ISSN: 2338-1345 – Vol. (5). 2-6 2017 Journal online http://journal.bakrie.ac.id/index.php/APJSAFE

Asia Pacific Journal of Sustainable Agriculture Food and Energy (APJSAFE)

Improving the Physicochemical Properties of Commercial Bovine Gelatin using Succinylation

Nur Aliyana Binti Alias[1], Benedict Oludare Omosebi[2], Wahyudi David[3] and Nurul Huda[4]*,

[1]*Food Technology Program, Universiti Sains Malaysia, Penang, 11800, Malaysia*
[2]*Department of Food Technology, Federal Institute of Industrial Research , Oshodi, Lagos State, Nigeria.*
[3]*Department of Food Science and Technology, Universitas Bakrie, Jakarta, Indonesia*
[4]*School of Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200, Terengganu, Malaysia*

**Corresponding author's Email address: nhuda@unisza.edu.my*

Abstract- Commercial bovine gelatin was modified by using succinic anhydride and resultant changes in physicochemical properties such as Bloom strength, foaming properties and extent of succinylation were investigated. The result indicated that addition of succinic anhydride at varying concentrations of 0.04, 0.08, 0.12 and 0.16 g/g of sample increased the degree of succinylation from 0% to 14%, while Bloom strength increased from 131.97% to148.60% but at higher concentration decreased from 133.50% to128.0%. Foaming capacity increased from 171.67% to 210.70% but a substantial constant decrease in foaming stability with time.

Keywords- Gelatin, Succinic anhydride, gel strength, foaming capacity, physicochemical properties,

INTRODUCTION

Gelatin is a biopolymer which is tasteless, highly purified and a collagenous protein ingredient. Gelatin is derived by the partial hydrolysis of collagen, the principal protein constituent of animal skin, bone and connective tissue. Presently gelatin is used in the food, pharmaceutical, cosmetic and photographic application (Karim and Bhat, 2009). In food, gelatin is mainly used to improve elasticity, consistency and stability of foods and provides a melts in mouth function with a thermo-reversible gel property. Research by Grand View Research (2014) stated that pigskin was the largest used raw material for the manufacturing of gelatin which accounts for more than 40% of the global gelatin production in 2013. Karim and Bhat (2009) and Gómez-Guillén et al., (2011) also reported that the annual world output of gelatin is nearly 326000 tons, with pig skin-derived gelatin accounting for the highest (46%) output followed by bovine hides (29.4%), bones (23.1%) and other sources (1.5%).

The quality of food grade gelatin depends to a large extent on its rheological characteristic such as viscosity and viscoelastic properties (Karim and Bhat, 2009). Nowadays, modification of gelatin has been done by many researchers to improve gelatin functionality especially for low quality gelatin and therefore, increase gelatin acceptability for other application. Modification of gelatin can be done enzymatically, physically and chemically. Chemical modification has been an acceptable alternative for improving protein functional characteristic as well as tailoring a protein to meet a specific characteristic for a given food system.

Succinylation is one chemical modification method to improve the functionality of protein (Mahaja et. al., 2010; Shilpashree et al, 2015). Beside through succinylation, chemical modification of protein can be conducted through acetylation (; Miedzianka et al. 2012) and glycation (Liu et al., 2012). In succinylation, succinic anhydride reacts with the ε-amino group of lysine and the N-terminal α-amino group of proteins, in their nonprotonated forms converting them from basic to acidic group (Klapper and Klotz, 1972). In general, functional properties such as solubility and emulsification capacity were improved by succinylation (Kabirullah and Wills, 1982). Groninger, (1973) studied that emulsification capacity of succinylated myofibrillar protein was related to the degree of succinylation of the protein. Oppenheimer et al., (1967) showed that succinylation of chicken protein resulted in a product that had increased viscosity, but molecular size similar to that unmodified myosin. Succinylation also improves the emulsion activity, emulsion stability and increase water absorption capacities of lentil globulin (Bora, 2002).

This project was carried out to determine the effect of different level of succinic anhydride modification on the physicochemical properties such as Bloom strength and foaming properties of commercial bovine gelatin.

MATERIALS AND METHODS

Materials

Commercial gelatin from bovine source purchased from Leverage Business Sdn Bhd (Penang, Malaysia) was used as the main sample. Succinic anhydride 99% was purchased from Acros Organic (New Jersey, USA). Ninhydrin (Fluka), 95% ethanol, acetic acid and glycine from Sigma (USA) were used for determination extent of succinylation.

