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A Comparative Study on Processed Chih-Shao

SHUENN-JYI SHEU AND YIH-JOE JAN

Department of Chemistry, National Taiwan Normal University
88, Sec. 4, Tingchow Road, Taipei, Taiwan, R.O.C.

ABSTRACT

Samples of *Chih-Shao* processed by wrap-moistening, stir-baking to char, stir-baking with wine and stir-baking with vinegar were analyzed by high-performance liquid chromatography and found to differ greatly from each other. The total extraction yield increased most in the wrap-moistening article (17.31% increase) followed by the stir-baking to char sample (11.92% increase). The highest increases in individual constituents were found to be gallic acid when processed by wrap -moistening for 12 hours, and the other five compounds including oxypaeoniflorin, albiflorin, paeoniflorin, benzoic acid and paeonol when processed by stir-baking to char for 30 or 40 minutes.

The results of the present investigation show significant changes in the extraction yields of various chemical constituents of *Chih-Shao* subsequent to various methods of preparation.

Key words : *Chih-Shao*, processing; quantitative analysis.

INTRODUCTION

Chih-Shao (Peony, *Paeoniae Radix rubra)* is the dried or steam-dried whole root of *Paeonia obovata* Maxim., *P. veitchii* Lynch. and *P. lactiflora* Pall. of the ranunculaceae family. It effects to disperse blood and as a diuretic[1] and contains several monoterpenoids, phenols and aromatic acids as their major bioactive components[1,2]. *Chih-Shao* has been traditionally processed by various methods including simple stir-baking, stir-baking with wine or vinegar and wrap-moistening, etc.[3]. It is generally believed that differently processed samples would yield different therapeutic effects. To investigate the differences elicited by the variable of herb processing of *Chih-Shao* as well as the significance of herb processing itself, this study used samples processed in four different ways: wrap-moistening, stir-baking to char, stir-baking with wine and stir-baking with vinegar. The yields of marker substances including gallic acid, oxypaeoniflorin, albiflorin, paeoniflorin, benzoic acid[4-7] and paeonol[1] (Fig.1) were determined by high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

1. *Marker Substances and Materials*

The marker substances and internal standard were purchased as follows: benzoic acid and

Correspondence to : Shuenn-Jyi Sheu

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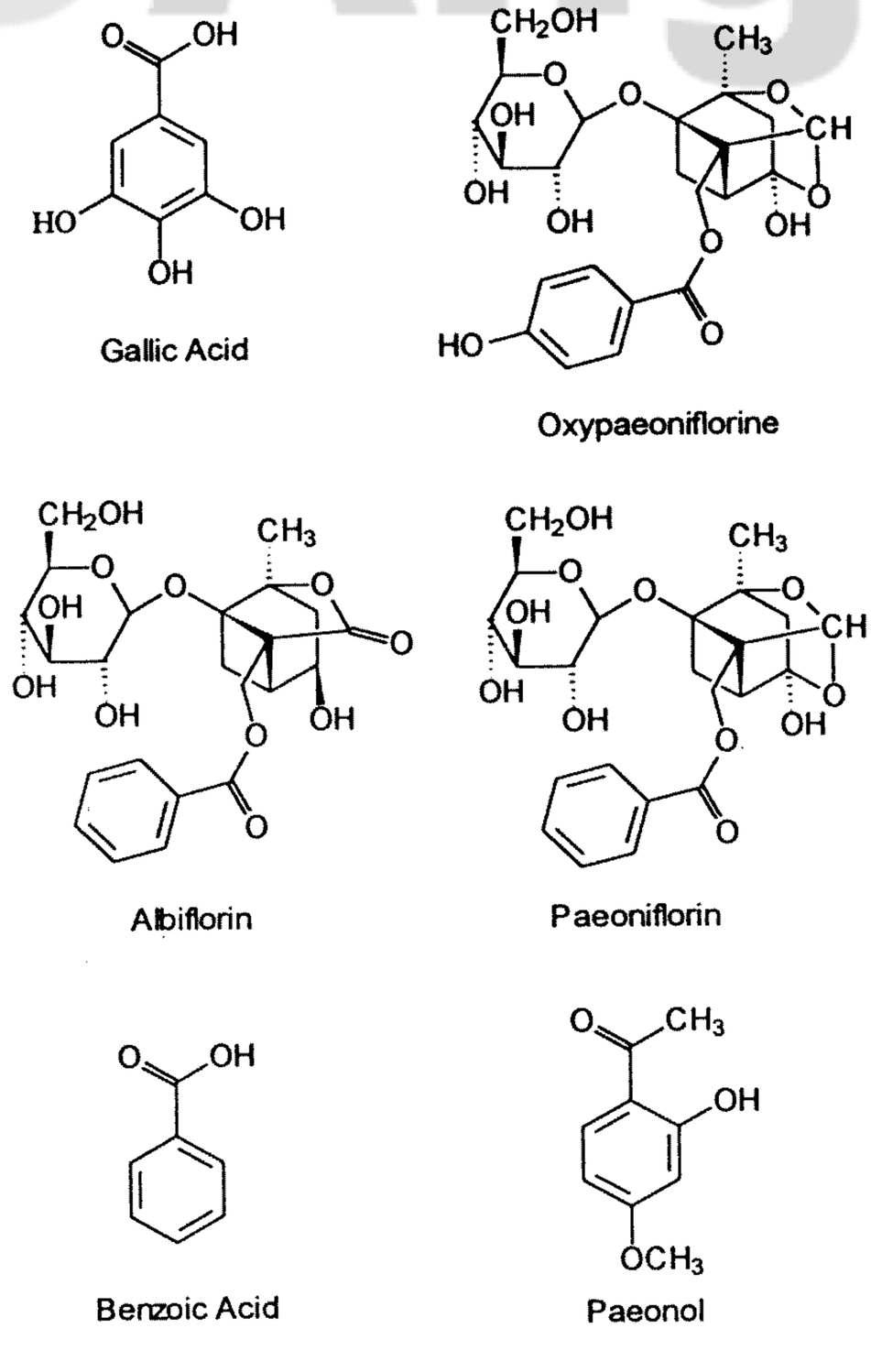

