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Original Research Article

Investigation of Maillard reaction involvement in the steam processing of Panax Notoginseng root

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Abstract

Purpose: *To explore the possible mechanism of Maillard reaction (MR) involvement in the steam processing of Panax notoginseng (PN) root.*
Methods: *PN root was soaked in water for 24 h and then steamed at 100 °C using an autoclave for 1, 2, 3, 4, 5 and 6 h, respectively. Several indicators associated with MR during steam processing were measured. The pH and absorbance at 420 nm (A420) of samples were measured using a pH meter and an ultraviolet-visible spectrophotometer, respectively. The contents of 5-hydroxy-methyl-furfural (5-HMF) and sugars were determined by high performance liquid chromatography (HPLC) while amino acids were evaluated using an automatic amino acid analyzer.*
Results: *During PN root steam processing (0 - 6 h), pH value gradually decreased from 6.35 ± 0.02 to 5.88 ± 0.03 while A420 value gradually increased from 0.23 ± 0.01 to 0.44 ± 0.02. The levels of reducing sugars (maltose and glucose) and amino acids (aspartic acid, glutamate, cysteine, lysine and arginine) in PN root decreased after steaming for 6 h. However, the content of 5-HMF in PN root increased with increase in steaming time.*
Conclusion: *The results indicate that MR occurs during steam processing of PN root, and the reaction mechanism might be closely related to the reaction between the reducing sugars and amino acids.*

Keywords: *Panax notoginseng, Steaming, Reducing sugars, Amino acids, Maillard reaction*

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INTRODUCTION

MR, known as non-enzymatic browning reaction, is the principal chemical reaction in food processing. It is a reaction between carbonyl compounds (reducing sugars, ketones or aldehydes) and amino compounds (amino acids, proteins or any nitrogenous compound) [1,2], and it often occurs during the steam processing of many traditional Chinese medicines [3]. Studies have shown that MR can generate some new compounds, such as 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4(H)-pyran-4-one (DDMP), 5-HMF, and maltol, et al. These new compounds (MR products) are responsible for the changes in color, aroma or nutritive value of processed foods, and they were reported to have

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antioxidant, antimicrobial and antimutagenic effects [4,5].

PN (*Panax notoginseng*) is an important traditional Chinese medicinal plant from the Araliaceae family, and is widely distributed in Guangxi and Yunnan provinces of China [6]. The root of PN, named *Sanqi* or *Tianqi* in Chinese, has been used as a traditional Chinese Medicine (TCM) for more than 400 years. Previous studies have demonstrated that PN root comprised numerous chemical constituents, such as saponins, flavonoids, cyclopeptides, sterols, polyacetylenes, saccharides, amino acids, and volatile oil [7,8]. Two different forms of PN root (raw and steamed) have been used in clinics in China due to their different therapeutical functions [9,10]. Investigations have shown that raw PN root possesses multiple pharmacological effects on cardiovascular and immune systems as well as excellent anti-inflammatory, anti-atherosclerotic, haemostatic and anti-tumor effects, etc. [7,11,12]. On the other hand, steamed PN root is mainly used as a tonic to 'nourish' blood and for blood cell-increasing function in anemic conditions [9,10].

It has been reported that MR were widely involved in the processing of food and TCM. However, there is no report about the MR during the steam processing of PN root, and the mechanisms of the reaction remain unclear. In the present study, several indicators associated with MR, such as the pH value, A_{420} value, and the contents of 5-HMF, sugars and amino acid were analyzed in order to investigate the mechanism of MR.

EXPERIMENTAL

Chemicals and reagents

Glucose, fructose, maltose, 5-HMF, and sucrose standards were purchased from National Institute for Food and Drug Control (Beijing, China). Amino acids standards were obtained from Hitachi, Ltd. (HQ, Japan). Acetonitrile was obtained from Sigma Chemicals Co. (St. Louis, MO, USA). All other chemicals and reagents used in the study were of analytical grade.

Plant material and processing

The PN root was purchased from Yunnan Qidan Pharmaceutical Co., Ltd. (Wenshan, China) in March, 2017 (batch No. D1703016), and identified by Prof. Chun-Jie Wu in College of Pharmacy, Chengdu University of Traditional Chinese Medicine (Chengdu, China). A specimen (sPN-20170515) was deposited in the herbarium of College of Pharmacy, Chengdu University of Traditional Chinese Medicine (Chengdu, China). PN root was soaked with water for 24 h, and then steamed at 100 °C for 1, 2, 3, 4, 5 and 6 h by an XFS-280CB autoclave (Xinfengyiliao, Zhejiang, China), respectively. The steamed samples were dried at 50 °C, and then powdered using a HX-200 pulverizer (Huaxi, Zhejiang, China).