Succinylation of gelatin

Succinylation of commercial gelatin was performed according to the method of Groninger (1973) with some modifications. Commercial gelatin (4 g) was dispersed in 160 ml distilled water (2.5 % w/v) and left to room temperature for gelatin to dissolve. Gelatin then melted at 60 °C to until completely dissolved and cooled to room temperature. Succinic anhydride was added in small increments with constant stirring (Eurostar Digital, IKA-WERKE, Germany) at level of 1, 2, 3 and 4 % weight of sample. pH of the mixture was maintained at 7 by adding 1 N NaOH in order to prevent further modification. Control was prepared with same procedure without the addition succinic anhydride. The mixture was freeze dried prior to analysis.

Determination of extent of succinylatiion.

The extent of succinylation was conducted as described by Kinsella et al., (1976). Ninhydrin solution (1 ml) was added to aqueous protein solution (1 ml). 4 ml of distilled water was added to each tube. The mixture then was placed in boiling water bath at 100 °C for 5 min. The absorbance of sample was read at 580 nm against ninhydrin solution blank. The extent of succinylation was calculated as follow formula:

$$\text{Succinylation \%} = \frac{A_{580\ (native)} - A_{580\ (succinylated)}}{A_{580\ (native)}}$$

Determination of Bloom strength.

Bloom strength of gelatin was determined according to the method Gelatin Manufacturer Institute of America (GMIA, 2014). 6.67% (w/v) of gelatin solution was prepared in bloom jar at room temperature. The mixture was left at room temperature for 10 minutes to allow gelatin to absorb water and swell. Gelatin solution then left to melt at 60 °C until gelatin completely dissolve and cooled to room temperature before kept in a refrigerated water bath at 10 °C for 16 to 18 hour for gel maturation. The gel strength was determined by using texture analyser TA.XT2 (Stable Microsystems, Surrey, UK) with a load cell of 5 kg equipped with flat bottom plunger 0.5 mm in diameter (SMS P/0.5). Gel strength (in grams) was obtained after plunger penetrates into gel to a depth of 4 mm at rate 0.5 mm/s.

Determination of foaming properties.

Foaming stability and foaming expansion of gelatin solution were measured according to the method described by Shahidi et al., (1995). 2% (w/v) of sample was prepared and swollen. Sample then dissolved at 60°C and homogenized at 10,000 rpm (IKA T25 digital, Germany) for 2 minutes to produce foam. The mixture was poured into 100ml measuring cylinder and the total volume was read. The sample was allowed to stand at 0, 20, 40 and 60 minutes. Foaming stability and foaming expansion were calculated using following formula:

$$\text{Foaming Stability \%} = \frac{V_t}{V_o} \times 100\%$$

$$\text{Foaming expension \%} = \frac{V_T}{V_o} \times 100\%$$

Where, VT = total volume after whipping, Vo = original volume before whipping and
Vt = total volume after leaving at room temperature for specific time.

Statistical Analysis

SPSS software (SPSS 17.0 Statistical Package for Social Science) was used to evaluate the chemical analysis, and physical analysis data. Comparison of means among the different samples was conducted using Duncan's multiple range test.

RESULTS AND DISCUSSIONS

Determination extent of succinylation

The Figure 1 shows the extent of succinylation of gelatin. The extent of succinylation of amino groups in the gelatin depends markedly on the amount of succinic anhydride being added. Incremental addition of succinic anhydride at 0.04, 0.08, 0.12 and 0.16 g/g of sample succinylated 3.68%, 8.10%, 10.92% and 13.14% respectively of the ε-amino groups. The result indicates that level of succinylation increased as the quantity of succinic anhydride increased.

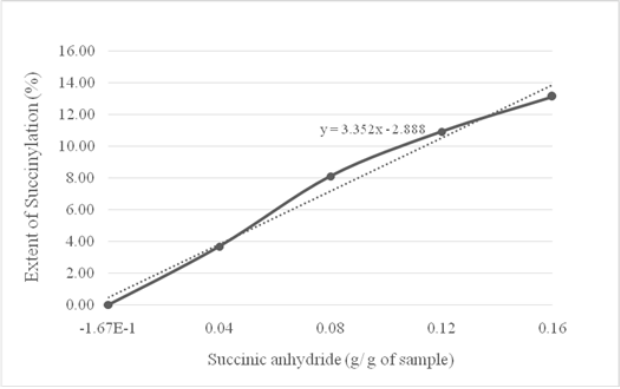

Figure 1. Extent of succinylation of gelatin

The protein acylation reactions presumably follow the carbonyl addition pathway and the rate of reaction depends on the rate of nucleophilic attack (Means and Feeney, 1971). High reactivity of protein group are readily acylated

