Microstructure of the protein hydrolysate; crude and purified, was conducted using Scanning Electron Microscope (SEM) (Jeol-6360, USA). The powdered sample was applied on the surface of the sticker on a specimen holder. Then, the sample was coated with 99% pure gold using JFC 1600 Auto fine coater, before being analysed using SEM [14]. Crude protein hydrolysate was viewed using 90X magnification, while purified protein hydrolysate was viewed using 250X magnification.

Water Binding Capacity

Water binding capacity analysis was conducted according to a modified method originally by Medcalf & Gilles (1965) [15]. A suspension of batter according to the formulation, with the incorporation of protein hydrolysate was weighed and placed into a centrifuge tube. The mixture was agitated at 25°C for 1 h. Then, it was centrifuged at 3000 rpm for 10 min. Free water was removed and drained for 10 min. The weight of the pallet left in the centrifuge tube was recorded.

Batter Pick Up

Batter pick up was defined as the adhesion of batter on the sample during immersion coating before frying. Thus, the weight of raw sample and the amount of coating picked up by the sample before frying were weighed in gram. This

method was a modification from the original method by Mukprasirt, Herald, & Flores (2000) [16]. The formula was shown in Equation 1

$$Percentage\ of\ batter\ pick\ up\ (\%) = \frac{Weight\ after\ coating}{Weight\ before\ coating}\ X\ 100$$

[Equation 1]

Flow Behaviour

In order to determine viscosity of batter, time dependency was investigated using a 40 mm parallel plate rotational rheometer (DHR 2- TA Instrument) at 25°C±1°C. This method was modified from the method reported by Seyhan, Serpil & Gulum (2005) [7]. The batter was equilibrated for 2 min and tested using 1mm gap. Time dependency of batter was conducted by measuring the apparent viscosity under constant shear rate of 100 s^{-1} for 300 s.

Determination of Fat Content

In crude fat determination using Soxhlet method [17], about 1g of sample was weighed and wrapped into a filter paper before placing it into a thimble. Pre-dried extraction cup was weighed and filled with 40ml of petroleum ether. Then the extraction cup was placed into the extraction unit of the Soxhtec machine. 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 h. Then, the extraction product was cooled in desiccators for 1 h before weighing. The percentage of fat was determined by Equation 2,

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

[Equation 2]

Oil Uptake

Percentage of oil uptake in coated sample relative to uncoated sample was calculated based on lipid content (LC) determination shown in Equation 3,

$$Percentage\ of\ oil\ uptake\ (\%) = \frac{LC\ (after\ coating) - LC\ (before\ coating)}{LC\ (before\ coating)} \times 100$$

[Equation 3]

Determination of Moisture Content

Gravimetric method, using oven drying to determine moisture content of samples [17]. The principle of the method was measuring the weight of the sample before and after the drying process. The samples were placed in crucible and dried in an oven (Memmert, Germany) at 105°C until constant weight was achieved.

Water Retention

The percentage of water retention was calculated by using water content (WC) of sample before coating and after coating according to the formula shown in Equation 4.

$$\%\ \text{water retention} = \frac{WC\ (after\ coating) - WC\ (before\ coating)}{WC\ (before\ coating)}\ X\ 100$$

[Equation 4]

Microstructure of Fried Crust

Microstructure determination was conducted using Scanning Electron Microscope. The de-fatted sample was applied on the surface of the sticker on a specimen holder. Then, the sample was coated with 99% pure gold using JFC 1600 Auto fine coater, before being analysed using SEM. The specimen was viewed using 40X magnification [14].

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 samples were arranged in one level arrangement according to the percentage of incorporation of powdered protein hydrolysate. Thus, the data obtained was analyzed using one-way ANOVA to compare samples in different percentages of incorporation in the batter. Comparisons of means were carried out using Tukey HSD and the data were presented in the form of mean ± standard deviation.

