



Effect of Non-phosphate Additive on Quality of *Litopenaeus vannamei* (Boone, 1931)

Yuansheng Chen¹, Fujia Yang¹, Yajing Guo¹, Minglong Ni², Mei Wang¹, Fen Yan^{1*}, Caili Fu¹

¹ College of Biological Sciences and Engineering, Fuzhou University, Fuzhou, Fujian - 350 108, China

² Guangdong Food and Drug Vocational College, Guangzhou, Guangdong - 510 520, China

Abstract

Litopenaeus vannamei (Boone, 1931) was treated with non-phosphate additives viz., chitosan, collagen peptide and sodium chloride. Using single factor and orthogonal tests, the optimal proportions of chitosan, collagen peptide and sodium chloride were found to be 0.6, 2.0 and 0.5%, respectively. Besides, the effect of soaking time on the water retention capability indicated best results for 80 min. Under optimal conditions, the rate of increase in soaking weight, thawing loss, cooking loss, product yield, and the moisture content of the shrimp were observed as $9.80 \pm 1.14\%$, $6.09 \pm 2.87\%$, $32.96 \pm 2.31\%$, $73.65 \pm 3.56\%$ and $71.38 \pm 0.52\%$, respectively. The water retention effect and texture of the samples treated with non-phosphate additive, commercial composite phosphate, and non-treated (control) were determined. The results revealed that non-phosphate additive could effectively improve the water-holding capacity of aquatic products, reduce the hardness, elasticity, adhesiveness and chewiness of shrimp significantly, and maintain the natural color and flavor.

Keywords: *Litopenaeus vannamei*, non-phosphate additive, chitosan, collagen peptide

Introduction

During frozen storage, the water retention capacity of aquatic products get reduced due to the different degrees of physics, chemistry reactions and organizational deterioration (Zhang et al., 2014), reflecting in the loss of food fluids and flavor when thawing and heating (Remya et al., 2015). In addition, the

shrimp muscle tissues get damaged and fat oxidation occurs when exposed to air. Maillard reaction also occurs between the carboxides and free amino acid released from the tissues, astaxanthin gets degraded, and so on (Ginson et al., 2017). These reactions are associated with paleness of seafood, meat fibrosis, and loss of the original product elasticity and freshness (Kaewmanee et al., 2009; Murthy et al., 2016).

In this context, water retention agents play a major role to maintain the taste, texture and flavor of shrimp. At present, most of the water retention agents used for aquatic products in market are of phosphate origin (Wang et al., 2014). Although the water-holding capacity of the aquatic products is enhanced substantially after adding phosphate additive, the product becomes bitter, which affects the taste and character of the product. Moreover, in order to obtain more profit, the market vendors often add phosphates at exceeding levels to maintain the quality of products, which can cause calcium and phosphorus imbalance in human body (Wachirasiri et al., 2016; Glorieux et al., 2017).

Chitosan is a product obtained by the deacetylation of chitin mainly from the shell of crustaceans in the food industry (Chen et al., 2008). As a natural, non-toxic, highly effective food preservative with film-forming, moisturizing and other excellent properties, chitosan can often form a layer of thin film on the surface of aquatic products thus effectively preventing moisture evaporation from it (Liu et al., 2015; Wang et al., 2015; Renuka et al., 2016). Collagen peptide, a small molecular weight peptide from the hydrolyzed collagen derived from animal protein (Hema et al., 2017b) is edible and often used as a filler, gel and food coating in food industry, which can prevent effectively food browning, oxidation, dampness, stiffness etc. (Hema et al., 2017a). Moreover, on account of the hydrophilic

Received 17 March 2018; Revised 02 August 2018; Accepted 09 April 2019

*E-mail: yanfen@fzu.edu.cn

groups *viz.*, hydroxyl and carboxyl group they possess enhanced water retention property (Sato, 2017; Hema et al., 2017c). Sodium chloride is often used as a preservative to retain freshness and inhibit the growth of corruption with a dehydration and high permeability. Beyond that, sodium chloride can also improve the water retention of aquatic products significantly by interacting with muscle protein to form a powerful 3D mesh structure to maintain moisture (Zhou et al., 2014; Åsli et al., 2016).

In the present study, an attempt was made to prepare a non-phosphate additive using chitosan, collagen peptides and sodium chloride, conforming to the requirements of food safety. It was further compared for its effect of retaining water against commercially used water-retaining agents of phosphate origin. The traditional water retention indices such as soaking weight, thawing loss and cooking loss, can only reflect the water-holding capacity of the samples. In the present study in addition to this, characterize when the specific state of the water and the process of change in the samples were evaluated. Low- Field Nuclear Magnetic Resonance technique (NMR), a new spectral detection technique was applied to rapidly detect the H proton signal inside the shrimp without damaging it (Liu et al., 2015; Kirtil & Oztop, 2016). Proton density reflects the measurement value of proton content which has a positive correlation with water content. It also explores the relationship between the moisture distribution and the water-holding capacity by transversing relaxation time which reflects the degrees of freedom in the sample (Behroozmand et al., 2015).

Materials and Methods

Fresh *Litopenaeus vannamei* and sodium chloride (food grade) were purchased from local market. Foodgrade tilapia collagen peptide chitosan (food grade, degree of deacetylation $\geq 90\%$) and compound phosphate were collected from local industry. The other reagents used for the study were of analytical grade (Sinopharm Chemical Reagent Co. Ltd).

Uniform sized live shrimp were shipped to the laboratory and iced for sudden death. Samples were cleaned further to headless, peeled and deveined form and stored at 4°C. They were treated with additive solution in 1:5 ratio (shrimp: additive solution (w/w)) solution at 0 ~ 4°C for 10 ~ 120 min, and stirred slowly every 15 min.

