Fishery Technology 50 (2013) : 126 - 132

Effect of pH and Ionic Strength on Functional Properties of Fish Gelatin in Comparison to Mammalian Gelatin

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Abstract

Owing to socio-cultural and safety concerns, fish gelatin is considered to be a potential alternative to mammalian gelatin. The purpose of the current study was to investigate how functional properties of fish gelatin are influenced at different environmental conditions such as pH and NaCl concentrations. The functional properties of gelatin extracted from tiger-toothed croaker (TTC) (*Otolithes ruber*) and pink perch (PP) (*Nemipterus japonicus*) skin were examined at different pH (2 - 10) and salt (2 -10% of NaCl) concentrations in comparison to porcine (PG) and bovine gelatin (BG). Functional properties, in general, were found better in mammalian gelatin compared to fish gelatin in all the pH and ionic strength conditions. However, emulsifying capacity of TTC was found higher compared to PG, especially in acidic conditions. In acidic and high NaCl concentration (8-10%) conditions, the water holding capacity of both fish and mammalian gelatin was found similar. Though the fish gelatin showed lower bloom strength, it was found quite stable to variations in pH (2 - 10) compared to mammalian gelatin.

Keywords: Fish gelatin, emulsifying capacity, foaming property, water holding capacity, gel strength

Received 28 June 2012; Revised 02 February 2013; Accepted 26 February 2013

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Introduction

In the food industry, gelatin is used as an ingredient to achieve multiple functions such as gelation, emulsification, foaming, water holding and stability. Significant amounts of gelatin are being used in desserts, candies, bakery products, ice cream and dairy products. However, the quality of food grade gelatin and its application in food systems depends on its functional properties. Most commercial gelatins are derived from by-products of mammalian sources. Due to religious sentiments and safety concerns against mammalian gelatin, there has been intense interest in gelatin derived from fish (Schrieber & Gareis, 2007; Ninan et al., 2009; 2010 and 2011). While mammalian gelatins have been extensively studied for their functional properties at different conditions, such studies of fish gelatin are limited (Grossman & Bergman, 1992; Gudmundsson & Hafsteinsson, 1997; Ninan et al., 2009; 2010 and 2011). The functional properties of protein preparations depend, not only on factors connected with the preparations themselves, such as kind of protein, type of preparation and the way by which it has been produced, but are also influenced by environmental factors (Hermmansson & Akesson, 1975; Binsi et al., 2009). For many food applications, good functional properties are required, and these could be attained by altering the pH and salt concentrations.

As reviewed by Asghar & Henrickson (1982), electrolytes have a decisive influence on the biophysical properties (swelling, solubility, gelation, viscosity and water-binding capacity) of protein at different ionic strengths and pH levels (Hermmansson, 1975). One possible means of manipulating the characteristics of a given gelatin is to trigger interactions by the addition of salts (Elysee-Collen & Lencki, 1996). Salt ions may either bind directly to the peptide backbone of collagen, or affect collagen folding indirectly by interacting with structurally bound water molecules.

The effect of different salts on the functional properties of mammalian gelatins is well known (Harrington & Von Hippel, 1961). Gelatin is surface-active and is capable of acting as an emulsifier in oil-in-water emulsions (Lobo, 2002). Though the occurrence of bovine spongiform encephalopathy (BSE) and foot/mouth diseases led to the increasing interest of fish gelatins, it is surprising to note that so far fish gelatin has not found wide spread application in the food industry. Recently, functional characteristics of gelatin extracted from skin and bone of tiger-toothed croaker (*Otolithes ruber*) and pink perch (*Nemipterus japonicus*) have been evaluated and it has been reported that their skin is a prospective source to produce gelatin in good yield with desirable functional properties comparable to commercially available mammalian gelatins (Koli et al., 2012).

The purpose of the current study was to investigate how functional properties of fish gelatin are influenced by different environmental conditions such as pH and NaCl concentrations. The effects of pH and ionic strength on emulsification, foaming, water holding capacities, gel strength and melting point of gelatins extracted from tiger-toothed croaker (TTC) and pink perch (PP) were studied in comparison to the properties of commercially available porcine (PG) and bovine gelatins (BG).