RESULTS AND DISCUSSION

Protein Purification

Crude protein hydrolysate was purified using Fast Protein Liquid Chromatography (FPLC). Figure 1 illustrates that purified proteins were collected at two different retention times; 11-15 min (20 ml were pooled)[peak A (purified protein hydrolysate A)] and 17-19 min (15 ml were pooled)[peak B (purified protein hydrolysate B)]. Elena (2010) [18] deduced that when pH is higher than pI, protein is negatively charged so it is attracted to positively charged stationary phase (anion exchange solid phase). Since the column used was packed with Q-Sepharose which contains 6% of cross-linked agarose beads with quaternary ammonium (a strong anion exchange beads-positively charged), the protein extracted (pooled in peak A and peak B) was in the group of negatively charged amino acid. Main negatively charged amino acids are aspartate and glutamate [19].

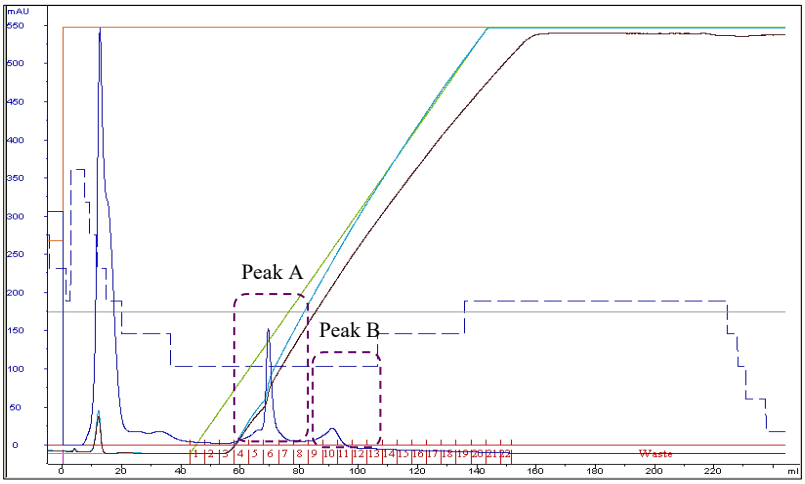
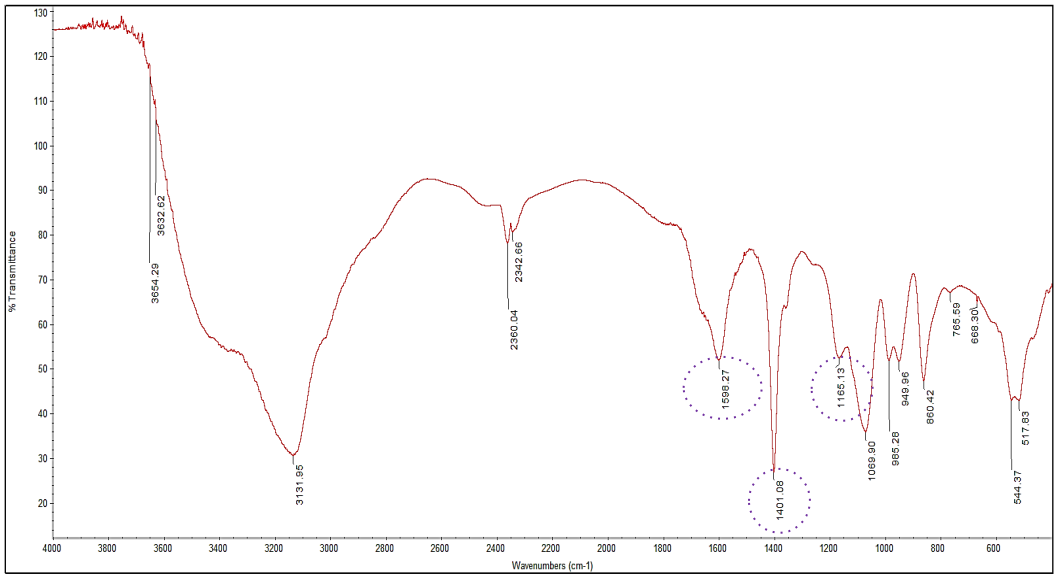


Figure 1. The Chromatogram obtained from purification of Yellowstripe scad protein hydrolysate