Formulation of non-phosphate additive: Optimization. Concentration of chitosan, collagen peptide, and sodium chloride were considered as three different test factors on the basis of preliminary experiments to explore the effect on quality of *Litopenaeus vannamei*. The mass fraction gradient of each of the factor were: chitosan: 0, 0.3, 0.6, 0.9, and 1.2%, collagen peptide: 0, 0.5, 1.0, 1.5, 2.0, and 2.5%, sodium chloride 0, 0.1, 0.3, 0.5, and 0.7%, respectively. The water retention capacity indices *viz.*, soaking weight, thawing loss, cooking loss, product yield, and moisture content were considered to determine the optimal quality score of each component. Meanwhile, a blank control group was also taken for comparison, and each index was measured six times in parallel.

Three levels were set for each factor to conduct the orthogonal test of $L_9 (3^4)$ according to the results of single factor test, and the factors and levels are shown in Table 1. To determine the optimum formula of the composite non-phosphate additive, indices *viz.*, soaking weight, thawing loss, cooking loss were considered with six replicators.

Table 1. The Factors and levels of orthogonal methodology

Levels	Factors concentration		
	Chitosan (%)	Collagen peptide (%)	Sodium chloride (%)
1	0.60	1.50	0.30
2	0.90	2.00	0.50
3	1.20	2.50	0.70

The optimum formula of the composite non-phosphate additive was obtained based on the results of single factor and orthogonal test. After verification, the shrimp were soaked in solution at 0 ~ 4°C for 20, 40, 60, 80, and 100 min, respectively and were stored at -20°C for 10 days. Water retention capacity indices were measured (n=6) to determine the optimal soaking time.

About 60 no. of shrimps were divided randomly into three groups, and each group was soaked under optimal soaking time in the following three solutions.

Group I: In distilled water: Control group

Group II: In non-phosphate additive: Treatment group,

Group III: In composite phosphate group (31 g/L): Positive control group.

The water retention indices and textural properties of the three sample groups were measured. The density of the proton was calculated by NMR technology to explore the distribution and state of water in the samples.

Soaking weight was determined according to the methodology of Gao (2007) with some modifications. Fresh shrimp samples (m_0) were weighed accurately and soaked in the solution in ratios of shrimp: non-phosphate additive as (1:5) for 60 min at 4°C. The mixtures were stirred gently every 15 min. After treatment, the samples were drained by placing on a stainless steel rack for 15 min (4°C). The samples (m_1) were re-weighed accurately after wiping the surface gently with the filter paper to remove excess soaking liquid. Soaking weight was then calculated and expressed as percentage increase in weight:

$$X_1(\%) = \frac{m_1 - m_0}{m_0} \times 100$$

The treated shrimps were packed in the storage bags, sealed and kept flat on trays. The samples were quick frozen at -80°C until the core reached -18°C and then frozen at -20°C for 10 days. Thereafter, the frozen samples were thawed for 5 h at 4°C, drained 30 min until the shrimps completely thawed, and excess liquid was wiped with a filter paper gently. Thawing loss was calculated as follows:

$$X_2(\%) = \frac{m_0 - m_2}{m_0} \times 100$$

With m_0 and m_2 being the initial weight before soaking and weight after freeze thawing, respectively.

The thawed shrimp (m_2) was kept in cooking bag and sealed with sealing machine. It was further cooked at 100°C for 3 ~ 5 min until the sample turned red and hardened. Further it was cooled to room temperature. The samples (m_3) were weighed accurately after removing the cooked fluid and the excess liquid from the surface. Cooking loss rate was then calculated as follows:

$$X_3(\%) = \frac{m_2 - m_3}{m_2} \times 100$$

Product yield is the most important parameter used to measure the water retention capacity of additive and it determines the water loss rate of samples in the whole process (Feng-Ying et al., 2013). The higher the product yield lower is the processing loss of shrimp. Product yield rate was calculated as follows:

$$X_4(\%) = \frac{m_3}{m_0} \times 100$$

Where m_0 and m_3 is the initial weight before soaking and weight after being removed the cooked fluid, respectively.

Moisture content was investigated using a fast moisture meter (model) with the programmed procedure. Steamed shrimps (0.5 g) were minced to determined moisture content ($n=3$).

Cooked shrimp was cooled to room temperature and the second node of the shrimp body back was kept as the test points to analysis texture characteristics by American TA-XT plus texture instrument (Ávila et al., 2014). Textural parameters *viz.*, hardness, elasticity, adhesiveness and chewiness were analyzed. Cylindrical probe of 5 mm Ø, at a unit speed of 0.8 mm s⁻¹ were considered for the study. The shape variable was set 50% and the time interval was 5 s for each operation was repeated six times.

The moisture distribution and the water-hold capacity of the shrimp were measured using NMR according to the method of Qiu et al. (2017) with some modifications.

Data were subjected to analysis of variance and mean comparison was carried out using Duncan's multiple range test (Johnson & Bhattacharyya, 2006), with a significance at 5% level ($p<0.05$) using SPSS statistical software package.

Results and Discussion

The effects of chitosan concentration on the water retention of shrimp are presented in Fig.1. As presented in Fig. 1a, the weight of shrimp decreased with increase in chitosan concentration at 0 ~ 0.9%, but was higher than the control lot ($8.54 \pm 1.09\%$). When the chitosan concentration was 1.2%, the soaking weight increase dropped to $5.71 \pm 0.50\%$, mainly due to the chitosan solution formed film on the surface of the shrimps. Moreover, the viscosity

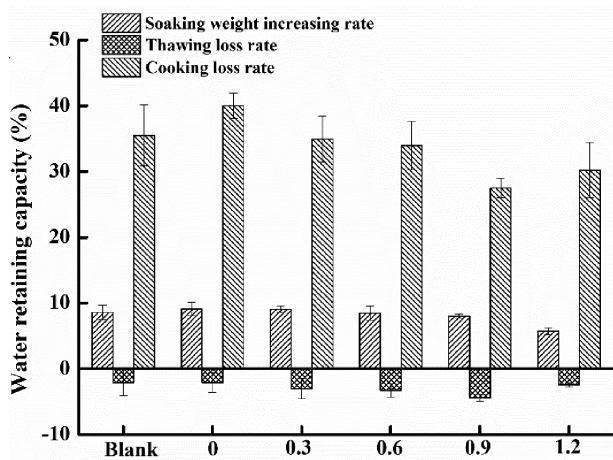


Fig. 1a. Chitosan concentration (%)

