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Research Article

Enzymatic and Thermal Extraction of Gelatin from Fish Scales and study its Sensory Properties

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ABSTRACT
Fish processing produce large quantities of wastes consisting of bones, scales and skin. This waste is rich source of gelatin. Gelatin is a protein of animal origin that is easy to digest. It contains all the essential amino acids except tryptophan. The current study was designed to produce gelatin from the scales of some river and marine fish (Carp, Grass Carp and canthopagrus). The results showed that the scales contain good percentage of protein and ash. Gelatin extracted thermally and enzymatically from the scales of fish, it was found that the moisture, protein, fat and ash were close to the commercial gelatin. It was also noted that the yield of gelatin extracted thermally was 7.16 to 8.21% higher compared to enzymatically. Also, the pH of gelatin scales was close to the commercial gelatin and the specification of Gelatine Manufacturers of Europe (GME) . The results observed that the gelatin of scales was free of the mineral elements Ni, Pb, Cd, Fe, Co, Se and Zn comparative to fish scales. However, the Cu ranged 0.003-0.007 ppm in scales of gelatin and it was identified that the mineral elements in scales of gelatin are less than the specification prepared by GME. The scales of gelatin showed highest absorption at 220 nm while commercial gelatin showed highest absorption at 225 nm. Carp gelatin extracted thermatically and enzymatically showed clear superiority in foam susceptibility, stability, solubility and viscosity compared to the other types of gelatin and commercial gelatin. The Fourier transform Infrared spectroscopy (FTIR) examination showed that the thermally extracted Carp gelatin contained Amide A and I groups, whereas, the enzymatically extracted gelatin contained Amide A, I, II, III groups comparative to the commercial gelatin that contained the groups Amide A, I, III. Gelatin of scales showed that they contain good ratios of Glycine amino acid, lack Methionine amino acids and good percentage of essential amino acids.

Keywords: Fish scales, fish gelatin, gelatin properties, FTIR of gelatin

INTRODUCTION

Gelatin is a protein of animal origin that is easy to digest. It contains all the essential amino acids except tryptophan. This protein is formed by the thermal denaturation of collagen or partial hydrolysis of collagen. It contains polypeptides of high molecular weight and is important in forming colloids. This protein has wide popularity due to its crystallization properties in the food industry. Its properties to form gel, composition, transparency, and odorless and tastelessness are one of the main characteristics of gelatin. But the strength of gel and viscosity and thermal and soluble reverse is one of the most important criteria as it affects the quality of gelatin and its applications It is also one of the most important features that the food, pharmaceutical and photographic industries demand.

Gelatin is traditionally produced from mammals, as the hides of cows and pigs constitute 46% of the total global production, while the bones and hooves constitute 23% and 29%, respectively .The global production of gelatin from fish constitutes 1.5% which comes from the internal organs, bones, fins and crusts. As the latter is a possible alternative source of gelatin coming from cows and pigs due to the religious beliefs of both Muslims and Hindus as well as the risk of transmitting Mad cow disease, technically known as Bovine Spongiform Encephalopathy (BSE) and Foot and Mouth disease (FMD). Because of religious beliefs and health concerns, researchers have tended to study gelatin obtained from the skin, bones and scales of fish, and they have become a topic of concern to them. Studies indicated that the annual total catch of fish

reached 100 million tons and that 50% of it is disposed of as waste because of its low commercial value and functional features and technological characteristics. These wastes are either dumped to the sea which may result in an environmental and marine problem or traditionally used to produce fertilizers and Cattle feed because of high content of nutritional value compounds (Manikkam *et al*., 2016).

Fish processing produce large quantities of wastes annually and 30% of these wastes are bones, scales and skin although this waste is a great source of gelatin, but the most preferred sources are crusts and fish bones due to the high yield and contain a high proportion of the amino acid proline as compared to the skin of fish. Therefore, the current study aimed to extract gelatin from the shells of some marine and river fish thermally and enzymatically, and to study its qualities and content of amino acids as well as the possibility of benefiting from it in the food or biological industries.

MATERIAL AND METHODS

Fish Scales

Fish samples Carp (C) and Grass Carp (G.C.) were collected from one of the fish farms in Basra Governorate, while the Acanthopagrus (A.) were purchased from fishers in Al-Faw district in southern Iraq. The samples were placed in ice box and transferred to the laboratory. Scales were removed using the knife, then washed and prepared for the extraction process.

Gelatin enzymatic extraction

Gelatin was enzymatically extracted according to the method described by Tong and Ying (2013) with some modifications, the NaOH and HCL was used to remove non-collagen protein substances from fish scales. Then the scales were submerged in the distilled water (pH: 6) at a ratio of 1:10 w/v. The bromelain enzyme (Sigma) was added at a concentration of 0.01%. Incubated in the water bath at 45°C for 2 hours. Then put the mixture in a boiling water bath for 5 minutes to dampen the enzyme action, filtered the solution through the cheese cloth, then through the What man filter paper.

Gelatin thermal extraction

The experiment was conducted according to Zakaria and Abu-Baker (2015) take 14.3 g of scales, then washed with tap water for 1 hour, add 100 ml of 0.4 w/v NaOH for 4 hours to remove non-collagen protein substances, the scales was washed with tap water until neutralization, after that add 100 ml of HCL 0.4 v/v and submerged for 4 hours, then washed with tap water until a neutralize is reached. The scales were immersed in distilled water at a ratio of (1:1) g / mL and heated at a temperature of 70°C for 1.5 hours, Separated the solution from the scales by cheese cloth, then concentrate the solution using a rotary evaporator for 30 minutes, and dried at 50°C for 18 hours an hour. Gelatin was stored by freezing until the procedure of tests.

Assay of chemical content of scales and gelatin

The chemical content of the scales and gelatin (moisture, protein, fat and ash) was estimated according to the method elaborated by Egan *et al*. (1988).

PH

Gelatin pH was estimated using the pH meter (Sartorius, Germany) following the method mentioned by Das *et al*. (2017).

Gelatin Yield

The gelatin yield for the scales was estimated using the method mentioned by Zakaria & Abu Bakar (2015):

Determination of mineral elements in scales and gelatin

The samples were digested using a mixture of concentrated sulfuric acid and per chloric acid (60%) at a ratio of 3: 1. After the completion of the digestion process, the concentration of trace elements was determined using the Atomic absorption apparatus (Phoenix-986, AA Spectrophotometer, UK), following the method given by Kalra (1998).

Determination of gelatin properties

UV Spectrophotometer

The light transmittance was measured using the Spectrophotometer (Apel, UK) (Li *etal*. 2018). The extract was prepared at a concentration of 0.1 mg / ml and absorbance was measured at a length of 200-400 nm.

