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Research Article

Research on the Technology of Inhibiting Browning in Chestnut Paste Processing

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Abstract: Chestnut (*Castanea mollissima*) is a multipurpose species that is cultivated for timber, nut, tannin and contributes positively to the forestry landscape. It is important in chestnut paste processing to prevent the chestnuts browning. For the past few years, there were many articles about inhibiting browning in fruits and vegetables processing. However, there were few articles about inhibiting browning in chestnuts processing. This study is mainly studied on the processing technology of preventing browning during the chestnut paste processing. The single factor experiment and the orthogonal experiment were used to determine the proportion of the best color-protect effect of the chestnuts. In this study, the optimum recipe of composite color-protect solutions for chestnut kernels was 0.25% EDTA-2Na, 0.10% Citric acid, 0.15% Vc and 0.25% chitosan. The optimum recipe of composite color-protect solutions for chestnut paste was 0.15% EDTA-2Na, 0.13% Citric acid and 0.30% Vc.

Keywords: Browning, chestnut paste, color protection

INTRODUCTION

Chestnut (*Castanea mollissima*) is a multipurpose species that is cultivated for timber, nut, tannin and contributes positively to the forestry landscape. There are abundant nutrients in china chestnuts (Pereira-Lorenzo *et al*., 2006). It is distributed mainly in the Northern Hemisphere, in Southern Europe from Turkey to Atlantic Islands and in the United States, in Asia mostly in China, Korea and Japan (Pereira-Lorenzo and Ramos-Cabrer, 2004). Many previous investigations documents that there are 62~70 g starch, 5.1~10.7 g protein, 2~7.4 g fat and 40~45 g carbohydrate in 100 g chestnuts (Breisch, 1995; Ensminger *et al*., 1995). In China, the output of chestnuts is the first of the world. With the domestic economic development, the requirement of chestnut paste will increase rapidly in the next decades. However, it is important in chestnut paste processing to prevent the chestnuts browning (Zhou *et al*., 2015b). Therefore, preventing the chestnut paste from browning will enhance the profitability of chestnuts processing industry.

Nowadays, there are accumulating investigations about the mechanism of browning and the preventing methods (He *et al*., 2008; López-Nicolás *et al*., 2007; Segovia-Bravo *et al*., 2009). There is a common view that the browning mechanism of fruit and vegetables is related with enzymatic browning which is also a technical problem in chest processing industry (Zhao *et al*., 2014; Zhou *et al*., 2015b). When the chestnuts are processed, the structure of chestnuts are destroyed, resulting in browning for oxygen combining with the polyphenol and enzymes related with browning (Finley and Given Jr., 1986). For the past few years, there were many articles about inhibiting browning in apples (Chen *et al*., 2015), bananas (Quevedo *et al*., 2009), apricots (Durmaz and Alpaslan, 2007), cherry (Pasquariello *et al*., 2015), strawberries (Sulaiman and Silva, 2013) and potatoes (Limbo and Piergiovanni, 2006). However, there were few articles about inhibiting browning in chestnuts processing.

This study investigates the inhibiting browning technology of different process stage in chestnuts processing and the effects of the different inhibiting browning technology.

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Table 1: Coded values and experimental range of variables for chestnut kernels

	Factors			
Level	A EDTA-2Na	B Citric acid	C Vc	D Chitosan
1	0.15%	0.10%	0.10%	0.25%
2	0.20%	0.15%	0.15%	0.30%
3	0.25%	0.20%	0.20%	0.35%

Table 2: The results of orthogonal experiments of composite color-protect solutions for chestnut kernels

