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Investigation on influencing factors of 5-HMF content in *Schisandra*[*]

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Abstract: In order to investigate the influencing factors of 5-hydroxymethyl-2-furaldehyde (5-HMF) content in *Schisandra*, confirm the theory of 5-HMF deriving mainly from *Schisandra* processing course, and give some suggestions about the *Schisandra* processing method, the 5-HMF contents in decoctions of *Schisandra* under different heating temperature, decocting time, soaking time, processing methods and treatment with different solvents before decocting the *Schisandra* were measured by RP-HPLC method. The results showed that there is great difference of 5-HMF level in decoctions from differently processed *Schisandra* and unprocessed *Schisandra*; decocting time of 60 min has some effects on 5-HMF level in decoctions and there is certain quantity 5-HMF in processed *Schisandra* itself and very little 5-HMF in unprocessed *Schisandra*. Heating time, heating temperature and treating solvents all have effect on 5-HMF level in decoction of *Schisandra*. 5-HMF in *Schisandra* was mainly from processing course. Both long heating time and high heating temperature can increase 5-HMF level in *Schisandra*. The production of 5-HMF in *Schisandra* may have some relationships with some polar components, which can dissolve in water, ethanol and acetone, especially in ethanol. To control processing temperature, processing time and treatment with some solvent is very important for controlling 5-HMF level in *Schisandra*.

Key words: *Schisandra* process, Heating temperature, Heating time, 5-Hydroxymethyl-2-furaldehyde (5-HMF)
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INTRODUCTION

Normally, 5-hydroxymethyl-2-furaldehyde (5-HMF) is considered an irritant to eyes, upper respiratory tract, skin and mucous membranes and damages striated muscles and viscera by combining to protein and thus causing the accumulation of poisons in the body (Chi *et al*., 1998; Pamplona *et al*., 1995), and may also lead to mutagenicity and carcinogenicity (Khan *et al*., 1995; Janzowski *et al*., 2000). It often takes place when fructose or glucose solutions of high concentrations encounter high temperature or acid environment (Miyazawa and Funazukuri, 2006). 5-HMF has been identified in honey (Nozal *et al*., 2001; Lu *et al*., 2006), fruit juice (Burdurlu and Karadeniz, 2003), raisins (Palma and Taylor, 2001), beer (Castellari *et al*., 2001), oak wood (García-Romero *et al*., 1998), milk (Morales and Jimenez, 1999) and instant coffee (Charlton *et al*., 2002). A sequence of nonenzymatic browning reactions (the so-called Maillard reaction) are initiated during heat treatment of foods containing reducing sugars and amino acids. 5-HMF is a common intermediate product in the Maillard reaction (Burdurlu and Karadeniz, 2003). Human are potentially exposed to 5-HMF through pharmceutical preparations, cigarette smoke, and the consumption of a number of commonly available beverages and foods. The content of 5-HMF in the honey, beer and glucose injection has been limited strictly (Lo Coco *et al*., 1995; Li *et al*., 2004). But in recent years, there are more and more reports about 5-HMF found in TCM (traditional Chinese medicine) and it has a positively effect to human being (Dai *et al*., 2001; Hou *et al*., 2005; Miyazawa *et al*., 2003; Sharma *et al*., 2004; Xu *et al*.,

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2004). It was reported that 5-HMF in the decoction of *Shengmaiyin* and believed that 5-HMF was derived from the combination of *Schisandra* and *Ophipogon* and has contributed to the cure of cardiovascular disease (Zhu *et al*., 1998a). Similar studies have been performed on 5-HMF origin and it was reported that 5-HMF is mainly derived from *Schisandra chinensis* only and that processing method plays an important role in 5-HMF production of *Schisandra* (Li and Lu, 2005).