Properties of Protein Hydrolysate

The FTIR spectra shown in Figure 2 (a), 2 (b) and 2 (c) were categorized by sample; crude hydrolysate, purified protein hydrolysate A and purified protein hydrolysate B respectively. The spectra deduced that primary amines were present in all the samples. Crude protein indeed had more functional groups detected while purified samples had finer spectrum. Primary amines were seen in N-H bending (amide II) in the region 1640-1560 cm^{-1}. Crude protein hydrolysate had amide bending vibration at 1598 cm^{-1} while broad band found in purified protein hydrolysate A at 1636.52 cm^{-1} and purified protein hydrolysate B at 1636.44 cm^{-1}. Many previous studies reported that bands found in this region were amide II vibration assigned to C=O stretching, N-H bending vibration or a combination of C-H stretch and in plane N-H deformation of peptide [20,21]. A broad band was seen in three samples at 1401 cm^{-1} which signified C-H_3 bending in protein, symmetrically or asymmetrically [22]. Symmetric stretching of P-O_2 group in nucleic acid and phospholipid was seen at band 1069.90 cm^{-1} (crude protein hydrolysate), 1073.57 cm^{-1} (purified protein hydrolysate A) and 1069.32 cm^{-1} (purified protein hydrolysate B) [23]. These functional groups are vital groups in protein that exhibit water binding capacity [24].

(a)



