

Improving the Physicochemical Properties of Stored Sardine Surimi Using Succinylated Gelatin

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Abstract: Commercial gelatin was modified using succinic anhydride at varying concentrations and added to low quality surimi (sardine surimi that had been stored for 2 years). The effects of the addition of succinylated gelatin on the water holding capacity (WHC), expressible moisture (EM), folding test, texture profile analysis and colour of the surimi gels then were analyzed. WHC increased from 63.95 to 67.38%, EM reduced from 33.52 to 26.51%, hardness increased from 1182.00 to 1779.00 g as well as cohesiveness from 0.26 to 0.43, springiness from 0.40 – 0.97 mm, chewiness from 0.31 to 0.51 kg and gumminess from 0.16 to 0.48 kg.mm as the level of succinic anhydride increased from 0 to 4%. However for folding test, improvement only appears in gelatin added with 4% succinic anhydride. Colour and whiteness of sardine surimi also improved from 62.32 to 64.45 and from 60.83 to 61.87, respectively. These results suggest that addition of succinylated gelatin has the potential to improve the physicochemical properties of stored surimi.

Key words: Commercial gelatin • Succinic anhydride • Chemical modification • Physicochemical properties

INTRODUCTION

Surimi is the Japanese term for minced fish. To produce surimi, the water soluble components of the meat, including sarcoplasmic proteins, are removed by leaching with potable water. In general, the types of fish used to produce surimi are underutilized, have high functional properties (e.g., gel forming ability) and white flesh with subtle odour and flavour, are low in fat and are abundant in nature, which permits mass production with consistent quality. Development and manufacturing of surimi have become highly successful and surimi production is increasing [1]. Good quality surimi with excellent gelling ability can be produced from low-value white-fleshed fish species. Dark-fleshed fish species also have potential as a source of surimi, but additives may be needed to improve the physicochemical properties of the surimi. For example, addition of collagen or gel strength enhancers such as starch, egg white, whey protein, or soybean protein can help produce good textural characteristics [2].

Gelatin is a tasteless, highly purified and collagenous protein that is used in the food pharmaceutical, cosmetic and photographic industries [3]. It is not a naturally occurring protein; rather, it is derived by partial hydrolysis of collagen, the principle constituent of animal skin, bone and connective tissue. In the food industry, gelatin is used mainly to improve elasticity, consistency and stability of foods. It imparts a melt-in-the-mouth quality and has the ability to form a thermo-reversible gel. Grand View Research [4] reported that pig skin was the most commonly used raw material for the manufacture of gelatine, accounting for more than 40% of global gelatin production. Karim and Bhat [3] and Gómez-Guillén *et al.* [5] reported that the annual world output of gelatin is nearly 326,000 tons, with pig skin-derived gelatin accounting for the 46% of 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 characteristics, such as viscosity and viscoelastic.

Gelatin can be modified in a number of ways to improve its functionality. The effects of succinic anhydride treatment on properties of fish myofibrillar protein [6], sunflower protein isolate [7], soy protein isolate [8], oat protein isolate [9], soy protein [10] and peanut flour [11] have been reported. In general, functional properties such as solubility and emulsification capacity were improved by succinylation [7]. Groninger [6] found that emulsification capacity of succinylated myofibrillar protein was related to its degree of succinylation. Oppenheimer *et al.* [12] showed that succinylation of chicken protein resulted in a product with increased viscosity, but the molecular size of the protein remained similar to that unmodified myosin. In another study, Bora [13] reported that succinylation improved the emulsion activity and emulsion stability and increased the water absorption capacity of lentil globulin.

This project was carried out to determine the effect of succinylated gelatin (at level 0, 1, 2, 3 and 4%) addition on the physicochemical properties of two years stored sardine surimi such as on water holding capacity (WHC), expressible moisture (EM), folding test, texture profile and colour of surimi gels.

MATERIALS AND METHODS

Materials: Commercial bovine gelatin was purchased from Leverage Business Sdn Bhd (Penang, Malaysia) and used as the main sample. For succinylation of gelatin, succinic anhydride 99% was purchased from Acros Organic (Morris Plains, NJ, USA). Sardine surimi that had been stored for 2 years at -18 °C was used to prepare surimi gels.

