Octopus Skins as a Source of Gelatin: Preparation, Evaluation and Utilization

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ABSTRACT

The octopus is a soft-bodied marine organism from the phylum Mollusca and kingdom Animalia. It is found in the rock and sand about 100–150 meters deep. Fish wastes are inadequate amounts and sizes of fish to guarantee sales, and un-edible parts such as ink, mucus, viscera, and skins have low commercial value. In many countries, throwing away or disposing of fish waste incurs unnecessary costs. The current study aimed to utilize octopus skin as a source of gelatin. Octopus skins are a good source of gelatin. Gelatin was extracted from octopus skins by five different treatments, namely acid, alkali, lime, direct extraction, and a combined extraction, and the yield was 1.76 %, 10.32%, 1.43%, 1.12%, and 6.85%, respectively. Regarding chemical and physical properties, alkali and combined treatment gelatin were superior to other extracted gelatins as compared with commercial bovine gelatin. Extracted gelatin was applied to prepare orange jelly and Rosella candy. Finally, Octopus skin gelatin with good physical-chemical properties can be a potential alternative to commercial bovine gelatin and be useful as an additive in various food applications. Therefore, recycling octopus skins into marketable products can reduce the amount of waste generated.

Keywords: *Gelatin, octopus vulgaris, partial hydrolysis, glycoprotein and gelatin extraction.*

INTRODUCTION

The octopus (Octopus vulgaris) is a soft-bodied marine organism that belongs to the phylum Mollusca and kingdom Animalia. It is found in marine rock and sand about 100 to 150 meters deep (Arvanitoyannis & Kassaveti, 2008). Fish wastes are defined as fish species having low commercial value, unconsumed body parts, undersized or damaged commercial species. Fish waste involves skin, bones, entrails, shells, or ink secreted by some species of fish, which causes a negative significant impact on the environment. It is essential to consider finding suitable ways of managing and utilizing fish waste (Kim & Mendis, 2006).

Every year, fish wastes produced worldwide exceed 20 million tons, equivalent to 25% of the total production of marine fishery catch, and include «non-target» species (FAO, 2011). In the European Union, fish wastes contribute 5.2 million tons annually (Mahro & Timm, 2007). Some countries, such as Peru and Chile, use fish waste for fish oil production, contributing to 52% and 13% of the total world production of fish oils, respectively. Iceland and Norway contribute approximately 7% of the world›s fish oil production. Using fish wastes as alternative sources for the supply of animal feeds, such as fish meal and fish oil, could reduce the fishing pressure of fish waste disposal on the species targeted. Also, it will contribute to sustainable aquaculture production (Davies *et al*., 2009). Despite the low value, fish wastes can be a source of bioactive compounds, including protein hydrolysates, lipids, astaxanthin, and chitin, which are used in pharmaceutical industries (Rochet & Trenkel, 2005). Muller & Werner (2003) defined gelatin as the mixture of collagen peptides produced by partial hydrolysis of collagen from the skin, bones, and tendons of animals such as cattle, chickens, pigs, and fish. A high percentage of gelatin is derived from pork, cattle, and bones. Gelatin made from fish avoids some of the religious restrictions on gelatin consumption. Mariod (2011) prepared and characterized edible halal gelatins from two Sudanese edible insects, melon and sorghum bugs. Karim & Bhat (2009) reported differences in mammalian gelatins (bovine, type B) and porcine, type A, due to using different sources and treatments during gelatin extraction. All methods of gelatin extraction must follow the basics of processes and treatments, which are pretreatments, hydrolysis, extraction, refining, and recovery. Fish

gelatin is obtained from fish skin, swimming bladder, and bones (Beranova, 2003; Rosengren*et al.,* 2003).

The octopus skin contains different components, such as glycoproteins, collagen, and amino acids (Choi & Regenstein, 2000; Mai *et al*., 2006). Gelatin is formed as an end product after the hydrolysis of collagen peptides by boiling the stock under a temperature range between 50 and 70°C for a certain period (3–5 hours) (Rochet &Trenkel, 2005; Ramshaw *et al.,* 2009). The extraction of gelatin from fish skins could be an alternative source that is Kosher for Jews and Muslims. It was reported that insect gelatin could be an alternative source acceptable for Muslim products, but challenges include the availability of insects to get enough gelatin. In Sudan, many edible insects, such as sorghum and melon bugs, are consumed (Mariod& Adam, 2013). Beranova (2003) stated that some plant materials from the ocean or water sources can be substituted for gelatin; they are not a source of gelatin but can be a substitute for gelatin. They have slightly different properties from animal gelatins. However, they are a good option for people who do not want to use a substance obtained from animals. Two of these gelatin substitutes are agar and carrageenan. The present study aimed to optimize the extractions of high quality gelatin from octopus skin and its utilization in producing some food products.

MATERIALS AND METHODS

Materials

Fifty kilograms of octopus skin were collected from a fish market in Dar es Salaam (Tanzania). The skins were removed by hand scissors, weighed and kept frozen at -15^{o}C. The percentage of skin to total body weight obtained was 23.26%. The frozen skin was packaged into ice boxes and transported to the Laboratory of Food Analysis, Faculty of Agriculture, University of Alexandria, Egypt. Bovine gelatin used as standard gelatin, obtained from El-Nasr company for gelatin manufacture in Alexandria, Egypt. All chemicals, reagents, and other materials used in this study were purchased from El-Gomhoria Company and local markets in Alexandria City, Egypt.

Methods

Several repeated trials were made to extract high-quality gelatin from octopus skin. The skin was removed from the freezer and allowed to defrost. The 50 kg of skin were dived into five treatments in which 10 kg were used for each treatment. The defrosted octopus skin was washed with running tap water for two hours to remove dirt, ink, and mucus.