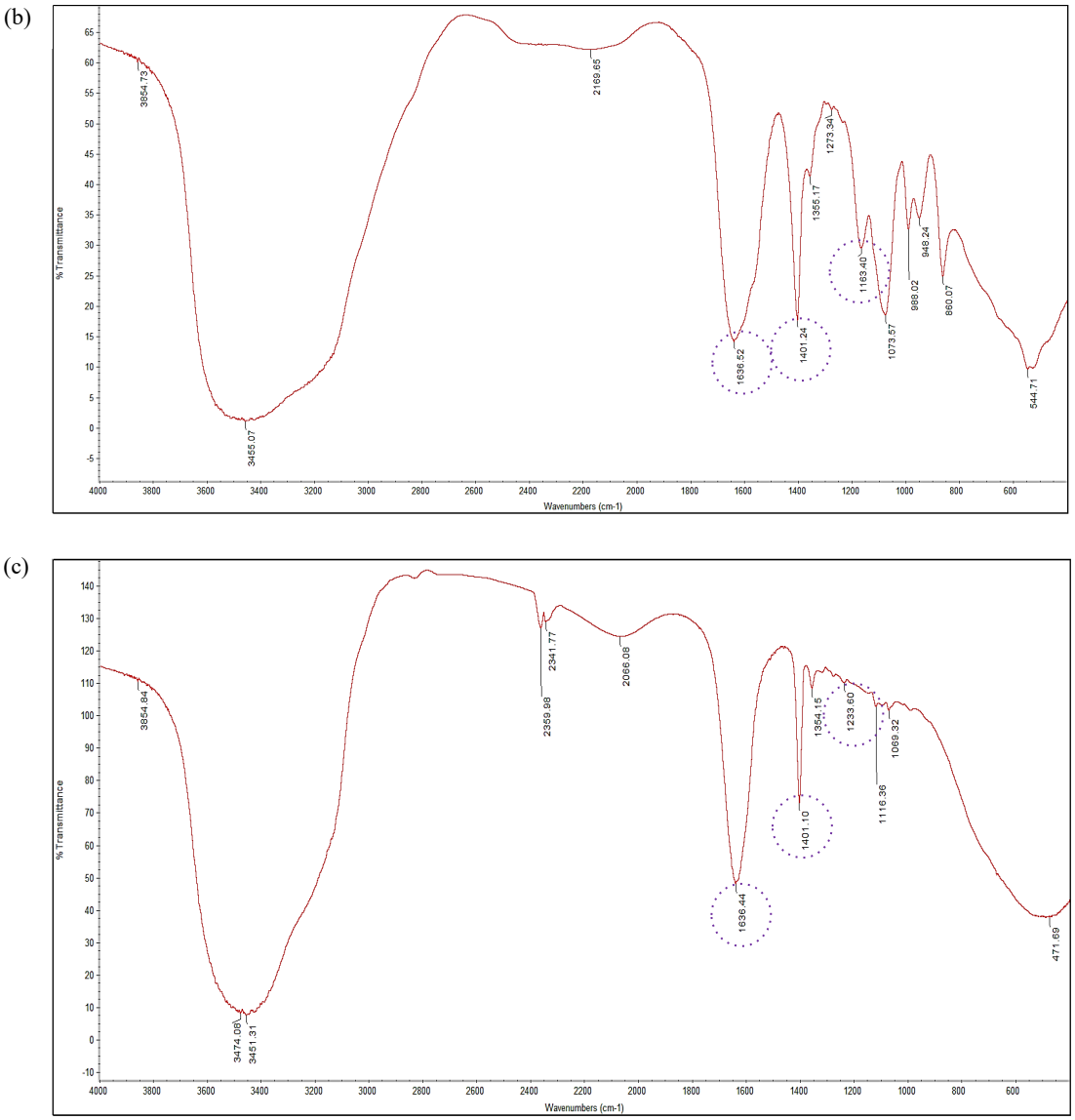


Figure 2. FTIR spectrum for (a) crude protein hydrolysate; (b) purified protein hydrolysate (purified protein hydrolysate A); (c) purified protein hydrolysate (purified protein hydrolysate B)

Microstructure of Protein Hydrolysate

Crude protein hydrolysate (Figure 3 (a)) that was subjected to freeze drying after hydrolysis process was found to have "collapsed-building" structure (unsymmetrical), however, purified protein hydrolysate A and B (Figure 3 (b) and (c)) tend to clump together but they more symmetrical "collapsed-building" shape as compared to crude hydrolysate [25]. Purified protein hydrolysates were very much finer (smaller size) as compared to crude protein hydrolysate. Hence, crude hydrolysate was viewed using 90X magnification while purified hydrolysate (purified protein hydrolysate A and B) were viewed using 250X magnification. Paraman *et al*. (2008) [26] reported that differences in functional properties of protein hydrolysate might be attributed by the diversity of extraction and drying methods (size and shape of powder) as protein concentrate with smaller size and rounded shape showed better functional properties such as higher solubility and emulsifying properties as compared to big and irregular samples. This can also be seen from the FTIR spectrum (Figure 2 (a)), at which crude protein hydrolysate had more functional groups (other compounds) than purified protein hydrolysate (Figure 2 (b) and (c)). Purified protein hydrolysate was seen to be hydroscopic when it is exposed to surrounding, probably because of the presence of polar amino acids [19].

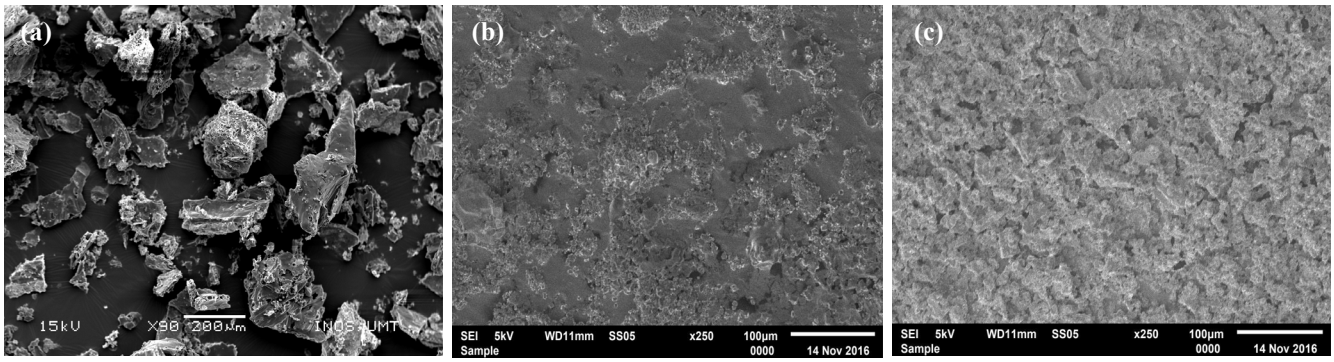


Figure 3. SEM image for (a) crude protein hydrolysate; (b) purified protein hydrolysate (purified protein hydrolysate A); (c) purified protein hydrolysate (purified protein hydrolysate B)