compared with other amino acid residue and group that generally higher pK and are usually more protected from the reaction than amino groups (Franzen and Kinsella, 1976). The amino and hydroxyl groups of a protein are readily acylated compared with the other amino acid residues available for the reaction (Lawal, 2005). The ε-amino group of lysine is most readily acylated because of its high nucleophilic character, relatively low pK and its steric availability for reaction (Franzen and Kinsella, 1976; Wanasundara and Shahidi, 1997).

Bloom Strength

The most important attribute of gelatin is its Bloom strength. Bloom strength is the weight in grams that is required for a specified plunger to depress the surface of standard, thermostated gel to a defined depth under standard conditions (Schrieber and Gareis, 2007). Table 1 shows the Bloom strength of gelatin with and without the addition of succinic anhydride.

Table 1. Bloom strength of gelatin with different level of modification

Sample	Bloom strength (g)	Improvement effect (%)	Percentage effect (%)
Control	131.97 ± 0.55^{c}	-	-
3.68% SG	134.20 ± 0.50^{b}	2.23	1.69
8.10% SG	148.60 ± 0.50^{a}	16.63	12.60
10.92% SG	133.50 ± 0.44^{b}	1.53	1.16
13.14% SG	128.03 ± 0.56^{d}	-3.94	-2.99

SG = Succinylated Gelatin
Values are presented as mean ± SD (n = 3)
Different subscript letters within the same row indicate significant differences ($p < 0.05$)

As noticed in table, addition of succinic anhydride into gelatin increases the Bloom strength up to certain point. Addition of succinic anhydride into gelatin slightly increases the Bloom strength for 3.68% SG (134.20 ± 0.50 g) and 8.10% SG (148.60 ± 0.50 g) with percentage effect 1.69% and 12.60%. However, increase in the concentration of succinic anhydride beyond 8.10% SG resulted in the decline of the Bloom strength. The Bloom strength of gelatin start to decrease at 10.92% SG (133.50 ± 0.44 g) and 13.14% SG (128.03 ± 0.56 g), hence lowering percentage effect. According to Schrieber and Gareis (2007), the Bloom strength of commercial gelatin types are within the range 50 to 300 Bloom. Bloom strength is dependent on the hydrogen bonds between water molecules and free hydroxyl groups of amino acids, size of protein chains, concentration and molecular weight distribution of the gelatin (Arnesen and Gildberg, 2007). Decreased in Bloom strength might be due to excessive cross-linking that might lower gel strength through impeding intermolecular aggregation that reduced the gel network formation (Jongjareonrak et al., 2006).

Foam Capacity

Gelatin and soluble collagen exhibit suitable foaming properties, even without gelling, because of their ability to reduce the surface tension at the liquid or air interface by increasing the viscosity of the aqueous phase (Schrieber and Gareis, 2007). Table 2 shows the effect of succinylation on foam capacity of gelatin.

Table 2. Foaming capacity of gelatin with different level of modification

Sample	Foaming Expansion (%)	Improvement effect (%)	Percentage effect (%)
Control	171.67 ± 0.76^{e}	-	-
3.68% SG	185.67 ± 0.38^{d}	16.00	9.32
8.10% SG	194.17 ± 0.29^{c}	22.50	13.11
10.92% SG	198.23 ± 0.25^{b}	26.56	15.47
13.14% SG	210.70 ± 0.66^{a}	39.03	22.73

SG = Succinylated Gelatin
Values are presented as mean ± SD (n = 3)
Different subscript letters within the same row indicate significant differences ($p < 0.05$)

The foam capacity of all samples increased significantly ($p < 0.05$) after treated with succinic anhydride. At 7.5% SG, the foam capacity was 185.67 ± 0.38, which means it increased 9.32% from control gelatin. The percentage effect of foam capacity of succinylated gelatin increased to 13.11% when with 8.10% SG was added, 15.27% when 10.92% SG was added and 22.73% when 13.14% SG was added . The result emphasized that succinylation was able to improve foam capacity. These observation agree with those reported for canola 12S globulin (Paulson and Tung, 1987), milk protein (Shilpashree et al, 2015), and mung bean protein isolate (El-Adawy, 2000).

