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Chemical and functional properties of bovine and porcine skin gelatin

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Abstract: The ability to compare bovine and porcine skin gelatin based on their amino acid composition, polypeptides pattern, bloom strength, turbidity and foaming properties were investigated. Amino acid composition of both gelatin showed that the content of glycine, proline and arginine in porcine gelatin were higher than bovine gelatin. However, the polypeptides pattern between both gelatin is closely similar. The bloom strength of porcine gelatin was higher than bovine gelatin from pH 3 to pH 10. Both gelatin possessed highest bloom strength at pH 9. The lowest bloom strength of bovine gelatin was at pH 3 while porcine gelatin at pH 5. The highest turbidity of bovine gelatin obtained at pH 7 while porcine gelatin at pH 9. Foam expansion and foam stability of bovine gelatin were higher than porcine gelatin at all concentrations.

Keywords: Gelatin, polypeptides, bloom strength, turbidity, foam expansion, stability

Introduction

Gelatin is an important hydrocolloid which has widespread used in food applications. In generally, mammalian gelatin has been utilized due to its high melting, gelling point and thermo-reversibility (Gudmundsson, 2002). It is a high molecular weight and water-soluble protein. All the amino acids are present in gelatin except tryptophan and have low in methionine, cystine and tyrosine due to the degradation during hydrolysis (Jamilah and Harvinder 2002; Chapman and Hall, 1997). The amino acid compositon and sequence in gelatin are different from one source to another but always consists of large amounts of glycine, proline and hydroxyproline (Gilsenan, and Ross-Murphy, 2000). It is repeated with typical sequence, Gly-X-Y where glycine is the most abundant amino acid in gelatin; X and Y are mostly proline and hydroxyproline, respectively. 25% of dry gelatin contains proline and hydroxyproline that stabilize its structure (Russell *et al*., 2007).

The chemical properties of gelatin are affected by amino acid composition, which is similar to that of the parent collagen, thus influence by animal's species and type of tissues. The differences in molecular weight distribution were also affected its chemical properties which result from the variation in the nature or extraction conditions (Zhou and Regenstein, 2006). Bovine and porcine skin gelatins are widely utilized in food manufacturing because the sources are more available. Gelatin from bovine skin produced from alkaline treatment is known as type B gelatin while porcine skin gelatin produced from acidic treatment is known as type A gelatin. They may possess different characteristics which determine whether one of them to be chosen by food manufacturer. Some manufacturer consider to use gelatin from bovine source while other preferred porcine gelatin. This study was to compare amino acid content, molecular weight distribution and chemical properties of bovine and porcine skin gelatins showing the importance of bovine or porcine gelatin in food applications. The present study was also to differentiate between both gelatins based on the studied parameters.

Materials and Methods

Gelatins from bovine skin (type B) and porcine skin (type A) were purchased from Sigma Co. (St. Louis, USA). AccQ Tag™ Eluent A and a derivatization reagent, AccQ-Fluor™ Reagent Kit were purchased from Waters (Massachusetts, USA). Regenerated cellulose (0.45µm) membrane filter and Minisart RC 15 filter were purchased from Sartorius Stedim Biotech (Goettingen, Germany). Acetonitrile and methanol were of HPLC grade.

Amino acid analysis

The bovine and porcine gelatin were weighed approximately within the range of 0.1 to 0.2 g and mixed with 5 ml of 6 N concentrated hydrochloric acid. The gelatin solutions were hydrolyzed in oven at 110°C for 24 hours (Nemati *et al*., 2004). The chromatographic system consisted of HPLC Waters (Model 2695, Massachusetts, USA) equipped with online degasser, auto injector and a multi-wavelength Waters fluorescence detector (Model 2475, Milford, Massachusetts, USA) was used. Waters AccQ Tag column (3.9 x 150 mm) was used with temperature

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for amino acid separation. The column was set at 36°C, and the injection volume was 5 µl. The AccQ Tag^{tm} Eluent A concentrate and 60% acetonitrile were filtered using a 0.45 µm regenerated cellulose membrane filter prior to injection onto HPLC system. A flow rate was set at 1 mLmin^{-1}. Waters Empower^{tm} Pro software was used for data acquisition. The methodology was referred from Waters AccQ Tag^{tm} method for hydrolysate amino acid analysis (Astephen and Wheat, 1993).

