Optimization of thermophysical properties of Pacific white shrimp (*Litopenaeus vannamei*) previously treated with freezing-point regulators using response surface methodology

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Abstract Three freezing-point regulators (glycine, sodium chloride and D-sorbitol) were employed to optimize thermophysical properties of Pacific white shrimp (*Litopenaeus vannamei*) using response surface methodology (RSM). The independent variables were glycine content (0.250–1.250 %), sodium chloride content (0.500–2.500 %) and D-sorbitol content (0.125–0.625 %) and analysis of variance showed that the effects of glycine, sodium chloride and D-sorbitol on the thermophysical properties were statistically significant ($P < 0.05$). The coefficient of determination, R^2 values for initial freezing point (T_i), unfreezable water mass fraction (W_u), apparent specific heat (C_{app}) and Enthalpy (H) were 0.896–0.999. The combined effects of these independent variables on T_i , W_u , C_{app} and H were investigated. The results indicated that T_i , C_{app} and H varied curvilinearly with increasing of glycine, sodium chloride and D-sorbitol content whereas W_u increased nearly linearly. Based on response plots and desirability functions, the optimum combination of process variables for Pacific white shrimp previously treated with freezing-point regulators were 0.876 % for glycine content, 2.298 % for sodium chloride content and 0.589 % for D-sorbitol content, correspondently the optimized thermophysical properties were T_i , -5.086 °C; W_u , 17.222 %; C_{app} , 41.038 J/g °C and H , 155.942 J/g, respectively. Briefly, the application of freezing-point regulators

depressed T_i and obtained the optimum W_u , C_{app} and H , which would be obviously beneficial for the exploitation of various thermal processing and food storage.

Keywords Pacific white shrimp · Thermophysical properties · Freezing-point regulator · Response surface methodology

Introduction

Pacific white shrimp (*Litopenaeus vannamei*) is one of the most important seafood traded worldwide as well as considerably representative aquatic products for its positive culinary quality and high nutritional values (Oosterveer 2006; Qian et al. 2013). However, fresh shrimp has a short shelf life, which causes substantial practical problems for its transportation and distribution, and the nutritional compositions of shrimp may be destroyed by various factors, i.e. enzymes and microbiological activities (Jeyasekaran et al. 2006). For an extended preservation of their nutrition and freshness during storage, distribution and export, they must be quickly frozen to about -18 °C immediately after caught so as to suppress the physiological, biochemical, microbiological and mass transfer processes developing in them (Bak et al. 1999). However, as is known to all, the shrimps are easy to lose nutritional compounds during freezing and thawing not chilled individually even though they are placed in appropriate packaging materials. That is why, good knowledge of thermophysical properties, in the real industrial situation of their cooling in a quick refrigerating system, is not less important than the sensory or physicochemical data for a separate product itself.

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Thermophysical properties (i.e. initial freezing point, end point of freezing, unfreezable water mass fraction, apparent specific heat, enthalpy, etc.) of food are important parameters, and so far have been applied in many aspects of food and biotechnical industry (Karunakar et al. 1998; Marcotte et al. 2008; van der Sman 2008; Liu et al. 2009; Otero et al. 2010; De Oliveira and Zanoelo 2012; Santana et al. 2013), in which initial freezing point, as one of the most important thermal properties in frozen state, has been widely used as the prerequisite to predicting other thermal and physical properties in freezing or thawing food (Rahman et al. 2002; Kovačević et al. 2007). Also, it is used to analyze the quality changes in the freezing and thawing of frozen foods (Karunakar et al. 1998; Rahman et al. 2002; Lu et al. 2007). In addition, Fikiin and Fikiin (1999) and Liu et al. (2009) reported that unfreezable water mass fraction, specific heat and enthalpy could accurately predict the unsteady-state temperature distribution and energy consumption in cooling and freezing. In particular, slight temperature fluctuations are easy to result in the freezing and thawing of products and further give rise to the loss of amount of soluble nutritional substances under controlled freezing-point storage ($-2\sim 0\text{ }^{\circ}\text{C}$) (Yamane 1996; Fukuma et al. 1998; Wang and Zeng 2009; Wang et al. 2010). Besides, the study of the heat and mass transfers during freezing, for the assessment of the product behavior under proposed process conditions and for the efficient design of process and equipment, requires accurate values of thermophysical properties of products (Hamdami et al. 2004a).