Foaming properties

The foam properties (Foamability and Foamstability) were estimated according to the method described by Zakaria and Abu Bakar (2015). The Foamability and Foamstability were calculated through the following equations:: -

$$\%\ \boldsymbol{foamability} = \frac{\textbf{Volume of solution befor mixing} - \textbf{Volume of solution befor mixing}}{\textbf{volume of solution after mixing}} \times \mathbf{100}$$

As for the foamstability , it is calculated after 15 minutes have passed since the foam remains.

$$\%\ foamstability = \frac{\textbf{Volume of solution befor 15 min.} - \textbf{Volume of solution befor15 min.}}{\textbf{volume of solution befor 15 min.}} \times 100$$

Viscosity

Viscosity was estimated according to the method recommended by Sath and Salunkhe (1981) for gelatin extracted thermally and enzymatically from fish scales and Co. Gelatin, by preparing a 1% gelatin solution using Ostwald Type D tube, and special tables were adopted to extract the specific density and viscosity at different temperatures, and viscosity was calculated: -

$m_2 / m_1 = d_2t_2 / d_1t_1$

Whereas: t_1 = distilled water time range, t_2 = constant in distilled water time tables,

m_1 = viscosity of water from the table, m_2 = required viscosity, d_1 = specific density of tables, d_2 = specific density

Solubility

The method put forward by Hindi and Al-Doure (1987) was adopted to calculate the solubility of gelatin samples by calculating the ratio of protein in prepared gelatin samples (P1). The percentage of solubility was calculated according to the formula: -

% Solubility = (P1 / P2) x 100

FTIR estimate for gelatin

The infrared spectra of compounds were recorded in the confined area between (400-4000) cm^{-1} using a Japanese-made Jasco-4200 device using the potassium bromide tablet method. The examination was conducted at the Polymer Research Center - University of Basra - Iraq, according to the method recommended by Muyonga *et al*. (2004).

Gelatin content of amino acids

The gelatin content of fish and scales was extracted enzymatically and thermally from amino acids at the Ministry of Science and Technology - Baghdad - Iraq, by Rattenbury, (1981). Amino acid analyzer SW, Yang Co., Korean amino acid was used.

Statistical analysis

The treatment were randomly distributed following the complete randomized design (CRD) and the averages were compared according to the LSD at 5% significance level.

RESULTS AND DISCUSSION

Chemical content and yield

The chemical content of C. and G.C. A. from moisture, protein, fat and ash is presented in Tab.1. The moisture results showed superiority of G.C. compared to other fish. It reached 23.71%, followed by C. then A. while C. fish showed a clear superiority in protein percentage, reaching 55.1%, followed by G.C. and A. i.e. 53.21 and 53%, respectively. It was also noted through the table a decrease in the fat content of fish scales A. and G.C. and C., with a clear increase in the ash percentage, as it reached 25.27, 22.65 and 22.04% respectively. The results indicated that the moisture and protein content, the percentage of fat and ash in river scales of fish was low as compared to marine fish. The reason for the variation in the chemical composition of fish scales may be due to the difference in varieties, environmental conditions and the nature of feeding. These results were consistent with Naqvi *et al*. (2014) when studying the chemical content of three different weights of fish *Ctenopharyngdon idella* wild and bred in farms, he observed an increase in the protein and fat content of farm fish scales with low moisture content, and higher moisture content of wild fish as compared to the farm fish. Masood *et al*. (2015) mentioned that the moisture content of some fish scales ranged between 42.85 - 58.3%, while the protein ranged from 62.28 - 78.07 %.

Tab. (1) also showed the percentage of the gelatin extract obtained thermally and enzymatically, as a clear superiority was observed in the thermal gelatin yield. It reached 8.21, 7.37 and 7.16% for G., G.C. and A. respectively, compared to the gelatin enzymes with a clearance rate of 7.61, 6.21 and 6.44%, respectively. These results were consistent with that of Tong & Ying (2013), noting a decrease in the gelatinous yield of Bighead Carp Fish by using pepsin at pH 4 compared to other methods. Jakhar *et al*. (2016) reported that the gelatin production of Catta, Rohu and Mrigal gelatin peels was 6.52, 7.21 and 6.38%, respectively. Arman *et al*. (2017) found that the gelatinous yield of Roha fish scales by Alkali Process and Iso-butanol Process methods was 6.8 and 12.79%, respectively.

The results presented in the table indicated that the moisture content of gelatin extracted thermally and enzymatically is close to that of co. gelatin, as it ranged from 6.225-10.331%, 5.209-8.831% and 8.65%, respectively. These results were consistent with what was indicated by a number of studies. Courts (1977) stated that the moisture content of gelatin ranges between 9-14 %. Gelatin manufacturers of Europe (GME) stated that the gelatin moisture content was no more than 15% (GME Monograph, 2005). Bordignon *et al*. (2012) reported the moisture content of gelatin at room temperature does not exceed 13%. As for Jakhar *et al*. (2016), note that the moisture content of Calta and Rohu fish was 8.47 and 9.20%, respectively.

The study showed an increase in the protein content of gelatin fish scales extracted thermally

and enzymatically compared to co. gelatin, as the protein percentage ranged 85.45 - 92.1%, 88.708 - 93.16 % and 90%, respectively. The results of the study indicated a superiority in the protein content of gelatin fish scales C. in comparison to the other species under study. The reason for the increase in the protein content in fish C. may be attributed to the high protein content in the scales. Zhang *et al*. (2014) mentioned that fish scales are rich in protein if it ranges between 41 - 84%, as it can contribute to the production of gelatin. Jakhar *et al*. (2016) found that the protein content in Cattle and Rohr fish gelatin is (69.25 and 65.85)%, respectively. Arman *et al*. (2017) noted that the protein content of Rohu and Snakehead gelatin scales was 82.04%.

The current study also showed that the fat content of fish scales used in the study was lower compared to co. Gelatin, as this result agreed with the findings of Jakhar *et al*. (2016). An increase in the ash content of gelatin fish scales was observed extracted thermally and enzymatically. It ranged (1.61 - 2.154) % and (1.5 - 2.271)%, respectively, compared to co. Gelatin which reached 0.84%. It is worth noting that the gelatin content of ash was close to the GME specifications, the standard indicated that the ash content was not more than 2% (GME Monograph, 2005). Jakhar *et al*. (2016) mentioned that the content of Catla and Rohu scales gelatin content was 1.03 and 1.18% respectively. Kumar *etal*. (2017) observed that the content of Croake Fish skin gelatin from ash was 1.98%. GME indicated that the gelatin content of ash not more than 2%, is acceptable in the food industry.

