ISSN: 2338-1345 – Vol. (5) 10-15 2017 Journal online http://journal.bakrie.ac.id/index.php/APJSAFE

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

The Effect of Succinylation on the Physicochemical Properties of Commercial Fish Gelatin

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Abstract- There has been growing interest in improving the physicochemical properties of gelatin especially fish gelatin due to its inferior rheological and functional properties compared to mammalian gelatin. The effect of chemical modification (succinylation) on the physicochemical properties fish gelatin by over varying degree of succinylation (0.0 – 11.11%). was investigated. For succinylated fish gelatin, physicochemical properties such as gel strength (Bloom), emulsion activity and foaming activity increased from 126.06% to 178.66%, 41.8% to 68.9%, and 14.5% to 65.1% respectively. However succinylation decrease emulsion and foaming stability of succinylated fish gelatin from 67.6% to 44.7% and 97.7 to 69.6% respectively. Therefore, the result showed the improvement of physicochemical properties of succinylated fish gelatin was influenced by degree of succinylation.

Keywords- Fish gelatin, gel strength, emulsion and foaming properties, and succinylation

INTRODUCTION

Gelatin is one of the most versatile gelling agents in food applications due to its special texture and the 'melt-in-mouth' perception. Gelatin has been used as an additive for improving elasticity, consistency, and stability of foods (Arvanitoyannis 2002). The global demand for gelatin has been increasing over the years. Recent reports indicate that the annual world output of gelatin is nearly 326,000 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%) (GME, 2008). Gelatin from different sources has varying properties, mainly related not only to the amino acid composition but also to the α-chain, β- or γ-component, and molecular weight distribution (Johnston-Banks 1990). The intrinsic differences between mammalian and fish gelatins employed may determine different properties of gel (Benjakul et al. 2009).

Protein modification using chemical modification is an important tool for tailoring food proteins into products with different functional properties. Chemical modification, the acylation of protein with acetic or succinic anhydride is one of the most convenient and most frequently used methods for altering the functional properties of many plant proteins. A great number of food proteins have been investigated with regard to the improvement of their functional properties by acylation. Several chemical modification approaches, acylation with acetic anhydride and succinylation with succinic anhydride has been widely used to improve functional properties of various food proteins (Schwenke andRauschal, 1980). By introducing acetyl groups into the protein molecules, acetylation improved emulsifying capacity, emulsion stability, foam capacity and stability and water absorption of pea (Johnson and Brekke, 1983), soy (Kim and Rhee, 1989), winged bean (Narayana and Rao, 1984 and mungbean (El- Adawy, 2000), among other proteins. Moreover, chemical modification procedures used in the manufacture of processed food include general non-specific modifications.

Earlier researchers have observed, an increase in the nitrogen solubility, emulsifying activity and stability and foaming capacity of the protein with acylated leaf proteins (Franzen and Kinsella, 1976). Similar observation have been reported for succinylated and acetylated winged bean protein (Narayana and Rao, 1984). Rationale for chemical modification of proteins is multiple but can be categorizedinto four main reasons: (i) Waste control: For example the re-use of fish gelatin from waste requires less natural resources for their production. Chemical modification can be used to increase the functional properties of the proteins; (ii) Health considerations:An example of this is the replacement of meat or soy proteins by (other) vegetable proteins (Moure et al., 2006). Similar

to proteins obtained as by-product from waste material, unmodified plant proteins often have limited functional applicability. Hence, chemical modification of plant proteins can be used to improve molecular functionality; (iii) Cost effectivity by extending molecular functionality of a protein: Chemical modification can be employed to enhance the functional properties of a protein, such that less material is required to obtain a product with similar structural characteristics; (iv) Structure-function relationships: Chemical modification is often used to investigate the contribution of specific molecular parameters, such as surface hydrophobicity, to functionality of the protein at ingredient level (Soderling, 1975).

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

MATERIALS AND METHODS

Materials

Commercial fish skin gelatin (168 bloom strength) obtained from SKW Biosystems, Grasse, France (Jelly strength 6.67%), succinic anhydride 99%, 3M NaOH, 2M HCl, ninhydrin solution, standard amino acid (glycine), 99% of ethanol, soybean oil, water bath, magnetic stirrer and spectrophotometer.