Although many studies on thermophysical properties of food are available in the literature, data on thermophysical properties evolution of Pacific white shrimp during low temperature phase transition are scarce as compared to other foods (i.e. fruits, baked products, etc.). Moreover, they are in general estimated using either semi-theoretical equations that are based on the thermodynamic principles of freezing point depression or on reliable empirical equations that are derived from regression analysis of experimental data and more reliable for specific food products for which they are developed so that they may not be applicable to a wide range of different foods and conditions (Fikiin and Fikiin 1999; Ngadi et al. 2003). So far, published information has been scarcely available concerning the optimization of thermophysical properties of Pacific white shrimp previously treated with freezing-point regulators including glycine, sodium chloride and D-sorbitol. Miles et al. (1997) and Ribero et al. (2007) reported that the soluble components such as sodium chloride, monosaccharides (e.g. glucose, fructose and galactose) would contribute to the freezing point depression which effectively extended the non-frozen temperature-zone between the freezing point of water and that of an individual material. In addition, special heat, enthalpy and

unfreezable water mass fraction depend strongly on temperature and compositions including various sugars, ions and acids and soluble proteins in foods (Karunakar et al. 1998; Ngadi et al. 2000; Kovačević et al. 2007; van der Sman 2008). In order to realize the optimization of thermophysical properties of Pacific white shrimp, response surface methodology (RSM) was used to optimize studies. RSM was one of the most efficient tools to optimize complex and evaluate interactive effects among the variables. It has been successfully demonstrated that it could be used in optimizing process variables (Weska et al. 2007; Shi et al. 2008; Cui et al. 2013).

Therefore, the objective of this study was to investigate the effects of freezing-point regulators including glycine, sodium chloride and D-sorbitol on the initial freezing point, unfreezable water mass fraction, apparent specific heat and enthalpy of Pacific white shrimp during phase transition and obtain the optimized thermophysical properties which maybe provide available thermophysical property parameters for various thermal processing, chilled, refrigerated and controlled freezing-point storage, food control and development of equipments on shrimps.

Materials and methods

Raw materials

Fresh Pacific white shrimps (*Litopenaeus vannamei*), of the size of 55–65 shrimps/kg, were procured from local aquatic products market. The shrimps were placed in a large polyethylene bag with sufficient oxygen and transported to laboratory within 1 h. The average length and weight of shrimp were $\sim 14\text{ cm}$ and $\sim 17\text{ g}$, respectively. The proximate compositions were moisture content 74.52 % (wet basis), protein 22.08 % (wet basis), fat content 1.04 % (wet basis) and ash 1.26 % (wet basis). Before the experiment, the shrimps were washed with clean water and beheaded. The shells were then peeled off manually by wearing sterile hand gloves, and the edible portions were ground to obtain uniformity in clean condition. Then, the uniform shrimp meat was treated with three freezing-point regulators (refer to Tables 1 and 2). Finally, the previously treated shrimp meat was placed into $11\times 100\text{ mL}$ glass flask (Kimble Bomex Glass Co., Ltd., Beijing, China) and held in a refrigerator ($4\text{ }^{\circ}\text{C}$) for no less than 10 h before experiment, in which one without pretreatment was used as control.

Total water content (W_t)

Total water content of shrimp meat was analyzed in triplicate by the method of Chinese standard (GB/T5009.3-2010).

