Malaysian Journal of Analytical Sciences (MJAS)
Published by Malaysian Analytical Sciences Society

CHARACTERIZATION AND PHYSICOCHEMICAL STUDY OF B - CYCLODEXTRIN- ANGELWING CLAM HYDROLYSATE COMPLEXES

(Pencirian dan Kajian Fisikokimia bagi β-Siklodekstrin-Mentarang Hidrolisat Kompleks)

Nurul Fasihah Razak*, Atikah Mansor, Nur Adila Basari, Normah Ismail

Department of Chemical and Food Technology,
Politeknik Tun Syed Nasir Syed Ismail, 84600 Pagoh, Johor, Malaysia

**Corresponding author:nurul.fasihah@ptsn.edu.my*

Received: 12 December 2021; Accepted: 23 November 2022; Published: 27 December 2022

Abstract

The formed β-cyclodextrin- angelwing complexes were characterized using Fourier transform infrared spectrometry (FTIR) and sodium dodecyl sulfate-polyacrylamide electrophoresis (SDS-PAGE) to mask the bitterness of angelwing clam protein hydrolysate. The shift of O-H stretching to a lower wavenumber and the reduction of the intensity of band C=O and C-H indicated the formation of β-cyclodextrin- angelwing clam hydrolysate complexes. SDS-PAGE analysis showed a lower number of band distributions, revealing the effects of β-cyclodextrin on the mobility of protein during gel electrophoresis. The physicochemical properties of angelwing clam hydrolysate (BH), kneading method hydrolysate (KMH), and physical mixture hydrolysate (PMH) were studied. When compared to functional properties, BH outperformed KMH and PMH. Meanwhile, KMH and PMH showed a higher water holding capacity and a lower oil holding capacity than BH. While the solubility, foaming properties and emulsifying properties of KMH and PMH were lower than BH, the values were still comparable with others of higher sources of protein hydrolysate. Therefore, KMH and PMH can be one of recent potential functional food ingredients.

Keywords: physicochemical analysis, functional properties, angelwing clam, β-cyclodextrin, inclusion complex

Abstrak

β-siklodekstrin-mentarang-hidrolisat kompleks yang terbentuk dicirikan menggunakan spektrometer transformasi inframerah Fourier (FTIR) dan elektroforesis gel poliakrilamida-sodium dodekil sulfat (SDS-PAGE). Peralihan peregangan bilangan gelombang O-H yang lebih rendah dan pengurangan intensiti pada gelombang C=O dan C-H menunjukkan pembentukan kompleks hidrolisat β-siklodekstrin-mentarang hidrolisat. Analisis SDS-PAGE menunjukkan jumlah pengedaran bilangan jalur yang lebih rendah membuktikan pengaruh β-siklodekstrin pada pergerakan protein dalam elektroforesis gel. Sifat fizikokimia mentarang hidrolisat (BH), Kaedah menguli hidrolisat (KMH) dan campuran fizikal hidrolisat (PMH) telah dikaji. Dalam membezakan ciri-ciri fungsi, BH menunjukkan ciri-ciri fungsi yang lebih baik daripada KMH dan PMH. KMH dan PMH menunjukkan daya tahan air yang lebih tinggi dan daya tahan minyak yang lebih rendah berbanding BH. Keterlarutan, sifat berbuih dan sifat pengemulsi KMH dan PMH adalah lebih rendah daripada BH, walaubagaimanapun nilainya masih boleh dibandingkan dengan sumber hidrolisat protein yang tinggi. KMH dan PMH dianggap sebagai salah satu potensi ramuan makanan.

Kata kunci: analisis fizikokimia, sifat kefungsian, mentarang, β-siklodekstrin, kemasukan kompleks

Introduction

Cyclodextrin molecules are cyclic oligosaccharides referred to as α, β, γ-cyclodextrin. They are composed of six, seven, and eight glucopyranose units, respectively linked by -1,4 linkage. Alpha cyclodextrin typically forms a complex with low molecular weight molecules and a compound with an aliphatic side chain. While γ-cyclodextrin can accommodate larger molecules such as steroids, the cost is expensive. β-cyclodextrin is the most accessible, cheap, and effective, as well as its cavity size is suitable for a wide range of guest molecules [1, 2]. Previous studies reported that among various flavor encapsulation techniques, molecular inclusions in β-cyclodextrin molecules are the most effective [1, 3]. Different types of guest molecules such as drugs, steroids, ionic liquids, and dyes have been used as host and guest interactions with β- cyclodextrin to change the properties of the guest molecules into the desired form. However, there is a lack of studies involved in the interactions of host and guest molecules between β-cyclodextrin and protein hydrolysates. Protein hydrolysates have been used for various purposes in foods such as flavor enhancers, protein supplements, and beverage stabilizers [4]. According to previous research, angelwing clam hydrolysate has the potential to be a good source of food ingredients because it consists of high protein content at 74.41%, low fat content at 3.43%, and high total essential amino acids, ranging from 32.94% to 54.17% [5].

Since the protein hydrolysate produced from angelwing clam species has a bitter flavor, its application for human consumption is limited [6]. Therefore, the study of inclusion complexes between β-cyclodextrin and angelwing clam protein hydrolysate is essential to mask the bitter taste. The inclusion complex of these host and guest systems occurred through various interactions such as hydrogen bonding, van der walls interaction, hydrophobic interactions, and electrostatic attraction, all of which alter the photochemical and photophysical properties of the guest molecules [7]. Thus, the physical, chemical, and biochemical properties of guest molecules will be changed [2]. The inclusion of cyclodextrins exerts a profound effect on the physicochemical properties of guest molecules [8]. However, it is still unknown whether entrapment in the internal cavity of β-cyclodextrins could affect their physicochemical properties since there is no information regarding the physicochemical properties of complex formation between protein hydrolysate and β-cyclodextrins. Thus, the current study focused on determining the characteristics and the effects of protein hydrolysate on its physical and chemical properties after forming a complex with β-cyclodextrin.

