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ORIGINAL PAPER

Enhancement of Functional Properties of Wami Tilapia (*Oreochromis urolepis* hornorum) Skin Gelatin at Different pH Values

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Abstract The effects of several agents in two different concentrations and pH values (5.0 and 8.0) on the functional properties of tilapia (*Oreochromis urolepis* hornorum) skin gelatin were evaluated and compared using a control tilapia skin gelatin and a commercial mammalian gelatin. The addition of the agents (sucrose 4 % and 8 % (*w*/*v*), glycerol 5 % and 10 % (*v*/*v*), NaCl 0.3 and 0.8 mol/L, $MgCl_2$ 0.3 and 0.8 mol/L, $MgSO_4$ 0.3 and 0.8 mol/L, KCl 0.3 and 0.8 mol/L, and transglutaminase 10 and 15 mg/mL) slightly increased the turbidity. There were different ratios of rheological properties depending on the agent, concentration, and pH. The addition of all agents increased the viscosity of the gelatin solution, mainly at pH 5.0. The addition of glycerol (10 % (*v*/*v*)) raised viscosity up to 7.45 cP. The setting time was prolonged by incorporating the agents. The gelatin samples with the addition of MgSO4 0.8 mol/L showed higher gel strength than the mammalian gelatin, exhibiting values of 298 and 295g_f at pH 5.0 and 8.0, respectively.

Keywords Tilapia · Skin · Gelatin · Agents · Functional properties

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Introduction

The fish and fisheries industries produce large amounts of wastes worldwide, including rejects, discards, and by-products. Although a part of this discard is reprocessed, several tons still end up as waste, requiring disposal (Arvanitoyannis and Kassaveti 2008; Arvanitoyannis and Ladas 2008). However, over the past few years the interest in fish by-products has gradually increased. These by-products are excellent raw material for the preparation of foods with high protein content (Gómez-Guillén et al. 2002; Boran et al. 2010) and high collagen content, which can be used for manufacturing gelatin (Johns and Courts 1977). This alternative exploitation can reduce costs, thereby maximizing the industries' profits and minimizing environmental pollution problems.

Collagens of warm water fish, e.g., tilapia species, contain higher amino acid contents (proline and hydroxyproline) than that of cold water fish (Gudmundsson and Hafsteinsson 1997), hence giving gelatins better functional properties (Leuenberger 1991; Gilsenan and Ross-Murphy 2000). One of this species is Wami tilapia (*Oreochromis urolepis* hornorum), native from the Wami River system in Africa (Nagl et al. 2001) which, regarding this aspect, has been marginally studied thus far.

The increasing interest in the use of fish gelatin has emerged with advanced technology for the substitution of mammalian gelatin in food production (Gildberg et al. 2002;

Mohtar et al. 2010). Although the gelatins of mammals and poultry have been extensively studied, little is known about fish gelatins (Norland 1990; Gudmundsson and Hafsteinsson 1997; Sarabia et al. 2000), but such interest has increased (Alfaro et al. 2009; Boran et al. 2010; Aewsiri et al. 2011). Recently, gelatin from red tilapia skin (*Oreochromis niloticus*) was evaluated as a substitute for mammalian gelatins in the production of microparticles for the encapsulation of essential fatty acids (Bueno et al. 2011). However, one of the main restrictions concerning the use of fish gelatin refers to its poorer rheological properties when compared to mammalian gelatin (Choi and Regenstein 2000; Cho et al. 2004; Sai-Ut et al. 2011), which in turn reduces its applications (Leuenberger 1991).

The gels, in solution, are formed when the gelatin molecules are partially reorganized, acquiring a collagen-like structure (Johns and Courts 1977). This gelling process involves a structural re-arrangement to form a gelatin triple helix structure (Gudmundsson and Hafsteinsson 1997; Montero et al. 2002; Jongjareonrak et al. 2010). This transition temperature is called gelling point; in the same way, the melting point is related to the denaturation point of this structure. The gelling and melting temperatures of gelatin are dependent on the proline and hydroxyproline ratios of the original collagen molecule (Ledward 1986; Gilsenan and Ross-Murphy 2000; Haug et al. 2004), and on the pretreatment that is used (Montero et al. 2002; Duan et al. 2011).

Gelatin modifier substances, such as salts, glycerol and enzymes, can be applied to improve rheological properties of gelatin (Fernández-Díaz et al. 2001; Aewsiri et al. 2011). Electrolytes, in general, influence the biophysical properties (swelling, solubility, gelling, viscosity, and water retention capacity) of proteins, depending on the ionic force and pH of the system (Asghar and Henrickson 1982). Non-electrolytes, such as sugar and glycerol usually increase the strength of gelatin gels (Fernández-Díaz et al. 2001).

It is known that the addition of different salts influences the melting temperature and rigidity of gelatins obtained from warm-blooded animals (Harrington and von Hippel 1962). Therefore, salts can be used to induce interactions in gelatins, modifying its characteristics (Elysée-Collen and Lencki 1996). Cross-linking agents can be also used to improve the rheological properties of gelatins. Among them, glutaraldehyde is a good alternative due to its reactivity with the amino group and its low cost. However, its toxicity has been previously reported (Chiou et al. 2006). Another possibility is the use of the transglutaminase enzyme, which forms covalent cross-linking between glutamine and lysine residues, making the molecule structure more stable.

