

## Physicochemical Characteristics of Gelatin Extracted from Catfish (*Clarias gariepinus*) and Carp (*Cyprinus carpio*) Skins

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### ABSTRACT

Gelatins from the skin of two different species of fresh water fish, namely African catfish (*Clarias gariepinus*) and Common carp (*Cyprinus carpio*) have been successfully extracted by different concentrations of sodium hydroxide for variable times (6,8,10hrs) followed by different concentrations of sulphuric acid. The maximum yield of gelatin was observed in carp samples (15.73%) compared to catfish gelatin samples (9.9%). Carp skin gelatin exhibited the best clarity (1.68%) and foam stability (2.04%) compared to catfish skin gelatin (1.90%) whereas, African catfish gelatin possessed the highest fat binding capacity. No significant differences were observed in the gel strength of catfish gelatin and bovine gelatin (235.73g and 252.00g, respectively.) Similarly, no significant differences were observed concerning WHC and foam formation among all tested gelatins. Orange jelly was prepared from bovine and extracted fish gelatins. Results indicated no significant differences concerning the taste, texture and color between bovine and common carp gelatin jelly except for overall acceptance where bovine gelatin recorded the highest score(4.83) followed by common carp gelatin(4.16). None of the organoleptic properties of the tested jellies reached the limit of rejection. The fresh water fishes investigated in the present study may be potential alternative sources of gelatin.

**Key words:** Fish gelatin, Fresh water fish, Extraction and characterization, Alternative resource of gelatin.

### Introduction

Gelatin is an important functional biopolymer widely used in foods to improve elasticity, consistency, and stability. It can be obtained not only from the skin and bones of land animals, but also from fish and insects. In recent years gelatins from fish and edible insects provide an alternative source that is acceptable for halal (Muslim) and kosher (Jewish) products.

Gelatin from marine sources (warm-and cold-water fish skin, bones and fins) is a possible alternative to bovine gelatin (Wassawa *et al.*, 2007).

Gelatin is obtained through hydrolysis of collagen, which is the principal protein found in skin and bones. It is an ingredient widely used in food industry, pharmaceutical, medical, cosmetic and photographic industries due to its unique functional and technological properties (Karim and Bhat, 2009).

Recent reports indicate that the annual world output of gelatin is increasing, especially in Asia, and it is mostly obtained from pig and cow skins and bones (Gomez-Guillen and Montero, 2001; GME, 2008). However, the use of gelatin from those resources is restricted due to the outbreaks of bovine spongiform encephalopathy (BSE) or “mad cow disease” and religious reasons. Therefore, there is an increasing interest in the production of fish gelatin as an alternative for mammalian counterpart (Gudmundsson *et al.*, 2002).

In recent years, extraction and characteristic of gelatin properties has been reported from various sources such as the skins of black tilapia (*Oreochromis mossambicus*) and red tilapia (*Oreochromis nilotica*), Nile perch (*Lates niloticus*) skin and bone gelatin (Muyonga *et al.*, 2004), sin croaker and shortfin scad (Cheow *et al.*, 2007), “kerapu” (*Epinephelus sexfasciatus*), “jenahak” (*Lutjanus argentimaculatus*), “kembung” (*Rastrelliger kanagurta*), and “kerisi” (*Pristipomodes typus*) (Irwandi *et al.*, 2009), carp (*Cyprinus carpio*) (Duan *et al.*, 2011), catfish (Liu *et al.*, 2008) and red tilapia (*Oreochromis nilotica*), walking catfish (*Clarias batrachus*) and striped catfish (*Pangasius sutchi fowler*) (Jamilah *et al.*, 2011).

The major physical properties of gelatin are gel strength and melting point, which are governed mainly by the amino acid composition (proline + hydroxyl proline content), molecular weight distribution and also the ratio of  $\alpha/\beta$  chains in the gelatin (Karim and Bhat, 2009). The amino acid content in a gelatin is dependent on the origin of the raw materials. Many studies have indicated that collagen extracted from warm water fish species contains more amino acids than that of cold water fish (Gudmundsson, 2002). However, the later has weaker gelling properties due to the low content of proline and hydroxy proline compared to the bovine and porcine derived gelatins.

There is very limited information of collagen derived from fresh water fish as an alternative gelatin source. The aim of the present research is to identify the best extraction condition and determine some

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physicochemical characteristics of gelatin extracted from the skin of some fresh water fish (catfish and common carp) as raw materials compared with commercial bovine gelatin found in local market in Alexandria- Egypt.

## Materials and Methods

### Materials:

The raw materials used in the present study are the skins of two cultured freshwater fishes Common carp (*Cyprinus carpio*) and African catfish (*Clarias gariepinus*). Samples were brought from Berciq freshwater farm located in Kafr El-Dawar , El-Behera , Egypt in October 2013.

### Pretreatment of fish skins prior to main extraction

The gelatin extraction procedure followed was essentially as described by Grossman and Bergman (1992) with slight modifications. The cleaned and drained fish skins were given a pretreatment with an alkaline solution followed by an acid solution. Cleaned skins were taken in conical flask and treated with different concentrations of sodium hydroxide (1:6 w/v) for variable times.

The samples were then rinsed with tap water and drained using cheesecloth. The above treatment was repeated for 2 times. The samples were treated with different concentrations of sulphuric acid (1:6 w/v) for variable times. The samples were then rinsed with tap water and drained using cheesecloth. The acid treatment was also repeated two times. The treated samples were squeezed manually using cheesecloth to remove excess water prior to the extraction. The conditions followed for the pretreatment are given in Table(1).

**Table1:** Process variables adopted for the pretreatment of fish skins.

Process variables	Conc.( mol/L)		
NaOH concentration	0.1	0.15	0.2
H2SO4 concentration	0.1	0.15	0.2
Pretreatment time (minutes)	40	50	60

### Gelatin extraction

The pretreated fish skins were taken in flasks for gelatin extraction with varying volumes of deionized water, extraction time and temperature (Table 2). The flasks were covered with parafilm and the extraction was carried out in a water bath. Finally, the gelatin solutions were filtered through 4 layers of cheesecloth, and dried prior to further work.

**Table 2:** Process variables adopted for the extraction of gelatin.

Process variables	Range		
Skin/water ratio	1:4	1:5	1:6
Extraction time	6h	8h	10h
Extraction temperature	40°C	50°C	60°C

### Physicochemical analysis

#### Yield:

Yield of gelatin extracts produced from each fish was determined according to the following equation:

$$\% \text{ yield} = \text{weight of gelatin/weight of skin} \times 100\% \text{ (AOAC, 2000).}$$

*Proximate analysis:* proximate analysis (moisture, ash, protein and fat contents) were carried out according to AOAC (2000).

