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Thermal and physical properties of Pacific white shrimp (*Litopenaeus vannamei*) meat as affected by additives and freeze-thaw process

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Abstract

Thermal and physical properties were used to describe the stability of Pacific white shrimp quality during the freeze-thaw process by adding sucrose and sodium citrate. Thermal properties of shrimp myofibrillar protein, namely transition temperature (T_{max}) and enthalpy (ΔH) of protein were measured by differential scanning calorimetry (DSC). The results indicated that ΔH of both myosin and actin decreased when shrimp meat was subjected to three freeze-thaw cycles due to denaturation. Adding sucrose resulted in slightly higher T_{max} than the control after freeze-thawing and in retention of high ΔH of both myosin and actin, while adding citrate exhibited similar effects, but without a difference in T_{max} of actin. The increase in fine and uniform continuous structure as well as higher thawing yield (~90%), cooking yield (almost 90%) and less shear force were observed in additive treated samples after three freeze-thaw cycles. The colour of cooked shrimp with additives had lower L^*, a^* and b^* which was coincident with the amount of water uptake. This suggested that sucrose and citrate can be employed as protective additives to prevent the reduction of protein stability, especially for myosin during the freeze-thaw process.

Introduction

Freezing is a commercial method for preserving shrimp's quality (Boonsumrej *et al*., 2007; Sriket *et al*., 2007a). Proteins are the major constituents in shrimp muscle, of which myofibrillar protein is the most abundant fraction (Sriket *et al*., 2007b). However, quality changes by denaturation and aggregation of myofibrillar proteins as well as sublimation of ice crystals and recrystallisation might occur during frozen storage of shrimp products (McCormick, 1994; Boonsumrej *et al*., 2007). Consequently, the physical properties may become less desirable including texture, expressed colour and decreased yield of the frozen product (Kingwascharapong and Benjakul, 2016a). One of the methods for maintaining food quality during the freeze-thaw process is the addition of food additives such as sugars and salts. These compounds have been chosen as cryoprotectants because they have the capacity to prevent freeze denaturation of fish muscle protein. Sugar is widely used as a cryoprotectant in surimi (Herrera and Mackie, 2004; Hayashi *et al*., 2007). The addition of sucrose significantly prevented the reduction of thermal stability of myofibrillar protein in common carp surimi after multiple freeze-thaw (FT) cycles (Chen *et al*., 2013). Sugar containing hydroxyl groups can interact with water molecules, thus contributing to retardation of ice crystallisation (Uedaira *et al*., 1990). However, the application of sugar in shrimp has scarcely been reported.

It was found that sodium citrate suppressed the freeze denaturation of fish myofibrils at low concentration (Kuwahara and Konno, 2010). Also, our previous study showed a similar result in shrimp (Jommark *et al*., 2018). Sodium citrate contains carboxyl groups, which have a major effect on ionic strength, and can extract myosin from the myofibrillar protein structure (Feiner, 2006). This additive offers potential for protein stabilisation but has not yet been studied for its effects on shrimp protein.

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Thermoanalytical methods, such as differential scanning calorimetry (DSC), are convenient and reliable for studying the changes of protein from native to denatured state. DSC measures the enthalpy associated with transitions and chemical reactions and determines the temperature at which these processes occur. DSC can detect the heat denaturation of a protein in complex protein systems as an endothermic peak in its thermogram (Judge *et al*., 1989). Also, it can be applied to observe thermal changes and denaturation of muscle proteins in meat (Stabursvik and Martens, 1980; Xiong and Brekke, 1990). In the present work, the changes in endothermic peaks on DSC thermograms of shrimp muscle with and without selected additives during multiple freeze-thaw (FT) cycles were conducted. The resulting physical properties such as texture, colour and yield were also evaluated to support the results.

Materials and methods

Determination of thermal properties of Pacific white shrimp

Preparation of samples

The samples were prepared from Pacific white shrimp (*Litopenaeus vannamei*) with the size of 60 shrimp/kg by mixing the chopped shrimp meat without shell, surface pigment or intestine with 6% (w/w) sucrose or 5% (w/w) citrate, using a homogeniser (Ace AM-3 Nissei, Tokyo, Japan). Each sample (30 mg) was put into an aluminium pan, hermetically sealed, frozen at -20°C and thawed at 25°C. Samples were either frozen and then thawed daily for three cycles (FT3) or not frozen (FT0) prior to analysis by differential scanning calorimetry (DSC).

Determination of thermal transition temperature and enthalpy of Pacific white shrimp

The thermal transition temperature of Pacific white shrimp was measured by a differential scanning calorimeter or DSC (TA-50 Shimadzu, Kyoto, Japan) equipped with thermal analysis software. The temperature and heat flow were calibrated with α-alumina powder as a reference. N_2 at a flow rate of 20 mL/min was used as carrier gas. Then the DSC measurement was performed following the method of Hashimoto *et al*. (2004). The sample was cooled from ambient temperature to -10°C and heated to a maximum of 120°C at a rate of 5°C/min. The maximum transition/denaturation temperature (T_{max}) and enthalpy (ΔH) of the shrimp meat were determined by measuring from the mid-point of the endothermic peak of the resulting thermogram and area under the transition curve, respectively, using thermal analysis software version TA-60WS (Shimadzu Co., Ltd, Tokyo, Japan).