Materials and Methods

Materials

Angelwing clams were purchased from Pantai Remis, Selangor, Malaysia. The clams were placed on ice and transported to the laboratory. Upon arrival, the clams were washed and their flesh was separated manually before they were then stored at -20°C prior to further processing. Meanwhile, bromelain (1.5 AU/g) was obtained from Novozymes Sdn. Bhd., Malaysia and stored at 4°C until further used.

Preparation of angelwing clam hydrolysates

Angelwing clam hydrolysate was prepared according to the method described by Normah and Nurul Fasihah (2014). 500 grams of angelwing clam flesh was mixed with 531.33 mL of distilled water and then minced in a blender [5]. The mixture was transferred into 1 L beaker, then placed in a water bath. The water bath was set at 45°C and the pH of the mixture was adjusted to 6 using 1.0 N NaOH or 1.0 N HCl. Once the temperature and pH were constant, bromelain (enzyme substrate ratio of 3%) was added and hydrolysis was performed for two hours. The mixture was continuously stirred at the speed of 200 rpm using a stirring propeller. The pH was kept constant throughout the hydrolysis by the addition of 1.0 N NaOH. At the end of the hydrolysis, the reaction was terminated by heating the mixture at 90 ºC for 15 minutes in a water bath. Then, the mixture was centrifuged (Hettich ZENTRIFUGEN, UNIVERSAL 320R) at 10000 rpm and 4 ºC for 20 minutes. The supernatant was collected and freeze dried using biomedical freeze dryer (Alpha 1-4, Martin Christ). The resulting hydrolysate was named as Angelwing clam hydrolysate (BH).

Preparation of angelwing clam hydrolysate –β-cyclodextrin complexes

Angelwing clam hydrolysate and β-cyclodextrin were mixed in a ratio of 1:0.8 (v/w) using the following methods:

Physical mixing method

For the physical mixing method, the hydrolysate was prepared according to the procedure mentioned earlier, except that β-cyclodextrin was added to the supernatant at a ratio of 1:0.8 (v/w) at a temperature of 38.5±1 °C. The mixture was consistently agitated at 150 rpm for 12 minutes using an incubator shaker (Innova 4080 INCUBATOR SHAKER, United States). The product was then freeze dried using biomedical freeze dryer (Alpha 1-4, Martin Christ) [3]. The resulting hydrolysate was named as physical mixed hydrolysate (PMH).

Kneading method

The procedure for physical method was imitated. However, after agitation, the sample was placed in a mortar and grounded for 45 minutes. The product was freeze dried. The resulting hydrolysate was named as kneaded method hydrolysate (KMH).

Characterization

Angelwing clam hydrolysate- β-cyclodextrin were characterized using Fourier transform infrared spectroscopy (FTIR) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Fourier Transform Infrared spectroscopy

The functional group analysis was performed using FTIR (Avatar 360, Thermo Nicolet, USA) as according to Normah and Nurul Fasihah [9]. The sample was ground in an agate mortar until the particle size was measured approximately at 2.5 micron and the surface of the solid appeared shiny. 0.08 g of powdered potassium bromide (KBr) was added into the mixture and grounded for about 30 seconds. The mixture was scraped into the middle and grounded for another 15 seconds. The sample and KBr should be finely grounded to avoid the mixture from scattering infrared radiation excessively. The mixture was then placed in an evacuable mold and subjected to a pressure of 10 to 20 MPa. Perkin Elmer spectrum software was used to control the spectrometer and data were collected over the wavenumber range 4000-400 cm^{-1} with a resolution of 4 cm^{-1} and collection spectra of 16.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis

One gram of hydrolysate was mixed with 10 ml deionized water and filtered using a membrane filter with a pore size of 0.45 µm. 6.5 µL of the solution was then mixed with 2.5 µL NuPAGE®LDS sample buffer (4X) and 1 µL NuPAGE® Reducing Agent (10X). The mixture was heated at 70 ºC for 10 minutes. 15 µL sample was loaded into each well of the SDS-PAGE system each comprised of a 12% resolving gel and a 5% stacking gel. Benchmark™ protein ladder with the range of 220 to 10 kDa was used as a marker. Electrophoresis was performed using the XCell Surelock electrophoresis cell (Bio-Rad Laboratories, Hercules, CA, USA). Electrophoresis was run for 50 minutes at 100-125 mA/gel. After the running process, the gels were washed with 100 mL of ultrapure water and were stained with Coomassie Brilliant Blue dye**.**

Solubility

Solubility, in this study, was determined according to the method by Klompong et al. [10]. A total of two hundred milligrams of samples were dispersed in 20 ml of deionized water, and the mixture's pH was adjusted between pH 2 and 10. The mixture was stirred at room temperature for 30 minutes and centrifuged at 7500 ×g for 15 minutes (5420, KUBOTA, JAPAN). The protein content of each supernatant was determined using Kjedhal (Vapodest 50s, Gerhardt, Germany). The following equation was used to calculate the nitrogen solubility index (NSI):

$$\text{NSI (\%)} = \frac{\text{Protein content in supernatant}}{\text{Total protein in sample}} \text{ x } 100 \quad (1)$$

Water holding capacity

The water holding capacity (WHC) was determined using the method outlined by Diniz and Martin [11]. Triplicate samples (0.5 g) were analyzed. Each sample was dissolved in 10 ml of distilled water in centrifuged tube and vortexes for 30 seconds. The dispersions were allowed to stand at room temperature for 30 minutes, and then centrifuged at 2800 ×g for 25 minutes (5420, KUBOTA, JAPAN). The supernatant was filtered, and the volume was accurately measured. The difference between the initial volumes of distilled water added to the protein sample and the supernatant volume was retrieved. The result was reported as the amount of water absorbed per gram (g) of protein hydrolysate in milliliter (mL).