Determination of pH and A_{420}

The powder (0.5 g) of the samples were extracted with 100 mL of deionized water for 1 h by reflux, and the supernatants were filtered after cooling. The pH values were measured using a PHS-3C pH meter (Leici, Shanghai, China) and the A_{420} values were determined using an *ultraviolet-visible spectroscopy* (Puxitongyong, Beijing, China) at 420 nm according to a previous report [3].

Determination of 5-HMF content

Sample preparation: A 10 mL volume of 40 % (v/v) methanol was added to 0.5 g of the PN root powder and then extracted by an AS5150A ultrasonic extractor (Auto science, Tianjin, China) for 30 min at room temperature, then the solutions were filtered through a 0.22 μm nylon filter membrane before analysis. The 5-HMF (0.036 mg/mL) standard was dissolved by methanol.

The content of 5-HMF was determined by using an Agilent Technologies 1260 system (Agilent Crop., MA, USA). The chromatography was performed on a PHENOMENEX C_{18} (250 × 4.6 mm, 5 μm) column. Acetonitrile (solvent A) and water (solvent B) were used as gradient elution system with a linear gradient: 0 - 16 min (2.5 %, A), 16 - 25 min (2.5 - 8.0 %, A), 25 - 35 min (8.0 - 2.5 %, A). The flow rate, injection volume, column temperature and the detection wavelength were 0.8 mL/min, 10 μL, 35 °C, and 283 nm, respectively.

Determination of (reducing) sugar contents

Sugar contents analysis were performed on an Agilent Technologies 1260 system (Agilent Crop., MA, USA) coupled with an 2000ES ELSD (Alltech, USA). The chromatography was performed on a CAPCELLPAKNH2 UG80 (250 × 4.6 mm, 5 μm) column. The mobile phase was a mixture of acetonitrile and water (82 : 18, *v/v*) at a flow rate of 0.5 mL/min. The column temperature, ELSD detector temperature, gas flow, and the injection volume were 35 °C, 105 °C, 2.5 L/min and 10 μL, respectively. The

mixture of standard solutions were prepared by dissolving fructose (5.65 mg), glucose (7.49 mg), sucrose (5.76 mg) and maltose (5.63 mg) with 5 mL deionized water. The mixed sugar standard solutions were filtered through a 0.22 μm membrane filter before analysis. The sample powder (0.5 g) was ultrasonically extracted with 30 mL deionized water at room temperature for 30 min. Then 20 % zinc acetate (2 mL) and 20 % potassium ferrocyanide (2ml) were added, homogeneously mixed and filtered. The filtrate was then added water to 50 mL [13]. The solution was centrifuged (4000 rpm, 10 min) and filtered through a 0.22 μm membrane before analysis.

Determination of amino acid contents

An L-8900 fully-automated amino acid analyzer (Hitachi, Tokyo, Japan) was used to determine the contents of 17 amino acids in raw PN root and steamed PN root (steamed for 6 h). Samples (5 g) were weighed and the qualitative analysis was based on the standard method [14].

Statistical analysis

All the experiments were performed in triplicate and the results are presented as mean ± SD. ANOVA (with the aid of SPSS17.0 software, SPSS Inc., Chicago, IL, USA) was used for group comparison. $P < 0.05$ was considered statistically significant.

RESULTS

Changes in pH and absorbance

Changes in pH and absorbance are shown in Figure 1 and Figure 2, respectively. The pH value gradually decreased (from 6.35 ± 0.02 to 5.88 ± 0.03), whereas A_{420} value increased almost linearly (from 0.23 ± 0.01 to 0.44 ± 0.02) with increase in steaming time in the steam processing.

