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Partial replacement of sodium pyrophosphate by xylitol and mannitol in white-leg shrimp (*Litopenaeus vannamei*) on its quality during frozen storage preservation

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Abstract

Polyphosphate is widely used as a food additive for soaking seafood products to improve their water holding capacity and sensory properties. Xylitol and mannitol are well-known as versatile cryoprotectants which are beneficial for frozen storage. This research evaluated the possibility of a combination of xylitol and mannitol with pyrophosphate to reduce the thawing loss, and cooking loss as well as to retain the myofibrillar protein content during 12 months of frozen preservation. Results showed that the combination of sodium pyrophosphate, xylitol and mannitol (1.5%:0.5%:0.5%) created a synergistic effect with the lowest thawing loss (0.10±0.03 to 1.64±0.03%) and cooking loss (2.13±0.04 to 8.79±0.04%), the highest myofibrillar protein content (99.72±0.13 mg/g) on frozen shrimp throughout 12 months of frozen preservation. Xylitol and mannitol would be promising alternatives to partially replace polyphosphate in frozen shrimp production.

1. Introduction

Frozen seafood products must be defrosted before thermal treatment or additional handling. Defrosting intends to recover the properties of raw items, unfortunately, it also negatively influences the quality characteristics of finished commodities. Polyphosphate substances are commonly implemented in seafood processing especially in the soaking step to enhance the functional attributes throughout frozen preservation as the polyphosphates could maximize moisture-keeping potential in raw materials, reduce drip loss at defrosting, and limit the cooking loss of frozen items (Vichasilp and Wangtueai, 2018). Polyphosphates also altered the organoleptic traits, like juiciness, owing to an acceleration of moisture-keeping capacities. Nevertheless, the over-abuse of polyphosphate residue results in a delicate structure, opaque figure, and spongy flavour in the oral evaluation (Long *et al.,* 2011). Excessive water entrapment by utilizing polyphosphates could result in customer boycott.

The negative effects of freezing fish products were texture degradation, drip loss and low water holding capacity (Etemadian *et al.,* 2012). Frozen storage for a prolonged duration induces a rubbery texture in meat and fish products (Tanushree *et al.,* 2018). At freezing temperatures, massive ice crystals could hurt muscle tissue, induce quality degradation, facilitate drip loss, freeze burn, loose texture, worsen colour, and causes protein condensate (Mulot *et al.,* 2019; Yang *et al.,* 2019; Zhang and Ertbjerg, 2019; Yang *et al.,* 2020). The minimal quality fluctuation in frozen products could be achieved by soaking appropriate cryoprotectants that could defend muscle texture against frozen demolition (Nikoo *et al.,* 2016). Hydrocolloids are used as cryoprotectants in different food applications such as gelling, thickening, stabilizing, and emulsifying. The incorporation of cryoprotectants can restrain protein dehydration so as to prevent protein freeze denaturation of myofibrillar proteins during frozen storage, hence preserving the gel-forming ability of fish cake (Anwar *et al.*, 2013). Cryoprotectant like pyrophosphate has a great capacity for water fulfilment and surrogate, the glass evolution, and the accumulative retardation of ice crystals in muscle texture (Zhang, Hao, Cao *et al.,* 2018). Different variations of cryoprotectants in chemical structure, dimension of the carbon chain, molecular mass, hydration intake, hydroxyl group, and the established hydrogen bond created the antifreeze activity to a great extent (Fahy and Wowk, 2013; Kan *et al.,* 2015). Cryoprotectants like xylitol, mannitol and sodium pyrophosphate were commonly supplemented owing to their cheap cost, safety and excellent cryoprotective

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effects (Wu *et al.,* 2020). Improvement of rheological properties and gel-forming capacity depends on the type and concentration of cryoprotectants supplemented (Benjakul *et al.,* 2003).