As for pH, gelatin fish scales under study had a pH ranging between 6.07 - 6.12, compared to co. gelatin which reached 5.84. These results were in agreement with the GME specification, as they indicated that the gelatin pH prepared with a concentration of 1%, its pH ranged between (3.8-. 6). Dincer *et al*. (2015) found that pH of gelatin scales Farmed Sea Bass was 5.52.

Tab.(1): Chemical content of fish scales and gelatin extracted in different ways

Chemical Composition of Scales Fish						
Fish type	**Moist.**	**protein**	**Lipid**	**Ash**	**pH.**	**Yield**
C.	22.61^{ab}	55.1^{a}	0.23^{a}	22.04^{b}	-	-
G.C.	23.71^{a}	53.21^{b}	0.33^{b}	22.65^{ab}	-	-
A.	21.53^{b}	53^{b}	0.17^{c}	25.27^{a}	-	-
chemical composition of Gelatin fish scales thermally extracted						
C.	6.225^{c}	92.1^{a}	0.15^{a}	1.61^{b}	6.1^{a}	8.21^{a}
G.C.	10.331^{a}	85.45^{b}	0.20^{a}	2.095^{ab}	6.07^{a}	7.37^{b}
A.	9.716^{b}	86.21^{ab}	0.13^{a}	2.154^{a}	6.08^{a}	7.16^{c}
Mean	8.757^{a}	87.92^{b}	0.16^{ab}	1.953^{ab}	6.08^{a}	7.58^{a}
chemical composition of Gelatin fish scales enzymatically extracted						
C.	5.209	93.16^{a}	0.13^{a}	1.5^{b}	6.12^{a}	7.61^{a}
G.C.	8.831	88.708^{b}	0.27^{b}	2.161^{ab}	6.07^{a}	6.21^{b}
A.	8.21	89.411^{ab}	0.098^{ab}	2.271^{a}	6.07^{a}	6.44^{c}
Mean	7.41^{b}	90.426^{a}	0.166^{a}	1.977^{a}	6.08^{a}	6.75^{b}
Co. Gelatin	8.65^{ab}	90^{ab}	0.5^{b}	0.84^{b}	5.84^{b}	-

Mineral elements

Tab.2 shows the scales and gelatin content of C. and G.C. And A. The extract by thermal method from some heavy elements was compared to the gelatin and GME Monograph (2017).The Cu content in scales reached 0.007 and 0.007 ppm. While Ni 0.007 and 0.013 ppm. Fe 0.223 and 0.118 ppm , and Co 0.008 and 0.010. While fish scales are free out of Pb, Cd and Zn. The results showed an increase in the Fe content of fish scales compared to other mineral elements, as it reached the highest concentration in the scales of C. fish. . The reason for the variation in the content of mineral elements from the scales may be due to the difference in the environment in which fish live, which may affect the salt balance necessary for marine and river fish life. As for the gelatin fish scales under study, it is noted that they contain Cu in concentrations 0.006, 0.003 and 0.007 ppm, whereas it

reached in co. gelatin 0.003 ppm, while the other elements were not noticed in the gelatin of fish scales and co. Gelatin. gelatin GME indicated that the gelatin content of Cu, Pb, Ca, Fe, and Zn is less or equal to 30, 5, 0.5, 30, 30 ppm, respectively (GME Monograph, 2017). The reason for the decrease in mineral elements in the gelatin of fish scales under study may be due to the fact that the chemical treatments at the base and acid during the early stages of gelatin extraction were able to get rid of these elements. The indices were lower than mentioned by GME Monogaph (2017) and shown in the above tab.2 . Alfaro *et al.* (2014) indicated that all types of gelatin have a similar chemical content in the moisture content and small amounts of mineral elements and protein. Wang & Regenstein (2009) mentioned that extracting gelatin from the Silver Carp scales in the presence of HCL, Citric Acid and EDTA has the ability to reduce 90% of the Ca, but it is accompanied by a loss of protein.

Tab. (2): Content of Scales and Gelatin from Mineral Elements (Ppm)

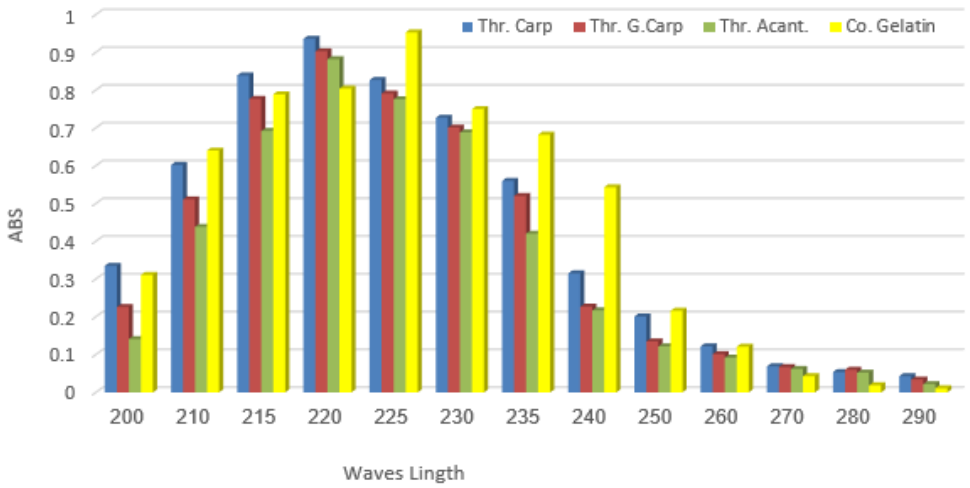
	Cu	Ni	Pb	Cd	Fe	Co	Se	Zn
Fish Scales								
C.	0.007^{a}	0.007^{b}	0	0	0.223^{a}	0.008^{a}	0.103^{ab}	**0**
G.C	0.005^{a}	0	0	0	0.111^{c}	0.008^{a}	0.106^{a}	**0**
A.	0.007^{a}	0.013^{a}	0	0	0.188^{b}	0.010^{a}	0.080^{b}	**0**
Mean	0.006^{a}	0.008	0	0	0.174	0.008	0.096	**0**
Fish Scales Gelatin								
C.	$^{a}0.006$	0	0	0	0	0	0	0
G.C.	0.003^{a}	0	0	0	0	0	0	**0**
A.	0.007^{a}	0	0	0	0	0	0	**0**
Mean	0.005^{a}	0	0	0	0	0	0	**0**
Co.Gelatin	0.003^{a}	0	0	0	0	0	0	**0**
GME Monograph(2005)	≤ 30	-	≤ 5	≤ 0.5	≤ 30	-	-	**≤ 30**