The purpose of this work was to study the influence of some agents (salts, transglutaminase, glycerol, and sucrose) at different pH values (5.0 and 8.0) on the functional properties (turbidity, gel strength, gelling point, melting point, time of gel formation, and viscosity) of the gelatin extracted from wami tilapia skin.

Materials and Methods

Raw Material and Chemicals

Fresh skins of filleted wami tilapia (*Oreochromis urolepis* hornorum) with average weight of 700±100 g were obtained from a company located in Pato Branco, Paraná, Brazil. The skins were stored at −18 °C until its use (approximately 24 h later). Mammalian gelatin (MG) type A was purchased from Vetec, Rio de Janeiro, Rio de Janeiro, Brazil. The following ingredients were used: transglutaminase enzyme (transglutaminase + lactose + maltodextrin, 100 U/g, ACTIVA MP®, Ajinomoto, Limeira, São Paulo, Brazil), glycerol (Merck, São Paulo, São Paulo, Brazil), sucrose and the salts: NaCl, $MgCl_2$, $MgSO_4$, and KCl (Synth, São Paulo, São Paulo, Brazil). All reagents used were of analytical grade (P.A.).

Pre-treatment and Gelatin Extraction

The gelatin extraction was carried out according to defined parameters previously established elsewhere (Alfaro et al. 2009). The skins were washed in running water to remove the adhered surface material and cut in segments of approximately 4×4 cm. Next, the material was immersed in NaCl 0.2 % (*w*/*v*) solution for 5 min, under continuous stirring. The cut and washed skins were alkali treated (1:10 *w*/*v*) in NaOH 0.3 % (*w*/*v*) solution for 80 min at 10 °C. The skins were then washed with running water to remove alkali in excess until pH above 8.0. After that, the skins were acid treated (1:10 *w*/*v*) in H_2SO_4 0.3 % (*w*/*v*) solution for more 80 min at 10 °C and washed with running water until pH was close to the neutrality. The skins were then submitted to a second acid treatment (1:10 *w*/*v*) in citric acid 0.7 % (*w*/*v*) solution for 80 min and washed with running water until pH was close to neutrality. The extraction was conducted in a BIOSTAT B bioreactor (B. Braun Biotech International, Germany) in deionized water for 6 h at 45 °C. The ratio of 2 mL of solution for 1 g of skin was kept. After the extraction, the material was filtered in a Büchner funnel with a Whatman filter no. 4, lyophilized, milled, and hermetically stored at environmental temperature.

Gelatin Samples

Of the gelatin samples, 6.67 % (*w*/*v*) were prepared and dissolved in deionized water at 45 °C under constant mechanical shaking for 30 min in solutions with pH values of

5.0 and 8.0, adjusted with the addition of HCl 0.1 mol/L and NaOH 0.1 mol/L. The different samples prepared were MG, tilapia skin gelatin (TSG), and tilapia skin gelatin containing the following agents and respective concentrations: sucrose 4 % (*w*/*v*) (SUC1) and 8 % (*w*/*v*) (SUC2); glycerol 5 % (*v*/*v*) (GLY1) and 10 % (*v*/*v*) (GLY2); NaCl 0.3 mol/L (Na1) and 0.8 mol/L (Na2); MgCl2 0.3 mol/L (Mg1) and 0.8 mol/L (Mg2); $MgSO_4$ 0.3 mol/L (MgS1) and 0.8 mol/L (MgS2); KCl 0.3 mol/L (K1) and 0.8 mol/L (K2); and transglutaminase 10 mg/ml (TG1) and 15 mg/ml (TG2). At least three independent samples were prepared for each experimental condition tested.

Turbidity

The turbidity of the gelatin sample was measured according to the method described by Cole and Roberts (1996) using a Quimis turbidimeter (model Q-179P, TURB, Diadema, São Paulo, Brazil) immediately after the sample preparation.

Viscosity

The viscosity of the gelatin sample was determined according the British Standard Institution method (BSI 1975). Gelatin samples were prepared as described above, melted in a water bath at 45 °C, and 10 mL of gelatin solutions were transferred to an Ostwald-Fenske (no. 100) viscometer. The viscometer was placed in a water bath at 60 °C for 10 min for temperature stabilization, then the efflux time was recorded using a stopwatch. Viscosity of the gelatin samples was calculated in centipoise (cP).

Dynamic Viscoelastic Properties

Viscoelastic properties were determined by a Rheostress Haake RS-150 rheometer (Haake, Karlsruhe, Germany), using the Rheowin Job Manager software. For the dynamic viscoelastic study, a 35-mm-diameter cone plate measuring system and a cone angle of 1° with a gap=0.14 mm was used. The gelatin samples were prepared as described above, cooled to 7 °C and then heated to 40 °C. The experiments were carried out at an applied stress of 3.0 Pa, frequency of 1 Hz, and temperature scan rate of 0.5 °C/min. The gelling process was monitored by means of the elastic modulus G′ and viscosity modulus G″. The gelling point was determined by the intersection point of the moduli G′ and G″ during the cooling of the sample, according to the method by Gudmundsson (2002). The melting point was determined in the same manner as the gelling point during the subsequent heating process. The phase angle (δ) was plotted as a function of temperature to observe the viscoelastic behavior of the sample.

The gel formation time (setting time) was determined as the time in minutes between the last temperature of the maximum phase angle and the first temperature of the minimum phase angle. The samples were kept at 7 °C for approximately 5 min before heating up to 40 °C, to assess the elastic modulus G′ and viscosity modulus G″ at standard temperature.