Materials and Methods

Commercial gelatins *viz*., bovine skin and porcine skin gelatin (type B) were purchased from Raymon Patel Gelatin Private Limited, Vasad, Gujarat, Code R- 185007-06, having pH values of 5.2 and 5.5 respectively. Fish skin gelatin was extracted from tiger-toothed croaker (TTC) (*Otolithes ruber*) and pink perch (PP) (*Nemipterus japonicus*) skin following the procedure described by Gudmundsson & Hafsteinsson (1997) with some modifications. This method was optimized and the parameters that gave best yield were, sodium hydroxide concentration (0.20%); sulphuric acid concentration (0.20%); citric acid concentration (1.0%); pre-treatment time (40 min); skin/water ratio for pre-treatment (1:7); extraction time (18 h); extraction temperature (45°C); skin or bone/ distilled water ratio for extraction (1:3); drying temperature (70°C) and drying time (18 h). The resulted fish gelatin is of type B having pH 4.5.

The gelatin samples for studying the gel strength and melting point were prepared by adjusting the pH values to 2, 4, 6, 8 and 10 with either 1.0 M HCl or 1.0 M NaOH using a pH meter (Macro-pro pH meter) by maintaining the final gelatin concentration as 6.67% (w/v). To study the effect of ionic strength, the samples were prepared in 2, 4, 6, 8 and 10% (w/v) NaCl solutions. All the tests were done in triplicates. For determining other functional properties, the pH values of different gelatin samples were adjusted to 2, 4, 6, 8 and 10 with either 1.0 M HCl or 1.0 M NaOH using a pH meter (Macro-pro pH meter). Solutions of 2, 4, 6, 8 and 10% (w/v) NaCl, gelatin samples were dissolved (6.67 % w/w) and used to study the effect of ionic strength on functional properties of gelatins.

Emulsifying capacity and stability were assessed by the method of Yasumatsu et al. (1972) and the method of Miller & Groniger (1976) was used to determine foaming properties. Water holding capacity (WHC) was determined using the centrifugation method (Diniz & Martin, 1997) and the results are reported as ml of water absorbed per gram of gelatin sample. Gelatin gel was prepared in bloom jar (150 ml capacity) by dissolving 6.67% (w/v) dry gelatin powder in distilled water at 60°C and its bloom value (gel strength) was determined according to the method described by Wainewright (1977) using TA.XT2i Texture Analyzer (Stable micro systems, Godalming, Surrey, U.K) according to BS 757 (BSI, 1975). The melting point was measured by a method described by Wainewright (1977).

Results and Discussion

The amphoteric nature and hydrophobic zones on the peptide chain give gelatin, limited emulsifying and emulsion-stabilizing properties. Emulsifying capacity (EC) and stability (ES) of different gelatins at different pH conditions are shown in Fig. 1. Emulsifying capacity of BG was the highest (65.10%) at pH 8, followed by TTC, PG and PP. Emulsifying stability was also highest in BG 55.96% at pH 8, followed by PG, TTC and PP. However, emulsifying capacity of TTC was found higher compared to PG, especially in acidic conditions indicating its potential for use in acidic food emulsions. The mechanism to generate the emulsion system is attributed to adsorption of peptides on the surface of freshly formed oil droplets during homogenization and formation of a protective membrane that inhibits coalescence of the oil droplet (Dickinson & Lorient, 1994). Another advantage of proteins as emulsifiers in foods is their ability to protect lipids from iron catalyzed oxidation (Hu et al., 2003). At pH values below their isoelectric point (pI), proteins form positively charged interfacial membranes around oil droplets, which electro-statically repel any Fe^{2+} and Fe^{3+} ions present in the aqueous phase, thereby preventing oxidation of polyunsaturated lipids within the droplets (Surh et al., 2005). As the gelatin