Table 1 Range of values for D-Saturated optimum design

Independent variables	Symbol	Coded levels				
		− 1	− 0.2912	0	+ 0.1925	+ 1
Glycine (%)	X_1	0.250	0.600	0.750	0.850	1.250
Sodium chloride (%)	X_2	0.500	1.200	1.500	1.700	2.500
D-sorbitol (%)	X_3	0.125	0.300	0.375	0.425	0.625

Apparent specific heat (C_{app})

A differential scanning calorimeter (DSC, 200PC, Netzsch Co. Ltd., Germany) with automatic data analysis software was employed to determine the apparent specific heat. Prior to measurements, the DSC was calibrated for temperature and energy sensitivities using indium (melting point 156.6 °C, $\Delta H_m = 28.60$ J/g), mercury (melting point − 38.8 °C, $\Delta H_m = 11.44$ J/g) and cyclohexane (melting point − 86.0 °C, $\Delta H_m = 79.40$ J/g) (Xu 2007). Small samples (8–10 mg each) were hermetically closed in aluminum DSC pan and very precisely weighed. Then, samples were frozen in situ in the calorimeter with liquid nitrogen cooling and stabilized at − 40 °C and finally heated from − 40 °C to 20 °C at a rate of 2 °C/min. An empty pan was used as a reference and the baseline was obtained from a scan realized with two empty pans and sapphire was used as a standard with a known specific heat value. Six replications of every sample were performed and the apparent specific heat could be obtained using the following equation (Liu 2000; Lu et al. 2007; Xu 2007) and mean values were displayed in the Table 3.

$$C_{app} = \frac{m_{std}}{ms} \cdot \frac{DSCs - DSCb}{DSC_{std} - DSCb} \cdot C_{p, std} \quad (1)$$

Where C_{app} and $C_{p, std}$ are the specific heats of sample and sapphire, respectively (J/g °C), ms and m_{std} are the

weights of sample and sapphire, respectively (g), and $DSCs$, DSC_{std} and $DSCb$ are the heat flow rates of sample, sapphire and baseline, respectively (mW).

Enthalpy (H)

Enthalpy was calculated by integrating the experimental data of apparent specific versus temperature (Hamdami et al. 2004a; Tocci and Mascheron 2008), with $H=0$ for the reference temperature at − 40 °C. The equation among the enthalpy, the apparent specific heat and temperature is presented below.

$$H = \int_{-40}^T C_{app}(T) dT \quad (2)$$

Where H is the enthalpy of sample (J/g), T is the temperature of sample (°C), and $C_{app}(T)$ is the specific heats of sample at T temperature (J/g °C).

Unfreezable water mass fraction (W_u)

The unfreezable water mass fraction could be calculated from the difference between total water content and the amount of melted ice detected by DSC fusion

Table 2 Experiment data from D-Saturated optimum design

Run number	Variables levels			Responses (Y)			
	X_1	X_2	X_3	T_i	W_u	C_{app}	H
1	− 1	− 1	− 1	− 4.21	13.84	95.07	178.22
2	+ 1	− 1	− 1	− 4.34	14.51	80.14	172.14
3	− 1	+ 1	− 1	− 6.00	16.42	48.64	164.90
4	− 1	− 1	+ 1	− 3.43	11.77	102.80	181.71
5	− 1	+ 0.1925	+ 0.1925	− 5.63	14.08	82.62	173.42
6	+ 0.1925	− 1	+ 0.1925	− 2.59	10.76	114.30	182.40
7	+ 0.1925	+ 0.1925	− 1	− 6.50	15.28	62.06	166.93
8	− 0.2912	+ 1	+ 1	− 6.91	13.26	76.68	169.84
9	+ 1	− 0.2912	+ 1	− 5.53	20.58	38.20	152.32
10	+ 1	+ 1	− 0.2912	− 4.11	18.99	47.88	157.30

T_i Initial freezing point (°C); W_u Unfreezable water mass fraction (%); C_{app} Apparent specific heat (J/g °C); H enthalpy (J/g)

