Effects of different drying methods on the rheological, functional and structural properties of chicken skin gelatin compared to bovine gelatin

Rasli, H. I. and *Sarbon, N. M.

School of Food Science and Technology, Universiti Malaysia Terengganu
21030, Kuala Terengganu, Terengganu, Malaysia

Abstract

This study investigates effects from different drying methods (vacuum oven dried vs. freeze dried) on the rheological, functional and structural properties of chicken skin gelatin compared to bovine gelatin. Vacuum oven dried chicken skin samples showed a higher gelatin yield (12.86%) than freeze-dried samples (9.25%). The latter showed a higher melting temperature (32.64^{o}C) and superior foaming capacity (176%) as well as foaming stability (166.67%). Vacuum oven dried samples demonstrated greater fat binding capacity (5.5 ml/g) and emulsion stability (55.79%). There were no significant differences (p >0.05) in emulsion and water holding capacity for three gelatins. Bovine gelatin did hold the lowest of all functional properties studied. A Fourier Transform Infrared (FTIR) spectrum analysis of chicken skin gelatin under both drying methods presented structures similar to those of bovine gelatin. Collectively, this findings indicated no significant differences (p >0.05) in rheological, functional and structural properties for chicken skin gelatins prepared by either drying method. Hence, to save costs and maintain gelatin quality, vacuum oven drying offers potential as an alternative means of production.

Introduction

Gelatin were derived from the partial hydrolysis of denatured protein constituents from collagen extracts in which the main raw materials are animal tissues including skin, bones and connective tissue (Schrieber and Gareis, 2007). Gelatin proteins are unique due to their solubility in water and capacity to form a thermo-reversible gel with melting temperatures that approximate the human body (Norziah *et al*., 2008). Gelatin has a typical amino acid composition with large amounts of proline, hydroxyproline, alanine and glycine, with the latter constituting approximately one third of the molecule (Mitchell, 1976). In addition, gelatin production increases yearly by nearly 326,000 tons, with pig skin derivatives at the lead (46%) followed by bovine hide (29.4%), bone (23.1%) and other sources (1.5%) (GME 2008). The food industry utilizes gelatins as stabilizers, thickeners and textural adjuncts that improve elasticity and consistency (Zhou and Regenstein, 2005).

Concerns have arisen regarding safety because of bovine spongiform encephalopathy (BSE: mad cow disease) (Gudmundsson, 2002) as well as religious (halal) issues. The latter have grown in importance and thus prompted research and development of alternative gelatin sources including fish wastes (skin, bone and scales) and poultry skin and bones. Hindus do not consume bovine products while Muslims abhor swine but will consume products from other animals slaughtered according to Islamic law. Hence, the development of gelatin alternatives has become an imperative of major import to the global food processing industry, especially as demands for Halal certification rapidly rise (Karim and Bhat, 2009).

Sheu and Chen (2002) reported high amounts of generated residue for chicken skin, estimating that each broiler carcass contained approximately 15% skin. According to Mokhtar and Chia (2000), the available poultry population in Sabah, Malaysia alone was 2.4 million with an estimated waste of 178,000 metric tons annually. Ockerman and Hansen (1988) reported that most excess chicken skin was processed in combination with other poultry waste to produce inedible rendered fat and food by-products of various qualities. This processing included wet or dry high temperatures at extraction temperatures greater than 100°C. As a consequence, a number of studies have been devoted to the preparation, extraction and characterization of gelatin derived from chicken skin. In Canada, a few studies investigated the collagen characterization of chicken skin with or without telopeptides (Cliché *et al*., 2003). Sarbon *et al.* (2013) compared the characterization of extracted chicken skin gelatin with commercial gelatin to assess its

*Corresponding author.
Email: *norizah@umt.edu.my*
Tel: +609 668 4968; Fax: +609 668 4949

potential as an alternative to mammalian gelatin.

The rheological properties of gelatin gel account for gelatin quality. These consist of flow and deformation metrics in particular, as well performance during transitions from solid to fluid and vice versa (Tabilo *et al.*, 2005). Hence, rheological properties of extracted gelatins generally include gel strength, viscosity of the gelatin solution, and viscoelastic properties (G′, G″ and phase angle) during heating and melting. These properties were previously studied for gelatins derived from sin croaker (Johnius dussumieri); from shortfin scad (Decapterus macrosoma) (Cheow *et al.*, 2007); and from chicken skin gelatin (Sarbon *et al.*, 2013).

Functional properties of gelatin are of significant importance to the food industry as they enhance elasticity, consistency and the stability of food products in addition to their use as outer films (coatings) to protect foods from light and oxygen (Montero and Gomez-Guillen, 2000). These functional properties have been studied by different methods and metrics including emulsion capacity, emulsion stability, water holding capacity, foam formation and stability, and fat binding capacity (Binsi *et al.*, 2009).

In addition, gelatin structure is directly related to physical properties that influence quality and potential application (Yang and Wang, 2009). Collagen is a triple helix that forms fibers arranged in bundles held together by a connective tissue matrix (Badii and Howell, 2006). When subjected to acid or alkaline hydrolysis, a mild degradation occurs whereby collagen's fibrous structure is irreversibly broken down due to the rupture of covalent bonds. Denaturation of soluble collagen due to the breakdown of hydrogen and probably electrostatic bonds in hot water (40ºC) destroys the triple helical structure only to produce one, two or three random chain gelatin molecules that lend high viscosity to water solutions (Flory and Weaver, 1960). Hence, several different reactions that occur during any extraction process using different methods may bring about differences in gelatin structures and properties when obtained from different sources.