The statistical analysis was performed by ANOVA using the Statistica v.8.0 software and the means were compared by the Tukey test (5 % probability) to determine the significant difference between the different agents using Microsoft Excel. The average and SD were calculated from at least nine measurements from three independent experiments (at least three measurements for each experiment).

Bloom Gel Strength

Bloom gel strength was determined according to the AOAC official method 948.21 (AOAC 2000). The gelatin samples were prepared as described above and immediately transferred into standard 150-ml Bloom jars (Schott, Mainz, Germany). The characteristic dimensions of the flat-bottom jar were 85 mm of total height and 65 mm of shoulder height at an outer diameter of 66 mm, 59 mm inner diameter, and 41 mm inner diameter at the neck. The samples were refrigerated at 7 °C for 18±1 h, 42±1 h, and 66±1 h. After cooling maturation, the gel strength, expressed in Bloom value, was determined using a texturometer (Stable Microsystems, Surrey, England), TA-XTplus model, with a load cell of 5 kg, with a cross-head speed for pre-test, test, and post-test of 1.5 mm/s, 1.0 mm/s, and 1.0 mm/s, respectively, using a 12.7-mm diameter 11 flat-faced cylindrical steel plunger. The plunger was forced to penetrate 4 mm into the sample at 8–10 °C to determine the maximum force (in g_f).

Results and Discussion

Turbidity and Viscosity

The functional properties of gelatins are determining factors for its applicability. The turbidity is the reduced transparency due the presence of material in suspension. The agents tested in the specified concentrations caused slight modification in the turbidity. However, there was no similar behavior between them (Fig. 1). With regard to the two pH values evaluated, it was verified that the smaller turbidity was when $MgSO_4$ 0.3 mol/L and KCl 0.3 mol/L was added while the addition of sucrose 8 % (*w*/*v*) resulted in considerable turbidity increase. Differences in the turbidity as a function of the pH of the solution were not observed. Depending on the gelatin application, the turbidity can be an important attribute (Cole 2011).

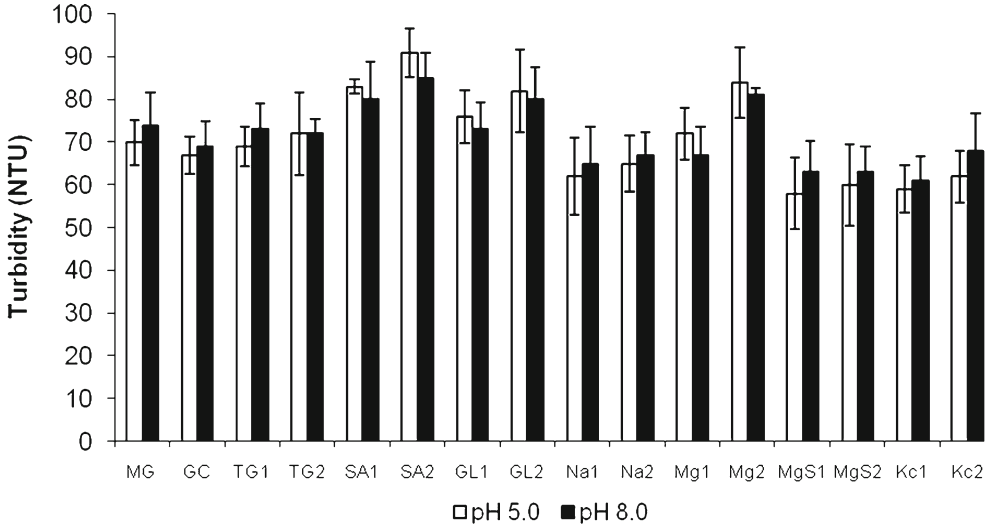

Fig. 1 Turbidity of tilapia skin gelatins at pH 5.0 and 8.0. *MG* mammalian gelatin, *GC* gelatin control (tilapia skin gelatin); TG1 = GC + transglutaminase (10 mg/g); TG2 = GC + transglutaminase (15 mg/g); SUC1 = GC + sucrose (4 %*w*/*v*); SUC2 = GC + sucrose (8 %*w*/*v*); GL1 = GC + glycerol (5 %*v*/*v*); GL2 = GC + glycerol (10 %*v*/*v*); Na1 = GC + NaCl (0.3 mol/L); Na2 = GC + NaCl (0.8 mol/L); Mg1 = GC + $MgCl_2$ (0.3 mol/L); Mg2 = GC + $MgCl_2$ (0.8 mol/L); MgS1 = GC + $MgSO_4$ (0.3 mol/L); MgS2 = GC + $MgSO_4$ (0.8 mol/L); Kc1 = GC + KCl (0.3 mol/L); Kc2 = GC + KCl (0.8 mol/L). Average and SD calculated from at least nine measurements from three independent experiments (at least three measurements for each experiment)

The viscosity values obtained, even for wami tilapia skin gelatin (control) (Fig. 2) were much higher than that observed for red tilapia skin gelatin (3.20 cP) but lower than that reported for black tilapia skin gelatin (7.12 cP) (Jamilah and Harvinder 2002). All the agents induced a viscosity increase in the gelatin solution, independently of the concentration and pH evaluated. At pH 5.0, glycerol (both concentrations) caused the largest viscosity rise, reaching values of 6.24 and 7.45 cP (Fig. 2).