Determination of polypeptides pattern

The polypeptides pattern of the gelatins was determined using a sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) using 4% stacking gel and 12% separating gel (Laemmli, 1970). The protein concentration of the samples was determined using Bradford assay (Bradford, 1976). The gelatin (5 mg/ml) was mixed with treatment buffer (0.125 M Tris-Cl pH 6.8; 4% SDS; 20% glycerol, 10% 2-mercaptoethanol) at a ratio of 1 to 1 (v/v). About 20 µg of proteins were loaded onto the gel. Electrophoresis was conducted using a Mini-PROTEAN® Tetra Electrophoresis System (Bio-Rad Laboratories, Hercules, CA, USA) at a constant voltage of 150V. Gels were stained using 0.05% Coomassie brilliant blue R250 dissolved in 15% (v/v) methanol and 5% (v/v) acetic acid and de-stained using a solution containing 30% (v/v) methanol and 10% acetic acid. The protein marker (Sigma Co., St. Louis, USA) ranged from 8 to 220 kDa was used.

Determination of gel strength

The bloom strength of BSG and PSG at pH 3 to 10 was determined according to the standard method (GMIA, 2006). Samples were weighed into the bloom bottles and dissolved in distilled water to a final concentration of 6.67% (w/v). The bloom strength was determined using a texture analyzer (Stable Micro Systems, Surrey, England) with a 30 kg load cell, equipped with 1.27 cm diameter flat-faced cylindrical plunger. The maximum force (in grams) taken when the plunger had penetrated 4 mm onto gelatin gel's surface, was recorded.

Determination of turbidity

The turbidity of PSG and BSG solution (6.67% w/v) at different pH (3-10) was determined according to preferred method (Aewsiri *et al*., 2008). The samples were dissolved in distilled water at 60°C and the pH of solution was adjusted with either 6 N NaOH or HCl. The turbidity was determined by measuring the absorbance at 360 nm using U-2810 UV-Vis Hitachi spectrophotometer (Tokyo, Japan).

Determination of foaming properties

Foaming property was measured according the method described (Aewsiri *et al*., 2008). BSG and PSG solutions were prepared at different concentrations (2 to 5% w/v). The mixtures were homogenized for 1 min at room temperature using a homogenizer at 13500 rpm. The homogenate was allowed to stand for 0 and 30 min. Foam expansion (FE) and foam stability (FS) were determined using the following equations 1 and 2 where V_T is total volume, V_O is the original volume before whipping and V_t is total volume after leaving at room temperature for different times (30 and 60 min).

$$FE\ (\%) = V_T/V_O \times 100 \quad (1)$$

$$FS\ (\%) = V_t/V_O \times 100 \quad (2)$$

Statistical analysis

All measurements on each sample were carried out in duplicate. Results showed the mean ± standard deviation and submitted to analysis of variance (ANOVA) using SPSS Statistics 17.0 (SPSS Inc., Chicago, IL). Mean values were compared using the Duncan's test at $P < 0.05$.

Results and Discussion

Amino acid composition

The amino acid composition affects the gelatin's physical and chemical properties. The amino acid analysis of gelatin showed molecular structure of gelatin was different according to composition of amino acids. Amino acid composition of BSG and PSG were different especially for glycine, proline and arginine (Table 1). The amino acid composition was expressed as residues per 1000 amino acid residues. Both BSG and PSG had high amount of glycine followed by proline and arginine. However, PSG contained higher amount of glycine, proline and arginine compared to BSG. Both gelatins had low amount of tyrosine. Histidine was not detected in both gelatins.

Polypeptides pattern

The polypeptide patterns of BSG and PSG are shown in Figure 1. The polypeptides bands were similar for both gelatins. The findings were in agreement with Gudmundson, 2002. The distinct bands with molecular weight approximately of 220 and 100 kDa could be represent β and α chain, respectively. The polypeptides with molecular weight

below than 100 kDa in BSG and PSG did not obtain as expected, meaning that the studied gelatins had high molecular weight protein.

Table 1. Amino acid composition of bovine and porcine skin gelatin

Amino acid	BSG (residues per 1000 total amino acid residues)	PSG (residues per 1000 total amino acid residues)
Nonpolar hydrophobic		
Alanine	33	80
Valine	10	26
Leucine	12	29
Isoleucine	7	12
Phenylalanine	10	27
Methionine	4	10
Proline	63	151
Total	139	335
Polar uncharged		
Glycine		
Serine	108	239
Threonine	15	35
Tyrosine	10	26
Total	2	7
	135	307
Polar acidic		
Aspartic acid	17	41
Glutamic acid	34	83
Total	51	124
Polar basic		
Lysine	11	27
Arginine	47	111
Histidine	Not detected	Not detected
Total	58	138

BSG: bovine skin gelatin; PSG: porcine skin gelatin; samples were run duplicates; each involves 2 batches of gelatins.