Therefore, the objectives of this study were to (i) prepare gelatin from chicken skin; (ii) investigate effects from different drying methods on rheological (dynamic oscillatory measurement) and functional properties (water holding capacity, fat-binding capacity, foam capacity and stability, emulsifying capacity and stability); (iii) examine the secondary structure of chicken skin gelatin; (iv) compare all of these metrics with those of bovine gelatin.

Materials and Methods

Raw materials

Fresh chicken skins were obtained from TD Poultry Sdn. Bhd. Kuala Terengganu, Malaysia, and kept in the ice during transport to Universiti Malaysia, Terengganu. The skins were thoroughly washed and weighed (wet weight) before storage at -18ºC. Chemical used for analysis like sodium hydroxide, sulphuric acid and citric acid were of analytical grade. Commercial bovine gelatin was purchased locally.

Chicken skin preparation

The frozen skins were thawed in a chiller (4–5ºC) overnight and thoroughly washed to remove impurities, after which they were cut into 2–3 cm pieces and dried in a cabinet drier at 40ºC overnight. The dried skins were then ground up and defatted following the Soxhlet method (AOAC, 2006).

Gelatin extraction

Gelatin was extracted using the method as described by Sarbon *et al.* (2013) with slight modification. Defatted chicken skin was pretreated sequentially in solutions of sodium hydroxide (0.15% w/v), sulphuric acid (0.15% (v/v), and citric acid (0.7% w/v) respectively. Each solution was shaken and stirred slowly at room temperature for 30 min before centrifugation at 3500 x g for 10 min. The supernatant was removed and each wash was repeated three times to remove non-collagenous proteins and pigments. The pellets were then thoroughly rinsed in distilled water to remove residual salts and centrifuged at 3500 x g for 15 min and then placed in distilled water overnight at 45ºC, followed by filtration in a Büchner funnel with Whatman filter paper (no. 4). The filtered solution was evaporated under vacuum at 45ºC (thus, reducing volume to 1/10), and then dried using two methods: (i) vacuum oven dried; (ii) freeze dried. The dried matter ('gelatin powder') was ground, weighed and stored for further use. Gelatin yield was calculated based on raw material wet weight and expressed as a percentage:

$$\text{Yield (\%)} = \frac{\text{Weight of gelatin powder (g) x 100}}{\text{Weight of wet (g)}}$$

Dynamic oscillatory measurement

Dynamic Oscillatory measurement was determined following the method described by Sarbon *et al.* (2013) with slight modification using a rheometer (Rheometer DHR-2, USA). Gelatin solutions (6.67% w/w) were measured in a 40 mm parallel plate with a 1000 μm gap at a frequency of

1 rad/s at temperature steps of 5°C with a controlled strain of 5%. Gelatin samples were cooled on a Peltier plate from 40 to 10°C and reheated to 40°C at a rate of 5 °C/min. When the elastic modulus (G′) began to dramatically increase in value the gelation temperature was recorded. The melting point was immediately determined where the loss modulus (G″) began to increase during reheating after each sample reached 10°C. The melting function of temperature was recorded when the elastic modulus (G′) began decreasing and the loss modulus (G″) began increasing.

Water holding capacity (WHC)

Water holding capacity (WHC) was determined by using the method described by Diniz and Martin, (1997) with slight modification. Gelatin samples (about 0.5 g) were weighed and dissolved in 10 ml of distilled water while in centrifuge tubes then mixed in a vortex mixer for about 30 min and then centrifuged at 2800 × g for 25 min. The supernatant was filtered with Whatman No.1 paper after which the retrieved volume was measured. The difference between the initial volume of distilled water added to the protein sample and the volume of the supernatant was determined. Results were reported as ml of water absorbed per gram of gelatin sample. Water holding capacities were calculated as follows:

$$\text{Water holding capacity (ml/g)} = \frac{\text{Initial volume} - \text{volume of supernatant}}{\text{Weight of gelatin (g)}}$$

The analysis was done in triplicate, averaged and analysis was repeated for bovine gelatin.

Fat binding capacity

To determine fat-binding capacity, we used the method described by Shahidi et al. (1995). About 0.5 g of chicken skin gelatin was added to 10 ml of palm oil (vesawit) in a 50 ml centrifuge tube and vortexed for 30 s for each of three samples. The oil dispersion was centrifuged at 2800 x g for 25 min after which the free oil was decanted and the fat binding capacity determined by weight using the following formula:

$$\text{Fat binding capacity (ml/g)} = \frac{\text{initial volume} - \text{volume of supernatant}}{\text{Weight of gelatin (g)}}$$

The analysis was done for three separate samples were then averaged and analysis was repeated for bovine gelatin.

Foam formation capacity and foam stability

Foam formation capacity and foam stability were determined by partially modifying the method described by Sathe et al. (1982). Approximately 1 g for each of three samples was weighed and added to 50 ml of distilled water and then dissolved at 60°C. Foam was prepared by homogenization at 10,000 x g for 5 min. The homogenized solution was poured into a 250 ml measuring cylinder and foaming capacity and stability were measured as follows:

$$\text{Foam capacity (\%):} \quad \frac{(\text{Volume of foam liquid - initial volume of liquid})}{\text{Initial volume of liquid}} \times 100$$

$$\text{Foaming stability (\%):} \frac{(\text{Volume of foam after 30 min - initial volume of liquid})}{\text{Initial volume of liquid}} \times 100$$

Results from three samples were then averaged ad analysis was repeated for bovine gelatin.

