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ORIGINAL ARTICLE

Effect of drying method on functional properties and antioxidant activities of chicken skin gelatin hydrolysate

Wan Hasyera Wan Omar[1] · **N. M. Sarbon**[1]

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Abstract The aim of this study is to investigate the functional and antioxidant properties of chicken skin gelatin hydrolysate (CSGH) as affected by the drying method used in the preparation of gelatin (freeze-dried and vacuum dried). CSGH obtained from freeze-dried gelatin showed better functional properties such as emulsifying activity index (EAI), water holding and oil binding capacity at different pH compared to CSGH produced from vacuum dried gelatin. Meanwhile, the CSGH of the vacuum dried gelatin exhibited a better emulsifying stability index (ESI), foaming capacity and stability. CSGH from freeze-dried gelatin showed better antioxidant, DPPH radical scavenging and metal chelating activity.

Keywords Chicken skin gelatin · Hydrolysate · Drying methods · Functional properties · Antioxidant activities

Introduction

Gelatin is a high molecular weight protein that is obtained by partial hydrolysis of collagen, which acts as a stabilizer, thickener, texture and as an ingredient for improvement to the elasticity, consistency and stability of food (Zhou and Regenstein 2005). Most available gelatins are manufactured from mammalian resources, such as pig skin, cattle bones and cattle hide. However, due to several reason such as bovine spongiform encephalopathy (BSE) or transmissible spongiform encephalopathy (TSE) and halal issue, many researchers are exceedingly interested in finding alternative gelatin sources. There are also alternative gelatins produced from other sources, such as eel (*Monopterus albus*) skin (Rosli and Sarbon 2015), cobia (*Rachylcentron canadum*) skin (Razali et al. 2015), sin croaker (*Johnius dussumieri*), shortfin scad skin (*Decapterus macrosoma*) (Cheow et al. 2007) and chicken skin gelatin (Sarbon et al. 2013). In addition, Jayathilakan et al. (2012) reported that waste products from poultry and egg production industries depends largely on waste management. Therefore, the utilization of skin from chicken can be one of the alternative to produce gelatin.

Gelatin hydrolysate have been used in food industry for a wide range of products such as instant teas, beverages, low fat spreads, low fat cheese, canned meat, marshmallow, cereal bars and pastilles. These zero bloom gelatins do not gel but are widely used in confectionery as substitutes for carbohydrates, as a protein sources, whipping and a binding agent for cereal bars. Higher molecular weight hydrolysate are used in the manufacture of soups, sauces and prepared meals to impart a creamy smooth consistency. These act as binding agents in low fat spreads. Gelatin hydrolysate also have been used in several energy drinks for athletes (Philips and Williams 2011). The gelatin hydrolysate from different sources, such as the skin of sole and squid (Giminez et al. 2009), and skin of cobia (*Rachylcentron canadum*) (Razali et al. 2015), have been evaluated for the functional properties and antioxidant activities. All protein hydrolysates possess properties such as solubility, emulsifying properties, thickening abilities, water holding capacity, and oil binding ability. Sarbon et al. (2013) have successfully extracted and characterized chicken skin gelatin as new potential gelatin sources. Chicken skin gelatin was reported to possess better

✉ N. M. Sarbon
norizah@umt.edu.my

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

characteristics as compared to mammalian gelatin (Sarbon et al. 2013). However, to date no study reported on the production of chicken skin gelatin hydrolysate in terms of its functional and antioxidant properties.

Alcalase is an alkaline enzyme produced from *Bacillus licheniformis*. It has been chosen as it was reported to be one of the highly potential bacterial proteases used to prepare protein hydrolysates due to higher proteolytic activity as compared to acidic and neutral enzymes (Klompong et al. 2007). According Roslan et al. (2014), alcalase is active at the pH range from 6 to 10 and temperature range from 50 to 70 °C. In contrast, acidic proteases such as pepsin, trypsin and chymotrypsin are active in acidic environment (pH 1–pH 4). Alcalase is an endopeptidase which attacks internal peptide bonds of a protein while exopeptidases such as aminopeptidase, carboxypeptidase A and flavourzyme detach the terminal amino acids from the protein chain (Peksa and Miedzianka 2014).

There has been increasing demand for natural antioxidants due to concerns about the potential health risks of synthetic antioxidants. In addition, the bioactive peptides with antioxidant properties derived from many sources of proteins by enzymatic hydrolysis have become of interest for the pharmaceutical, health food and processing or preservation industries (Hagen and Sandnes 2004). Several studies have described the antioxidant activity of gelatin hydrolysates from yellowfin sole (Jun et al. 2004), and cobia (*Rachylcentron canadum*) (Razali et al. 2015). The antioxidant activity is dependent on the molecular weight, amino acid composition and their sequences. Gelatin with a shorter molecular weight containing a shorter chain has been reported to possess higher antioxidative activity (Zhang et al. 2012).

The drying method in gelatin production can be divided into low temperature (freeze-dried, −50 °C) and high (oven dried, 45 °C) temperature (Rasli and Sarbon 2015). Between the two drying methods which is more economically viable, freeze-drying has been developed as a dehydration process for high quality products and has proven for high value products (Ratti 2008), while vacuum drying allows the drying temperature to be reduced and a higher quality to be obtained than the conventional air drying process at atmospheric pressure (Jaya and Das 2003). Heat can modify the functional properties due to the aggregation of the denatured molecule that occurred. The low molecular weight peptides make hydrolysis easily to occur as compared to the high molecular weight peptides. In addition, at high temperature which is more likely resulted in the production of low molecular peptides, the occurrence of conformational change was irreversible and polymerization by the formation of intermolecular disulphide bonds. Therefore, the aggregation of the denatured molecules is mediated by hydrophobic and sulfhydryl-disulphide interchange reaction (Klompong et al. 2007).