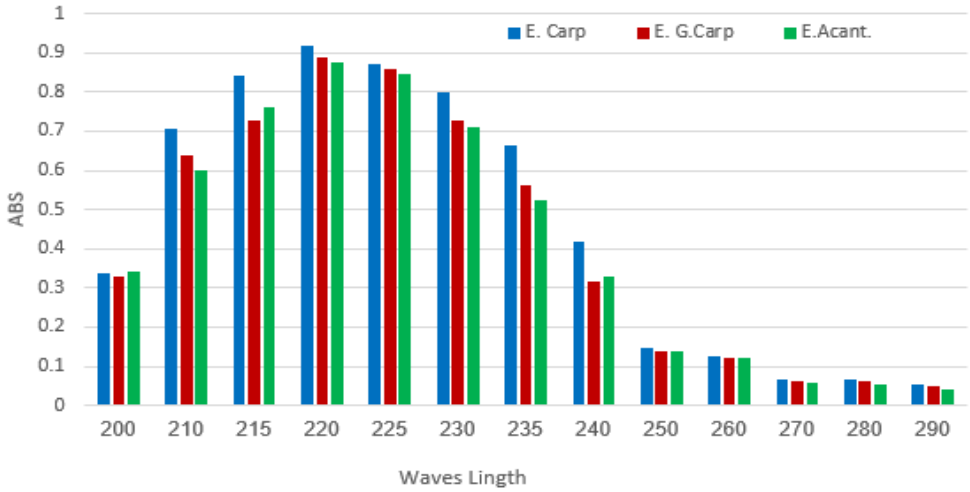
UV Spectrophotometer

The use of the UV radiation spectrum is one of the approved methods for determining gelatin protein, as the Chromophore group that gives high absorption of wavelengths between (210-240) nm is evidence of peptide bonds to the gelatin protein parallels and the absence of cyclic amino acids (Hermanto *et al*., 2013).

Fig. (1) and (2) show the light absorbance of gelatin scales of C. and G.C. and A. extracted by thermal and enzymatic method at a range of wavelengths (210-290) nm, as a gradual increase in absorbance values is observed with an increase in the wavelength of gelatin scales. The maximum absorbance of UV at the wavelength 220 nm reached (0.919, 0.887 and 0.874) and (0.936, 0.903 and 0.882) respectively, whereas Co. Gelatin showed its highest absorbance at wavelength 225 nm as it reached 0.935. The reason for the variation in absorbance values at wavelengths may be due to the different gelatin sources and the extraction method. These results were consistent with Hermanto *et al.* (2013) that the wavelengths between (210-240) nm are evidence of peptide bonds for gelatin protein fragment and the absence of cyclic amino acids. Moreover, the highest absorption of UV in the scales of some fish was at 224 nm. Li *etal.* (2017) found that gelatin skin of Alaska Pollock fish obtained by acidic methods and hot water and gelatin of pig skin reached the highest absorption at wavelength 215 nm, confirming the presence of gelatin protein peptides and its fragment, whereas absorption at wavelength of 280 nm is absorption cyclic amino acids tryptophan, tyrosine and Phenylalanine Kumar *et al.* (2017) also confirmed that the UV absorption spectrum that lies between 220-240 nm is evidence for the absence of cyclic amino acids in gelatin synthesis, while the UV absorption spectrum between 260-

280 nm is evidence of the presence of some cyclic amino acids such as Phenylalanine, Tyrosine and Tryptophan





Gelatin properties

Foamability and Stability

One of the most important characteristics of protein properties is the foamability and stability, as gelatin is one of the most foam-forming proteins. Tab. 3 shows the percentage of foamability and stability for fish scales gelatin of C. and G.C. and A., obtained from thermal and enzymatic treatment of scales and was compared with Co. Gelatin. A difference in the foamability according to the gelatin source and extraction method was observed, with note in an approximation of the percentage of foam between the gelatin of scales in the same species. As the gelatin extracted by the thermal method showed the highest foamability formation compared to the gelatin extracted enzymatically. A clear superiority of fish gelatin of C. was observed, as it reached 65.4 and 65% for both extraction methods respectively. As for Co. Gelatin, the foamability reached 70%. The reason for the difference in the values of the foamability is due to the sources of gelatin and the percentage of protein in fish scales under study.

As for the foamstability for scales gelatin, fish C. showed highest stability 34.3 and 32 % for thermal and enzymatically extract respectively. While, gelatin of scales fish G.C. reached 30.09 and 28 %, but the gelatin of scales fish A. was 30.64 and 25 %. Co. gelatin also showed significant superiority in foam stability as compared to the gelatin of fish scales used in the study. The cause of high foam stability in gelatin may be attributed to the high protein content, which leads to the formation of a coherent layer around the air bubble that helps in foam ability and increase its stability. These results indices are less than that of presented by Zakaria and Abu Bakar (2015). They found that the foam ability amounted to 60 and 25% for gelatin scales and bones of Black Tilapia fish respectively. And its foam stability for gelatin scales and bones of

Black Tilapia fish amounted 10 and 3.45% respectively. The foam ability and foam stability in gelatin of Black Tilapia fish scales is much better than its bones gelatin. Some studies also showed that the foamability and foam stability are affected by the protein concentration, as the foamability becomes more stable by increasing the protein concentration due to the increase in the thickness of the interlayer membranes (Ahmad & Benjakul, 2011; and Kumar *et al*., 2017).

Tab.(3) shows the percentage of solubility of gelatin obtained from the fish scales of C., G.C. and A., extracted by the thermal and enzymatic methods and the results were compared with the Co. Gelatin. The gelatin of the fish scales under study showed a significant superiority in the solubility of gelatin extracted thermally as compared to the enzymatically gelatin. C. fish gelatin was distinguished by its high solubility as compared to gelatin of other fish scales as it reached 98.33 and 97.47% of the gelatin extracted thermally and enzymatically, respectively, while co.Gelatin reached 99%. The reason for the high gelatin solubility of fish scales may be attributed to the fact that it contains hydrophilic amino acids that are highly soluble, and the solubility of gelatin may be affected by the degree of protein degradation that leads to increased hydrophilic groups. The cause of the difference in the solubility of gelatin can also be attributed to the difference in molecular weights or concentrations of polar and nonpolar groups of amino acids (Zayas, 1997). Firlianty (2016) studied four types of freshwater fish and he found that the solubility of gelatin of fish skin ranged between (99.21-99.40) % compared to Co. Gelatin which reached 99.60%. It was indicated that the solubility of gelatin is greatly affected by the ph. The solubility of cow's gelatin decreases at pH 5, for example, while the solubility of gelatin of fish skin decreases at pH 8.