Gelatin preparation by acid treatment

The skin was soaked in 5% HCl (ratio, 1:2 w/v) for 4 hours at room temperature (20-25°C). Then, the skin was washed with water to remove the impurities, then blended by electrical blender. the pH adjusted by washing with cold water until pH reached 6–6.5, which is optimum for the hydrolysis of collagen as explained by Ockerman & Hansen, (2000). Extraction was done by cooking the skin treated by acid treatment with water at ratio of 1:2 w/v at 60–70°C for two hours in water bath. Then, the suspension was filtered by sieving to remove residue skin and impurities. Finally, the suspension was stored at 5°C overnight (12 hours) and the excess fluid is separated by decantation. The gelled mass was spread on the drying nets and dried in a hot-air oven at a 50°C overnight (12 hours). The gelatin is carefully cooled until it reaches room temperature at 20°C. The gelatin sheets were then ground to obtain gelatin powder. The powder is stored in a closed container and kept at room temperature (Abu Tor, 1988; Ockerman & Hansen, 2000).

Gelatin preparation by alkali treatment

The skin was soaked with 4% NaOH (ratio, 1:2 w/v) for 4 hours at room temperature (20–25 °C), then washed under running water. The pH was adjusted to be pH 6-6.5 by using NaOH, which is optimum for collagen hydration. Extraction was done by cooking the stock in water (1:2 w/v) at 60–70 °C for 2 hours in a water bath. Clarification and drying were done as mentioned in preparation of gelatin by acid (Abu Tor, 1988; Ockerman & Hansen, 2000)

Gelatin preparation by lime treatment

Ten kilograms of octopus skin were washed under running water for 2 hours then soaked in a 10% lime liquor ratio (1:2 w/v) for 4 hours at room temperature (20–25°C), then washed under running water to remove impurities. The skin was blended with an electrical blender. The pH was adjusted to 6.0-6.5, and extraction was done at 60–70°C in a water bath for 4 hours. Clarification

and drying were done as mentioned in preparation of gelatin by acid (Abu Tor, 1988; Ockerman & Hansen, 2000).

Gelatin preparation by combined treatment

The octopus skin was removed from the freezer and defrosted. Gelatin was prepared according to the method explained by Ockerman & Hansen (2000). Ten kilograms of skin were washed with running water for 1 hour to remove the impurities and soaked at room temperature (20–25°C) in an aqueous solution of 0.4% NaOH (1:2 w/v) for 4 hours. The skins were washed under running tap water for 1 hour and then soaked in a 0.4% aqueous hydrochloric acid solution (1:2 w/v) for another 4 hours at room temperature. The skin was rewashed by running tap water to pH 6-6.5, then blended and mixed with distilled water at a ratio of 1:2 (w/v). The gelatin was extracted by cooking the stock for 3 hours at 60 - 70°C, Clarification and drying were done as mentioned in preparation of gelatin by acid (Abu Tor, 1988; Ockerman & Hansen, 2000)

Gelatin preparation by direct extraction method

The skin after defrosting was soaked in water for 4 hours at a temperature of 20–25°C and washed again with running tap water. The pH was adjusted to 6-6.5. The extraction was undertaken by keeping the vessel in the range of 60–70°C in a water bath for 7 hours; the ratio of skin to distilled water was 1:2 (w/v). Clarification and drying were done as mentioned in preparation of gelatin by acid (Abu Tor, 1988; Ockerman & Hansen, 2000)

Gelatin yield

Gelatin yield was calculated according to the following equation:

$$\text{Quantity Yield (\%)} = \frac{\text{Weight of gelatin extracted}}{\text{Weight of skin used}} \times 100$$

Preparation of orange jelly

Orange juice was extracted from the orange and filtered through a sieve to get clear orange juice. Then, gelatin powder in a ratio of 15% (w/v) was added to the extracted juice and kept at a temperature of 10-25°C for one hour to allow the gelatin to dissolve completely into the juice. Then, heated up to 60°C, 100g of sugar were added, accompanied by gentle stirring, and kept overnight at 5°C to solidify (Mariod& Adam, 2013).

Preparation of rosella candy cubes

Four hundred milliliters of rosella juice were mixed with gelatin powder in a 30% (w/v) ratio at 25°C for 2 hours to allow the gelatin to dissolve. Thereafter, the mixture was heated up to 60°C, 100g of sugar and cola flavour were added, and the mixture was stirred to get uniform porridge juice. The mixture was poured into a plate, cooled overnight at 5°C to solidify, and then cut into cubes**.**

Analytical methods

Gross chemical composition

Crude protein (NX 6.25), crude fat and ash content were determined according to AOAC (2000). Carbohydrate was calculated by the difference [%Carbohydrate = 100 – (%Moisture + %Protein + %Fat+ %Ash)]. Minerals (Calcium, copper, manganese, magnesium, zinc and iron) were determined by Atomic Absorption Spectrophotometer (9 Series - AA Spectrometers, PerkinElmer) as described by the AOAC(2000).

Amino acids were analyzed and determined by High-Performance Liquid Chromatography using an LC-300 HPLC system (Sykam, Chroma Tech, Germany) equipped with two detectors set at 570 and 440 nm (λmax). A volume of 0.1 ml of sample was separated on a cation exchanger resin column (50 mm × 4 mm × 5 μmi.d No. 2619 resin). The column operated at 17°C using citrate buffer at pH 2.2 with a 1.0 mL/min flow rateas described by Schmidt *et al.* (2006).

Total phenolic were assayed with Folin–Ciocalteu reagent using tannic acid as standard as described by the AOAC (2000). The mixture was kept in the dark at 25°C for 2 hours before measuring at 765nm using (US-Vis- spectrophotometer, Laxco- Alpha-1102)

Protein pattern by electrophoresis

Gel electrophoresis (SDS-PAGE) was used for the separation and identification of protein, as described by Marion *et al.* (2010). The apparatus used was a Gel casting system, 10cmX10cm, FB-GC10-1 (model 422 Electro-Eluter); the electrical field was set at 200volt for 2 hours at 15°C. The stacking gel used was 4%, and resolving gels were 7.5% and 12%. The separated bands were identified by comparison with standard protein marker (Bio-Pad Laboratory, USA).