NO	A	B	C	D	ΔE
1	1	1	1	1	3.10±0.07
2	1	2	2	2	3.21±0.16
3	1	3	3	3	4.70±0.22
4	2	1	2	3	4.34±0.18
5	2	2	3	1	4.71±0.14
6	2	3	1	2	4.86±0.15
7	3	1	3	2	3.41±0.24
8	3	2	1	3	2.99±0.47
9	3	3	2	1	2.79±0.33
K_1	11.01	10.85	11.46	10.59	
K_2	13.91	10.91	10.35	11.49	
K_3	58.17	12.36	12.81	12.54	
k_1	3.67	3.67	3.82	3.53	
k_2	4.63	3.80	3.45	3.83	
k_3	3.23	4.12	4.27	4.18	
R	1.40	0.43	0.82	0.65	

Table 3: The results of variance analysis

	SS	df	MS	F	P
A (EDTA-2Na)	36.9654	2	18.4827	32.4283	0.0001
B (citric acid)	4.6394	2	2.3197	4.0700	0.0348
C (Vc)	12.0878	2	6.0439	10.6042	0.0009
D (chitosan)	7.5488	2	3.7744	6.6223	0.0070
Error	10.2592	18	0.5700		

Table 4: Coded values and experimental range of variables for chestnut paste

	Factors		
Levels	A EDTA-2Na	B Citric acid	C Vc
1	0.15%	0.10%	0.30%
2	0.20%	0.13%	0.40%
3	0.25%	0.16%	0.50%

Table 5: The results of orthogonal experiments of composite color-protect solutions for chestnut paste

NO	A	B	C	ΔE
1	1	1	1	3.37±0.210
2	1	2	2	3.81±0.381
3	1	3	3	7.27±0.108
4	2	1	2	7.68±0.209
5	2	2	3	5.92±0.340
6	2	3	1	6.25±0.065
7	3	1	1	6.40±0.070
8	3	2	3	7.11±0.122
9	3	3	2	7.02±0.220
K_1	43.3500	52.3500	50.1900	
K_2	59.5500	50.5200	55.5300	
K_3	61.5900	61.6200	58.7700	
k_1	4.8167	5.8167	5.5767	
k_2	6.6167	5.6133	6.1700	
k_3	6.8433	6.8467	6.5300	
R	2.0267	1.2333	0.9533	

MATERIALS AND METHODS

Materials and chemicals: Chestnuts were obtained from Wuhan, Hubei province in China. Ethylenediamine tetraacetic acid disodium salt (EDTA-2Na) was purchased in Tianjin HengXing chemical Reagent co., LTD (Tianjing, China). Citric acid, Vitamin C (Vc) and chitosan were purchased in Xiamen Blue Bay Science and Technology co., LTD (Xiamen, China). All the chemicals were of analytical grade.

Preparation technology of chestnut paste: The chestnut shells and kernel coating were stripped after the chestnuts being heated up to 100°C for 1 min and 30°C for 15 min. The kernels were soaked in different color-protection solutions, combined with water (1:0.6, g/mL) and mashed with blender (ACA, AF-YM03, USA) to chestnut paste. The chestnut paste was soaked in different color-protection solution and packed.

Single factor experiment of color-protection solution for chestnuts kernels: The chestnut kernels were soaked in EDTA-2Na, Citric acid, the aqueous solution of Vc and the aqueous solution of chitosan respectively with different concentration gradient (0, 0.1, 0.15, 0.20, 0.25, 0.30, 0.35%, respectively). After 30 min, the color changes of different samples were measured with colorimeter (Minolta CR-321, Japan).

Determination of the ratio of color-protection solution with orthogonal experiments for chestnuts kernels: Based on the results of single factor experiments of color-protection solution for chestnuts kernels, a three-level-four-factor orthogonal experiments were used to determine the best combination variables for color-protection effect of chestnuts kernels. Table 1 shows the range of each factor. The color changes of chestnut kernels with combined color-protection solutions presented as Table 2 were measured with colorimeter. The optimal recipe of color-protection solution for chestnut kernels was determined by variance analysis (Zhou *et al*., 2015a).