Oil holding capacity

The oil holding capacity (OHC) was determined using the method described by Shahidi et al. with a slight modification. A 10 mL of soybean oil was added to 500 mg samples into a centrifuge tube [12]. The mixture was centrifuged for 25 minutes in 3800 ×g (5420, KUBOTA, JAPAN). The amount of oil absorption was determined by measuring the volume of oil before centrifugation and the amount of oil supernatant after centrifugation. The analysis was done in triplicate.

$$\text{OHC (mg/mL)} = \text{volume before centrifuge (mg/mL)} - \text{volume of supernatant (mg/mL)} \quad (2)$$

Emulsifying properties

The emulsifying activity index (EAI) and the emulsion stability index (ESI) were determined using the method described by Klompong et al. (2007) with a slight modification [10]. A total of 300 milligrams of sample was dissolved in 30 ml of deionized water. The solution was then mixed with 10 ml sunflower oil, and the pH was adjusted between 2 and 10. The mixture was homogenized (IKA T25 digital ULTRA-TURRAX) at the speed of 14000 rpm for 1 minute. 15 µl of aliquot emulsion was pipetted from the bottom of the container at 0 and 10 minutes after homogenization. After that, the sample was then mixed with 5 ml of 0.1 % sodium dodecyl sulphate solution. The absorbance of the diluted solution was measured at 500 nm using a UV-VIS spectrophotometer (Perkin Elmer Instrument Lambda 35, USA). This data calculated EAI and ESI using the following equation:

$$\text{EAI } (m^2/g) = (2 \text{ x } 2.303 \text{ x } A_{500})/(0.25 \text{ x protein weight (g)}) \quad (3)$$

where; A_{500} = the absorbance at 500 nm.

$$ESI \text{ (min)} = A_0 \, x \, \Delta t \, / \Delta A \quad (4)$$

Where; $\Delta A = A_0 - A_{10}$,

$\Delta t = 10 \, min, A_0$ is the absorbance at zero min and A10 is the absorbance at 10 min, at 500nm.

Foaming properties

Foaming capacity and stability of hydrolysate were measured using the method of Sathe and Salunkhe [13]. A set of 3-gram samples were dissolved in 100 ml of distilled water, with its pH was adjusted between 2 and 10. The protein solution was whipped at 16000 rpm for 3 minutes using a homogenizer (IKA T25 digital ULTRA-TURRAX) and poured into a 100 ml graduated cylinder. The total sample volume was taken at 0 minutes for foam capacity, and up to 60 minutes for foam stability. Foam capacity and stability were then calculated using the equation below:

$$\text{Foam capacity (\%)} = [(A - B)/B] \text{ x } 100$$

Where; A = the volume after whipping (mL) at 0 min and B = the volume before whipping (mL) at 0 min.

$$\text{Foam stability (\%)} = [(A - B)/B] \times 100$$

Where; A = the volume after standing (mlL) at 60 min and B = the volume before whipping (mL).

Statistical analysis

The data were analyzed using Analysis of Variance (ANOVA) to determine statistical significance at the 5% level. The Duncan Multiple Range Test (DMRT) was used to identify mean differences. Statistical analysis was performed using the Statistical Package for Social Science (IBM SPSS Statistics for Windows, Version 20.0. [14].

Results and Discussion

Fourier transform infrared spectroscopy

The complexation of Angelwing clam hydrolysate (BH) and β-cyclodextrin was investigated using FTIR. The formation of a complex between the mixture of β-cyclodextrin and BH is desired to gain a better product with a less bitter taste. As illustrated in Figure 1, the O-H stretching vibration of all samples exhibited a different profile. The peak of KMH and PMH were 3368.04 cm^{-1} and 3370.62 cm^{-1}, respectively, meanwhile β-cyclodextrin and BH were at 3400.70 cm^{-1} and 3375.25 cm^{-1}, respectively. The broad hydroxyl (O-H) peak shown in β-cyclodextrin indicated that β-cyclodextrin contained water molecules. However, it was found that this pattern was narrowed in the complexes of KMH and PMH, showing that this pattern gave a good indication of the formation of the inclusion complex. This study is in line with the study by Crupi et al., who observed a decrease in the frequencies of O-H bands during the formation of inclusion complexes, indicating a reduction of water molecules in the cyclodextrin cavity due to the entrance of guest molecules inside the cavity [15]. However, BH showed high frequencies at 3375.25 cm^{-1}. This is probably because the protein contents in BH have a high amount of hydrogen bonds formed between dipolar groups of carboxylic acid and amide [16].

The stretching regions of the C=O group can be found approximately 1650 cm^{-1} to 1540 cm^{-1}. As shown in Figure 1, the frequencies of β-cyclodextrin and BH were at 1647.33 cm^{-1} and 1640 cm^{-1}, respectively. KMH and PMH showed lower frequencies compared to their BH at 1642.01cm^{-1} and 1643.11 cm^{-1}, respectively. The intensity of this band appeared to be reduced, indicating that the carbonyl group was used for complexation.

