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ORIGINAL ARTICLE

A comparative study on the physical, chemical and functional properties of carp skin and mammalian gelatins

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Abstract Carp species forms the bulk of the aquaculture production in India and studies have shown that the filleting waste of these species, particularly skin can be a good source of gelatin. This study is a comparison of the gelatins from these unexploited sources with that of mammalian gelatins to get a better understanding of their physicochemical and functional properties with respect to mammalian gelatins. The study showed that mammalian gelatins had significantly superior physical properties viz., higher viscosity, melting & setting temperature and faster setting time. The odour scores were significantly higher ($P<0.05$) for bovine and porcine skin gelatins (3.1–3.12), indicating that they had a distinguishable odour and hence can be considered as inferior to fish skin gelatins in organoleptic qualities. The gel strengths of rohu and common carp skin gelatins were significantly lower than mammalian gelatins. Among the carp skin gelatins, grass carp skin gelatin was found to have better compatibility with gelatin from bovine and porcine skins.

Keywords Carp skin gelatin · Mammalian gelatin · Physical properties · Functional properties · Organoleptic quality

Introduction

Gelatin from marine sources (fish skin, bone and fins) has been looked upon as a possible alternative to bovine and porcine gelatin, especially since the outbreak of the Bovine Spongiform Encephalopathy in the 80's. Search for new gelling agents to replace mammalian gelatin led to patents for fish gelatin production (Grossman and Bergman 1992; Holzer 1996) as well as several published methods for fish gelatin production (Gomez-Guillen and Montero 2001; Gudmundsson and Hafsteinsson 1997; Nagai and Suzuki 2000; Arnesen and Gildberg 2002). The commercial interest in fish gelatin has this far, however, been relatively low. This is due to sub-optimal physical properties compared to mammalian gelatin. Common problems connected with fish gelatin from cold water species, representing the majority of the industrial fisheries, are low gelling and melting temperature and low gel modulus (Leuenberger 1991). Fish collagens are of interest to the food processing industry as they are used to produce gelatin which is extracted from the collagen (Jayathilakan et al. 2011) The trash fish, leather jacket is a good source of Type I collagen which has got good thermal properties (Muralidharan et al. 2011).

Warm water fish gelatins however, have properties quite similar to mammalian samples. Gelatin from the skin of yellow fin tuna (*T.albacares*) had a high gel strength (426 Bloom) in comparison with bovine and porcine gelatins while gelling and melting points were lower (Cho et al. 2005). Jamilah and Harvinder (2002) reported bloom strength of 180.8 Bloom for gelatin extracted from black tilapia skin. The gelatin from channel catfish skin showed high gel strength of 276 Bloom (Liu et al. 2008). Similarly gelatin from the skin of grass carp showed high contents of imino acids (proline and hydroxyproline of around 19.47 %) and medium gel strength 267 Bloom (Kasankala et al. 2007). Type A gelatins extracted from skins and bones of young and adult Nile perch had Bloom values of 81–229 and 134–179 g, respectively (Muyonga et al. 2004). George et al. (2010a) has reported that the gelatin extracted from the skin of Rohu and Common carp had Bloom values of 188.6 g and 181.3 g respectively. Carp skin gelatin based films had significantly lower water vapour permeability and

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oxygen permeability than mammalian gelatin films which indicated the superior barrier properties of the latter (George et al. 2010b). These reports indicate that gelatin from fish resources of tropical waters have comparable properties with that of gelatin from mammalian origin. Production and utilization of fish gelatin not only satisfies the needs of consumers, but also serves as a means to utilize some of the byproducts of the fishing industry (Karim and Bhat 2009). The fishery waste generated from the processing of cultured Indian Major Carps can be a potential source for the production of gelatin. Hence the objective of this study is to compare carp skin gelatin with mammalian gelatin with respect to the physical, chemical and functional properties.