Determination of physical properties of Pacific white shrimp

Preparation of samples

Fresh Pacific white shrimp with the size of 60 shrimp/kg were purchased from a farm in Nakornprathom Province, Thailand and kept on ice bath at a ratio of shrimp to ice of 1:2 for 72 - 96 h in a refrigerator at 4 ± 1°C prior to study. Deveined and peeled shrimp with heads removed were weighed and soaked for 6 h at 4 ± 1°C in one of the following solutions, at a ratio of shrimp to solution of 1:2 (w/v): two trial (additive) solutions - 5% (w/v) sucrose or 4.5% (w/v) citrate; a commercial treatment - 2.0% (w/v) tetrasodium pyrophosphate; or a control - distilled water. A second control sample was not soaked in any additives or water. Eight shrimp were prepared for each treatment. The samples without and with additives were weighed after soaking and packed in Ziplock bags, subjected to freezing at -60°C (Sanyo Ultra Freezer MDF-1155, Tokyo, Japan) for 2 d to assure complete freezing, then stored at -20°C until thawing. The frozen shrimp were thawed using running water (22 - 25°C) for 20 min until the core temperature reached 0 - 2°C. For microstructure observation, the thawing of samples was done daily for 0 or 3 cycles. For other properties, including thawing yield, cooking yield, shear force and colour, the thawing was performed every 15 d for 0, 1 and 3 cycles prior to the analyses. The control samples are herein designated as FT0C and FT3C for 0 and 3 FT cycles, respectively. The samples with citrate were FT0CIT, FT1CIT and FT3CIT; samples with sucrose were FT0SUC, FT1SUC and FT3SUC; samples with water were FT0CW, FT1CW and FT3CW; and the samples with tetrasodium pyrophosphate were FT0TSPP, FT1TSPP and FT3TSPP for 0, 1 and 3 FT cycles, respectively.

Determination of muscle structure

The structure of raw and thawed shrimp (after 3 FT cycles) was determined by microscopy. Samples were prepared from the third abdominal segment into a cylindrical shape of frozen section compounds. The embedded sample was sliced with a freezing microtome at -20°C (CM1850 Leica, Nussloch GmbH, Germany) into 5 μm thick sections and stained with 1% Eosin Y (Hagiwara *et al*., 2003). The longitudinal section was visualised at 400× magnification under a microscope (BX51 Olympus,

Tokyo, Japan) connected to a digital camera (DP27 Olympus, Tokyo, Japan). The photomicrographs were scanned using DP2-BSW software version 2.1 (Olympus Co., Ltd., Tokyo, Japan).

Determination of thawing yield

The weight of freeze-thawed shrimp after treatment with additives was determined and compared to the weight of fresh, unfrozen shrimp, which was considered as 100% (AOAC, 2012). Relative percentage of thawing weight was calculated as:

$$\text{Relative percentage of thawing weight (\%)} = (A/B) \times 100 \quad \text{(Eq. 1)}$$

where A = weight after thawing, and B = initial weight (without treatment or freezing/thawing).

Determination of cooking yield

The cooking yield was measured by weighing the shrimp before and after cooking based on the method of Chantarasuwan *et al*. (2011) with slight modification. The shrimp was cooked in boiling water for 2 min, immediately cooled in iced water for 1 min, and drained for 5 min at 4°C. The cooking yield was calculated as:

$$\text{Cooking yield (\%)} = (A/B) \times 100 \quad \text{(Eq. 2)}$$

where A = weight of drained shrimp after cooking and cooling, and B = initial weight (without soaking and cooking).

Determination of shear force

The shrimp meat samples were evaluated for shear force by cutting the second segment crosswise with a Warner-Bratzler blade at a speed of 5 mm/s using a texture analyser (TA-HD Stable Micro System, Surrey, UK) following the method described by Brauer *et al*. (2003). The peak was recorded as the maximum shear force required for cutting the sample. Five shrimp were used in each treatment.

Determination of colours

The colour of the third abdominal segment of raw and cooked shrimp was measured in the *L**, *a** and *b** mode of CIE (Young and Whittle, 1985), using a Chroma meter (CM-3500d Minolta, Tokyo, Japan). The *L**, *a** and *b** indicate lightness, redness/greenness and yellowness/blueness, respectively. Five shrimp were used in each treatment.

Statistical analysis

All tests with three replicates were expressed as the mean ± standard deviation. Statistical analyses were performed using SPSS for Windows (version 11.5, SPSS Inc., Chicago, IL, USA). The differences between means were evaluated by univariate analysis of variance (ANOVA) and compared by Duncan's new multiple range test (DMRT) at 5% significance level ($p < 0.05$).