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 of distilled water (2.5 % w/v) and left at room temperature to allow the gelatin to dissolve. Gelatin was then melted at 60 °C until it was completely dissolved and the solution was cooled to room temperature. Succinic anhydride was added in small increments with constant stirring (Eurostar Digital, IKA-WERKE, Germany) at levels of 0, 1, 2, 3 and 4% of the weight of the gelatin in order to obtain different degrees of succinylation. pH of the mixtures was maintained at 7 by adding 1 N NaOH in order to prevent further modification. The mixtures were freeze dried prior to analysis.

Preparation of Surimi Gels: Surimi gels were prepared according to method described by Babji and Gna [14]. Frozen sardine surimi was thawed at 4 °C for 24 h prior to surimi gel preparation. To produce the gels, 2% succinylated gelatin (added with 0, 1, 2, 3 and 4% of succinic anhydride) were mixed with 3% salt and 95% surimi for 2 min in a cutter mixer and then the mixtures was stuffed into 2 cm of casing and bound at both ends. Stuffed casings were incubated in two different water baths (Wisebath Model WB-22, Korea) at 36 °C for 30 min followed by 90 °C for 10 min. Surimi gels were then immediately cooled in ice water for 30 min and stored at 4 °C overnight prior to analysis.

Water Holding Capacity (WHC): The water holding capacity of surimi gels was determined according method of Jin *et al.* [15] with slight modification. First, 10 g of sample were homogenised with 40 ml of distilled water at 7000 rpm (IKA T25 digital, Germany). Next, a 10 ml aliquot of homogenate was placed in a 50 ml centrifuge tube, weighed and centrifuged (Kubota Model 4000, Japan) at 1500 g for 10 min. The supernatant was discarded and the sediment was weighed. The WHC was calculated using the following formula:

$$\text{WHC}\% = \frac{\text{Weight before centrifuge} - \text{weight after centri}}{\text{weight before centrifuge}}$$

Expressible Moisture (EM): The EM of surimi gel was determined following the method described by Rawdkuen *et al.* [16]. Surimi gels were cut into pieces 0.5 cm thick. A slice was weighed (X) and then placed between filter paper (one piece on top, two pieces on the bottom). A standard weight (5 kg) was placed on the top of the sample for 2 min, after which the sample was weighed again (Y). The EM was calculated as follows:

$$\text{Expressibe moisture \%} = \frac{X - Y}{Y} \times 100\%$$

Folding Test: The folding test was conducted following the method described by Lanier [17]. The gel samples were cut into 0.3 cm thick portions. A surimi gel slice was held between the thumb and forefinger and folded to observe the way in which it broke. Grading of the folding test was as follows: 1 = easily 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 surimi gels was performed according to the method of Bourne [18] using a Textural Analyser TA.XT2 (Stable Microsystems, Godalming, UK) with a compression platen (SMS P/75) and a 30 kg load cell. The surimi gel was cut into a cylindrical shape with length of 2.5 cm. The settings 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 the first parameter measured with a probe during the first compression. Cohesiveness (ratio) was calculated as the measure of 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, or the force applied to the semi-solid sample, was calculated as hardness \times cohesiveness. Chewiness (kg.mm) was calculated as the product of gumminess and springiness. It is a measure of the energy required to chew a solid sample to a steady state of swallowing.

Colour Analysis and Whiteness: Analysis of colour and whiteness of surimi gels was conducted using a colorimeter (Minolta Spectrophotometer CM-3500D, Osaka, Japan). The instrument was calibrated with zero calibration (CM-A100) followed by a white calibration plate (CM-A120). L^* , a^* and b^* were the parameters used to determine colour. L^* represents lightness (0 = lightest, 100 = darkest), a^* represents redness (red +60 to green -60) and b^* represent yellowness (yellow +60 to blue -60). The whiteness of surimi gels was calculated using the following formula:

$$100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{0.5}$$

Statistical Analysis: All analyses were performed using data from duplicate experiments, each of which included analysis of three samples. The data were analysed using one way analysis of variance. SPSS version 22 was used for data analysis and differences were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

WHC and EM: Table 1 shows the WHC and EM data for the sardine surimi gels containing different amounts of succinylated gelatin. As the degree of succinylation increased, the WHC increased and the EM decreased.

