

# Characteristics and Functional Properties of collagen extracted from Nile tilapia (*Oreochromis niloticus*) skin

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## Abstract

Tilapia skin might be used as a new source of collagen, therefore collagen obtained from Nile tilapia skin can be utilized as food product because of its good functional properties. Two extraction methods were applied to obtain acid-soluble collagen (ASC) and pepsin-soluble collagen (PSC) from Nile tilapia skin. In this study, collagen was characterized in terms of FT-IR Spectrum, amino acid analysis, SDS-PAGE and Thermal denaturation temperatures (Td). Also, some major functional properties including solubility, oil and water absorption, emulsifying activity, stability, foaming ability, foam stability, and gelation properties were determined. The results showed that the yields of ASC and PSC were 4.30% and 1.84 % (on a wet weight basis), respectively. Amino acid composition indicated that the imino acid content of ASC and PSC were found to be 172.10 and 164.18 residues per 1000 residues, respectively. The FTIR Spectrum confirmed that the presence of a triple-helical structure characteristic of ASC and PSC. SDS-PAGE results indicated that ASC and PSC were type I collagen. The denaturation temperature of ASC and PSC were 26° C and 25° C respectively. The solubility of ASC and PSC were highly solubilized in the pH range of 2–3. ASC and PSC had good water and oil absorption capacity. Gelation studies revealed that ASC and PSC have good gelation properties. Consequently, Nile tilapia skin could be used as a good source of collagen in the food industries.

## 1. Introduction

Collagen is a main protein in all animal tissues, which comprises about 30% of total protein. It is found in bones, tendons, ligaments, eye lenses, skin, and corneas. More than 29 different of collagen from various animals have been identified. In addition, each type of collagen has a specific molecular structure and amino acid sequence [1]. The production of collagen in the body decreased through a bad diet and age. While collagen injections are not a preference for most people, so the subsequent best alternative to obtain collagen is through diet. Consequently, pure collagen has been mixed in many food products [2].

Using Acid extraction and pepsin to extract the collagen was a common method [3]. Collagen is mainly obtained from mammalian skin, such as pig and cow skin. However, because of mouth -and foot disease, mad cow disease, in addition, religious barriers. Therefore, the Collagen of fish waste has received increasing attention [4]. Fish processing manufacturing produces every year a huge amount of fish waste that represents about 25% of the total fish production. Generally, the waste mainly consists of scales, bones, skin and fins, which represent around 70% of fish Coppola et al., 2020 [5].

Tilapia is of great importance in the aquaculture sector. It is one of the commercially produced species. Tilapia skin is a waste that contains approximately 27.8% collagen so it can be

used for the extraction of collagen Zeng et al., 2009 [6]. According to [7] In Egypt, production of tilapia comprised 61.44% of the total fish production. Hence the use of fish skin as new source of collagen turns low economic value raw material into a food industries [8].

Collagen has been generally applied in cosmetics; medicine and food due to its good functional properties. These include foaming ability, solubility, emulsification and oil absorption capacity [2]. Also, the high imino acid content affects the functional properties which are thermal stability and solubility of collagen [9]. Consequently, the high imino acid of collagen has broader applications in food manufacturing.

As far as we know, little information is found in the literature regarding the functional properties of collagen [10]. Consequently, it is required to evaluate the functional properties of collagen extracted from fish skin, for applying the collagens in bread, cakes, drinks, ice cream and other food application. Consequently, this study aimed to extract ASC and PSC from Nile tilapia skin. Also, the characterization and function properties of collagen were investigated to use it in food industries; the process can also find high value-added processing of waste using this new approach.

## 2. Materials and Methods

## 2.1 Fish skin preparation

Life Nile tilapia purchased from fishery market in El-Minia governorate then transported in the icebox to the Food Science Laboratory Faculty of Agriculture Minia University. The average weight and length of the fish were  $478.6 \pm 33.84$  g and  $26.75 \pm 2.47$  cm, respectively. Fish samples were washed with tap water. Tilapia skins were obtained manually and cut into small pieces ( $0.5 \times 0.5$  cm) then washed with cold distilled water, packed in a polyethylene bag and frozen at  $-20^\circ\text{C}$  until use.