Results and discussion

Thermal properties of Pacific white shrimp treated with sucrose and citrate

Protein denaturation involves structural or conformational changes from the native structure. The native-to-denatured change in the protein state is a cooperative phenomenon that is accompanied by significant heat uptake, seen as an endothermic peak in the DSC thermogram. The denaturation or maximal transition temperature (T_{max}) as well as transition enthalpy (ΔH) of shrimp proteins were observed from endothermic peaks of myosin and actin as shown in Table 1.

Table 1 Transition temperature (T_{max}) and enthalpy (ΔH) of shrimp meat without additive (C), with 5% citrate (CIT) and 6% sucrose (SUC) after 0 and 3 freeze-thaw (FT) cycles.

FT cycle	Additive	Peak I (myosin)		Peak II (sarcoplasmic)		Peak III (actin)	
		T_{max} (°C)	ΔH (mW/mg)	T_{max} (°C)	ΔH (mW/mg)	T_{max} (°C)	ΔH (mW/mg)
0	C	48.64 ± 0.40^{Ans}	$0.49 \pm 0.08^{b*}$	66.38 ± 0.26^{Ans}	$0.51 \pm 0.10^{b*}$	79.77 ± 0.59^{Ans}	$0.51 \pm 0.10^{b*}$
	CIT	49.41 ± 0.65^{Ans}	$0.91 \pm 0.06^{a*}$	67.19 ± 1.77^{Ans}	$0.92 \pm 0.06^{a*}$	77.91 ± 5.36^{Ans}	$0.93 \pm 0.04^{a*}$
	SUC	49.56 ± 0.43^{Ans}	0.94 ± 0.05^{ans}	67.12 ± 0.34^{Ans}	0.94 ± 0.05^{ans}	80.35 ± 0.39^{Ans}	0.98 ± 0.00^{ans}
3	C	48.16 ± 0.01^{B}	0.19 ± 0.10^{b}	66.12 ± 0.34^{A}	0.20 ± 0.10^{b}	79.41 ± 0.43^{B}	0.16 ± 0.15^{b}
	CIT	49.85 ± 0.86^{A}	0.79 ± 0.45^{a}	66.33 ± 1.94^{A}	0.79 ± 0.04^{a}	79.09 ± 0.55^{B}	0.81 ± 0.03^{a}
	SUC	49.27 ± 0.09^{A}	0.81 ± 0.09^{a}	66.60 ± 0.73^{A}	0.82 ± 0.09^{a}	80.54 ± 0.43^{A}	0.82 ± 0.09^{a}

Data are mean ± standard deviation of triplicate determinations ($n = 3$). Different uppercase or lowercase superscript letters of T_{max} or ΔH in the same FT cycle indicate significant ($p < 0.05$) differences between additives. Significant ($p < 0.05$) differences between FT0 and FT3 cycles are indicated by an asterisk (*), while "ns" is not significant.

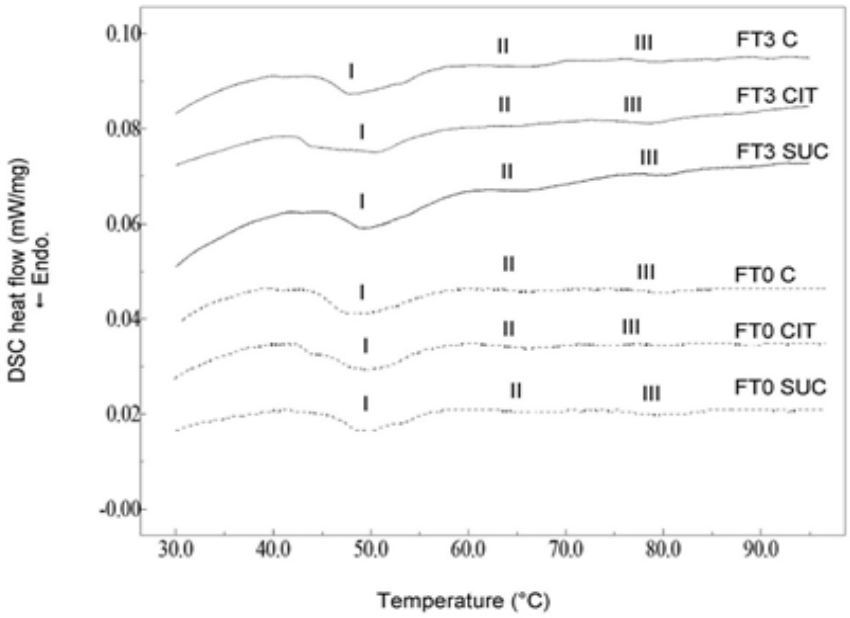

Figure 1. DSC thermograms of shrimp meat without additive (C), with 5% citrate (CIT) and with 6% sucrose (SUC), before (FT0) and after (FT3) three freeze-thaw cycles, indicating the endothermic peaks of myosin (I), sarcoplasmic proteins (II) and actin (III).