Figure 1. The structures of marker substances

paeoniflorin from Nacalai Tesque (Kyoto, Japan), gallic acid and 2,5-dihydroxybenzoic acid from E. Merck (Darmstadt, Germany). Acetonitrile and methanol were of LC grade (Mallinckrodt, Kentucky, USA). *Chih-Shao* was obtained from the Chinese herbal market in Taipei, Taiwan. Albiflorin, oxypaeoniflorin[5] and paeonol were isolated from *Chih-Shao*, and confirmed by comparing their IR, PMR, CMR and MS to references. Purity checking and peak identification of all marker substances and test samples were done with a photodiode array detector.

II. *Preparation of Processed Samples*[3]

(I). Wrap-moistening

Chih-Shao intact herbs (60g) in the form of a round strip was wrapped with gauze, sprayed thoroughly with water, and then placed in a hermetic container for 6,10,12,14,18 or 24 hours, respectively. The drug was cut into thin slices and then air dried until constant weight was maintained.

(II). Stir-baking to char

The herb cuttings (30g) were heated in a baking pan to 180±5°C, and stir-baked for 10, 20,30,.40, or 60 min.

(III). Stir-baking with wine

The herb cuttings (30g) were placed in a baking pan to which 3 ml of rice wine was added. The sample was stir-baked to yellow (120± 5°C) for 10,20,40,50 or 60 min.

(IV). Stir-baking with vinegar

Herb cutting (30g) was placed in a baking pan into which 3 ml of vinegar containing about 4.5% acetic acid was added. The herb was allowed to absorb the vinegar thoroughey then stir-baked at 120±5°C for 10,20,40,45 or 60 min.

III. *Assay of Processed Articles*

Aliquots of 0.5g from each of the pulverized processed-samples was extracted by refluxing for 45 minutes with 5 ml of 70% ethanol, 30% ethanol and water, respectively. Extraction was repeated three times. The extract was spiked with 1 ml of 2,5-dihydroxybenzoic acid solution as internal standard (1.00g of 2,5-dihydroxybenzoic acid was diluted into a 500 ml methanol solution) and then diluted to 25 ml. 10μl of the test solution was injected into HPLC system.

IV. *HPLC Conditions*

The experiments were performed on an ABI HPLC equipped with an ABI 400 solvent delivery system and an ABI 1000s photodiode array detector (λ=254nm). Satisfactory separation of the marker substances was obtained with a reversed-phase column (Cosmosil C_{18}, 25cm × 4.6mm i.d., Nacalai Tesque, Japan) eluted with a linear solvent gradient of A:B (A, 2.5% acetic

acid/ acetonitrile=95/5(v/v); B, acetonitrile/methanol/water=35/35/30(v/v)) varied as follows: 0 min 100:0; 10-25min 80:20; 26-30min 77:23; 40 min 70:30 53-57min 0:100 and 60 min 100:0 at a flow rate of 1.0ml/min.