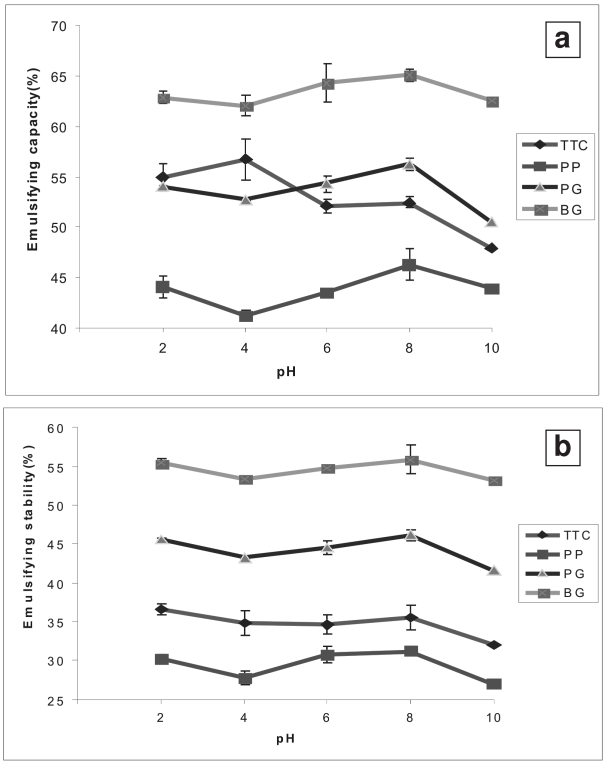


Fig. 1. Influence of pH on the emulsifying capacity (a) and emulsifying stability (b) of gelatins (TTC- tiger-toothed croaker skin gelatin; PP- pink perch skin gelatin; PG-porcine skin gelatin; BG- bovine skin gelatin) at different pH levels

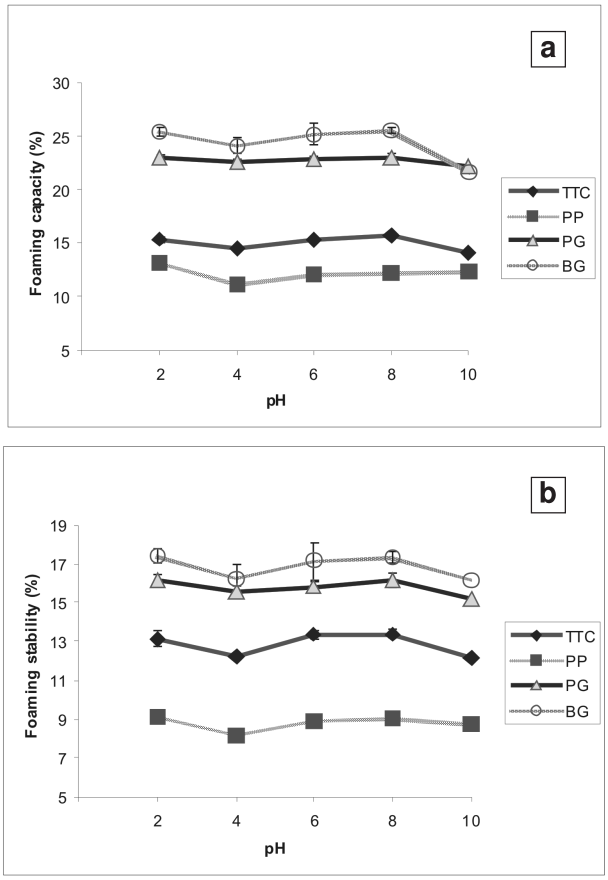


Fig. 2. Influence of pH on the foaming capacity (a) and foaming stability (b) of gelatins (TTC- tiger-toothed croaker skin gelatin; PP- pink perch skin gelatin; PG-porcine skin gelatin; BG- bovine skin gelatin) at different pH levels

extracted from TTC belongs to Type B (Koli et al., 2012) with pH value ~5, it can be utilized in acidic food systems to protect unsaturated lipids by providing cationic interfacial membrane.