Therefore, the purpose of this study is to prepare chicken skin gelatin using different drying methods (freeze-dried and vacuum oven dried). Enzymatically hydrolysed chicken skin gelatin was prepared using different drying methods to evaluate their effect on functional properties and antioxidant activities.

Materials and methods

Materials

Fresh chicken skins were obtained from a local supplier, TD Poultry, Chendering, Kuala Terengganu, Malaysia. It was chilled in ice while being transported to the laboratory. The visible fat was mechanically removed, the skin was washed, stored at −80 °C until used for further experiments. The enzyme Alcalase was obtained from Sigma Aldrich. All the chemicals used were of analytical grade.

Methods

Sample preparation

The frozen chicken skins were thawed in the chiller (4–5 °C) overnight. After thoroughly rinsing in excessive water to remove impurities, the skins were cut into small pieces and freeze-dried. The completely dried skins were ground before being defatted using the Soxhlet method (AOAC 2006).

Extraction of chicken skin gelatin

Gelatin was extracted from the chicken skin according to the method of Sarbon et al. (2013) with a slight modification. The defatted dried chicken skin was treated with 0.15% (w/v) sodium hydroxide, 0.15% (v/v) sulphuric acid and 0.7% (w/v) citric acid solution consecutively with skin to acid/alkali ratio of 1:14. The mixture was shaken well and slowly stirred using a magnetic stirrer at room temperature (30 min) before centrifuging (Multi-purpose centrifuge Gyrozen 158R, Deejan, Korea) at 6500×*g* for 10 min. This step was repeated three times. Every 30 min, the alkaline solution was changed to remove non-collagenous proteins and pigments. Then, the pellets were washed with distilled water to remove any residual salts followed by centrifuging at 6500×*g* for 15 min. The final water extraction was carried out in a water bath (Protech model-903, Germany) at controlled temperature (45 °C) for overnight. The extract must immerse in distilled water. The resulting mixture was filtered, evaporated under a vacuum

(45 °C) and freeze-dried (Freeze dry system, Freezone 6, LABCONCO, Kansas City, Missouri) or vacuum-dried (Vacuum Oven ADP-21, Yamato, Mexico) until it completely dried. Then, the samples were further enzymatically hydrolysed for further analyses.

Preparation of chicken skin gelatin hydrolysate (CSGH)

Chicken skin gelatin hydrolysate (CSGH) was prepared following Razali et al. (2015) with a slight modification. About 6.25 g of CSG (2.5%, w/v) was dissolved in distilled water and subjected to enzymatic hydrolysis for 3 h. Then, about 0.31 g of Alcalase was added in the solution with an enzyme-substrate ratio 1:20 (w:w) at optimum condition of pH 8, at a temperature of 50 °C. The pH of the reaction was kept constant by adjusting the value (pH 8) using 1 N NaOH and 1 N H_2SO_4 solution to the reaction medium. The enzymes were inactivated by heating at 90 °C for 10 min, and the sample was centrifuged at 3000×*g* for 15 min. The supernatant as hydrolysate was freeze-dried (LABCONCO, Kansas City, Missouri) in order to obtain CSGH powder. The hydrolysate was conducted in triplicate. The yield of CSGH was calculated as below:

$$\text{Yield of CSGH}\,(\%) = \frac{\text{Weight of CSGH}}{\text{Weight of gelatin}} \times 100$$

Functional properties of chicken skin gelatin hydrolysate (CSGH)

Foaming capacity and stability properties The foaming properties were determined using the method described by Razali et al. (2015) with some modification. Approximately, 20 ml of sample solution (0.2 g/ml) was adjusted to pH 2, 4, 6, 8 and 10. The mixture was homogenized (Homogenizer with generator, Eurostar power control-visc, IKA-WERKE, Germany) at a speed of 1600 rpm in order to incorporate the air in the sample solution for 2 min at room temperature. The whipped sample was immediately transferred into a 25 ml cylinder and the total volume was taken after 30 s. The foaming capacity was calculated as follows:

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

While, for determination of the foaming stability, the whipped sample was allowed to stand at room temperature for 3 min and the volume of the whipped sample was then recorded. All determinations were derived from the means of three measurements. The foaming stability was calculated as follows:

$$\text{Foam stability}\,(\%) = \frac{\text{Volume of foam after 30 min} - \text{Initial volume of liquid} \times 100}{\text{Initial volume of liquid}}$$

Emulsifying activity index (EAI) and stability index (ESI) properties The emulsifying properties of CSGH were determined using the method described by Razali et al. (2015). About 10 ml of corn oil and 30 ml of the sample solution (0.3 g/ml) was mixed. The mixture was homogenized using a homogenizer (Homogenizer with generator, Eurostar power control-visc, IKA-WERKE, Germany) with a speed of 20,000 rpm for 1 min. An aliquot of the emulsion (50 µl) was pipetted from the solution at the bottom of the container at 0 and 10 min after homogenization. Next, the mixture was mixed with 5 ml of 0.1% sodium dodecyl sulphate (SDS) solution. The absorbance of the diluted solution was measured at 500 nm using a spectrophotometer (Cary 50, Varian, Inc., United States).

In addition, the absorbance was measured immediately at 0 min (A_0) and 10 min (A_{10}) after the emulsion formation, the emulsifying activity index (EAI) and the emulsion stability index (ESI) were measured as follows:

$$\text{EAI}\,\left(\text{m}^2/\text{g}\right) = \frac{2 \times 2.303 \times \text{A}_{500}}{0.25 \times \text{weight of protein}\,(\text{g})}$$

$$\text{ESI}\,(\text{min}) = \text{A}_0 \times \text{AT}/\Delta\text{A}$$

where, A_{500} = Absorbance at 500 nm, A_0 = ESI at 0 min, AT = ESI at 10 min, $\Delta A = A_0 - AT$ and $\Delta t = 10$ min. All determinations were the means of three measurements.