Table 1: Expressible moisture (EM) and water holding capacity (WHC) of surimi gels containing succinylated gelatin

Type of Gelatin Added	WHC (%)	EM (%)
Control	63.95 \pm 0.39 ^a	33.52 \pm 0.50 ^a
1% succinic anhydride	64.79 \pm 0.14 ^{a,b}	32.38 \pm 0.38 ^a
2% succinic anhydride	65.39 \pm 1.65 ^{a,b}	31.70 \pm 1.87 ^a
3% succinic anhydride	65.92 \pm 2.77 ^{a,b}	28.45 \pm 2.21 ^b
4% succinic anhydride	67.38 \pm 0.49 ^b	26.51 \pm 1.05 ^b

Values are mean \pm SD ($n = 3$). Different superscript letters within the same column indicate significant differences ($p < 0.05$).

WHC is a measure of the loose water that is released under application of force [19]. Increased WHC means an increase in water retention in the gels and a decreased percentage of water loss. The WHC of sardine surimi gels increased from 63.95% in unsuccinylated gels to 67.38% in the gels with highest level of succinylation. The WHC of sardine surimi gel containing unsuccinylated gelatin (63.95%) was lower than that of fresh sardine surimi gel (97.01%) reported Huda *et al.* [20]. The lower WHC observed in the current study was related to the protein denaturation that occurred in the surimi during 2 years of frozen storage. Benjakul *et al.* [21] reported that water was released from muscle tissue more easily with increased duration of frozen storage time. EM is amount of liquid squeezed from a protein system by the application of force and it is a measure of the amount of loose water released under the measurement conditions [19]. Addition of increasing amounts of succinylated gelatin reduced the EM of surimi gels from 33.52% to 26.51%. During thermal gelation of surimi gels, the protein matrix forms and water is absorbed regularly throughout the network [16]. The observed decrease in EM indicated that the added gelatin held water molecules in the surimi gel matrix and thereby improved the WHC of the gels.

Folding Test: The folding test is a simple and quick method to determine the quality of gel springiness [22]. Table 2 shows the results of the folding test of sardine surimi gels containing succinylated gelatin. The folding test result was the same (score 2) for all gels tested except for the one with the highest level of succinylation (score 3). The latter cracked gradually when folded in half rather than immediately when folded in half.

Table 2: Results of folding test for surimi gels containing succinylated gelatin

Type of Gelatin Added	Folding Test
Control	2.00 \pm 0.00 ^b
1% succinic anhydride	2.00 \pm 0.00 ^b
2% succinic anhydride	2.00 \pm 0.00 ^b
3% succinic anhydride	2.00 \pm 0.00 ^b
4% succinic anhydride	3.00 \pm 0.00 ^a

Values are mean \pm SD ($n = 3$). Different superscript letters within the same column indicate significant differences ($p < 0.05$).

Table 3: Texture profile analysis results for surimi gels containing succinylated gelatin

Type of Gelatin Added	Hardness (g)	Cohesiveness (ratio)	Springiness (mm)	Gumminess (kg)	Chewiness (kg.mm)
Control	1182.00±0.01 ^c	0.26±0.03 ^b	0.40±0.07 ^b	0.31±0.04 ^b	0.16±0.01 ^b
1% succinic anhydride	1473.33±0.01 ^b	0.27±0.03 ^b	0.46±0.06 ^b	0.45±0.01 ^a	0.16±0.10 ^b
2% succinic anhydride	1653.30±0.19 ^{ab}	0.27±0.02 ^b	0.50±0.05 ^b	0.46±1.00 ^a	0.22±0.02 ^b
3% succinic anhydride	1736.70±0.20 ^a	0.29±0.07 ^b	0.84±0.17 ^a	0.49±0.07 ^a	0.26±0.08 ^b
4% succinic anhydride	1779.00±0.09 ^a	0.43±0.06 ^a	0.97±0.02 ^a	0.51±0.07 ^a	0.48±0.07 ^a

Values are mean±SD ($n = 3$). Different superscript letters within the same column indicate significant differences ($p < 0.05$).