Three major transition peaks corresponding to myosin, sarcoplasmic proteins and actin are shown in Figure 1. Two important transition peaks are usually apparent in the myofibrillar protein fraction, namely myosin and actin (Sriket *et al*., 2007b; Ramirez-Guerra *et al*., 2012). For the unfrozen control (FT0C), the lower transition temperature with a peak temperature of about 48.64°C represented the denaturation of myosin, while the higher peak temperature of approximately 79.77°C was associated with denaturation of actin. These values agreed with results of Sriket *et al*. (2007b) and Ramirez-Guerra *et al*. (2012), who reported that T_{max} values of the myosin and actin peaks of Pacific white shrimp to be 49.00 - 50.13 and 67.30 - 71.17°C, respectively. The transition peak between myosin and actin may possibly be associated with sarcoplasmic protein and collagen, but only sarcoplasmic protein was able to be quantified, at around 66°C. The transition temperature for Pacific white shrimp sarcoplasmic protein found in the present work was rather high as compared to 51.9°C found for black tiger shrimp (*Penaeus monodon*) by Jantakoson *et al*. (2012), and 54.9°C found for freshwater prawn (*Macrobrachium rosenbergii*) by Srinivasan *et al*. (1997).

Of the unfrozen samples (FT0), the samples with additives (FT0CIT and FT0SUC) showed results for T_{max} similar to the control (FT0C), but approximately double the enthalpy of denaturation (ΔH) for both myosin (0.91 and 0.94 vs. 0.49 mW/mg) and actin (0.93 and 0.98 vs. 0.51 mW/mg) (Table 1). This indicates that adding citrate or sucrose could possibly provide protein stabilisation of the unfrozen protein.

When the shrimp muscle was subjected to the FT process for three cycles in order to induce protein denaturation, the T_{max} of myosin in FT3C (48.16°C) was unchanged ($p \geq 0.05$) when compared to FT0C (48.64°C), but ΔH was noticeably lower (0.19 vs. 0.49 mW/mg), which indicates the greater susceptibility of frozen myosin to denaturation. When water is lost from muscle as induced by FT, the muscle fibrils become more concentrated, resulting in higher aggregation (Kingwascharapong and Benjakul, 2016a), cell disruption, and structural damage of muscle (Sriket *et al*., 2007b). Increased numbers of FT cycles were found to be accompanied by reduced T_{max} as well as enthalpy of denaturation and enhanced susceptibility to thermal aggregation (Chen *et al*., 2013). For FT samples, FT3SUC was slightly higher ($p < 0.05$) than the control in both the T_{max} of myosin (49.27°C) and actin (80.54°C), while in FT3CIT, only the T_{max} of myosin denaturation (49.85°C) was higher ($p < 0.05$). A previous report on using sugar in osmotically dehydrated tilapia fillets indicated an increase in denaturation temperature for both myosin and actin (Medina-Vivanco *et al*., 2007). Adding sucrose maintained the enthalpy of denaturation of both myosin and actin (ΔH in Table 1) for FT3SUC close to the level for FT0SUC ($p \geq 0.05$), while for the citrate treatments, slightly lower enthalpy of both myosin and actin was noted ($p < 0.05$) for FT3CIT as compared to FT0CIT. Nevertheless, the enthalpy

values of FT3SUC and FT3CIT were approximately four times higher than those of FT3C (Table 1). These results indicated the effectiveness of both additives in protecting myofibrillar proteins against freeze denaturation. For the effects on sarcoplasmic protein and collagen, similar results to those of myosin and actin were observed. This also indicated the preventive effect of both additives against deterioration of carotenoproteins, the main pigment in shrimp (Niamnuy *et al*., 2008), during the FT process.

The results regarding denaturation temperature and energy indicated that sucrose and citrate acted to stabilise the native structure of myosin when the FT process was applied. A previous report stated that compounds used as cryoprotectants required some structural properties with respect to the capacity to prevent freeze denaturation of fish muscle protein (Jaczynski *et al*., 2006). The cryoprotectant molecules may associate or bind with protein molecules at one of the functional groups either by hydrogen or ionic bonds, meaning that each protein molecule is coated by cryoprotectant (Somjit *et al*., 2005). The following requirements for exhibiting cryoprotective effect have been proposed: a molecule has to possess one essential group of the various functional groups, such as -COOH, -OH or $-OP_2H_2$ and more than one supplementary group of the type -COOH, -OH, NH_2, SH, $-SO_3H$ or $-OP_2H_2$; the functional groups must be suitably spaced and properly oriented about each other; and the molecule must be comparatively small (Jaczynski *et al*., 2006). Sucrose is one of the cryoprotectants commercially used in the surimi industry (MacDonald and Lanier, 1991), and it can preserve the structural stability of myosin to some extent and slows down the exposure of buried hydrophobic residues on the protein surface and consequently retards the kinetics of protein aggregation (Benjakul *et al*., 2003; Herrera and Mackie, 2004). As sugar contains hydroxyl groups, it can interact with water molecules, thus contributing to retardation of ice crystallisation (Uedaira *et al*., 1990) as well as leading to decreased aggregation and denaturation (Ooshiro *et al*., 1976; Somjit *et al*., 2005). Thus, the denaturation temperature of the shrimp protein in the sucrose treated samples tended to shift slightly higher, and with increased denaturation enthalpy as compared to the control, as can be seen from Table 1. Sodium citrate contains three carboxyl groups in the structure, which has a major effect on ionic strength. It can extract myosin from myofibrillar structures in meat and contributes only to the swelling of the muscle fibre structure but not to protein solubilisation (Feiner, 2006). Ooizumi *et al*. (1984) also confirmed the great protective effect of sodium citrate against denaturation of Chub mackerel myofibrils, and that it was dependent on the proportion of carboxyl groups in its structure.