Foaming capacity (FC) and stability (FS) of different gelatins at different pH levels are shown in Fig. 2. Foaming capacity and foaming stability of BG were the highest (25.23% and 17.43%), followed by PG, TTC and PP. Mammalian gelatin showed better FC and FS than fish gelatin at all the pH levels. Here also, TTC exhibited better characteristics compared to PP. FS depends principally on the nature of film and reflects the extent of protein-protein interaction within the matrix (Mutilangi et al., 1996). The maximum water holding capacity (WHC) of fish gelatin was at pH 2 to 3. The WHC of all gelatin types decreased with increasing pH levels (Figure 3). Unlike other functional properties, fish gelatin exhibited better WHC than mammalian gelatin, especially in acidic pH ranges. The results give scope for fish gelatin as a potential alternative to mammalian gelatin, particularly in acidic food systems requiring high WHC. Mammalian gelatin showed higher bloom strength than fish gelatin at all pH levels. Bovine gelatin has the highest bloom strength value of 350.83 g at pH 6, followed by PG with 345.42 g (Fig. 4a). Though the fish gelatin (both TTC and PP) showed lower bloom strength, it was found quite stable to variations in pH (2 to 10) compared to inconsistent mammalian gelatin, which varied between 180 and 350 g. The stability over a fairly wide pH range is very useful for food applications of gelatin. Further, the ability to form weak gels may find new applications for fish gelatin as "non-gelling" gelatins and it could possibly be used in refrigerated products and in products where low gelling temperatures are required (Gudmundsson, 2002).

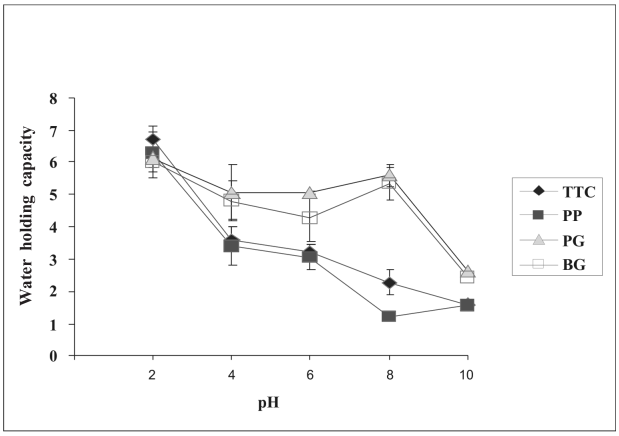


Fig. 3. Influence of pH on the water holding capacity of gelatin (TTC- tiger-toothed croaker skin gelatin; PP- pink perch skin gelatin; PG-porcine skin gelatin; BG- bovine skin gelatin) at different pH levels

Bovine gelatin showed the highest melting point of 28.66 (at pH 6), followed by PG, PP and TTC (Fig. 4b). All the four gelatins displayed the highest values at pH 6 and lowest melting points at extreme pH levels. In general, mammalian gelatins show higher melting points than fish gelatin (Karim & Bhat, 2009). The low melting temperatures of fish gelatin gels have been reported to possess melt-in the mouth property with better release of aroma and stronger flavour during mastication (Choi & Regenstein, 2000). The fish gelatin gels with low melting points of 15 to 18°C at different pH levels may provide a good mouth feel leading to pleasant sensation. Emulsifying capacity (EC) and stability (ES) of different gelatins at different salt concentrations are shown in Fig. 5. Emulsifying capacity of BG was the highest showing 64.53% at 2% NaCl concentration followed by TTC, PG and PP. At low (2 and 4%) NaCl concentrations, TTC has more EC than PG, whereas PP has better EC at 8%.