Table 4: Colour analysis results for sardine surimi gels containing succinylated gelatin

Type of Gelatin Added	L*	a*	b*	Whiteness
Control	62.32±0.19 ^a	0.30±0.09 ^a	14.72±0.28 ^a	59.54±0.27 ^a
1% succinic anhydride	63.43±0.10 ^b	0.17±0.02 ^b	14.02±0.24 ^b	60.83±0.06 ^c
2% succinic anhydride	63.60±0.19 ^b	-0.24±0.03 ^d	13.66±0.32 ^{bc}	61.11±0.08 ^b
3% succinic anhydride	64.12±0.50 ^c	-0.39±0.03 ^c	13.30±0.16 ^{cd}	61.74±0.10 ^d
4% succinic anhydride	64.45±0.04 ^d	-0.13±0.01 ^c	13.79±0.15 ^b	61.87±0.07 ^d

Values are presented as mean±SD ($n = 3$). Different superscript letters within the same column indicate significant differences ($p < 0.05$).

During frozen storage, myofibrillar protein undergoes denaturation, which leads to loss of protein functionality, especially gel forming ability [21]. Results of this analysis showed that only the highest level of succinylation improved the folding test score of low quality surimi and the improvement was minimal. However, the folding test is very subjective and is only a preliminary test to differentiate high and low grade surimi; it lacks the sensitivity to distinguish different properties of surimi samples such as gel strength [23].

TPA: Table 3 shows the TPA results for surimi gels containing succinylated gelatin. Values of all parameters increased with increasing level of succinylation and in all cases the values were significantly higher in the gels with the highest level of succinylation compared to unsuccinylated gels ($p < 0.05$). Significant differences in hardness 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 [24]. Cohesiveness is a measure of the work required to break down the internal bonds in surimi [25], thus the cohesiveness value affects the hardness value of a surimi gel. The sample with the highest cohesiveness will have the highest hardness [26]. The addition of succinylated gelatin improved the cohesiveness of surimi gels, with the greatest improvement recorded for gels with the greatest amount of succinylation.

Springiness is defined as the rate at which a deformed surimi gel recovers its initial conditions [25]. Addition of succinylated gelatin improved the springiness of the low

quality surimi by 15.0% to 142.5% relative to the control. Springiness is a typical characteristic of viscoelastic materials. With fish meat used as the raw material, the conditions used to prepare surimi gel and the formation of the net structure affect the springiness of the resulting gels [27].

Gumminess is the product of hardness and cohesiveness. Gumminess values of all surimi gels containing succinylated gelatin were significantly increased relative to those containing unsuccinylated gelatin ($p < 0.05$). Chewiness is a parameter that provides a global assessment of texture quality and a complementary parameter of hardness [26]. Chewiness is the energy required to chew surimi gel until it is ready to swallow [25]. Chewiness values of all treatments were in the range of 0.20 to 0.48 kg.mm and did not differ significantly ($p > 0.05$), except for the treatment with the highest level of succinylation, which had a significantly higher chewiness value. These chewiness values were low compared to those previously reported for sardine surimi [20].

Colour and Whiteness: Lightness and whiteness of surimi are related to consumer acceptability. Table 4 shows the colour and whiteness values of surimi gels containing succinylated gelatin. Addition of succinylated gelatin affected the colour characteristics and whiteness of the gels.

The lightness of all surimi gels containing succinylated gelatin was significantly ($p < 0.05$) higher than that of surimi gels containing unsuccinylated gelatin, whereas the redness and yellowness of all surimi gels containing succinylated gelatin were significantly ($p < 0.05$) lower than those of the control. Whiteness is an important attribute of surimi gels, as consumers prefer

whiter products [28, 29]. The whiteness of all surimi gels containing succinylated gelatin was significantly ($p < 0.05$) higher than that of surimi gels containing unsuccinylated gelatin and whiteness increased with increasing level of succinylation. Kaewudom *et al.* [29] previously reported that additives affect the whiteness of surimi gels depending on the type and amount of additive incorporated.

CONCLUSION

Incorporation of succinylated gelatin into stored surimi increased the functional properties of the surimi gels. Although the additive did not greatly affect the folding test score test of the surimi gels, addition of succinylated gelatin significantly increased ($p < 0.05$) the WHC and decreased the EM of sardine surimi. The TPA parameters also were improved by the addition of different levels of succinylated gelatin, as were the L^* and whiteness values. These colour results suggest that the succinylated gelatin acted on the myofibrillar protein in sardine surimi. In summary, the quality of low quality sardine surimi can be improved by the addition of succinylated gelatin.

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