Water Holding Capacity

The ability of components in batter to hold water is expressed by water holding capacity value. Water holding capacity in batter incorporated with 10% of protein hydrolysate (crude, purified protein hydrolysate A and purified protein hydrolysate B) had no significant difference (p>0.05) among each other (Table 2). Batter incorporated with 10% of purified protein hydrolysate A had slightly higher water holding capacity as compared to crude and purified protein hydrolysate B. All the batters had more than 60% of water holding capacity. Higher water holding capacity indicates better role play in reducing oil uptake.

Table 2. The physical analysis of fried squid incorporated with protein hydrolysate

Sample	Water holding capacity	Batter pick-up	Fat content	Oil uptake	Moisture content	Moisture retention
Crude	63.40±3.65^{a}	15.94±1.22^{a}	49.71±1.21^{a}	-17.68±2.014^{b}	55.80±4.22^{b}	35.42±7.31 ^{b}
Purified protein hydrolysate A	63.66±1.70^{a}	16.34±0.85^{a}	37.44±3.51^{b}	-37.94±1.916^{a}	65.64±4.28^{a}	59.28±7.88 ^{a}
Purified protein hydrolysate B	61.91±2.48^{a}	17.53±1.41^{a}	39.22±3.02^{b}	-35.07±5.007^{a}	64.93±3.70^{a}	57.57±9.29 ^{a}

*All values given are means of triplicate results. Standard deviation (mean ± SD) is included for each average. Means with different letters are significantly different (p < 0.05) within the same column.

Water holding capacity is vital to reduce oil uptake as it retards water from being replaced by oil during frying (Holownia *et al.,* 2000). Seyhan *et al.* (2005) [7] also agreed that protein hydrolysate has high water binding capacity, able to retain water which will reduce fat absorption during frying. Batter with higher amount of soluble protein gave higher water absorption [27]. Water holding capacity of batter also influences the viscosity of batter [7].

Viscosity

Viscosity of batter was determined using time dependency, with constant shear stress (100 s^{-1}) in 300 s. Batter viscosity affects batter pick up, subsequently influencing texture and appearance of coated product [16]. Figure 4 illustrated the time dependency of batter incorporated with crude protein hydrolysate, purified protein hydrolysate A and purified protein hydrolysate B. However, the batter incorporated with crude protein hydrolysate was thicker than batter incorporated with purified protein hydrolysate A and B. This batter exhibited thixotropic behaviour under constant shear rate (time dependency) as the viscosity of batter reduced with increasing mixing time. Viscosity in batter also depends on structural association, solubility of other ingredients and molecular weight of components [7,28]. Crude protein had the highest viscosity among the three protein hydrolysate, probably due to the presence of other compounds (as shown in Figure 4) that can hold moisture content.