Determination of pH value

The pH value was measured by pH meter (AD1020, Adwa - Professional pH-ORP-Temp Bench Meter with GLP, Hungary) at 20°C as described by AOAC (1980).

Determinations of physicochemical properties of gelatin

The gelatin solution used for physicochemical tests was prepared according to the procedures described by the AOAC(1980).About 7.5g of gelatin was soaked in 105g of distilled water for 2 hours at room temperature. Thereafter, the swollen gelatin was heated using a water bath at 45°C, accompanied by gentle stirring until the gelatin was completely dissolved.

Gel strength

The gel strength of the gelatin solution (6.66% w/v) was measured in units of blooms by using the Bloom geometer apparatus (TA-XT2 Texture Analyzer-Stable 194 Microsystems, Godalming, UK) at the gelatin plant of the El-Nasr Company in Alexandria by the procedures described by the AOAC (1980). A 6.66% gelatin solution was carefully poured into a standard bloom bottle 200 of dimension 6 × 8 × 20.1 cm (bottom diameter × height). Then, the bloom bottle was kept in a water bath at 10°C±0.1 overnight. Finally, bloom strength was measured by placing the bloom bottle centrally under the standard probe. The probe proceeded to penetrate the gel to a depth of 4 mm. The maximum force read is the resistance force for penetration obtained and expressed as the gel's grams bloom (Bloom strength - g).

Viscosity

The viscosity of gelatin solution (6.66%w/v) was assessed by Programmable Rheometer (DV-III ULTRA) Viscometer at a temperature of 60°C. The instrument was optimized by adding 40 ml of distilled water in the special cylinder of the instrument whereby the scale should indicate zero. Then, 40 ml of the gelatin solution was poured into the cylinder. Viscosity values of gelatin solution were recorded at 60°C and calculated by the equation:

$$\text{Equation of viscosity (Mps)} = S \times E \times U \times R \times K \times 10$$

Whereby:, S: Scale indication, E: Sensitivity, U: Speed velocity, R: Speed reduction, K: System constant and 10 – Transformation from ml pascal to ml poise (British Standard 757:1975).

Foaming property

Fifty milliliters of the gelatin solution (6.66%w/v) were poured in a 100 ml graduated cylinder (2.5 cm diam.) and closed tightly. After shaking for one minute, the cylinder was placed in a water bath at 45°C. The time in minutes needed to obtain 45 ml length of solution in the graduated cylinder was recorded and the height of foam in ml was measured (British Standard 757:1975).

Melting point

The gelatin solution (6.66%w/v) in a test tube with a thermometer inserted was allowed to gel in the refrigerator. The tube was placed in a water bath, the temperature of which was gradually raised until the contents of the tube started to melt (gel to sol), at which instant the temperature was recorded (British Standard 757:1975).

Clarity

A gelatin solution prepared was used to determine the clarity or degree of turbidity as described by the AOAC (2000)

Determination of colour by Hunter lab

The colour of gelatin was determined in terms of Chroma, Hue, and Values by using the Hunter lab (The LabScan XE, Australia). The colour of gelatin was measured by CIE L*(chroma), a*(hue), and b* (value) by absorbance spectrophotometric as described by Nixsensor (2020).

Microbiological examination

Total bacterial count

Ten grams of sample were dissolved into 90ml distilled water in water-bath at 45°C. One ml of solution was added to nutrient agar (N.A) medium, incubated at 32°C for 48 hours. The total count of bacterial colonies was recorded (CFU/g) as the number of colonies per gram of sample (Anderson& Shi, 2006).

Salmonella sp.

One ml of solution (prepared for the total count) was added to a 9 cm-diametric Petrie dish which contains *Salmonella Shigella* Agar (SSA) medium, incubated at 32°C for 48 hours. The total count of bacterial colonies was recorded and reported as the number of colonies per gram (CFU/g) (Banerjee *et al.,* 2014).

Coliform bacteria count

Ten ml of sample were dissolved into 90ml of double distilled water in water-bath at 45°C. One ml of solution was added to solidified MacConkey

medium, incubated at 32°C for 48 hours. The total count of bacterial colonies was recorded and reported as the number of colonies per gram (CFU/g) (Casadevall*et al*., 2000).

Sensory evaluation

Texture, taste, colour, odour and overall acceptability of the samples were evaluated by 25 panelists, a scale of 1 -9 was used according to Rustad (2003).

Statistical analysis

All data were statistically analyzed through analysis of variance (ANOVA) and Duncan's multiple comparison range tests at a 5% level of probability (Jones, 2004).

RESULTS AND DISCUSSION

Gross chemical composition of raw octopus skin

The data presented in Table (1) reveal the intriguing potential of raw octopus skin with a chemical composition of moisture (62.23±1.04%), protein (80.249%), fat (0.821%), ash (0.593%), carbohydrate (18.258%) based on dry weight, and pH 6.8±0.02. Rahman *et al.*, (2008) reported that, the chemical composition of pork skin was 31.9% protein, 22.6% fat, and 44.4% moisture. This suggests that with its lower fat and higher protein content, octopus skin could potentially replace pork skin in gelatin production, offering a higher-quality alternative gelatin. Furthermore, approximately 30% of fish waste, including skin and bones with high collagen content, can also be utilized to produce fish gelatin (Gómez-Guillén *et al*., 2011).

Mineral contents of octopus skin

The skin of octopus contains copper (4.804 ± 0.079), zinc (11.465 ± 0.452), iron (92.639 ± 0.168), calcium (13.385 ± 0.330), manganese (5.926 ± 0.179) and magnesium (58.262 ± 0.947) mg/100g dry sample as shown in Table (1). Rahman *et al.* (2008) showed that animals contained higher concentrations of copper, calcium, and magnesium in muscles than in other body parts, especially the skin. Also, octopus skin contained fewer amounts of magnesium, copper, and zinc than octopus ink.