Water holding capacity (WHC) properties The water holding capacity (WHC) properties of CSGH were determined using the method described by Razali et al. (2015) with a slight modification. Approximately (0.01 g/ml sample) 0.1 g of the sample was dispersed in 10 ml of distilled water by adjusting the pH to 2, 4, 6, 8 and 10. The sample was kept at room temperature for 30 min. Then, the mixture was centrifuged (Multi-purpose centrifuge Gyrozen 158R, Deejan, Korea) at 5000×*g* for 30 min. The supernatant was filtered using filter paper and the volume was measured. The volume of water absorbed, which was the difference between the initial volume of distilled water added to the sample and the volume of supernatant, was determined. The final result showed the volume (ml) of the water absorbed per weight of hydrolysate. All determinations were based on the means of

three measurements. The water holding capacity was determined using the following formula:

$$\text{Water holding capacity (ml/g)} = \frac{\text{Volume of water absorbed (ml)}}{\text{Weight of hydrolysate (g)}}$$

Oil binding capacity (OBC) properties The oil binding capacity (OBC) of CSGH was determined using the method described by Razali et al. (2015) with a slight modification. About (0.01 g/ml sample) 0.1 g of CSGH was added with 10 ml of corn oil in a 50 ml centrifuge tube by adjusting the pH to 2, 4, 6, 8 and 10 and was vortexed for 30 s. Next, the oil dispersion was centrifuged (Multipurpose centrifuge Gyrozen 158R, Deejan, Korea) at $3000\times g$ for 30 min. The free oil was decanted and the oil binding capacity was determined by weight difference. All determinations were from the means of three measurements. The calculation of the oil binding capacity was calculated using the following formula:

$$\text{Oil binding capacity (ml/g)} = \frac{\text{Oil absorbed (ml)}}{\text{Weight of hydrolysate (g)}}$$

Antioxidant activities of chicken skin gelatin hydrolysate (CSGH)

1,1-Diphenyl-2-picryhydrazyl (DPPH) radical scavenging activity The DPPH radical scavenging activity of CSGH was determined using the method described by Razali et al. (2015). About 500 µl (4.5 mg/ml) of sample solution was mixed with 500 µl of ethanol and 125 µl 0.02% (w/v) of DPPH in 99.5% ethanol. Then, the mixture was shaken vigorously and incubated in a dark place. After 60 min, the absorbance was measured at 517 nm using a spectrophotometer (Cary 50, Varian, Inc., United States). Butylated hydroxytoluene (BHT) and ascorbic acid acts as a positive control. All determinations were based on the means of three measurements. The calculation of the DPPH radical scavenging activity was calculated as follows:

$$\text{Radical-scavenging activity} = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100$$

where A_{blank} = absorbance of the control (blank), A_{sample} = absorbance of the sample

Metal chelating activity The metal chelating activity of CSGH was determined using the method described by Razali et al. (2015). About 2.5 µl of 2 mM $FeCl_2$ was added to the sample in 0.5 ml methanol. The reaction was then initiated by the addition of 5 mM ferrozine (0.1 ml). The mixture was vigorously shaken and left to stand at room temperature for 10 min. Then, the absorbance was measured at 562 nm using a spectrophotometer (Cary 50, Varian, Inc., United States). The BHT and ascorbic acid acts as a positive control. The metal chelating activity was calculated using the following formula:

$$\text{Metal chelating effect } (\%) = [(\text{Abs}_1 - \text{Abs}_2)/\text{Abs}_1] \times 100$$

where Abs_1 is the absorbance of the control and Abs_2 is the absorbance of CSGH. All determinations were based on the means of three measurements.

Reducing power assay The reducing power of CSGH was determined according to the method of Razali et al. (2015). A 500 µl (4.5 mg/ml) of sample was dissolved in ethanol and mixed with 1.25 ml of 0.2 M phosphate buffer (pH 6.6) and 1.25 ml of potassium ferracyanide (1%). Then, the mixture was incubated at 50 °C for 30 min, followed by the addition of 2.5 ml (w/v) trichloroacetic acid. The mixture was centrifuged (Multipurpose centrifuge Gyrozen 158R, Deejan, Korea) at $1650\times g$ for 10 min. About 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% (w/v) ferric chloride. After a 10 min reaction, the absorbance of the resulting solution was measured at 700 nm using a spectrophotometer (Cary 50, Varian, Inc., United States). BHT was used as a positive control. The reducing power of the sample was measured according to the absorbance. As an increase in the absorbance, caused an increase in the reducing power activity. All determinations were based on the means of three measurements.

Hydroxyl radical scavenging activity The hydroxyl radical scavenging activity of CSGH was determined according to the method described by Yu et al. (2004). A 60 µl of $FeCl_3$ (1 mM) was added to 90 µl of aqueous, 1, 10-phenanthroline (1 mM). Then, 2.4 ml of 0.2 M Na_2HPO_4 (pH 7.8), 150 µl of H_2O_2 (0.17 M) and 1.5 ml (13.5 mg/ml) of hydrolysate solution was added to the mixture. Then, the reaction was started by the addition of H_2O_2. The absorbance at 560 nm was measured with a spectrophotometer (Cary 50, Varian, Inc., United States) after incubation at room temperature for 5 min. The ascorbic acid was used as a positive control. All determinations were based on the means of three measurements. The inhibition percentages were determined using following formula:

$$\text{Hydroxyl radicals scavenging activity } (\%) = \frac{A_{\text{control}} - A_{\text{sample}} \times 100}{A_{\text{control}}}$$

where A_{control} = Absorbance of the control, A_{sample} = -Absorbance of the sample in the presence of other reagents in the reaction mixture.