Xylitol is a sugar alcohol utilized as a sweetener. It originated from the hydrogenation of xylose (Granstrom *et al.,* 2007). Xylitol is also produced via enzymatic biocatalysts and microbial fermentation using bacteria, fungi, and yeast (Mayer *et al.,* 2002). Xylitol has an asymmetric carbon atom. Xylitol is used to improve the quality attributes of semi-dried jerky (Jang *et al.,* 2015). Similar to xylitol, mannitol is also important sugar alcohol widely used in the food industry. Mannitol is often derived from the high-pressure hydrogenation of fructose or the reduction of mannose. It is also obtained from enzymatic biosynthesis and fermentation by microorganisms like bacteria, fungi, and yeast. Mannitol is commonly applied in lyophilisation as a bulking agent, a sweetener in diabetes, a carrier and stabilizer in tablets, and a diuretic in neuropharmacology (Kumar *et al.,* 2017; Lang *et al.,* 2020). Mannitol is also an antioxidant, which is beneficial for preventing the growth of colon cancer (Gaspar *et al.,* 2004; Ghoreishi and Shahrestani, 2009; Song and Vieille, 2009). Crystalline mannitol revealed a very low hygroscopicity, making it feasible in commodities that were durable at high moisture content. Mannitol showed a protective effect on the functional attributes of microorganisms after freezing and freeze-drying (Dianawati *et al.,* 2013).

White leg shrimp (*Litopenaeus vannamei*) is one of the most important seafood products in the export of Vietnam. Vietnamese government sets US$10 million targets for shrimp exports by 2025. Vietnamese government recommends the diversified application of resources, high-quality seeds and a sensitive balance between intensive and extensive culture to sustainably attain its national production and export targets (Kim *et al.,* 2020). The processor is concerned about the drip loss, cooking loss and protein denaturation in frozen white leg shrimp during freezing and frozen storage. The purpose of this study was to focus on the effect of using xylitol and mannitol to partially replace pyrophosphate in the additive soaking step. Synergistic effect of the combination of pyrophosphate: xylitol: mannitol is expected to minimize the drip loss and cooking loss while maintaining the myofibrillar protein content in the frozen white leg shrimp during 12 months of frozen storage.

2. Materials and methods

2.1 Materials

White-leg shrimps were supplied by Vinh Chau district, Soc Trang province, Vietnam. Shrimps were cultured following the Global Good Agriculture Practice standard to ensure food hygiene, safety and traceability. After being collected, they were preserved by flake ice at 0-4^{o}C in the ice chest and transferred to the laboratory as quickly as possible. Food additives including sodium pyrophosphate, xylitol, and mannitol were all food-grade with purities over 99% supplied by Sigma-Aldrich (USA). Chemical reagents such as Tris(hydroxymethyl) aminomethane, potassium chloride, and peracetic acid were all analytical grades purchased from Rainbow Co. Ltd, Ho Chi Minh City, Vietnam.

2.2 Researching method

A total of 240 kg of white-leg shrimps at size 50-60 pcs/kg was selected for experiments. They were thoroughly cleaned in freshwater by air-bubble blowing with 50 ppm peracetic acid for sanitation. They were then deheaded peeled tailed-on, and deveined. They were separated into 8 groups and soaked for 45 mins, stirred 3 times in 8 different formulas of additives: (G1) water as control, (G2) 2.5% sodium pyrophosphate, (G3) 2.5% xylitol, (G4) 2.5% mannitol, (G5) 1.5% sodium pyrophosphate + 1.0% xylitol, (G6) 1.5% sodium pyrophosphate + 1.0% mannitol, (G7) 1.5% sodium pyrophosphate + 0.5% xylitol + 0.5% mannitol, (G8) 0.5% sodium pyrophosphate + 1.0% xylitol + 1.0% mannitol. After being soaked, each group was separated into 10 batches, each batch included 3 samples. All samples were frozen to -18^{o}C by IQF (individual quick frozen) method.

Experiment #1: 5 frozen batches including 120 samples were kept in frozen storage for 0, 3, 6, 9, and 12 months. In every 3-month interval, 24 samples were taken to verify the thawing loss.

Experiment #2: 5 frozen batches including 120 samples were kept in frozen storage for 0, 3, 6, 9, and 12 months. In every 3-month interval, 24 samples were taken to verify the cooking loss. The thawed samples which were executed in experiment #1 were also utilized for experiment #2.