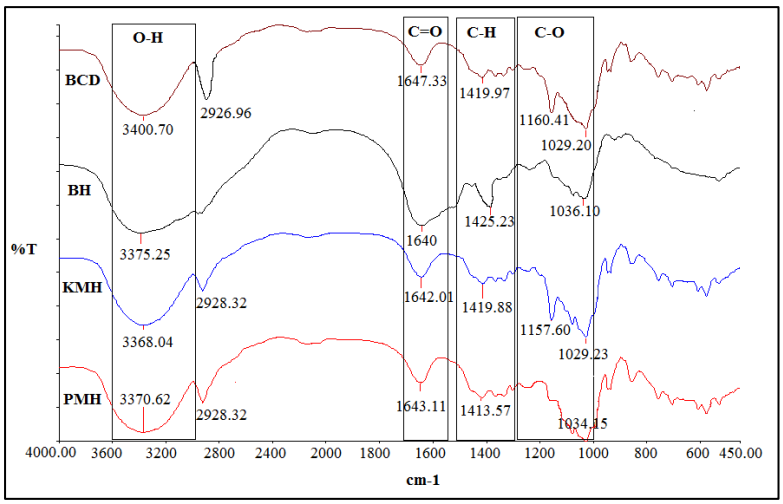

Figure 1. FTIR spectrums of (BCD) β-cyclodextrin, (BH) angelwing clam hydrolysate, (KMH) kneaded method hydrolysate and physical mixed hydrolysate (PMH)

The stretching region of alkane (C-H) were found between 1300 cm^{-1} and 1512 cm^{-1}. The wavenumbers for BH and β-cyclodextrin were 1425.23 cm^{-1} and 1419.97 cm^{-1}, respectively. While KMH and PMH had the peak frequencies at 1419.88 cm^{-1} and 1413.57 cm^{-1}, respectively. The intensity of this band appeared to be reduced for KMH and PMH. Thus, this indicated that the C-H group was also used in the complexation process.

The spectrums within the range of 1160 cm^{-1} to 1020 cm^{-1} were associated with the C-O stretching peaks. It can be seen in β-cyclodextrin and BH at frequencies of 1029.20 cm^{-1} and 1036.10 cm^{-1}, respectively. KMH and PMH peaks had lower frequencies compared to BH at 1029.23 cm^{-1} and 1034.15 cm^{-1}, respectively. The frequencies of modified hydrolysate and its constituent molecule showed a declining pattern, indicating the formation of an inclusion complex. The lower frequencies of KMH and PMH compared to BH were due to the new formation of intermolecular hydrogen bonding, which weakened the strength of interatomic C-O bonds [15]. Thus, C-O stretching of KMH and PMH is shifted to a lower wavenumber than BH [2].

The same pattern as for KMH and PMH in FTIR spectrum analysis reveals the formation of an inclusion complex between BH and β-cyclodextrin. However, the current study was insufficient to determine which method was better. Even though the patterns in the spectrum of KMH and PMH were similar, kneading is a more effective method for forming complexes between angelwing clam hydrolysate and β-cyclodextrin than physical mixing. This is supported by previous research which demonstrated that the morphology of BH- β-cyclodextrin complex was more distinct in KMH rather than PMH [6]. Although the FESEM image of the PMH confirmed the presence of crystalline hydrolysate, BH particles were observed either partially mixed or loosely adhered to the surface of β-cyclodextrin. On the contrary, a drastic change was observed in the morphology of BH and β-cyclodextrin in KMH complex, with both molecules losing their original shape [6].

Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Figure 2 shows the SDS-PAGE patterns for Flesh, BH, KMH and PMH. There was a noticeable difference in SDS-PAGE profiles between samples. Following electrophoresis, BH showed more than eleven subunits at 160 kDa, 90 kDa, 80 kDa, 70 kDa, 50 kDa, 40 kDa, 30 kDa, 25 kDa, 20 kDa, 15 kDa, 10 kDa and <10 kDa. Whereas KMH showed only two subunits at 70 kDa and 60 kDa. Meanwhile, PMH showed more than three subunits at 50 kDa, 20 kDa and <10 kDa (see Figure 2). BH showed many bands distributions at lower molecular weights, which indicated the presence of hydrophobic peptides due to the high hydrophobic of proteins. This is bound to more SDS reagent and thus a faster migration in polyacrylamide gels [17]. However, KMH and PMH had the least bands distributions at lower molecular weights. This is the effect of inclusion complex formed by the hydrophobic peptide inserted into β-cyclodextrin cavity, which then reduced the denaturation effect on protein by SDS reagent.