Single factor experiment of color-protection solution for chestnuts paste: The chestnut paste was soaked in EDTA-2Na (0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06%, respectively), Citric acid (0, 0.05, 0.07, 0.09, 0.11, 0.13, 0.15%, respectively), the aqueous solution of Vc (0, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60%, respectively) and the aqueous solution of chitosan (0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30%) respectively. After storing at room temperature for 5 d, the color changes of different samples were measured with colorimeter (Minolta CR-321, Japan) (Table 3).

Determination of the ratio of color-protection solution with orthogonal experiments for chestnuts paste: Based on the results of single factor experiments of color-protection solution for chestnuts paste, a three-level-three-factor orthogonal experiments were used to determine the best combination variables for color-protection effect of chestnuts paste. Table 4 shows the range of each factor. The color changes of chestnut paste with combined color-protection solutions

presented as Table 5 were measured with colorimeter. The optimal recipe of color-protection solution for chestnut paste was determined by variance analysis.

Color measurement: Color changes of the samples were analyzed by measuring their reflectance using a colorimeter. The color values were expressed using ΔE as the Eq. (1):

$$\Delta E = [(\Delta L)^2+(\Delta a)^2+(\Delta b)^2]^{1/2} \quad (1)$$

where, ΔL represents the luminosity (0 = black; 100 = white), Δa represents the redness (a*>0) or greenness (a*<0) and Δb represents the blueness (b*>0) or yellowness (b*<0). Each sample was measured three times and the result was presented as an average.

Statistical analysis: All experiments were performed in triplicate and randomized. Each data was presented as mean±standard deviation.

RESULTS AND DISCUSSION

The results of Single factor experiment of color-protection solution for chestnuts kernels: The color-protection effects of different solutions with different concentration gradient were presented in Fig. 1. As presented as Fig. 1, although EDTA-2Na, Citric acid, Vc and chitosan were used for color-protection solutions of chestnut kernels, there were different effects in different concentration. When the chestnut kernels were soaked in EDTA-2Na with the concentration of 0.20% (g/mL), the ΔE was 2.65±0.15, smaller than other concentration. When the chestnut kernels were soaked in Citric acid with the concentration of 0.15% (g/mL), the ΔE was 4.41±0.21, smaller than other concentration. When the chestnut kernels were soaked in Vc with the concentration of 0.15% (g/mL), the ΔE was 4.21±0.32, smaller than other concentration. When the chestnut kernels were soaked in chitosan with the concentration of 0.30% (g/mL), the ΔE was 3.02±0.17, smaller than other concentration.

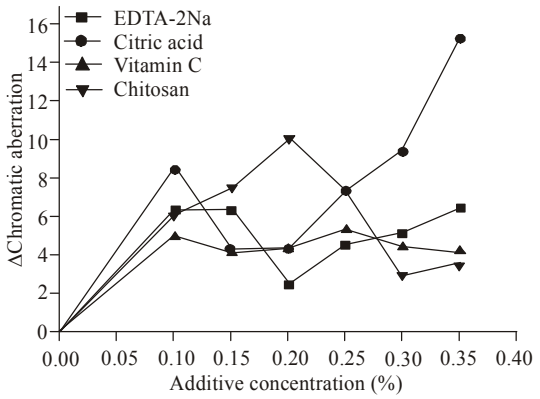

Fig. 1: The affection of color change in chestnut kernels with different color-protect solutions. n = 3

The reason that protect effect of these four color-protect solutions for chestnuts kernels was different was the different color-protect mechanism of these four solutions. The color-protect mechanism of EDTA-2Na and Citric acid was to inhibit the enzyme related browning by chelate the metal ions of the enzyme (Durge *et al*., 2013). Vc could inhibit browning by oxidation-reduction (Drach *et al*., 2011). Chitosan could inhibit browning by prolong the storage period of fruits which were plucked (Bastos *et al*., 2012; Pasquariello *et al*., 2015). Previous investigations documented that the browning of chestnuts was induced by the polyphenol oxidase (PPO) which was a metalloproteinase (Xu, 2005; Zhou *et al*., 2015b). Therefore, the color-protect effects of EDTA-2Na and Vc were more effective than other two solutions in the present article. Moreover, it is interesting that the color-protect effect of Vc in lower concentration was better than high concentration, which was indicating that Vc can inhibit oxidization at low concentration and promote oxidization at high concentration (Beker *et al*., 2011; Gao *et al*., 2014).

