ISSN: 2338-1345 – Vol. 5 (2) 3-8 2017 Journal online http://journal.bakrie.ac.id/index.php/APJSAFE

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

Effect of Added Succinylated Fish Gelatin on the Textural Profile of Stored Sardine Surimi Gel

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Abstract- Gelatin is a biopolymer with unique functional properties that are very useful in food, pharmaceutical, cosmetic, and photographic applications. Low quality gelatin such as that from fish has limited application because its gels have inferior rheological and functional properties. However, researchers are looking for ways to use this abundant resource. In this study, the effect of adding succinylated fish gelatin to stored sardine surimi was investigated. Changes in the physicochemical properties of surimi gels depended on the degree of succinylation of the fish gelatin. As the degree of succinylation increased, expressible moisture and water holding capacity of surimi gels increased from 0.56 to 18.07% and from 2.86 to 20.45%, respectively. The lightness value of surimi gels also increased. Addition of succinylated fish gelatin had no effect on gel folding test results, but it did improve texture of the gels. With increasing succinylation of fish gelatin, hardness of the gels in creased from 11.11 to 77.78%, springiness from 0 to 20.83%, cohesiveness from 0 to 67.5%, gumminess from 9.38 to 200%, and chewiness from 8.7 to 265.22%. In summary, addition of succinylated fish gelatin improved the physicochemical properties of surimi gels.

Keywords- Fish gelatin, surimi, succinic anhydride, texural profile, water holding capacity

INTRODUCTION

Gelatin is one of the most versatile gelling agents used in food applications due to its special texture and its 'melt-in-mouth' quality. Gelatin has been used as an additive to improve 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, 2012). Gelatins from different sources have varying properties that are related to the amino acid composition, α-, β-, or γ-chain components, and molecular weight distribution (Johnston-Banks, 1990). The intrinsic differences between mammalian and other sources of gelatin affect the properties of their gels (Benjakul et al., 2009).

Protein alteration using chemical modification is an important tool for tailoring food proteins into products with different functional properties. Succinylation of protein with succinic anhydride is one of the most convenient and frequently used methods for altering the functional properties of many plant proteins, and the effects of succinylation on the functional properties of numerous food proteins have been investigated. Acylation with acetic anhydride is also widely used to improve functional properties of various food proteins (Schwenke and Rauschal, 1980). Introducing acetyl groups into the protein molecules has been shown to improve emulsifying capacity, emulsion stability, foam capacity and stability, and water absorption of pea (Johnson and Brekke, 1983; Mendoza-Martinez et al., 1988), soy (Kim and Rhee, 1989), winged bean (Narayana and Rao, 1984), rapeseed (Ponnampalam et al., 1990) and mung bean (El- Adawy, 2000) proteins. An increase in the nitrogen solubility, emulsifying activity and stability, and foaming capacity of acylated leaf proteins was reported (Franzen and Kinsella, 1976), and Narayana and Rao (1984) described similar results for succinylated and acetylated winged bean protein.

Surimi is the Japanese term for minced fish. During preparation of surimi, the water soluble components, including sarcoplasmic proteins, are removed by leaching with potable water. Generally, the types of fish used to produce surimi have high functional properties (e.g., gel forming ability), have white flesh with subtle odour and flavour, are low in fat, and are abundant in nature, which allows for mass production with consistent quality. Good quality surimi can be produced using low-value white fleshed fish species, and it generally has excellent gelling ability. The less-popular dark fleshed fish species also have potential for the production of surimi, but it requires additives to improve its physicochemical properties. The addition of collagen or gel strength enhancers such as starch, egg white, whey protein, or soybean protein can be used to improve the physicochemical properties of surimi (Zayas, 1997).

This objectives of this study was to determine the effect of succinylated fish gelatin addition on the physicochemical properties of two years stored sardine surimi such as on colour, expressible moisture (EM), water holding capacity (WHC), folding test and texture profile.