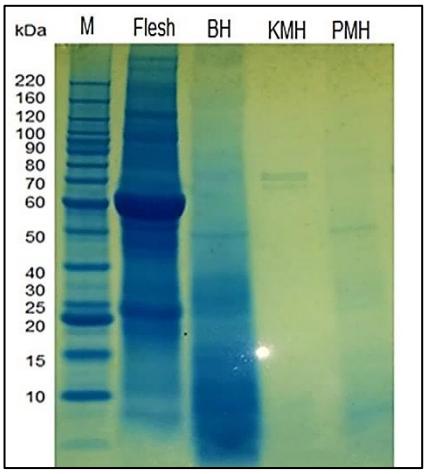

Figure 2. Molecular weight distribution of angelwing clam hydrolysate (BH), hydrolysate from kneading method (KMH) hydrolysate from physical mixing method (PMH)

The difference in SDS-PAGE profiles between samples also revealed the formation of the inclusion complex in KMH and PMH. Different bands distribution in Figure 2 shows that β-cyclodextrin influenced the mobility of proteins in polyacrylamide gel electrophoresis. The

least band presented in KMH indicated the low fragmentation of protein by anionic surfactant of sodium dodecyl sulfate (SDS). This is attributed to that β-cyclodextrin groups were possibly grafted covalently onto the surface of proteins and formed intramolecular inclusion complexes with nearby hydrophobic residues of the protein, thus reducing the denaturation of protein [18]. This study also corroborated with Rozema and Gellman, who found that β-cyclodextrin is one of the carbohydrate molecules that is known to stabilize protein folding and function as an artificial chaperone that refolds protein structure and inhibits aggregation [19].

Solubility

The pH-solubility profiles of BH, KMH and PMH proteins are shown in Figure 3. It was found that the protein solubility of BH, KMH and PMH in water at different pH (pH 2 to pH 10) showed the same pattern curve. Protein solubility of BH, KMH and PMH were significantly different ($p < 0.05$) and soluble over a wide pH range with a solubility of greater than 50%. BH, KMH and PMH presented minimum protein solubility at pH 4 with values of 86.03%, 65.27% and 70.79%, respectively, and a maximum protein solubility at pH 10 with values of 99.02%, 70.33% and 82.28%, respectively. This indicates that in general, protein's solubility decreases as the pH increases until it reaches the isoelectric points and then increases [20]. Proteins are amphoteric molecules, which means that when exposed to an acidic medium at pH lower than their isoelectric point, they behave as positive ions and repel each other, resulting in their good dispersity. ON the other hand, as the pH value approaches the isoelectric point, solubility decreases. Similarly in alkaline solutions, at pH values higher than the isoelectric point, protein molecules transform into negative ions, increasing their solubility [21]. However, not all protein hydrolysates have an isoelectric point at pH 4. Previous studies showed that peanut protein had an isoelectric point at pH 4.5, as seen from its drastically reduced solubility at this pH [22]. This result indicated that protein solubility is directly related to the pH of the solution and the isoelectric point of protein.

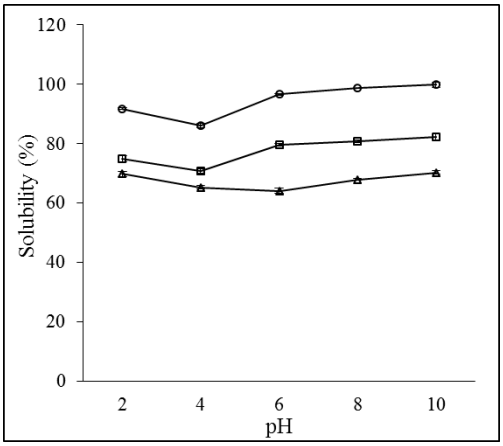

Figure 3. Solubility of Angelwing clam hydrolysate (BH =●), physical mixed hydrolysate (PMH =▲) and kneaded method hydrolysate (KMH = ■). Bars represent standard deviation from triplet determination

Solubility in BH was higher than KMH and PMH due to the lower molecular weight of peptides during hydrolysis which was generated by reducing the secondary structure in protein [23]. The solubility of KMH and PMH was slightly lower compared to BH which proved the formation of an inclusion complex between the guest and host compounds of BH and β-cyclodextrin. According to previous studies by Rahman [24] and Sanjoy et al. [25], the solubility of the inclusion complex is higher than the solubility of β-cyclodextrin if the guest molecules are highly soluble in water. However, the solubility of the inclusion complex is lower than β-cyclodextrin, but will have a higher solubility than its guest molecule if the guest molecule has poor solubility in water. This explains the reason for a lower solubility between KMH and PMH. To conclude, these studies revealed that a physicochemical property of a complex is different from that of β-cyclodextrin and its guest molecule.

Water holding capacity and oil holding capacity

According to Table 1, the water holding capacity of BH, KMH and PMH were significantly different ($p < 0.05$), with BH having lower water holding capacity than KMH and PMH at 4.60 mL/g, 5.349 mL/g and 5.572 mL/g, respectively. According to Amiza et al. water holding capacity values were influenced by the

solubility of the samples [23]. However, Tang et al. reveals the high protein solubility was not necessarily related to the high-water holding capacity. The lower water holding capacity of BH was probably related to its lower molecular weight, leading to a smaller interface surface area [22]. Besides, decreased water holding capacity might be attributed to the hydrolytic degradation of the protein structure, in which physical entrapment plays an important role in the adsorption of water [23].

Table 1: Water holding capacity and oil holding capacity of hydrolysate and the modified

Functional Properties	**BH**	**PMH**	**KMH**
Water Holding Capacity (mL/g)	4.60^{b}±0.24	5.35^{a}±0.17	5.57± 0.25
Oil Holding Capacity	5.16^{a}±0.05	3.96^{b}±0.04	3.44^{c}±0.01

Values are expressed as means ± standard deviation from triplicate determinations. Different letters within rows indicate significant difference at $p < 0.05$

The higher value of WHC in KMH and PMH indicated the effects of β-cyclodextrin on water holding capacity. The changes in water holding capacity in the samples were probably due to the inclusion complex between BH and β-cyclodextrin, which had changed the molecule structure of KMH and PMH. This study corroborated previous studies indicating that the binding strength of proteins was mainly dependent on their molecular structure [26]. Therefore, the changes in molecular structure produced from the complexes PMH may have significantly altered its water holding capacity in KMH and PMH.