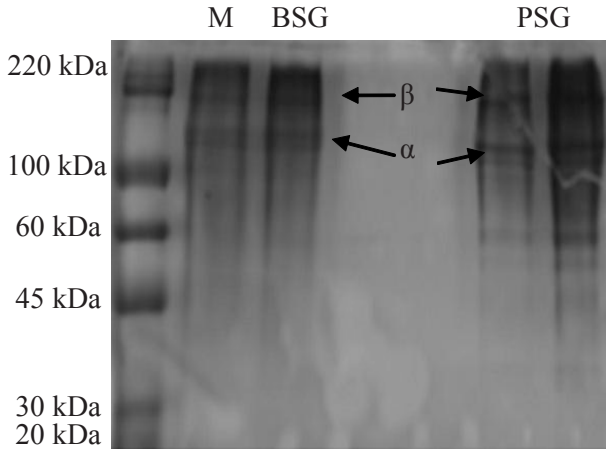

Figure 1. Polypeptide patterns of bovine and porcine skin gelatin. M: protein marker, BSG: bovine skin gelatin; PSG: porcine skin gelatin; α and β chains: protein component of gelatin.

Effect of pH on gel strength

The gel strength is one of important criteria which determine the quality of gelatin as required by manufacturer. It measures the hardness, stiffness, firmness and compressibility of the gel at a particular temperature. It is associated with the contents of proline and hydroxyproline in gelatin. The gel strength might be dependent on the isoelectric point and could be controlled by adjusting the pH (Gudmundsson and Hafsteinsson, 1997). Formation of compact and stiffer gels can be achieved by adjusting the pH of the gelatin close to its isoelectric point, in which the charge of proteins are more neutral and thus the gelatin polymers are closer to each other (Gudmundsson and Hafsteinsson, 1997).

The effect of pH on the bloom strength of BSG and PSG is shown in Table 2. The strength of BSG and PSG increased with increasing pH although for PSG, the strength increased inconsistently. The highest bloom strength of BSG and PSG were observed at pH 9. The lowest bloom strength of BSG was observed at pH 3 while for PSG, the lowest bloom strength at pH 5. Maximal rigidity of porcine gelatin was achieved at pH 9 while minimum rigidity attained at pH below 4 and above 10 (Aewsiri *et al*). The bloom strength of PSG was higher than that of BSG at all pHs. It showed the PSG was stiffer than BSG. The higher bloom strength of PSG as compared to BSG is due to the high degree of cross-linking and amount of glycine and proline contained in it. In addition, the hydrogen bonds between water molecules and free hydroxyl groups of amino acid will influence gelatin strength (Arnesen and Gildberg, 2002). From the results, the high amount of tyrosine and serine in PSG which had a free hydroxyl group contributes to the formation of hydrogen bonds which leads to increased gel strength. The bloom strength is also said to be affected by the content of hydroxyproline and molecular weight (Aewsiri *et al*, 2008).

Table 2. Gel strength of BSG and PSG at different pHs

pH	Force (g)	
	BSG	PSG
3	193.49 ± 2.09^{a}	330.57 ± 0.81^{b}
4	234.00 ± 0.46^{b}	372.95 ± 0.45^{e}
5	251.03 ± 2.92^{c}	326.47 ± 0.07^{a}
6	251.40 ± 1.62^{c}	357.87 ± 1.95^{d}
7	266.69 ± 6.67^{d}	350.42 ± 1.05^{c}
8	267.63 ± 5.80^{d}	389.04 ± 0.29^{f}
9	270.35 ± 8.02^{d}	415.10 ± 1.21^{g}
10	247.09 ± 1.80^{c}	348.57 ± 2.36^{e}

Different letters within same column denote significant differences ($P < 0.05$). BSG: bovine skin gelatin; PSG: porcine skin gelatine. Mean ± SD from duplicate determinations.