MATERIALS AND METHODS

Materials

Materials used in this study were fish skin gelatin (168 bloom strength) obtained from SKW Biosystems (Grasse, France; jelly strength 6.67%), succinic anhydride 99% (Acros Organic, Morris Plains, NJ, USA), and sardine surimi that had been stored for 2 years at –18 oC

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 of succinic anhydride were added in small increments to reach succinylation degree of 0.00, 0.55, 1.66, 2.77, 5.55 and 11.11 %. The solution was stirred and the pH maintained at 8.0 by adding 3M NaOH. After reaction was complete, the succinylated fish gelatin was kept at -18°C before freeze-dried.

Surimi Gel Preparation

Surimi gels were prepared according to the method of Babji and Gna (1994). For each treatment, ~200 g of surimi, 6 g of salt, and 4 g of fish gelatin were mixed for 2 min in a cutter mixer (Robot Coupe®, Model Blixer®, 3B, France) and stuffed into a 25 mm diameter cellulose casing. In two separate water baths (Model WB-22, Korea), the stuffed samples first were cooked in warm water at 36 °C for 30 min to allow low temperature setting, followed by high temperature setting at 90 °C for another 10 min. After cooking, all gels were immediately cooled in iced water for 30 min and stored at 4 °C overnight prior to analysis.

Colour Measurement.

A colorimeter (Minolta Spectrophotometer CM-3500D, Osaka, Japan) was used to measure the colour (L*, a*, b* values) of surimi gel samples based on the CIE Lab Scale. The instrument was calibrated with zero calibration (CM-A100) followed by a white calibration plate (CM-A120). L* represents the lightness (L* = 100 is the lightest and L* = 0 is the darkest), a* represents the redness (red +60 to green –60), andb* represents the yellowness (yellow +60 to blue –60) of the sample.

Expressible Moisture (EM)

EM was measured according to the method of Benjakul et al. (2001). Gel samples were cut into pieces 5 mm thick, which were weighed and placed between two pieces of Whatman paper No. 41. The standard weight (5 kg) was placed on top of each sample for 2 min, and then the sample was weighed again. EM was calculated as follows:

$$\frac{Weight\ of\ pre-pressed\ sample\ (g) - Weight\ of\ pressed\ sample\ (g)}{Weight\ of\ pre-pressed\ sample\ (g)} \times 100$$

Water Holding Capacity (WHC)

WHC was measured according to Pietrasik (1999). A gel sample (25 x 15 mm) of known weight was placed in a tube and centrifuged (Sorvall RC 5B Plus) at 365 x g for 20 min at 4 °C. WHC was expressed as the ratio of gel weight after centrifugation to the initial gel sample weight.

Folding Test

The folding test was performed following the procedures of Lanier (1992). Samples were cut into 3 mm thick portions. Each slice was held between the thumb and forefinger and folded to observe how it broke. The scale used was as follows: 1 = breaks by finger pressure, 2 = cracks immediately when folded in half, 3 = cracks gradually when folded in half, 4 = no cracks showing after folding in half, 5 = no cracks showing after folding twice.

Texture Profile Analysis (TPA)

TPA of the surimi gels was performed following Bourne (1978) and using a Textural Analyser TA.XT2 (Stable Microsystems, Godalming, UK) with a compression platen (SMS P/75) and 30 kg load cell. The surimi gel was cut into a cylindrical shape with length of 2.5 cm. The settings for analysis were as follows: speed, 1.0 mm/sec; test speed, 1.0 mm/sec; post-test speed, 1.0 mm/sec; distance, 15 mm; time before second compression, 2 sec; trigger force, 5 g. The parameters evaluated were hardness, cohesiveness, springiness, chewiness, and gumminess. Hardness (kg) was measured first with a probe during the first compression. Cohesiveness (ratio) was calculated as the area of work during the second compression divided by the area of work during the first compression. Springiness (mm) was measured as the force at maximum compression during the second compression cycle. Gumminess refers to the force applied to the semi solid-sample and was calculated as hardness × cohesiveness. Chewiness (kg.mm) was calculated as the product of gumminess and springiness.

Statistical Analysis

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

RESULTS AND DISCUSSIONS

Colour Analysis

Colour is one of the main factors that consumers consider when evaluating product quality. More intense lightness is indicated by a higher L* value, which is a desirable attribute and has high consumer acceptance (Resurreccion, 2003; Huda et al., 2013). Table 1 shows that the colour values of sardine surimi gels containing succinylated fish gelatin became lighter as the degree of succinylation increased (0.00–11.11%), and the lightness was significantly higher ($p < 0.05$) compared to the unmodified control sample.