Statistical analysis

All analyses were conducted in triplicate. The collected data were analysed statistically using Minitab version 16 for Windows (Minitab, inc., USA). The independent *t* test and one-way analysis of variance (ANOVA) were used to find the differences using different drying methods for all responses with a significant level of $p < 0.05$. All data were reported in mean ± standard deviation with different letter denoting significance.

Results and discussion

Extraction of chicken skin gelatin

The yield of extracted chicken skin gelatin produced using different drying methods presented in Table 1. The recovery gelatin (wet weight basis) from freeze-dried and vacuum dried method 13.63 and 14.85%, respectively. These results show that the yield of gelatin from the vacuum dried method was higher than freeze-dried method ($p < 0.05$). Higher losses during drying in freeze drier. The sample which was in frozen state directly changed to powder form may cause some of the sample stick on the freeze drier wall chamber. This is because the freeze dried sample was too fine with light weight as compared to sample dried in vacuum oven.

In addition, the higher temperatures of gelatin extraction provided higher energy for destroying the bonds of hydrogen stabilising the collagen localised in the skin matrix. Therefore, the collagen underwent the denaturation to a higher extent and contributed to the higher yield (Kaewruang et al. 2013). In addition, the acid/alkali pre-treatment acts as a role to make the collagen structure weak, solubilise the non-collagen protein and hydrolyse some of the peptide bonds, while, at the same time, keeping the consistency of the collagen fibres (Sarbon et al. 2013). Thus, the extraction of the gelatin affects the yield of the gelatin.

Table 1 Yield of gelatin and gelatin hydrolysate of chicken skin based on dry basis from two different drying methods

Different drying method	Yield (%)	
	Gelatin	Gelatin hydrolysate
Freeze-dried	13.63 ± 0.20[b]	87.34 ± 0.50[b]
Vacuum oven dried	14.85 ± 0.08[a]	92.45 ± 0.40[a]

Means of different drying methods were significantly different ($p < 0.05$). Data are reported in mean ± standard deviation (n = 3)

Significant superscript letters 'a', 'b' shows that there was a significant difference between the two drying methods

The higher yield in the vacuum dried method was supported by the previous study of Rasli and Sarbon (2015) who reported that the gelatin obtained from vacuum drying produced a higher yield (12.86%) compared to the gelatin using the freeze-dried method (9.25%). Besides, Rafieian et al. (2013) revealed that the optimum conditions for producing gelatin included higher extraction temperature (86.8 °C).

Production of chicken skin gelatin hydrolysate (CSGH)

Similar to the yield of gelatin extraction, the yield of gelatin hydrolysate from the vacuum dried method was higher than the yield obtained using the freeze-dried method ($p < 0.05$). Table 1 shows the comparison of the yield of gelatin hydrolysate obtained using the vacuum dried and freeze-dried method. The hydrolysis process caused the breakdown of protein into amino acids and peptides. Lee et al. (2012), who studied the gelatin hydrolysate of duck skin by-products, revealed that the yield of enzymatic hydrolysates from alkali pre-treated gelatin with various enzymatic enzymes, such as Alcalase, was 98.30%. However, there were various other factors, such as the type of source used, time of hydrolysis process and type of enzyme used, which were closely related to the yield of hydrolysate. The low molecular peptides make the hydrolysis easy to occur and thus increased the yield of hydrolysate.

Functional properties of chicken skin gelatin hydrolysate (CSGH)

Foaming properties of chicken skin gelatin hydrolysate (CSGH)

Figure 1a, b present the percentage of the foaming capacity and foaming stability of chicken skin gelatin hydrosate (CSGH) prepared by vacuum and freeze dryer at different pH (pH 2, 4, 6, 8 and 10). The foaming capacity of CSGH prepared from the freeze-dried gelatin at pH 2, 4, 6, 8 and 10 was 123.33, 158.13, 154.13, 79.05 and 133.81%, respectively. Meanwhile, the foaming capacity of CSGH prepared from vacuum dried gelatin was 101.67, 163, 188.63, 206.83 and 163.53%, respectively. CSGH from the vacuum dried gelatin showed a higher foaming capacity compared to CSGH from the freeze-dried gelatin ($p < 0.05$). The results revealed that the higher foam ability, expressed as the ratio of the foam volume to liquid volume, was higher in the gelatin produced using a higher temperature. However, result was contrary to the previous study by Kwak et al. (2008) on the functional properties of shark (*Isurus oxyrinchus*) cartilage gelatin using different