RESULTS AND DISCUSSION

Gallic acid and benzoic acid are both aromatic acids and can easily be dissociated into carboxylate ion in a neutral aqueous solution. Therefore, in order to prolong the retention times and obtain a satisfactory separation, the addition of suitable amounts of acetic acid into the eluent was found to be necessary. After a series of experiments, an analytical condition as listed in the experimental section was chosen. There were over 0.999 for correlation coefficients, less than 1% for the relative standard deviations (n =5) and around 97.8-103.0% for the recoveries of all the constituents. The retention times and calibration curves (Y=ratio of peak area, X=μg) of the marker substances were as follows: gallic acid, 10.22min, $Y=2.3162\ X-2.3462\times10^{-1}$; oxypaeoniflorin 21.21 min $Y=(1.1406\times10^{-1})X+2.9667\times10^{-2}$; albiflorin, 31.40 min $Y=(2.1536\times10^{-1})X+7.3657\times10^{-2}$; paeoniflorin, 37.89 min, $Y=(1.8641\times10^{-1})X+7.9384\times10^{-2}$; benzoic acid, 49.75 min, $Y=(4.2899\times10^{-1})X+9.3129\times10^{-2}$; paeonol, 58.20 min, $Y=1.6562\ X+2.4908\times10^{-1}$; 2,5-dihydroxybenzoic acid, 22.34 min.

Chih-Shao exhibited considerable diversity between individual samples, as well as samples of different sizes, odors and colors which showed differences in constituents. To compare the differences before and after processing, each sample was divided into two protions, one to serve as control and the other for processing.

For herbs processed by stir-baking to char and stir-baking with wine or vinegar,commercial samples of herb cuttings were used to achieve a quick ,even processing effect. But in consideration of the possible loss of constituents in herbs processed by wrap-moistening which requires a longer processing time, whole herbs in the form of a round strip were processed as indicated in the experimental section.

Usually the goal of processing by wrap-moistening is to soften the herbs to enhance extraction. For this study the herbs were thoroughly moistened by spray-wetting with water and placed in an hermetic container for various lengths of time. The herbs were subsequently allowed to dry, weighed, extracted with suitable solvents (solvents that have higher extraction rate for the herb) and assayed as shown in Table 1. Increase rates are shown in Table 2.

Tables 1 and 2 show significant changes in the individual constituents of *Chih-Shao* when subjected to processing at different lengths of time. The overall extraction yield was optimal for processing by wrap-moistening for 12 hours, when extraction with water resulted in a 14.75% increase in yield, extraction with 30% ethanol gave a 12.21% increase and extraction with 70% ethanol yielded a 17.31% increase. Although differences existed between individual extraction yields, the six marker substances all had the highest increase when the herb was moistened 12 hours prior to extraction. Gallic acid, which was the most abundant in content, increase 16.55% in yield rate when extracted with water, 15.87% when extracted with 30% ethanol and 31.17% when extracted with 70% ethanol. Paeoniflorin, oxypaeoniflorin and albiflorin, the important bioactive components of the peony, increased 13.75%, 12.00% and 9.68%, respectively when extracted with water. By prolonging the processing time over a 12 hour period, the extraction yields of the various constituents dropped gradually, with oxypaeoniflorin and albiflorin having the highest drop. The increase rates became 4.17% for the former and 3.45% for the latter after processing for 24 hours when extracted with water.

Chih-Shao was processed by stir-baking to char, stir-baking with wine and stir-baking with vinegar, then assaying with HPLC. The results of highest extraction yields and the optimal processing times are given in Table 3.

Data in Table 3 show that all the processed

Table 1. Assay results of the processing Chih-Shao by wrap-moistening (mg constituent/g herb)