Table 1. Colour (*L, *a, *b) of surimi gels containing succinylated fish gelatin.

Sample	*L	*a	*b
0.00%	61.19 ± 0.45^{e}	–0.51 ± 0.51^{ab}	13.48 ± 0.39^{d}
0.55%	61.44 ± 0.11^{cd}	–0.29 ± 0.83^{a}	13.90 ± 0.14^{bcd}
1.66%	61.63 ± 0.30^{cd}	–0.48 ± 0.10^{ab}	13.69 ± 0.95^{cd}
2.77%	62.03 ± 0.07^{b}	–0.37 ± 0.19^{a}	13.97 ± 0.57^{abc}
5.55%	62.34 ± 0.38^{ab}	–0.49 ± 0.04^{ab}	14.53 ± 0.16^{ab}
11.11%	62.54 ± 0.55^{a}	–0.70 ± 0.27^{a}	12.65 ± 0.27^{a}

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

Addition of gelatin or collagen and its interaction with water made it swell and caused an increase in light scattering, which led to the increased value of lightness (Pereira et al., 2011). The change in colour of surimi is mainly imparted by the colour of the succinylated fish gelatin. The addition of succinated fish gelatin only slightly affected the redness and yellowness of the surimi gels. This colour change can be roughly distinguished by the naked eye. However, these differences may be eliminated by using a small amount of fish gelatin (Schilling et al. 2003).

Expressible Moisture (EM) and Water Holding Capacity (WHC)

EM is correlated with WHC, as high EM indicates poor WHC (Chaijan et al., 2006). Table 2 lists the EM values and WHC of all samples. EM decreased with increasing level of succinylation, and in some cases the difference was significant ($p < 0.05$). Succinylation significantly increased ($p < 0.05$) the WHC at all levels of modification compared to surimi containing unsuccinylated gelatin.

EM is an indication of the amount of liquid that can be squeezed from a protein system by the force applied (Jauregui et al., 1981). Niwa (1992) also suggested that EM is inversely associated with WHC. Surimi gel containing 11.11% succinylated fish gelatin had the lowest EM (10.20%), whereas the control had the highest EM. According to Gosset and Baker (1983), succinylation of protein can increase the charge of the protein. Thus, when pH of the mixture reaches 8.0 or higher, succinate anions bind with two carboxyl groups, leading to a net gain in negative charge and a decrease in EM value. The WHC usually reflects the extent of denaturation of proteins and the water content (Shaviklo, 2006).

Table 2. Expressible moisture (EM) and water holding capacity (WHC) of surimi gels containing succinylated fish gelatin.

Sample	EM (%)	WHC (%)
0.00%	12.45 ± 0.02[a]	17.6 ± 0.01[a]
0.55%	12.38 ± 0.01[a]	18.1 ± 0.01[b]
1.66%	11.32 ± 0.01[ab]	18.3 ± 0.01[b]
2.77%	10.76 ± 0.01[ab]	18.6 ± 0.01[c]
5.55%	10.25 ± 0.00[b]	19.2 ± 0.01[d]
11.11%	10.20 ± 0.01[b]	21.2 ± 0.01[e]

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

Addition of succinylated fish gelatin improved the WHC of surimi gels by causing dissociation and unfolding of the protein. This likely exposes more hydrophilic groups than hydrophobic, thereby increasing the number of hydrophilic binding sites. Increased WHC due to other chemical modifications, such as acylation, has been reported for chickpea protein (Liu and Hung, 1998), rapeseed flour (Dua et al., 1996), mungbean protein (El-Adawy, 2000). WHC is directly correlated with the myofibrillar protein content (Smith, 1991), as myofibrillar protein contributes to the juiciness of meat and hence a higher WHC and lower EM (Kinsella, 1982).

Folding Test

The folding test is a simple and quick method to determine the quality of gel springiness (Nowsad et al., 2000). No facturing occurs during the test for high quality surimi (Ramirez et al., 2011). No significant difference ($p > 0.05$) was detected among the samples. All of the surimi gels cracked when folded in two (score 2.0), which shows that the addition of succinylated fish gelatin did not improve the folding test score of two years stored sardine surimi. It is possible that the degree of succinylation was not high enough to affect this folding test of the surimi samples. During long time storage, probably sardin surimi lost its ability to produce gel with high folding test score.