Emulsifying capacity and stability

Emulsifying capacity and stability were determined by the method described by Neto et al. (2001). An emulsion was prepared with 5 ml of gelatin solution at a concentration of 10 mg/ml in distilled water for each of three samples. The solution was homogenized with 5 ml of palm oil for 1 min after which the emulsion was centrifuged at 1100 rpm for 5 min. The height of the emulsified layer and total content was measured as emulsion capacity. Emulsion stability was determined by heating the gelatin solution in a water bath at 55oC followed by centrifugation at 2000 x g for 5 min and then measured. Emulsifying capacity and stability were calculated as follows:

$$\text{Emulsifying capacity (\%)} = \frac{\text{Height of emulsion layer}}{\text{Height of the total content}} \times 100$$

$$\text{Emulsifying stability (\%)} = \frac{\text{Height of emulsion layer after heating}}{\text{Height of emulsion layer before heating}} \times 100$$

Results were then averaged and analysis was repeated for bovine gelatin.

Fourier transform infrared (FTIR) spectroscopy

Structural properties of gelatin were measured via FTIR (Nicole, Thermo Electrin, USA) using a Deuterated triglycine sulphate (DTGS) detector. The sample holder (Multi-bounce horizontal attenuated total reflectance unit (HATR) with a plate of zinc

selenite (Zn Se) crystal was cleaned thoroughly with acetone after which the background spectrum was collected (without test sample) using 4000–650 cm^{-1} resolution for 32 scans. Gelatin samples were then placed on the plate for analysis. A single-beam for each sample was measured against a single air-beam background reading before conversion to absorbance units. The analysis was done for three samples from each drying method.

Statistical analysis

Results are expressed as a mean (± SD) for each metric. Statistical comparison of means with the one-way ANOVA was done using Minitab 14.0 to measure significance differences (p <0.05) between samples.

Results and Discussion

Gelatin extraction

No significant differences (p >0.05) in yields between freeze dried (9.25%) and vacuum oven dried (12.86%) samples were noted. However, yields from both methods were lower than those (16%) previously reported by Sarbon *et al.* (2013) for freeze dried gelatin. This difference may due to the conversion of collagen to gelatin, a process that depends on temperature, extraction time, pH, and pretreatment conditions (Karim and Bhat, 2009). Furthermore, a lower yield following freeze dried gelatin (vs. vacuum oven dried) may have come from losses that occur during processing where portions of powdered samples may have adhered to a container or tray.

Dynamic oscillatory measurement

The storage (G′) and loss (G″) modulus for both gelling from 40 to 10°C and melting from 10 to 40 °C showed a gelling-point for freeze dried samples at 23.68°C, vs. vacuum oven drying and bovine (data not shown) at 22.84°C and 19.09°C, respectively. Freeze dried and vacuum oven dried chicken skin gelatins both had higher gelling points than bovine gelatin, possibly due to the higher content of imino acid and hydroxyproline in chicken skin as previously reported by Sarbon *et al.* (2013) (13.42% and 12.13%, respectively, compared to bovine gelatin at 12.66% and 10.67%, respectively). Previous study found that the higher elastic modulus (G′) as well as thermo-stability are related to imino acid composition where hydroxyproline plays a unique role in stabilizing the triple helix (Sarbon *et al.*, 2013). Similarly, the elastic modulus (G′) obtained in this study was higher than that for bovine gelatin. A

maximum G′ and G″ value were registered by using vacuum oven dried of chicken skin gelatin which were 1743.32 Pa and 56.76 Pa at 10°C followed by bovine gelatin which were 1153.21 Pa (G′) and 41.15 Pa (G″) respectively. While for feeze dried chicken skin gelatin presented G′ and G″ value at 127.17 Pa and 2.672 Pa, respectively. An increase in G′ value during thermal gelation process is an indicative of elastic structure development (Binsi *et al.*, 2009). According to Gómez-Guillén *et al.* (2011), gelling temperature for gelatins derived from warm water fish ranged from 18–19°C compared to cold-water fish gelatins (4–12°C). Both concluded that higher gelling points represented thermal stability due to Pro-rich regions in collagen and gelatin molecules, which are higher in mammals and fresh warm water fish compared to cold water fish.

The melting points for freeze dried vs. vacuum oven dried samples were 32.64°C and 29.12°C (p <0.05) respectively; higher than bovine gelatin's (28.8°C). Higher melting points reflect higher gel strength (Boran *et al.*, 2010). Sarbon *et al.* (2013) previously reported the gel strength of chicken skin gelatin at 355 g vs. bovine gelatin at 229 g. The melting point obtained for the present study was also higher than those previously reported for gelatins extracted from fish skins (range: 16.80–28.67°C). Even lower melting points for fish gelatins have been reported for bigeye snapper (16.8°C, Binsi *et al.*, 2009). Choi and Regenstein (2000) suggested that melting points increase with maturation time and also observed that levels of proline and hydroxyl proline contributed to melting point characteristics.