Materials and methods

Materials and methods

The fish gelatin samples used for the study were Rohu skin gelatin (RG), Common carp skin gelatin (CG) and grass carp skin gelatin (GG). The mammalian gelatins used were Type A Porcine Skin Gelatin of 300B (G2500) and Type B Bovine Skin Gelatin of 225B (G9382). Fish skin for the extraction of gelatin was collected in fresh condition from the filleting waste of cultured Carps viz., Rohu (*Labeo* rohita), Common carp (*Cyprinus carpio*) and Grass carp (*Ctenopharyngodon idella*). Fish skin gelatins were extracted using the procedure as essentially described by Gudmundsson and Hafsteinsson (1997) with slight modifications as outlined by George et al.(2010a) and freeze dried using a Freeze Drier (Martin Christ, Gamma 1-16 LSC, Germany). The mammalian gelatins were supplied by M/s Sigma Aldrich Inc., St. Louis, USA.

Proximate composition and pH The moisture, protein, fat and ash contents of the gelatin samples were determined by the AOAC (1995) methods. For protein determination, a Nitrogen conversion factor of 5.4 was used as per Eastoe and Eastoe (1952). The pH of gelatin solution was determined using the British Standard Institution method, BSI 757(1975).

Viscosity Viscosity was measured as per the method described by Cho et al. (2006). The viscosity (cP) of 10 ml of the Gelatin solution of 6.67 % (*w*/*v*) was determined using Brookfield digital viscometer (Model DVE Brookfield Engineering Laboratories Inc., Middleboro, MA) equipped with a No.1 spindle at 33±0.2 C.

Sensory evaluation Determination of odour by sensory evaluation was conducted as per the method of Muyonga et al. (2004) using a ten member panel. Only individuals who were able to detect off odour in gelatin samples having a slight putrid odour were selected. Samples for sensory evaluation were prepared by dissolving 0.5 g of gelatin in 7 ml of distilled water, to obtain a solution containing approximately 6.67 % gelatin. The samples were prepared in test tubes with screw caps and dissolved as described for the Bloom samples. The samples were held in a water bath at 50 °C, with the screw caps lightly closed. Panelists were instructed to remove the screw caps, sniff the contents and identify the odour they perceived as well as indicate the odour intensity, using a six point scale (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).

Foam formation ability, foam stability, water-holding and fat-binding capacities Form formation ability (FA), foam stability (FS), Water-holding capacity (WHC) and fat-binding capacity (FBC) of the gelatins were determined based on the methods described by Cho et al. (2004). One gram of gelatin was placed in 50 ml distilled water and swollen. The sample solution was dissolved at 60 °C and the foam was prepared by homogenizing at 10,000 rpm for 5 min in a homogenizer (Euro Turrax, T20 B IKA Labortechnik, Staufen, Germany). The homogenized solution was poured into a 250 ml measuring flask. The foam formation ability was calculated as the volume ratio of foam liquid. The foam stability was calculated as the ratio of the initial volume of foam to the volume of foam after 30 min.

One gram of gelatin was placed in a centrifuge tube and weighed (tube with gelatin). For measuring water-holding capacity and fat-binding capacity, 50 ml distilled water or 10 ml sunflower oil added, respectively, and held at room temperature for 1 h. The gelatin solutions were mixed with vortex mixer for 5 s every 15 min. The gelatin solutions were then centrifuged at 450 g for 20 min in a REMI Cooling centrifuge (Model CPR 24, REMI Instruments, Maharashtra, India). The supernatant was filtered with Whatman No. 1 filter paper and the volume recovered was measured. The difference between the initial volume of distilled water/sunflower oil added to the gelatin sample and the volume of the supernatant was determined, and the results were reported as ml of water/oil absorbed per gram of gelatin sample.

Melting point, setting point and setting time Determination of melting point was based on the method by Wainewright (1977). Gelatin solutions 6.67 % (*w*/*w*) were prepared and a 5-mL aliquot of each sample was transferred to a small culture test tube of 12×75 mm. the samples were degassed in vacuum chamber (Heraeus vacutherm—Germany). The tubes were then covered with parafilm and heated in a water bath (Julabo TW 20, Germany) at 60 °C for 15 min. It was then cooled immediately in ice chilled water and matured at

10 °C for 16–18 h. Five drops of a mixture of 75 % chloroform and 25 % red dye were placed on the surface of the gel. The gels were then put in a water bath (circulating bath—Haake D3) at 10 °C and the water heated at the rate of 0.2 °C per minute. The temperature at which the drops began to move freely down the gel was taken as the melting point.