Experiment #3: Another 5 frozen batches including 120 samples were kept frozen in storage for 0, 3, 6, 9, and 12 months. In every 3-month interval, 24 samples were taken to evaluate the myofibrillar protein content.

2.3 Physicochemical analysis

Thawing loss (%) was evaluated following the procedure described by Wangteui *et al.* (2021). Frozen shrimps were thawed in the fridge at 4^{o}C for 24±2 hrs. The abundant water on the sample surface was removed using absorbent paper.

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$$\text{Thawing loss (\%)} = [(W_1-W_2)*100\%/W_1]$$

Where W_1: weight of frozen shrimp (g), W_2: weight of thaw-drained shrimp (g)

Cooking loss (%) was estimated by weighing the thawed shrimp (at 4°C for 24±2 hrs) and the cooked shrimp (at 98±0.5°C for 4 min) (Wangteui *et al.*, 2020).

$$\text{Cooking loss (\%)} = [(W_2-W_3)*100\%/W_2]$$

Where W_2: weight of thaw-drained shrimp (g), W_3: weight of cooked shrimp (g).

Myofibrillar protein content (%) was determined following the procedure described by Zhang, Fang, Hao *et al.* (2018). Thawed shrimp was finely minced by a grinder. An amount of 5 g of minced shrimp was mixed with 10 mL of 20 mM Tris-maleate solution (pH 6.5) and 5 mL of 0.05 M KCl in the stomacher for 2 min. The mixture was centrifuged at 3,000 rpm for 4 mins by centrifugator (Sigma 3-30KHS, Sigma Laborzentrifugen GmbH, Germany), and the received protein deposit was re-dissolved in the aforementioned solutions, blended, and extracted once more. The obtaining deposit was supplemented with 10 mL of the same solutions, blended, and centrifuged at 3,000 rpm for 4 mins. The upper layer was considered as the collected myofibrillar protein (mg/g).

2.4 Statistical analysis

The experiments were run in triplicate with different groups of samples. The data were presented as mean ± standard deviation. Statistical analysis was performed by Statgraphics Centurion version XVI. The mean value ($\bar{x}$) and standard deviation (2*s*) of a set of data were obtained by analysis of random samples estimating the population statistics. 95% of results would be expected to lie within the range $\bar{x} \pm 2s$. The lower and upper bounds of this range were described at the 95% confidence limits of the results. The differences between the treated samples were analyzed using a one-way analysis of variance (ANOVA). A significant value is set at a 95% confidence interval ($p \leq 0.05$). If significant differences were found, then post hoc analysis was performed using Duncan's multiple range tests.

3. Results and discussion

3.1 Thawing loss of frozen shrimp

The effect of different cryoprotectants on thawing loss of frozen shrimp during 12 months of storage was presented in Table 1. There was an increasing trend of thawing loss by the time of frozen storage. The highest thawing loss (1.09±0.02 to 13.19±0.03%) was noticed in the control sample. With 2.5% sodium pyrophosphate in the soaking solution, the thawing loss of frozen shrimp was recorded (0.78±0.00 to 8.28±0.02%) during 12 months of storage. Meanwhile, the combination of sodium pyrophosphate, xylitol and mannitol (1.5%:0.5%:0.5%) created a synergistic effect with the lowest thawing loss (0.10±0.03 to 1.64±0.03%) on frozen shrimp.

The highest thawing loss noticed in the control sample was related to the accelerated extracellular gap, the contraction of the myofilament grid, and the negative modification of textural proteins, mostly initiated by the evolution of abnormal ice-crystals in texture tissue (Zuo *et al.,* 2016). Phosphates have a great ability to improve water-binding properties in seafood (Klinmalai *et al.,* 2021). Sodium pyrophosphate participated as polyanion to preserve the moisture retention of frozen seafood and donated to the protein durability over the negative modification through frozen preservation. The incorporation of pyrophosphate improved the electrostatic attraction of protein particles, wherein moisture might be captured strictly with proteins and/or phosphate through ionic strength (Ma *et al.,* 2015).