## 2.2 Extraction of acid-soluble collagen (ASC)

The collagen has been prepared using the methods of [11] and [12]. All preparations were carried out at the temperature not higher than  $7^\circ\text{C}$ . The skins were stirred in 0.1 M NaOH at a ratio of 1:8 (w/v) for 24 hours to elimination non-collagen compounds then the solution was changed every eight hrs. The skins were washed with distilled water until a neutral pH of 7 was achieved. The skin was defatted with 10 volumes of 10% butyl alcohol for 48 hrs. The alcohol solution was changed every eight hours. The defatted skins were washed with cold water and collagen was extracted by acetic acid solution, as 'acid-soluble collagen (ASC)'.

## 2.3. Extraction of Pepsin- Soluble Collagen (PSC)

Undissolved matter from acid-soluble collagen was further treated with 0.5 M acetic acid containing 1% pepsin (w/w) at a ratio of 1:10 (w/v) for 2 days at  $4^\circ\text{C}$  according to the method of [13]. The obtained Collagen was called pepsin soluble collagen (PSC). Both ASC and PSC were used for analysis.

$$\text{Yield of collagen} = \frac{\text{Weight of collagen (g)}}{\text{Weight of skin (g)}} \times 100\%$$

## 2.4 Characterization of the extracted collagen

### 2.4.1 Amino Acid Analysis

Collagen samples were first hydrolyzed in 6 N HCl at  $110^\circ\text{C}$  for 24 hr. and the hydrolysates were analyzed by HPLC an amino acid analyzer [14].

### 2.4.2 Fourier Transform Infrared Spectroscopy (FTIR)

All spectra were obtained using an FTIR (Omicron spectra) spectroscopy [15].

### 2.4.3 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the methods of [16].

### 2.4.4. Evaluation of collagen denaturation temperature

The denaturation of collagen in solution was performed according to a method described by [17], [18]. A Brookfield viscometer beaker was filled with 0.1% (m/v) collagen solution in 0.1 M acetic acid. Collagen solution

viscosities were measured at a temperature from  $10^\circ\text{C}$  up to  $50^\circ\text{C}$ . Fractional viscosities were computed for each temperature. The fraction change was calculated from the viscosity measurement obtained with the below equation. Where C is the collagen concentration (mg/mL),  $\epsilon_1$  is the viscosity at  $10^\circ\text{C}$ ,  $\epsilon_2$  is the viscosity at measured temperature

Fraction change =  $[(\epsilon_2/C) - (\epsilon_3/C)] / [(\epsilon_1/C) - (\epsilon_1/C) - (\epsilon_3/C)]$   
The denaturation temperature was the temperature at which the fractional viscosity value was 0.5.

## 2.5 Determination of functional properties

### 2.5.1 Effect of pH range (1-10) on solubility:

Evaluation the effect of pH range (1-10) on the solubility of collagen according to the method of [3], [19]

### 2.5.2 Effect of NaCl on solubility:

The effect of NaCl on the solubility of collagen was determined according to the method described by [3] at different concentrations (0, 2, 4, 6 and 8%) of NaCl and the protein content in the supernatants was evaluated by the method of [19].

### 2.5.3. Water Absorption Capacity of Collagen (WAC)

WAC was evaluated using the method of [20]. A certain quality (M) of collagens and a certain volume ( $V_0$ ) of distilled water mixed in the vortex mixer 30 s, standing 30 min at  $25^\circ\text{C}$ , then 5000 r/min centrifuge 30 min, the supernatant volume was recorded ( $V_t$ ) Water absorption was calculated as:

$$\text{Water absorption capacity (ml/g)} = \frac{V_0 - V_t}{M}$$

### 2.5.4. Oil Absorption Capacity of Collagen (OAC)

OAC was measured using the method of [21]. A certain quality (M) of collagens and a certain volume ( $V_0$ ) of oil mixed in the vortex mixer 30 s, standing 30 min at  $25^\circ\text{C}$  then centrifuge 5000 r/min for 30 min. The supernatant was measured ( $V_t$ ) Oil absorption was calculated as:

$$\text{Oil absorption capacity (ml/g)} = \frac{V_0 - V_t}{M}$$

### 2.5.5. Determination of Emulsifying Properties:

Emulsifying activity (EA) was determined according to the method of [21], [10] and the volume of the emulsified mixture was recorded.