The mechanism of fat absorption is mostly attributed to the physical entrapment of the oil. The oil holding capacity (OHC) obtained from BH was significantly ($p <0.05$) higher (5.16 mL/g) than KMH (3.44 mL/g) and PMH (3.96 mL/g) as shown in Table 1. This agrees with a previous study in which the oil holding capacities of BH possessed a higher oil holding capacity [27]. The high oil holding capacity of BH could be due to its higher surface hydrophobicity and degree of hydrolysis as suggested by Kristinsson and Rasco [28]. On the contrary, low oil absorption might be because the presence of a large proportion of hydrophilic groups and polar amino acids on the surface of the protein molecules after the complex was formed with β-cyclodextrin [13].

Foaming properties

The foaming capacity and stability were significantly ($p<0.05$) higher than KMH and PMH at all pH values (pH 2 to pH 10). The results suggested that BH had a more flexible protein structure in aqueous solutions and interacted strongly at the air–water interface to form more stable foams when compared to the KMH and PMH. In addition, hydrophobic had improved the foaming capacity and had made the protein more flexible [29]. Hydrophobic residues of protein in KMH and PMH were found less in SDS-PAGE studies. This is proved by less band distribution in polyacrylamide gels at lower molecular weights. This occurs due to sodium dodecyl sulphate (SDS) preferentially binds to hydrophobic regions of proteins. Therefore, hydrophobic proteins bind a higher amount of SDS than hydrophilic proteins, resulting in faster migration on SDS-PAGE [17]. This study revealed that β-cyclodextrin gave an effect on foaming properties by forming an inclusion complex with hydrophobic amino acids residues, thus causing hydrophobic residues to be hidden and less exposed.

The effect of pH on foaming capacity and foaming stability of BH, PMH and KMH are shown in Figures 4 (a) and (b). The lowest foam capacity and stability was observed at pH 4 among BH (74.33%, 70.33%), PMH (37.00%, 31.17%) and KMH (29.50%, 26.16%). The relationship between foaming properties and pH for BH, PMH and KMH was similar to the relationship between protein solubility and pH. In this study, the foaming

properties of the hydrolysate samples were found to decrease at pH 4, and to increase at pH 6. However, there was a slight decrease in alkaline pH. The changing foaming properties at different pH values showed that net charge had an influence on the adsorption of proteins at the air-,water-interface [9]. However, BH showed an increase in foaming properties as the pH continued to increase. This is agreed in previous studies, which demonstrated that as the pH increased, deprotonation of the acidic and basic groups occur, and when the net charge is increased, the foaming properties of protein are also enhanced [30].

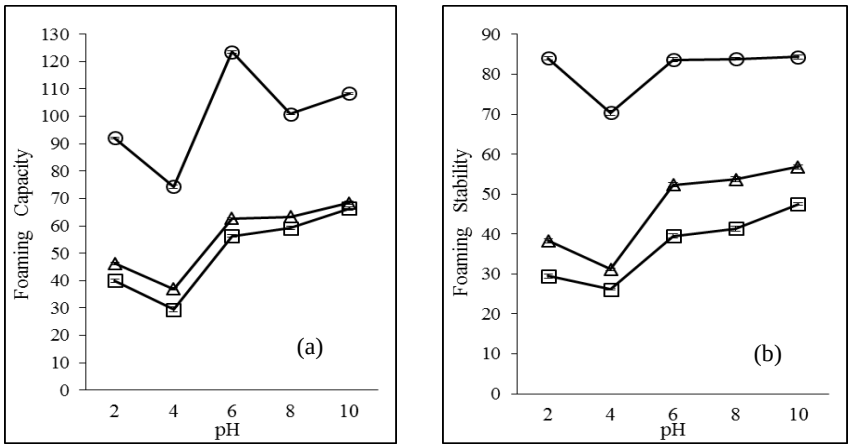

Figure 4. (a) Foaming Capacity and (b) Foaming Stability of Angelwing clam hydrolysate (BH=○), hydrolysate produced by physical mixing (PMH=Δ) and hydrolysate produced by kneading method (KMH= □). Bars represent standard deviation from triplicate determination

Emulsion properties

Emulsifying activity index (EAI) and emulsion stability index (ESI) of hydrolysate are shown in Figure 5. BH had a higher emulsion activity index at all pH values (pH 2 to pH 10) compared to KMH and PMH. This supports Mutilangi et al. as the higher solubility of hydrolysate results in higher EAI [29] because hydrolysate with higher solubility could rapidly diffuse and adsorb at the interface [10]. However, both KMH and PMH showed lower EAI across all pH ranges compared to BH, due to the complex formation between β-cyclodextrin and BH. This complex formation reduced the ability of the oil to form stable oil-in-water emulsions because β-cyclodextrin is not a surfactant, therefore, does not have the tendency to lower the surface tension between two liquids and thus, does not promote the oil-in-water emulsion. However, BH contains surface active material and promote oil-in-water emulsion due to the presence of hydrophilic and hydrophobic groups with their associated charge [31, 32]. Besides that, the lower EAI values in PMH and KMH are probably because of the extensive degradation of protein caused by further methods used to debitter the hydrolysate, such as kneading and rapid shaking in an incubator shaker.