#### Gelatin color:

Color of gelatin gels were measured based on the method described by Jamilah *et al.* (2011) using a HunterLab MiniScan XE Plus Spectrocolorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA). Samples were read three times and reported as L\*, a\* and b\* parameters indicating lightness, redness/greenness and yellowness/ blueness.

### Determination of amino acid composition

Amino acids were determined according to the methods described by Moore (1958). Sample of 20-25mg was placed in glass hydrolysis tube containing 10 ml of 6N HCL with 0.1% mercaptoethanol . The tube was sealed and heated in an oven at 110°C for 24 hrs. The hydrolyzed sample was then cooled to room temperature and filtered through Whatman No 1 filter paper. The tube and precipitate on the paper was washed with distilled water and the filtrates were then completed to 25 ml in a volumetric flask. Five ml of the filtrate were transferred to a 25 ml beaker and placed under vacuum in a desiccators over potassium hydroxide. The resulted dried residue was dissolved in one ml of sodium citrate buffer of PH 2.2 and stored at 4°C until analyzed by Beckman Amino Acid Analyzer Model 119 CL.

#### *Determination of gel strength*

Gelatin at rate of  $7.50 \pm 0.01$  g was weighed into the Bloom bottle and  $105 \pm 0.2$  ml deionized water was added and stirred (BS 757:1975). For determining the gel strength, the plunger of the Texture Analyzer was set to move a distance 4 mm into the gel with a speed of 0.5 mm/sec. The value given by the Texture Analyzer was the gel strength (BS 757:1975).

#### *Determination of Viscosity*

Gelatin solutions at a concentration of 6.67%(w/v) were prepared by dissolving the dry powder in distilled water and heating at  $60^\circ\text{C}$ . Viscosity (cP) of 10 ml of the solution was determined using Brookfield Digital Viscometer( Model DV E Brookfield Engineering, USA) equipped with a No.1 spindle at  $30 \pm 0.5^\circ\text{C}$  (Cho *et al.*, 2006).

#### *Determination of gelatin pH*

The pH values of raw fish skins and gelatin solutions were measured using 1% (w/v ) solution of gelatin prepared in distilled water at  $60^\circ\text{C}$ , cooled to room temperature and the pH was measured using (Adwa AD1030 PH/ MV & Temperature Meter . Made in Romania, Europe) as described by Varelziz *et al.* (1997).

#### *Determination of setting point and setting time*

The method used for the determination of setting point (SP) and setting time (ST) of gelatin was that described by Muyonga *et al.*, (2004).

#### *Determination of foaming properties*

Foam formation ability (FA) and foam stability (FS) of gelatin were determined by the procedure of Cho *et al.*, (2004). The foam stability was calculated as the ratio of the initial volume of foam to the final volume of foam after 30 min.

#### *Determination of fat binding capacity and water holding capacity*

Fat binding capacity (FBC) and water holding capacity (WHC) were measured as follows :

One g of gelatin powder was placed in a centrifuge tube and weighed. Then, ten ml sunflower oil was added, and held at room temperature for 1 h. During this period, the gelatin solutions were mixed with a Vortex mixer (CM-101 Plus, REMI Instruments, Maharashtra, India) for 5 s every 15 min. The gelatin solutions were then centrifuged at 450 g for 20 min with cylinder bottom centrifuge of 20 ml capacity (Model CPR 24, India). The upper phase was removed by tilting the centrifuge tube to  $45^\circ$  angle and draining on to a filter paper for 30 min. The FBC was calculated as the weight of the contents of the tube after draining divided by the weight of the dried gelatin, and expressed as the weight % of dried gelatin (Cho *et al.*, 2004).

For measuring WHC, one g of gelatin powder was placed in a centrifuge tube and weighed (tube with gelatin). Distilled water (50 ml) was added, and held at room temperature for 1 h. During this period, the gelatin solutions were mixed with a Vortex mixer (CM-101 Plus, REMI Instruments, Maharashtra, India) for 5 s every 15 min. The gelatin solutions were then centrifuged at 450 g for 20 min (Heraeus Multifuge 3SR Plus, Thermo Scientific, MK, Buckinghamshire, England). The upper phase was removed and the centrifuge tube was drained for 30 min on a filter paper after tilting to  $45^\circ$  angle. WHC was calculated as the weight of the contents of the tube after draining divided by the weight of the dried gelatin, and expressed as the weight % of dried gelatin (Cho *et al.*, 2004).

#### *Clarity*

Gelatin at rate of 7.50 g ( $\pm 0.01$ ) was weighed into a 150 ml bottle and 105 ml ( $\pm 0.2$ ) water was added. The absorbance at 620 nm was measured at room temperature against deionized water (ISO 7027:1999).

#### *Determination of gelatin odor*

Oder evaluation was conducted using a seven member expert panel according to the method of Muyonga *et al.*, (2004). The samples were prepared in test tubes with screw caps, by dissolving 0.5 g of gelatin in 7 ml of distilled water, thus obtaining a solution containing approximately 6.67% gelatin. The tubes were then held in a water bath at  $50^\circ\text{C}$  for dissolving, with the screw caps closed. Panelists were instructed to remove the screw caps, sniff the contents and identify the odour they perceived as well as to indicate the odour intensity, using a five point scale. i.e., 0 = no odour, 1 =very mild and only perceivable on careful assessment, 2 = mild but easily perceivable, 3 = strong but not offensive, 4 = strong and offensive, 5 = very strong and very offensive.

**Sensory Evaluation:-****Gelatin Jelly Preparation:**

Gelatin jelly were prepared as described by Zhou & Regenstein, (2007) by dissolving gelatins in a flavored orange juice (prepared from orange flavor instant drink mix, Baity Foods Ltd., Egypt.) heated to 45 – 50 °C. Sensory evaluation of the jelly from bovine and prepared fish skin gelatin were subjected to panelist. Samples were served along with water and unsalted crackers to 5 trained members from the Dept. of Food science. Panelists were instructed to rinse with water and consume crackers after tasting the next samples. Empty cups were provided for expectoration of the samples (Kramer and Twigg, 1970). The panelists received 5 samples at each testing period. Sensory scores for colour, taste, odour, texture and overall acceptance were determined using a five-point hedonic scale as follow: 5 = nondetectable off flavour and 1 = extreme off flavor, 5 = typical fresh colour and 1 = faint colouration and 5 = firm and juicy and 1 = soft and fibrous for texture. (IFT/ SED, 1981). The gelatin jelly prepared had a gelatin concentration of (3%w/w) according to Zhou & Regenstein (2007). The final composition of the jelly is shown in table (3).