Sample	Extracting solvent	GA	OX	AL	PA	BA	PN	Total
Crude drug	a	5.15	0.25	0.41	1.02	0.88	1.38	9.09
	b	6.40	0.17	0.32	0.91	0.71	1.21	9.72
	c	7.10	0.11	0.22	0.83	0.43	1.04	9.73
Moistening	a	5.27	0.25	0.43	1.07	0.91	1.44	9.37
for 6 hr	b	6.59	0.17	0.33	0.94	0.74	1.26	10.03
	c	7.33	0.11	0.23	0.85	0.45	1.09	10.06
Crude drug	a	4.81	0.42	0.48	1.21	0.93	1.54	9.39
	b	6.29	0.31	0.38	1.12	0.84	1.31	10.25
	c	7.56	0.23	0.24	1.01	0.53	1.23	10.80
Moistening	a	5.19	0.45	0.51	1.31	0.98	1.64	10.08
for 10 hr	b	6.82	0.33	0.40	1.21	0.89	1.39	11.04
	c	8.24	0.25	0.26	1.10	0.57	1.34	11.76
Crude drug	a	3.24	0.58	0.55	1.32	0.99	1.41	8.09
	b	4.41	0.45	0.50	0.99	0.87	1.30	8.52
	c	5.92	0.25	0.31	0.80	0.55	1.12	8.95
Moistening	a	4.25	0.63	0.59	1.45	1.05	1.52	9.49
for 12 hr	b	5.11	0.48	0.53	1.11	0.93	1.40	9.56
	c	6.90	0.28	0.34	0.91	0.60	1.24	10.27
Crude drug	a	6.04	0.46	0.48	1.12	0.84	1.27	10.21
	b	6.79	0.32	0.36	1.01	0.70	1.09	10.27
	c	7.39	0.23	0.29	0.86	0.40	0.84	10.01
Moistening	a	6.81	0.49	0.51	1.22	0.89	1.36	11.28
for 14 hr	b	7.74	0.34	0.38	1.13	0.74	1.17	11.50
	c	8.84	0.25	0.31	0.97	0.43	0.91	11.35
Crude drug	a	5.48	0.44	0.51	1.21	0.91	1.16	9.71
	b	6.29	0.36	0.40	1.09	0.82	1.01	9.97
	c	7.04	0.28	0.31	0.92	0.48	0.78	9.81
Moistening	a	6.15	0.46	0.54	1.31	0.96	1.24	10.66
for 18 hr	b	7.11	0.38	0.42	1.20	0.86	1.08	11.05
	c	8.03	0.30	0.33	1.03	0.51	0.84	11.04
Crude drug	a	6.12	0.37	0.49	1.34	1.12	1.14	10.58
	b	7.04	0.31	0.38	1.19	0.99	1.01	10.92
	c	8.11	0.24	0.29	1.01	0.57	0.75	10.97
Moistening	a	6.85	0.38	0.51	1.45	1.18	1.21	11.58
for 24 hr	b	7.95	0.32	0.39	1.30	1.03	1.08	12.07
	c	9.21	0.25	0.30	1.13	0.60	0.80	12.29

GA: gallic acid, OX: oxypaeoniflorin, AL: albiflorin

PA: paeoniflorin, BA: benzoic acid, PN: paeonol

a: 70% ethanol, b: 30% ethanol, C: water.

Table 2. Percentage increase (%) of the extraction yield from wrap-moistening samples compared with the original crude drugs

Processing time (hr)	Extracting solvent	GA	OX	AL	PA	BA	PN	Total
6	a	+2.33	0.00	+4.88	+4.90	+3.41	+4.35	+3.08
	b	+2.97	0.00	+3.13	+3.30	+4.23	+4.13	+3.19
	c	+3.24	0.00	+4.55	+2.41	+4.65	+4.81	+3.39
10	a	+7.90	+7.14	+6.25	+8.26	+5.38	+6.49	+7.35
	b	+8.43	+6.45	+5.26	+8.04	+5.95	+6.11	+7.71
	c	+8.99	+8.70	+8.33	+8.91	+7.55	+8.94	+8.89
12	a	+31.17	+8.62	+7.27	+9.85	+6.06	+7.80	+17.31
	b	+15.87	+6.67	+6.00	+12.12	+6.90	+7.69	+12.21
	c	+16.55	+12.00	+9.68	+13.75	+9.09	+10.71	+14.75
14	a	+12.75	+6.52	+6.25	+8.93	+5.95	+7.09	+10.17
	b	+14.00	+6.25	+5.56	+11.88	+5.71	+7.34	+11.98
	c	+14.75	+8.70	+6.90	+12.79	+7.50	+8.33	+13.39
18	a	+12.23	+4.55	+5.88	+8.26	+5.49	+6.90	+9.78
	b	+13.04	+5.56	+5.00	+10.09	+4.88	+6.93	+10.83
	c	+14.06	+7.14	+6.45	+11.96	+6.25	+7.69	+12.54
24	a	+11.93	+2.70	+4.08	+8.21	+5.36	+6.14	+9.45
	b	+12.93	+3.23	+2.63	+9.24	+4.04	+6.93	+10.53
	c	+13.56	+4.17	+3.45	+11.88	+5.26	+6.67	+12.03