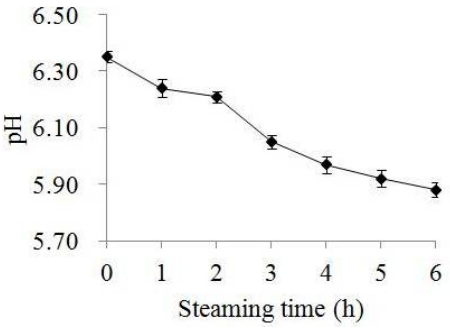

Figure 1: pH change during steam processing of PN root

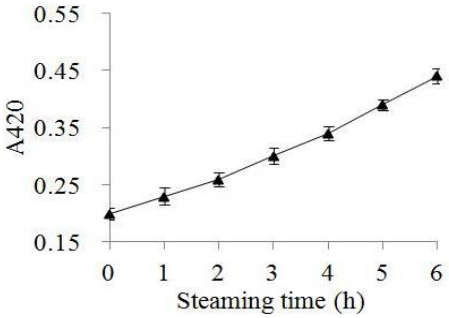

Figure 2: Change in absorbance during steam processing of PN root

Change in 5-HMF content

The calibration curve, precision, repeatability, stability and recovery test for the HPLC method were examined and validated. A regression equation ($y = 3.438 \times 10^8 x + 1200606$, $R^2 = 0.9998$) was obtained by a series of 5-HMF standard solutions .The results demonstrated that the regression equation presented a good linearity in the ranges of 0.019 to 0.384 μg. The peak area RSD values of the 5-HMF in the precision, repeatability and stability tests were 0.86, 0.91 and 0.76 % (< 3.0 %), respectively. The results implied that the HPLC method presented a good repeatability, precision and stability. The mean recovery was 99.1 %, and RSD value was 1.12 % (< 3.0 %), indicating that the HPLC method was also accurate.

HPLC chromatograms of the 5-HMF standard and PN root samples are shown in Figure 3, while the correlation between 5-HMF content and steaming time are depicted in Figure 4. The results showed that the content of 5-HMF increased with the steaming time during the steam processing. Compared to the raw PN root, in the PN root steamed for 1 h, the content of 5-HMF increased significantly to 0.38 ± 0.04 mg/g, and then increased almost linearly.

Changes in (reducing) sugar contents

The HPLC analysis was validated by calibration curves, repeatability, precision, stability and recovery tests. The results of standard calibration curves for the four sugars including regression equation, coefficient of association (R^2) and linear range were shown in Table 1. The regression equations for fructose, glucose, sucrose, and maltose were y = 0.9951x + 5.3496, y = 1.0177x + 5.5898, y = 0.9717x + 5.6072 and y = 0.9404x + 5.6021, respectively. In addition, the regression equations showed good linear relationship ($R^2 > 0.998$) with linearity ranges of 0.45 - 5.65, 0.60 -7.49, 0.46 - 5.76, and 0.45 - 5.63 μg, respectively.

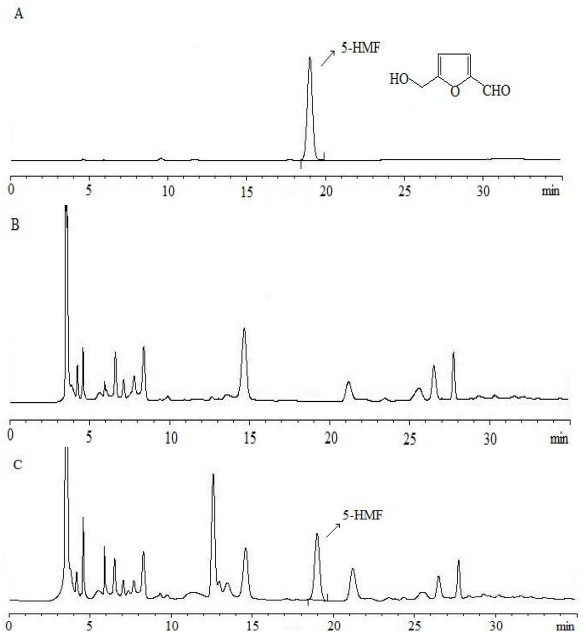

Figure 3: HPLC chromatograms of 5-HMF standard (A), raw PN root (B) and steamed PN root for 6 h (C).

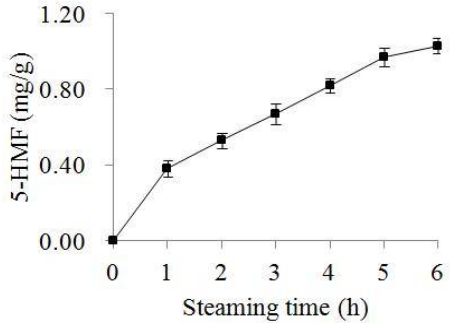

Figure 4: Change in 5-HMF content during steam processing of PN root

The results of precision, stability, repeatability and recovery tests were shown in Table 2. RSD values of the four sugars for the precision tests were 0.89, 0.71, 0.76 and 0.92 % (< 3.0 %), respectively, implying that HPLC system showed a good precision. The RSD values of the four sugars for the other three tests were also below 3.0 %, which represented that the samples were stable in 24 h. All these results indicated that the HPLC method for sugar determination were repeatable and accurate.