Table 3 Regression equation coefficients for T_i , W_u , C_{app} and H

Regression coefficients	T_i	W_u	C_{app}	H
Constant				
b_0	− 5.197	13.393	87.242	173.019
Linear				
b_1	− 0.501a	1.961a	− 9.738b	− 5.860a
b_2	− 1.113a	1.560b	− 18.621a	− 6.537a
b_3	− 0.247b	0.273b	− 3.037b	− 2.411b
Quadratic				
b_{11}	− 0.431a	3.857a	− 31.570a	− 10.950a
b_{22}	1.444a	− 1.660b	13.317a	5.004a
b_{33}	− 0.740b	0.441b	− 3.025b	− 0.251NS
Interaction				
b_{12}	0.492a	0.296b	4.612a	0.732a
b_{13}	0.074NS	1.331a	− 6.885a	− 3.542a
b_{23}	− 0.710a	− 0.026NS	− 0.017NS	− 0.619b
R^2	0.999	0.997	0.939	0.896

NS not significant

T_i initial freezing point (°C); W_u unfreezable water mass fraction (%); C_{app} apparent specific heat (J/g °C); H enthalpy (J/g)

a Significant at $P < 0.01$

b Significant at $P < 0.05$

endotherm (Cogne and Andrieu 2003; Hamdami et al. 2004a, b). The expression is presented as follow.

$$W_u = W_t - \frac{H_{fw}}{L} \quad (3)$$

Where W_u is the unfreezable water mass fraction (%), W_t is the total water content (%), and H_{fw} is the enthalpy of water fusion (J/g), L is latent heat of fusion (J/g).

Initial freezing point (T_i)

When a frozen food is heated quasi-statistically, the temperature at which the last infinitesimal trace of ice melts is called the initial freezing point (Miles et al. 1997). Samples are heated from frozen to non-frozen state and together with phase transition, amount of latent heat is released so that the apparent specific heat capacity will peak at the initial freezing-point temperature, thereafter the apparent specific heat will tend a constant with the increasing of temperature (Becker and Fricke 1999; Tocci and Mascheron 2008). Therefore, based on the relations between the apparent specific heat and temperature, the position of the thawing peak in the experimental specific heat capacity versus temperature plot is determined as initial freezing-point temperature (T_i) of Pacific white shrimp.

Experiment design and statistical analysis

In order to describe the nature of response variables in the experimental region and find the optimum thermophysical properties, a D-saturating optimum design (Arora 2011) was applied with glycine content (0.250–1.250 %), sodium chloride content (0.500–2.500 %) and D-sorbitol content (0.125–0.625 %) being the independent experimental variables. As presented in Table 1, each at five levels, coded—1, − 0.2912, 0, + 0.1925 and +1, respectively. SAS (Statistical Analysis System v9.1, SAS Institute Inc., Cary, NC, USA) was used to analyze the experimental data and to fit the regression model. Secondary order polynomial model given in Eq. (4) was fitted to predict the response variables. Each experimental run was replicated thrice.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (4)$$

The coefficients of the polynomial were represented by b_0 (constant term), b_1 , b_2 and b_3 (linear coefficient), b_{11} , b_{22} and b_{33} (quadratic coefficient), and b_{12} , b_{13} and b_{23} (interactive coefficient). Y was the responses (i.e. T_i , W_u , C_{app} and H); X_1 , X_2 and X_3 were glycine content (%), sodium chloride content (%) and D-sorbitol content (%), respectively. The significance of regression coefficients were examined at 5 % level of significance. Response surface plots were generated with Data Processing System v9.50 (Tang 2006).

Parameters optimization and verification

In general, several response variables describing the thermophysical properties of Pacific white shrimp are to be optimized. In present research, T_i , W_u , C_{app} and H were optimized by constituting desirability functions (Eren and Kaymak-ertekin 2007; Shi et al. 2008) to obtain maximum W_u and minimum T_i , C_{app} and H . Thereafter verification of the optimum thermophysical parameters of Pacific white shrimp previously treated with freezing-point regulators was preformed. The shrimp samples made were experimentally analyzed and the results were statistically compared with the predicted values of the mathematical model.