According to TCM theory, there is no clear difference between poison and medicine. Some strongly effective TCMs are medicine at low dose and poison in large amount. Maybe 5-HMF in TCM plays the same role as strongly effective TCM plays. It is very important to control 5-HMF level in view of the activity and side effects of the herbs which can produce 5-HMF in the processing course. In order to control the quality of *Schisandra* to a level of a high curative effect with low side effects, optimize the process technique, and confirm the theory of 5-HMF deriving mainly from *Schisandra* processing course, the origin and influencing factors on 5-HMF level in *Schisandra* were investigated in this paper by measuring the 5-HMF contents under different heating temperature, decocting time, soaking time, processing methods and extracting with different solvents before decocting the *Schisandra*. The result can provide some suggestions about the *Schisandra* processing method and somehow influence the knowledge of TCM, moreover, it can give us some inspirations about the 5-HMF origin of *Schisandra*.

MATERIALS AND METHODS

Materials and reagents

Unprocessed *Schisandra* is the dry ripe fruit of *Schisandra chinensis* (*Turcz*.) *Baill*. Medicinal *Schisandra* is made from *Schisandra chinensis* (*Turcz*.) *Baill* and *Schisandra sphenanthera Rehd*. *Et Wils*. by different process. All crude herbal medicine and medicinal medicine were authenticated by Professor Kongrong Chen from the College of Zhejiang Traditional Chinese Medicine and all are measured up to pharmacopoeia (Ch.P.C., 2005).

HPLC grade acetonitrile (Merck, Germany), ultra-pure water (milli-Q), 5-HMF (Sigma, USA), 0.45 μm micro-filter (nylon), ethanol (CP), petroleum ether (60~90 °C), acetone (AR), deionized water.

Instruments

Agilent HP1100 HPLC system equipped with a G1322A solvent degasser, a G1354A quaternary gradient solvent pump, a G1313A multiple autosampler, a G1316A thermostatted column compartment, a G1314A UV-Vis detector, a Diamonsil C_{18} column (250 mm×4.6 mm, 5 μm; Dikma) with an ODS guard column (4 mm×3 mm i.d., 5 μm; Phenomenex) and Agilent HP1100 chromatography workstation was used for analyses; TC-15 canular thermostated container (Zhejiang Xinhua Medical Instrument Factory) for decocting TCM, analytical balance (0.1 mg, AL204, Mettler Toledo GmbH) for weighing standards and TCM.

Analytical methods

5-HMF of standards and samples were all analyzed by RP-HPLC (Li and Lu, 2005). The precision, stability, accuracy and recoveries of the samples and their relative standard deviations were measured to observe the HPLC performance and system suitability of the method.

Preparation of calibration curve

Stock solution of 5-HMF was prepared by dissolving 13.62 mg 5-HMF in a 50 ml volumetric flask with ultra-pure water and stored at 4 °C. Seven concentrations of 5-HMF standard solutions (2.724×10^{-2}, 5.448×10^{-2}, 8.172×10^{-2}, 1.0896×10^{-1}, 1.362×10^{-1}, 1.6344×10^{-1}, 2.724×10^{-1} mg/ml) were prepared by appropriately diluting the stock solution with mobile phase and used to evaluate the linearity by HPLC. The calibration curve was established by plotting the peak areas against the concentrations of 5-HMF standard solutions.

Sample preparation

Processed *Schisandra* samples of different origins and weights (6.25, 12.5, 18.75, 25, 50 g) were soaked in 250 ml deionized water for 2 h and weighed, then decocted for 1 h at a moderate boiling temperature and reweighed. Deionized water was added to reach the required weight before decoction. The decoction was filtered and the filtrate was passed through a 0.45 μm micro-filter for HPLC analysis.

Samples were soaked for different times: 12.5 g of *Schisandra* of different origins was soaked in 250 ml deionized water for 1, 2, 4, 6, 8, 10, 12 h and then the lixiviums were filtered; the filtrate was passed through a 0.45 μm micro-filter for HPLC analysis.

Decocting time of the samples was different: 12.5 g of *Schisandra* of different origins was soaked in 250 ml deionized water for 2 h and weighed, then decocted for 10, 20, 40, 60, 80, 100, 120 and 140 min at moderate boiling temperature and reweighed with deionized water to make up the weight before decoction. The decoction was filtered and the filtrate was passed through a 0.45 μm micro-filter for HPLC analysis.

Decocting temperatures were as follows: 12.5 g unprocessed *Schisandra* samples were soaked in 250 ml deionized water for 2 h and weighed, then decocted for 1, 2, 4, 6, 8, 10, 12 h at 20, 40, 60, 80 and 100 °C and reweighed with deionized water added to make up to the weight before decoction. The decoction was filtered and the filtrate was passed through a 0.45 μm micro-filter for HPLC analysis.