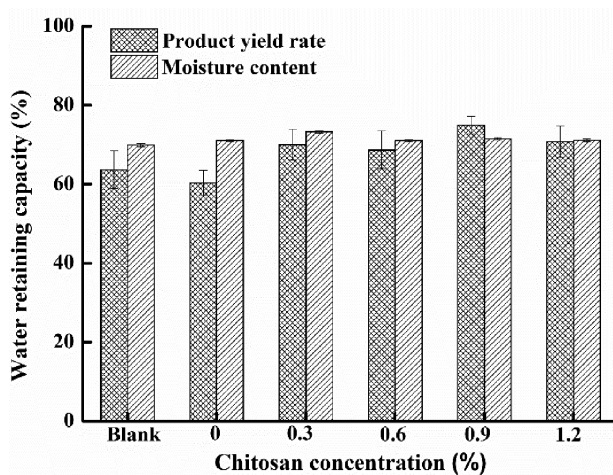


Fig. 1b. Effect of chitosan concentration on water retention of shrimp (a: soaking weight increasing, thawing loss, cooking loss, b: product yield, moisture content)

of the solution increased with increases in concentration, which can prevent the water molecules entering (Rinaudo, 2007). The thawing loss of shrimp was negative, indicating that weight gain after soaking was not lost during the 10 days of frozen storage and thawing process. When the concentration of chitosan was 0.9%, the thawing loss rate decreased to $-4.400552 \pm 0.48\%$, which was significantly lower than the control ($p < 0.05$). When the concentration of chitosan was 0 ~ 0.9%, both the thawing loss and cooking loss of the samples decreased with increase in chitosan. The cooking loss rate increased to $30.22 \pm 4.20\%$ when the concentration of chitosan was 1.2%. When the

concentration of chitosan was higher, the shrimp surface showed a filming inhomogeneity.

Product yield rate is the most important parameter index to measure the water retention effect. Fig. 1b revealed that the product yield rate increased with the increase of chitosan concentration, and all were higher than 65% after treatment. The product yield rate reached a plateau ($74.94 \pm 4.20\%$) when the concentration was 0.9%, which was significantly higher than that of the blank control group ($63.65 \pm 4.74\%$) ($P < 0.01$), and meanwhile the moisture content was $71.52 \pm 0.28\%$. In summary, 0.9% was chosen as the optimal concentration of chitosan for further exploration.

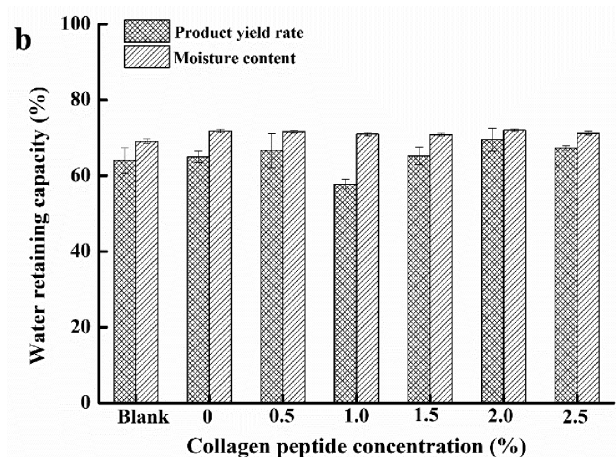
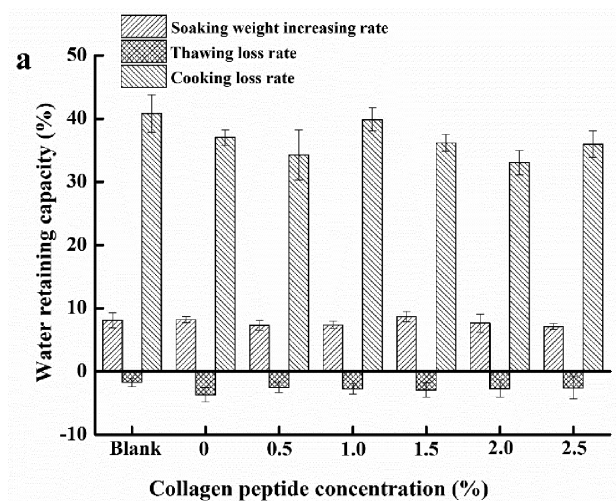


Fig. 2a and b. Effect of collagen peptide concentration on water retention of shrimp (a: soaking weight increasing rate, thawing loss rate, cooking loss rate, b: product yield rate, moisture content)

Fig. 2 (a) showed that rate of increase in soaking weight was proportional to the collagen peptide reaching a plateau when the concentration of collagen peptide was 1%, which was higher than that of the blank control group ($8.09 \pm 1.20\%$). The thawing loss rate and cooking loss rate of all treatment groups were lower than the blank control group. It is known that the water molecules can penetrate into the shrimp tissues quickly when shrimps are treated by distilled water and the muscle cells swell to form ice crystals during frozen storage. However, when the shrimp thaws ice crystals stored inside the cell melt, resulting in the damage and rupture of tissue cells, the flowing out of water and the loss of internal nutrients such as salts, vitamins, water-soluble proteins (Gao, 2007). After treatment with soaking solution, on account of higher viscosity of the solution was higher due to the presence of chitosan, which reduced the penetration rate of collagen peptide molecules were observed. Therefore, the weight increased as collagen peptide concentration increased. Besides, the collagen peptide can combine with the muscle protein to enhance the water retention of muscles when infiltrating into the muscle gap with low molecular weight and a large number of active groups such as carboxyl and hydroxyl group (Sato, 2017). The cooking loss rate was reduced to a minimum of $33.01 \pm 1.92\%$ ($p < 0.05$) when collagen peptide was 2%, meanwhile the thawing loss rate was $-2.71 \pm 1.38\%$, product yield rate was $69.55 \pm 3.02\%$ ($p < 0.05$) (Fig. 2b), which meant that the water retention capacity of the additive was better than the control group. In conclusion, the concentration of collagen peptide 2% was suitable for further exploration.