Results and discussion

Characteristics of fresh shrimp

In this work, the thermophysical properties of fresh shrimp were analyzed before optimum design in order to effectively

study the effects of glycine, sodium chloride and D-sorbitol on thermophysical properties of Pacific white shrimp and in the meantime keep the pace of results. In general, Pacific white shrimp can be regarded as aqueous and nonaqueous components, in which total water content and unfreezable water mass fraction reached $74.282\% \pm 0.306\%$ and $12.422 \pm 0.186\%$, respectively, and the initial freezing point of Pacific white shrimp ($-1.504 \pm 0.262\text{ }^{\circ}\text{C}$) was lower than that of pure water ($0\text{ }^{\circ}\text{C}$) due to dissolved substances in the moisture within the shrimp meat. Such as various sugars, ions and acids and soluble proteins will contribute to the freezing point depression, while the insoluble components including fat and insoluble proteins will not (Miles et al. 1997; van der Sman and Boer 2005; Ribero et al. 2007). Furthermore, a portion of the water within the shrimp meat crystallizes and the remaining solution becomes more concentrated at the initial freezing point. Thus, the freezing point of the unfrozen portion of the shrimp meat is further reduced. As the temperature continues to decrease, the formation of ice crystals increases the concentration of solutes in solution and further depresses the freezing point (Fricke and Becker 2002; Rahman et al. 2002).

Statistical analysis

Table 2 presents the experimental results on the effect of the independent variables including glycine content, sodium chloride content and D-sorbitol content on the four response functions (T_i , W_u , C_{app} and H). Table 3 shows the regression coefficients of the variables and corresponding R^2 values of the fitted model. The results of statistical analysis showed that fitted model was adequate with satisfactory R^2 values. The R^2 value for T_i , W_u , C_{app} and H were 0.999, 0.997, 0.939 and 0.896, respectively, indicating that the regression models explained the variability in the response variables.

Initial freezing point (T_i)

It was observed from Table 3 that T_i was related to the negative linear effect of glycine content ($P < 0.01$), sodium chloride content ($P < 0.01$) and D-sorbitol content ($P < 0.05$). The quadratic terms of glycine content ($P < 0.01$) and D-sorbitol content ($P < 0.05$) had a negative effect whereas sodium chloride content ($P < 0.01$) had positive effect on T_i . Fig. 1a describes the dependence of T_i with glycine and sodium chloride content at constant D-sorbitol content. It was evident that at a fixed sodium chloride and D-sorbitol content, the T_i decreased slowly with glycine content whereas it decreased sharply with sodium chloride content (as observed in Table 3). The interaction effect between glycine and sodium chloride content was very significant ($P < 0.01$). Fricke and Becker (2002) and Pighin et al. (2008) reported that various ions and acids contributed to the freezing point depression. And in our research, the addition of glycine and sodium chloride notably

increased the concentrations of soluble components and the amount of bound water in shrimp meat so that the initial freezing point shifted to lower temperature.

The variation of T_i with sodium chloride and D-sorbitol content at constant glycine content is displayed in Fig. 1b. It was evident that at a fixed glycine and D-sorbitol content, the T_i varied roughly with sodium chloride content. This indicated that the linear and quadratic effect of sodium chloride content was very significant ($P < 0.01$) on initial freezing point depression. It could be observed that the T_i decreased slowly with increase in D-sorbitol content, which may be attributed to the multi-hydroxyl structure of D-sorbitol. In addition, the interaction effect was also very significant ($P < 0.01$) between sodium chloride and D-sorbitol content.