Succinylation causing increasing negative charge. Lawal et al, (2005) noted that increasing negative charge of succinylated protein would especially promote protein-protein interaction which facilitate improved foaming capacity. Shilpashree et al, (2015) reported that increase in foam capacity of protein could be due to significantly increased water holding capacity of protein. According to Bora (2002) that, increase in water holding capacity is due to unfolding of protein due to electrostatic repulsion between the added carboxyl groups and the neighbouring native carboxyl groups, exposing buried amino acid residues and making them available for interactions with aqueous medium.

Foaming stability

The effect of succinylation on foam stability of gelatin is shown in Figure 2. The foam stability of decreased constantly with time in all treatments. However, the foam stability of control gelatin was slightly higher compared to the succinylated gelatin samples. Foam stability is reduced with succinylation because of negative charges imparted during modification causing the protein molecule to unfold.

Modification lead to increased net charge density which prevent protein-protein interaction in foam lamellae,

hence causing foam destabilization and poor stability (Cheftel et al., 1985). Similar result was found for oat protein isolate (Mirmoghtadaie et al., 2009) which explained that decrease in foaming stability was due to excessive increase in charge reduce protein-protein interaction, hence prevent the formation of an elastic film at the air-liquid interface.

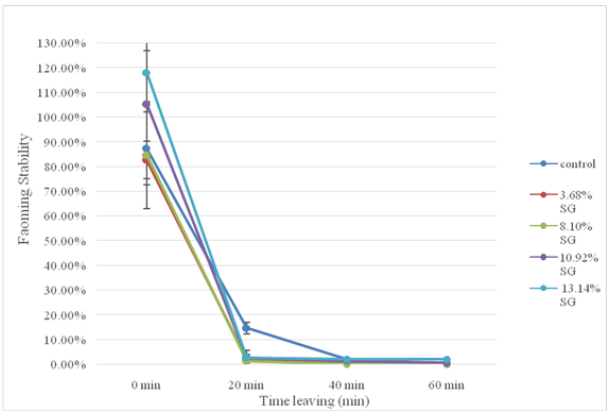

Figure 2. Foaming stability of succinylated gelatin

The unfolding and dissociate protein might exposed more hydrophilic groups than hydrophobic, thereby increasing hydrophilic binding site (El-Adawy, 2000). Protein with low hydrophobicity showed poor stability. According to Townsend and Nakai (1983), hydrophobicity of protein are associated with good balance of both hydrophobic and hydrophilic group necessary for effective stabilization of air bubbles. Foam stability is directly affected by protein concentration which will influence the thickness, mechanical strength and cohesiveness of film (Zayas, 1997).

CONCLUSIONS

Gelatins were succinylated by adding different concentration of succinic anhydride based on the sample weight. From the result, it was shown that extent of succinylation increased as concentration increased. Also result showed that succinylation was able to improve the Bloom strength of gelatin but the improvement declined at concentration beyond 8.10% succinylated gelatin level. The addition of succinic anhydride in gelatin influenced the foaming properties, whereby foaming capacity increased at increasing level of modification. However, foaming stability of succinylated gelatin decreased with time.

ACKNOWLEDGEMENT

The authors acknowledge with gratitude the support given by Universiti Sains Malaysia,and Universiti Sultan Zainal Abidin to conduct reserach in the area of food science and technology.

REFERENCES

Arnesen, J. A. and Gildberg, A. 2007. Extraction and characterisation of gelatine from Atlantic salmon (*Salmo salar*) skin. Bioresource Technology, 98(1):53-57.

Bora, P. S. 2002. Functional properties of native and succinylated lentil (*Lens culinaris*) globulins. Food Chemistry, 77(2): 171-176.

Cheftel, J. C., Cug, J. L. and Lorient, D. 1985. Amino acids, peptides and proteins. In. Fennema, O.R. (Ed.). Food chemistry: Second edition, revised and expanded, pp. 245-370. New York: Marcel Dekker, Inc.

El-Adawy, T. A. 2000. Functional properties and nutritional quality of acetylated and succinylated mung bean protein isolate. Food Chemistry, 70(1): 83-91.

Franzen, K. L. and Kinsella, J. E. 1976. Functional properties of succinylated and acetylated soy protein. Journal of Agricultural and Food Chemistry, 24(4): 788-795.