The method used for the determination of setting point and setting time of gelatin was that described by Muyonga et al. (2004). Gelatin solutions of 10 % (*w*/*w*) were prepared in thin wall (12 mm×75 mm) test tubes in the same way as described for the Bloom samples. The dissolved samples from the warm water bath were transferred to another water bath held at 40 °C (circulating bath—Haake D3). The bath was then cooled slowly at the rate of 0.2 °C per minute. A thermometer was inserted into the sample and lifted out at 30 s intervals. The temperature of the mixture at which the gelatin solution no longer dripped from the tip of the thermometer was recorded as the setting temperature. Setting time was determined on samples prepared in the same way as those for the determination of the setting temperature. Samples were transferred to a water bath maintained at 10 °C (circulating bath—Haake D3). A rod was inserted in the gelatin solution and raised at intervals of 15 s. The time at which the rod could not detach from the gelatin sample was recorded as the setting time.

Statistical analysis

All data were analysed using the analysis of variance (ANOVA) and Duncan's multiple test were carried out to determine the significance difference between the means. Statistical package used in the study was SAS, Version 6 (1989). All the data represented are the means of triplicates.

Results and discussion

Proximate composition and pH The proximate compositions and pH of gelatins are given in Table 1. Crude Protein content was significantly higher ($P<0.05$) for grass carp skin gelatin and mammalian gelatins The crude protein content reported for fish skin gelatin from different sources is in the range of 87–89 % (Jongjareonrak et al. 2006; Muyonga et al. 2004) . Moisture content in all the samples were below 10 % which is less than the limit prescribed for edible gelatin i.e. 15 % (GME 2008). The ash content in all the samples were in the range of 0.97–1.18 %, much less than the recommended maximum limit of 2. % set for edible gelatin (GME 2008). The pH varies between 4.05 and 4.42 in fish skin gelatins. Grass carp gelatin shows significantly higher values for pH ($P<0.05$) than the other two gelatins. The values of pH for gelatin samples are outside the range prescribed for Type A Gelatin and Type B Gelatin. This is because the pretreatment method employed during the extraction process involves both alkali and acid treatments. In mammalian gelatins, porcine skin gelatin has a pH of 7.5 since it is a Type A gelatin and bovine skin gelatin has a pH of 5.02 since it is a Type B gelatin.

The main difference in the amino acid profile of carp skin gelatins and mammalian skin gelatins is the lower imino acid content in the latter. The imino acid content in carp skin gelatin ranges from 19.16 % to 20.86 % whereas for bovine and porcine skin gelatin it is 22.91 and 23.7 % respectively (George et al. 2010a). Overall, fish gelatins have lower concentrations of imino acids (proline and hydroxyproline) compared to mammalian gelatins, and warm-water fish gelatins (big eye-tuna and tilapia) have a higher imino acid content than cold-water fish (cod, whiting and halibut) gelatins (Eastoe and Leach 1977). Four amino acids viz., glycine, proline, hydroxyproline and alanine account for two out of every three amino acid residues in mammalian gelatins (Balian and Bowes 1977). These four amino acids accounted for 63.51 % and 62.59 % of the total amino acid residues in bovine and porcine skin gelatins respectively whereas for carp skin gelatins the corresponding values were less than 50 %, except in the case of grass carp skin gelatin which had 52.46 % of the above mentioned amino acids (George et al. 2010b). The stability of the collagens and gelatins is also proportional to the glycine content, apart from total imino acid content (Lehninger et al. 1993).

Studies by George et al. (2010b) has shown grass carp gelatin had gel strength of 230.2 Bloom which is comparable to the reported value for bovine skin gelatin (227.2 B). It was also reported that the bloom values of rohu and common carp skin gelatins were 188.6 and 181.3 Bloom respectively which was significantly lower than mammalian gelatins.