The crossover modulus observed during gelling and melting for freeze dried samples was (0.12 Pa, 0.20 Pa) vs. vacuum oven dried samples (0.09 Pa, 0.08 Pa) compared to bovine gelatin (0.05 Pa, 0.05 Pa). These results were indicative of higher elasticity and loss (G' and G") modulus for freeze-dried chicken skin gelatin than for vacuum oven dried samples and the bovine product during gelling and melting. The higher crossover value clearly showed greater gel strength for chicken skin gelatin vs. bovine gelatin. This result agreed with previous study by Sarbon *et al.* (2013) which found gel strength for chicken skin gelatin at (355 ±1.48 g) vs. bovine gelatin (259 ±0.71 g). The dynamic viscoelasticity profile of chicken skin gelatin was higher (G' and G" at 10 °C 8273 Pa and 6639 Pa, respectively) than bovine gelatin (4330 Pa and 4122 Pa, p <0.05, respectively) (Sarbon *et al.*, 2013). The G' value in the present study sharply increased due to a greater quantity of available energy that was elastically stored, thus allowing for the rapid formation of junction zones that reinforced

the gel's network (Sarbon *et al.*, 2013). Furthermore, previously Sarbon *et al.* (2013) assigned higher values for G' and G'' as the result of gelatin equilibration for 10 min at 10°C. Cheow *et al.*, (2007) reported similar viscoelastic properties for fish skin gelatin after treatment maturation: for sin croaker (G' increased from 44 to 1270 Pa); for shortfin scad (G'' from 118 to 1690 Pa) after equilibration at 5°C for 2 hours; while G' for bovine gelatin increased from 2160 to 4200 Pa. Nevertheless, the present results only reflect the crossover modulus during gelling and melting without equilibration.

Water holding capacity

Figure 1 shows results for the water holding capacity of freeze-dried (15.6 ml/g) chicken skin gel samples and for vacuum oven dried (15.37 ml/g) samples compared to bovine gelatin (13.87 ml/g). However, no significant differences between the three were noted (p >0.05).

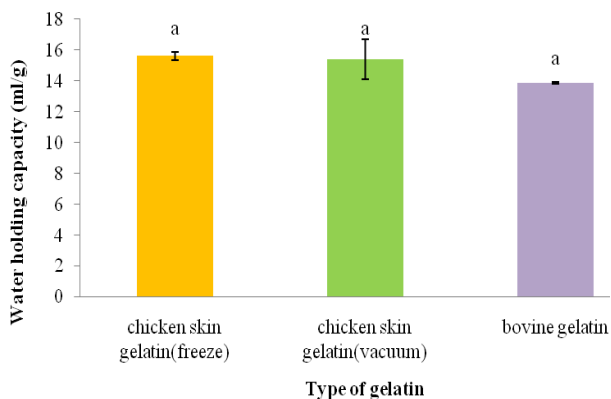Figure 1. Water holding capacity (ml/g) for different gelatins. The letter (a) indicates no significant difference (p >0.05)

Lower water holding capacity mainly depends on a reduced content of hydrophilic amino acids and hydroxyproline (Ninan *et al.*, 2011), which suggests that chicken skin gelatin has them in higher amounts compared to bovine gelatin. This finding also agreed with previous study by Sarbon *et al.* (2013) in which chicken skin gelatin contained high amounts of glutamate (5.84%), arginine (5.57%), histidine (0.30%) and hydroxyproline (12.13%) vs. bovine gelatin which contained lower amounts of glutamate (5.43%), arginine (5.09%) and hydroxyproline (10.67%).

Fat binding capacity

Fat-binding capacity (FBC) is a functional property that is closely related to gelatin texture which depends on interactions between oil components and gelatin. Figure 2 presents that FBC for vacuum oven dried sample was higher (5.50 ml/g) than freeze dried
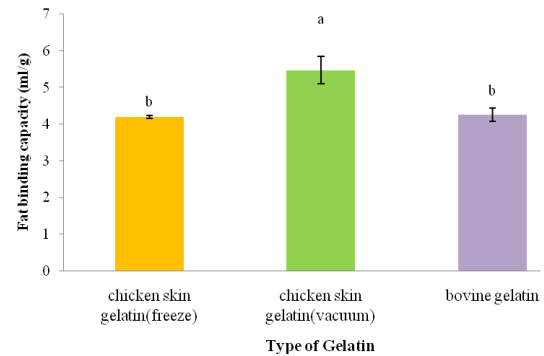

Figure 2. Fat binding capacity for different gelatins. Different letters (a, b) indicate significant differences (p < 0.05)

sample (4.2 ml/g), showing a significant difference (p <0.05). Furthermore, FBC for bovine gelatin was 4.3 ml/g, which was similar to freeze dried chicken skin gelatin samples. Chicken skin gelatin's higher FBC may derive from an higher hydrophobic amino acid content including alanine (10.08%), leucine (2.63%) and proline (13.42%) as the reported previously, whereas bovine alanine, leucine and proline were measured at 8.41%, 1.89% and 12.66%, respectively (Sarbon *et al.*, 2013). Vacuum dried chicken skin gelatin's higher FBC is likely due to exposed hydrophilic groups compared to freeze dried samples. This mechanism was supported by Cho *et al.* (2004) who posited that hydrophobic residues were exposed during the hot-air drying process during their study of process optimization and observation of the functional properties of shark cartilage gelatin *(Isurus oxyrinchus)*. Study of three different samples showed that the hydrophobic amino acid, tyrosine, comprised 1.17% of the gelatin, much higher than that of porcine gelatin (0.10%) and additive grades from porcine skin (0.24%) (Cho *et al.*, 2004). Their results suggested that higher FBC was likely attributable to higher tyrosine content. Furthermore, present observations strongly suggest that higher FBC also corresponds with lower water holding capacity as demonstrated by Ninan *et al.* (2011) who found that Rohu skin gelatin had the highest fat binding capacity and lowest water holding capacity.