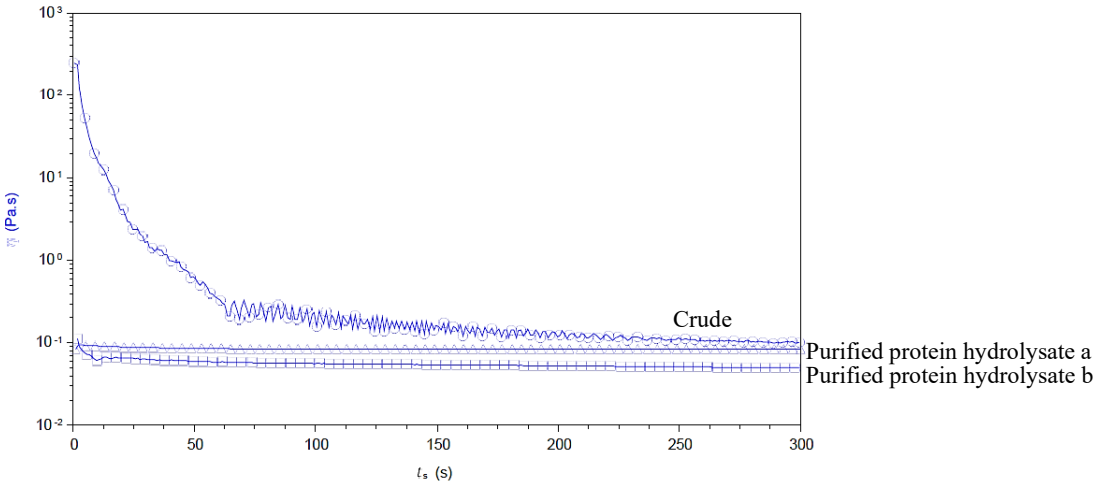


Figure 4. Time dependency of batter incorporated with different percentage of protein hydrolysate

Batter Pick Up

Table 2 shows the batter pick up analysis determines the quality of fried product in terms of the oiliness of the product. Batter pick up incorporated with crude protein hydrolysate, purified protein hydrolysate A and B showed no significant difference (p>0.05) among each other. The viscosity of these batters showed no significant difference although batter incorporated with 10% of purified protein hydrolysate B was seen to be a little higher than the others. Seyhan *et al.* (2005) [7] also reported that batter pick up is related to viscosity of batter.

Oil Uptake Analysis

Table 2 illustrates the percentage fat content in fried crust and the percentage of oil uptake by the crust against 0% of incorporation. The incorporation of the different protein hydrolysate showed significant difference (p < 0.05) in fat content and oil uptake. Crude protein hydrolysate showed the lowest oil uptake with the reduction of 17% while purified protein hydrolysate A had the highest with the reduction of 38% followed by purified protein hydrolysate B (35%). Protein hydrolysate showed positive oil reduction due to its ability in film forming and cross linking of protein could reduce oil absorption up to 30% [8].

The incorporation of protein hydrolysate in batter as an ingredient to reduce oil uptake is due to the ability of protein in forming gel [8]. Previous study by Creusot *et al.* (2011) [29] also reported the effectiveness of protein in gel formation could reduce oil absorbed during frying. The difference found in these protein hydrolysates could be related to the components of protein, as oil uptake is inversely proportional to moisture retention [30]. The mechanism of oil uptake is closely related to moisture retention in the sample during frying. Thus, the properties of protein hydrolysate disclosed by FTIR in Figure 2, was vital to determine the functional side chains that could hold water, subsequently reducing oil absorption during frying.

Water Retention Analysis

Moisture content plays a vital role in oil uptake because oil is absorbed when moisture leaves the pores on the surface via vapour during frying (water replacement theory) [17]. Moisture content and water retention (Table 2) by crust in fried squid showed significant difference (p<0.05) as the incorporation of protein hydrolysate varied. Moisture content and water retention shown by the incorporation of protein hydrolysate from purified protein hydrolysate A and B (Table 2) were significantly higher than that of crude protein. Water retention in purified protein hydrolysate A was the highest (59%) followed by purified protein hydrolysate B (57%) and crude protein (35%) (Table 2).

The increase in water retention and reduction in oil absorption in fried crust was contributed by the protein hydrolysate in the batter which acts as a barrier to limit the movement between oil and moisture [31,32]. Seyhan *et al.* (2005) [7] also agreed that protein can control moisture loss by water binding capacity, subsequently reducing oil uptake. Purified protein hydrolysate A and B contributed more in water retention probably due to the presence of more functional groups that can hold water. This is because the amount of water associated with protein is a function of amino acid composition and conformation of protein molecules [33,34]. Water is bound to protein in many forms; structural water (bound directly to protein molecule), monolayer water (bound to surface of protein), unfreezable water (around to each polar group), water associated via hydrophobic hydration, imbibition or capillary water and hydrodynamic water (transported along with protein molecule) [24].