**Table 3:** Composition and pH of Gelatin jelly\*

	BG	TG	CG
Gelatin (g)	3.0	3.0	3.0
Water (g)*	87.0	87.0	87.0
Sugar (g)*	9.0	9.0	9.0
Others (g)*	<1.0	<1.0	<1.0
Final pH	3.8	3.6	3.7

\*BG=Bovine Skin Gelatin W(225B); TG= Catfish Skin Gelatin; CG = Common carp Skin Gelatin; The amount of water and sugar are calculated based on the ingredient label of the flavored orange juice.

**Statistical analysis:**

The obtained data were subjected to analysis of variance (ANOVA) an Duncan's multiple range tests to differentiate between treatments means (P<0.05) using SAS program (SAS,1995).

**Results and Discussions****Yield of gelatin**

The yield of gelatin from the different species of fresh water fish are shown on Table (4).The highest yield was obtained from common carp( B2, B3 and B1) being 15.733%, 15.030% and 14.623%, respectively. The yield of gelatin from African catfish were (A2, A3 and A1) were 9.900%, 8.590% and 7.576%, respectively. The aforementioned result were higher than that reported by Jamilah and Harvinder (2002), where the yield of extracted gelatin of red tilapia and black tilapia were 7.81% and 5.39%, respectively; higher than that of sin croacker (14.3%) as reported by Cheow *et al.*, (2007), as well as that of squid (7.5%) as reported by Uriarte *et al.*, (2011). Similarly, it was also higher than those reported by Gomez-guillen *et al.*, (2002) for Sole (7.3%), megrim (7.4%), cod (7.2%) and megrim (6.5%); Muyonga *et al.*, (2004) for young Nile perch (12.5%) and salmon 11.3%; and also for cod (10.1%) as reported by Arnesen and Gildberg (2007).

Those results may be explained as the different kind of skin, acid concentration, pH condition, the rate of collagen break down were among the possible reasons for the high gelatin yield from the two species of fresh water fish. Gomez-Guillen *et al.*, (2001) noted that the different marine species has different structural and physical properties of gelatin. While Jamilah and Harvinder (2002), Songchotikunpan *et al.* (2008), and Tabarestani *et al.*, (2010) suggested that the wide diversity among the fish species present intrinsic differences in the collagen molecules present in their skin. Moreover, the higher susceptibility of the collagenous material from fish skin to degradation is due to the lower content in intra- and interchain non-reducible crosslinks. While Karim and Bhat (2009) noted that the yield and quality of gelatin are influenced by the species and age of the fish, extraction process and pretreatment temperature.

**Table 4:** Yield\* of *Clarias gariepinus* and *Cyprinus carpio* gelatins \*\*

SAMPLE	A1	A2	A3	B1	B2	B3
YEILD	7.576±0.21e	9.900±0.29 c	8.590±0.20 d	14.623±0.62b	15.733±0.32 a	15.030±0.17b

\* Means of triplicates ± S.D.

\*\*A1 *Clarias gariepinus* gelatin extracted at(40°C, 6 hrs, skin/water ratio 1:4), A2 *Clarias gariepinus* gelatin extracted at(50°C, 8 hrs, skin/water ratio 1:5), A3 *Clarias gariepinus* gelatin extracted at(60°C, 10 hrs, skin/water ratio 1:6), B1 *Cyprinus carpio* gelatin extracted at(40°C, 6 hrs, skin/water ratio 1:4), B2 *Cyprinus carpio* gelatin extracted at(50°C, 8 hrs, skin/water ratio 1:5), B3 *Cyprinus carpio* gelatin extracted at(60°C, 10 hrs, skin/water ratio 1:6).

**Color measurement of gelatin by Hunter lab**

The gelatin obtained from the different species of fresh water fish and its appearance are shown in Table (5). The lightness (L\*) value of gelatin extracted from African catfish skin (91.77) and common carp (90.44) were higher compared to the commercial gelatin (61.73). However, a\* (redness) and b\* (yellowness) value of

commercial gelatin was higher than those of later fresh water fish gelatin. Ockerman and Hansen (1999) noted that the appearance of gelatin from striped snakehead visually are close to that of commercial one whereas pangas catfish and Asian redtail catfish gelatin are similar to pig gelatin. The color of the gelatin depends on the raw material. However, it does not influence other functional properties. The lighter color of gelatin may have more commercial satisfaction.

**Table 5:** Color of extracted Gelatin as measured by Hunter Lab from the skin of Catfish and Common carp\*

Fish skin gelatin	L*	a*	b*
Catfish	91.77±0.42	-0.35±0.01	2.24±0.05
Common carp	90.44±0.23	-0.42±0.01	1.81±0.04

\* Results are means ± standard deviation (n = 3). L\* = The lightness a\* = Redness b\* = Yellowness

*Proximate composition of gelatin*

Table (6) and (7) shows the proximate composition of gelatin extracted from two different fresh water fish. Protein content of African catfish A1 ,A2 ,A3 and common carp B1, B2 and B3 were 88.7%; 87.31% and 87.69% and 87.25%; 87.02% and 87.48%, respectively. All were significantly different than that of commercial gelatin (78.9%). Ash content of gelatin from the two different fresh water fishes studied were lower to that suggested by Jones (1997) which reached a maximum of 2.6%; for instance brown stripe red snapper is 1.9% (Jongjareonrak *et al.*, 2006), sin croaker and shortfin scad 1.49% and 1.15%, respectively (Cheow *et al.*, 2007) and Nile perch 0.4% (Songchoticupan *et al.*, 2008). Benjakul *et al.* (2009) noted that high quality of gelatin should contain no more than 0.5% ash. Jongjareonrak *et al.*, (2006) suggest that the high protein content and the less moisture, ash and fat contents are determined by raw material or may be contributed by the residual of chemicals after processing, or also the possibility of mixing with other ingredients.

**Table 6:** Proximate chemical composition\* of *Clarias gariepinus* gelatin\*\* on wet bases (%)

Treatments	Moisture	Lipid	Protein	Ash
A1	8.55±0.40 <sup>a</sup>	0.59±0.01 <sup>b</sup>	88.7±0.88 <sup>a</sup>	1.19±0.02 <sup>a</sup>
A2	8.30±0.12 <sup>bc</sup>	0.52±0.04 <sup>c</sup>	87.31±1.30 <sup>b</sup>	1.20±0.02 <sup>a</sup>
A3	8.22±0.05 <sup>bc</sup>	0.50±0.02 <sup>c</sup>	87.69±0.49 <sup>ab</sup>	1.19±0.02 <sup>a</sup>

\* Means of triplicates ± S.D.

\*\*A1 *Clarias gariepinus* gelatin extracted at(40°C, 6 hrs, skin/water ratio 1:4), A2 *Clarias gariepinus* gelatin extracted at(50°C, 8 hrs, skin/water ratio 1:5), A3 *Clarias gariepinus* gelatin extracted at(60°C, 10 hrs, skin/water ratio 1:6).