Table 1. Thawing loss (%) of frozen shrimp by various cryoprotectants during frozen storage

Soaking	Frozen storage (months)				
	0	3	6	9	12
Water as control	1.09 ± 0.02^{aE}	3.18 ± 0.03^{aD}	5.72 ± 0.01^{aC}	9.41 ± 0.04^{aB}	13.19 ± 0.03^{aA}
2.5% sodium pyrophosphate	0.78 ± 0.00^{abE}	2.03 ± 0.04^{bD}	3.26 ± 0.03^{bC}	5.67 ± 0.03^{bB}	8.28 ± 0.02^{bA}
2.5% xylitol	0.60 ± 0.03^{bE}	1.28 ± 0.02^{cD}	2.50 ± 0.00^{cC}	4.26 ± 0.04^{cB}	6.75 ± 0.03^{cA}
2.5% mannitol	0.43 ± 0.01^{bE}	1.04 ± 0.01^{cD}	2.28 ± 0.04^{cC}	4.01 ± 0.02^{cB}	6.20 ± 0.01^{cA}
1.5% sodium pyrophosphate + 1.0% xylitol	0.25 ± 0.01^{bcE}	0.63 ± 0.03^{cdD}	1.45 ± 0.02^{dC}	2.97 ± 0.04^{dB}	5.07 ± 0.02^{dA}
1.5% sodium pyrophosphate + 1.0% mannitol	0.19 ± 0.00^{bcE}	0.57 ± 0.02^{cdD}	1.36 ± 0.01^{dC}	2.43 ± 0.03^{dB}	4.72 ± 0.00^{dA}
1.5% sodium pyrophosphate + 0.5% xylitol + 0.5% mannitol	0.10 ± 0.03^{cE}	0.31 ± 0.04^{dD}	0.65 ± 0.03^{eC}	1.02 ± 0.01^{eB}	1.64 ± 0.03^{eA}
0.5% sodium pyrophosphate + 1.0% xylitol +	0.51 ± 0.02^{bE}	1.19 ± 0.01^{cD}	2.41 ± 0.04^{cC}	4.15 ± 0.02^{cB}	6.42 ± 0.01^{cA}

Values are presented as mean±SD of three replications. Values with different lowercase alphabet superscripts within the same row are significantly different (α = $p \leq 0.05$) by Duncan's multiple range test. Values with different uppercase alphabet superscripts within the same column are significantly different (α = $p \leq 0.05$) by Duncan's multiple range test.

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Moreover, phosphate could also reduce the deformation and contraction of texture filaments, influence the evolution of massive ice crystals in the internal and external cellular region, and additionally decrease the physical vulnerability resulting in textural tissues.

Xylitol and mannitol were demonstrated to be effectively minimized the thawing loss in shrimp muscle superior to the control sample and even the sample treated with sodium pyrophosphate (Bin *et al.,* 2020). The cryoprotection effect of xylitol and mannitol in the minimization of thawing loss could be due to their moisture-storing capacity. Xylitol showed high water retention in semi-dried jerky (Jang *et al.,* 2015).

3.2 Cooking loss of frozen shrimp

There was a significant difference ($P<0.05$) in cooking loss on frozen shrimp treated by different cryoprotectants at the time of storage. Under frozen storage time, the cooking loss showed an accelerating trend. The highest cooking loss (8.72±0.06 to 25.28±0.05%) was recorded on the control sample. High cooking loss of the frozen water-soaked sample was unbeneficial and could be reflective of quality degradation due to moisture deficiency (Klinmalai *et al.,* 2021). Soaking with 2.5% sodium pyrophosphate effectively minimized the cooking loss (6.32±0.07 to 19.03±0.03%) on frozen shrimp. Meanwhile, a synergistic effect was noticed by the incorporation of sodium pyrophosphate, xylitol and mannitol (1.5%:0.5%:0.5%) to provide the lowest cooking loss (2.13±0.04 to 8.79±0.04%) (Table 2).