$$\text{EA (\%)} = v_1 / v_0 \times 100\%$$

Where  $v_0$  is the total volume of the mixture and  $v_1$  is the volume of emulsified mixture;

Emulsion stability (ES) was calculated according to [22].

$$\text{ES (\%)} = \frac{\text{High of emulsified layer after heating}}{\text{Height of emulsified layer before heating}} \times 100$$

### 2.5.6. Evaluation of foaming characteristics:

Foam ability (FA) and foam stability (FS) of collagen solutions were determined, as described by [23].

### 2.5.7 Gelation properties:

Gelation properties were investigated using the method described by [24], with a slight modification at various concentrations 1–4% of collagens. The least gelation concentration was measured as the concentration when the sample from the inverted test tube did not slip or fall.

### 2.6. Statistical Analysis:

Data were analyzed with the General Linear Model program using SAS (25). Mean values were compared by Duncan's.

## 3. Results and discussion

### 3.1 Yield of extracted Nile tilapia skin collagen

The yields of ASC and PSC from Nile tilapia skin were 4.30 and 1.84% (based on the wet weight), respectively (Table 1). The yield of ASC is higher than PSC. This may be due to that collagen was incomplete solubilization in 0.5 M acetic acid extraction through the acid-soluble method. So, when the residue was extracted by using the pepsin method, PSC was wholly solubilized. This result was in agreement with the yields of PSC and ASC of silver catfish skin (2.27 % and 4.27%) reported by (26). On the other hand, the difference in collagen yield maybe because of the differences in preparation methods and different fish species. Yao *et al.*, 2012 (27). In the present study, the enzyme extraction used the residual from acid extraction which was low content of collagen as compared to the raw materials. [28] reported that the yield of ASC was higher than the PSC of squid fish skin. On the other hand, [3] reported that the pepsin digestion cleaved at the telopeptide region without totally breaking the structure of collagen's triple helix, which contributed to the lower yield of PSC.

**Table 1** Yield of extracted collagen of ASC and PSC of skin Nile tilapia.

Samples	Yield (%)
Acid soluble collagen (ASC)	4.30
Pepsin soluble collagen(PSC)	1.84

### 3.2 Amino acid compositions:

The ASC and PSC showed similar amino acid profiles with slight variations in (Table 2). It was maybe due to the elimination of some portion of telopeptides hydrolyzed by the pepsin [29]. Collagen has the highest content of glycine [30]. It was 218.3 and 226 residues/1,000

residues in ASC and PSC, respectively. This result is lower than the Nile Tilapia (*Oreochromis niloticus*) skin [30], the skin of Nile tilapia [31] and the Red Snapper skin [32]. Similarly, the glycine contents of channel catfish skin for ASC and PSC were 239 and 233 residues/1,000 residues [33], furthermore, there are high contents of proline, alanine, glutamic acid, and hydroxyproline. Generally, there was no tryptophan in collagen [34] while tyrosine, histidine, cysteine and Isoleucine were very low, this is in agreement with [30] and [32].

The imino acid content (hydroxyproline and proline) of ASC and PSC was found to be 172.1 and 164.1 residues per 1000 residues, respectively. They are similar to surf smelt skin (172 residues/1000 residues) according to [35] and higher than the codfish skin (154 residues per 1000 residues) [36], and lower than the ASC and PSC from the pufferfish (329 and 334 residues/1000 residues) [34]. Furthermore, mammalian collagen, such as pig (220 residues per 1000 residues) was higher imino acid content than fish collagen Wu *et al.*, 2016 [37]. [38] Showed that the thermal stability of collagen increases through imino acid content. Additionally, imino acid content is the most important used for determining the thermal stability of collagen triple helix.