Succinylation method

Succinylation method that was performed as detailed in the method of Franzen and Kinsella (1976). About 4.5 g of fish gelatin was prepared in 120 ml distilled water at room temperature then was stirred until all gelatin were dissolved. Solution then heated in water bath at 60 ° C to ensure the entire gelatin is dissolved. The solution was filter using filter paper to remove the residue. Known amounts (0.025, 0.075, 0.125, 0.25, 0.5 g) of succinic anhydride were added in small increments (g/g sample). Total quantity of succinic anhydride used based on g/g sample. The solution was stirred and the pH maintained at 8.0 by adding 3M NaOH. After reaction was complete, the protein was kept at -18°C before freeze-dried.

Determination of extent of succinylatiion.

The extent of succinylation was quantified by determining the free amino group by using spectrophotometric analysis following reaction with ninhydrin (Moore and Stein, 1954). Series of glycine stock solution 0.001M (7.5mg in 100ml distilled water) was prepared. 1 ml of ninhydrin solution added to 1% of sample (w/v) (0.1g in 10 ml). The solution heated at 100°C for 5 minutes and cool immediately at 25°C. 5 ml of distilled water was added and homogenize. The absorbance of the sample was read at 580 nm against a blank. The degree of succinylated was calculated:

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

Determination of Bloom strength.

Wainewright (1977) method was used to determine the bloom strength of modified bovine and fish gelatin. Gel of 6.67% w/v was prepared in 150 ml capacity bloom jars (Schott Duran, Mainz, Germany). Dry modified gelatin samples were dissolved by mechanically stirring in deionized water and heated up at 60ºC for 1 hour in water bath (Schutzart DIN 40050-IP 20, Germany) before cooling down to room temperature which is around 25ºC ± 1ºC for at 15 min. Then, the gel was set by storing the solution in refrigerator at 4-7ºC, the maturation temperature for 16-18 hours before bloom strength analysis. The analysis was done by using Texture analyzer (TA.XT2i Texture Analyzer; Stable Micro Systems, Godalming, Surrey) according to British Standard BS 757 (BSI. 1975). Load cell of 5 kg was used with cross head speed 1 mm/s, equipped with a 0.5 in. diameter, flat-bottomed plunger. The bloom jar containing gelatin gel was placed at the centre below the plunger. The penetration test was done by plunger penetrate to a 4 mm depth into the gel for determination of maximum force (g). Graph was built to see the difference between each sample.

Emulsification properties

Emulsify activity (EA) of protein were studied by the procedure of Yasumatsu et al (1972). To 5 ml of each succinylated sample preparation, 5 ml of the soybean oil was added and the mixture was homogenized for 3 min. the emulsion obtained were centrifuged at 500 rpm for 10 min. the height of emulsion layer was noted in the graduated centrifuge tube. Emulsifying activity was expressed as:

$$\frac{Height\ of\ emulsified\ layer}{Height\ of\ the\ total\ content\ in\ tube}\ x\ 100$$

Emulsion stability (ES) was determined in a similar manner as above but involved heating the emulsion before centrifugation at 80 ° C for 30 minutes in water bath. It was then kept cooled under running water for 15 minutes; the emulsion stability was expressed as the percentage of the emulsifying activity remaining after heating.

$$\frac{Height\ of\ emulsified\ layer\ after\ heating}{Height\ of\ the\ total\ content\ in\ tube}\ x\ 100$$

Foaming properties

Foaming capacity (FC) and foaming stability (FS) was estimated given by Lawhon et al. (1972) and Ahmed and Schmidt (1979) respectively. About 20 ml of of each succinylated sample preparation was whipped at 1600 rpm for 5 min. the mixture was poured immediately into 100 ml graduated cylinder and the foam volume was recorded. Foam capacity (FC) was calculated using formula:

$$\frac{Volume\ after\ whipping - Volume\ before\ whipping}{volume\ before\ whipping} \times 100$$

Foaming stability (ES) was determined in a similar manner as above but It was then kept after different time intervals ranging from 30s to 60 minutes. Foam stability (FS) was calculated using formula:

$$\frac{Foam\ volume\ after\ time\ t}{Initial\ foam\ volume} \times 100$$

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 extent of succinylation can be expected to vary with different proteins. Extent of succinylation allows estimating number of amino acid that had not reacted with succinic anhydride and total number of reactive amino group. The degree of succinylation of free amino group in fish gelatin increased with the addition of incremental amount of succinic anhydride ($p<0.05$). Trend of increment at modification 0.55%, 1.66% show small level of increament. Whereas at 2.77%, 5.55% and 11.11% it shows high degree of succinylation. The maximum level of succinylation was about 26.7% at modification concentration of 11.11%. The highest concentration of succinic anhydride added gave the highest percentage of extent of succinylation at 26.7% (Figure 1).