Tab. (3) shows the viscosity of gelatin scales of fish extracted thermally and enzymatically and its comparison with co.gelatin. It was observed that there are significant differences at the 5% probability level between the gelatin of fish used in the study and the methods of extraction. As the co. gelatin exceeded the viscosity values compared to the gelatin extracted thermally and enzymatically, it reached 39.5 cP. It is followed by thermally extracted gelatin and enzyme with an average of 28.58 and 23.78 cP respectively. Also, gelatin of fish C. extracted thermally and enzymatically showed a significant increase in gelatin viscosity values compared to gelatin of G.C fish. And A. The reason for the difference in viscosity between gelatin of fish scales studied was due to the high percentage of protein that is accompanied by a rise in hydrophilic amino acids. These results indices were higher than that of Alfaro *et al*. (2014), keeping in view that the viscosity of gelatin in the skin and bones of African Catfish was 1.13 and 0.66 cP, respectively. Whereas, Koli *et al*. (2012) found that Tiger-Toothed Croaker skin gelatin and fish bones had viscosity 10.53 and 8.30 cP. Odonyo (2013) also found that the viscosity of Nile Tilapia and Pangas Catfish were 36.5 and 19.3 cp, respectively, while co. gelatin had 39.5 cP . Dancer *etal*. (2015) pointed out the viscosity of gelatin of fish scales for *Dicentrarchus labrax* reached 32.92 cP. Gomez-Guillen *et al*. (2002) mentioned the properties of hydroxproline and hydrogen bonds play an active role in stabilizing the helical structure of collagen, which positively affects the viscosity and strength of gelatinization of gelatin.

Tab.(3): % foamability, foamstability, solubility and viscosity of gelatin of fish scales

	Gelatin								
properties	C. Th.	G.C. Th.	A. Th.	Mean	C. E.	G.C. E.	A. E.	Mean	Co. Gelatin
Formability%	65.4^{a}	60.05^{b}	59.9^{b}	61.78^{b}	65^{a}	58.28^{b}	58.28^{b}	60.52^{c}	70^{a}
Foamstability%	34.3^{a}	30.09^{b}	30.64^{b}	31.67^{b}	32^{a}	28^{b}	25^{c}	28.33^{c}	40^{a}
Solubility %	98.33^{a}	97.43^{b}	97.12^{b}	97.62^{b}	97.47^{a}	96.5^{b}	96.4^{b}	96.79^{c}	99.7^{a}
Viscosity %	32.44^{a}	28.1^{b}	25.22^{c}	28.58^{b}	30.24^{a}	21.12^{b}	20^{c}	23.78^{c}	39.5^{a}

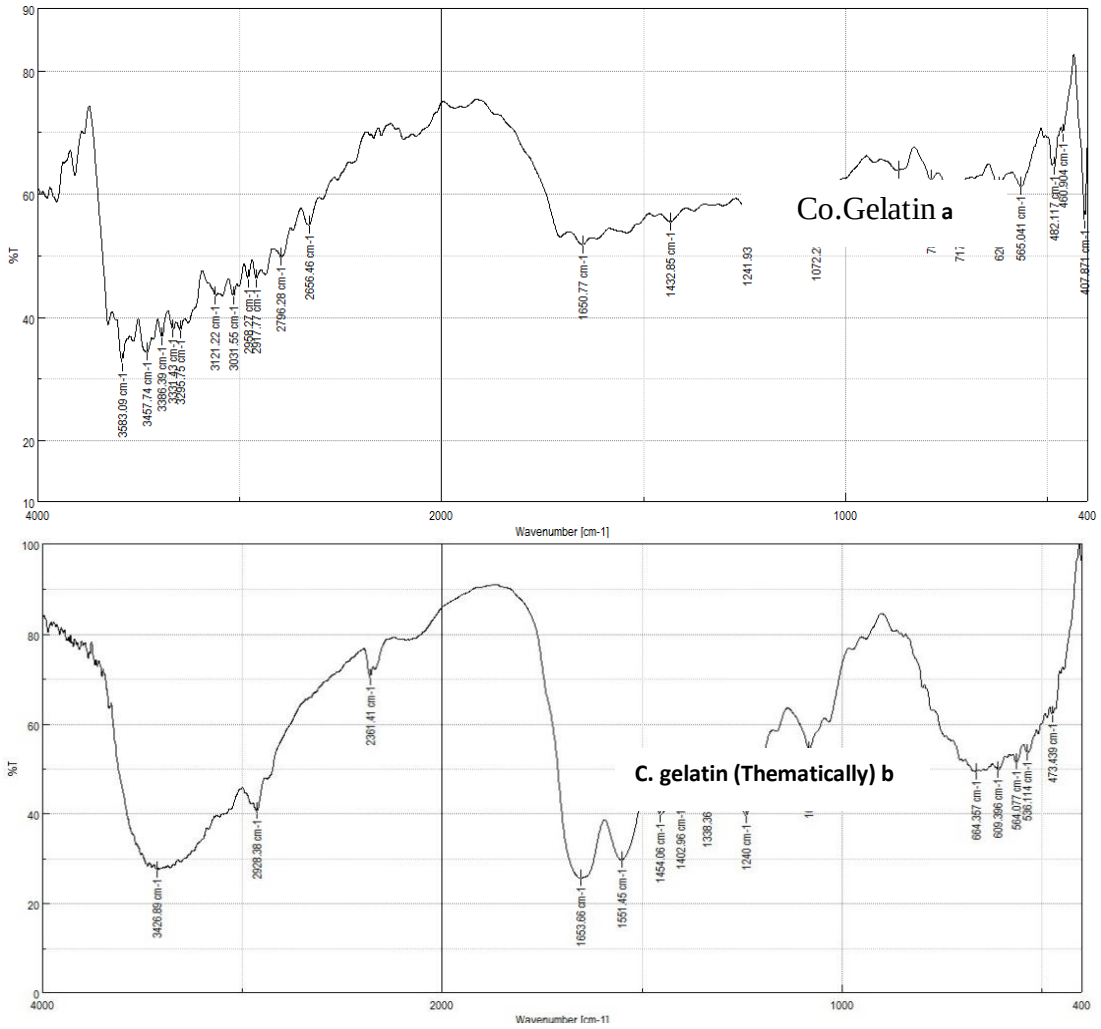
Th = thermally extract, E. = enzymatically extract