**Table 7:** Proximate chemical composition\* of *Cyprinus carpio* gelatin\*\* on wet basis (%)

Treatments	Moisture	Lipid	Protein	Ash
B1	8.41±0.14 <sup>ab</sup>	0.65±0.02 <sup>a</sup>	87.25±0.09 <sup>b</sup>	1.12±0.02 <sup>b</sup>
B2	8.18±0.09 <sup>c</sup>	0.63±0.04 <sup>ab</sup>	87.02±0.17 <sup>b</sup>	1.11±0.03 <sup>b</sup>
B3	8.16±0.16 <sup>c</sup>	0.59±0.01 <sup>b</sup>	87.48±0.60 <sup>ab</sup>	1.10±0.03 <sup>b</sup>

\* Means of triplicates ± S.D.

\*\*B1 *Cyprinus carpio* gelatin extracted at(40°C, 6 hrs, skin/water ratio 1:4), B2 *Cyprinus carpio* gelatin extracted at(50°C, 8 hrs, skin/water ratio 1:5), B3 *Cyprinus carpio* gelatin extracted at(60°C, 10 hrs, skin/water ratio 1:6).

*Amino acid composition*

Amino acid composition of different species of fresh water fish is presented in Table (8). Since the hardness of gelatin gel was in direct correlation with proline (pro) and hydroxyproline (hyp) (Holzer, 1996) content. As can be seen, Glycine and proline found in African catfish gelatin were 21.69g/100g and 11.65g/100g, respectively and it was higher compared to that of common carp which were 19.20g/100g and 2.94g/100g, respectively. Gómez-Guillèn *et al.*, (2002) report that the amino acids composition of gelatin extracted from the skin of sole, megrim, cod, hake and squid had more than 30% Gly and ~17% imino acids. In the present study the glycine content of gelatin in both species ranged between 19.20-21.69g/100g whereas proline & hydroxyproline ranged between 10.38-19.66g/100g. However, Jamilah and Harvinder (2002) report that the proline contents of the gelatins extracted from red and black tilapia is very low and almost undetectable.

Glutamic acid is the third order from amino acid after glycine and proline. In the present study, the glutamic acid of African catfish 13.15g/100g and common carp was 12.96g/100g. On the other hand, alanine in common carp gelatin (3.16 g/100g) was higher compared to that of African catfish (1.81 g/100g). On the contrary, arginine of African catfish gelatin (7.19 g/100g) was higher compared to that of common carp gelatin (5.24 g/100g).

The amino acid composition play a main role in the physical properties of gelatin. The amino acid analysis of gelatin is variable, particularly for the minor constituents, depending on raw material and process used, but proximate values by weight as described by (Stevens, 1992) are:-

glycine 21 %, proline 12 %, hydroxyproline 12 %, glutamic acid 10 %, alanine 9 %, arginine 8%, aspartic acid 6 %, lysine 4 %, serine 4 %, leucine 3 %, valine 2 %, phenylalanine 2 %, threonine 2 %, isoleucine 1 %,hydroxylysine 1 %, methionine and histidine <1% with tyrosine < 0.5 %.

### pH

The pH of the extracted fish gelatins is given in Table (9). The pH varies between 3.99 and 4.07. African catfish gelatin shows significantly higher values ( $p < 0.05$ ) than that of common carp gelatin. The values of pH for gelatin samples in the present study are outside the range prescribed for catfish A gelatin (pH 4.03 - 4.07) and common carp B gelatin (pH 3.99 - 4.03). This may be due to the pretreatment method employed during the extraction process which involves both alkali and acid treatments. Functional properties of gelatins viz., gel strength and melting point are dependent on pH.

Choi and Regenstein (2000) observed that the gel strength of the fish and pork gelatins decreased markedly below pH 4 and slightly above pH 8. For the melting point also similar dependencies were observed in relation to pH. The pH reported for gelatin extracted from the skin of red and black tilapia was 3.05 and 3.91 respectively (Jamilah & Harvinder, 2002).

**Table 8:** Amino acid composition of gelatin from Catfish and Carp skin\*

Amino acid	Catfish gelatin	Carp gelatin	FAO provisional pattern	
Leucine	3.28±0.02	2.07±0.02	9.00	Essential amino acids
Isoleucine	0.17±0.09	0.73±0.05		
Threonine	4.09±0.04	3.42±0.12	2.80	
Valine	3.15±0.07	2.94±0.07	4.20	
Methionine	2.43±0.01	3.25±0.05	2.20	
phenylalanine	1.92±0.01	0.67±0.02	2.80	
Lysine	3.31±0.12	5.15±0.04	4.20	
Histidine	0.69±0.10	0.39±0.11		
Arginine	7.19±0.03	5.24±0.09		
Aspartic acid	2.30±0.03	2.60±0.01		
Serine	6.66±0.08	4.12±0.03		
Glutamic acid	13.15±0.07	12.96±0.05		
Glycine	21.69±0.02	19.20±0.02		
Alanine	1.81±0.28	3.16±0.02		
Proline	11.65±0.04	2.94±0.03		
Ammonia	0.18±0.00	1.04±0.07		
Cystine	1.12±0.04	0.86±0.06		
Tyrosine	4.20±0.02	3.70±0.04		
Hydroxyl proline	8.01±0.72	7.44±0.42	2.80	
Total	96.11±4.61	81.88±6.28		
Proline+ Hydroxyl proline	19.66±0.76	10.38±0.45		

\*Results obtained are means of duplicate readings ± SD.

**Table 9:** PH\* of *Clarias gariepinus* and *Cyprinus carpio* gelatin\*\*

Sample	A1	A2	A3	B1	B2	B3
PH	4.07±0.01 <sup>a</sup>	4.04±0.02 <sup>a</sup>	4.03±0.02 <sup>a</sup>	4.03±0.03 <sup>a</sup>	4.03±0.03 <sup>a</sup>	3.99±0.02 <sup>b</sup>

\* Means of triplicates ± S.D.

\*\*A1 *Clarias gariepinus* gelatin extracted at(40°C, 6 hrs, skin/water ratio 1:4), A2 *Clarias gariepinus* gelatin extracted at(50°C, 8 hrs, skin/water ratio 1:5), A3 *Clarias gariepinus* gelatin extracted at(60°C, 10 hrs, skin/water ratio 1:6), B1 *Cyprinus carpio* gelatin extracted at(40°C, 6 hrs, skin/water ratio 1:4), B2 *Cyprinus carpio* gelatin extracted at(50°C, 8 hrs, skin/water ratio 1:5), B3 *Cyprinus carpio* gelatin extracted at(60°C, 10 hrs, skin/water ratio 1:6).

### Physicochemical Properties of Gelatin

#### Gel strength

The gel strength of African catfish gelatin as shown in Fig. (1) exhibited a significant higher gel strength value (235.73g) than common carp (185.32g). No significant differences were observed between the gel strength of both AG and CG.