Microbiological examination of octopus skin

Microbiological examination of octopus skin reveals that it was free (not detected) from salmonella ssp/25g, while total plate count was 1.6 x 107 cfu/g and coliforms were 1.2 x 104 cfu/g. The presence limit of about 10,000 bacterial colonies is acceptable as reported by Schrieber& Gareis (2007), and Duan *et al.,*(2011). However, raw octopus skin testing is acceptable for gelatin manufacturing because raw materials will be exposed to different treatments.

Table 1:Gross chemical composition and mineral contents

Gross chemical compounds	
Component	Dry bases (%)*
Protein	80.249
Fat	0.821
Ash	0.593
Carbohydrate	18.258
Mineral contents	
Mineral	Amount (mg/100g)*
Copper	4.804 ± 0.79
Zinc	11.465 ± 4.52
Iron	92.639 ± 1.68
Calcium	13.385 ± 3.30
Manganese	5.926 ± 1.79
Magnesium	58.262 ± 9.47

*Mean± SD

Effect of pretreatment on gelatin yield

The average percentage yield of gelatin extracted by alkali treatment was 10.32 ± 0.78% based on a wet basis. The alkali treatment method produced significantly ($P \leq 0.05$) the highest yield of gelatin compared to acid treatment, lime treatment, direct extraction, and combined treatment, as shown in Table (2). Alkali treatment gave significantly ($P \leq 0.05$) the highest amount of gelatin compared to gelatin extracted by acid treatment. It was observed that octopus skins tend to swell more in the alkaline solution than in the acidic or lime solution. Therefore, octopus skins gave a higher yield in alkali treatment, possibly due to the increased opening of cross-links during swelling. This scenario correlated with the high gelatin yield from the octopus's skin. Karim & Bhat (2009) reported that the yield and quality of gelatin are influenced by the species and age of the fish, the extraction process, and the pretreatment temperature. The yield of sharkskin gelatin by using alkali treatment was 19.7 ± 0.04%, tuna skin gelatin was 11.3 ± 0.03%, and rohu skin gelatin was 17.2± 0.03% (Ahmad &Benjakul, 2011).

The average percentage yield amount of gelatin extracted by acid treatment was 1.76 ± 0.32% on a wet basis. The acid treatment method yields less gelatin than alkali treatment(Table 2). From an economic viewpoint, this method is not accepted for extracting gelatin from octopus skin. Both quantity and quality are key factors to consider in gelatin extraction; the best method of gelatin extraction is the one that will yield a high amount of gelatin with high quality. For the highest yield of gelatin, one must consider several factors and parameters, such as pH, temperature, and the nature of the species. During extraction, pH and temperature are critical parameters to be considered; the yield amount was affected by pH and temperature. Muyonga & Cole (2004) and Benjakul *et al*. (2009) showed that the yield of gelatin extracted from duck feet was 7.01±0.31% on a wet weight basis. Yields of gelatin were reported to vary among sources (i.e., different types of body parts of poultry), mainly due to differences in collagen content.

The lime treatment method produced significantly (*P≤0.05*) the lowest yield 1.43 ± 0.56% of gelatin with a dark colour and the lowest quality compared to alkali and acid treatments (Table 2). This method is not accepted for extracting gelatin from an octopus's skin for application purposes. The lime solution is probably a weak base that tends to open fewer crosslinks during swelling, producing the lowest amount of gelatin. A lower amount of extractable gelatin was obtained if the collagen contained a high degree of crosslinking *via* covalent bonds, leading to decreased collagen solubility.

The amount of gelatin yield is a crucial factor in gelatin extraction, and it is influenced by several key factors. Among these, the method of extraction which plays a significant role, as demonstrated by our findings. The procedures taken during extraction and the nature of the raw material or species used also contribute to the final yield. It is worth to note that higher yields of gelatin can be obtained from sources with higher collagen content, highlighting the need for careful selection of the extraction method and raw material in gelatin production.

The average percentage yield of gelatin extracted by direct extraction was 1.12 ± 0.15% based on a wet basis. The direct extraction produced significantly (*P≤0.05*)the lowest amount of gelatin compared to others treatments as shown in Table (2). This method is not accepted for extracting gelatin from octopus skin for application purposes because it produces low quality and low quantity. Less gelatin yield by direct extraction indicates that pretreatment is essential in gelatin extraction because it opens and breaks down the cross-links of collagen, thereby making it easier for the hydrolysis of collagen to form gelatin more easily.

The yield quantity of gelatin extracted by alkali treatment followed by acid treatment (combined) was 6.85%. The combined treatment produced higher-quality and higher-yielding amounts of gelatin than the gelatin prepared by the other treatments investigated here. This method is accepted to extract the gelatin from an octopus›s skin from a technological and economic point of view. From the previous results, two samples with the highest yield percentages, alkali, and combined treatments, will be used in subsequent experiments along with commercial bovine gelatin.

Table 2: Yield of gelatin prepared by different methods*

Method	Yield (%)**
Acid treatment	1.76 ± 0.32^{b}
Alkali treatment	10.32 ± 0.78^{a}
Liming treatment	1.43 ± 0.56^{c}
Direct extraction	1.12 ± 0.15^{bc}
Combined treatment	6.85 ± 0.49^{ab}

*Mean ± SD
**Means in a column not sharing the same superscript are significantly(P≤0.5) different.

Gross chemical composition extracted gelatin

Alkali treatment gelatin contained a moisture content of 8.9±0.05%, while combined treatment gelatin (9.2±0.34%) and bovine gelatin contained a moisture content of 9.6±1.02%, as shown in Table (3). Most gelatin samples had between 9% and 12% moisture content (Karim & Bhat, 2008). The moisture content of gelatin was influenced by humidity. The South African National Specification requires less than 16% moisture content in gelatin. For this reason, it is necessary to monitor gelatin moisture content during production (Karim & Bhat, 2009).