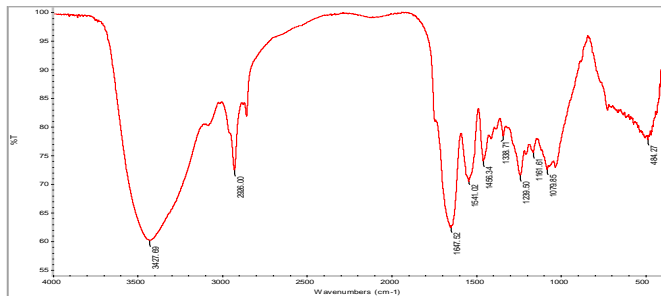
**Table 2** Amino acid composition of ASC and PSC from the skin of Nile tilapia skin (residues/1000 residues)

Amino acid	Acid soluble collagen (ASC)	Pepsin soluble collagen(PSC)
Glutamic acid	61.2	58
Aspartic acid	41.5	44
Serine	48	38.71
Glycine	218.3	226
Histidine	5.10	4.2
Arginine	63.5	62
Threonine	41	37
Alanine	140.1	180
Proline	93.4	89.38
Tyrosine	21.7	23
Valine	33.37	29
Methionine	21.2	18.3
Cysteine	0.73	0.8
Isoleucine	16.1	18
Hydroxyproline	78.7	74.8
Leucine	21.3	17
Phenylalanine	48.3	45
Lysine	46	34.81
Imino acids	172.10	164.18

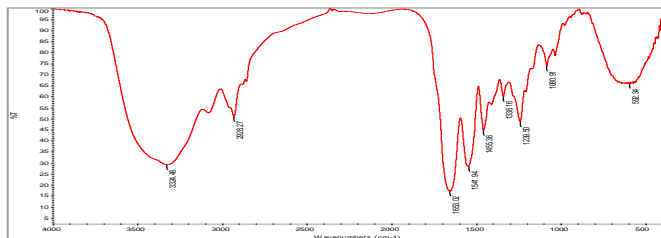
### 3.3 Fourier Transform Infrared Spectrophotometer (FTIR):

Both ASC and PSC presented characteristic peaks of amide A and B in addition to amide I, II, III (Figures 1 and 2). Amide A band positions of ASC and PSC were obtained at wave-number 3427.69 and 3324.48 cm<sup>-1</sup> respectively which similar to amide A in red snapper fish collagen, it was obtained at wave number 3417.86 cm<sup>-1</sup> [32]. This band is related to N–H stretching vibrations included in the hydrogen bonds of the peptide chains and

usually occurs in the range of 3400–3440  $\text{cm}^{-1}$  and when hydrogen bonds were introduced there are become lower to around 3300  $\text{cm}^{-1}$  [37]. On the other hand, the Amide B band positions of ASC and PSC were detected at 2926.00 and 2928.27  $\text{cm}^{-1}$ , respectively. The amide B band is related with an asymmetrical stretch of  $\text{CH}_2$  [13]. Amide B band of samples similar to Nile tilapia [31], silver carp [29], and channel catfish [39]. The main peaks in the spectra of ASC and PSC from Nile tilapia skin were similar to puffer fish collagen [34]. The amide I band of ASC and PSC was detected at 1647.52 and 1653.02  $\text{cm}^{-1}$ , respectively. The amide I band is generally associated with the carbonyl group ( $\text{C}=\text{O}$ ) stretching vibration, it normally occurred in the range 1600–1700  $\text{cm}^{-1}$  and is an indication of the secondary structure of the peptide [32]. Amide II of ASC and PSC was detected at 1541.02 and 1541.94  $\text{cm}^{-1}$ , respectively. Amide II band was related to CN stretching and NH bending vibration [37].