The gel strength of African catfish and common carp in the present study were considerably high when compared to those reported in other studies, such as gelatins from red tilapia (128.1 g) , Black tilapia skin 180.7g (Jamilah and Harvinder, 2002), Salmon 108g (Zhou *et al.*, 2006).

Gudmonsson and Hafsteinsson (1997) noted that the gel strength probably depend on the isoelectric point and control of pH. The gel strength of commercial gelatin has a range value of 200-300 g and melting point is >30°C. The gel strength of cold marine species is 100 g or less and melting temperature is <17°C, whereas warm water species is higher than 200 g and melting temperature 24 - 29°C.

#### Viscosity

Viscosity is the second most important commercial physical property of gelatin. Fig (2) shows the different viscosities of the two fish species of fresh water fish compared to commercial bovine gelatin. The viscosity obtained from African catfish and common carp were 6.02 cP and 5.96 cP, respectively with no significant differences between them. These results were significantly less than that of commercial gelatin

(6.77cP). The viscosity increases with increasing gelling temperature, melting temperature, melting point and gel strength (Schrieber and Garies, 2007).

Grossman and Bergman (1992) report that the viscosity of tilapia gelatin, walking catfish and striped catfish are 7.70 cp, 6.28 cp and 8.21cp, respectively. However, Yang *et al.*, (2007) report less viscosity (<3.0 cp) for channel catfish gelatin.

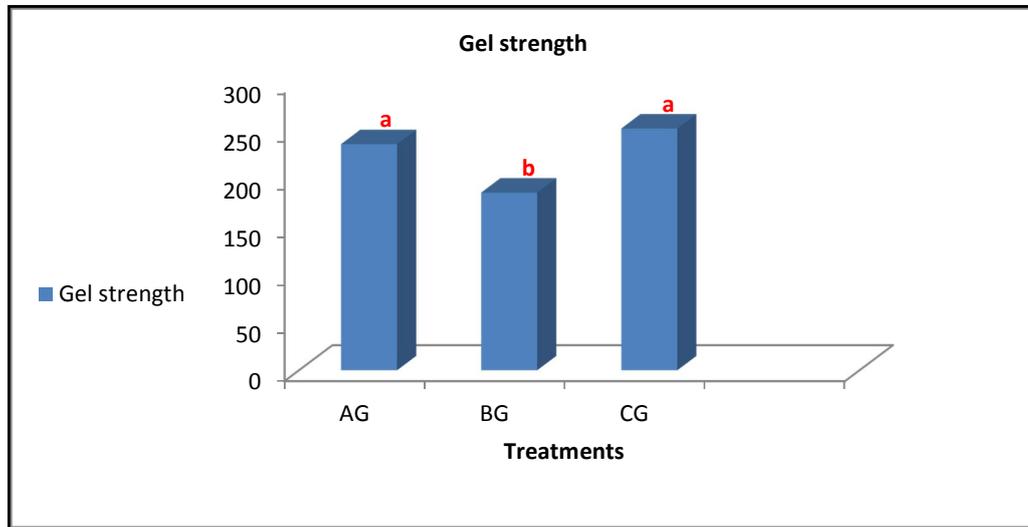


Fig.1: Gel strength of African catfish gelatin, common carp gelatin and mammalian Gelati (\*AG=African catfish gelatin, BG=Common carp gelatin and CG= Commercial gelatin(Bovine)

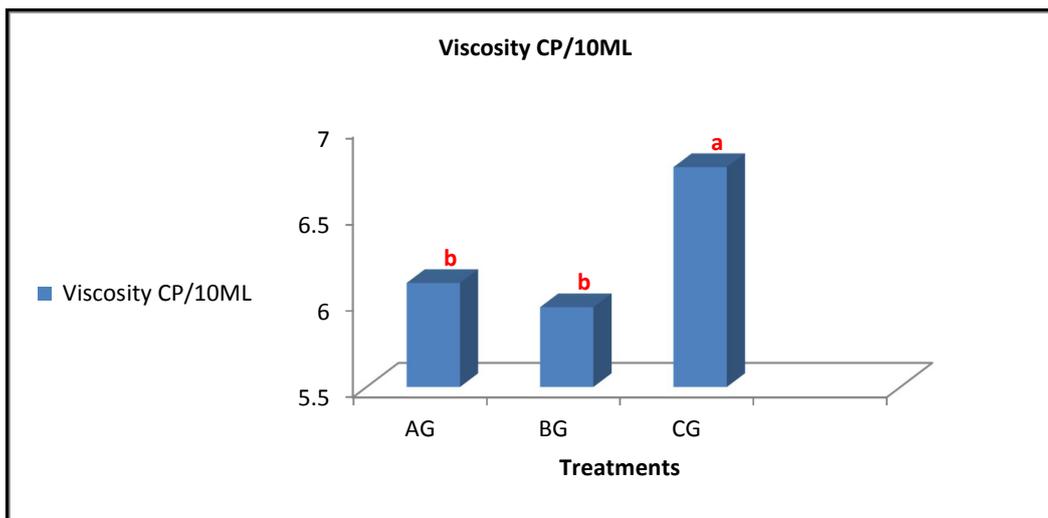


Fig.2: Viscosity of African catfish gelatin, common carp gelatin and mammalian Gelatin (\*AG=African catfish gelatin, BG=Common carp gelatin and CG= Commercial gelatin(Bovine).

*Clarity*

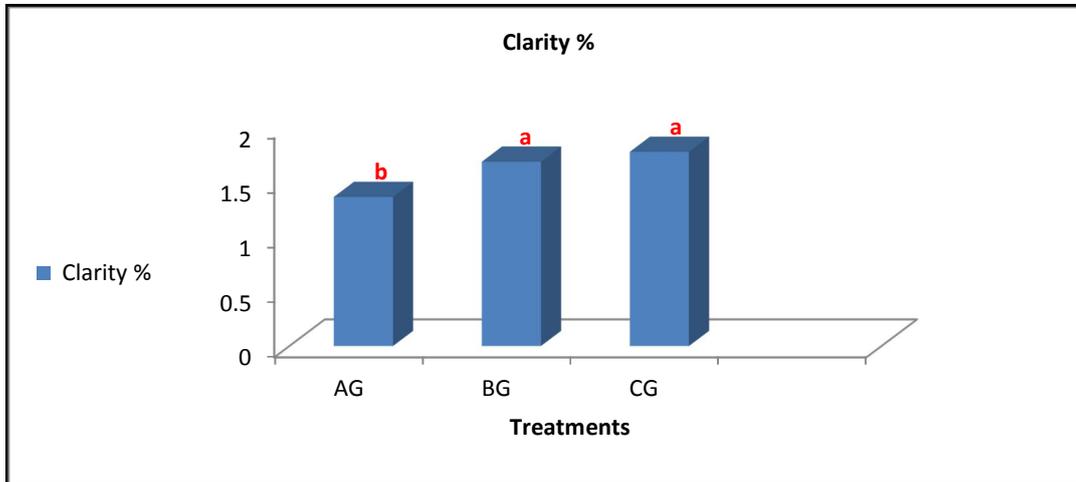
Carp skin gelatins in the present study BG exhibited better clarity than AG. As a matter of fact, no significant differences were observed between gelatin clarity of common carp and bovine gelatin (Fig.3.). High temperature extraction can result in higher molecular weight aggregates which will increase the turbidity of the gel and affect the clarity (Montero *et al.*, 2002). Clarity is important in commercial applications and this property is frequently assessed for determining the quality of gels.