GMIA (Gelatin Manufactures Institute of America). 2014. Gelatin handbook. Available from: http://www.gelatin-gmia.com/images/GMIA_Gelatin_Manual_2012.pdf [Accessed on 26 Nov. 2014].

Gómez-Guillén, M. C., Giménez, B., López-Caballero, M. A., and Montero, M. P. 2011. Functional and bioactive properties of collagen and gelatin from alternative sources: A review. Food Hydrocolloids, 25(8): 1813-1827.

Grand View Research. 2014. Gelatin Market Analysis by Raw Material (Pig Skin, Bovine Hides, Bones), by Application (Food & Beverage, Nutraceuticals, Pharmaceuticals, Photography, Cosmetics) and Segment Forecasts to 2020. http://www.grandviewresearch.com/industry-analysis/gelatin-market-analysis[Accessed on 26 Nov. 2014].

Groninger Jr, H. S. 1973. Preparation and properties of succinylated fish myofibrillar protein. Journal of Agricultural and Food Chemistry, 21(6), 978-981.

Jongjareonrak, A., Benjakul, S., Visessanguan, W. and Tanaka, M. 2006. Skin gelatin from bigeye snapper and brownstripe red snapper: Chemical compositions and effect of microbial transglutaminase on gel properties. Food Hydrocolloids 20 (8): 1216-1222.

Kabirullah, M., & Wills, R. B. H. 1982. Functional properties of acetylated and succinylated sunflower protein isolate. International Journal of Food Science and Technology, 17(2), 235-249.

Karim, A. A., & Bhat, R. 2008. Gelatin alternatives for the food industry: recent developments, challenges and prospects. Trends in Food Science and Technology, 19(12): 644-656.

Kinsella, J. E. 1976. Functional properties of protein in food: a survey. Critical Reviews in Food and Nutrition. 7:219-280.

Klapper, M. H. and and Klotz, I. M. 1972. Acylation with dicarboxylic acid anhydrides. Methods in Enzymology, 25:531-536.

Lawal, O. S. 2005. Functionality of native and succinylated Lablab bean (*Lablab purpureus*) protein concentrate. Food Hydrocolloids, 19(1), 63-72.

Liu, J., Ru, Q. and Ding, Y., 2012. Glycation a promising method for food protein modification: Physicochemical properties and structure, a review. Food Research International 49, 170–183.

Mahajan, A., Neetu and Ahluwalia, A. S. 2010. Effect of processing on functional properties of Spirulina protein preparations. African Journal of Microbiology Research, 4 (1):55-60.

Means, G. E., and Feeney, R. E., 1971. Chemical Modification of Proteins. Holden-Day, Inc.: San Francisco.

Miedzianka, J., Peksa, A. and Aniołowska, M. 2012. Properties of acetylated potato protein preparations. Food Chemistry 133: 1283–1291.

Mirmoghtadaie, L., Kadivar, M., and Shahidi, M. 2009. Effects of succinylation and deamidation on functional properties of oat protein isolate. Food Chemistry, 114 (1), 127-131.

Oppenheimer. H., Baranv. K.. Hamoir. G. and Fenton. J. 1967. Succinylation of myosin. Archives of Biochernistry and Biophysics. 120:108-118.

Paulson, A. T., and Tung, M. A. 1987. Solubility, hydrophobicity and net charge of succinylated canola protein isolate. Journal of Food Science, 52(6), 1557-1561.

Schrieber, R., and Gareis, H. 2007. Gelatine handbook. Weinhem: Wiley-VCH GmbH & Co.

Shahidi, F., Han Xiao-Qing, H., and Synowieck, J. 1995. Production and characteristics of hydrolysates from capelin (Mallotus villosus). Food Chem. 53:285-293.

Shilpashreea, B.G., Arora, S., Prince Chawla, P. and Tomar, S.K. 2015. Effect of succinylation on physicochemical and functional properties of milk protein concentrate. Food Research International, 72:223–230.

Townsend, A. A., and Nakai, S. 1983. Relationships between hydrophobicity and foaming characteristics of food proteins. Journal of Food Science, 48(2), 588-594.

Wanasundar, P. K. J. P. D. and Shahidi, F. 1997. Functional properties of acylated flax protein isolates. Journal of Agriiculture and Food Chemistry, 45:2431-2440.

Zayas, J. F. 1997. Functionality of proteins in food. Berlin: Springer,