The protein content of gelatin prepared by alkali treatment was 85.2±2.05%, gelatin extracted by combined treatment was 87.9±2.78%, and bovine gelatin (89.3±1.04%) (Table 3). According

to Abu Tor (1988), acid-treated gelatin had a higher protein content than alkali-treated gelatin. Dried gelatin contains more protein, around 98–99% (Sabaté*et al*., 2012).

Alkali treatment gelatin contained a higher ash content of 2.09±0.01%, combined treatment gelatins contained 1.13±0.01%, and bovine gelatin contained 0.94±0.02%, as shown in Table (3). Ash content is an inorganic matter of gelatin that depends on the source of gelatin (Aquilina *et al*.,2004; Ahmad &Benjakul, 2011). The highest ash content of gelatin from lizardfish (1.7%) was found due to the addition of 0.8% (w/v) of NaCl during preparation (FAO/WHO-JECFA, 2003). The ash content of gelatin from Nile tilapia was 0.26%, and that of Nile perch was 0.15%, which was lower than that of commercial gelatin (0.82%). The high ash content in bovine gelatin was due to the high quantity of minerals in the skins. Benjakul *et al.* (2009) stated that high-quality gelatin should contain no more than 0.5% ash.

The pH of alkali treatment gelatin was 6.8, combined treatment gelatin was 5.6, and bovine gelatin was 5.9. The pH of gelatin depends on the methods by which it is processed. According to Europe Warehouse Products (2020), edible gelatin›s pH ranges from 4.5 to 7.0, so all processed gelatin can be used for human consumption. Pure gelatin has a pH of approximately 4.95 (Choi & Regenstein, 2000)

Mineral contents of Processed gelatins

The data in Table (3) reveal that the concentrae tion of mineral contents in gelatins varies and depends on the types of gelatins, sources, treatments used, and soaking time during extraction. The gelatin extracted from the octopus exhibited more zinc, copper, and iron than animal gelatin (bovine gelatin)**.** Gelatin extracted by alkali treatment had a higher mineral content than combined treatment. According to Abu Tor (1988), acid was more efficient than alkali for demineralizing minerals, and thereby, combined treatments gave lower mineral content than gelatin extracted by alkali treatment alone. The mineral content of gelatin has an impact on the color of gelatin.

Soaking time was a more important factor in the demineralization and mineral content of gelatin. If stock is soaked for a long period of time, it will lower the mineral content, and vice versa. It was observed that octopus gelatins contain more minerals, especially copper, zinc, iron, and manganese while being low in calcium compared to cow (bovine) gelatin.

Amino acid composition of extracted gelatin

The total amino acids present in gelatin extracted through alkali treatment were 85222 mg/100 g**,** combined treatment gelatin was 87351 mg/100 g, and bovine gelatin was 90081 mg/100g (Table 4). Bovine gelatin had the highest amino acid content,

Table 3: Chemical composition and mineral contents of gelatins prepared by different methods versus bovine gelatin*

Chemical composition	Moisture(%)	Protein (%)	Ash (%)
Alkali treatment	8.9±0.05^{b}	85.2±2.05^{b}	2.09±0.01^{a}
Combined treatment	9.2±0.34^{ab}	87.9±2.78^{ab}	1.13±0.01^{ab}
Bovine	9.6±1.02^{a}	89.3±1.04^{a}	0.94±0.02^{b}
Mineral contents	**Treatment****		
	Alkali gelatin	**Combined gelatin**	**Bovine gelatin**
Copper (Cu)	89.638±0.504^{ab}	128.833±2.36^{a}	69.060 ± 4.17^{b}
Zinc (Zn)	53.002±0.187^{ab}	88.702±4.79^{a}	20.635 ± 2.09^{b}
Iron (Fe)	65.606±0.307^{ab}	90.540±3.61^{a}	55.055 ± 1.108^{b}
Calcium (Ca)	21.078±0.761^{ab}	19.984±0.857^{b}	31.946 ±0.588^{a}
Manganese (Mn)	23.849±1.404^{ab}	30.372±1.609^{a}	16.143 ± 1.371^{b}
Magnesium (Mg)	172.928±1.301^{b}	259.996±4.937^{a}	199.755 ±6.96^{ab}

*Mean ± SD

**Means in a row not sharing the same superscript are significantly ($P \leq 0.5$) different.

Table. 4: Amino acid composition of gelatin alkali and combined versus bovine gelatin.

Amino acids	Alkali gelatin		Combined gelatin		Bovine gelatin	
	Amount [mg/100g]	% Total amino acids	Amount [mg/100g]	% Total amino acids	Amount [mg/100g]	% Total amino acids
Aspartic acid	6991	8.2	8836	10.11	7912	7.87
Thrionine	677	0.79	612	0.70	480	0.54
Serine	34456	40.44	35921	41.10	25926	29.07
Glutamic acid	2014	2.36	2106	2.41	2852	3.20
Glycine	9083	10.66	7979	9.13	14421	16.20
Alanine	1977	2.32	1136	1.30	1362	1.53
Cystine	1656	1.94	2014	2.30	3406	3.81
Valine	6015	7.06	1783	2.04	2904	3.25
Methaionine	1190	1.4	1830	2.09	3015	3.38
Iso-Leucine	3092	3.6	2797	3.20	5135	5.75
Leucine	1219	1.43	1355	1.55	2493	2.79
Tyrosine	3525	4.14	1870	2.14	2322	2.60
Penyl Alanine	9780	11.48	5331	6.10	6920	7.76
Histidine	1440	1.69	4990	5.71	4200	4.70
Lysine	1321	1.55	8005	9.16	5163	5.78
Arginine	786	0.92	786	0.90	1570	1.76
Total Amino Acids	85222	100	87351	100	90081	100

followed by gelatin extracted by combined treatment and gelatin extracted by alkali treatment. All three types of gelatin contained high serine content compared with other amino acids in each type of gelatin, namely, alkali treatment gelatin (40.44%), combined treatment gelatin (41.10%), and bovine gelatin (29.07%).