Therefore, in the present work, the use of sucrose and citrate in shrimp muscle both showed a noticeable effect of protein stabilisation, especially for myosin, during frozen storage or the freeze-thaw process.

Physical properties of Pacific white shrimp as affected by treatment of sucrose and citrate

Muscle structure

The effects of sucrose and citrate on the microstructure of FT0 and FT3 samples was observed from light micrographs as shown in Figure 2. The muscle structure of FT0C (Figure 2a) exhibited well-organised and compact, uniform structure. The structure of FT0CIT and FT0SUC samples after being soaked with 5% citrate and 6% sucrose solutions contained slightly larger spaces between fibres, possibly in which water was held (Figures 2b and 2c). The hydroxyl groups of sugars can interact with water molecules, thus contributing to retardation of ice crystallisation (Uedaira *et al*., 1990). FT0CIT (Figure 2b) seemed to have larger spaces than that of FT0SUC (Figure 2c). The three carboxyl groups in sodium citrate structure contribute to ionic strength and swelling of the muscle fibre (Feiner, 2006). After freeze-thawing for three cycles, the FT3C sample (Figure 2d) became denser with many larger voids as gapping formed, indicating the damaged structure. This result is similar to an earlier study on microstructure of longitudinal sections of white shrimp subjected to five FT cycles, in which shrinkage of fibres was noticed as evidenced by the gapping formed (Sriket *et al*., 2007a). The increase in the intra-myofibrillar protein spaces has been associated with the effect of the freezing process and this change became more evident with increased number of freeze-thaw cycles (Sigurgisladottir *et al*., 2000; Sriket *et al*., 2007a; Ramirez-Guerra *et al*., 2012), resulting in shrinkage and drip loss (Hale and Waters, 1981). The microstructure of FT3CIT and FT3SUC became increasingly fine and as a continuous pattern (Figures 2e and 2f). The asddition of sucrose enhanced the uniform and regular structure (Figure 2f) similar to the microstructure of fresh raw shrimp (Figure 2a). The structure of FT3CIT (Figure 2e) displayed similarity to the structure of FT0CIT before freezing (Figure 2b). These microstructure results support the earlier suggestion that sucrose and citrate can enhance the stability of muscle protein.

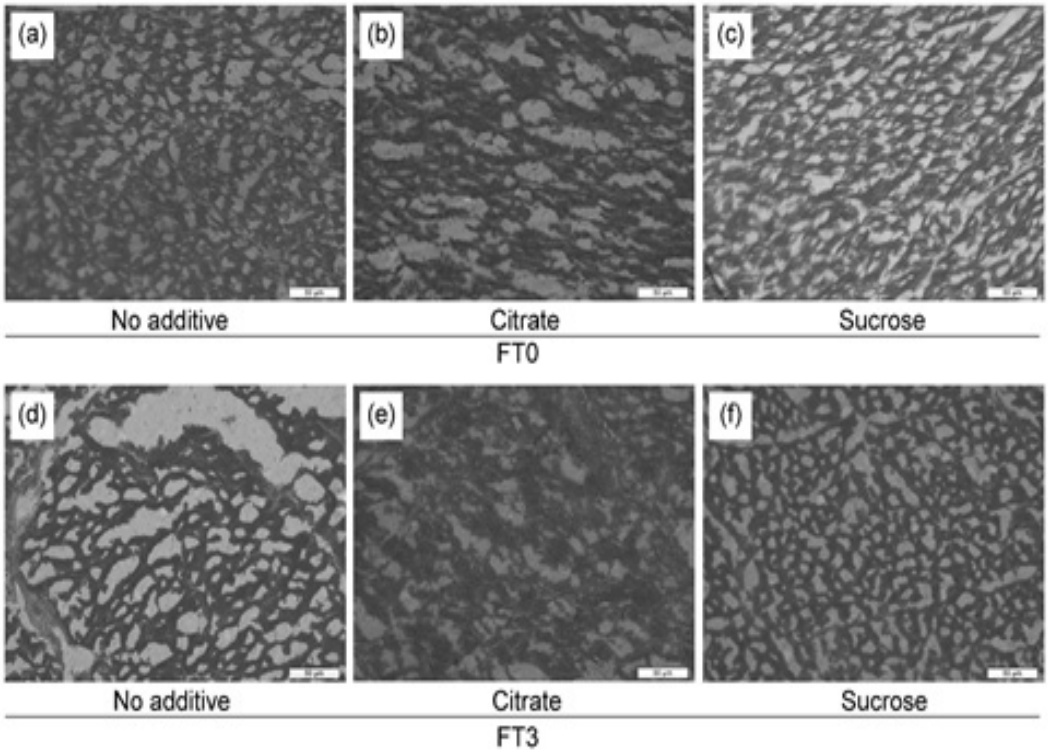

Figure 2. Light micrographs of longitudinal sections of the third abdominal segment of raw (a-c) and freeze-thawed (d-f) shrimp muscle without treatment (no additive) and treatment with solutions of 5% citrate and 6% sucrose, respectively before (FT0) and after (FT3) three freeze-thaw cycles (magnification 400×).