Foaming capacity

Figure 3 demonstrates significant differences (p <0.05) for foaming capacity between chicken skin (freeze and oven dried) and bovine gelatins. The foaming capacity of freeze dried samples was significantly higher (176%) than both vacuum oven dried (80%) and bovine (61.17%) gelatins. The lower foaming capacity for vacuum oven dried samples suggested temperature mediated interactions between protein and water that inhibited foam
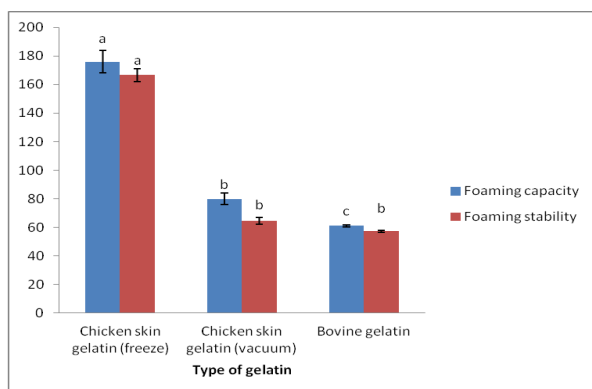
Figure 3. Foaming capacity and stability for different gelatins. Different letters (a, b, c) indicate significant differences (p <0.05)

formation. Different drying methods may also result in different particle size within the gelatin that also affect foam formation as particle size is a factor for foaming ability. The freeze dried gelatin powder was found to have finer particles than vacuum dried gelatin powder.

This result agreed with Kwak *et al*. (2007) who reported that the foaming capacity of freeze dried shark *(Isurus oxyrinchus)* cartilage gelatin was higher than gelatins dried by either hot-air or spray. The results obtained also indicated that bovine gelatin's foaming capacity was lower than either chicken skin gelatin samples; most likely due to the latter's higher content of hydrophobic amino acids such as proline, isoleucine, leucine and phenylalanine (Sarbon *et al*., 2013). A comparison of fish skin and bovine gelatin foaming capacity was also reported by Jellouli *et al*. (2011). Their study of trigger fish skin gelatin showed higher amounts of hydrophobic amino acids (319 per 1000 residue) than Halal bovine gelatin (313 per 1000 residue). In general, proteins are rapidly adsorbed by a newly-created air liquid interface during bubbling and undergo unfolding and molecular rearrangement at the interface. Thus, they exhibit better foaming ability than proteins that adsorb slowly and resist unfolding (Damodaran, 1997). Hence, the foaming capacity of protein improves by increasing its capacity to decrease surface tension and by making it more flexible, thereby exposing the protein to more hydrophobic residue (Mutilangi *et al*., 1996).

Foaming stability

Figure 3 depicts results for foaming stability. Freeze dried chicken skin gelatin (166.67%) was significantly higher (p <0.05) than either vacuum oven dried samples (64.67%) or bovine gelatin (57.4%) with no significant difference found between the latter two (p >0.05); albeit, bovine gelatin showed the least foaming stability and ability. The higher foaming stability of chicken skin gelatin was possibly

due to the greater presence of highly hydrophobic groups consequent to molecular reactions in the foam lamella (Benjakul *et al*., 2010)

According to Benjakul *et al*. (2010) indicated protein stability is a combined effect of cohesive forces between molecules in the foam lamella as well as hydrophobic groups and the prevention of drainage from the lamella. These effects are achieved by gelatin molecules anchored in the interface by the presence of long hydrophobic chains within the lamella. Moreover, gravitational drainage of liquid from the lamella and a disproportion of gas bubbles vis-à-vis inter-bubble gas diffusion contributes to foam instability (Aewsiri *et al*., 2009). Previously, Sarbon *et al*. (2013) reported similar findings by showing that freeze dried chicken skin gelatin had higher amounts of hydrophobic groups such as proline (13.42%) and alanine (10.08%) compared to bovine gelatin (12.66% proline, 8.41% alanine).

The lower foaming stability of vacuum oven dried chicken skin gelatin may be due to the lower content of negatively charged amino acids (Aspartic and Glutamic) (Liceaga-Gesualdo and Li-Chan, 1999). According to Aletor and Abiodun (2013), who studied the effects of drying on functional properties and protein solubility for edible tropical leafy vegetables, freeze-dried samples had a significantly higher level of foaming capacity and foaming stability (p <0.05), but lower water absorption capacity, swelling power, solubility and bulk density (loose and packed) than sun-dried samples.

Emulsion capacity

Figure 4 showed results from the emulsion capacity assay on vacuum oven chicken skin gelatin (56.51%), freeze dried samples (55.97%) and bovine gelatin (51.4%).
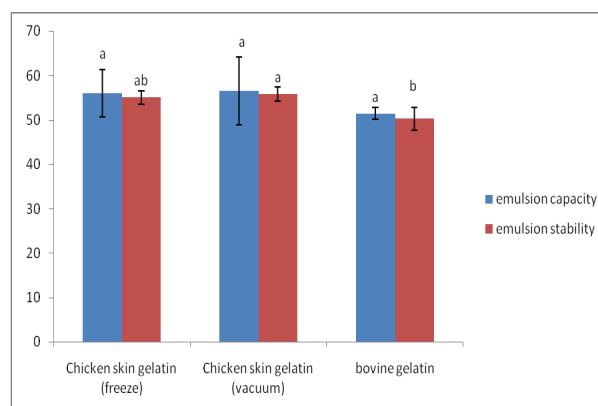

Figure 4. Emulsion capacity and stability for different gelatins. Different letters (a, b) indicate significant differences (p <0.05)

No significant differences were found between all samples. Emulsion capacity is a property that is closely associated with protein hydrophobicity (Kato and Nakai, 1980). The present results indicated that chicken skin and bovine gelatin possess hydrophobic content in agreement with previous study by Sarbon *et al.* (2013) which also found no significant differences in hydrophobic tryptophan residue between chicken skin (0.21) and bovine gelatins (0.22).