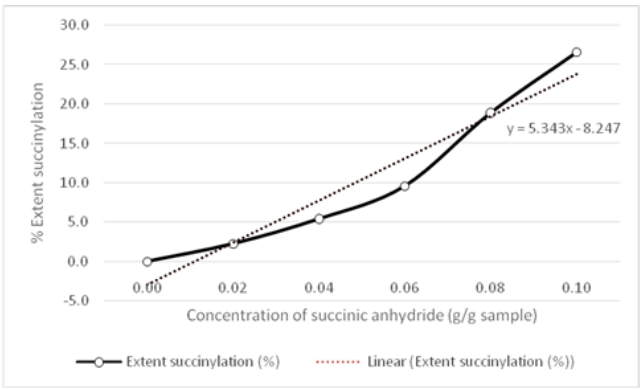

Figure 1. Extent of succinylation of fish gelatin

These result indicated that most of reactive group which are amino group of lysine and N-terminal amino acid were accessible for succinic anhydride at pH 8.0 or at the initial succinylation of exposed amino residue may have altered protein conformation thus enhanced availability of otherwise inaccessible lysine groups. The reaction of cysteine with ninhydrin is an exception to the general reaction of α-amino-acids with this reagent and the absorption curve of the reaction is similar to that of proline and hydroxyproline (Moore & Stein 1961).Some investigations indicated that there was no significant difference in the effect of acetic and succinic anhydride on the acylation of winged bean flour (Narayana and Rao, 1984). Consequently, the acylation of chickpea proteins indicated that the effect of acylation reagents (acetic and succinic anhydride) on the level of modification varied not only with the type of reagent and as concentration but also with the different types of proteins involved in the system.

Bloom strength

The relative gel strength of a gelatin product is indicated by its "Bloom" value, a measure of resistance to compression of a 6.67% gel prepared and held under prescribed conditions. Higher Bloom gelatins form stronger gels than lower Bloom gelatins. Bloom strength of gelatin is one of the most important parameters to evaluate the grade and physical quality of a gelatin (Schrieber and Gareis, 2007). Gel strength is one of the important properties of gels, and the specific application of a gel is determined by the range of gel strength values. Table 1 shows the Bloom strength of fish 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 (%)
0%	126.06±6.47^{e}	0	0
0.55%	140.86±2.18^{d}	14.8	11.74
1.66%	154.73±2.60^{c}	28.67	22.74
2.77%	164.90±5.65^{c}	38.84	30.81
5.55%	168.33±6.02^{b}	42.27	33.53
11.11%	178.66±1.52^{a}	52.6	41.73

*abcde Values are mean of each triplicate of three repeated samples with ± standard deviation. Different letters in the same column indicate significant differences ($p<0.05$).

According to Table 1, there is significant different ($P<0.05$) in bloom value after succinylation treatment. As concentration of succinic anhydride increased the gel strength of fish gelatin also increased. The introduction of succinic anhydride at level 0.55%, resulted in increase in gel strength by 140.9g compared with that of unmodified fish gelatin at 126.1g. Gel strength continuously increased as concentration increased from 0% to 11.11% and maximum value gotten at 11.11% (g/g sample) which is the highest concentration. With addition of 0.5 g of succinic anhydride gel strength increase by 11.74% compared with untreated fish gelatin. The stronger gel could be due to the denaturation of triple helix, increased crosslink of junction zones, as well as the formation of hydrogen bond between hydroxylated amino acid and incorporated water (Arnesen & Gildberg, 2007).

Emulsion activity (EA)

The effect of succinylation on emulsifying activity (EA), of the succinylated fish gelatin is shown at Table 2. There is significant difference between samples after succinylation ($p<0.05$). A significant increase in emulsifying activity (EA) was observed in the highly succinylated (11.11%) that is 68.9%. Increase in the emulsifying activity after succinylation is an indication of increased solubility. Similar observations have been reported with other food proteins such as cottonseed flour (Choi et al, 1983), wheat (Barber and Warthesen, 1982), soy (Franzen and Kinsela, 1976) and canola (Paulson and Tung, 1988). At concentrations ranging from 0.55 to 11.11%, all succinylated proteins had slightly higher emulsifying activities (1.67% to 64.83%) than that of the unmodified protein. Franzen and Kinsella (1976) found a positive relationship between the EA and solubility of a protein. A high solubility of succinylated protein could contribute to its higher EA. However, this contribution is not always proportionally related to the solubility. Moreover, different level of succinylations did not greatly influence the EA of succinylated proteins.