**Figure 1:** Fourier transform infrared spectra (FTIR) of acid-soluble collagen (ASC)



**Figure 2:** Fourier transform infrared spectra (FTIR) of pepsin-soluble collagen (PSC)

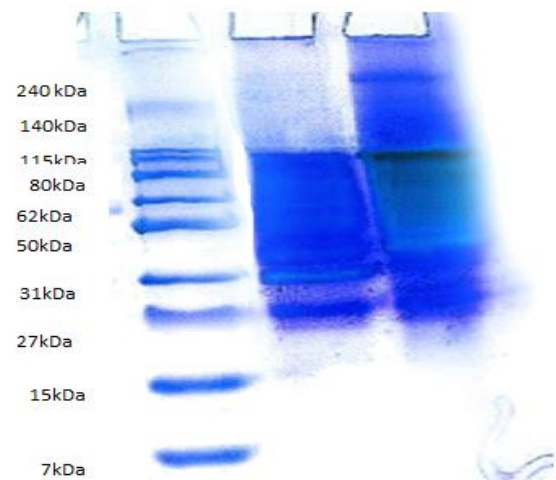
Also, the amide III band (1220–1320  $\text{cm}^{-1}$ ) was related to the mixture of the stretching vibration between  $\text{C}-\text{N}$  and the  $\text{N}-\text{H}$  bending vibration. The amide III band of ASC and PSC was found at wave-number 1239.50  $\text{cm}^{-1}$  [34]. The amide I, amide II, amide III bands wavenumbers are exactly associated with the collagen formation, Therefore, from these results, it was confirmed that the presence of a triple-helical structure-property of Nile tilapia in both the ASC and PSC.

### 3.4 SDS – PAGE analysis

ASC and PSC contain two  $\alpha$  chains ( $\alpha_1$  and  $\alpha_2$ ) showed in (Figure 3). The molecular weight of  $\alpha_1$  and  $\alpha_2$  of PSC was 146kDa and 86kDa, respectively. On the other hand, the molecular weights of  $\alpha_1$  and  $\alpha_2$  chains of ASC were 135 and 102kDa, respectively. The patterns were similar to the molecular masses of  $\alpha_1$  and  $\alpha_2$  therefrom

loach fish skin were 127 kDa and 115 kDa, respectively [13]. High molecular weight components, containing  $\gamma$  and  $\beta$  chains, were also higher in ASC than PSC (Fig 3). Because of the cross-link containing telopeptides was broken by pepsin, and then the  $\beta$ -chain is turned to two  $\alpha$ -chains. This pattern was proven by earlier research conducted by [40].

[41] reported that collagens consist of intra molecular and inter cross-linked components of  $\gamma$  and  $\beta$ . Also [42] showed that more amounts of molecular cross-linked components and  $\beta$  chains were detected in Tilapia waste collagen. These results indicated that ASC and PSC may be described as type I collagen. Chen *et al.*, 2019[43] reported that the ASC contained more dimers and trimers compared to PSC This result is in agreement with skin collagens from pufferfish by [34] and loach fish [13].



**Figure 3:** SDS-PAGE patterns of ASC and PSC from the skins of Nile tilapia fish. Lane 1 protein markers; lane 2, PSC; lane 3, ASC

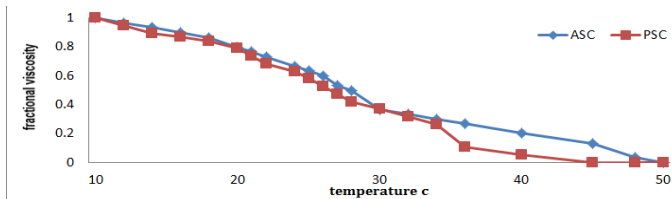
### 3.5 Denaturation temperature (Td)