Effect of pH on turbidity

The turbidity of BSG and PSG was influenced by pH as shown in Table 3. BSG had higher turbidity than that of PSG at all pHs. The turbidity of BSG was highest at pH 7 and decreased at alkaline pHs. PSG showed the highest turbidity at pH 9 although its absorbance was slightly lower at pH 6 and 7. The maximum turbidity of gelatins occurred at their isoelectric point (Poppe, 1997). For PSG, its isoelectric point might be at pH 9, as it showed a maximum turbidity while for BSG, pH 7 might not be its pI because BSG was type B alkaline-processed gelatin. This type of gelatins has isoelectric point ranged between pH 4.8-5.0 as reported (Aewsiri *et al*., 2008). At pH close to isoelectric point, aggregation of protein molecules occurs and reduces its interaction with water molecules (Vojdani, 1996).

Table 3. The turbidity of BSG and PSG solutions (6.67% (w/v)) at different pH

pH	Absorbance (360 nm)	
	BSG	PSG
3	0.919 ±0.003[b]	0.055 ± 0.001[a]
4	1.011 ±0.003[d]	0.065 ± 0.000[b]
5	1.023± 0.002[f]	0.078 ± 0.001[e]
6	1.018 ± 0.002[e]	0.069 ± 0.001[c]
7	1.057 ± 0.002[g]	0.073 ± 0.001[d]
8	0.932 ± 0.002[c]	0.083 ± 0.001[f]
9	0.903 ± 0.000[a]	0.087 ± 0.001[g]

Different letters within same column denote significant differences (P < 0.05). BSG: bovine skin gelatin; PSG: porcine skin gelatin. Mean ± SD from duplicate determinations.

Determination of foaming properties

Table 4 showed foam expansion (FE) and foam stability (FS) of BSG and PSG at different concentrations. FE and FS of BSG and PSG were increased at concentrations of 2 and 3% (w/v) but decreased at higher gelatin concentrations (4 and 5% w/v). Zayas (1997) described that increasing tuna fin gelatin concentration and porcine skin gelatin (%w/v) lead to the increased of FE and FS. This complies with the results presently obtained. However, the reduced value of FE and FS at 4 and 5% (w/v) PSG and BSG concentration is might be due to the improper homogenization of the gelatin. In overall, FE and FS of BSG were higher than PSG at all tested concentrations (%w/v).

Table 4. The foaming properties of bovine and porcine skin gelatin at different concentrations

Concentration of gelatin (%)	FE (%)	FS (%)
	0 min	30 min
BSG (2%)	93.00 ± 2.65[b]	91.67 ± 3.21[b]
(3%)	94.67 ± 1.53[b]	93.67 ± 1.53[b]
(4%)	91.00 ± 1.00[b]	89.33 ± 1.53[b]
(5%)	72.33 ± 5.51[a]	86.50 ± 5.51[a]
PSG (2%)	90.00 ± 1.00[c]	87.67 ± 2.08[c]
(3%)	93.00 ± 1.00[c]	88.67 ± 2.52[c]
(4%)	70.00 ± 10.44[b]	68.33 ± 9.29[b]
(5%)	53.00 ± 1.00[a]	51.33 ± 0.58[a]

Different letters in the same column within the same gelatin denote significant differences (P<0.05). FE: foam expansion; FS: foam stability. Mean ± SD from duplicate determinations.

FS of BSG and PSG decreased when incubation time increased because during foam ageing, gravitational forces might cause water to drain and air cells came closer together. High viscosity at higher concentrations was useful in preventing gravity deformation of the film in protein foams. The bulk viscosity of BSG and PSG affected the FS which in turn extends the the stability of foams. Foaming properties of protein could be influenced by protein source, intrinsic properties of protein, the compositions and conformation of the protein in solution and at the air/ water interface (Zayas, 1997).

Conclusions

The bovine and porcine skin gelatin could be distinguished based on amino acid composition in which the glycine and proline of PSG were higher than BSG. In addition, the gel strength of PSG is higher than BSG while the foaming properties of BSG are more stable than PSG. Maximum turbidity of BSG was achieved at pH 7; in contradict with PSG that achieves it maximum turbidity at pH 9. However, the polypeptides pattern of both gelatin could not be differentiated using one dimensional electrophoresis (SDS-PAGE). PSG that has high bloom strength is suitable to be used in the production of jellied meats and marshmallow. Both PSG and PSG are also used as foam stabilizer like has been exploited in marshmallows industry. Low Bloom strength BSG is also suitable for used as clarifying agent in fruit juice products.

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