This increase in viscosity can be a result of the alteration in the disposal of the water surrounding the gelatin molecules, with the consequent break/formation of hydrogen bonds and exposition of hydrophobic sites of the protein chain due to interactions occurred with the agents. These

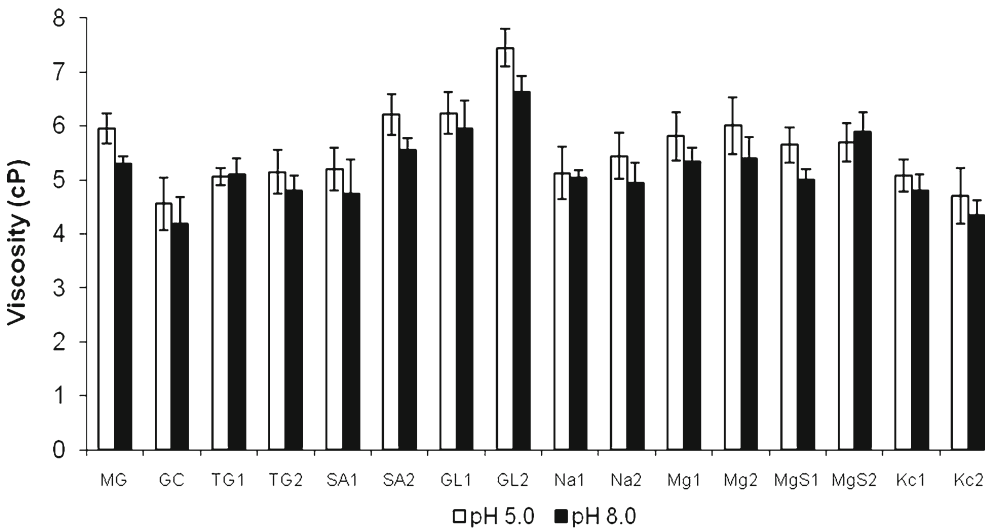

Fig. 2 Viscosity of tilapia skin gelatins at pH 5.0 and 8.0. *MG* mammalian gelatin, *GC* gelatin control (tilapia skin gelatin); TG1 = GC + transglutaminase (10 mg/g); TG2 = GC + transglutaminase (15 mg/g); SUC1 = GC + sucrose (4 %*w*/*v*); SUC2 = GC + sucrose (8 %*w*/*v*); GL1 = GC + glycerol (5 %*v*/*v*); GL2 = GC + glycerol (10 %*v*/*v*); Na1 = GC + NaCl (0.3 mol/L); Na2 = GC + NaCl (0.8 mol/L); Mg1 = GC + $MgCl_2$ (0.3 mol/L); Mg2 = GC + $MgCl_2$ (0.8 mol/L); MgS1 = GC + $MgSO_4$ (0.3 mol/L); MgS2 = GC + $MgSO_4$ (0.8 mol/L); Kc1 = GC + KCl (0.3 mol/L); Kc2 = GC + KCl (0.8 mol/L). Average and SD calculated from at least nine measurements from three independent experiments (at least three measurements for each experiment)

alterations generate conformational changes, leading to a sharp unfolding of the gelatin chains, causing an increase in its viscosity. All gelatins (except for that with $MgSO_4$ 0.8 mol/L addition) presented lower viscosity at pH 8.0 (Fig. 2). The proximity to the isoelectric point possibly enabled a higher aggregation among the protein fractions, leading to the reduced viscosity of the solution. Piotrowska et al. (2008) observed an increased viscosity of Baltic cod skin gelatin when transglutaminase enzyme was added, until the concentration of 0.25 mg/mL.

Viscoelastic Properties

To evaluate the viscoelastic properties, the gelatins were maintained at 7 °C for a few minutes before heating. This temperature was chosen as standard due the fast maturation caused by the cold (Choi and Regenstein 2000; Fernández-Díaz et al. 2003), provoking alterations in its elasticity. The samples varied in different ratios depending on the agent, concentration, and pH of the medium. The gelatin presented a considerable increase in its elasticity modulus (G′) when $MgSO_4$, transglutaminase and sucrose were added to both concentrations and pH 5.0 (Fig. 3). Similar conditions were observed in the behavior of the viscous modulus (G″), except in the samples containing sucrose which, contrary to what occurred in the elasticity modulus (G″), exhibited reduction in relation to the control sample.

The action of these agents on the viscoelastic properties of the gelatin was confirmed after subsequent heating, which verified that the melting temperature (Fig. 4) increased considerably when they were incorporated. However, the glycerol addition generated a perceptible increase in the viscosity modulus (G″) of the samples but practically did not modify the elasticity modulus (G′) and the melting point of the gelatin (Fig. 3).