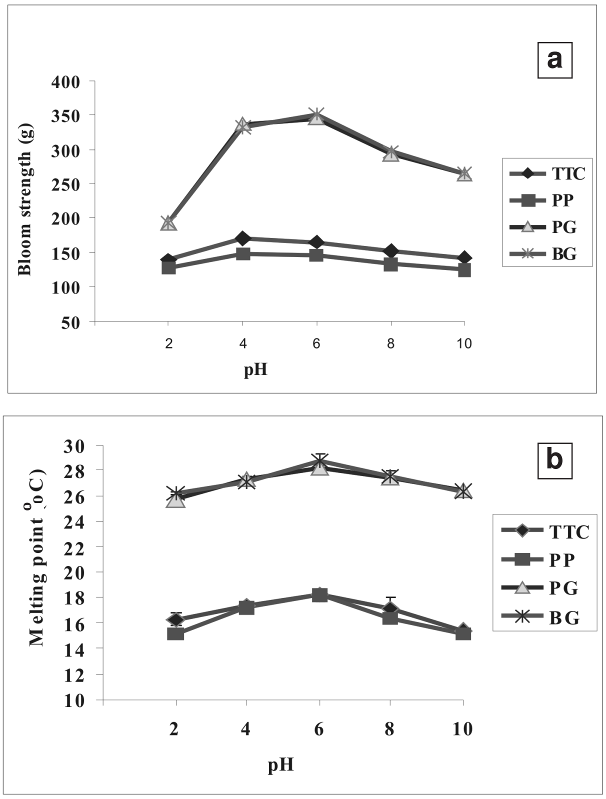


Fig. 4. Influence of pH on the bloom strength (a) and melting point (b) of gelatins (TTC- tiger-toothed croaker skin gelatin; PP- pink perch skin gelatin; PG-porcine skin gelatin; BG- bovine skin gelatin) at different pH levels

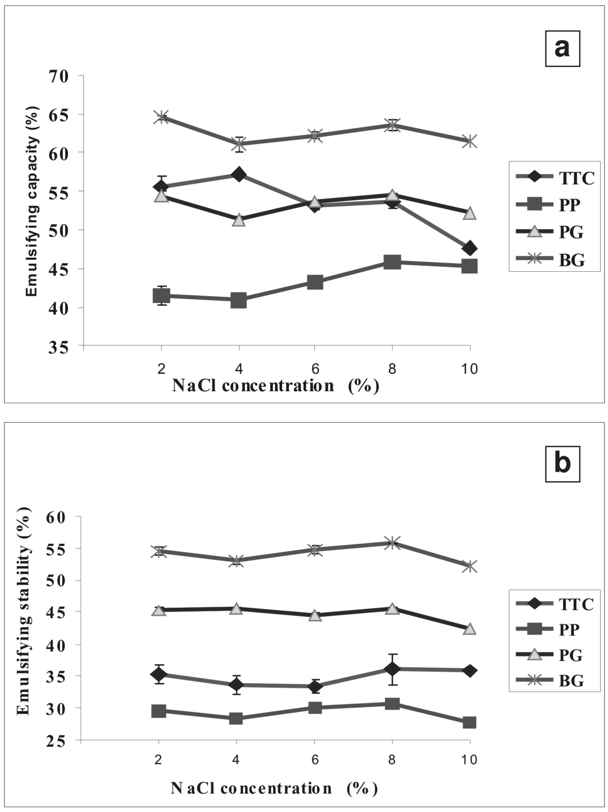
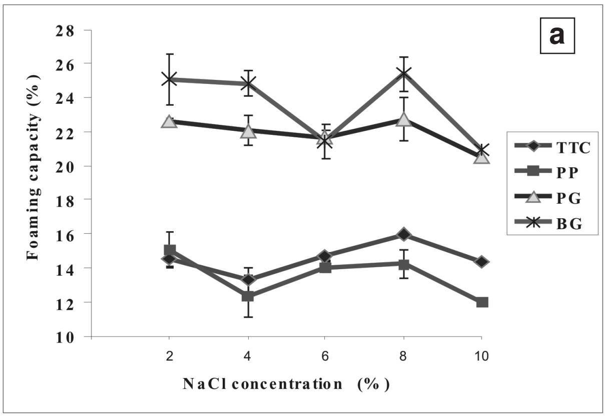


Fig. 5. Influence of NaCl on the emulsifying capacity (a) and emulsifying stability (b) of gelatins (TTC- tiger-toothed croaker skin gelatin; PP- pink perch skin gelatin; PG-porcine skin gelatin; BG- bovine skin gelatin) at different NaCl concentrations

Emulsifying stability was also the highest in BG 55.80% at all the salt concentrations, followed by PG, TTC and PP. Overall, mammalian gelatin exhibited better EC and ES than fish gelatin. All gelatin types showed consistent emulsification properties at different ionic strengths demonstrating their suitability for varied salt concentrations.