Unfreezable water mass fraction (W_u)

In general, 100 % of the freezable water will be frozen at a temperature of $-40\text{ }^{\circ}\text{C}$, while the remaining water that cannot be formed into ice at even lower temperature is defined as the unfreezable water (Miles et al. 1997; Hamdami et al. 2004a). Furthermore, it was reported by Wolfe et al. (2002) and van der Sman and Boer (2005) that the unfreezable water consisted of free water and bound water, in which the latter was viewed as the water of hydration of soluble biopolymers such as proteins and carbohydrates and also the special water with respect to freezing that had a crystal-like structure and was unavailable for solving solutes. In this study, it was clear from Table 3 that the W_u had a positive linear effect with glycine content ($P < 0.01$), sodium chloride and D-sorbitol content ($P < 0.05$). The quadratic terms of glycine content ($P < 0.01$), sodium chloride and D-sorbitol content ($P < 0.05$) were also found to be statistically notable while considering the W_u . Glycine and sodium chloride content ($P < 0.05$) as well as glycine and D-sorbitol content ($P < 0.01$) had a positive interaction effect on the W_u , and on the contrary the interaction effect between sodium chloride and D-sorbitol content was not significant ($P > 0.05$).

Figure 2a presents the variation of the W_u with glycine and sodium chloride content at a fixed D-sorbitol content. It was observed that at a fixed sodium chloride and D-sorbitol content, the W_u increased slowly with glycine content whereas it increased nearly linearly with sodium chloride content at constant glycine and D-sorbitol content. This fact is confirmed by the observations of the values of coefficients (Table 3). The interaction effect between glycine and sodium chloride content was significant ($P < 0.05$) on the increase of unfreezable water. Similar observations were made earlier by Herrera and Mackie (2004) and Lu et al. (2007). This might be attributed to the increase of amino acid and ion concentration and the acceleration of hydration among free water, glycine and other soluble ingredients, which made the water “unfreezable”.

Fig. 1 Response surface diagram for T_i of Pacific white shrimp as affected by glycine, sodium chloride and D-sorbitol content. Where **a** is glycine content and sodium chloride content; **b** is sodium chloride and D-sorbitol content

The variation of W_u with glycine and D-sorbitol content at constant sodium chloride content is presented in Fig. 2b. It was evident that at a fixed D-sorbitol and sodium chloride content, the W_u increased sharply with glycine content whereas it increased roughly linearly with D-sorbitol content at a fixed glycine and sodium chloride content. As clearly displayed in Table 3, the interaction effect between glycine and D-sorbitol content was very significant ($P < 0.01$). These substances interact strongly with water and participate in the water lattice through hydrogen bonds, which lead to formation of extended region of hydrogen-bonded structured water in the vicinity of the cryoprotective molecule and increase of

unfreezable water (Smolinska et al. 1995; Auh et al. 2003; Kovačević et al. 2007).

Apparent specific heat (C_{app})

It was clear from Table 3 that the C_{app} had a negative linear effect with glycine content ($P < 0.05$), sodium chloride ($P < 0.01$) and D-sorbitol content ($P < 0.05$), and moreover the quadratic terms of the three regulators showed the same significance. Besides, glycine and sodium chloride content ($P < 0.01$) had a positive interaction effect; in contrast sodium chloride and D-sorbitol content ($P < 0.01$) had a negative interaction effect.

Fig. 2 Response surface diagram for W_u of Pacific white shrimp as affected by glycine, sodium chloride and D-sorbitol content. Where **a** is glycine content and sodium chloride content; **b** is glycine and D-sorbitol content

Figure 3a presents the variation of the C_{app} with glycine and sodium chloride content at a fixed D-sorbitol content. It was observed from the figure that at a fixed sodium chloride and D-sorbitol content, the C_{app} decreased with glycine content down to a critical value beyond which it increased whereas it decreased slowly with sodium chloride content at constant glycine and D-sorbitol content. This fact is corroborated by the observations of the values of coefficients are shown in Table 3. The interaction effect between glycine and sodium chloride content was very notable ($P < 0.01$).