FTIR infrared spectrum

Fig. (2 a, b, c) refer to the FTIR infrared spectrum of gelatin of C. fish extracts thermally and enzymatically and their comparison with the Co. Gelatin. It is observed in the profile (2 b, c) that there is a wide bundle at frequency (3402.78 and

3426.89) cm^{-1} for Gelatin extracted thermally and enzymatically, respectively. As for the profile (2a) that represents Co. Gelatin, a bundle has been observed that ranged between (3295.75 - 3583.09) cm^{-1}. These frequencies indicate the presence of the Amide A group that is associated with water by a hydrogen bond, as this peak arises from the vibrations extending the group NH (Das *et al.*, 2017 ; Jridi *et al.*, 2013). The profile also showed the presence of a bundle at frequencies (1632.92, 1653.66 and 1650.77) cm^{-1} in gelatin of C. fish extracted thermally, enzymatically and Co. Gelatin, respectively. It indicated the presence of Amide I groups that are caused by the vibration of C = O that are related Dual hydrogen bond with COO (Das *etal.*, 2017). Whereas, Liu *et al.* (2012) showed that the Amide I groups result from the vibration of the extension C = O and conjugated with the C-N and CCN extensions. A profile of the enzymatically extracted gelatin also showed the presence of the 1551.45 cm-1 bundle that indicate the presence of the Amide II group. These bundle did not appear in both Co. Gelatin and gelatin extracted thermally from the gelatin of scales fish C. These results were in agreement with Zakaria & Abu Bakar (2015), provided that the presence of a frequency of 1539.55 cm^{-1} indicated the presence of amide II groups in black Tilapia scales.

The gelatin C. extracted enzymatically and co.gelatin showed the presence of the 1240 and 1241.93 cm^{-1} bundle. It indicated the presence of Amide III groups. Also, the bundle 1403.92, 1402.96 and 1432.85 cm^{-1} was observed in both the fish scales gelatin C. and Co. Gelatin, respectively. These frequencies indicate the presence of methyl groups.



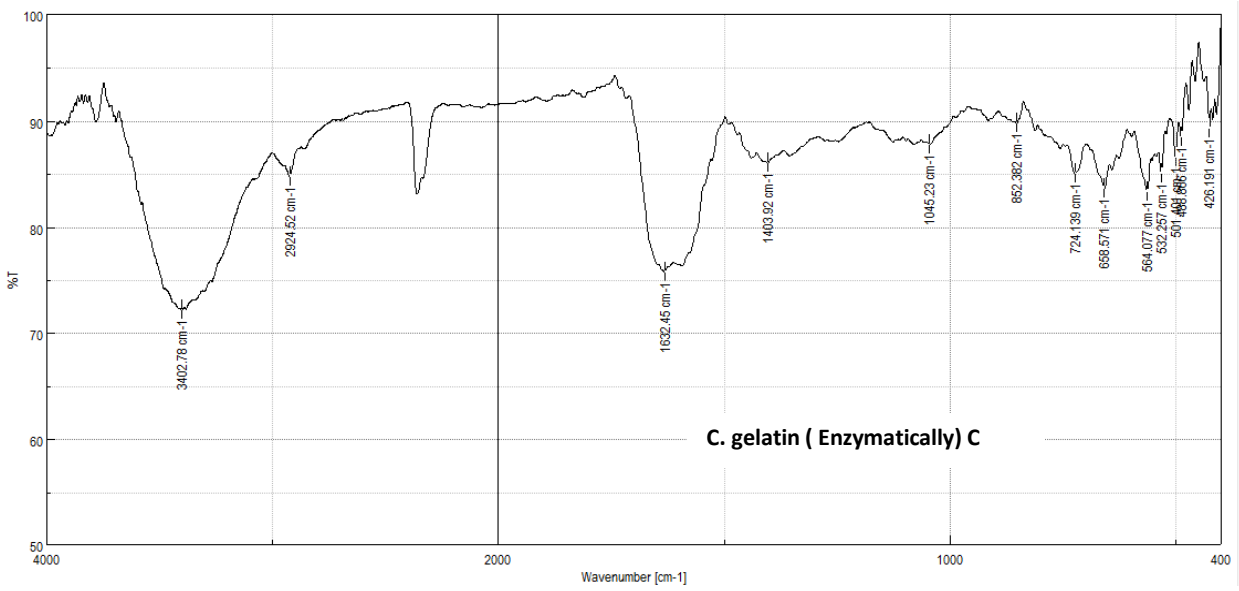


Fig.2: FTIR spectrum for gelatin C. fish and co.gelatin

Amino Acid

The amino acids are the basic structure of the protein, and the most prominent feature is the presence of the amino acids that gain the gelatin protein characteristic in the food and pharmaceutical industries. Tab. (4) shows the gelatin content of fish scales from amino acids, as it was observed that the gelatin scales contained good proportions of the hydroxyproline amino acid ranging between 6.79 - 8.84%. The enzymatically extracted gelatin showed a significant increase at the probability level P≤0.05. It was found that the highest percentage of H. Pro was present in gelatin of fish scales C. as compared to other species. While, gelatin scales extracted enzymatically exceeded in its content of amino acid Pro as compared to the gelatin extracted thermally. It is noted that the content of amino acid ranged between 23.06 - 25.15 %. It may be attributed to the rise in amino acid may be due to the type of fish used in the study, as it is a warm water fish having a high content of these acids. These results were in agreement with Karin & Bhat (2009), indicating that warm-water fish such as *Tilapia Oreochromis* ssp contains a high content of the amino acid Ala, H. Pro and Pro. While, some studies mentioned that the mammalian gelatin content of the amino acid H. Pro and Pro is 30%, while the gelatin content of warm and cold water fish is 22-25% and 17%, respectively (Muyonga *et al*., 2004). Tong & Ying (2013) found that the content of Bighead Carp scales gelatin from Amino Acid (Pro and H. Pro) was 20.75% compared to pig gelatin which was 24.01%. Gudmundsson (2000) noted the presence of Amino Acid in fish gelatin makes it possess physical properties similar to mammalian gelatin.