Capeda *et al.* (1998) demonstrated that emulsion capacity increased as concentration of the sample increased and that the emulsion capacity of spray-dried protein was greater than freeze-dried at concentrations less than 2.5 mg/ml. Similarly, higher emulsion capacity has been recorded by increasing gelatin concentrations so that higher degrees of polypeptide unfolding occur from shearing during emulsification (Binsi *et al.*, 2009). Binsi's study also mentioned a difference in emulsion capacity between spray and freeze dried protein, likely due to the smaller particle size of spray dried powder. Together with this strongly suggest that higher concentrations of gelatin molecules possess higher hydrophilic content which then interact causing less availability of the gelatin at the oil–water interface.

Emulsion stability

A significant difference in emulsion stability (p <0.05) between chicken skin and bovine gelatin as effected by different drying method was determined (see Figure 4). Vacuum dried samples measured 55.79% while freeze dried samples averaged 55.06% (no significant difference (p >0.05). However bovine samples averaged 50.28% (p <0.05). This result obtained may due to higher hydrophobic content in chicken skins in which was in the same agreement as studied by Sarbon *et al.* (2013). This finding supported by Zayas (1997) who posited that protein surface hydrophobicity specifically influenced emulsifying properties, especially emulsion stability. Hence, bovine gelatin's lower emulsion stability is likely due to its lower content of hydrophobic groups (Sarbon *et al.*, 2013). Moreover, smaller peptides of higher solubility can migrate to the interface and effectively form a film around oil droplets, and thus increase emulsifying efficiency (Kittiphattanabawon *et al.*, 2012).

Likewise, Jellouli *et al.* (2011) reported the emulsion stability index of grey triggerfish skin gelatin was more stable than bovine gelatin (p <0.05). Moreover, larger and longer peptides may enhance the effective stabilization of protein film at the interface for oil-in-water emulsions prepared with high molecular weight fish gelatin (120 kDa).

The latter proved more stable than lower molecular weight fish gelatin (50 kDa) as reported by Surh *et al.* (2006). In addition, Yamauchi et al. (1980) suggested that higher protein concentrations facilitated greater protein adsorption at the interface.

Structural properties of gelatins by FTIR

Figure 5 shows the structural properties observed via FTIR for all gelatin samples. All samples shared a similar spectrum with absorption bands situated in amide regions. Amide I and Amide II bands for freeze dried samples were 1634.02 cm^{-1} and 1539.05 cm^{-1}; for vacuum oven samples were 1635.06 cm^{-1} and 1539.32 cm^{-1}; and for bovine gelatin were 1633.94 cm^{-1} and 1538.95 cm^{-1}, respectively.
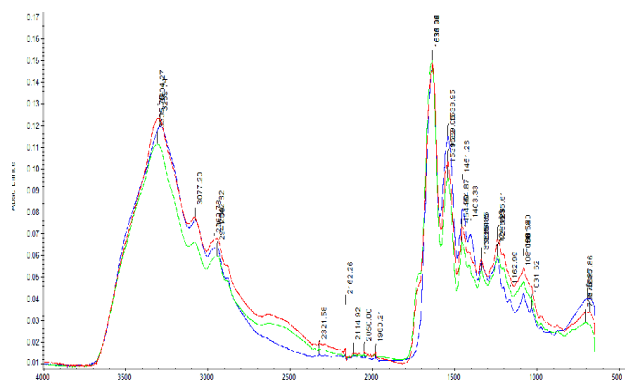

Figure 5. FTIR spectra for three types of gelatin where there is no significant different (p>0.05)

The Amide I vibration primarily represents C=O stretching coupled to contributions from CN stretching, CCN deformation and in-plane NH bending (Bandekar, 1992). Absorption in the Amide I region is the most useful infrared spectroscopic finding in the study of secondary protein structure (Surewicz and Mantsch, 1988). In addition, an absorption peak at 1633 cm^{-1} is characteristic of gelatin's coil structure (Yakimets *et al.*, 2005). Amide I and II bands from 1700–1600 and from 1560–1500cm^{-1}, respectively, was reported by Yakimets *et al.* (2005) for gelatin film of bovine gelatin.

Of the three samples presently studied, vacuum oven dried chicken skin gelatin's Amide I band appeared highest, indicating the higher frequency was related to a higher processing temperature. This can most likely be attributed to the greater loss of molecular order in the triple helix due to an uncoupling of intermolecular cross-links (Kittiphattanabawon *et al.*, 2012). By comparison, the Amide I band reported for bigeye snapper skin gelatin was 1630 cm^{-1} (Benjakul *et al.*, 2009).

Amide I absorption is determined by secondary structures adopted by polypeptide chains that reflect a backbone conformation and hydrogen-

bonding patterns (Barth and Zscherp, 2002). Strictly speaking, Amide I contours for proteins or polypeptides comprise overlapping component bands that represent α-helices, ß-sheets, turns and random structures (Kong and Yu, 2007). Amide II vibrations, on the other hand, may be attributed to 'out-of-phase' combinations of CN stretching and in-plane NH deformation of peptide groups (Bandekar, 1992). Vacuum oven and freeze dried Amide III bands observed were 1236.66 cm^{-1} and 1235.61 cm-1, respectively, with no significant difference (p >0.05). The Amide III bands for bovine gelatin ranged from 1031.62–1241.66 cm^{-1}, representing a combination of peaks due to C-N stretching and N-H deformation from amide linkages, as well as absorptions that arose out of wagging vibrations from the CH_2 groups of the glycine backbone and proline side-chains (Almeida *et al*., 2012).