Protein pattern of extracted gelatin by electrophoresis

Bovine gelatin had the highest molecular weight (45 KDa), followed by gelatin extracted by combined treatment (31 KDa), and finally, that which was extracted by alkali treatment(28 KDa). The protein pattern and molecular structure of gelatin depend on the methods of extraction, the conditions during extraction, and the nature of the materials used. Nagarajan *et al*. (2012) showed that the tiger tooth croaker fish head gelatin protein pattern had bands near the molecular weights of 116 and 97 kDa. Gelatin extracted under higher hydrolysis temperatures (85–90°C) typically reveals multiple bands observed up to 45 kDa, indicating the formation of low molecular weight peptides in the gelatin during preparation.

Physicochemical properties of extracted gelatin

The gel strength

Gel strength is the most critical functional property of gelatin. The gel strength obtained was 158 blooms for alkali treatment gelatin; combined treatment gelatin had 89 blooms; and the gel strength of bovine (control) was 216 blooms, as shown in Table (5). The gel strength of the final gelatin depends on the drying period, the temperature of extraction, and the moisture content. The gelatin finally loses its gel strength when the final gelatin products are dried at a higher temperature than 40°C. Beranova (2003) showed that moisture content is an essential factor to be considered because high moisture content results in a gel with low strength. Sarbon et al. (2013) reported that the gel strength of gelatin is categorized into three

groups, namely: low (<150), medium (150–220), and high (220–300) bloom values. Proline is an amino acid responsible for the collagen structure's stability. According to Balti et al. (2011), gelatin with low levels of proline and hydroxyproline has a lower gel strength.

Viscosity

The viscosity value of gelatin prepared by alkali treatment was 23.8 cP, combined treatment gelatin was 14.4 cP, and bovine gelatin was 28 cP (Table 5). There is a strong relationship between viscosity and gel strength since as gel strength increases, viscosity increases (Venien & Levieux, 2005). The gelatins that have a higher viscosity are considered high-quality gelatins compare with the produced gelatin (Abo Tor, 1988). Kasankala *et al.* (2007) showed that the viscosity of gelatin could be affected by many factors, such as molecular weight, temperature, and concentration.

Clarity

Clarity is a physical parameter that measures the turbidity and transparency of gelatin. The clarity of the gelatin solution was measured at the same concentration used for measuring gel strength (6.67 %). The turbidity (clarity) of a gelatin solution of gelatin extracted by alkali treatment was 126 NTU, gelatin extracted by combined treatment had 86 NTU, and bovine gelatin had 49 NTU (Table 5). Turbidity or clarity of liquid or gel loses due to the presence of suspended particulates. The more total suspended solids in the liquid, the higher turbidity (Silva *et al*., 2014).

Foaming properties

The foam height and time to reach 45 ml of all gelatins have been recorded, as shown in Table (5). The highest height recorded in bovine gelatin was 58 cm (7:32; min: sec to reach 45 ml height) followed by alkali treatment gelatin (53 cm, 3:08) and the least foam height found in combined treatments gelatin (49cm,6:09). Ahmad &Benjakul (2011) showed that excessive increases in charge reduce foam stability by reducing protein-protein interactions and preventing the formation of an elastic film at the air-liquid interface.

Melting point

The melting point of bovine gelatin was 34.3ºC, combined treatment was 32.6ºC, and alkali treatment gelatin was 33.1ºC (Table 5). Melting temperature depends on gel strength, gelatin concentration, purity, and pH of gelatin (Choi & Regenstein, 2000). For a given gelatin concentration at a fixed pH, as gelatin gel strength (Bloom) increases, the melting temperature decreases (Ktari *et al*., 2014).

Table 5:Physical properties of gelatin

Property		Treatment*		
		Combined	Bovine	Alkali
Gel strength (Bloom)		158	89	216
Viscosity (cP)		23.8	14.4	28
Clarity (NTU)		126	86	49
Melting Point (ºC)		33.1	32.6	34.3
Foam	Height (ml)	53	49	58
	Time (Min:sec)	3:08	6:09	7:32

Colour of extracted gelatins

The colour of gelatins can described in different formats, such as the CIE L*a*b* colour scale, XYZ, CIE Lab, and RGB. In the present work, the color of gelatin was measured by Hunter Lab which expressed in terms of CIE L*(chroma), a*(hue) and, b* (value), and absorbance spectrophotometric graphs, as shown in Table (6). All three types of gelatins used in the present study showed a significant ($P \leq 0.05$)difference in L*, a*, and b* values. Gelatin extracted by alkali treatment had 50.17 ± 0.40*, 7.96 ± 0.21* and 6.37 ± 0.02*, values for L*, a*, and b* respectively. Gelatin extracted by combined treatment had 56.80 ± 0.40*, 9.39 ± 0.01* and 6.24 ± 0.02*, respectively, and bovine gelatin had 81.90 ± 0.02*, 2.32 ± 0.01* and 17.45 ± 0.01* respectively. Also, Table **7** show that there is a strong relationship between color intensity and the mineral content of gelatin. The mineral content that showed the most significant ($P \leq 0.05$)differ-

Table 6: Colour analysis of gelatin extracted from octopus's skin*

Gelatin	L*	a*	b*
Alkali treatment	50.17 ± 0.40^{b}	7.96 ± 0.21^{ab}	6.37 ± 0.02^{ab}
Combined treatment	56.80 ± 0.40^{ab}	9.39 ± 0.01^{a}	6.24 ± 0.02^{b}
Bovine (control)	81.90 ± 0.02^{a}	2.32 ± 0.01^{b}	17.45 ± 0.01^{a}

*Mean ± SD

**Different letters in a column indicate significantly different values at $P \leq 0.05$

ence was copper, zinc, and iron. Gelatin with higher mineral content, such as alkali, and combined gelatin treatments had low lightness intensity compared to gelatin with lower mineral content, such as bovine, which had the highest L* lightness intensity. For yellow colour intensity (b*), the yellow colour increases with decreasing mineral content; gelatin with low mineral content has a higher yellow colour intensity, and vice versa (Nixsensor, 2020).