The established HPLC method was performed for the determination of the four sugars in the samples steamed for different times. The HPLC chromatograms of the standards and samples are shown in Figure 5, while the contents of fructose, glucose, sucrose and maltose depicted in Table 3. The results showed that the content of maltose reduced rapidly from 9.32 ± 0.08 to 0.00 (mg/g) after the first hour, and then could not be detected. The content of fructose gradually increased from 1 to 6 h, on the contrary, the glucose and sucrose contents gradually decreased in the whole steam processing.

Table 1: Regression equation, coefficient of association (R^2), linear range for glucose, fructose, maltose and sucrose

Content	Regression equation	R^2	Linear range (μg)
Fructose	Y=0.9951X+ 5.3496	0.9987	0.45-5.65
Glucose	Y=1.0177X+ 5.5898	0.9991	0.60-7.49
Sucrose	Y=0.9717X+ 5.6072	0.9989	0.46-5.76
Maltose	Y=0.9404X+ 5.6021	0.9989	0.45-5.63

Table 2: Precision, repeatability, stability, and recovery data for the HPLC system

Content	Precision	Stability	Repeatability	Recovery tests	
	RSD (%)	*RSD (%)*	*RSD (%)*	*Mean recovery (%)*	*RSD (%)*
Fructose	0.89	0.72	0.43	100.06	1.23
Glucose	0.71	1.25	1.05	98.52	1.12
Sucrose	0.76	1.12	1.13	98.87	1.06
Maltose	0.92	0.75	0.67	99.89	1.17

Table 3: Sugar content during steam processing of PN root

Steaming time (h)	Fructose (mg/g)	Glucose (mg/g)	Sucrose (mg/g)	Maltose(mg/g)
0	8.82 ± 0.13	17.72 ± 0.14	66.10 ± 0.07	9.32 ± 0.08
1	9.41 ± 0.05	14.06 ± 0.12*	64.48 ± 0.15*	--
2	10.65 ± 0.11	13.86 ± 0.09*	60.32 ± 0.10*	--
3	12.74 ± 0.15	12.30 ± 0.11*	59.01 ± 0.13*	--
4	12.23 ± 0.10	10.57 ± 0.08*	58.22 ± 0.08*	--
5	15.36 ± 0.08	9.59 ± 0.18*	55.25 ± 0.06*	--
6	17.07 ± 0.17	9.56 ± 0.16*	54.45 ± 0.12*	--

*P < 0.05 compared with raw PN root

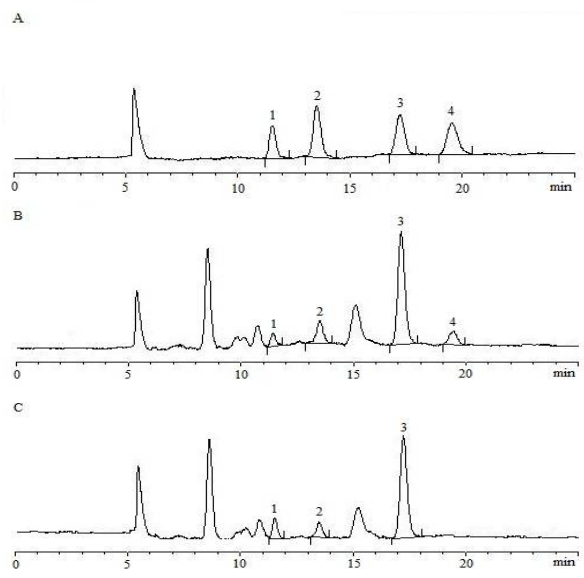

Figure 5: HPLC chromatograms of mixed sugar standards (A), raw PN root (B) and steamed PN root for 6 h (C). 1 - 4 represent fructose, glucose, sucrose, and maltose, respectively

Changes in amino acids

The results for contents of 17 amino acids in raw PN root and steamed PN root for 6 h were shown in Table 4. It was found that the content of 14 amino acids decreased for different extent, one amino acid (tyrosine) increased and two amino acids (phenylalanine and histidine) in steamed root for 6 h had no changes when compared to raw PN root. In addition, aspartic acid, glutamate, cysteine, lysine, and arginine were significantly decreased after steaming ($P < 0.05$).