Table 1 Proximate composition and pH of carp skin and mammalian gelatins

	Rohu	Common carp	Grass carp	Porcine	Bovine
Moisture (%)	8.1±0.12^{a}	8.5±0.11^{b}	7.2±0.20^{c}	8.4±0.20^{bd}	8.5±0.20^{bd}
Protein (%)	90.4±0.70^{a}	89.8±0.59^{a}	91.2±0.20^{ab}	91.3±0.14^{b}	91.2±0.15^{b}
Lipid (% dwb)	0.57±0.071^{a}	0.62±0.063^{b}	0.41±0.031^{c}	0.78±0.050^{d}	0.41±0.032^{c}
Ash (%)	1.2±0.04^{a}	1.1±0.02^{b}	1.1±0.07^{c}	1.02±0.04^{d}	0.97±0.072^{d}
pH	4.1±0.04^{a}	4.1±0.06^{a}	4.4±0.04^{b}	7.5±0.03^{c}	5.0±0.03^{d}

Results are presented as mean± standard deviation of $n=3$. Values within a row with different superscripts are significantly different ($P<0.05$)

Viscosity Among the gelatin samples, maximum viscosity was noted for porcine skin gelatin (7.89 cP) followed by grass carp gelatin (7.07 cP). Bovine skin gelatin and grass carp skin gelatin had similar values for viscosity. Rohu skin gelatin and common carp skin gelatin had significantly lower values of viscosity ($P<0.05$) compared to the other three samples (Fig. 1a). Viscosity is partially controlled by molecular weight and molecular size distribution (Sperling 2006). The viscosities of most of the commercial gelatins have been reported to be in the range of 2.0 to 7.0 cP for most gelatins and up to 13.0 cP for specialized ones (Johnston-Banks 1990).

Melting point The melting point of the gel samples are illustrated in Fig. 1(b). Mammalian gelatins showed significantly higher ($P< 0.05$) melting points (32.2–32.6 °C) than carp skin gelatins. Among the carp skin gelatins, Grass carp skin has the highest melting point (29.1 °C) followed by Rohu (28.1 °C) and Common carp (28.2 °C). The melting points of carp skin gelatins were found to be higher than those reported for many other fish species viz., 8–10 °C for cod skin gelatin (Gudmundsson and Hafsteinsson 1997); 24.3 °C for yellow fin tuna gelatin (Cho et al. 2005); 21.4–26.5 °C for Nile perch gelatin (Muyonga et al. 2004); 22.5–28.9 °C for tilapia skin gelatin (Jamilah and Harvinder 2002). The melting temperature of gelatin has been found to correlate with the proportion of the imino acids proline and hydroxyproline (both with a 5-membered pyrrolidine ring) in the original collagen (Ledward 1986; Piez and Gross 1960; Veis 1964). The imino acid content of grass carp gelatin was maximum (20.80 %) followed by common carp (19.50 %) and rohu (19.49 %) gelatins whereas the imino acid content of bovine and porcine skin gelatin is 22.9 and 23.7 % respectively (George et al. 2010b). The