As observed for the melting temperature, the addition of $MgSO_4$, transglutaminase, and sucrose propitiated the rise of the gelling temperatures (gelling point) of gelatins, except

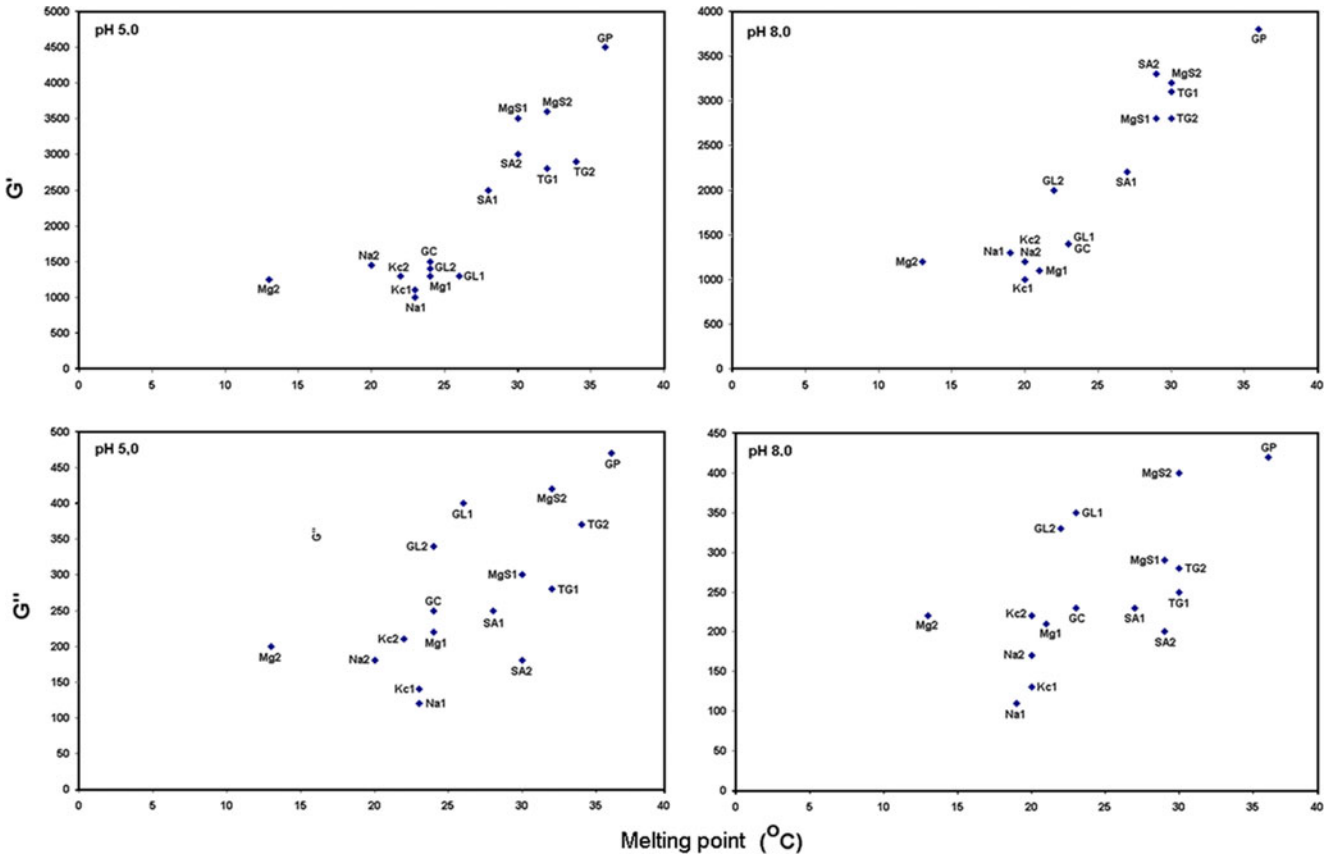

Fig. 3 Modulus of elasticity G′ and viscosity G″ of tilapia skin gelatins, measured at 7 °C in pH values of 5.0 and 8.0, plotted as a function of the melting point. *GP* mammalian gelatin, *GC* gelatin control (tilapia skin gelatin); TG1 = GC + transglutaminase (10 mg/g); TG2 = GC + transglutaminase (15 mg/g); SUC1 = GC + sucrose (4 %*w*/*v*); SUC2 = GC + sucrose (8 %*w*/*v*); GL1 = GC + glycerol (5 %*v*/*v*); GL2 = GC + glycerol (10 %*v*/*v*); Na1 = GC + NaCl (0.3 mol/L); Na2 = GC + NaCl (0.8 mol/L); Mg1 = GC + $MgCl_2$ (0.3 mol/L); Mg2 = GC + $MgCl_2$ (0.8 mol/L); MgS1 = GC + $MgSO_4$ (0.3 mol/L); MgS2 = GC + $MgSO_4$ (0.8 mol/L); Kc1 = GC + KCl (0.3 mol/L); Kc2 = GC + KCl (0.8 mol/L). Average and SD calculated from at least nine measurements from three independent experiments (at least three measurements for each experiment)