Tab.(3) also show that the fish scales gelatin contained good ratios of Gly, as they ranged from (21.21 - 23.12)%, in which the gelatin extracted from the scales of fish thickness was significantly superior at P≤ 0.05 in the enzymatic way as compared to other species under study. These results were lower than that obtained in some studies, as it was found that the ratio of Gly in the scales and gelatin of fish of the Farmed Sea Bass was (5905 and 24406.5) mg / 100g respectively (Dincer etal.,2015). And higher than the results of Wangtueai & Noomhorm (2009). He found that the Gly ratio of Lizard Fish Scales reached 18%. Arman *et al*. (2017) observed that the content of Rohu Fish's gelatin from amino acid Gly was 23.1%, while its ratio in Bighead Carp Fish's gelatin was 21.37% (Tong & Ying, 2013). The current study also showed that gelatin fish scales are free of the Met amino acid as confirmed by Muyonga *etal*. (2004) and Masood *etal*. (2015) that gelatin is an incomplete protein due to the absence of the Cys and Met amino acids.

The current study also showed that the total of Essential Amino Acid (EAA) ranged between 17.72 to 21.93% It showed gelatin extracted enzymatically from the scales of fishes C., G.C. and A. showed a significant increase, as it reached 19.28, 20.21 and 21.93%, respectively as compared to the gelatin thermally extracted for the aforementioned fish. The study also indicated that the amino acid Lys is the most prominent

among them ranging from 5.35-6.94%. As this acid possesses an important characteristic through its participation in the cross linking and telopeptide to form collagen as confirmed by Knott & Bailley (1998). The results were similar to the findings of Tong & Ying (2013), as it was found that the EAA content in Bighead carp fish scales reached 18.43%, in which the amino acid Lys increased, reaching 3.72%. Masood *et al*. (2015) observed that the content of the Lys amino acid in the scales of *Liza melinoptera*, *Liza macrolepis*, *Valamugil speigleri* and *Mugil cephalus* ranged (6.35 - 8.73) g / 100 g.

Tab. (4): The content of amino acids in gelatin scales of fish extracts thermally and enzymatically

Amino acid	C. Th.	G. C. Th.	A. Th.	Mean	C. E	G. C. E	A. E	Mean
H. pro	8.11^{a}	7.87^{b}	7.25^{c}	**7.743^{b}**	8.84^{a}	8.33^{b}	6.81^{c}	**7.993^{a}**
Asp	3.36^{a}	2.98^{b}	2.87^{b}	**3.07^{b}**	3.7^{a}	3.23^{b}	2.49^{c}	**3.14^{a}**
Thr	2.09^{ab}	1.99^{b}	2.76^{a}	**2.28^{a}**	2.1^{b}	1.85^{c}	2.58^{a}	**2.176^{b}**
Ser	3.13^{a}	3.05^{a}	2.43^{b}	**2.87^{b}**	3.31^{a}	3.25^{a}	2.57^{b}	**3.043^{a}**
Glu	5.62^{a}	5.39^{b}	4.51^{c}	**5.173^{b}**	5.15^{b}	6.31^{a}	4.71^{c}	**5.39^{a}**
Pro	16.09^{ab}	15.79^{b}	16.2^{a}	**16.023^{b}**	16.31^{a}	16.34^{a}	16.25^{a}	**16.3^{a}**
Gly	22.55^{a}	22.25^{b}	22.35^{ab}	**22.383^{a}**	23.12^{a}	22.59^{b}	21.21^{c}	**22.306^{a}**
Ala	7.82^{b}	7.56^{c}	8.05^{a}	**7.81^{a}**	7.19^{b}	7.64^{a}	7.67^{a}	**7.5^{b}**
Val	2.15^{c}	2.27^{b}	2.57^{a}	**2.33^{a}**	1.29^{c}	2.89^{a}	2.28^{b}	**2.153^{b}**
Met	ND	ND	ND	ND	ND	ND	ND	ND
Ile	1.48^{b}	1.54^{b}	2.01^{a}	**1.676^{b}**	1.51^{b}	1.42^{b}	2.67^{a}	**1.866^{a}**
Leu	0.41^{c}	2.47^{a}	1.36^{b}	**1.413^{b}**	2.41^{a}	2.34^{a}	1.86^{b}	**2.203^{a}**
Tyr	1.67^{c}	2.96^{a}	1.82^{b}	**2.15^{a}**	1.12^{b}	1.01^{b}	1.54^{a}	**1.223^{b}**
Phe	1.98^{b}	1.75^{c}	2.33^{a}	**2.02^{b}**	2.72^{b}	1.77^{c}	3.23^{a}	**2.573^{a}**
Lys	6.94^{a}	5.78^{b}	5.61^{b}	**6.11^{a}**	6.8^{a}	5.48^{b}	5.37^{b}	**5.883^{b}**
His	1.43^{b}	1.452^{b}	1.55^{a}	**1.476^{b}**	1.33^{b}	0.96^{c}	2.58^{a}	**1.623^{a}**
Arg	14.2^{b}	13.97^{b}	16.22^{a}	**14.796^{a}**	12.71^{c}	14.44^{b}	15.24^{a}	**14.126^{b}**
SEM	**99.03**	**99.072**	**99.89**	**99.33**	**99.6**	**99.85**	**99.06**	**99.498**
EAA	18.15^{b}	17.72^{c}	20.01^{a}	**18.62^{b}**	19.28^{c}	20.21^{b}	21.93^{a}	**20.47^{a}**
GLY+PRO	38.61^{a}	38.04^{b}	38.55^{ab}	**38.4^{a}**	39.23^{a}	38.93^{ab}	37.46^{b}	**38.54^{a}**
H.pro+Pro	24.2^{a}	23.66^{b}	23.45^{b}	**23.77^{b}**	25.15^{a}	24.67^{b}	23.06^{c}	**24.29^{a}**

CONCLUSIONS

Through this study, it was concluded that fish scales, especially river ones, contain a good percentage of proteins represented by collagen and gelatine. The experiments showed an affinity with bovine gelatine, and it was noted that extraction methods (enzymatic and thermal) have a clear effect on the properties of the extracted gelatine, as well as it contains many important amino acids, especially essential amino acid. Despite the experiments conducted on gelatine in this study and the results obtained, it needs more experiments for its use in the food, industrial and pharmaceutical fields.

Authors' contributions

WKA and AAK develop a research plan and conduct laboratory experiments. RAI estimating mineral elements for scales and gelatin in the laboratory and perform statistical analysis for experiments.MA and ZA auditing of article (linguistically and scientifically), and follow up the statistical analysis. All authors read and approved the submission of the manuscript

CONFLICT OF INTEREST

We would like to confirm that there are no known conflicts of Interest associated with this publication and there has been no significant financial support for this work.