The variation of C_{app} with glycine and D-sorbitol content at constant sodium chloride content is presented in Fig. 3b. It was evident that a fixed glycine and sodium chloride content,

the C_{app} decreased nearly linearly with D-sorbitol content. However, the C_{app} decreased with glycine content down to a critical value beyond which it increased at constant D-sorbitol and sodium chloride content. Due to the release of latent heat at the initial freezing point (Wang and Kolbe 1991; Tocci et al. 1997; Becker and Fricke 1999; Hamdami et al. 2004a; Tocci and Mascheron 2008), the effect of low glycine content on C_{app} might be weaker than those of low sodium chloride and D-sorbitol content. This phenomenon needs however to be addressed in further studies. In addition, the interaction effect between glycine and D-sorbitol content was still very significant ($P < 0.01$) at constant sodium chloride content (Table 3).

Fig. 3 Response surface diagram for C_{app} of Pacific white shrimp as affected by glycine, sodium chloride and D-sorbitol content. Where **a** is glycine content and sodium chloride content; **b** is glycine and D-sorbitol content

Enthalpy (H)

Enthalpy is also a key thermal property and state function used to solve the non-linear heat diffusion equation when taking into account phase transition (Wang and Kolbe 1991; Hamdami et al. 2004a, b). From Table 3, it was seen that the H had a negative linear effect with glycine content ($P < 0.01$), sodium chloride content ($P < 0.01$) and D-sorbitol content ($P < 0.05$). The quadratic terms of glycine and sodium chloride content were found to be statistically significant ($P < 0.01$) but that of D-sorbitol content was not ($P > 0.05$) when considering the H . Glycine and D-sorbitol content ($P < 0.01$) as well as sodium chloride and D-sorbitol content ($P < 0.05$) had a negative interaction effect whereas glycine and sodium chloride content ($P < 0.01$) had positive effect on H .

Similarly to the changes of C_{app} , the effects of glycine and sodium chloride content on the variation of the H at a fixed D-sorbitol content are depicted in Fig. 4a. Easily observed that at a fixed sodium chloride and D-sorbitol content, the H decreased with glycine content down to a critical value beyond which it increased whereas it decreased slowly with sodium chloride content at constant glycine and D-sorbitol content. This fact is also corroborated by the observations of the values of coefficients are shown in Table 3. Besides, the phenomenon could be ascribed to the functional relations between C_{app} and H , which specific heat represents the rate of enthalpy change with the temperature (Hamdami et al. 2004a; Tocci and Mascheron 2008).

The variation of H in relation to glycine and D-sorbitol content at constant sodium chloride content is presented in

Fig. 4b. It was evident that at a fixed D-sorbitol and sodium chloride content, the H decreased with glycine content down to a critical value beyond which it increased; on the contrary the H slowly decreased with D-sorbitol content at constant glycine and sodium chloride content. Also, the interaction

effect between glycine and D-sorbitol content was very significant ($P<0.01$).

Figure 4c displays the effects of sodium chloride and D-sorbitol content on H at a fixed glycine content. It was observed from the figure that the H decreased linearly with D-sorbitol content at constant glycine and sodium chloride content whereas it gradually decreased with sodium chloride content at a fixed glycine and D-sorbitol content. Considering that most of the water in muscle was held within the myofibrils, in the space between myosin and actin (Tomberg 2005; Cui et al. 2013) and amount of sodium ions were incorporated to alter original hydration properties and increased protein solubilization, gradually the peak of latent heat and thermal absorption weakened together with the addition of sodium chloride (Lu et al. 2007; Pighin et al. 2008). Similarly, the interaction effect between sodium chloride and D-sorbitol content was also significant ($P<0.05$) on H .

Parameters optimization and verification

Secondary order polynomial models fitted for each response to obtain maximum W_u and minimum T_i , C_{app} and H of Pacific white shrimp. In this study to optimize the response variables the three freezing-point regulators were selected in the range of glycine content (0.250–1.250 %), sodium chloride content (0.500–2.500 %) and D-sorbitol content (0.125–0.625 %). Desirability function method was used to optimize the response and the optimum concentration were 0.876 % for glycine content, 2.298 % for sodium chloride content and 0.589 % for D-sorbitol content. At this point, the predicted values of responses (T_i , W_u , C_{app} and H) were -5.086 °C, 17.222 %, 41.038 J/g °C and 155.942 J/g, respectively.