Foaming capacity (FC) and stability (FS) of different gelatins at different NaCl concentrations are shown in Fig. 6. Foaming capacity of BG was the highest (25.40%), followed by PG, PP and TTC. At 8% ionic concentration, the fish gelatins showed maximum FC and FS. Low concentrations of salt enhance protein stability whereas high concentrations decrease it. Since foaming capacity appears to be due to solubilized protein, the differing effects of salt concentrations may be explained on this basis. At high ionic strength, foaming properties become depressed because ions may reduce the coulombic forces between poly-peptide chains in the protein molecules (Altschul & Wilcke, 1985).



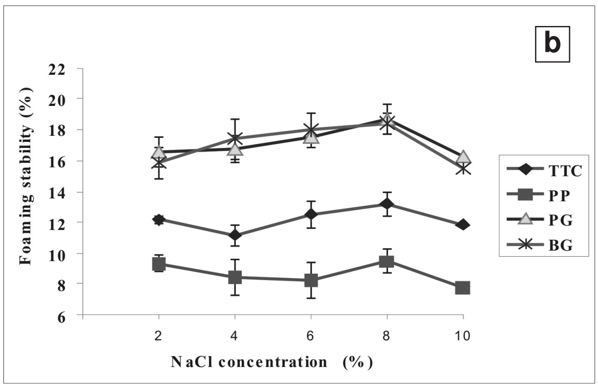


Fig. 6. Influence of NaCl on the foaming capacity (a) and foaming stability (b) of gelatins (TTC- tiger-toothed croaker skin gelatin; PP- pink perch skin gelatin; PG-porcine skin gelatin; BG- bovine skin gelatin) at different NaCl concentrations

Mammalian gelatin exhibited better FC and FS than fish gelatin at all ionic concentrations. Foam formation is generally controlled by transportation, penetration and reorganization of protein molecules at the air-water interface. A protein must be capable of migrating rapidly to the air-water interface, unfolding and rearranging at the interface to express good foaming ability (Halling, 1981). Foam stability mainly depends on the extent of protein-protein interactions within the matrix of the gels surrounding the air bubbles (Mutilangi et al., 1996). The higher foaming activity of mammalian gelatins may be related to their higher ability to establish protein-protein interactions *via* hydrogen bonds, resulting in denser network that favour foam stabilization. Foam stability also has been related to the flexibility of protein or peptide structure (Klompong et al., 2007). The water holding capacity of all gelatin types decreased with increasing ionic strength (Fig. 7) showing maximum WHC at 2%. BG showed maximum water holding capacity, followed by PG, PP and TTC.

Mammalian gelatin showed higher bloom strength than fish gelatin (Fig. 8a). Pig gelatin exhibited the highest bloom value of 210.23 g at 2% NaCl, followed by BG with 193.36 g. It was also observed that all gelatin types exhibited better bloom strength at this ionic strength (2%) and gradually it decreased

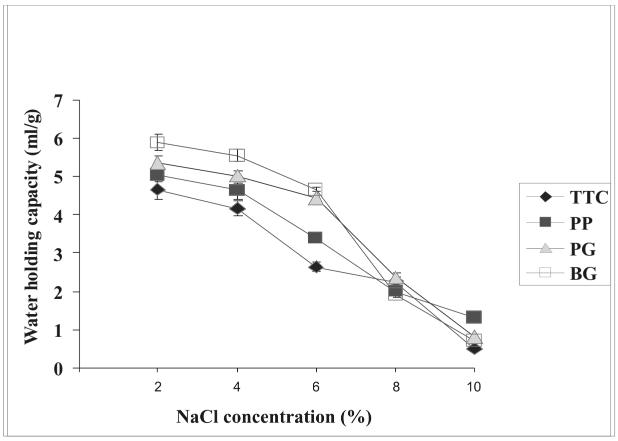


Fig. 7. Influence of NaCl on the water holding capacity of gelatins (TTC- tiger-toothed croaker skin gelatin; PP- pink perch skin gelatin; PG-porcine skin gelatin; BG- bovine skin gelatin) at different NaCl concentrations