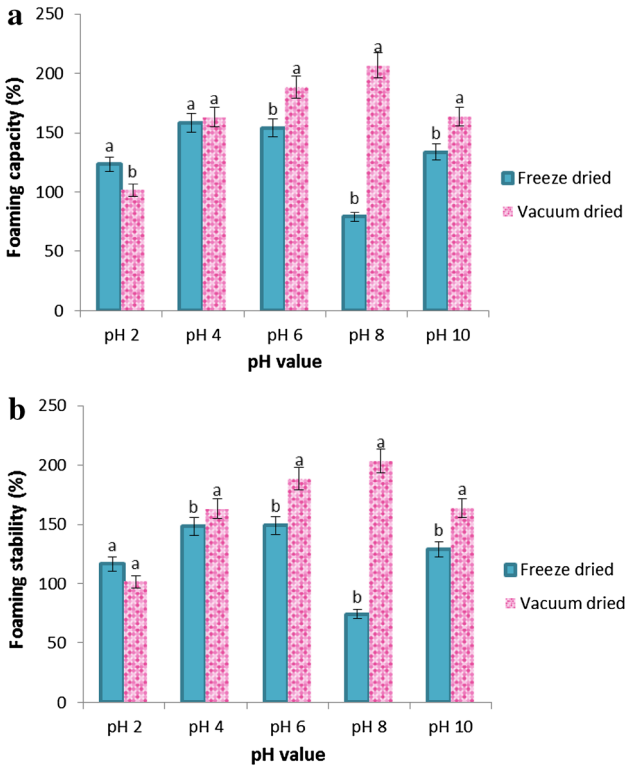

Fig. 1 a Foaming capacity (%) of CSGH for the different drying methods of gelatin at different pH (pH 2, 4, 6, 8, and 10). *Different letter a*, *b* shows significant differences ($p < 0.05$) between the two CSGH for the different drying temperatures of the prepared gelatin. b Foaming stability (%) of CSGH for the different drying methods of gelatin at different pH (pH 2, 4, 6, 8 and 10). *Different letter a*, *b* shows significant differences ($p < 0.05$) between the two CSGH for the different drying temperatures of the prepared gelatin

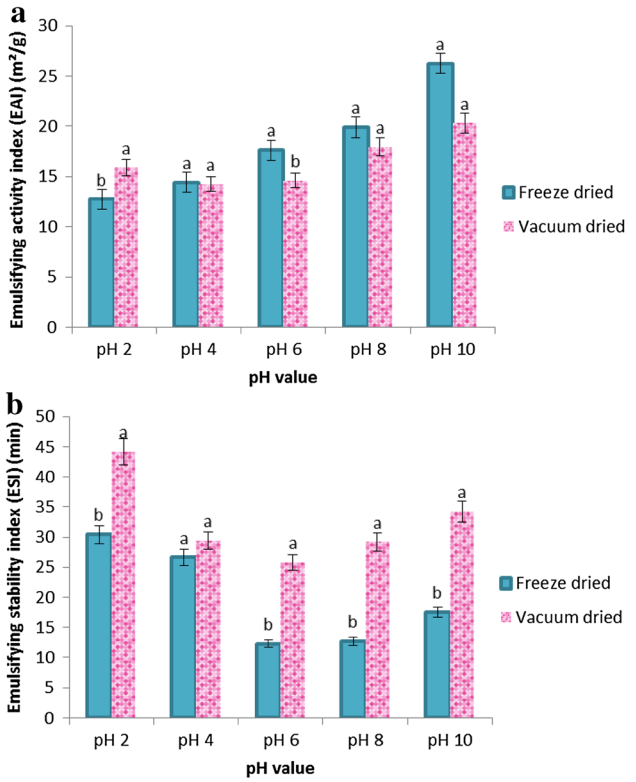

Fig. 2 a Emulsifying activity index (EAI) (%) of CSGH prepared by different drying methods of gelatin at different pH (pH 2, 4, 6, 8, 10). *Different letter a*, *b* shows significant differences ($p < 0.05$) between the two CSGH for the different drying temperatures of the prepared gelatin. b Emulsifying stability index (ESI) (%) of CSGH for the different drying methods of gelatin at different pH (pH 2, 4, 6, 8, 10). *Different letter a*, *b* shows significant differences ($p < 0.05$) between the two CSGH for the different drying temperatures of the prepared gelatin

drying methods (freeze-dried, hot-air drying and spray drying), which reported that the lower temperature of gelatin using the freeze-dried method exhibited a higher foam capacity compared to the others.

In addition, the foaming stability of CSGH from the freeze-dried at pH 2, 4, 6, 8 and 10 was 116.67, 148.33, 149.21, 74.21 and 128.97%, respectively (Fig. 1b). Trend for, CSGH of vacuum dried gelatin exhibited a higher foaming stability compared to those from freeze-dried gelatin ($p < 0.05$) at pH 4, 6, 8 and 10. CSGH from the vacuum dried gelatin showed a higher ability to form protein interaction compared to CSGH from the freeze-dried gelatin. Sarbon et al. (2013) revealed that chicken skin gelatin using a freeze dryer contained a high amount of hydrophobic groups, such as Proline (13.42%) and Alanine (10.08%), compared to bovine gelatin with 12.66 and 8.41%, respectively, of Proline and Alanine. Therefore, CSGH from high temperatures contain optimum hydrophobic regions to decrease the surface tension and thus increase the foaming properties.

Emulsifying properties of chicken skin gelatin hydrolysate (CSGH)

Figure 2a, b present the emulsifying activity index (EAI) and emulsifying stability index (ESI) of CSGH produced by vacuum and freeze drying at different pH (pH 2, 4, 6, 8 and 10), respectively. The EAI of CSGH from the freeze-dried gelatin at pH 2, 4, 6, 8 and 10, were 12.72, 14.42, 17.62, 19.90 and 26.25 m^2/g, respectively. Meanwhile, the EAI of CSGH from the vacuum dried gelatin was 15.88, 14.24, 14.59, 17.94 and 20.31 m^2/g, respectively. The overall results show that the CSGH from freeze-dried gelatin exhibited higher EAI compared to CSGH from the vacuum dried gelatin. There was a significant difference ($p < 0.05$) between the CSGH using different methods of preparing gelatin at pH 2 and 8. However, there was no significant difference ($p > 0.05$) for CSGH at pH 4, 8 and 10. This finding was supported by Nagajaran et al. (2012) who studied gelatin extracted from the skin of splendid squid (*Loligo formosana*) at different temperatures and

revealed that gelatin extracted at a lower temperature had a higher EAI compared to the gelatin extracted using a higher temperature.