Based on the optimized parameters, the fresh Pacific white shrimp were previously treated with formulated freezing-point regulators and then all the response variables of the final samples were analyzed. As presented in Table 4, the experimental and predicted values had no statistically significant difference ($P<0.05$) between them, and indicating that the freezing-point regulators

Fig. 4 Response surface diagram for H of Pacific white shrimp as affected by glycine, sodium chloride and D-sorbitol content. Where **a** is glycine content and sodium chloride content; **b** is glycine and D-sorbitol content; **c** is sodium chloride and D-sorbitol content

Table 4 Predicted and experimental values of the response variables at optimum formulated parameters^a

Response variables	Optimum pacific white shrimp	
	Predicted values	Experimental values
T_i (°C)	-5.086	-5.243 ± 0.458
W_u (%)	17.222	18.278 ± 0.277
C_{app} (J/g °C)	41.038	42.138 ± 1.323
H (J/g)	155.942	153.782 ± 2.752

^a The optimum combination of process variables was 0.876 % glycine content, 2.298 % sodium chloride content and 0.589 % D-sorbitol content

(glycine, sodium chloride and D-sorbitol) interacted strongly with free water and participated in the water lattice through hydrogen bonds, and this presumably led to formation of extended region of hydrogen-bonded structured water in the vicinity of the cryoprotective molecule, which may stabilize surface hydration of protein and protect them from freeze-denaturation during storage (Auh et al. 2003; Herrera and Mackie 2004; Kovačević et al. 2007), and in the meantime the thawing phase transition range of Pacific white shrimp shifted to the lower temperature, and the content of unfreezable water increased while the initial freezing point linearly decreased, and corresponding peak value of latent heat and apparent specific heat also decreased. In particular, the magnitude of the initial freezing point depression indicated the susceptibility of the food to microbial growth. The lower the initial freezing point, the more microbiologically stable the food. The slower the ice content increases as the temperature is lowered (Mossel et al. 1995; Miles et al. 1997). Therefore, 0.876 % glycine, combined with 2.298 % sodium chloride and 0.589 % D-sorbitol, as formulated freezing-point regulators, can be better used to optimize the thermophysical properties of Pacific white shrimp and keep the quality of Pacific white shrimp in the certain range.

In the present study the regression equations obtained is valid within the limits of experimental factors used and could be employed to find the optimum formulated parameters for the desired thermophysical properties of Pacific white shrimp previously treated with freezing-point regulators. However, the extrapolation of the responses beyond the experimental range of the independent variables applied in this study may not be valid. Likewise, any changes in the compositions of shrimp will influence all thermophysical properties, with the possibility of a different optimal solution. However it is worth noting that the use of the three freezing-point regulators effectively depressed the freezing point of Pacific white shrimp (from -1.504 to -5.086 °C), which will be applicable values for exploration of thermal processing and food storage, especially controlled freezing-point storage.

Conclusion

RSM was employed to determine the optimum thermophysical properties of Pacific white shrimp previously treated with freezing-point regulators to maximize W_u and minimize T_i , C_{app} and H . Analysis of variance had shown that the effects of glycine, sodium chloride and D-sorbitol on the thermophysical properties were statistically significant ($P < 0.05$), and secondary order polynomial models were obtained

for predicting W_u , T_i , C_{app} and H . The optimum combination of process variables was 0.876 % glycine content, 2.298 % sodium chloride content and 0.589 % D-sorbitol content and the corresponding optimized thermophysical properties were -5.086 °C for initial freezing point, 17.222 % for unfreezable water mass fraction, 41.038 J/g °C for apparent specific heat and 155.942 J/g for enthalpy, which would be obviously beneficial for the exploitation for various thermal processing, chilled, refrigerated and controlled freezing-point storage, food control and development of equipments on shrimps.

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