Water could be bound to protein through hydrogen bonds via carbonyl (oxygen atoms) and amide groups (hydrogen

atoms), hydrophobic interactions (non-polar amino acid chains) and electrostatic interactions or salt linkages (aspartate or glutamate) [24]. Aspartic and glutamic acid tends to bind the most water, along with some ionized proteins [33]. Figure 2 (b) and 2 (c) (FTIR spectra) showed that purified protein hydrolysate A and B had higher intensity of amino group (1640-1560cm^{-1}) than crude protein (Figure 2 (a)). This supported the result in which water retention in purified protein hydrolysate A and B were higher than crude protein hydrolysate as carboxyl and amino groups were mainly responsible for binding of water [24]. Negatively charged amino acids have a greater effect on water structure. Labuza (1977) [35] disclosed that the principal bonding to form gel in protein was due to hydrogen bonding between C=O and NH groups of peptide linkages; in which purified protein hydrolysate A and B had higher intensity than crude protein as shown in the FTIR spectrum. Thermal gelation and gelling properties of protein hydrolysate played a vital role to reduce water loss, subsequently reducing oil uptake [36].

Microstructure of Fried Crust

Microstructure of deep-fried crust incorporated with different types of protein hydrolysate was shown in Figure 5 (a), 5 (b) and 5 (c). Oil absorption is closely related to pores structure (size, depth and number) as oil will replace the empty space left by moisture that escaped through frying oil [37]. Figure 5 (a) illustrated microstructure of crust formed by incorporating crude protein, at which a small number of large pores and many small pores were seen. As the incorporation varied to protein hydrolysate from purified protein hydrolysate A (Figure 5 (b)), there were a few large pores but very small amounts of small pores, while purified protein hydrolysate B incorporation resulted in a higher number of small pores. Smaller and lesser pores on fried crusts were contributed by the functional properties of fish protein hydrolysate in gel formation [38]. Since purified protein hydrolysate A and B had higher intensity of functional groups that were responsible for gel formation, the porosity in the crust was seen to be smaller, subsequently reducing oil efficiently.

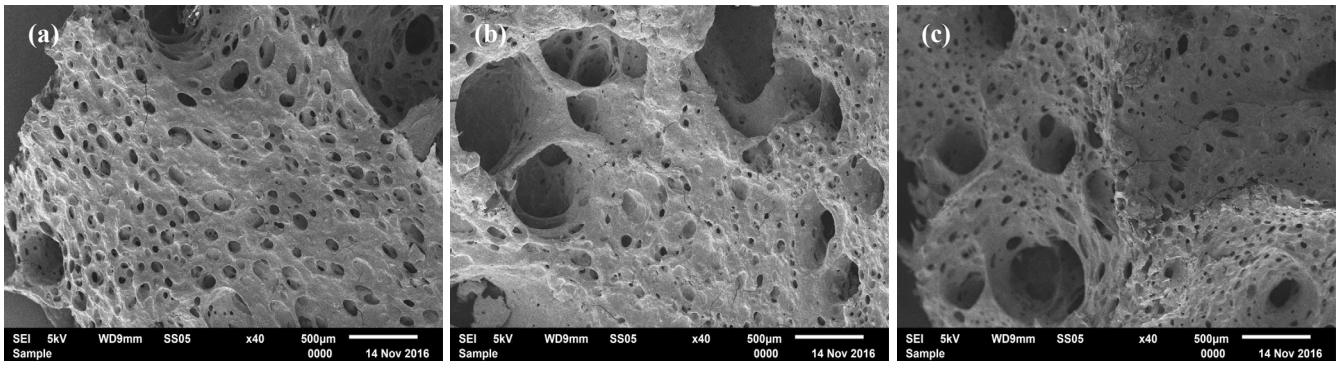


Figure 5. The effect of (a) crude protein hydrolysate; (b) purified protein hydrolysate A; (c) purified protein hydrolysate B incorporation in microstructure of crust

CONCLUSION

Purified protein had finer powder than crude protein hydrolysate. In this objective, the incorporation of protein hydrolysate into batter was set at 10%. Both crude and purified protein incorporated into the batter showed positive oil reduction in the fried sample. Oil uptake and moisture retention showed significant differences among crude and purified hydrolysate. Purified protein hydrolysate A showed the highest oil reduction (38%) while crude protein only reduced oil up to 18%.

ACKNOWLEDGMENT

Deepest appreciation to the Ministry of Higher Education (FRGS grant –Vot 59372) for financial support and UMT for lab facilities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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