Salt (sodium chloride) is widely used in the preservation of various meat products. In the present study rate of increase in soaking weight of shrimps added with sodium chloride were lower than that of control group (Fig. 3a). However, the volume of water molecules increased gradually during the frozen storage, which damaged the tissue cells due to rupturing. A large amount of nutrients was thus lost (Wang et al., 2016), as shown by the data of thawing loss rate (Fig. 3a).

In the selected range of salt concentration, the thawing loss rate of the treatment group decreased with increase in sodium chloride concentration. The thawing loss was up to $0.83 \pm 1.30\%$ and cooking loss rate was $40.34 \pm 2.32\%$ of the control samples

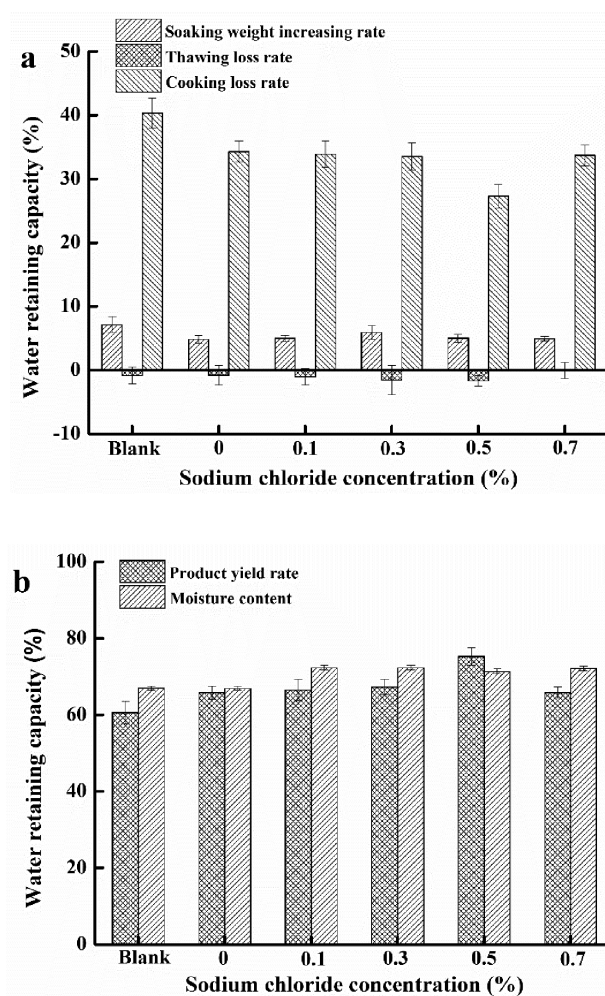


Fig. 3a and b. Effect of sodium chloride concentration on water retention of shrimp (a: soaking weight increasing rate, thawing loss rate, cooking loss rate, b: product yield rate, moisture content)

(Fig. 3a). The thawing loss rate ($-0.04 \pm 1.26\%$) was higher than control group when concentration of sodium chloride reached 0.7%. The extra cellular concentration increased with increase is concentration of sodium chloride. When the extracellular osmotic pressure is greater than that of the interior, the cells would dehydrate and lose fluids seriously (Cardoso et al., 2010). The results were similar to the study of Zhou (2011).

The moisture content can directly affect the taste and tenderness of the shrimps. The product yield increased initially and then declined with increasing sodium chloride concentration, and reached a plateau ($75.26 \pm 2.36\%$) ($p < 0.01$) at 0.5% (Fig. 3b). The moisture content of the blank group was only

66.97±0.44%, and its texture was coarse, dry and had poor taste. The moisture content of the shrimp was 71~72% after treatment, which was similar to the moisture content of fresh shrimp (72.66%). The results suggest including a non-phosphate additive to have good water retention effect, and improved moisture content of the shrimp.

In conclusion, the concentration of sodium chloride added at 0.5% was suitable for further optimization.

Chitosan concentration (A), collagen peptide (B), sodium chloride concentration (C) was taken as the factors and rate of increase in soaking weight, rate of thawing loss, and cooking loss were considered as the indexes to carry on L_9 (3^4) orthogonal test. The distribution of non-phosphate additive was studied on the basis of single factor test results. The orthogonal test results are shown in Table 2.

Influence of the factors on dependent parameters *viz.*, soaking weight increasing rate, thawing loss rate, and cooking loss rate were concluded based on range R: A (chitosan concentration) > B (collagen peptide) > C (sodium chloride concentration). The optimal combination for increased soaking weight rate was $A_1B_3C_2$ according to K (chitosan 0.6%, collagen peptide 2.5%, and sodium chloride of 0.5%). Similarly, the optimal combination for thawing loss was $A_1B_2C_2$ (chitosan of 0.6%, collagen peptide of 2.0%, and sodium chloride of 0.5%), and the optimal range for cooking loss was $A_1B_2C_3$ (chitosan of 0.6%, collagen peptide of 2.0%) and sodium chloride of 0.7%.