Conclusion

This study found that there were no significant differences (p >0.05) between freeze dried and vacuum oven dried chicken skin gelatins compared to bovine gelatin as effected by different drying method for characterized properties. Vacuum oven dried gelatin and freeze dried gelatin produced showed no significant different on fat binding and emulsion stability, water holding and emulsion capacities compared to bovine gelatin. Bovine gelatin did demonstrate lower functional properties compared to chicken skin gelatin. These included fat binding capacity, foaming capacity, foaming stability, and emulsion stability. Furthermore, FTIR analysis demonstrated similar structures for both freeze dried and oven dried chicken skin gelatins in contrast to bovine gelatin structure.

References

Aewsiri, T., Benjakul, S. and Visessanguan, W. 2009. Functional properties of gelatin from cuttlefish *(Sepia pharaonis)* skin as affected by bleaching using hydrogen peroxide. Food Chemistry 115: 243-249.

Aletor, R. and Abiodun, O. 2013. Assessing the effects of drying on the functional properties and protein solubility of some edible tropical leafy vegetables. Research Journal of Chemical Sciences 3: 20–26.

Almeida, P.F., Araújo, M.G.O. and Santana, J.C.C. 2012. Collagen extraction from chicken feet for jelly production. Acta Science Technology 34: 345–351.

AOAC (2006). Official methods of analysis of AOAC international (18th ed.). Virginia, USA: Association of Official and Analytical Chemists International.

Badii, F. and Howell, N.K. 2006. Fish gelatin: structure, gelling properties and interaction with egg albumen proteins. Food Hydrocolloids 20: 630–640.

Bandekar, J. 1992. Amide modes and protein conformation. Biochimica et Biophysica Acta – Protein Structure and Molecular Enzymology 1120: 123–143.

Barth, A. and Zscherp, C. 2002. What vibrations tell about proteins. Quarterly Reviews of Biophysics 35: 369-430.

Benjakul, S., Oungbho, K., Visessanguan, W., Thiansilakul, Y. and Roytrakul S. 2009. Characteristics of gelatin from the skins of bigeye snapper, *Priacanthus tayenus* and Priacanthus macracanthus. Food Chemistry 116 (2): 445–451.

Benjakul, S., Kittiphattanabawona, P., Visessanguan, W. and Shahidi, F. 2010. Comparative study on characteristics of gelatin from the skins of brownbanded bamboo shark and blacktip shark as affected by extraction conditions. Food Hydrocolloids 24: 164–171.

Binsi, P.K., Shamasundar, B.A., Dileep, A.O., Badii, F. and Howell, N.K. 2009. Rheological and functional properties of gelatin from the skin of Bigeye snapper *(Priacanthus hamrur)* fish: Influence of gelatin on the gel-forming ability of fish mince. Food Hydrocolloids 23: 132–145.

Boran, G., Mulvane, S.J. and Regenstein, J.M. 2010. Rheological properties of gelatin from silver carp skin compared to commercially available gelatins from different sources. Journal Food Science 75: 565–571.

Cepeda, E., Villara´n, M.C. and Aranguiz, N. 1998. Functional properties of faba bean *(Vicia faba)* protein flour dried by spray drying and freeze drying. Journal of Food Engineering 36: 303–310.

Cheow, C.S., Sarbon, N.M., Kyaw, Z. and Howell, N.K. 2007. Preparation and characterization of gelatins from the skins of sin croaker *(Johnius dussumieri)* and shortfin scad *(Decapterus macrosoma)*. Food Chemistry 101: 386–391.

Cho, S. M., Kwak, K.S., Park, D.C., Gu, Y.S., Ji, C.I., Jang, D.H., Lee, Y.B. and Kim, S.B. 2004. Processing optimization and functional properties of gelatin from shark *(Isurus oxyrinchus)* cartilage. Food Hydrocolloids 18: 573-579.

Choi, S.S. and Regeinstein, J.M. 2000. Physicochemical and sensory characteristics of fish gelatin. Journal of Food Science 65(2) : 194–199.

Cliché, S., Amiot, J., Avezard, C. and Gariepy, C. 2003. Extraction and characterization of collagen with or without telopeptides from chicken skin. Poultry Science 8: 503–509.

Damodaran, S. 1997. Protein-stabilized foams and emulsions. In Damodaran, S. and Paraf, A. (Eds). Food proteins and their applications, p. 57–110. New York: Marcel Dekker.

Diniz, A.M. and Martin, A.M. 1997. Optimization of nitrogen recovery in the enzymatic hydrolysis of dogfish *(Squalus acanthias)* protein: Composition of the hydrolysates. International Journal of Food Science and Nutrition 48: 191–200.

Flory, P.J. and Weaver, E.S. 1960. Helix, coil transition in dilute aqueous collagen solution. Journal of American Chemical Society 82: 4518–4525.

GME, 2008. Gelatin Manufacturers of Europe. Web. 2008.

Gómez-Guillén, M.C., Giménez, B., López-Caballero, M.E. and Montero, M.P. 2011. Functional and bioactive properties of collagen and gelatin from alternative sources: A review. Food Hydrocolloids 25: 1813–1827.