Thawing weight and cooking yields

Relative percentage of thawing weight and cooking yields of treated and untreated shrimp are shown in Figures 3a and 3b, respectively. In this section, the treatment with commercially used tetrasodium pyrophosphate (TSPP) was also conducted in order to compare efficacy. The unfrozen (FT0) shrimp treated with additives had higher relative percentage of thawing weight than the controls ($p < 0.05$), especially FT0TSPP which demonstrated the highest relative weight of 107%, as expected. For cooking yield of additive-treated FT0 samples citrate retained a yield as high as those samples with TSPP, while the sucrose treatment was the lowest ($p < 0.05$).

With increasing FT cycles, the relative percentage of thawing weight of all samples gradually decreased ($p < 0.05$) due to damaged cell tissue and protein denaturation. However, a significant decrease in cooking yield ($p < 0.05$) was only observed after three cycles. After the first FT cycle, the relative percentage of thawing weight of FT1TSPP and FT1CIT samples were similar and were the highest, while the FT1SUC sample was the lowest among additive-treated samples. When samples were subjected to three FT cycles, where severe damage and denaturation was expected, the citrate-treated sample retained both the highest thawing weight and cooking yield of approximately 90%, which were higher than the 80 - 85% yields observed when using TSPP. Both thawing weight and cooking yields of FT1SUC samples decreased after the first FT cycle to the level similar to the control (FT1C) sample. Increasing the number of FT cycles strongly affects the yield of shrimp, which is a main concern for producers. Thus, improving the initial yield before frozen storage by soaking in appropriate additives may be one of the methods to retain the finished product weight and subsequently the quality.

In the present work, the cryoprotectants' effect on cooking yield was markedly dependent on the type of cryoprotectant. The factors influencing yield might be related to the molecular weight and functional group, such as the hydroxyl group (-OH) in sucrose and carboxyl (-COOH) in citrate (Noguchi, 1974), as well as type of compound, such as ionic or non-ionic (Hayashi *et al*., 2007). Citrate is an ionic compound which could provide a negative charge on protein, leading to protein-to-protein molecule repulsion and resulting in the swollen muscle structure (Zayas, 1997). Therefore, water could be more readily taken up in shrimp muscle (Chantarasuwan *et al*., 2011). Even though sucrose exhibits a stabilising effect on protein as evidenced by its thermal properties and

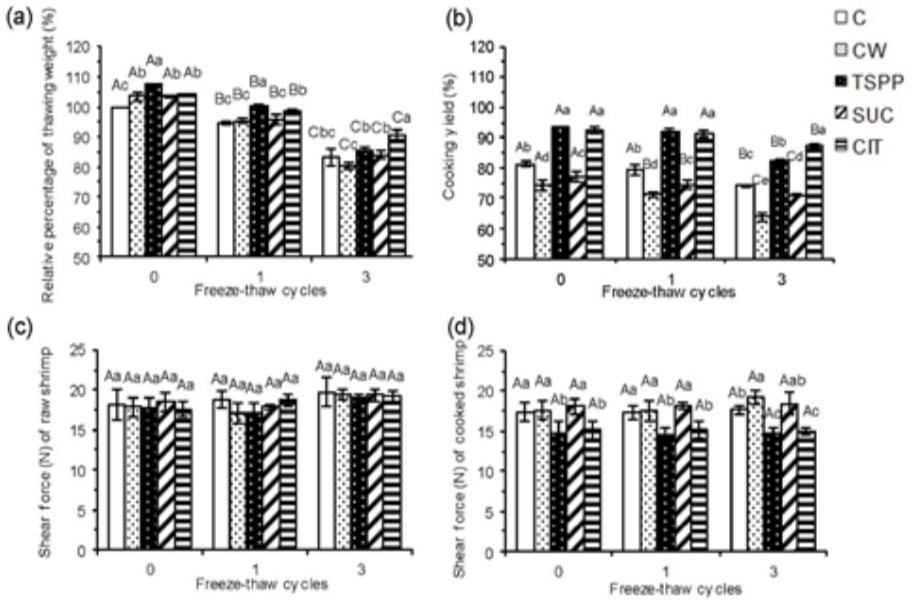

Figure 3. Relative percentage of thawing weight (a) and cooking yield (b) of shrimp and shear force of raw (c) and cooked (d) shrimp without treatment (C) and treatment with water (CW), 2% tetrasodium pyrophosphate (TSPP), 5% sucrose (SUC) and 4.5% citrate (CIT) after 0, 1 and 3 freeze-thaw cycles. Data are mean ± standard deviation of triplicate determinations (n = 3). Different uppercase superscript letters indicate significant ($p < 0.05$) differences between FT cycles for the same additive, and different lowercase superscript letters indicate significant ($p < 0.05$) differences between additives at the same FT cycle.

microstructure, the weak covalent bonds between sucrose and protein may be disrupted and unable to hold water within the protein structure, especially when it undergoes denaturation (Kingwascharapong and Benjakul, 2016b).