The results of orthogonal experiments for chestnuts kernels: The results of orthogonal experiments of composite color-protect solutions and variance analysis were presented in Table 2 and 3 respectively. The results of variance analysis showed that all four factors had significant impact on the chestnuts' color during the processing. As depicted in Table 2, the influence order of these four solutions was: EDTA-2Na>Vc>chitosan>Citric acid. The optimum levels of every factor were determined with k_1, k_2, k_3 values of every factor and the optimum levels were $A_3B_1C_2D_1$. In other words, the optimum recipe of composite color-protect solutions was 0.25% EDTA-2Na, 0.10% Citric acid, 0.15% Vc and 0.25% chitosan. There were some articles indicating that citric acid could promote the solubility and the preservative activity of chitosan. In the optimum recipe, the dosage of citric acid and chitosan was lower than the dosage in signal factor experiments, which illustrated that there were cooperation effects between citric acid and chitosan (Ducamp-Collin *et al*., 2008; Qiua *et al*., 2014).

The results of Single factor experiment of color-protection solution for chestnut paste: The color-protection effects of chestnut paste with different solutions at different concentration were presented in Fig. 2. As depicted in Fig. 2, the concentrations of EDTA-2Na, citric acid and Vc for the best color-protect effects of chestnut paste were 0.20, 0.13 and 0.40%, respectively. Different from other three solutions, chitosan promote browning of chestnut paste. Therefore, chitosan could not be considered in the orthogonal experiments.