A combination of three kinds of cryoprotectants could save the amount of each additive in soaking while improving moisture retention during thermal treatment (in cooking) as well as stability in frozen storage. Drip loss was a serious phenomenon in freezing, preserving and thawing resulting in the development of ice crystals and the denaturation of textural proteins (Ramirez-Guerra *et al.,* 2012). A great enhancement was observed in a cooking loss in xylitol- and mannitol-soaked seafood samples. Xylitol and mannitol could surrogate the hydrogen by creating massive hydrogen bridges with the structural proteins, hence maintaining seafood's texture without moisture in the cryoprotective status and retarding the annihilation of muscle texture (Bin *et al.,* 2020). Moreover, xylitol and mannitol also created boundaries against the movement and allocation of hydrogen surrounding the protein outer layer and influenced the evolution of ice crystals in muscle, hence depleting the cryo vulnerability (Kuwajima *et al.,* 2009).

3.3 Myofibrillar protein content of frozen shrimp

There was a downtrend in myofibrillar protein content on the frozen shrimp at the time of storage. At the end of 12-month storage, the lowest myofibrillar protein content was found on the control (60.14±0.13 mg/g). Meanwhile, the highest myofibrillar protein content (99.72±0.13 mg/g) was noticed on the frozen shrimp treated with sodium pyrophosphate, xylitol and mannitol (1.5%:0.5%:0.5%) (Table 3). The reduced myofibrillar content in the control sample implied that there was a remarkable negative modification of myofibrillar proteins through cryopreservation.

Food additives are often used with different concentrations in the production of seafood products. The concentration of myofibrillar proteins is one of the important factors for improving gel strength and elasticity of fish cake. A reduction in water-soluble protein increases the concentration of myofibrillar proteins, thus enhancing the functional properties of fish cake. The gelling process entails the association of long myofibrillar protein chains which produces a continuous three-dimensional network in which water and other components are trapped. As a result, a visco-elastic gel is obtained (Sánchez-González *et al.,* 2008). A

Table 2. Cooking loss (%) of frozen shrimp by various cryoprotectants during frozen storage

Soaking	Frozen storage (months)				
	0	3	6	9	12
Water as control	$8.72±0.06^{aE}$	$12.64±0.05^{aD}$	$15.87±0.03^{aC}$	$19.51±0.06^{aB}$	$25.28±0.05^{aA}$
2.5% sodium pyrophosphate	$6.32±0.07^{bE}$	$9.40±0.03^{bD}$	$11.28±0.04^{bC}$	$15.27±0.05^{bB}$	$19.03±0.03^{bA}$
2.5% xylitol	$4.85±0.05^{bcE}$	$6.85±0.06^{cD}$	$9.36±0.05^{cC}$	$11.64±0.03^{cB}$	$14.41±0.04^{cA}$
2.5% mannitol	$4.61±0.06^{bcE}$	$6.13±0.04^{cD}$	$8.97±0.03^{cC}$	$11.03±0.04^{cB}$	$13.98±0.06^{cA}$
1.5% sodium pyrophosphate + 1.0% xylitol	$3.97±0.02^{cE}$	$5.04±0.05^{cdD}$	$6.33±0.05^{dC}$	$8.75±0.06^{dB}$	$11.46±0.03^{dA}$
1.5% sodium pyrophosphate + 1.0% mannitol	$3.81±0.03^{cE}$	$4.93±0.04^{cdD}$	$6.04±0.02^{dC}$	$8.21±0.04^{dB}$	$11.04±0.02^{dA}$
1.5% sodium pyrophosphate + 0.5% xylitol + 0.5% mannitol	$2.13±0.04^{dE}$	$3.24±0.02^{dD}$	$4.37±0.04^{eC}$	$6.53±0.03^{eB}$	$8.79±0.04^{eA}$
0.5% sodium pyrophosphate + 1.0% xylitol +	$4.72±0.05^{bcE}$	$6.50±0.03^{cD}$	$9.12±0.05^{cC}$	$11.32±0.04^{cB}$	$14.15±0.03^{cA}$

Values are presented as mean±SD of three replications. Values with different lowercase alphabet superscripts within the same row are significantly different ($\alpha = p \leq 0.05$) by Duncan's multiple range test. Values with different uppercase alphabet superscripts within the same column are significantly different ($\alpha = p \leq 0.05$) by Duncan's multiple range test.