REFERENCES

1. Ahmad M., Benjakul S. 2011. Characteristics of gelatin from the skin of unicorn leatherjacket (Aluterus monoceros) as influenced by acid pretreatment and extraction time. Food Hydrocolloids.25(3):381-388.
2. Alfaro AD, Balbinot E, Weber, CI, Tonial, IB, Machado-Lunkes A. 2014. Fish Gelatine : Characteristics, Functional Properties, Applications and Future Potentials. Food Eng Rev, 6(4): 1-14.
3. Alfaro AD, Biluca F, Marquetti C , Tonial IB, Nilson Souza N. 2014. African catfish (*Clarias gariepinus*) skin gelatin: Extraction optimization and physical–chemical properties. Food Research International 65:416-422.
4. Das MP, Suguna PR, Karpuram P, Vijaylakshmi JV, Renuka M. 2017. Extraction and characterization of gelatin: A functional biopolymer . Int. J. Pharm. Pharm. Sci.,9(9): 239-242 .
5. Dincer MT, Agcay OY, Sargin H. Bayram H. 2015. Functional properties of gelatin recovered from scales of farmed sea bass (Dicentrarchus labrax) Mehmet .Turk J Vet Anim Sci, 39: 102-109 .
6. Firlianty IR. (2016). Physico-chemical characterization and skin gelatin rheology of four freshwater fish as alternative gelatin source. AACL Bioflux, V. 9(6):1196-1207.
7. (GME) Gelatin Manufactures of Europe Monograph. 2005. Standard methods for testing of edible gelatin. Version 12, Annex: Commission Decision 1999/724/EC.
8. Gomez-Guillen C, Turnay J, Fernandez-Diaz MD, Lizarbe MA, Montero P. 2002. Structural and physical properties of gelatin extracted from different marine species: a comparative study. Food Hydrocoll, 16: 25–34.
9. Jakhar JK, Kumar A, Vardia HK. 2016. Extraction of Gelatin from Skin and Scale of Indian Major Carps. Environment & Ecology 34 (4A): 1513—1518.
10. Jongjareonrak A, Benjakul S, Visessanguan W Tanaka M. 2006. Effects of plasticizers on the properties of edible films from skin gelatin of big eye snapper and brown stripe red snapper,‖ *European Food Research and Technology*, vol. 222, pp. 229–235.
11. Jridi M, Nasri R, Lassoued I, Souissi N, barek AM, Barkia A. Nasri M. 2013. Chemical and biophysical properties of gelatins extracted from alkali-pretreated skin of cuttlefish (*Sepia officinalis*) using pepsin. Food Research International, vol. 54, pp. 1680–1687.
12. Kasankala LM, Xue Y, WeilongY, Hong SD, He Q. 2007. Optimization of gelatin extraction from grass carp (*Catenopharyngodon idella*) fish skin by response surface methodology. Bioresource Technology, 98(17):3338–3343.
13. Koli MJ, Basu S, Nayak BB, Patange BS, Pagarkar UA ,Gudipati V. 2012. Functional characteristics of gelatin extracted from skin and bone of Tiger-toothed croaker (*Otolithes ruber*) and Pink perch (*Nemipterus japonicus*). Food Bioprod Process 90(3):555–562 .
14. Kong J, Yu S. 2007. Fourier transform infrared spectroscopic analysis of protein secondary structures. Acta Biochim Biophys, 39:549–59.
15. Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature, 227: 680-685.
16. Li Y, Chen F, Zou J, Zhi M., Huang G. 2018. Gelatin extraction of Alaska pollock (*Theragra chalcogramma*) dry skin. American Journal of Biochemistry and Biotechnology , 14 (1): 13-19.
17. Liu D, Liang L, Joe MR ,Zhou P. 2012. Extraction and characterization of pepsin-solubilised collagen from fins, scales, skins, bones and swim bladders of bighead carp (*Hypophthalmichthys nobilis*). J. Food Chemistry, 133: 1441–1448.
18. Masood Z, Yasmeen R., Haider MS, Tarar OM, Zehra LE.,Hossain MY. 2015. Evaluation of crude protein and amino acid content from scales of four mullet species (Mugilidae) collected from Karachi fish harbor, Pakistan . Indian J. of Geo-marine Sci., 44(5): 724-731.
19. Muyonga JH, Cole CGB, Duodu KG. 2004 . Fourier transform infrared (FTIR) spectroscopic study of acid soluble collagen and gelatin from skins and bones of young and adult Nile perch (*Lates niloticus*). J. Food Chem 86: 325-332 .
20. Naqvi MA, Tahir SA. 2014. Proximate composition of head and scales in wild and farmed *Ctenopharyngodon idella* under different weight categories. J. Glob. Innov. Agric. Soc. Sci., 2(4): 171-174.
21. Sha XM, Tu ZC, Wang H, Shi Y, Liu GX, Man ZZ, Huang T, Zhang L. 2013. Preparation and properties on gelatin from fish scale.

Advanced Materials Research., 647: 352-356 .
22. Tong Y,Ying T. 2013. Gelling strength improvement and characterization of a gelatin from scales of bighead carp (*Aristichthys nobilis*). Journal of Food, Agriculture & Environment., 11 (1): 146-150. C:\Users\Lenovo\Desktop\ilis.
23. Wang Y., Regenstein JM. 2009. Effect of EDTA, HCl, and citric acid on Ca salt removal from asian (Silver) carp scales prior to gelatin extraction. J Food Sci.,;74(6):C426-31.
24. Wangtueai S, Noomhorm A. 2009. Processing optimization and characterization of gelatin from lizardfish (*Saurida* spp.) scales. LWT - Food Science and Technology, 42, 825-834.
25. Wu J, Kong L, Zhang J, Chen W. 2019. Extraction and Properties of Acid-Soluble Collagen and Pepsin-Soluble Collagen from Silver Carp (*Hypophthalmichthys molitrix*) Scales: Prerequisite Information for Fishery Processing Waste Reuse. Pol. J. Environ. Stud. 2019;28(4):2923–2930 .
26. Yilmaz MT, Kesmen Z, Baykal B, Sagdic O, Kulen O, Kacar O, Yetim H , Baykal AT. 2013. A novel method to differentiate bovine and porcine gelatins in food products: NanoUPLC-ESI-Q-TOF-MS E based data independent acquisition technique to detect marker peptides in gelatin. Food Chemistry 141(3): 2450–2458.
27. Zakaria S,Abu Bakar NH. 2015. Extraction and characterization of gelatin from black tilapia (*Oreochromis niloticus*) scales and bones. Int'l Conf. on Advances in Science, Eng., Technology & Natural Resources (ICASETNR-15), Kota Kinabalu (Malaysia). Pp:77-80..
28. Zayas JF.(1997). *Functionality of proteins in food*. Springer, Berlin, Heidelberg