Gelatin extracted by alkali treatment, combined treatment, and bovine gelatin was scanned from 360 nm to 780 nm. Gelatin extracted by alkali treatment looked pink, gelatin extracted by combined treatment looked pink, and bovine gelatin looked yellowish.

Microbiological examination of gelatins

Two samples of gelatin extracted from octopus skin and one sample of bovine gelatin were tested microbiologically. All gelatin samples, were free from *Salmonella ssp* and Coliform bacteria. The total bacteria count was 2.0×10^2 CFU/g for alkali treatment gelatin, 4.0×10^2 CFU/g for combined treatment gelatin, and 2.0×10^2 CFU/g for bovine gelatin. The contamination of microbes may have occurred during and after preparation, which is known as post-contamination. Sabaté *et al*., (2012) stated that post-contamination involves contaminating the final product of gelatin; practicing hygiene during packaging and handling of extracted gelatin is essential for gelatin safety. According to the global market standard, the edible gelatin must have a total count of bacteria below 1.0 × 103 CFU/gm of the sample (Europe Warehouse Products, 2020). So, it is necessary to produce gelatin under strict sanitary conditions (Schrieber & Gareis, 2007). Edible and pharmaceutical gelatins should be free of pathogenic bacteria, with a plate count of not more than 1×10^5 per gram, and conform to the Food and Drug Administration (Jones, 2004).

Applications of gelatin in orange jelly and rosella candy

Orange jelly

Three orange jelly types were prepared using the same ratio of three different gelatin samples. Sensory evaluation showed that orange jelly containing gelatin extracted by combined treatment possessed significantly ($P \leq 0.05$)the highest acceptance. While orange jelly contained bovine gelatin, had the least acceptance, as shown in Table (7).The texture of orange jelly made from combined treatment gelatin was preferable to panelists, followed by alkali treatment gelatin, and the least for bovine gelatin. The texture of orange jelly depends on the gel strength of specific gelatins; gelatin with a low gel strength was more suitable to make good jelly than gelatin with a higher gel strength. The flavour and odour of jelly mainly depend on the type of

Table 7: Sensory evaluation of orange jelly and rosella candy prepared by different types of gelatins*

Rosella candy	Treatment**		
	Alkali gelatin candy	Combine gelatin candy	Bovine gelatin candy
Texture	6.64 ± 0.938^{b}	6.95 ± 0.304^{a}	6.88 ± 0.636^{ab}
Taste	5.62 ± 0.561^{b}	6.25 ± 0.292^{ab}	6.63 ± 0.858^{a}
Odour	6.01 ± 0.463^{b}	5.56 ± 0.601^{ab}	6.25 ± 0.661^{a}
Colour	7.63 ± 0.239^{a}	6.38 ± 0.653^{b}	7.57 ± 0.297^{ab}
Elasticity and stickiness'	6.85 ± 0.835^{ab}	7.43 ± 0.736^{a}	6.44 ± 0.761^{b}
Orange jelly Property	**Treatment****		
	Alkali	**Combine**	**Bovine**
Texture	7.33 ± 0.778^{ab}	8.33 ± 0.778^{a}	6.88 ± 2.003^{b}
Taste	6.67 ± 1.775^{ab}	7.02 ± 1.206^{a}	6.22 ± 2.038^{b}
Odor	6.23 ± 0.673^{ab}	6.37 ± 0.983^{a}	5.12 ± 2.067^{b}
Color	7.17 ± 0.389^{ab}	7.50 ± 0.798^{a}	6.33 ± 2.229^{b}

*Mean ± SD

**Means in a row not sharing the same superscript are significantly ($P \leq 0.5$) different

juice and ingredients used. The taste of jelly can always be modified according to the consumer's needs and desires. Orange jelly made from gelatin extracted by combined treatment was more accepted than that made from alkali and bovine gelatin.

Rosella candy

Three groups of rosella candy were prepared using identical amounts of rosella juice, water, and sugar with a different type of gelatin (Figure 1). Generally, no considerable differences in the degree of acceptance could be noticed among the three groups of candy. Each group of candy was superior in either one or two attributes. Rosella candy prepared from gelatin extracted by alkali treatment performed better in the category of colour, rosella candy prepared from gelatin extracted through combined treatment showed the highest acceptance in terms of texture, elasticity, and stickiness. Rosella candy prepared from bovine gelatin was more accepted by panelists regarding flavour and odour.

Regarding texture, panelists preferred rosella candy prepared from gelatin extracted by combined treatments, followed by rosella candy prepared from bovine gelatin. In comparison, the least acceptable rosella candy is prepared from gelatin extracted by alkali treatment. The candy texture depends on the gel strength of specific gelatin. Gelatin with a higher gel strength was much better for making candies than gelatin with a lower gel strength. Due to its higher gel strength, the rosella candy prepared from bovine gelatin exhibited more rigidity and solidity.

Rosella candy prepared from bovine gelatin was more acceptable to panelists regarding flavour and odour. The colour of the candy depends on the type of juice used to make it. Regarding elasticity and stickiness, rosella candy prepared from bovine was more elastic. Rosella candy prepared from gelatin extracted by combined treatment and alkali treatment had a higher degree of acceptance because it is easily mystified or chewed, which gives good feelings and emotions.