The results of the orthogonal test (Table 3) were analyzed by SPSS statistical software and it was found that factor A had a significant effect on the

Table 2. Results of orthogonal array methodology

Test	Factor				Evaluation Index		
	A	B	C	D Blank	Soaking weight increasing rate (%)	Thawing loss rate (%)	Cooking loss rate (%)
1	1	1	1	1	7.46	-3.40	31.76
2	1	2	2	2	7.05	-3.98	32.10
3	1	3	3	3	6.93	-3.20	34.39
4	2	1	2	3	6.42	-2.44	34.94
5	2	2	3	1	4.52	-2.68	35.76
6	2	3	1	2	4.37	-2.63	36.92
7	3	1	3	2	3.06	-2.29	36.42
8	3	2	1	3	2.37	-2.86	35.25
9	3	3	2	1	3.69	-2.52	36.38
K1	7.17	5.64	4.73	5.22	Soaking weight increasing rate Intuitive analysis		
K2	5.10	4.65	5.72	4.83			
K3	3.04	4.99	4.83	5.24			
R	4.11	0.99	0.99	0.41			
K1	-3.53	-2.71	-2.96	-2.87	Thawing loss rate Intuitive analysis		
K2	-2.58	-3.17	-2.98	-2.97			
K3	-2.56	-2.79	-2.72	-2.84			
R	0.97	0.46	0.26	0.13			
K1	32.75	34.38	34.64	34.64	Cooking loss rate Intuitive analysis		
K2	35.87	34.37	34.47	35.15			
K3	36.02	35.90	35.53	34.86			
R	3.27	1.53	1.05	0.51			

Table 3. Results of analysis of variance and significance test

Index	Sources of variance	Deviation sum of squares	Degrees of freedom	Ratio F	Critical value F	significance
Soaking weight increasing rate	A	25.32	2	77.669	19	*
	B	1.535	2	4.709	19	
	C	1.772	2	5.436	19	
	error	0.33	2			
	Total variation	28.957	8			
Thawing loss rate	A	1.837	2	63.345	19	*
	B	0.369	2	12.724	19	
	C	0.128	2	4.414	19	
	error	0.03	2			
	Total variation	2.364	8			
Cooking loss rate	A	20.411	2	51.413	19	*
	B	4.659	2	11.736	19	
	C	1.916	2	4.826	19	
	error	0.4	2			
	Total variation	27.386	8			

Note: * $\alpha = 0.05$, and $F_{0.05}(2, 2) = 19.00$ by checking the distribution table.

parameters evaluated ($P < 0.05$), while the effect of B and C on three indices were not significant. Some studies showed that chitosan could protect the muscle proteins, prevent the formation of ice crystals effectively during the freezing process, and prevent the cell and tissue structure from damage and fluid loss (Wang et al., 2015; Chouljenko et al., 2016) during thawing after being treated by the composite aquasorb. In addition, the chitosan might also form a membrane on the surface of the shrimp body to reduce the evaporation of water (Fernández-Martín et al., 2014).

Hence the factors influencing the water retention capacity followed the order $A > B > C$, and the optimal combination was determined as $A_1B_2C_2$ and the optimal formula of non-phosphate additive was: chitosan at 0.6%, collagen peptide of 2.0%, and sodium chloride of 0.5%. Under optimal conditions, the rate of increase in soaking weight, thawing loss and the cooking loss of shrimps were $6.89 \pm 1.36\%$, $4.01 \pm 1.98\%$ and $33.12 \pm 2.13\%$, respectively.

The shrimps were soaked in the optimized solution (chitosan of 0.6%, collagen peptide of 2.0%, and sodium chloride of 0.5%) for 20 ~ 100 min to explore the best soaking time.

Rate of increase in soaking weight was linear early related to the soaking time at 20 ~ 80 min, and was

about 10% at 80 min and 100 min (Fig. 4). As the soaking time extended, the composite aquasorb could penetrate into the muscles of the shrimps fully and reached equilibrium. The thawing loss rate gradually decreased during soaking, and was minimum of $4.15 \pm 0.99\%$ when the soaking time was 80 min. At this time, cooking loss was a minimum of $34.63 \pm 3.67\%$, and product yield rate was $68.31 \pm 4.83\%$. The water retention indices barely indicated changes on further extending the soaking time. Therefore, 80 min was chosen as the most suitable soaking time for further studies.

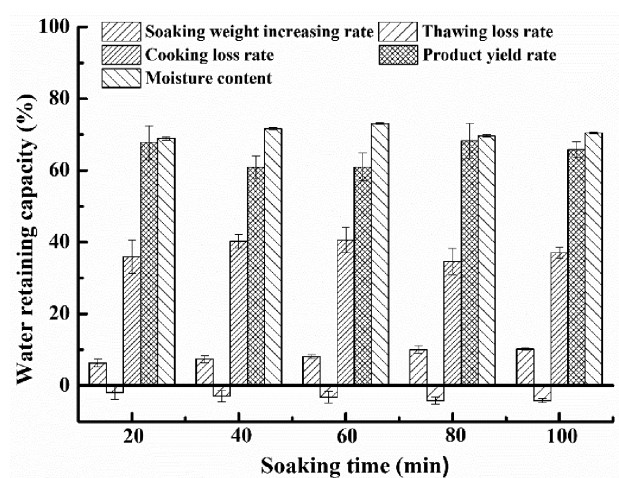


Fig. 4. Effect of soaking time on water retention of shrimp

The effect of the water quality of shrimp with the optimized non-phosphate additive, commercial composite phosphate (31 g L), and distilled water as blank control group were investigated, with 80 min as soaking time (Fig. 5)