DISCUSSION

It has been reported that MR begins with a condensation between carbonyl and amino groups. Subsequently, a series of reactions take place, such as Amadori rearrangement, enolization, Strecker degradation, aldol condensation and aldehyde-amide polymerization, leading to the formation of melanoidins and fragrant flavors [3,15]. In the present study, the contents of sugars and amino acids were determined in the raw and steamed PN root, and the results indicated that the heating-induced reduction in reducing sugars and amino acids may be due to the MR.

Previous studies have reported that 5-HMF was the intermediate products of MR and it formed from dehydration and degradation of carbohydrates in MR [14,15]. The 5-HMF was often generated in processed Chinese material medica and it usually be regarded as an indicator of the degree of MR [16]. In the present study, the level of 5-HMF increased gradually throughout the whole steam processing of PN root, indicating that the degree of MR was enhanced with the steaming time. It has been also reported that the fall in pH was related to the MR, and was due to the consumption of amino groups, as well as the productions of MR, such as formic acid, acetic acid and glyoxal [17,18]. In addition, A_{420} value is usually used as indicator of the content of melanoidins (the end-product of MR) [14,18]. In the present research, the pH value decreased while the A_{420} value increased, indicating that MR occurred during the steam processing of PN root.

Table 4: Contents of amino acids in raw PN root and steamed PN root for 6 h

Compound	Root of PN		Steamed root of PN		Reduction (%)
	Content (%)	*RSD (%)*	*Content (%)*	*RSD (%)*	
Aspartic acid	0.58 ± 0.02	0.32	0.48 ± 0.03*	0.41	0.10
Threonine	0.28 ± 0.01	0.52	0.27 ± 0.01	0.55	0.01
Serine	0.22 ± 0.03	0.26	0.21 ± 0.02	0.34	0.01
Glutamate	0.64 ± 0.02	0.41	0.49 ± 0.03*	0.67	0.15
Glycine	0.24 ± 0.04	0.70	0.21 ± 0.01	0.26	0.03
Alanine	0.32 ± 0.02	0.48	0.29 ± 0.03	0.35	0.03
Cysteine	0.26 ± 0.02	0.33	0.10 ± 0.02*	0.42	0.16
Valine	0.32 ± 0.03	1.16	0.29 ± 0.02	1.04	0.03
Methionine	0.14 ± 0.01	0.82	0.10 ± 0.03	0.78	0.04
Isoleucine	0.25 ± 0.03	0.69	0.22 ± 0.02	0.67	0.03
Leucine	0.44 ± 0.05	1.22	0.42 ± 0.01	1.36	0.02
Tyrosine	0.13 ± 0.04	0.29	0.14 ± 0.04	0.34	-0.01
Phenylalanine	0.34 ± 0.03	1.35	0.34 ± 0.02	1.01	0.00
Lysine	0.31 ± 0.03	1.28	0.22 ± 0.01*	1.33	0.09
Histidine	0.13 ± 0.03	0.54	0.13 ± 0.01	0.64	0.00
Arginine	0.85 ± 0.01	0.38	0.65 ± 0.05*	0.43	0.20
Proline	0.26 ± 0.03	0.22	0.21 ± 0.04	0.31	0.05
Total content	5.71 ± 0.05	0.46	4.77 ± 0.03	0.58	0.94

*$P < 0.05$ compared with raw PN root

It is also widely recognized that different combinations of reducing sugars and amino acids can produce a variety of MR products [19]. The mono- or disaccharides were more likely to take part in the MR. Many studies have reported that aspartic acid, glutamate, cysteine, lysine and arginine react with glucose or maltose to generate color and aroma volatile compounds, and their reaction mechanisms are already elucidated [20-24].

In the present study, the significant decrease in levels of reducing sugars (maltose and glucose) and amino acids (aspartic acid, glutamate, cysteine, lysine, and arginine) are observed, indicating that the mechanism of MR during the steam processing of PN root might be related to the reaction between these reducing sugars and amino acids.

CONCLUSION

The results of the present study demonstrate that MR is involved in the steam processing of PN root. The underlying mechanism may be closely related to the reaction between reducing sugars and amino acids. Thus, the findings support MR involvement in steam processing of PN root, and therefore, there is a need to further investigate the pharmacological activities and application of steamed PN root.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. In addition, Yong-Liang Huang and Chun-Jie Wu conceived and designed the study, Zhi-Jie Yu and Mei-Bian Hu collected and analyzed the data, Jiang-Hua Li, Yu-Jie Liu, and Rui-Zhen Huang performed the detail experiments, Jiang-Hua Li and Yu-Jie Liu wrote and revised the manuscript. All authors read and approved the manuscript for publication.

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