Table 2. Emulsion activity (EA) of succinylated fish gelatin

Sample	Emulsion Activity (%)	Improvement effect (%)	Percentage effect (%)
0%	41.8±0.01^{d}	0	0
0.55%	42.5±0.02^{d}	0.7	1.67
1.66%	46.0±0.01^{d}	4.2	10.05
2.77%	54.5±0.04c	12.7	30.38
5.55%	63.8±0.03^{b}	22	53.63
11.11%	68.9±0.21^{a}	27.1	64.83

*abcd Values are mean of each triplicate of three repeated samples with ± standard deviation. Different letters in the same column indicate significant differences ($p<0.05$).

Change in hydrophobic-hydrophilic balance can alter the functional properties of protein. Acylation enhances the emulsification because exposed hydrophobic residue associate with the oil and increase of negative surface charge on the droplets stabilize the emulsion via charge repulsion of the particles. Previous studied shows, succinylated sunflower protein isolate had improved emulsifying activity and stability (Canella et al., 1979), as the protein became more soluble, it formed a layer around the fat droplets to facilitate their association with the aqueous phase. Succinylation caused exposure of buried functional groups within the protein matrix, which enhanced interaction at the protein– oil interface (Lawal, 2005).

Emulsion stability (ES)

The emulsion stability of the fish gelatin decreased after succinylation as Table 3. shown. There is significant difference between the sample ($p<0.05$).The decrease in emulsion stability of the succinylated fish gelatin might be due to the effect of the high degree of succinylation on the fish gelatin. Increase in protein–protein repulsion, preventing protein–protein interactions so that no elastic protein film can form at the oil-aqueous phase interface, which leads to the formation of a less stable emulsion. This finding does not agree with those reported by El Adawy (2000), Lawal (2005), and Lawal et al. (2007) on Lablab bean, Mung bean, and Bambarra groundnut, respectively. The emulsion stability (ES) of succinylated fish gelatin was influenced by several factors such as protein concentration, solubilised pH and salt concentration. For a time stability (30 minutes) after heating the effect of protein concentration on the ES of unmodified fish gelatin decreases compared succinylated proteins.

Table 3. Emulsion stability (EA) of succinylated fish gelatin

Sample	Emulsion Stability (%)	Improvement effect (%)	Percentage effect (%)
0%	67.6±0.01^{a}	0	0
0.55%	61.8±0.02^{b}	-5.8	-8.58
1.66%	59.0±0.03^{b}	-8.6	-12.72
2.77%	53.8±0.02^{c}	-13.8	-20.41
5.55%	48.1±0.07^{cd}	-19.5	-28.85
11.11%	44.7±0.07^{d}	-22.9	-33.88

*abcd Values are mean of each triplicate of three repeated samples with ± standard deviation. Different letters in the same column indicate significant differences ($p<0.05$).

Foaming capacity (FC)

The effects of succinylation on the foaming capacity of the fish gelatin are shown at Table 4. Succinylation increased foaming capacity significantly as concentration of anhydride used increases ($p<0.05$). High foam capacity was observed at the highest modification concentration of 11.11%, this may however be due to an increase in the net charge of the protein molecules, which weakens hydrophobic interactions and increases protein flexibility. This allowed them to spread to the air water interface more quickly thus, encapsulating air particles and increasing foam formation.

Earlier researchers reported a similar increase in the foam capacity as the concentration of the proteins was increased (El-Adawy, 2000; Franzen and Kinsella, 1976). Acylation can cause unfolding of the protein, which increases protein-water interaction. Also, the increased net negative charge of succinylated proteins would especially promote protein-water interaction, which facilitates improved foaming capacity. Increased solubility of the fish gelatin, by acid treatment, enhanced foam ability, since soluble proteins contribute to foaming (Chan & Ma, 1999). Cheftel et al, (1985) reported that foam capacity may be improved because the negative charges imparted timing succinylation cause protein molecules to unfold. Increased net charge density may prevent protein-protein interactions in the foam lamellae causing foam destabilization and poor stability (Townsend and Nakai. 1983; Thompson and Cho, 1984). Acylated rapeseed protein isolate and cheese whey

protein concentrate (Thompson and Cho. I984) had decreased whippability while succinylated soy protein (Franzen and Kinsella, 1976) had improved foam capacity.