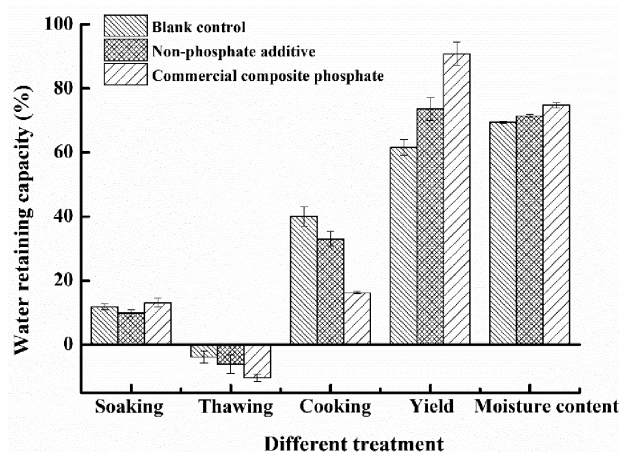


Fig. 5. Effects of different treatments on water retention capacity of shrimp

Studies by Gao, (2007) have indicated that compound phosphate (Sodium Pyrophosphate at 0.2%, Sodium Tripolyphosphate at 0.2% and Sodium Hexametaphosphate at 0.1%) can effectively improve the water retention of aquatic products and maintain high product yield rate in food industry. In this study, for the shrimps treated with non-phosphate additive, the soaking weight increasing rate was $9.80 \pm 1.14\%$, which is lower than the blank control group ($11.77 \pm 0.91\%$) and commercial composite phosphate group ($13.07 \pm 1.38\%$), thawing loss rate was $6.09 \pm 2.87\%$, cooking loss rate was $32.96 \pm 2.31\%$, and product yield rate was $73.65 \pm 3.56\%$ ($p < 0.01$), respectively. Though product yield rate of the shrimps treated with commercial composite phosphate was $90.83 \pm 3.61\%$, there was no remarkable difference between the moisture content of non-phosphate additive group and commercial composite phosphate group, which suggested non-phosphate additive has good water retention. Thorarinsdottir et al. (2010) indicated that the water retention mechanism of the complex phosphate is to destroy the myofibrillar structure, make myosin detach from the fibrous network, and form as gel to hold moisture when heating.

The texture analyzer is usually used to quantify the quality of shrimp to avoid the influence of human factors on the evaluation results, and the interpre-

tations are more reliable and objective (Ávila et al., 2014; Hackl & Ermolina, 2016). Hardness, elasticity, adhesiveness, chewiness as the important indices were used to evaluate the tenderness, adhesion and texture of meat.

The sensory evaluation of the treated shrimps indicated that the shrimps treated with non-phosphate additive presented a rich, fresh and natural flavor and natural color, while the shrimps expanded, hardened and got pale on treating with compound phosphate and exhibited transparent appearance after cooking. Hence the non-phosphate additive was better option and promised to serve as a substitution for compound phosphate aquasorb.

The texture characteristics of shrimp (hardness, elasticity, adhesiveness, chewiness) as influenced by treatment with compound phosphate and non-phosphate additive was evaluated (Fig. 6). The hardness of the shrimp was lower than that of the blank control group after the treatment of two additives. It meant that the muscle was tender and juicy. The hardness of samples added with the non-phosphate additive was 1133.61 ± 189.99 g, which was lower than that of the blank control group by 10% (Fig. 6a).

The elasticity of shrimp decreased after adding additive, indicating force between muscle protein increased and the quality of shrimp muscle were improved (Fig. 6b). The elasticity of the shrimp treated by commercial composite phosphate was 0.75 ± 0.19 . Compared with the blank control group and non-phosphate additive group, commercial composite phosphate group decreased by 29.9% and 25.23%, respectively.

The adhesiveness of shrimp ie., the force between the moisture and muscle protein improved significantly ($p < 0.05$) after adding additives (Fig. 6c). There was no significant difference between the adhesiveness of non-phosphate additive group (0.29 ± 0.07) and commercial composite phosphate group (0.29 ± 0.02).

As can be seen from Fig. 6 (d), the chewiness of the additive treated shrimp reduced significantly ($p < 0.05$). Compared to the chewiness of blank control group which was 624.67 ± 124.15 g, the non-phosphate additive group had a chewiness of only 308.89 ± 81.67 g (decrease by 50.55%) and it was higher than that of compound phosphate treated group (236.42 ± 40.46 g).

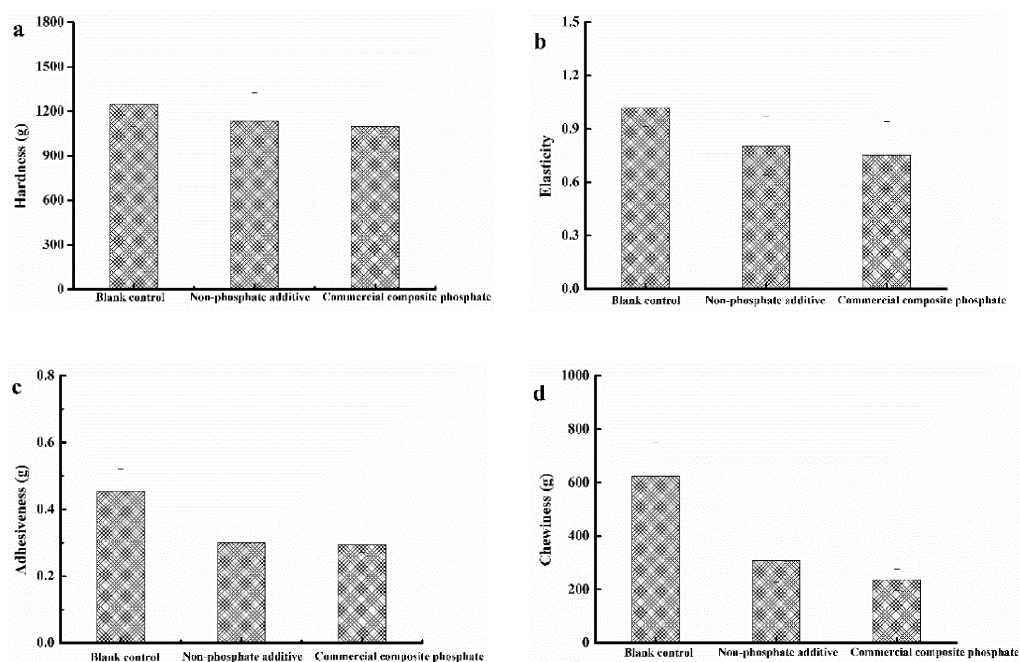


Fig. 6. Effect of different treatments on texture characteristics of shrimp (a: hardness, b: elasticity, c: adhesiveness, d: chewiness)

Changes in the texture characteristics of shrimp is mainly associated to the muscle proteins (Hackl & Ermolina, 2016). As aquasorb can lock more moisture, it can keep the shrimp tender and slow down the muscle protein degradation during storage. As a result, it will enhance the muscle force between protein molecules and prevent fluid loss by adding additive (Feng-Wu et al., 2011).