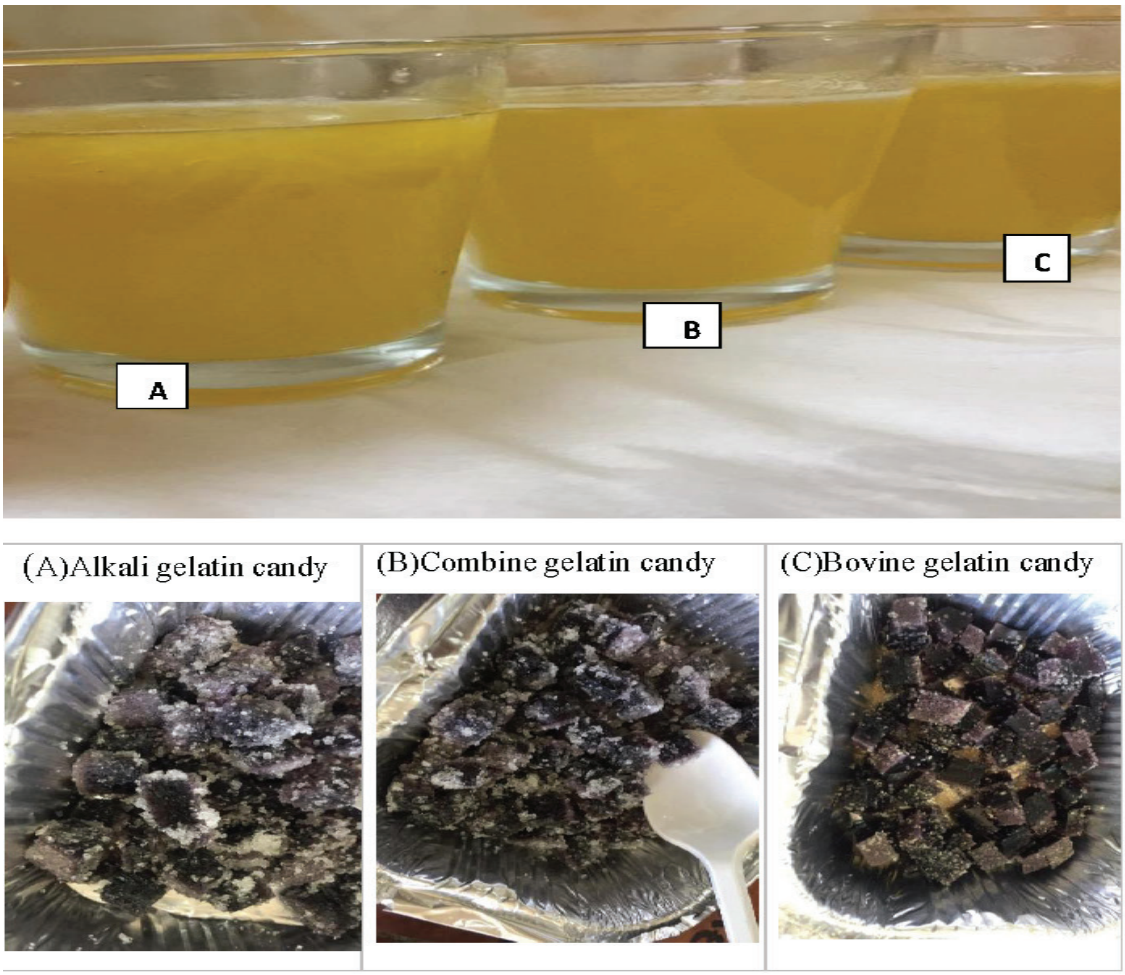

Fig. 1: Orange jelly and candies prepared with (A)alkali treatment gelatin, (B) combined treatment gelatin and (C) bovine gelatin

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جلد الأخطبوط كمصدر للجيلاتين: الإعداد و التقييم و الاستفادة

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الأخطبوط كائن بحري ذو جسم طرى،و يوجد في المناطق الصخرية و الرملية بالبحار على عمق من ١٠٠ إلى ١٥٠ متراً. و تعتبر مخلفات الأسماك و تلك التي لا تتوافر بكميات أو أحجام مناسبة من المنتجات التي لا تسوق، كذلك فإن الأجزاء غير القابلة للأكل مثل الحبر و الأحشاء و الجلود تعتبر ذات قيمة تجارية منخفضة. وفي العديد من البلدان يتم إلقاء هذه الأجزاء كمخلفات. و تعتبر جلود الأخطبوط مصدراً جيداً للجيلاتين.

في هذه الدراسة تم استخلاص الجيلاتين من جلود الأخطبوط بواسطة خمس طرق استخلاص مختلفة هي: الحامض ،القلوي، الجير، الاستخلاص المباشر، الاستخلاص بأكثر من طريقة، وقد تبين أن العائد كان ٧٦, ١٪،٣٢، ١٠٪, ٤٣، ١٪، ١٢، ١٪، ٨٥، ٦٪ على الترتيب. وبالنسبة للصفات الكيماوية والفيزيقية فلقد كان الجيلاتين المستخلص بالقلوي و المستخلص بأكثر من طريقة الأفضل مقارنة بجيلاتين البقر التجاري.

تم استخدام الجيلاتين المستخلص في تصنيع جيلى البرتقال و حلوى الروزيلا، أوضحت نتائج الدراسة أن الجيلاتين المستخلص من جلد الأخطبوط يتميز بصفات فيزيقية وكميائية جيدة مما يجعله بديلاً واعداً للجيلاتين المتحصل علية من الأبقار في تطبيقات غذائية مختلفة،ومن ثم فإن إعادة تدوير جلود الأخطبوط واستخدامها فى تصنيع جيلاتين يمكن تسويقه و استخدامه في التصنيع الغذائي من شأنه التقليل من كميات المخلفات الملوثة للبيئة. فضلاً عن كونه قيمة مضافة من الناحية الاقتصادية.