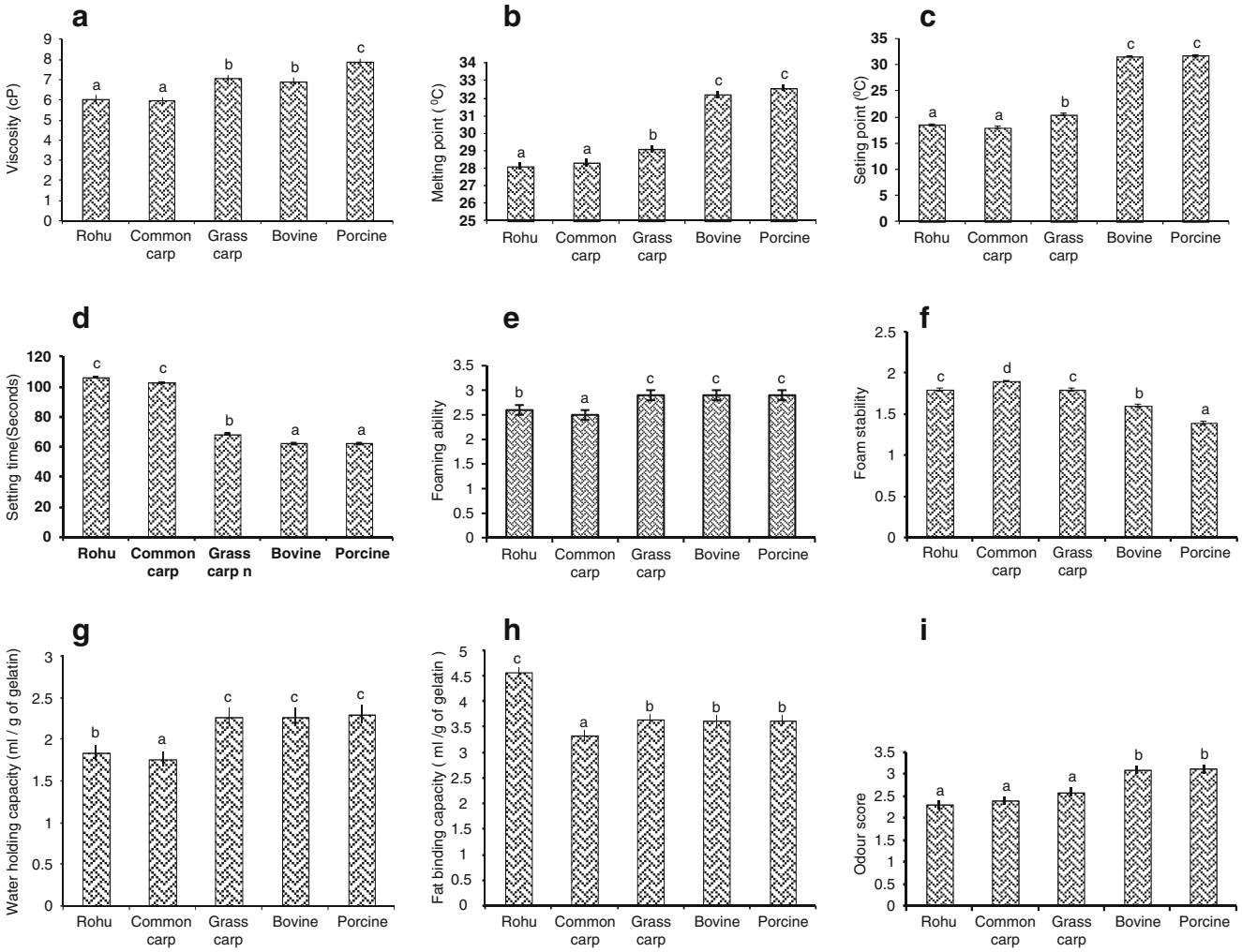

Fig. 1 Physico-chemical quality characteristics of gelatins from different mammalian and carp skins ($n=3$). Results are presented as mean± standard deviation of $n=3$. *Bars* super scribed with different letters are significantly different ($P<0.05$)

melting point values correspond to the imino acid content in the samples. Gomez-Guillen et al. (2002) correlated the thermal stability of gelatin to the number and stability of Proline rich region in collagen or gelatin molecules, which are high in fresh warm water fish and mammalian species.

Setting point and setting time Setting point and setting time of the gels are given in Fig. 1 (c) & 1 (d) respectively. Mammalian gels have significantly higher setting temperatures (31.6–31.8 °C) than carp skin gelatins. Common carp had the lowest setting temperature (17.9 °C) and the highest was for Grass carp (20.5 °C). Also the Grass carp gel showed a significantly faster setting time ($p<0.05$) of 68.6 s when compared to the other two gels. Muyonga et al. (2004) reported a setting temperature of 19.5 °C and a setting time of 60 s for the gelatin from the skin of adult Nile perch extracted at 50 °C which is similar to the values observed for Grass carp skin gelatin. Gudmundsson (2002) compared the rheological properties of fish gelatins (tuna, tilapia, cod and megrim) with conventional bovine and porcine gelatins. The gelling (setting) and melting points of tilapia gelatin (18.2 °C and 25.8 °C respectively) were the highest among the fish gelatins and was comparable to low molecular weight porcine and bovine gelatins. The gel setting time was significantly faster for mammalian gels. Grass carp skin gel had a setting time of 68.6 s which is comparable to mammalian gels. Bovine and porcine gelatins have considerably higher gelling and melting points than most fish gelatins, and the high gelling and melting points expand the range of gelatin application. Setting temperature of gelatin has also been found to correlate with the imino acid content which is typically ~24 % for mammals and 16–18 % for most fish species (Choi and Regenstein 2000; Gilsenan and Ross-Murphy 2000; Gudmundsson 2002; Leuenberger 1991).