Shear force

The shear force of raw shrimp was approximately 17.5 - 20 N and was not significantly different ($p \geq 0.05$) among treatments, even of different FT cycles, as shown in Figure 3c. Although increasing FT cycles slightly increased shear force from 17.5 - 18.5 N for all unfrozen treatments, to 19 - 20 N after three FT cycles, the means were not significantly different ($p \geq 0.05$). The effect may not be strong enough to affect the shear force of raw shrimp, but this is inconsistent with previous reports which described more compact muscle fibres, and noted that water loss might have occurred due to the thawing loss (Kingwascharapong and Benjakul, 2016a). However, the effect could be noticeably seen in the cooked shrimp, as additive-treated samples with more held water had looser texture or less shear force than those without additives (Figure 3d). Similarly, lower shear force was found in samples treated with TSPP and CIT at all FT cycles, which was consistent with the cooked yield (Figure 3b). Prolonging FT cycles did not significantly affect the shear force of cooked CIT and TSPP samples, indicating that the water-holding capacity of samples had not been affected.

Colours

The colours of raw and cooked shrimp after the freeze-thaw process for 0, 1 and 3 cycles are shown in Table 2. For raw shrimp, the lightness values (L^*) of FT0TSPP and FT0SUC samples were lower than the treatment with water (FT0CW) but not lower than FT0C or FT0CIT ($p < 0.05$). It was revealed that shrimp treated with additives had more water retained in muscle (Figure 3), thus light could pass through and increase transparency, resulting in lower L^*. The L^* of samples was not totally consistent with the relative percentage of thawing weight. Higher L^* values might also be caused by an increase in water released to the surface during the denaturation of muscle proteins (Sriket *et al*., 2007a). It was also found that the FT cycle did not affect the L^* value of raw shrimp in any of the treatments ($p \geq 0.05$). There was no significant difference in a^* values (redness/greenness) among raw samples for 0, 1 or 3 FT cycles ($p \geq 0.05$). Similarly, non-significant differences ($p \geq 0.05$) were observed among treatments for b^* value (yellowness/blueness), except that the freeze-thawed TSPP and SUC samples had significantly lower b^* values ($p < 0.05$).

For the cooked shrimp, shrinkage of the myofibrils increased light scattering, thus giving higher L^* values (Jeong *et al*., 2011). In other words, increases in L^* value indicate higher aggregated protein which has more opaqueness (Kingwascharapong and Benjakul, 2016a). The L^* value of the samples among additives

at the FT0 and FT1 cycles were not significantly different ($p \geq 0.05$). After freeze-thawing for three cycles, FT3TSPP and FT3CIT samples showed lower L^* values than the other treatments, and FT3TSPP showed lower L^* values than 0 or 1 FT cycles ($p < 0.05$) and were coincident with their higher cooking yields (Figure 3b). This might indicate lower aggregated muscle protein after cooking.

Accordingly, the results for both additives (TSPP and CIT) tended to support previous findings on their stabilising effects on protein. The a^* and b^* values also indicated that TSPP and CIT treated samples had less redness and yellowness as compared to other treatments ($p < 0.05$), which was coincident with the water uptake. It may be that carotenoprotein in shrimp leached out during soaking in the TSPP and CIT solutions, thus the pigments were less concentrated, as indicated by the lower a^* and b^* values of shrimp when subjected to cooking. The control and water treated samples had slightly lower cooking yield and higher protein aggregation with negligible loss of carotenoprotein, and thus were more likely to retain higher content of pigments. Dense structure caused by the intense aggregation of muscle proteins and less water retained also resulted in opaque (high L^*) and slightly higher redness (Table 2). Although

Table 2. Colour of raw and cooked shrimp without treatment (C) and treatment with water (CW), 2% tetrasodium pyrophosphate (TSPP), 5% sucrose (SUC) and 4.5% citrate (CIT) after 0, 1 and 3 freeze-thaw (FT) cycles.