Table 4. Foaming capacity (FC) of succinylated fish gelatin with different level of modification

Sample	Foaming Capacity (%)	Improvement effect (%)	Percentage effect (%)
0%	14.5±0.71[d]	0	0
0.55%	33.0±1.41[c]	18.5	127.59
1.66%	37.5±0.70[c]	23.0	158.62
2.77%	47.0±1.41[b]	32.5	224.14
5.55%	61.3±1.41[a]	46.8	322.76
11.11%	65.1±1.41[a]	50.6	348.97

*abcd Values are mean of each triplicate of three repeated samples with ± standard deviation. Different letters in the same column indicate significant differences ($p<0.05$).

Foaming stability (FS)

Foaming stability of succinylated fish gelatin decreased which is unstable over the time (1 hour) as the concentration of succinic anhydride increases ($p<0.05$) (Figure 2).

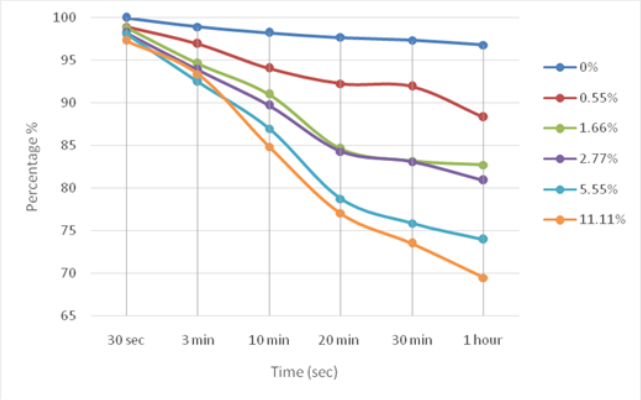

Figure 2. Foaming stability (FS) of succinylated fish gelatin vs time.

Decrease in foam stability following modifications might be as a result of increased charge density of succinylated proteins which inhibits the protein-protein interactions (Townsend and Nakai, 1983). This is because excessive increases in charge reduce protein–protein interactions and prevent the formation of an elastic film at the air–liquid interface, which reduces foam stability. In order for proteins to make foam, they must be soluble in the aqueous phase so that they can accommodate themselves at the interface, unfold to form cohesive layers of protein around air droplets as they are formed, and possess sufficient viscosity and mechanical strength to prevent rupture and coalescence.

While foaming is favoured by increasing viscosity, hydrophobicity and solubility, the increase of net charge density cause by succinylation tend to decrease foam stability since it prevent optimum protein-protein interactin required in cintinuous film around air bubles. Therefore number of percentage of foaming stability showed a drop with increasing degree of modification. This wasl reported for protein from faba beans, (Schwenke, 1983), and peas (Townsend and Nakai,1983) . In some protein preparation such as sunflower isolate and concentrate stable foam were obtained although succinylation was high. In these cases, the other instrinsic and extrinsic parameters obviously overcome the charge repulsion. Protein stability is high in the neighbourhood of isoelectric pH than at any other pH. This observation lends credence to similar results that have been reported earlier by Buckingham (1970). Increase in the foam stability at the region of isoelectric pH might be due to the formation of stable molecular layers in the air-water interface of the foams. Protein adsorption and viscoelasticity at an air-water interface is high at isoelectric pH because protein is not strongly repelled. In addition, the protein possesses low net charge near isoelectric pH, which may contribute to the formation of stable molecular layers in the air-water interface, a development that improves foam stability.

CONCLUSIONS

Gelatin can be modified by succinylation thus can improve physicochemical properties of fish gelatin such as gel strength, emulsification activity, foaming capacity through its amino, carboxyl and hydroxyl functional group in its structure. The changes in physicochemical properties depended on the level of modification of succinylation. For skin fish gelation physicochemical properties such as gel strength (Bloom), emulsion capacity, foaming capacity were increase at 41.73%, 64.83%, and 348.97%. But for emulsion stability was decrease at 33.88% and foaming stability also was decrease (unstable) over the 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.

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