Foaming ability and foam stability The foaming ability and foam stability of carp skin and mammalian gelatins are given in Fig. 1 (e) & 1 (f) respectively. Grass carp skin and mammalian gelatins exhibited better foam formation abilities (2.9) than Rohu and Common carp skin gelatin. The hydrophobic areas on the peptide chain are responsible for giving gelatin its emulsifying and foaming properties (Cole 2000; Galazka, et al. 1999). Foam stability was significantly higher for mammalian gelatins (1.4–1.6) than for carp skin gelatins (1.8–1.9). 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).

Water holding and fat binding capacities Water holding and fat binding capacities of carp skin and mammalian gelatins are given in Fig. 1 (g) & 1 (h) respectively. Rohu skin gelatin had the maximum fat binding capacity (4.57 ml/g) and common carp skin gelatin had the minimum water-holding capacity (1.76 ml/g). No significant differences were observed in the water holding and fat binding capacities of Grass carp and mammalian skin gelatins. Water-holding and fat-binding capacities are functional properties that are closely related to texture by the interaction between components such as water, oil and other components. Fat binding capacity depends on the degree of exposure of the hydrophobic residues inside gelatin . Rohu skin gelatin had the highest percentage of hydrophobic residue tyrosine (George et al. 2010b) among the five gelatins which could explain its higher capacity for fat binding. Water-holding capacity is affected by the amount of hydrophilic amino acids like hydroxyproline. In this study highest water holding capacity (2.27–2.30 ml/g) was observed for Grass carp skin and mammalian gelatins since these had significantly higher percentage of hydroxyproline (George et al. 2010b).

Sensory evaluation Odour score of the gels is given in Fig. 1 (i). The odour scores were significantly higher ($P<0.05$) for bovine and porcine skin gelatins (3.1–3.12) than carp skin gelatins, indicating that they had a distinguishable odour and hence can be considered as inferior to fish skin gelatins in organoleptic qualities. Choi and Regenstein (2000) observed that fish gelatins had less off odour and better aroma than pork gelatins on sensory evaluation. They 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. Muyonga et al. (2004) has reported that the gelatins prepared from the skin and bone of Nile Perch were found to be free of fishy odour and to have a mild putrid odour with a mean hedonic score of 2–2.5 with activated carbon treatment. Strong fishy odour was reported for freeze dried gelatin prepared from the skin of black tilapia (Jamilah and Harvinder 2002). Gudmundsson and Hafsteinsson (1997) reported that for gelatin extraction from cod skin, the odor was absent or barely detectable if sulphuric acid and sodium hydroxide were used in low concentrations. Grossman and Bergman (1992) have developed a method that can make the gelatin odorless.

Conclusion

A comparative study of mammalian skin gelatins and carp skin gelatins showed that mammalian skin gelatins had significantly higher viscosity, melting & setting temperature and faster setting time than carp skin gelatins. The odour scores were higher for mammalian gelatins, indicating that they had a distinguishable odour and hence can be considered

as inferior to carp skin gelatins in organoleptic qualities. Foam formation ability was similar for mammalian and Grass carp skin gelatins and mammalian skin gelatins exhibited significantly better foam stability than carp skin gelatins. No significant differences were observed in the water holding and fat binding capacities of Grass carp and mammalian skin gelatins. Among the carp skin gelatins, grass carp skin gelatin was found to have better compatibility with gelatin from bovine and porcine skins.

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