Sample	FT cycle	Additive	Colour		
			L^*	a^*	b^*
Raw	0	C	35.71 ± 0.83^{Ac}	-0.79 ± 0.66^{Aa}	0.44 ± 0.76^{Aa}
		CW	41.99 ± 1.59^{Aa}	-1.19 ± 0.49^{Aa}	0.58 ± 1.08^{Aa}
		TSPP	38.82 ± 0.72^{Ab}	-1.06 ± 0.46^{Aa}	-0.01 ± 0.78^{Aa}
		SUC	39.37 ± 0.53^{Ab}	-1.04 ± 0.60^{Aa}	1.36 ± 0.61^{Aa}
		CIT	35.55 ± 1.40^{Ac}	-1.52 ± 0.48^{Aa}	-0.93 ± 0.89^{Aa}
	1	C	36.18 ± 0.61^{Aa}	0.05 ± 0.34^{Aa}	-0.91 ± 2.12^{Aa}
		CW	39.50 ± 1.43^{Aa}	-0.34 ± 0.66^{Aa}	0.10 ± 0.95^{Aa}
		TSPP	39.11 ± 2.09^{Aa}	-0.29 ± 0.34^{Aa}	-1.98 ± 0.19^{Ba}
		SUC	38.07 ± 2.99^{Aa}	-0.29 ± 0.78^{Aa}	-2.29 ± 0.63^{Ba}
		CIT	36.52 ± 1.11^{Aa}	-0.36 ± 0.08^{Aa}	-2.14 ± 0.51^{Aa}
	3	C	35.40 ± 2.21^{Aa}	-0.47 ± 0.52^{Aa}	-1.03 ± 0.37^{Aa}
		CW	39.06 ± 3.02^{Aa}	-0.67 ± 0.32^{Aa}	-1.68 ± 0.86^{Aa}
		TSPP	36.67 ± 0.96^{Aa}	-0.33 ± 0.03^{Aa}	-1.99 ± 0.67^{Ba}
		SUC	38.41 ± 2.93^{Aa}	-0.22 ± 0.75^{Aa}	-1.39 ± 0.40^{Ba}
		CIT	35.02 ± 2.35^{Aa}	-0.31 ± 1.00^{Aa}	-2.03 ± 0.30^{Aa}
Cooked	0	C	57.66 ± 2.24^{Aa}	11.52 ± 1.60^{Aa}	13.18 ± 2.73^{Aa}
		CW	58.95 ± 1.50^{Aa}	10.55 ± 0.56^{Aa}	11.16 ± 1.53^{Aa}
		TSPP	56.03 ± 1.29^{Aa}	7.16 ± 1.93^{Ab}	9.40 ± 2.98^{Aa}
		SUC	55.97 ± 2.13^{Aa}	10.75 ± 1.12^{Aa}	11.99 ± 2.37^{Aa}
		CIT	55.44 ± 1.05^{Aa}	7.27 ± 1.70^{Ab}	8.94 ± 1.73^{Aa}
	1	C	56.58 ± 2.27^{Aa}	10.82 ± 1.41^{Aa}	12.28 ± 0.37^{Aab}
		CW	57.46 ± 2.88^{Aa}	11.09 ± 2.85^{Aa}	13.11 ± 2.96^{Aa}
		TSPP	54.70 ± 2.19^{ABa}	7.47 ± 0.83^{Ab}	9.21 ± 2.51^{Abc}
		SUC	56.08 ± 2.36^{Aa}	9.44 ± 0.59^{Aab}	12.26 ± 1.33^{Aab}
		CIT	54.82 ± 1.03^{Aa}	6.66 ± 0.26^{Ab}	7.09 ± 1.19^{Ac}
	3	C	56.58 ± 0.82^{Ab}	10.91 ± 1.14^{Aab}	12.88 ± 2.34^{Aab}
		CW	55.73 ± 0.89^{Ab}	12.62 ± 1.61^{Aa}	14.75 ± 2.27^{Aa}
		TSPP	51.95 ± 0.75^{Bc}	8.68 ± 0.12^{Abc}	8.47 ± 0.33^{Acd}
		SUC	59.82 ± 2.62^{Aa}	8.64 ± 2.20^{Abc}	10.99 ± 2.14^{Abc}
		CIT	53.79 ± 1.71^{Abc}	6.57 ± 1.00^{Ac}	6.00 ± 1.56^{Ad}

Data are mean ± standard deviation of triplicate determinations ($n = 3$). Different uppercase superscript letters indicate significant ($p < 0.05$) differences between FT cycles for the same additive, while different lowercase superscript letters indicate significant ($p < 0.05$) differences between additives at the same FT cycle.

colour changes can occur during frozen storage and thawing due to lipid oxidation and pigment degradation (Dias *et al*., 1994), changes in *a** and *b** of cooked shrimp as affected by the FT process were not detected in the present work. In summary, the effect of additives on colour was more pronounced in shrimp after cooking. The *L** might indicate lower aggregated protein, while *a** and *b** indicated greater distribution of water in the muscle of TSPP- and CIT-treated samples.

Conclusion

Adding sucrose and citrate to shrimp meat subjected to three cycles of freeze-thaw process resulted in slight increases in transition temperature and high retention of energy of transition of shrimp myofibrillar proteins. The microstructure, yield, shear force and colour measurements support the indication that sucrose and citrate could enhance the stabilisation of myofibrillar proteins during frozen storage. The findings indicated that sucrose and citrate act as cryoprotectants in shrimp, and might lead to their application as pre-treatment by soaking prior to processing in commercial production.

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