Gudmundsson, M. 2002. Rheological properties of fish gelatin. Journal of Food Science 67: 2172–2176.

Jellouli, K., Balti, R., Bougatef, A., Hmidet, N., Barkia, A. and Nasri, M. 2011. Chemical composition and characteristics of skin gelatin from grey triggerfish *(Balistes capriscus)*. Food Science and Technology 44: 1965–1970.

Karim, A.A. and Bhat, R. 2009. Review: Fish gelatin: properties, challenges, and prospects as an alternative to mammalian gelatins. Food Hydrocolloids 23: 563–576.

Kato, A. and Nakai, S. 1980. Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins. Biochimica et Biophysica Acta 624: 13-20.

Kittiphattanabawon, P., Benjakul, S., Visessanguan, W. and Shahidi, F. 2012. Effect of extraction temperature on functional properties and antioxidative activities of gelatin from shark skin. Food and Bioprocess Technology 5: 2646–2654.

Kong, J. and Yu, S. 2007. Fourier transforms infrared spectroscopic analysis of protein secondary structures. Acta Biochimica et Biophysica Sinica 39: 549–559.

Kwak, K.S., Cho, S.M., Ji, C.I., Lee, Y.B. and Kim, S.B. 2007. Changes in functional properties of shark *(Isurus oxyrinchus)* cartilage gelatin produced by different drying methods. International Journal of Food Science and Technology 44: 1480–1484.

Liceaga-Gesualdo, A.M. and Li-Chan, E.C. 1999. Functional properties of fish protein hydrolysate from herring *(Clupea harengus)*. Journal of Food Science 64: 1000–1004.

Mitchell, J.R. 1976. The rheology of gels. Journal of Texture Studies 7 (313): 161–169.

Mokhtar, I. and Chia, S.P. 2000. Livestock Pollution and Control in Sabah. Proceedings; First Sarawak-Sabah Environmental Convention. Natural Resources and Environment Board, Sarawak and Department of Environment, Sarawak.

Montero, P. and Gomez-Guillen, M.C. 2000. Extracting conditions for megrim (*Lepidorhombus boscii)* skin collagen that affect functional properties of resultant gelatin. Journal of Food Science 65: 434–438.

Neto, V.Q., Narain, N., Silva, J.B. and Bora, P.S. 2001. Functional properties of raw and heat processed cashew nut (*Anarcarduim occidentale* L.) kernel protein isolates. Nahrung/Food 45: 258–262.

Ninan, G., Jose, J. and Abubacker, Z. 2011. Preparation and characterization of gelatin extracted from the skins of rohu *(Labeo rohita)* and common carp *(Cyprinus carpio)*. Journal of Food Processing and Preservation 35: 143–162.

Norziah, M.H., Al-Hassan, A., Khairulnizam, A.B., Mordi, M.N. and Norita, M. 2008. Characterization of fish gelatin from surimi processing wastes: thermal analysis and effect of transglutaminase on gel properties. Food Hydrocolloids 23: 1610–1616.

Sarbon, N.M., Badii, F. and Howell, N. K. 2013. Preparation and characterization of chicken skin gelatin as an alternative to mammalian gelatin. Food Hydrocolloids 30: 143–151.

Sathe, S.K., Deshpande, S.S. and Salunkhe, D.K. 1982. Functional properties of lupin seed *(Supinus mutabilis)* proteins and protein concentrates. Journal of Food Science 7: 191–197.

Schrieber, R. and Gareis, H. 2007. Gelatine Handbook. Wiley-VCH, GmbH and Co.,Weinheim.

Shahidi, F., Han, X.Q. and Syanoiocki, J. 1995. Production and characteristics of protein hydrolysates from cpelin *(Mallotus villosus)*. Food Chemistry 53: 285–293.

Sheu, K.S. and Chen, T.C. 2002. Yield and quality characteristics of edible broiler skin fat as obtained from five rendering methods. Journal of Food Engineering 55: 263–269.

Surewicz, W. and Mantsch, H.H. 1988. New insight into protein secondary structure from resolution-enhanced infrared spectra: Protein Structure and Molecular Enzymology. Biochimica nad Biophysica Acta 952: 115–130.

Surh, J., Ward, L.S. and Mc Clements, D.J. 2006. Ability of conventional and nutritionally modified whey protein concentrates to stabilize oil-in-water emulsions. Food Research International 39: 761–771.

Tabilo Munizaga, G., Gustavo, V. and Barbosa-Canovas 2005. Rheology for the food industry. Journal of Food Engineering 67: 147–156.

Yakimets, I., Wellner, N., Smith, A. C., Wilson, R. H., Farhat, I., and Mitchell, J. 2005. Mechanical properties with respect to water content of gelatin films in glassy state. Polymer 46: 12577-12585.

Yamauchi, K., Shimizu, M. and Kamiya, T. 1980. Emulsifying properties of whey protein. Journal of Food Science 45: 1237–1242.

Yang, H. and Wang, Y. 2009. Effects of concentration on nanostructural images and physical properties of gelatin from channel catfish skins. Food Hydrocolloids 23: 577–584.

Zayas, J.F. 1997. Solubility of proteins: In Functionality of Proteins in Food, p. 6-22. Berlin: Springer-Verlag.

Zhou, P. and Regenstein, J.M. 2005. Effects of alkaline and acid pretreatments on Alaska pollock skin gelatin extraction. Journal of Food Science 70: 392–396.