Td of ASC Nile tilapia was 26 $^{\circ}$  C and PSC was 25 $^{\circ}$  C in (Figure 4), the difference Td of ASC and PSC due to the hydrolysis action of the enzyme. These values are similar to collagens of red stingray skin which recorded at Td= 23.82  $^{\circ}$ C ASC and 24.46  $^{\circ}$ C PSC Chen *et al.*, 2019[43]. The Td of miyu croaker swim bladders for ASC= 24.7  $^{\circ}$ C and PSC =26.7  $^{\circ}$ C [44]. The Td of tiger puffer skin for ASC =28  $^{\circ}$ C and PSC =25.5  $^{\circ}$ C [45] respectively. But lower than the Td of Nile tilapia skin (35.2  $^{\circ}$ C, ASC) [31].The Td of river puffer skin for ASC = 29.5  $^{\circ}$ C, PSC= 27.5  $^{\circ}$ C [45].Also, according to Li *et al.* 2013 [46], the collagen of land animal had a higher denaturation temperature than that fish collagen due to its higher amino acid content.

Chen *et al.*, 2019 [43] showed that the hydrogen bond in the collagen molecule was destroyed when the temperature rises therefore the triple helix structure dissociates then converts into random coil. Thus, the viscosity of collagen decreases. On the other hand, Nile tilapia collagens had a higher denaturation temperature



than cold water fish collagen such as Spanish mackerel (Td of ASC = 15.12 °C and Td of PSC=14.66 °C) li et al., 2013 [46].



**Figure 4:** Thermal denaturation curve of ASC and PSC from Nile tilapia skin, as shown by changes in fractional viscosity

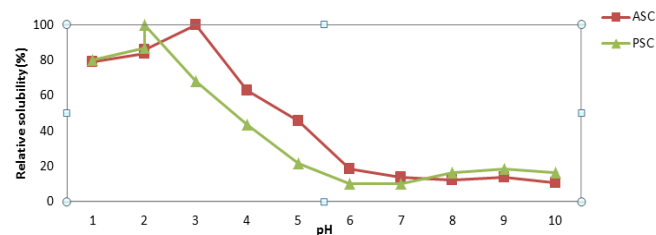
### 3.6 Determination of functional properties

#### 3.6.1 Effect of pH and NaCl concentration on collagen solubility

##### 3.6.1.1 Effect of pH on collagen solubility

The highest solubility of ASC and PSC was at pH 3 and pH 2.5 respectively (Figure 5). Both the collagens indicated the highest solubility in the acidic region pH (1–3). At above pH 3, the solubility started to decrease. A similar result was shown for Pufferfish skin collagen [47] and ASC from silver catfish [12]. Also, [34] revealed that the solubility of ASC and PSC of pufferfish skin achieved the maximum at pH 3 and then decreased above this pH.

[15] reported that most of the solubility of collagen increased in acidic conditions. Furthermore, the least solubility was detected at pH 6. This likely due to the isoelectric point (pI) of the proteins, as after getting to pI, the total net charge of proteins becomes zero leading to precipitation [3]. Also, PSC presented better solubility than ASC; this was due to a slight variation in the structure, composition and properties of trimer [48].



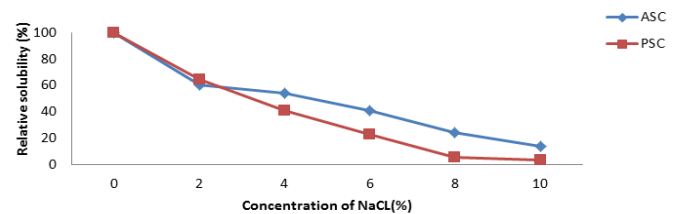
**Figure 5:** Effect of pH on solubility of acid-soluble collagen (ASC) and pepsin-soluble collagen (PSC)

##### 3.6.1.2 Effect of salt concentration on collagen solubility

Both ASC and PSC presented similar solubility at various NaCl concentrations (0-10%) in (Figure 6). The sharp decrease when the solubility was above 2% NaCl concentration for both ASC and PSC with NaCl concentration was increasing because the hydrophobic interactions increased. Also, the competition for the ionic

salts with water increased leading to protein precipitation [47]. These are in agreement with what was found by [5] who reported that the solubility of collagens from the Brown stripe red snapper fish skin was decreased at 3% NaCl or above.