Samples were decocted after extraction in different solvents: 50 g unprocessed *Schisandra* was soaked into 1000 ml of different solvents (ethanol, petroleum ether, acetone and deionized water) respectively for 12 h, filtered and the residue was dried at 60 °C, then 12.5 g dried residue was soaked in 250 ml deionized water for 2 h and weighed, then decocted for 4 h at moderate boiling temperature and reweighed with deionized water added to make up the weight before decoction. The decoction was filtered and the filtrate was passed through a 0.45 μm micro-filter for HPLC analysis.

RESULTS AND DISCUSSIONS

HPLC performance and system suitability of the method

Effective separation, acceptable sensitivity, and symmetric peak shapes were achieved in a short analytical period. The retention time of 5-HMF was about 12.5 min, and no interferences were observed. The number of theoretical plates of column (N) and the tailing factor (T) for analyzing 5-HMF were about 14300 and 0.96 respectively. The average extraction recovery of 5-HMF in decoction was 98.89%. The relative standard deviations of precision, stability, repeatability and recoveries of 5-HMF in decoction were 0.71%, 1.23%, 1.58% and 2.78% respectively. The extraction recovery data of 5-HMF is presented in Table 1. Representative chromatograms are shown in Fig.1.

Table 1 Extraction recovery data of 5-HMF

Sample	Found (mg)	Added (mg)	Extraction recovery (%)	Average (%)	*RSD* (%)
1	13.46	13.62	98.83	98.89	2.78
2	13.04	13.62	95.77		
3	13.64	13.62	100.12		
4	13.30	13.62	97.66		
5	13.25	13.62	97.31		
6	14.12	13.62	103.65		

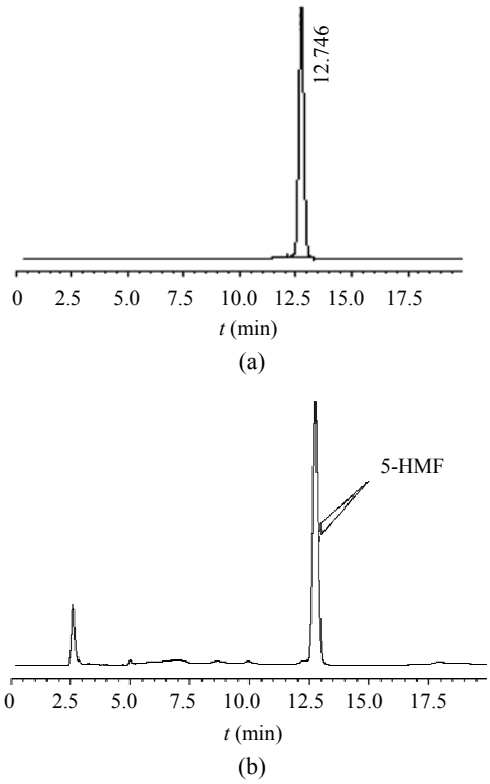

Fig.1 Chromatograms of standard and sample. (a) 5-HMF standard; (b) *Schisandra* sample decoction

Linearity

The peak area (Y) and concentration (X) of 5-HMF standard solutions were subjected to regression analysis to calculate the calibration equation and correlation coefficient. The calibration equation was Y=7376.8X+6.8016, R^2=0.9999. The results showed

excellent correlation between the peak area and the concentration of 5-HMF at the concentrations of 0.02724 to 0.2724 mg/ml.

Influence of soaking time on the 5-HMF level

There was no difference between the 5-HMF level in lixivium of the *Schisandra* after soaking for 2 h and 12 h, but a great difference between 5-HMF levels in lixiviums of different medicinal *Schisandra* and unprocessed *Schisandra* preparations. The quantity of 5-HMF in lixivium of medicinal *Schisandra* was much more than that of unprocessed *Schisandra* (Fig.2). The results showed that medicinal *Schisandra* contained a certain amount of 5-HMF and that unprocessed *Schisandra* contained less 5-HMF. 5-HMF in processed *Schisandra* decoction was from the processed *Schisandra* itself but not from decocting course. Different processing procedures may result in different 5-HMF level.