The distribution, content and migration process of water in shrimp were determined by NMR (Fig. 7).

As shown in Fig. 7. in T_{21} (1~10 ms), the part of water called bound water was tightly bound to the polymer polar group in the samples, and the fluidity was poor; in T_{22} (10-150 ms), it was considered to be semi-binding water that bound indirectly with macromolecules or directly with strongly bound water, which was related to juiciness and meaty taste; in T_{23} (150-1000 ms), it was considered to be mobile water, or free water, which was related to dripping water.

There was a small peak at T_{21} in non-phosphate additive group. The T_{22} (25.14 ms) of samples treated by two kinds of additives was earlier than that of the blank control group, meaning that the higher relaxation time component migrated to the

rather lower relaxation time component and the semi-binding water was converted into bound water, which is tightly bound to muscle (Carneiro et al., 2013) (Fig. 7). In addition, the free water mobility of the blank control group was higher (T_{23}) and the shrimps of blank control group could not hold the water and swelled rapidly again during the thawing and cooking process. The moisture content

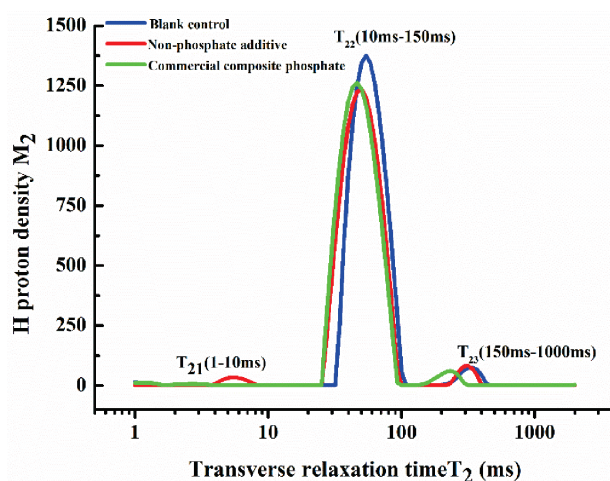


Fig. 7. Determination of water retention of shrimp by Low-Field Nuclear Magnetic Resonance

of three groups could be calculated according to the peak area and unit proton density respectively: $M_2 = 13097.64$ for blank control group, $M_2 = 13385.83$ for non-phosphate additive, $M_2 = 13534.33$ for commercial composite phosphate group. As a result, groups treated by additives had higher semi-binding water content and had a good water retention effects.

The optimum proportion of non-phosphate additive: chitosan of 0.6%, collagen peptide of 2.0%, and sodium chloride of 0.5% were derived by single factor and orthogonal test. The optimum soaking time observed was 80 min with maximum water retention capacity. The results showed that non-phosphate additive can serve as a substitute for phosphate aquasorb to improve the water-holding capacity of aquatic products, as well as enhanced sensory as well as textural properties.

Acknowledgments

The authors are grateful for the support of the National Key R&D Program of China (No. 2016YFD0400202), the Natural Science Foundation of China (No. 31571779), the Science and Technology Program of Fujian, China (Grant No. 2016N0017), Fujian Spark Program (Grant No. 2016S0042), Fuzhou Science and Technology Project (Grant No. 2015-G-70), and the Science Foundation of Guangdong Food and Drug Vocational College (No. 2016YZ033).

References

- Åsli, M., Ofstad, R., Böcker, U., Jessen, F., Einen, O. and Mørkøre, T. (2016) Effect of sodium bicarbonate and varying concentrations of sodium chloride in brine on the liquid retention of fish (*Pollachius virens* L.) muscle. *J. Sci. Food Agric.* 96: 1252-1259
- Ávila, R. D., Isabel, C. M., Ordóñez, J. A., De, L. H. L. and Herrero, A. M. (2014) Rheological behaviour of commercial cooked meat products evaluated by tensile test and texture profile analysis (TPA). *Meat Sci.* 98: 310-315
- Behroozmand, A. A., Keating, K. and Auken, E. (2015) A Review of the Principles and Applications of the NMR Technique for Near-Surface Characterization. *Surveys in Geophysics.* 36: 27-85
- Cardoso, C., Mendes, R., Vazpires, P. and Nunes, M. L. (2010) Effect of salt and MTGase on the production of high quality gels from farmed sea bass. *J. Food Eng.* 101: 98-105
- Carneiro, C. D. S., Mársico, E. T., Júnior, C. a. C., Álvares, T. S. & Jesus, E. F. O. D. (2013) Quality Attributes in Shrimp Treated with Polyphosphate after Thawing and Cooking: A Study Using Physicochemical Analytical Methods and Low-Field ^1H NMR. *J. Food Process Eng.* 36: 492-499
- Chen, C. H., Wang, F. Y., Mao, C. F., Liao, W. T. and Hsieh, C. D. (2008) Studies of chitosan: II. Preparation and characterization of chitosan/poly(vinyl alcohol)/gelatin ternary blend films. *Int. J. Biol. Macromol.* 43: 37-42
- Chouljenko, A., Chotiko, A., Reyes, V., Alfaro, L., Liu, C., Dzandu, B. & Sathivel, S. (2016) Application of water-soluble chitosan to shrimp for quality retention. *LWT - Food Sci. Technol.* 74: 571-579
- Feng-Wu, L. I., Xue-Qin, X. U. and Shan, J. H. (2011) Effect of phosphorus-free water retaining agent on quality of water retention of pre-conditioning frozen shrimp. *Sci. Technol. Food Industry.* 32: 342-345
- Feng-Ying, W. U., Shui-Juan, W. U. and Dai, C. Y. (2013) Water Retention Effect of Various Non-phosphorus Agents on Frozen Shrimp. *Academic Periodical of Farm Products Processing.* 13: 21-25
- Fernández-Martín, F., Arancibia, M., López-Caballero, E., Gómez-Guillén, C., Montero, P. and Fernández-García, M. (2014) Preparation and molecular characterization of chitosans obtained from shrimp (*Litopenaeus vannamei*) shells. *J. Food Sci.* 79: 1722-1731
- Gao, R. (2007) Study on the mechanism of polyphosphates hydrolysis in bighead carp and non-phosphate additive. Ocean University of China
- Ginson, J., Bindu, J. (2017) Review on Biochemical Composition and Microflora of Prawns. *Fish. Technol.* 54: 75-85
- Glorieux, S., Goemaere, O., Steen, L. and Fraeye, I. (2017) Phosphate Reduction in Emulsified Meat Products: Impact of Phosphate Type and Dosage on Quality Characteristics. *Food Technol. Biotechnol.* 55: 390-397
- Hackl, E. V. and Ermolina, I. (2016) Using Texture Analysis Technique to Assess the Freeze-Dried Cakes in Vials. *J. Pharm. Sci.* 105: 2073-2085
- Hema, G. S., Ganesan, B. and Shyni, K. (2017a) Collagen Hydrogel as Bio Interactive dressing for wound healing. *Fish. Technol.* 54: 252-257
- Hema, G. S., Joshy, C. G., Shyni, K., Chatterjee, N. S., Ninan, G. and Mathew, S. (2017b) Optimization of process parameters for the production of collagen peptides from fish skin (*Epinephelus malabaricus*) using response surface methodology and its characterization. *J. Food Sci. Technol.* 54: 488-496
- Hema, G. S., Shyni, K., Prasad, M. Manu. (2017c) Collagen Biosynthesis in Osteoblast Cells Treated with Fish Collagen Peptides. *Fish. Technol.* 54: 107-111
- Johnson, R. A. and Bhattacharyya, G. (2006) Statistics: principles and methods. *Technometrics.* 35: 237-238