Texture Profile Analysis (TPA)

Tables 3 shows the TPA results for surimi gels containing succinylated fish gelatin. Lee and Chung (1989) reported that TPA parameters are better for assessing the overall binding properties of surimi gel with or without added ingredients. However, higher values of texture profile parameters do not necessarily indicate better quality (Yu and Yeang, 1993).

Table 3. Texture Profile Analysis (TPA) results for surimi gels containing succinylated fish gelatin.

Sample	Hardness (g)	Springiness (mm)	Gumminess (g)	Cohesiveness (ratio)	Chewiness (g.mm)
0.00%	812.10 ± 1.00[e]	0.72 ± 0.02[c]	324.84 ± 34.46[d]	0.40 ± 0.05[c]	233.90 ± 22.90[a]
0.55%	888.77 ± 9.25[e]	0.72 ± 0.01[c]	355.51 ± 55.53[d]	0.40 ± 0.02[c]	253.46 ± 41.22[a]
1.66%	1137.22 ± 10.21[d]	0.79 ± 0.02[b]	591.35 ± 22.57[c]	0.52 ± 0.03[b]	467.17 ± 28.90[a]
2.77%	1286.20 ± 48.55[c]	0.83 ± 0.04[ab]	678.69 ± 14.23[b]	0.60 ± 0.10[ab]	563.31 ± 8.55[ab]
5.55%	1371.92 ± 57.12[b]	0.84 ± 0.02[a]	771.72 ± 2.37[b]	0.63 ± 0.04[a]	648.24 ± 40.36[ab]
11.11%	1435.89 ± 4.07[a]	0.87 ± 0.01[a]	962.04 ± 6.58[a]	0.67 ± 0.03[a]	836.98 ± 3.90[a]

[abcd]Values are mean of triplicate samples ± standard deviation. Different letters in the same column indicate significant differences ($p < 0.05$).

Significant differences in hardness, cohesiveness, gumminess, springiness, and chewiness of surimi gels were detected among the different treatments ($p < 0.05$). The control (0.00% succinylated fish gelatin) had the low hardness value, and hardness of the gels increased as degree of succinylation increased. The ability of succinylated fish gelatin to increase the hardness value may be of interest to the meat processing industry, as hardness determines the commercial value of a meat (Park et al., 1996; Huda et al., 2013).

Santana et al. (2012) showed that surimi gels containing hydrocolloid, including fish gelatine, had higher TPA values than surimi gel without hydrocolloid. Gel strength is the major physical property of gelatin gels, and it is related to hardness. It is governed by chain length, amino acid composition, and the ratio of α- to β-chains present in the gelatin (Cho et al., 2004). According to Schrieber and Gareis (2007), gel strength depends mainly on the proportion of fractions having a molecular weight of approximately 100,000 g mol−1. In addition, there is a strong correlation between gel strength and the α-chain content in gelatin. Gelatins containing more α-chains have greater gel strength (Karim and Bhat, 2009).

Surimi gel containing 11.11% succinylated fish gelatin had the highest springiness value among the samples tested, and the springiness decreased with decreasing level of gelatin (Table 3). Increasing the degree of succinylated fish gelatin added to the surimi also improved the gumminess, cohesiveness, and chewiness parameters (Table 3). Bourne (1978) measured chewiness as a function of water activity and found that chewiness increased with increased content of solids. Bourne (2002) noted that gumminess should be used to evaluate semi-solid foods and chewiness should be used for solid foods.

CONCLUSIONS

Surimi gels containing succinylated fish gelatin exhibited significantly decreased EM and increased WHC compared to the control. The lightness value of surimi gels

also increased as the degree of succinylation of fish gelatin added increased. In contrast, the addition of succinylated fish gelatin had no effect on folding test score (score 2.0 for all gels tested). Results of TPA showed that hardness, chewiness, cohesiveness, gumminess, and springiness all increased with increasing degree of succinylation. Addition of succinylated fish gelatin alters functional properties of stored sardine surimi proteins..

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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