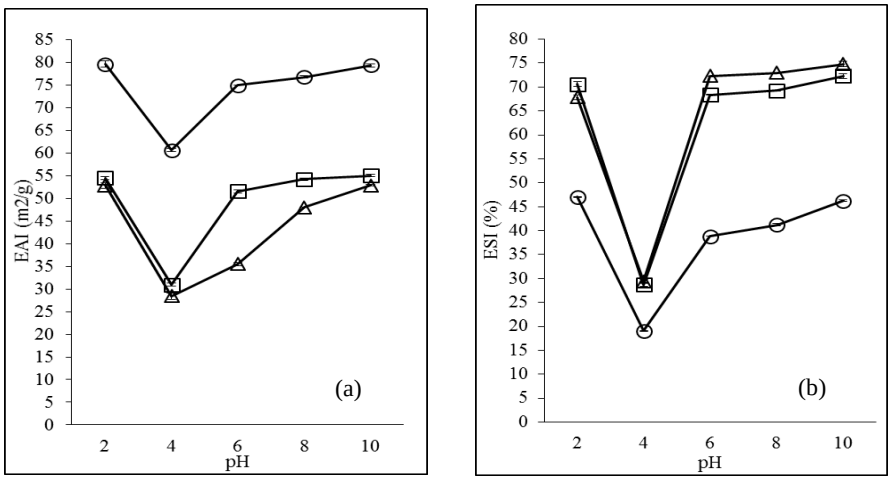

Figure 5. (a) Emulsifying activity index and (b) Emulsion stability index of Angelwing clam hydrolysate (BH=○), hydrolysate produced by physical mixing (PMH= Δ) and hydrolysate produced by kneading method (KMH= □)

At pH 10, the emulsion activity indexes of BH, PMH and KMH were significantly different ($p < 0.05$), with maximum values at 79.30%, 52.87% and 55.01%, respectively, whereas the emulsion stability index were at 46.55%, 74.81% and 69.63%, respectively. However, the minimum emulsion activity index in BH, PMH and KMH at pH 4 (isoelectric point) were 60.58%, 28.45% and 30.91%, respectively whereas the emulsion stability index was 19.01%, 29.41% and 28.71%, respectively. The results indicated that emulsion properties were pH-dependent, with alkaline pH significantly improving emulsion capacity over acidic pH. The tendency was similar to protein solubility (see Figure 3). For instance, protein solubility was low at pH of 4, implying that protein adsorption at the oil–water interface would be diffusion controlled. However, at pH range of 6 to 10, as protein solubility increased, the activation energy barrier prevented protein migration from taking place in a diffusion dependent manner. An increase in protein solubility facilitated interaction between the oil and aqueous phases [20]. The emulsion stability of BH was significantly lower than that of KMH and PMH due to its lower molecular weight (refer Figure 2). Although small peptides diffuse rapidly toward the interface are effective in producing emulsions, they are less efficient at stabilizing emulsions, because they may not readily agglomerate to produce a fat-globule membrane due to charge repulsions [33].

Conclusion

The characterizations of complex formation between BH and β-cyclodextrin are shown through FTIR analysis. The changes in the FTIR peak patterns of the complexes were compared with their corresponding peaks of pure BH and β-cyclodextrin. The difference in the peak patterns of O-H stretching profiles reveals the complexation formation in KMH and PMH. The low frequency in the C=O stretching vibration and C-H stretching vibration of KMH and PMH also reveals the formation of complex between BH and β-cyclodextrin. Low fragmentation of proteins in polyacrylamide gels in KMH and PMH is compared to BH, proving that the inclusion complex had formed by grafting the β-cyclodextrin molecule to the protein surface and reducing the denaturation of protein by SDS reagent. The physicochemical analysis shows that β-cyclodextrin influences the functional properties of hydrolysate. Despite that BH has a good quality of nitrogen solubility index, the solubility of BH is reduced when it forms a complex with β-cyclodextrin. As a result, the decreased solubility in KMH and PMH has an effect on their water and oil holding capacities, as well as foaming emulsifying properties. Even though, the functional properties of KMH and PMH are slightly reduced after the complex formation between BH and β-cyclodextrin,

their reducing values remain within the best ranges for all protein hydrolysates studied.