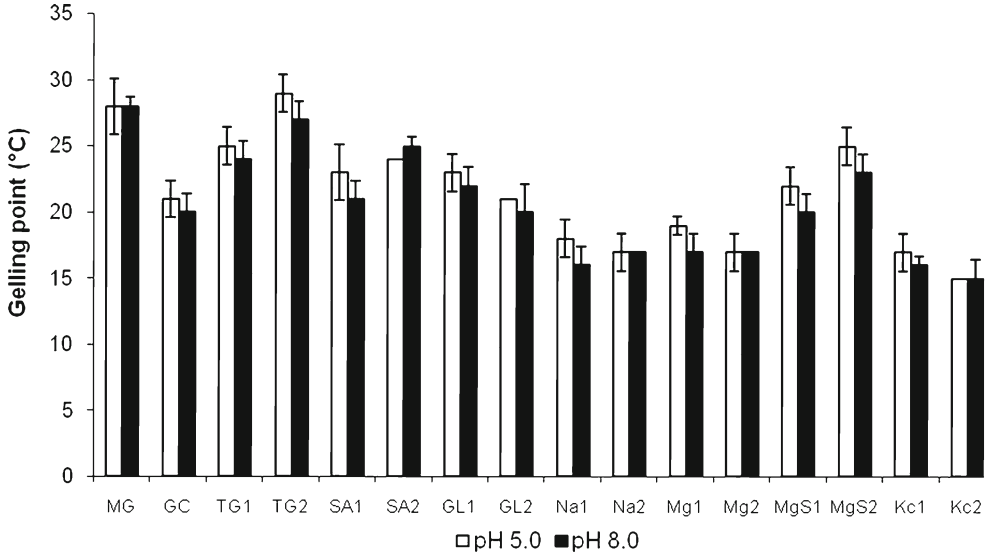

Fig. 4 Gelling point of tilapia skin gelatins at pH 5.0 and 8.0. *MG* mammalian gelatin, *GC* gelatin control (tilapia skin gelatin); TG1 = GC + transglutaminase (10 mg/g); TG2 = GC + transglutaminase (15 mg/g); SUC1 = GC + sucrose (4 %*w*/*v*); SUC2 = GC + sucrose (8 %*w*/*v*); GL1 = GC + glycerol (5 %*v*/*v*); GL2 = GC + glycerol (10 % *v*/*v*); Na1 = GC + NaCl (0.3 mol/L); Na2 = GC + NaCl (0.8 mol/L); Mg1 = GC + $MgCl_2$ (0.3 mol/L); Mg2 = GC + $MgCl_2$ (0.8 mol/L); MgS1 = GC + $MgSO_4$ (0.3 mol/L); MgS2 = GC + $MgSO_4$ (0.8 mol/L); Kc1 = GC + KCl (0.3 mol/L); Kc2 = GC + KCl (0.8 mol/L). Average and SD calculated from at least nine measurements from three independent experiments (at least three measurements for each experiment)

in the sample containing $MgSO_4$ 0.3 mol/L at pH 8.0 that presented values equal to the control (Fig. 4). Overall, the gelling temperatures tend to be lower at pH 8.0; however, no pronounced difference was observed.

The gelling and melting points of the samples containing transglutaminase increased considerably, mainly at pH 5.0 (Figs. 4 and 5). The reactivity of this enzyme is directly related to the amino acid profile of the protein since it

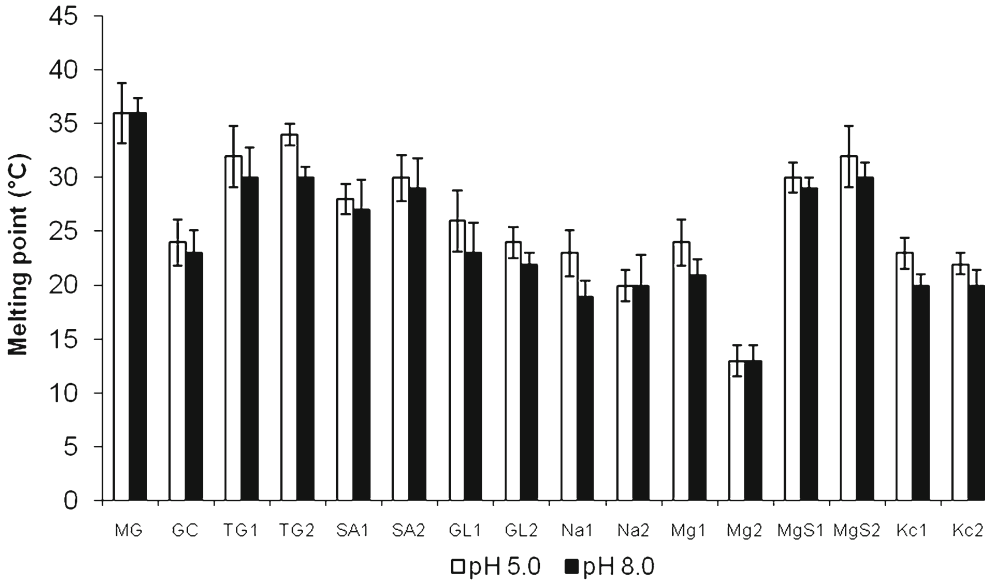

Fig. 5 Melting point of tilapia skin gelatins at pH 5.0 and 8.0. *MG* mammalian gelatin, *GC* gelatin control (tilapia skin gelatin); TG1 = GC + transglutaminase (10 mg/g); TG2 = GC + transglutaminase (15 mg/g); SUC1 = GC + sucrose (4 %*w*/*v*); SUC2 = GC + sucrose (8 %*w*/*v*); GL1 = GC + glycerol (5 %*v*/*v*); GL2 = GC + glycerol (10 % *v*/*v*); Na1 = GC + NaCl (0.3 mol/L); Na2 = GC + NaCl (0.8 mol/L); Mg1 = GC + $MgCl_2$ (0.3 mol/L); Mg2 = GC + $MgCl_2$ (0.8 mol/L); MgS1 = GC + $MgSO_4$ (0.3 mol/L); MgS2 = GC + $MgSO_4$ (0.8 mol/L); Kc1 = GC + KCl (0.3 mol/L); Kc2 = GC + KCl (0.8 mol/L). Average and SD calculated from at least nine measurements from three independent experiments (at least three measurements for each experiment)