- Kaewmanee, T., Benjakul, S. and Visessanguan, W. (2009) Protein Hydrolysate of Salted Duck Egg White as a Substitute of Phosphate and Its Effect on Quality of Pacific White Shrimp (*Litopenaeus Vannamei*). J. Food Sci. 74: 351-361
- Kirtil, E. and Oztop, M. H. (2016) ¹H Nuclear Magnetic Resonance Relaxometry and Magnetic Resonance Imaging and Applications in Food Science and Processing. Food Eng. Rev. 8: 1-22
- Liu, R., Wu, L., Zhang, Y., Zhang, H., Zhang, B., Huang, B. and Wei, Y. (2015) Water state and distribution in noodle dough using low-field nuclear magnetic resonance and differential scanning calorimetric. Transactions of the Chinese Society of Agricultural Engineering. 31: 288-294
- Murthy, L. N., Jesmi, D. and Rao, B. M. (2016) Effect of Different Processing Methods on the Texture of Black Tiger (*Litopenaeus monodon*) and Pacific White Shrimp (*Litopenaeus vannamei*). Fish. Technol. 53: 205-210
- Qiu, Q. Z., Wei, L., Hao, K. L., Xiao, H. C., Mei, J. and Ming, S. D. (2017) Low-field nuclear magnetic resonance for online determination of water content during sausage fermentation. J. Food Eng. 212: 291-297
- Remya, S., Basu, S., Venkateshwarlu, G. and Mohan, C. O. (2015) Quality of shrimp analogue product as affected by addition of modified potato starch. J. Food Sci. Technol. 52: 1-9
- Renuka, V., Mohan, C. O. and Kriplani, Y. (2016) Effect of Chitosan Edible Coating on the Microbial Quality of Ribbonfish, *Lepturacanthus savala* (Cuvier, 1929) Steaks. Fish. Technol. 53: 146-150
- Rinaudo, M. (2007) Chitin and Chitosan — Properties and Applications. Progress in Polymer Science. 38: 603-632
- Sato, K. (2017) The presence of food-derived collagen peptides in human body-structure and biological activity. Food Funct. 8: 4267-4770
- Thorarinsdottir, K. A., Arason, S., Bogason, S. G. and Kristbergsson, K. (2010) Effects of phosphate on yield, quality, and water-holding capacity in the processing of salted cod (*Gadus morhua*). J. Food Sci. 66: 821-826
- Wachirasiri, K., Wanlapa, S., Uttapap, D. and Rungsardthong, V. (2016) Use of amino acids as a phosphate alternative and their effects on quality of frozen white shrimp (*Litopenaeus vannamei*). LWT - Food Sci. Technol. 69: 303-311
- Wang, L. F., Feng, T. C., Wang, X. J., Zheng, J. H. and Bao-Sheng, L. I. (2014) Study on effects of food additives named mixed phosphates on water-holding porkballs. Science & Technology of Food Industry. 35: 320-323
- Wang, L. M., Jing, L. I., Long, Z. F. and Peng, H. (2016) Effects of Non-phosphate Macro-molecular Water-retention Agents on Meat Quality. Food Res. Development. 14: 221-224
- Wang, Y., Liu, L., Zhou, J., Ruan, X., Lin, J. and Fu, L. (2015) Effect of Chitosan Nanoparticle Coatings on the Quality Changes of Postharvest Whiteleg Shrimp, *Litopenaeus vannamei*, During Storage at 4°C. Food Bioprocess Tech. 8: 907-915
- Zhang, Y., Zhang, L., Sun, J., Qiu, J., Hu, X., Hu, J. and Bao, Z. (2014) Proteomic analysis identifies proteins related to carotenoid accumulation in Yesso scallop (*Patinopecten yessoensis*). Food Chem. 147: 111-116
- Zhou, M. (2011) Study on application of non-phosphate additive in frozen *Litopenaeus vannamei* and mechanism of water-holding.: Guangdong Ocean University
- Zhou, X., Zhang, J., Liu, S. and Ding, Y. (2014) Supplementation of sodium chloride in diets to improve the meat quality of Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity water. Aquacult. Res. 45: 1187-1195