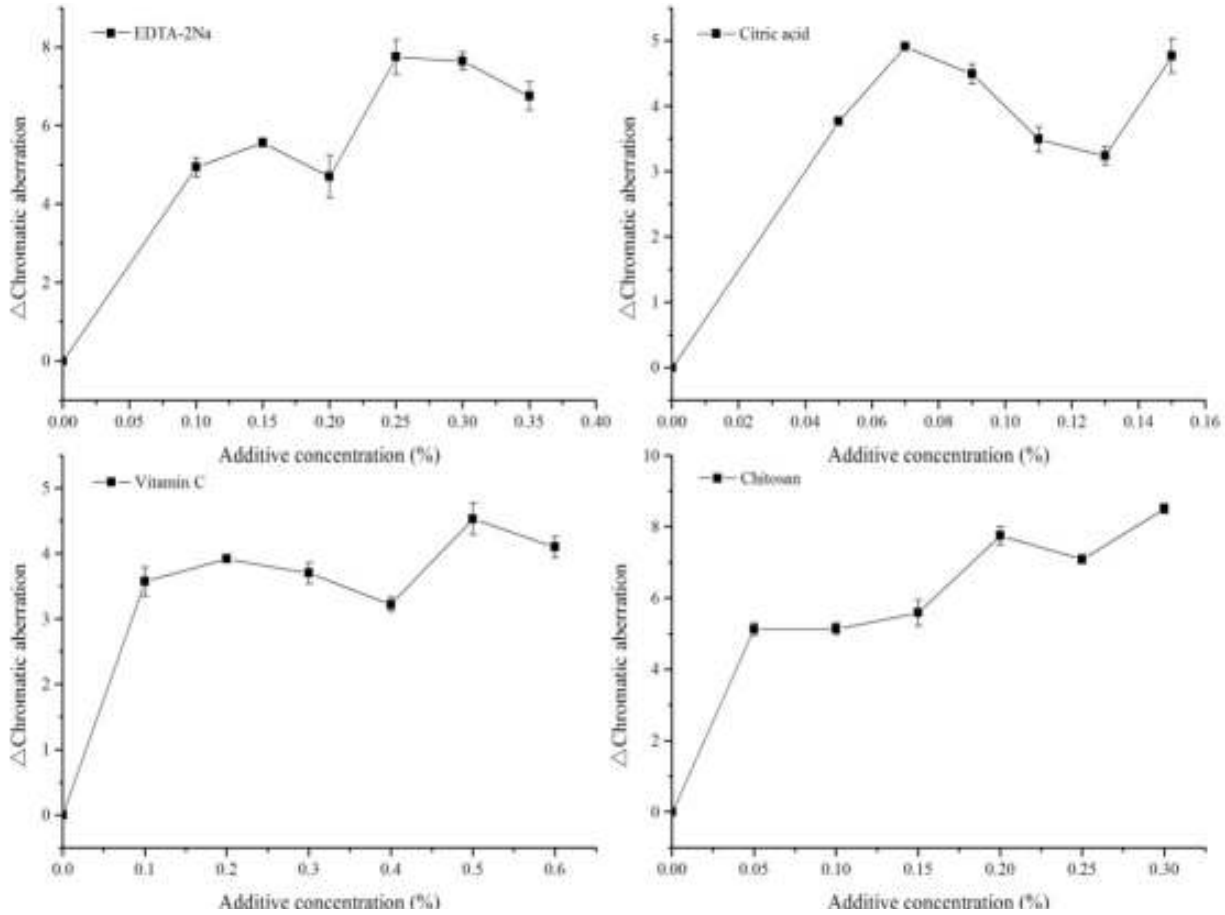

Fig. 2: The affection of color change in chestnut paste with different color fixatives. Mean value±standard deviation, n = 3

Table 6: The results of variance analysis

	SS	df	MS	F	P
A (EDTA-2Na)	22.1963	2	11.0981	230.0396	0.0001
B (citric acid)	7.8701	2	3.9350	81.5645	0.0001
C (Vc)	4.1715	2	2.0857	43.2326	0.0001
Error	0.8684	18	0.0482		

Accumulating investigations demonstrated that chitosan could inhibit browning (Martínez-Castellanos *et al*., 2009; Pasquariello *et al*., 2015; Xiao *et al*., 2011). However, according to the results of this study, it is interesting that chitosan could inhibit browning in chestnut kernels but promote browning in chestnut paste. Compared with chestnut kernels, there were more oxygen in the chestnut paste. Also, there were abound of active hydroxide radicals and amino groups in chitosan. Therefore, chitosan promote browning after being added to chestnut paste.

The results of orthogonal experiments for chestnuts paste: The results of orthogonal experiments of composite color-protect solutions and variance analysis for chestnut paste were presented in Table 5 and 6 respectively. The results of variance analysis showed that all three factors had significant impact on the chestnuts paste' s color during the processing. As depicted in Table 2, the influence order of these three solutions was: EDTA-2Na> Citric acid > Vc. The optimum levels of every factor were determined with k_1, k_2, k_3 values of every factor and the optimum levels were $A_1B_2C_1$. In other words, the optimum recipe of composite color-protect solutions was 0.15% EDTA-2Na, 0.13% Citric acid and 0.30% Vc. There were some articles indicating that citric acid could promote the solubility and the preservative activity of chitosan. In the optimum recipe, the dosage of citric acid and chitosan was lower than the dosage in signal factor experiments, which illustrated that there were cooperation effects between citric acid and chitosan.

During the process of chestnut paste, the structure of chestnuts was destroyed and PPO was released. There were metal ions in the active site of PPO. Therefore, the color-protect solutions whose mechanism was to inhibit the enzyme related browning by chelate the metal ions of the enzyme were more effective for chestnut paste than other solutions.

CONCLUSION

In this study, the optimum recipe of composite color-protect solutions for chestnut kernels was 0.25%

EDTA-2Na, 0.10% Citric acid, 0.15% Vc and 0.25% chitosan. The optimum recipe of composite color-protect solutions for chestnut paste was 0.15% EDTA-2Na, 0.13% Citric acid and 0.30% Vc.

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