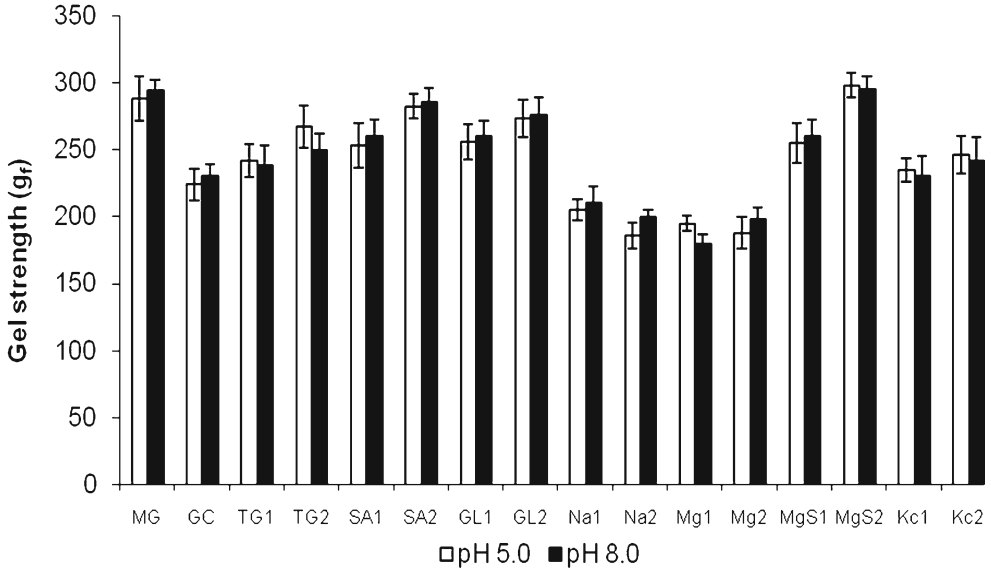

Fig. 6 Gel strength of tilapia skin gelatins at pH 5.0 and 8.0. *MG* mammalian gelatin, *GC* gelatin control (tilapia skin gelatin); TG1 = GC + transglutaminase (10 mg/g); TG2 = GC + transglutaminase (15 mg/g); SUC1 = GC + sucrose (4 %*w*/*v*); SUC2 = GC + sucrose (8 %*w*/*v*); GL1 = GC + glycerol (5 %*v*/*v*); GL2 = GC + glycerol (10 %*v*/*v*); Na1 = GC + NaCl (0.3 mol/L); Na2 = GC + NaCl (0.8 mol/L); Mg1 = GC + $MgCl_2$ (0.3 mol/L); Mg2 = GC + $MgCl_2$ (0.8 mol/L); MgS1 = GC + $MgSO_4$ (0.3 mol/L); MgS2 = GC + $MgSO_4$ (0.8 mol/L); Kc1 = GC + KCl (0.3 mol/L); Kc2 = GC + KCl (0.8 mol/L). Average and SD calculated from at least nine measurements from three independent experiments (at least three measurements for each experiment)

catalyzes covalent linking bonds between the glutamine and lysine residues, propitiating higher stability to the gel. According to Fernández-Díaz et al. (2001), the transglutaminase effect depends on the manner and amount of enzyme added.

The gelatin solutions, adding NaCl, KCl, and $MgCl_2$ in both concentrations and pH values, showed reduced elasticity modulus (G′) (Fig. 3). A possible explanation for this behavior may be the concentration tested for these salts, where high ionic force could lead to a pronounced unfolding of the protein chains, damaging the formation of junction zones and the subsequent formation of the gelatin helix. Figure 5 shows that these samples at pH 5.0 presented reduced melting temperature in relation to the control sample (CG), except for the gelatin solution containing $MgCl_2$ 0.3 mol/L, which presented equal melting temperature. The melting temperatures for wami tilapia skin gelatin (control) (Fig. 5) were close to that reported by Jamilah and Harvinder (2002) for red and black tilapia and Gudmundsson (2002) and Bueno et al. (2011) for Nile tilapia (22.45, 28.9, 25.8, and 24–26 °C, respectively).

The chloride salts led to reduced viscoelastic properties of the gelatin while the addition of $MgSO_4$ clearly maximized the rheological properties evaluated. According to the Hofmeister series, the interactions between proteins are more affected by some salts than others (Kunz et al. 2004). However, this effect was not verified when $MgCl_2$ was added, which could have occurred due the presence of Cl^-. The larger sulfate ion size may have hindered its approach to the positively charged centers of the protein, where the chloride ions, due to its lower diameter, could move toward it and interact more easily. The free permanence

Table 1 Setting time (minutes) for gelatins with several agents added in different concentrations and pH values