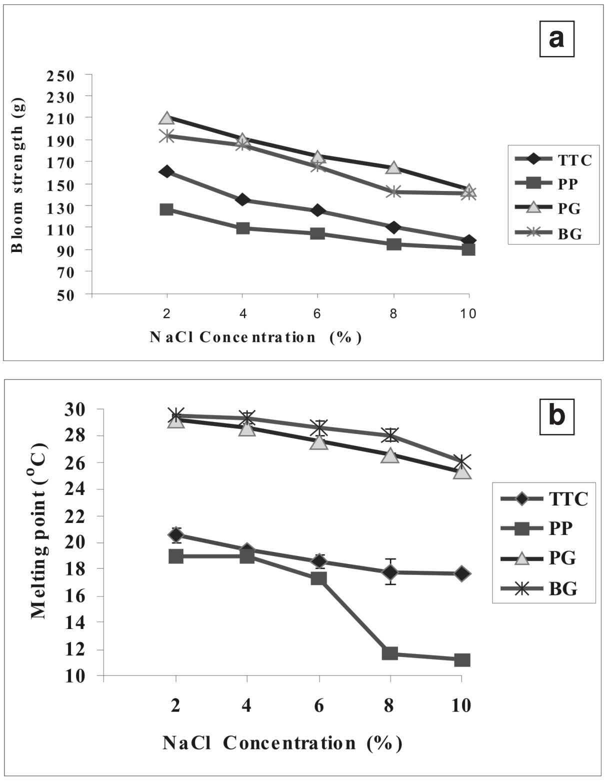


Fig. 8. Influence of NaCl on the bloom strength (a) and melting point (b) of gelatins (TTC- tiger-toothed croaker skin gelatin; PP- pink perch skin gelatin; PG-porcine skin gelatin; BG bovine skin gelatin) at different NaCl concentrations

with increasing ionic concentrations. These decreases caused by NaCl are ascribed to the fact that NaCl is capable of breaking both hydrophobic and hydrogen bonds, thus presumably preventing the stabilization of the gel junction sites, either directly by preventing hydrogen bond formation and/or by modifying the structure of the liquid water in the vicinity of these sites (Finch et al., 1974). Compared to mammalian gelatins, fish gelatins were found more sensitive to NaCl concentration. Bovine gelatin showed the highest melting point of 28.66°C (at 2%), followed by PG, TTC and PP (Fig. 8b). Higher melting points of mammalian gelatin gels were maintained at all ionic concentrations. As ionic strength increased, melting point decreased correlating with their gelation ability. Fish gelatin with lower melting temperature, as discussed in the above section, may be useful in product development to control the texture and flavour release during mastication.

On the basis of the present study, it was concluded that the fish (tiger-toothed croaker and pink perch) gelatin and mammalian gelatin (porcine and bovine) exhibited considerable differences in their functional properties at different pH and salt levels. Fish gelatin showed higher emulsifying capacity in acidic conditions, comparable water holding capacity as mammalian gelatin in acidic food environment and stable gel strength at different pH conditions demonstrating its suitability for diverse food applications. Compared to mammalian gelatin, fish gelatin showed low melting point in all the situations which may offer melt-in the mouth property and extend its utility. These results may be useful for finding new applications of fish gelatin extending the gelatin market to some religious groups that currently do not accept commercially available mammalian gelatin.

Acknowledgements

The authors would like to acknowledge the support rendered by Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, College of Fisheries, Shirgaon, Ratnagiri and Ulka Seafood Pvt. Ltd., Taloja, Panvel, Mumbai.

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