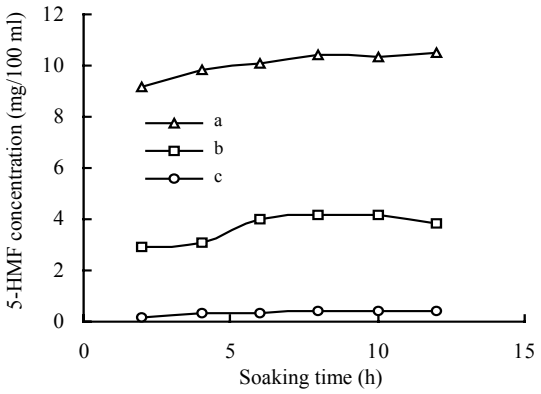

Fig.2 Influence of soaking time on the 5-HMF level in *Schisandra*

a: Medicinal *Schisandra* (made by factory A); b: Medicinal *Schisandra* (made by factory B); c: Crude S*chisandra* (unprocessed)

Influence of decocting time on the 5-HMF level

The 5-HMF level of the decoctions of processed *Schisandra* or pulverized *Schisandra* was evidently affected by decocting time and has positive relationship with decocting time of less than 60 min (Fig.3). Six minutes later, the level of 5-HMF in *Schisandra* decoctions did not increase further, probably because the 5-HMF content in the processed *Schisandra* was limited. After decoction for a certain time, the 5-HMF content in decoctions reaches its maximum. The result was consistent with the report that 5-HMF is not present in decoctions after three extractions (Zhu *et al*., 1998b). 5-HMF level in pulverized *Schisandra* was no more than that in unpulverized *Schisandra* but its solubility time has advanced. If 5-HMF is considered to be representative of active components, the theory that *Schisandra* has to be pulverized when used as medicine was reasonable because the pulverization of *Schisandra* can decrease the decocting time and protect the thermo-sensitive ingredients from being destroyed.

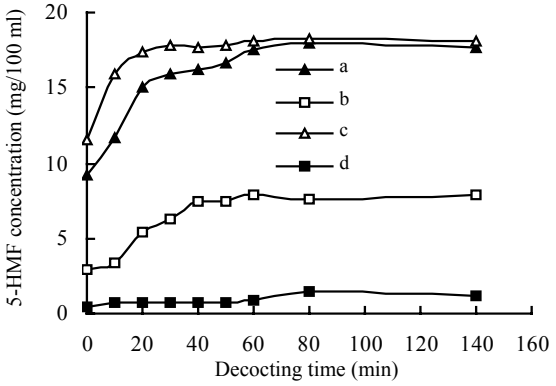

Fig.3 Effect of decocting time on the level of 5-HMF in decoctions

a: Medicinal *Schisandra* (made by factory A); b: Medicinal *Schisandra* (made by factory B); c: Medicinal *Schisandra* (made by factory A, comminuted); d: Crude *Schisandra* (unprocessed)

Influence of *Schisandra* origin on the 5-HMF level

The 5-HMF level of the decoctions showed evident differences between differently processed *Schisandra* group decoctions and the unprocessed *Schisandra* group decoctions (Fig.4). There was far less 5-HMF in unprocessed *Schisandra* decoctions than in processed *Schisandra* decoctions and 5-HMF levels were proportionate to the *Schisandra* content. There were also great differences in the 5-HMF levels among the three kinds of medicinal *Schisandra* decoctions. The results showed that the processing methods have great effect on the production of 5-HMF and that the changing of 5-HMF levels in the decoctions was mainly controlled by *Schisandra* processing methods. The results were confirmed in the report that 5-HMF appeared in the course of Ephedra Stapf processing (Xu *et al*., 2004). Maybe

the factors of processing time, processing temperature and herb species probably all affect 5-HMF levels in medicinal *Schisandra* during the course of the processing.