CSGH from the vacuum dried gelatin possesses a better ESI compared to CSGH from the freeze-dried gelatin (Fig. 2b). The ESI of CSGH from freeze-dried gelatin at pH 2, 4, 6, 8 and 10 was 30.44, 26.64, 12.27, 12.71 and 17.50 min, respectively. Meanwhile, the ESI of CSGH from vacuum dried gelatin was 44.12, 29.41, 25.82, 29.20 and 34.18 min, respectively. CSGH from vacuum dried gelatin exhibited a higher ESI compared to CSGH from the freeze-dried gelatin and it was found to be significantly different ($p < 0.05$) at pH 2, 6, 8 and 10. However, there was no significant difference ($p > 0.05$) for CSGH at pH 4. In general, the lowering of protein size lowers the emulsion stability because of the weak interfacial films around the emulsion droplets. Freeze drying of gelatin resulted in a smaller protein molecule than that from the vacuum oven dried method. The results obtained showed that the CSGH from the vacuum dried gelatin demonstrated a higher emulsifying stability index (ESI) compared to those produced from the freeze-dried method of preparing gelatin.

The different pH levels also contributed to the effects on the emulsifying properties of CSGH. The EAI values of CSGH increased as the pH value increased. Meanwhile, The ESI showed that the stability index decreased as the pH increased. Naqash and Nazeer (2013) reported that the EAI and ESI increased with an increase in the pH value, which was accompanied by their higher solubility. The lowest EAI of CSGH was at pH 2 for freeze-dried gelatin but at pH 4 the vacuum dried gelatin resulted from the solubility decrease at this pH.

Water holding capacity (WHC) of chicken skin gelatin hydrolysate (CSGH)

The water holding capacity (WHC) of CSGH prepared from gelatin prepared by vacuum and freeze drying at different pH (pH 2) to 10 was determined for the different drying methods of preparing gelatin. The WHC of CSGH of freeze-dried gelatin at pH 2, 4, 6, 8 and 10 was 63.75, 54.93, 28.75, 49.72 and 27.93 ml/g, respectively (Table 2). However, the WHC of CSGH from the vacuum dried gelatin was 8.44, 35.15, 15.56, 9.27 and 16.34 mg/ml, respectively. CSGH from gelatin prepared using a freeze drier showed a higher WHC at all the pH level than that from gelatin prepared using the vacuum oven method ($p < 0.05$) at all pH levels. The WHC was believed to be affected by the amount of hydrophilic amino acids (Cho et al. 2004). Therefore, a high amount of hydrophilic amino acid may contribute to an increase in the water holding capacity of the sample. Sarbon et al. (2013) reported that chicken skin gelatin was high in hydrophilic amino acid,

Table 2 Water holding capacity (WHC) and oil binding capacity (OBC) of CSGH for the different drying methods of gelatin at different pH (pH 2, 4, 6, 8, 10)

	PH 2		PH 4		PH 6		PH 8		PH 10	
	WHC	OBC	WHC	OBC	WHC	OBC	WHC	OBC	WHC	OBC
Freeze dried	63.75 ± 1.14^{a}	9.31 ± 1.22^{b}	54.93 ± 2.49^{a}	23.37 ± 2.52^{a}	28.75 ± 0.74^{a}	16.13 ± 0.23^{b}	49.72 ± 0.67^{a}	21.94 ± 0.50^{a}	27.93 ± 3.00^{a}	19.93 ± 1.58^{a}
Vacuum oven dried	8.44 ± 2.29^{b}	32.51 ± 0.90^{a}	35.15 ± 3.07^{b}	21.94 ± 0.56^{b}	15.56 ± 1.25^{b}	30.10 ± 2.59^{a}	9.27 ± 2.78^{b}	14.19 ± 1.04^{b}	16.34 ± 0.30^{b}	10.99 ± 0.57^{b}

Different superscript letters 'a', 'b' shows significant differences ($p < 0.05$) between the two CSGH for the different drying temperatures of the prepared gelatin

Table 3 Antioxidant activities (DPPH, metal chelating, reducing power and hydroxyl radicals scavenging activity) of CSGH for the different drying methods of gelatin

	DPPH	Metal chelating	Reducing power	Hydroxyl radical scavenging activity
Freeze dried	47.33 ± 1.91^{c}	92.25 ± 0.31^{a}	0.34 ± 0.01^{b}	7.32 ± 1.17^{c}
Vacuum oven dried	37.98 ± 2.87^{d}	86.44 ± 0.71^{b}	0.35 ± 0.00^{b}	28.74 ± 0.23^{b}
Ascorbic acid	90.52 ± 0.11^{a}	49.37 ± 0.03^{c}	–	79.89 ± 0.51^{a}
BHT	77.48 ± 2.82^{b}	2.78 ± 1.52^{d}	0.47 ± 0.00^{a}	–

Means of different drying methods were significantly different ($p < 0.05$). Data are reported in mean ± standard deviation (n = 3)
Significant superscript letters 'a–d' shows that there was a significant difference between the two drying methods

such as glutamine (5.84%), arginine (5.57%), histidine (0.30%) and hydroxyproline (12.13%). Therefore, CSGH from the freeze-dried method of prepared gelatin contained high hydrophilic amino acid besides the smaller molecule particles, thus resulting in a higher WHC. This result was supported by the study conducted by Rasli and Sarbon (2015). They found that the WHC of freeze-dried gelatin was slightly higher (15.6 ml/g) compared to vacuum dried gelatin (15.37 ml/g), which was due to difference in the hydrophilic content in both samples.