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Table 3. Myofibrillar protein content (mg/g) of frozen shrimp by various cryoprotectants during frozen storage

Soaking	Frozen storage (months)				
	0	3	6	9	12
Water as control	119.21±0.17^{aA}	96.05±0.11^{dB}	83.74±0.15^{dC}	72.59±0.16^{eD}	60.14±0.13^{eE}
2.5% sodium pyrophosphate	119.49±0.12^{aA}	101.14±0.18^{cB}	91.29±0.14^{cC}	79.64±0.10^{dD}	69.43±0.15^{dE}
2.5% xylitol	119.57±0.14^{aA}	105.09±0.17^{bcB}	98.07±0.12^{bcC}	87.40±0.17^{cD}	80.05±0.19^{cE}
2.5% mannitol	119.63±0.11^{aA}	105.36±0.16^{bcB}	98.71±0.15^{bcC}	88.06±0.13^{cD}	80.84±0.14^{cE}
1.5% sodium pyrophosphate + 1.0% xylitol	119.80±0.18^{aA}	109.17±0.13^{bB}	104.25±0.17^{bC}	97.18±0.14^{bD}	89.34±0.11^{bE}
1.5% sodium pyrophosphate + 1.0% mannitol	119.89±0.15^{aA}	109.84±0.10^{bB}	104.98±0.13^{bC}	98.02±0.11^{bD}	90.17±0.12^{bE}
1.5% sodium pyrophosphate + 0.5% xylitol + 0.5% mannitol	119.93±0.16^{aA}	116.54±0.19^{aB}	112.60±0.14^{aC}	106.29±0.15^{aD}	99.72±0.13^{aE}
0.5% sodium pyrophosphate + 1.0% xylitol +	119.61±0.13^{aA}	105.24±0.18^{bcB}	98.47±0.12^{bcC}	87.79±0.14^{cD}	80.51±0.17^{cD}

Values are presented as mean±SD of three replications. Values with different lowercase alphabet superscripts within the same row are significantly different ($\alpha = p \leq 0.05$) by Duncan's multiple range test. Values with different uppercase alphabet superscripts within the same column are significantly different ($\alpha = p \leq 0.05$) by Duncan's multiple range test.

combination of sodium pyrophosphate, xylitol and mannitol in soaking created a better effect on frozen shrimp to retain more myofibrillar protein content superior to mono application of separated additives. A remarkable enhancement was noticed in myofibrillar protein content in xylitol- and mannitol-soaked seafood muscles. These results were in accordance with the findings of Bin *et al.,* (2020). Xylitol and mannitol played a key role in additive-soaking to effectively minimize the vulnerability in seafood muscle texture induced by large ice crystals (Bin *et al.,* 2020). The hydrogen bridges are created between the xylitol/ mannitol and proteins, maintaining the facial electrostatic capacity and versatile protein matrix without frozen hydrogen, and extra defence for protein against cryodemolition (Tadanori *et al.,* 2002). Xylitol and mannitol with abundant hydroxyl groups interacted with myofibrillar proteins, resulting in the textural durability of myofibrillar proteins throughout cryopreservation (Kong *et al.,* 2013).

4. Conclusion

The increasing demand for frozen shrimp for export has promoted the implementation of cryoprotectants in frozen shrimp production. The combination of sodium pyrophosphate, xylitol and mannitol (1.5%:0.5%:0.5%) showed a synergistic effect to limit the thawing loss, and cooking loss and improve the myofibrillar protein content of frozen shrimp. By using low content of cryoprotectants in soaking, the overall acceptance of the treated products would be nearly the original ones. Findings in this research would be very important for seafood processors not only to save production costs but also to overcome the customer's complaints by using heavy content of food additives especially polyphosphates in soaking that might cause unnatural sensory behaviours.

Conflict of interest

The author strongly confirms that this research was conducted with no conflict of interest.

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