Also, the ASC and PSC showed the best solubility at low NaCl concentration (0, 1 and 2%), because the salt ions bind weakly to charged groups on the protein surfaces [49]. This result was similar to the solubility of the tilapia skin Zeng et al., 2009[6], and Pufferfish skin [47].



**Figure 6:** Relative solubility of acid soluble collagen (ASC) and pepsin soluble collagen (PSC) at various NaCl concentrations.

#### 3.6.2 Water Absorption Capacity (WAC) and Oil Absorption Capacity (OAC) of Collagen

WAC and OAC of collagens from Nile tilapia skin were shown in Table 3. ASC was significantly higher in WAC than PSC. The WAC of ASC was 23.924ml/g, but the PSC was 22.72 ml/g ( $P < 0.05$ ), which may be due to its higher ratio of hydrophilic areas [50]. This result is similar to PSC and ASC red stingray skin. there were 28.48 and 20.76 mL/g, respectively Chen et al., 2019[43]. The OAC of ASC was higher than PSC, 26.72 ml/g and 24.51 ml/g respectively. The OAC of a protein is associated with non-polar amino acids. Accordingly, the OAC values denoted that ASC has non-polar amino acids higher than PSC. These results are similar to the collagen of red stingray skin Chen et al., 2019[43].

**Table 3 :** Water and oil absorption capacities of skin Nile tilapia collagen

Sample	WAC(ml/g)	OAC(ml/g)
Acid-soluble collagen (ASC)	23.92 <sup>a</sup> ± 0.39	26.72 ± 0.09 <sup>a</sup>
Pepsin soluble collagen (PSC)	22.72 <sup>b</sup> ± 0.19	24.51 ± 0.56 <sup>b</sup>

\*\*Mean values in the same column not followed by the same letter are significantly different ( $P \leq 0.05$ )  $n=3 \pm$  SD.

Generally speaking, the hydrophobic interactions between the non-polar amino acids of protein molecules as well as the hydrocarbon chains of oil determine the OAC of the protein [51]. Additionally, [52] reported that the protein with higher OAC gave better shape retention in foods, affect the food flavor and also are important in the functional characteristics of ingredients used in the processing of confectionery and meat industries. These results indicated that ASC and PSC showed higher OAC than WAC. So the hydrophobic groups' content of

collagen was higher than the hydrophobic groups' content. Therefore, the collagens of Nile tilapia skin could be applied in the meat products.

### 3.6.3 Emulsifying Properties

Emulsifying properties play an essential role in food products, subsequently; they contribute directly to the texture and sensory properties of food [53]. Emulsification is an important property of protein; It includes the emulsify activity and emulsify stability. Emulsifying ability is the function that protein elevates oil and water mixture and then forms the emulsion [20]. The significant difference was found between emulsify activity of ASC and PSC (Table 4) and they were 51.50% and 54.25%, respectively. Similar to pigskin collagen polypeptides 53.4 % [10] and higher than pacific whiting skin 45.9 % [54] and it is lower than ASC and PSC from fish skin *Amiurus nebulosus*. They were 64.44% and 76.19% respectively [20], whereas Yak bone polypeptides were 57.3% [10].

Emulsify stability is the capacity that protein keeps oil-water and not separation as well as the resistance strain of external conditions [20]. ASC and PSC had similar emulsify ability and emulsify stability (Table 4). Also, emulsify stability can be used as emulsifier in food products. As the importance of emulsifying properties and foaming in the food industry, this result suggests that the collagens of Nile tilapia skin may be used for baking, beverages, and meat products.