Furthermore, the pH also affected the WHC of CSGH from the different drying methods of preparing gelatin (Table 2). The WHC of both CSGH showed a decrease in its capacity to hold the water as the pH increased. The CSGH from the freeze-dried gelatin decreased with an increase in the pH but fluctuated at pH 8. On the other hand, the CSGH of the vacuum dried method was the lowest at pH 8. The present result showed that the maximum WHC for the freeze dried prepared gelatin was between pH 2 and 4 while for the vacuum oven prepared gelatin it was at pH 4. These findings were similar to the study reported by Koli et al. (2003) in which the maximum water holding capacity (WHC) of fish gelatin was at pH 2 to 3 and the WHC of all gelatin types decreased with an increase in the pH levels. Moreover, the study on the effects of pH and ionic strength on the functional properties of gelatin revealed that the WHC decreased with an increase in the ionic strength for all gelatin types (Koli et al. 2003).

Oil binding capacity (OBC) of chicken skin gelatin hydrolysate (CSGH)

The oil binding capacity (OBC) was one of the functional properties that was highly related to the texture by the interaction between the components of oil and protein. Table 2 also presents the CSGH from gelatin prepared by vacuum and freeze drying method at different pH (pH 2, 4, 6, 8 and 10). The OBC of CSGH of freeze-dried gelatin at pH 2, 4, 6, 8 and 10 was 9.31, 23.37, 16.13, 21.94 and 19.93 ml/g, respectively. While the OBC of CSGH of vacuum dried gelatin was 32.51, 21.94, 30.10, 14.19 and 10.99 mg/ml, respectively. There were significant differences ($p < 0.05$) at pH 2, 6, 8 and 10 between the two samples. However, there was no significant difference ($p > 0.05$) in the OBC of CSGH at pH 4 between gelatin prepared using the freeze drier and the vacuum oven. The ability of the oil binding capacity was related to the degree of the exposure of the hydrophobic residues inside the gelatin hydrolysate. The present result was supported by Sarbon et al. (2013) in that the hydrophobic amino acid of chicken skin gelatin contained Alanine (10.08%), Leucine (2.63%), and Proline (13.42%). Thus the OBC of CSGH was highly influenced by the hydrophobic amino acid content in the gelatin. However, this result was in contrast with the study by Rasli and Sarbon (2015) who showed that chicken skin gelatin exhibited better fat binding capacity (FBC) with vacuum drying compared to freeze-drying.

Moreover, the pH has a high tendency to affect the OBC in the CSGH. Table 2 shows that the OBC of CSGH from the vacuum dried method of prepared gelatin decreased when the pH was higher than pH 6. The OBC of CSGH from the freeze-dried method of preparing gelatin increased at a pH higher than pH 4. These results indicate that the pH affects the CSGH for different drying methods.

Antioxidant activities of chicken skin gelatin hydrolysate (CSGH)

1, 1-diphenyl-2-pieryhydrazyl (DPPH) radical scavenging activity

Table 3 presents the percentage of DPPH radical scavenging activity of CSGH on the different drying methods of the prepared gelatin compared to the Butylated hydroxytoluene (BHT) and ascorbic acid, which act as positive control at a concentration 10 mg/ml. The inhibition of the DPPH radical obtained by freeze-dried gelatin, oven vacuum dried gelatin, BHT and ascorbic acid was 47.33, 37.98, 77.48 and 90.52%, respectively. The positive control of ascorbic acid showed the highest inhibition of DPPH radical scavenging compared to BHT, freeze-dried

and vacuum dried hydrolysate of the prepared gelatin ($p < 0.05$). The CSGH from the freeze-dried method of gelatin exhibited better DPPH scavenging activity compared to the CSGH from the vacuum dried method of the preparing gelatin. This may be because the gelatin prepared by the freeze drying produced smaller particles, and, hence, when subjected to the hydrolysis process, was easily solubilised and exposed the hydrophobic group, which contributed to the antioxidant activity. Therefore, CSGH from the freeze-dried gelatin has a high capability of stabilizing the radical compared to CSGH from the vacuum dried gelatin. The changes in size, amount, exposure of the terminal amino groups of the products obtained, and composition of the free amino acids or small peptides affect the antioxidative activity (Wu et al. 2003). As reported by Sarbon et al. (2013), the molecular weight of chicken skin gelatin using freeze drying was 285,000 Da compared to bovine gelatin, which was 350,000 Da. This finding shows that chicken skin gelatin contains a low molecular weight compared to bovine gelatin and possesses better antioxidant activity. Besides, the study reported by Sae-leaw et al. (2016) reported that the gelatin hydrolysate that prepared after the gelatin extraction resulted a lower DPPH scavenging activities due to the structure of the peptides of hydrolysate as compared to the gelatin hydrolysate that prepared during the gelatin extraction.

Metal chelating activity

Table 3 shows the percentage of metal chelating activity by CSGH from different drying methods of preparing gelatin compared to BHT and ascorbic acid, which acts as a positive control at a concentration of 10 mg/ml. The chelating activity of CSGH from freeze-dried gelatin, vacuum dried gelatin, BHT and ascorbic acid was 92.25, 86.44, 2.78 and 49.37%, respectively. Surprisingly, the CSGH from freeze-dried gelatin exhibited the highest degree of chelating activity and it was significantly different ($p < 0.05$) compared to the CSGH of the vacuum dried gelatin, BHT and ascorbic acid. The positive control, BHT and ascorbic acid showed lower scavenging activity than CSGH from the different drying temperatures on metal chelating. This is because the BHT is the primary antioxidant and ascorbic acid can be the primary or secondary antioxidant. Therefore, ascorbic acid is capable of scavenging radicals directly by converting hydroperoxides into stable products (Akoh and Min 2008).