*Setting Temperature and Setting Time.*

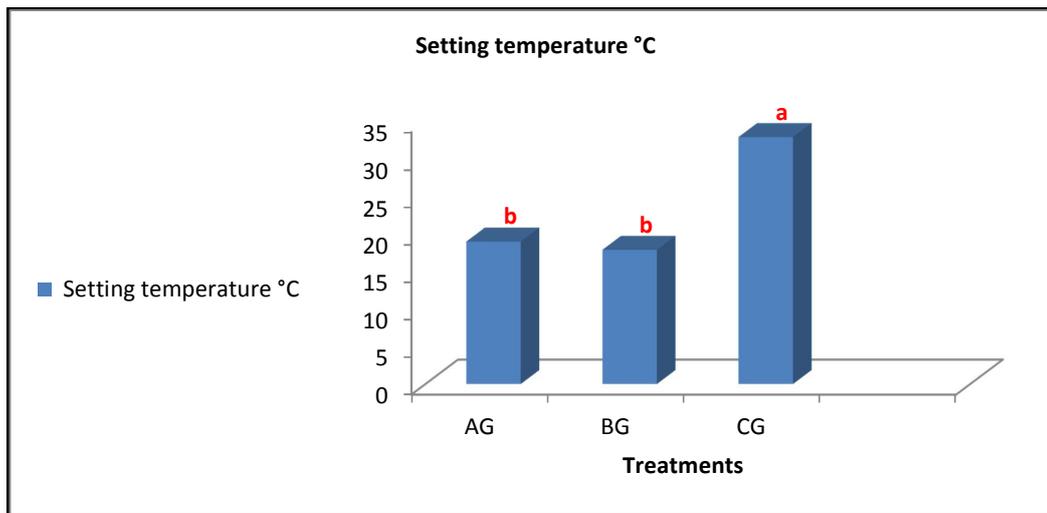
Results of setting temperature (Fig 4) indicated that bovine gelatin exhibited significantly higher setting temperatures (32.94°C) than African catfish gelatin(19.00°C) and common carp skin gelatin(17.90°C).Setting temperature of gelatin has also been found to correlate with the imino acid content which is ~24% for mammals and 16-18% for most fish species (Choi & Regenstein, 2000; Gilsenan & Ross-Murphy, 2000; Gudmundsson,

2002; Leuenberger, 1991). Collagens derived from fish species living in cold environments have lower contents of hydroxyproline and they exhibit lower thermal stability than those from fish living in warm environments. This is because hydroxyproline is involved in inter-chain hydrogen bonding, which stabilizes the triple helical structure of collagen (Darby & Creighton, 1993).

The gel setting time was significantly faster in bovine gelatin (63.96 seconds) compared to common carp and catfish skin gelatins with no significant differences between them (Fig 5). Common carp skin gel and African catfish had a setting time of (103.19) and (106.90) seconds, respectively.



**Fig.3:** Clarity of African catfish gelatin, common carp gelatin and mammalian gelatin Clarity (\*AG=African catfish gelatin, BG=Common carp gelatin and CG= Commercial gelatin(Bovine))



**Fig.4:** Setting temperature of African catfish gelatin, common carp gelatin and mammalian Gelatin (\*AG=African catfish gelatin, BG=Common carp gelatin and CG= Commercial gelatin(Bovine))

*Foaming Formation and Foam Stability*

The foaming formation (ability) and foam stability of African catfish, common carp and bovine gelatins are given in Fig. (6). Results show that there is no significant differences between both fish gelatins in respect to foam formation. The hydrophobic areas on the peptide chain are responsible for giving gelatin its emulsifying and foaming properties (Cole, 2000 and Galazka, *et al.*, 1999). Foam formation of both gelatins of fish species 2.68% & 2.45%, under study showed no significant differences compared to bovine gelatin (2.75%).

Foam stability (Fig7) was significantly higher for common carp (2.04%) than for mammalian and African catfish gelatins being 1.70 and 1.90, respectively. The reduced foam formation and stability may be due to aggregation of proteins which interfere with interactions between the protein and water needed for foam formation (Kinsella, 1977).

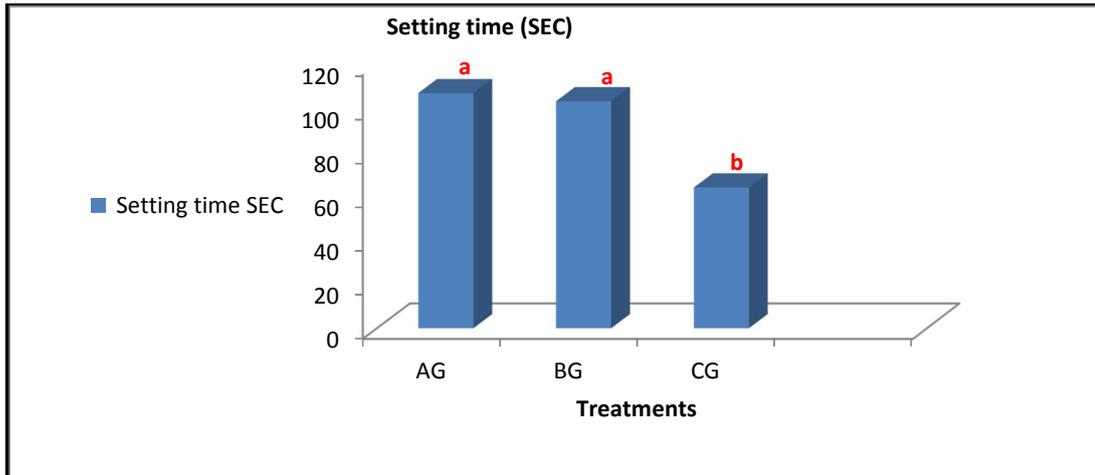


Fig.5: Setting temperature of African catfish gelatin, common carp gelatin and mammalian Gelatin (\*AG=African catfish gelatin, BG=Common carp gelatin and CG= Commercial gelatin(Bovine))