**Table 4:** Emulsifying activity EA (%) and stability ES (%) of ASC and PSC

sample	Emulsifying activity EA (%)	Emulsifying stability ES (%)
Acid-soluble collagen (ASC)	51.50 <sup>a</sup> ±0.70	57.92 <sup>a</sup> ±0.11
Pepsin soluble collagen (PSC)	54.25 <sup>b</sup> ±0.74	57.615 <sup>a</sup> ±0.54

\*±s Mean values in same column not followed by the same letter are significantly different (P≤0.05) n=3 ±=SD

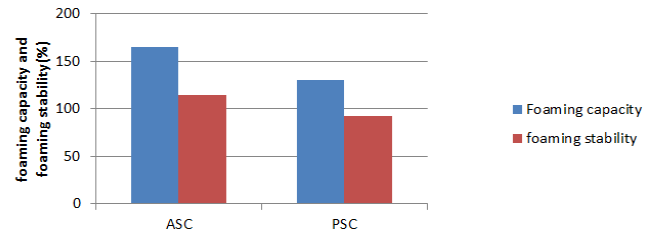
### 3.6.4 Foaming properties:

ASC exhibited a higher FA and much stronger FS than that PSC (Figure 7). This result is higher than FA of ASC (14%) and PSC (4%) extracted from *Amiurus nebulosus* Skin [20], and it's similar to the ASC and PSC of red stingray skin collagen 146.67 and 151.67 % respectively Chen *et al.*, 2019[43] and collagen polypeptides of Yak bone and pigskin 163% and 160% respectively[10].

Generally speaking, when the protein included more large size peptides, it maybe improves the configuration of a stable film around the gas bubbles [52]. Also, the interaction between water and protein important for foaming was stronger and the hydrophobic areas on the peptide chain are responsible for giving protein its emulsifying and foaming properties [55].

As a result of this foaming property, Nile tilapia skin collagen is applied in food processing as a foaming agent.

On the other hand, foaming properties is an important attribute in food products such as desserts, baked products. [53].



**Figure 7:** Foaming capacity and foaming stability of acid-soluble collagen (ASC) and pepsin-soluble collagen (PSC) from Nile tilapia skin.

### 3.6.5 Gelation properties:

The effective concentration of collagen on gelation capacity is showed in (Table 5) by taking the least gelation concentration (LGC) as the index of gelation capacity. Lower LGC of Both ASC and PSC collagens were 4%. usually, lower LGC means better gelation capacity. Hence increasing the protein concentration required to form a stable gel. On the other hand, [56] reported that the balance between hydrophobic interaction and repulsive electrostatic interaction control gel appearance and the gelation mechanism. Generally, Gelation occurs when native globular collagen proteins are denatured in the presence of heat [57]. Therefore, Protein gelation is imperative for the acceptability and preparation of food products.

**Table 5:** Gelation properties of ASC and PSC from the skin of Nile tilapia

Concentration (%)	ASC	PSC
1	-	-
2	±	±
3	±	±
4	+	+

-, Not gelled; ±, gelled slightly; +, gelled

## 4. Conclusion:

Collagen from Nile tilapia skin was successfully extracted by ASC and PSC methods. The yield of ASC was higher than PSC. The results obtained show that ASC and PSC have a typical type I collagen characteristics. Both ASC and PSC have a triple-helical structure characteristic. The denaturation temperature (Td) of PSC was slightly lower than that of ASC. ASC and PSC have good functional properties because there were high oil absorption capacity, 26.72 mL/g and 24.51 mL/g, respectively. Both ASC and PSC had excellent foam ability (165.5 and 130.5%, respectively) and foam stability (114.5 and 92.5%, respectively). ASC was better in water and oil absorption capacity and foaming ability than PSC, The Maximum solubility was in 0.5 M acetic acid for ASC and PSC at pH 3 and 2.5 respectively. ASC and PSC have good gelation properties, taking the least

gelation concentration at 4% for ASC and PSC. It could be concluded that the characteristics and functional properties of the isolated ASC and PSC indicate that Nile tilapia skin had a good yield of collagen and it could be served as a new source of collagen for food products.

## 5. Acknowledgment

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