Gelatin	Setting time (min)	
	pH 5.0	pH 8.0
Mammalian gelatin	10±0.7a	8±0.4a
Gelatin control (tilapia skin gelatin)	12±0.5b,c	11±0.9a,d,e,f,i
GC + transglutaminase (10 mg/g)	12±0.0b,c	12±0.7a,d,e,f,h,i
GC + transglutaminase (15 mg/g)	13±0.0b,g	11±0.0a,d,e,f,h,i
GC + sucrose (4 %*w*/*v*)	11±0.4a,c	10±0.0a,d,f,i
GC + sucrose (8 %*w*/*v*)	10±0.7a,d	10±0.8a,d,f
GC + glycerol (5 %*v*/*v*)	17±0.6e	16±0.9b,c,d,e,f,g,h,i
GC + glycerol (10 %*v*/*v*)	19±0.7f	18±0.6c,e,g,h,i
GC + NaCl (0.3 mol/L)	13±0.0b,g	13±0.0d,e,f,g,h,i
GC + NaCl (0.8 mol/L)	14±0.2g	13±0.1d,e,f,g,h,i
GC + $MgCl_2$ (0.3 mol/L)	16±0.0e	14±0.4e,f,g,h,i
GC + $MgCl_2$ (0.8 mol/L)	14±0.7g	13±0.0f,g,h,i
GC + $MgSO_4$ (0.3 mol/L)	19±1.4f	16±0.9g,h,i,j
GC + $MgSO_4$ (0.8 mol/L)	17±0.0e	15±0.4h,i,j
GC + KCl (0.3 mol/L)	15±0.7e,g	14±0.0i,j
GC + KCl (0.8 mol/L)	14±0.4g	12±0.5a,f,j

Values in the same column with different letters are significantly different ($P<0.05$) by the Tukey test

of sulfate ions in aqueous solution then allowed a sharp reactivity with the adjacent water molecules, increasing the number of electrostatic interactions, allowing greater opening of the protein chains, and increasing the probability of the formation of junction zones. According to Zhang and Cremer (2006), in recent studies involving water molecules and different salts, the Hofmeister effect was observed not only in the water structure, but also in ion–macromolecule interactions, and in the water molecules of the first hydration layer of the macromolecule. The addition of salts containing the chloride ion resulted in reductions in the viscosity modulus (G″). Generally, this module tended to present lower values at pH 8.0.

Bloom Gel Strength

The literature reports gel strengths of 128.11 g_f and 180.76 g_f for red and black tilapia skin gelatins, respectively (Jamilah and Harvinder 2002) and 202.7 g_f for Nile tilapia skin gelatin (Bueno et al. 2011), which are considerably lower values than those found for wami tilapia skin gelatin (control) (Fig. 6).

The gel strength of gelatin increased considerably when $MgSO_4$ 0.8 mol/L was added, reaching a slightly higher value than the mammalian gelatin (MG) and considerably higher than the fish gelatin control (GC) (Fig. 6). Sarabia et al. (2000) and Fernández-Díaz et al. (2001) observed an increase in the gel strength of gelatins from other fish species when this salt was added, attributing such effect to the promotion of a higher number of electrostatic interactions, with the formation of adjusted junction zones due to the correct unfolding of the structure.

Transglutaminase, sucrose, and glycerol also considerably increased the gel strength of the gelatin when it was incorporated. Jongjareonrak et al. (2006) reported an increase in gel strength of gelatin obtained from bigeye snapper skin and brown stripe red snapper when the transglutaminase enzyme was added. Choi and Regenstein (2000) evaluated the effect of sucrose addition on some gelatins, having observed that the increase in the added content results in increased gel strength. Naftalian and Symons (1974) suggested that this increase occurs due to the stabilization of hydrogen bonds by the sucrose. It is known that these bonds play an essential role in thermal stability, where the superior rheological properties of mammalian gelatins are attributed to its amino acid distribution, mainly the higher amount of amino acids (proline and hydroxyproline). It is believed that they are an important determinant for the formation of hydrogen bonds in aqueous solutions with the consequent rise of the gelling temperature (Norland 1990). According to Ledward (1986), the hydroxyproline content is a determinant factor due to its ability to form hydrogen bonds through OH groupings.

The setting time was statistically prolonged by the addition of the agents, particularly at pH 5.0 (Table 1). Only gelatins containing sucrose presented lower setting times at both pH values when compared with the control sample. In samples containing glycerol, considerably longer times were necessary for the gel formation. As previously mentioned, the glycerol addition may have generated a sharp unfolding of the gelatin chains, which consequently led to an increased time necessary for the subsequent jellification.

The time required for gel formation was longer for the control in all samples with salts added. It is well-known that salts can unchain interactions in gelatins, modifying its characteristics (Elysée-Collen and Lencki 1996). Among the salts evaluated, $MgSO_4$ (at both pH values) lead to higher gelling times. Generally, the setting time tended to be higher at pH 5.0. This difference may be due to the fact that gelatin, at pH 8.0, is found next to its isoelectric point, allowing more interactions between the protein chains and the occurrence of the gelling process at a lower time interval.

Conclusions

All agents increased the viscosity of the gelatin solution especially at pH 5.0. The incorporation of the agents triggered turbidity modifications. However, their behavior was not similar. The addition of $MgSO_4$, transglutaminase, and sucrose, in both concentrations and pH values, increased the gelling and melting points and gel strength.

The gel strength and the viscosity of the gelatin increased with the addition of glycerol. However, the gelling and melting temperatures did not increase with the addition of glycerol. Among the agents studied, the addition of $MgSO_4$ (at both pH values) favored obtaining wami tilapia skin gelatin with rheological properties close to the properties of mammalian gelatins. The addition of NaCl, KCl, and $MgCl_2$, in both concentrations and pH values, showed reduced rheological properties of the gelatin solutions.

Among the results obtained, the viscosity of 7.45 cP reached with the addition of glycerol (10 % (*v*/*v*)) and the gel strength of 298 g found with the addition of MgSO4 (0.8 mol/L) are emphasized.

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