References

1. Del Valle. E. (2004). Cyclodextrins and their uses: a review. *Process Biochemistry*, 39: 1033-1046.
2. Sambasevam, K. P., Sharifah, M., Norazilawati, M. S. and Nor Atiqah, I. (2013). Synthesis and characterization of the inclusion complex of β-cyclodextrin and azomethine. *International Journal Molecular Science*, 14: 3671-3682.
3. Hou, L., Wang, J. and Zhang, D. (2013). Optimization of debittering of soybean antioxidant hydrolysates with β-cyclodextrin using surface response methodology. *Journal of Food Science and Technology* 50: 521-527.
4. Nazeer, R. A., Prabha, K. R., Divya Kumar, N. S., Sampath, G. and Jai, R. (2011). Isolation of antioxidant peptides from clam, *Meretrix casta* (Chemnitz). *Journal of Food Science and Technology* 50: 777-783.
5. Normah, I. and Nurul Fasihah, R. (2014). Sensory, amino acids composition and flavour compounds of angelwing clam (*Pholas orientalis*) hydrolysates produced using two different enzymes. *Science Letters*, 8:1, 25-33.
6. Normah, I. and Nurul Fasihah, R. (2017). Evaluation of β-cyclodextrin masking effect on the bitterness of angelwing clam (*Pholas orientalis*) hydrolysate. *International Food Research Journal*, 24(4): 1500-1506.
7. Szejtli J. (1998). Introduction and general overview of cyclodextrin chemistry. *Chemistry Reviews*, 98:1743-1753.
8. Schmid G. (1989). Cyclodextrin glucanotransferse production: yield enhancement by overexpression of cloned genes. *Trends Biotechnologies*, 7: 244-248.
9. Normah, I and Nurul Fasihah, R. (2016). Bitterness and physichochemical properties of angelwing clam (*Pholas orientalis*) hydrolysate. *Malaysian Journal of Analytical Sciences*, 20: 594-600.
10. Klompong, V., Benjakul, S., Kantachote, D and Shahidi. (2007). Antioxidative activity and functional properties of protein hydrolysate of yellow stripe trevally (*Selaroids leptolepis*) as influenced by the degree of hydrolysis and enzyme type. *Food Chemistry*, 102: 317-327.
11. Diniz, F. M. and Martin, A. M. (1997). Effects of the extent of enzymatic hydrolysis on the functional properties of shark protein hydrolysate. *Lebensmittel Wissenschaft and Technologies* 30: 266-272.
12. Shahidi, F., Xiao-Qing, H. and Synowiecki, J. (1995). Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*). *Food Chemistry* 53: 285-293.
13. Sathe, S. K. and Salunkhe, D. K. (1981). Functional properties of the Great Northern bean (*Phaseolus vulgaris* L.) proteins: emulsion, foaming, viscosity and gelation properties. *Journal of Food Science*, 46: 71-74.
14. IBM Corporation (2011). IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.
15. Crupi, V., Ficarra, R., Guardo, M., Majolino, D., Stancanelli, R. and Venuti, V. (2007). UV-vis and FTIR-ATR spectroscopic technique to study the inclusion complexes of genistein with β-cyclodextrin. *Journal of Pharmaceutical and Biomedical Analysis*, 44: 110-117.
16. Simon, B. W., Adi, N. and Teti, E. (2011). Functional interaction components of protein isolates and glucomannan in food bars by FTIR and SEM studies. *African Journal of Food Science*, 5: 12-21.
17. Robinson, N. C. and Tanford, C. (1975). The binding of deoxycholate, triton X-100, sodium dodecyl sulfate, and phosphatidylcholine vesicles to cytochrome b5. *Biochemistry*, 14: 369-378.
18. Aachmann, F. L., Otzen, D. E., Larsen, K. L. and Wimmer, R. (2003). Structural background of cyclodextrin-protein interactions. *Protein Engineering,*16: 905-912.
19. Rozema, D and Gellman, S. H. (1996). Artificial chaperone-assisted refolding of carbonicanhydrase. *Biological Chemistry*, 271: 3478-3487.

20. Muo, X and Hua, Y. (2012). Composition, structure and functional properties of protein concentrates and isolates produced from walnut (*Juglans regia* L.). *International Journal of Molecular Sciences*, 13: 1561-1581.
21. Yin, Y. R., Liu, W. Q and Xiao, K. J. (1996). The study of Sesame protein solubility and emulsification. *Food Science*, 17: 3-6.
22. Tang, L., Sun, J., Zhang, H. C., Zhang, C. S., Yu, L. N. and Bi, J. (2012). Evaluation of physicochemical and antioxidant properties of peanut protein hydrolysate. *PLoS One* 7(5): e37863.
23. Amiza, M. A., Ow, Y. W. and Faazaz A. L. (2013). Physicochemical properties of silver catfish (*Pangasius* sp.) frame hydrolysate. *International Food Research Journal*, 20(3):1255-1262.
24. Rahman, M. S. (2007). Hand book of food preservation. 2nd edition, Boca Raton, FL, USA, CRC Press: pp. 519.
25. Sanjoy Kumar D., Rajabalaya, R., Sheba, D., Nasimul, G., Jasmina, K and Nanda, A. (2013). Cyclodextrins-the molecular container. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 4: 1694-1720.
26. Chaplin, M. F. (2003). Fibre and water binding. *Proceeding of Nutritional Society*, 62: 223-227.
27. Popovic, l., Peričin, D., Vaštag, Z. and Popovic, S. (2011). Optimization of enzymatic hydrolysis of cucurbitin using response surface methodology: improvement of the functional properties. *International Journal of Food*, 7, 5: 1556-3758.
28. Kristinsson, H. G. and Rasco, B. A. (2000). Fish protein hydrolysates: Production, biochemical and functional properties. *Critical Reviews in Food Science and Nutrition*, 40: 43-81.
29. Mutilangi, W. A. M., Panyam, D. and Kilara, A. (1996). Functional properties of hydrolysates from proteolysis of heat-denatured whey protein isolate. *Journal of Food Science*, 61: 270-274.
30. Wagner, J. R., Sorgentini, D. A and Anon M. C. (2000). Relation between solubility and surface hydrophobicity as an indicator of modifications during preparation processes of commercial and laboratory-prepared soy protein isolates. *Journal of Agricltural Food Chemistry*, 48, 3159-3165.
31. Mentink, L. (1999). β-cyclodextrin emulsion: Mechanism of emulsification and application to the preparation of green metalworking fluids. Netherlands, Springers: pp. 693-696.
32. Gbogouri, G. A., Linder, M., Fanni, J, and Parmentier, M. (2004). The influence of hydrolysis degree on the functional properties of salmon byproducts hydrolysates. *Journal of Food Sciences*, 69: 615-622.
33. Cho, D-Y., Jo, K., Cho, S. Y., Kim, J. M., Lim, K., Suh, H. J and Oh, S. (2014). Antioxidant effect and functional properties of hydrolysates derived from egg-white protein. *Korean Journal Food Sciences of Animal Resources*, 34(3): 362-371.