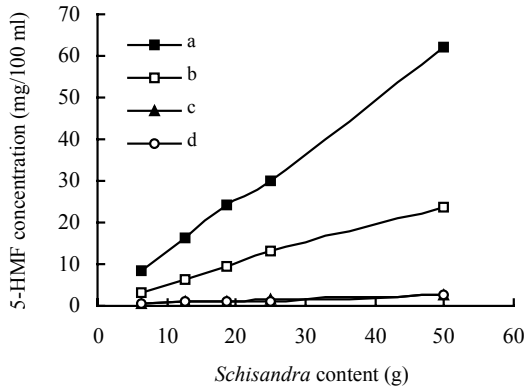

Fig.4 Influence of *Schisandra* origin on the production of 5-HMF
a: Medicinal *Schisandra* (made by factory A); b: Medicinal *Schisandra* (made by factory B); c: Medicinal *Schisandra* (made by factory C); d: Unprocessed *Schisandra*

Influence of decocting temperature on the 5-HMF level

The effect of temperature on 5-HMF level was significant when crude *Schisandra* was heated for 4 h at 100 °C. There was no difference in 5-HMF levels when the unprocessed *Schisandra* was decocted below 80 °C or was heated to below 100 °C within 2 h (Table 2). The results showed that high temperature and long heating time may affect 5-HMF levels evidently in the course of processing *Schisandra*. 5-HMF in the decoctions or lixiviums under 80 °C or within 2 h resulted in 5-HMF in *Schisandra* diffusing to water, and 5-HMF in the decotions was the result of chemical reaction in *Schisandra* when the temperature was up to 100 °C or heating time was long. If 5-HMF has a positive active function at low doses and a poisonous function at high doses according to TCM theory, it may be reasonable to steam the crude *Schisandra* for 4 h before using it as medicinal *Schisandra*.

Table 2 Influence of decocting temperature on the 5-HMF level in crude *Schisandra* decoction

Heating time (h)	5-HMF concentration (mg/100 ml)				
	25 °C	40 °C	60 °C	80 °C	100 °C
1	0.08	0.11	0.24	0.18	0.13
2	0.18	0.21	0.32	0.35	0.34
4	0.33	0.40	0.33	0.56	0.72
6	0.33	0.41	0.35	0.63	1.45
8	0.45	0.43	0.46	0.71	2.19
10	0.43	0.52	0.45	0.90	2.68
12	0.45	0.46	0.47	0.98	3.21

Influence of different extracts on the 5-HMF level

There are great differences in 5-HMF levels in the decoction of the unprocessed *Schisandra* residue extracted by different solvents. 5-HMF levels in decoctions of the unprocessed *Schisandra* residue treated by petroleum ether were much higher than that treated by acetone, ethanol or water. 5-HMF levels in decoctions of *Schisandra* residues treated by acetone, ethanol or water were lower than that in decoctions of untreated *Schisandra* (Table 3). The results showed that 5-HMF may derive from some polar components present in *Schisandra*. Since there is a report about that tannin in fruit may trigger browning reaction and that browning reaction has relation with 5-HMF (Roig *et al*., 1999). It was also reported that 5-HMF level in immature fruit juice is higher than in ripe fruit juice and that the tannin content in immature fruit is higher than that in ripe fruit (Poll, 1985). Whether there is relationship between 5-HMF and tannin or not requires further investigation.

Table 3 Influence of treating solvent on the 5-HMF level

Treating solvent	5-HMF concentration (mg/100 ml)
Petroleum ether	8.06
Ethanol	0.11
Acetone	0.73
Water	0.91
Without extract	1.53

CONCLUSION

Processed *Schisandra* has much more 5-HMF content than unprocessed *Schisandra*. 5-HMF in *Schisandra* decoctions results mainly from the method of processing and was not from decocting course. Both long heating time and high heating temperature can increase 5-HMF level in *Schisandra*. The theory that *Schisandra* has to be pulverized when being used as medicine was reasonable because pulverizing *Schisandra* can decrease the decocting time and protect the thermo-sensitive ingredients from

being destroyed. 5-HMF may derive from some polar components which are soluble in water, ethanol and acetone, especially ethanol. To control processing temperature and processing time or pretreatment by some solvents is very important if want to control 5-HMF level in *Schisandra*. Whether 5-HMF has relation with tannin has not been determined.

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