+: percentage increase, 0.00:no change

Other abbreviations are as in Table 1.

samples produced a large increase in extraction yield of the various constituents only if an optimal length of time could be selected. Gallic acid, the most abundant component among all the marker substances, had the best yield after wrap-moistening for 12 hours. For the other five components, however, all the best extraction yields were achieved when herbs were stir-baked to char for 30 or 40 minutes. The change in total extraction yield mainly paralleled that of gallic acid which occupied as high as 70% of the total content of the herb. Therefore, even if five of the six components had their best increase rate by stir-baking to char, in terms of the total extraction yield, samples by "wrap-moistening" still increased the most, followed by "stir-baking to char".

Among the various processing methods, "wrap-moistening" required the longest length of time, whereas "stir-baking to char" required the shortest. As the processing time was prolonged over its optimal lengh, the extraction yields of the various constituents decreased slowly with the "stir-baking to char" method having the highest drop.

In summary, the results of the present investigation show significant changes in the extraction yields of various chemical constituets of *Chih-Shao* subsequent to various methods of preparation. This may represent the chemical basis which confirms the commonly accepted thesis that different processing methods may result in different therapeutic properties for *Chih-Shao* and may be applicable to other traditional Chinese herbs as well.

Table 3. Highest increase rates (%) in extraction yield of Chih-Shao under various processing methods and periods

Processing method	Extracting solvent	GA	OX	AL	PA	BA	PN	Total
Stir-baked to char	a	11.04 (30min)	12.50 (30min)	11.76 (30min)	15.52 (30min)	13.51 (40min)	11.03 (40min)	11.92 (30min)
	b	9.09 (30min)	8.33 (30min)	9.68 (30min)	13.73 (30min)	9.62 (30min)	14.88 (40min)	9.73 (30min)
	c	9.93 (30min)	10.00 (40min)	13.04 (30min)	15.38 (30min)	11.11 (30min)	11.93 (40min)	10.65 (30min)
Stir-baked with wine	a	9.89 (50min)	5.56 (50min)	6.67 (50min)	7.55 (50min)	10.90 (50min)	7.38 (50min)	8.72 (50min)
	b	12.81 (50min)	3.33 (50min)	7.89 (40min)	7.69 (50min)	7.04 (50min)	6.92 (50min)	10.15 (50min)
	c	8.59 (50min)	3.23 (60min)	6.45 (50min)	8.97 (50min)	5.56 (50min)	8.04 (50min)	8.02 (50min)
Stir-baked with vinegar	a	7.85 (40min)	4.55 (40min)	4.88 (40min)	5.93 (40min)	6.02 (40min)	5.13 (40min)	6.88 (40min)
	b	8.01 (40min)	5.00 (40min)	8.57 (40min)	6.11 (40min)	5.26 (40min)	5.93 (40min)	7.30 (40min)
	c	5.31 (40min)	7.14 (40min)	3.57 (20min)	6.84 (40min)	4.65 (40min)	5.04 (40min)	10.98 (40min)
Wrap-moistened with water	a	31.17 (12hr)	8.62 (12hr)	7.27 (12hr)	9.85 (12hr)	6.06 (12hr)	7.80 (12hr)	17.31 (12hr)
	b	15.87 (12hr)	6.67 (12hr)	6.00 (12hr)	12.12 (12hr)	6.90 (12hr)	7.69 (12hr)	12.21 (12hr)
	c	16.55 (12hr)	12.00 (12hr)	9.68 (12hr)	13.75 (12hr)	9.09 (12hr)	10.71 (12hr)	14.75 (12hr)

The abbreviatons are as shown in Table 1.

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赤芍炮製品之比較研究

許順吉　詹逸州

國立台灣師範大學化學研究所

摘　要

赤芍經悶潤、炒炭、酒炒、醋炒後以高效液相層析儀分析，發現彼此間有很大差異。總萃取率以悶潤炮製品增加17.31%爲最多，炒炭炮炙品增加11.92%居次。個別成分增加情形分別爲：gallic acid以悶潤12小時增加最多，而oxypaeoniflorin, albiflorin, paeoniflorin, benzoic acid和paeonol等五成分的萃取則均以炒炭30或40分鐘爲最佳。