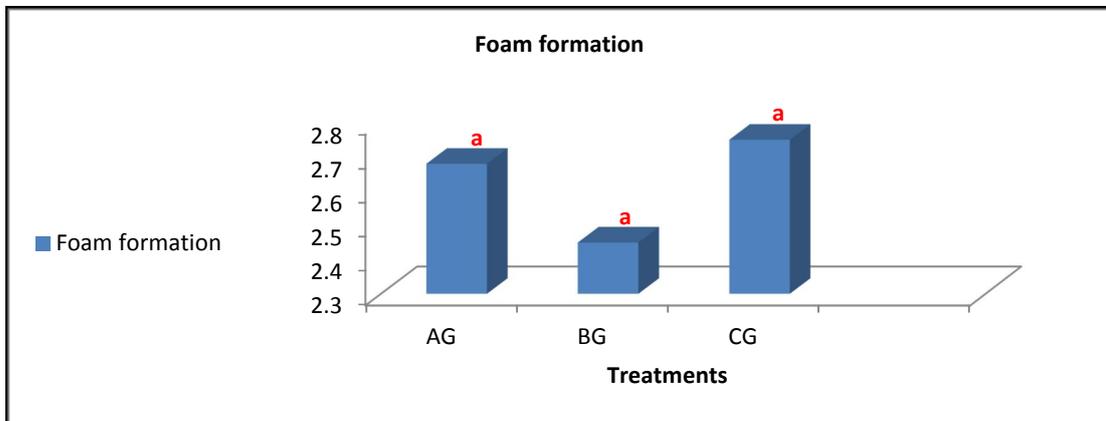


Fig.6: Foam formation of African catfish gelatin, common carp gelatin and mammalian gelatin(\*AG=African catfish gelatin, BG=Common carp gelatin and CG= Commercial gelatin(Bovine))

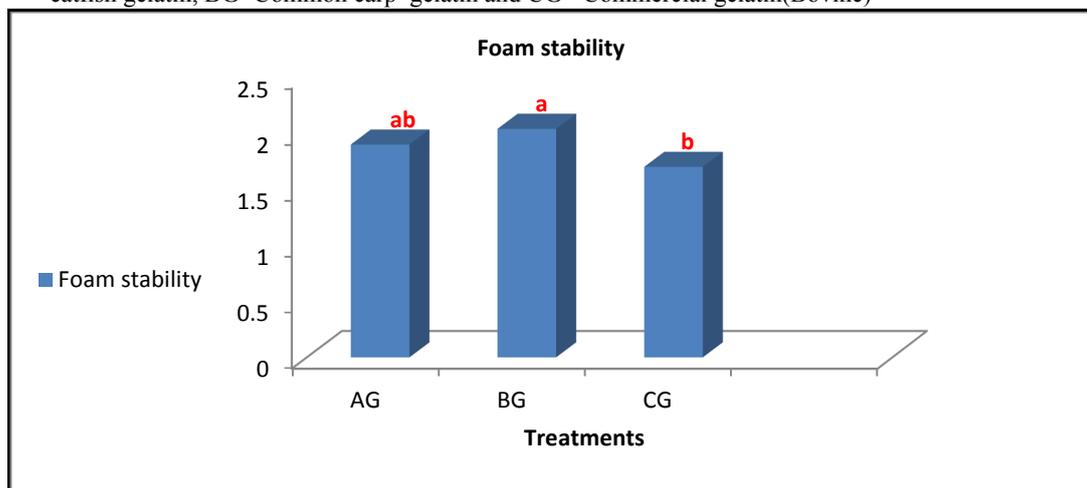
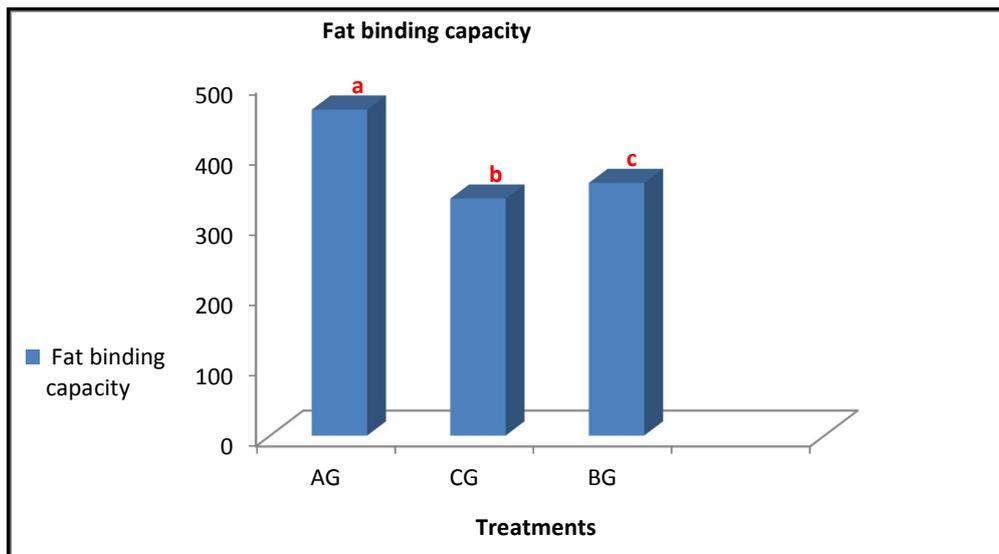


Fig7: Foam stability of African catfish gelatin, common carp gelatin and mammalian gelatin(\*AG=African catfish gelatin, BG=Common carp gelatin and CG= Commercial gelatin(Bovine))

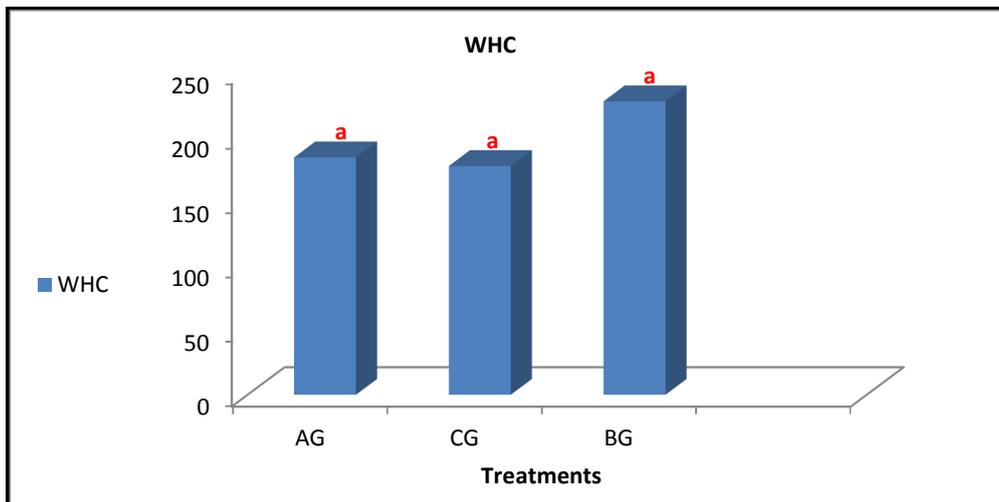
*Fat binding capacity and Water holding capacity*

Fat binding capacity of Common carp, African catfish and mammalian gelatins are given in Figure (8). It can be observed that catfish gelatin exhibited the highest fat binding capacity and was significantly different from both carp and bovine gelatins. Fat binding capacity depends on the degree of exposure of the hydrophobic residues inside the gelatin. The high value of fat binding capacity of African catfish skin gelatin may be due to the highest percentage of hydrophobic residue tyrosine (Cho, *et. al.*, (2004).