The metal chelating activity reveals that CSGH produced different drying temperatures of gelatin may act as chelators or metal ions and can prevent the lipid oxidation via the metal chelating activity. According to Ktari et al. (2012), the reaction of this activity starts when the ferrozine quantitatively forms a complex with Fe^{2+}. The transition metal ions, such as iron or copper, may catalyse the formation of reactive oxygen species, which accelerates lipid oxidation. Therefore, the carboxyl and amino groups in the side chains of the acidic (Glutamic acid, Glx; Aspartic acid, Asx) and basic (Lysine, Lys; Histidine, His; Arginin, Arg) amino acids were thought to play an important role in chelating metal ions (Saiga et al. 2003). Sarbon et al. (2013) reported that chicken skin gelatin contained Lys (4.66%), His (0.3%) and Arg (5.57%), which indicated that the chicken skin gelatin had higher basic amino acids, and thus exhibited higher metal chelating activity. The CSGH from the freeze-dried gelatin was high in metal chelating activity, which may be due to the high carboxyl and amino groups being highly exposed during the drying process compared to the CSGH from the vacuum dried gelatin. As reported by Kittiphattanabawon et al. (2010), both the brownbanded bamboo shark (BBS) and blacktip shark (BTS) gelatin at different temperatures were able to chelate Fe^{2+} and catalyse the generation of reactive oxygen species, such as hydroxyl radicals (OH) ($p > 0.05$). Other than that, gelatin hydrolysate was found to have good antioxidant activities compared to unhydrolyzed gelatin.

Reducing power

The reducing power of CSGH prepared from freeze-dried and vacuum dried gelatin was compared to BHT as a positive control and synthetic antioxidant. Table 3 depicts that the reducing power of BHT (positive control) (0.473 nm) was significantly higher ($p < 0.05$) than the CSGH from the freeze-dried (0.343 nm) and vacuum dried gelatin (0.348 nm). However, there was no significant difference ($p > 0.05$) between the CSGH samples. The BHT showed the highest absorbance on reducing power compared to CSGH from the freeze-dried and vacuum dried gelatin. Since BHT acts as a positive control, the result indicated that the standard BHT solution exhibited a higher reducing activity than both samples of CSGH.

CGSH produced by both the methods had the ability to produce Fe^{2+} and Fe^{3+}. Therefore, CSGH from gelatin produced by both drying methods for preparing gelatin have the ability to donate electrons and scavenge, and hence enable oxidation to occur. The higher reducing power was also closely related to the lower molecular weight and higher protein solubility (Galla et al. 2012). This finding was similar to the study conducted by Kittiphattanabawon et al. (2010) in which low molecular weight peptides, which were likely produced at high temperature, exhibited higher antioxidant activities. However, Mahmoodani et al. (2012) revealed that the gelatin hydrolysate from pangasius catfish (*Pangasius sutchi*) skin exhibited high and low molecular weight bands ranging from 66 to 45 kDa by using freeze dried method. This

previous study supported that only a bit differences resulted between the hydrolysate from vacuum oven and freezed dried methods.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activities of CSGH produced from gelatin produced by different method and ascorbic acid solution, (10 mg/ml concentration) are presented in Table 3. The hydroxyl scavenging activity of CSGH of freeze-dried gelatin, vacuum dried gelatin, and ascorbic acid was 7.32, 28.74 and 79.90%, respectively. There was a significant difference ($p < 0.05$) between the ascorbic acid and CSGH of gelatin produced by different methods. The result indicated that CSGH from the oven vacuum dried method of gelatin showed better hydroxyl radical scavenging compared to the CSGH from the freeze-dried gelatin. Saiga et al. (2003) stated that the hydroxyl radicals could be formed from superoxide anion and hydrogen peroxide in the presence of transition metal ions, such as Fe^{2+} and Cu^{2+}. Therefore, chelating metal ions may inhibit the formation of hydroxyl radicals. From the results, the CSGH from the oven vacuum dried gelatin exhibited high hydroxyl radical scavenging activity and the ability to protect hydroxyl radical induced damage.

This was supported by Kittiphattanabawon et al. (2010), who found that the antioxidative activities were related to the content of α-amino groups. They found that the increase in the α-amino groups content interact well from the higher degraded proteins. As reported by Sarbon et al. (2013), amino acids of chicken skin gelatin were Glutamine (5.84%), Arginine (5.57%), Alanine (10.08%) and Proline (13.42%). This finding shows that the chicken skin gelatin have high α-amino groups and possessed high hydroxyl scavenging activity. In addition, the higher temperature that was applied in the gelatin production was revealed to contain a higher amount of degradation peptides, similar to that in Nile perch gelatin (*Lates niloticus*) (Muyonga et al. 2004), and yellowfin tuna skin (*Thunnusn albacores*) (Cho et al. 2006). Therefore, this finding supported that the CSGH of vacuum dried gelatin exhibited better hydroxyl scavenging activity.

Conclusion

The present study revealed that the chicken skin gelatin hydrolysate (CSGH) prepared from gelatin by vacuum and freeze drying influenced the yield of CSGH prepared from freeze-dried gelatin showed better functional properties, such as in emulsifying activity index (EAI), water holding capacity, and oil binding capacity at different pH (pH 2, 4, 6, 8, and 10) compared to the CSGH prepared from vacuum dried method. It also exhibited better antioxidant activity (DPPH and metal chelating activity). Meanwhile, the CSGH prepared from vacuum dried gelatin exhibited better properties in terms of the emulsifying stability index (ESI), foaming capacity and foaming stability with better hydroxyl radical scavenging activity. In conclusion, the CSGH from the freeze-dried gelatin showed better functional properties and antioxidant activities than hydrolysate from the vacuum dried prepared gelatin.

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