Water holding capacity of Common carp, African catfish and mammalian gelatins are given in Figure (9). As a matter of fact no significant differences were observed in the water holding capacity, among African catfish, common carp and bovine gelatins. Water-holding capacity is affected by the amount of hydrophilic amino acids like hydroxyproline. In the present study the highest water holding capacity (227.32 %) was observed for bovine gelatins, this may be due to significantly higher percentage of hydroxyproline of bovine gelatin as compared to both fish species (183.97 and 177), respectively. Water-holding and fat-binding capacities are functional properties that are closely related to texture by the interaction between water, oil and other components.



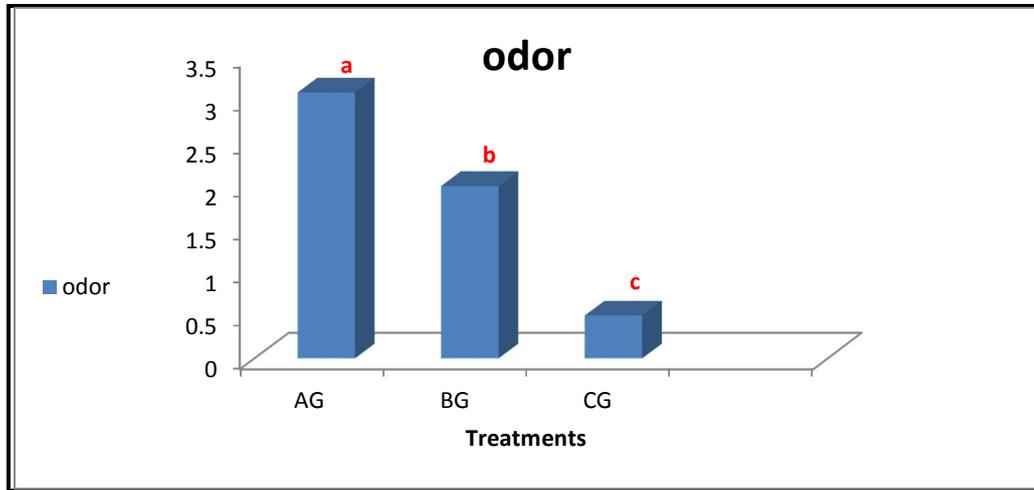
**Fig. 8:** FBC of African catfish gelatin, common carp gelatin and mammalian Gelatin (\*AG=African catfish gelatin, BG=Common carp gelatin and CG= Commercial gelatin(Bovine))



**Fig.9:** WHC of African catfish gelatin, common carp gelatin and mammalian Gelatin (\*AG=African catfish gelatin, BG=Common carp gelatin and CG= Commercial gelatin(Bovine)).

*Odor of different fish gelatins:-*

Odor scores as judged by panelists of both fish gelatins are given in Fig(10). The odor scores were significantly higher for African catfish and common carp skin gelatins (3.08 and 2.00), respectively, indicating that they had a distinguishable fishy odor and hence can be considered as inferior to bovine gelatin in organoleptic qualities. On the other hand, Choi & Regenstein (2000) observed that fish gelatins had less off odor and better aroma than pork gelatins on sensory evaluation.



**Fig.10:** Odor of African catfish gelatin, common carp gelatin and mammalian gelatin (\*AG=African catfish gelatin, BG=Common carp gelatin an CG= Commercial gelatin(Bovine))

*Sensory Evaluation of fish skin gelatin jelly compared to bovine gelatin jelly*

Table (10) and Fig (11) show the orange jelly prepared from both fish and bovine gelatins. Results indicated that panelists could not differentiate between color of different orange jellies prepared with fish skin gelatin and bovine gelatin as there were no significant differences between them. As a matter of fact, odor of the jelly prepared by bovine gelatin was significantly different (4.50) from that prepared from the fish gelatins, but still the later were moderately accepted ( Table10).

As for the taste, fortunately no significant different were observed between the three types of jellies. Taste is the most important sensory attribute. Regarding this aspect, there were no significant difference between all samples, but still the bovine jelly exhibit numerical higher scores (3.83) compared to fish gelatin jellies. Concerning the texture, there were no significant differences between all samples. Both fish gelatin jellies exhibited higher numerical scores compared to bovine jelly being (4.25 and 4.16), respectively. As for the overall acceptance, the bovine gelatin jelly (GAC) and common carp gelatin jelly (GA2), were the most acceptable and the most highly scored, as compared to catfish gelatin jelly (GA1).

However, Choi & Regenstein (2000) noted that flavored fish gelatin dessert gel product had less undesirable off-flavors and off-odors, with more desirable release of flavor and aroma than the same product produced with pork gelatin possessing equal Bloom values, but a higher melting point.

As a matter of fact, none of the sensory parameters reached the level of rejection, this may also assure the fact that fish gelatins can be used successfully in preparing jelly products. Also, the texture of the prepared jellies with different gelatins in the present study showed no significant differences thus emphasizing the fact that fish gelatin in the present study could be used successfully in manufacturing jelly-type products.

**Table 10:** Sensory assessment\* of orange jelly processed from different fish skin gelatins and bovine gelatin

Treatments	Color	odor	taste	texture	acceptance
GAC	4.83±0.40a	4.50±0.54 a	3.83±0.98 a	3.50±1.04 a	4.83±0.25 a
GA2	2.83±0.75 a	3.33±0.51 b	3.00±0.63 a	4.25±0.41 a	4.16±0.40 b
GA1	3.66±0.81 a	3.33±1.21 b	2.75±1.08 a	4.16±0.75 a	3.50±0.54 c

\* Means with different superscripts in a row or column are significantly different at (p<0.01)

GA1 = African cat fish skin gelatin. GA2 = Common carp skin gelatin.

GAC = bovine gelatin.



**Fig.11** : Gelatin jelly sample ( GA1 = African cat fish skin gelatin. GA2 = Common carp skin gelatin. GAC = bovine gelatin).

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