

## Original Article

# Effect of different extracting methods on quality of *Chrysanthemum Morifolium* Ramat. Infusion

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The flower of *Chrysanthemum Morifolium* Ramat. (CM) is a useful food and folk medicine in China. Effects of *Chrysanthemum Morifolium* extracting (CME) conditions, including extraction temperature, extraction time and the ratio of flower to water on infusion quality were investigated. The extractability, liquor color differences and the major flavonoids of luteolin-7-O- $\beta$ -D-glucoside and luteolin of various CME methods were evaluated. The results indicated that CME temperature was the most important factors affecting quality of CM infusion. Based on the extractability and chemical compositions, the best CME conditions were the 90~100°C, 20-30min and the ratio of flower to water of 1:40 (w/v). High-performance liquid chromatography (HPLC) of *Chrysanthemum Morifolium* chemical compositions including luteolin-7-O- $\beta$ -D-glucoside and luteolin was also discussed in the present paper.

**Key Words:** *Chrysanthemum Morifolium*, extraction, flavonoids, HPLC, extracted solids yield, color differences

## Introduction

The flower of *Chrysanthemum Morifolium* Ramat. (CM) is widely used as a healthy beverage and medicine in China. Its anti-oxidation, vasoactive effects and anti-ischemia/reperfusion injury action have been proved in animal tests and clinic studies.<sup>1</sup> The healthy benefits of CM are confirmed to be connected with the flavonoids, such as luteolin, luteolin-7-O- $\beta$ -D-glucoside, acacetin-7-O- $\beta$ -D-glucoside, apigenin-7-O- $\beta$ -D-glucoside, etc.<sup>2-4</sup> Luteolin is a natural flavone and it is distributed extensively in the vegetable kingdom. In clinic, luteolin can relieve a cough, make expectoration easy, antiphlogosis. In vivo, it has the functions of antiviral, strong antimicrobial activity and reduces cholesterol and serum lipid. Luteolin-7-O- $\beta$ -D-glucoside could be biotransformed to their aglycone forms-luteolin (3', 4', 5, 7-tetrahydroxyflavone) in intestine by action of microorganism when the extract of CM was orally administered.<sup>5</sup>

Luteolin was isolated from *Chrysanthemum Morifolium* Ramat in China. A close relationship was found between concentration of luteolin and quality of CM.<sup>6-7</sup> But researchers also found that the content of luteolin-7-O- $\beta$ -D-glucoside was higher than that of luteolin in CM and it will be more interesting to use luteolin-7-O- $\beta$ -D-glucoside as an indicator of CM quality.<sup>8</sup> Because of its healthy benefits, CM has now been used as a herbal in production of healthy drinks. However, little information on the effects of extraction conditions on quality of CM extracts has been available and the topic will be interesting for the drink industry.

The purpose of the present work is to investigate the effects of extraction conditions, including temperature, extraction duration and the ratio of CM flower to water, on the extractability and major chemical compositions of the extracts, so as to offer guideline for extraction *Chrysanthemum Morifolium* drink.

## Materials and methods

### Material

Dry flower of *Chrysanthemum Morifolium* Ramat. for extraction test was collected from Tongxiang of Zhejiang province and ground (0.25mm) before extraction. Luteolin and luteolin-7-O- $\beta$ -D-glucoside for HPLC references were obtained from National Institute for the Control of Pharmaceutical and Biological Products and Department of Pharmaceutical Analysis and Drug Metabolism of Zhejiang University. The other chemical reagents used were of HPLC grade (Tianjin Shild Biometric Technical Co. Ltd., China).

### Instrumentation

The HPLC system was Shimadzu LC-2010A coupled with a four pumps gradient system (shimadze Co. Ltd., Kyoto, Japan), a thermostatted column compartment, a variable-wavelength UV detector (VWD) and Agilent Chemstation software.

### Study design

Extraction temperature, extraction time and ratio of CM to water were important factors affecting extractability of CM and fifteen treatment combinations were investigated by Box-Behnken design with 3 levels each of the above 3 factors (Table 1). A response surface design was carried out on the software of the Version 8.0 SAS System for Windows (SAS Institute Inc.100 SAS Campus Drive, Cary, NC 27513-2414, USA).

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The flower was extracted with 240 ml distilled water in water bath at a controlled temperature. The weight of samples, extraction temperature and extraction time tested were shown in Table 1.

#### HPLC analysis

The extracts were filtered through a "Double-ring" No.102 filter paper (Xinhua Paper Industry Co. Ltd, Hangzhou, China) and 0.22  $\mu$ m Milipore filter before it was injected into HPLC. The HPLC conditions were as follows:

Injection volume:	10 $\mu$ l
Column:	Shimadzu CLC-ODS column 0.15m $\times$ 6.0 $\phi$ (Shimadzu Co.Ltd.,Kyoto, Japan ).
Temperature:	28 $^{\circ}$ C
Mobile phase:	Solvent A: acetonitrile/acetic acid/water (60:1:193,v), Solvent B: acetonitrile/acetic acid/water (60:1:139,v) Gradient: 70% (v) Solvent A and 30% (v) solvent B to 100% (v) Solvent B by linear gradient during the early 45min and then 100% (v) Solvent B till 55min.
Flow rate:	1ml $\cdot$ min $^{-1}$
Detector:	Shimadzu SPD ultraviolet detector, 350nm

#### Infusion color difference analysis

It was carried out according to Liang *et al.*<sup>9</sup> The extracted CM solution was cooled to room temperature and then filtered on "Double-ring" No.102 filter paper (Xinhua Paper Industry Co. Ltd., Hangzhou, China). The liquor color difference analysis was carried out on TC-PIIG automatic color difference meter (Beijing Optical Instru-

ment Factory, Beijing, China). The white plate supplied by TC-PIIG automatic color difference meter was used as background. To diminish the error arising from different determination conditions, such as different equipment and temperature, distilled water was used as control and the infusion color difference indicator of  $\Delta L$ ,  $\Delta a$ ,  $\Delta b$  and  $\Delta E$ , representing the lightness-darkness( $\Delta L$ ), redness-greenness( $\Delta a$ ), yellowness-blueness( $\Delta b$ ) and total color difference( $\Delta E$ ) between the CM infusion and the distilled water were read and printed out directly by the TC-PIIG automatic color difference meter.

#### Solids concentration Measurement

The solids concentrations of the extracted CM solutions were determined by drying 20ml of the liquor in a weighed glass dish with 5cm diameter at 80 $^{\circ}$ C for 24h. The dry matter was weighted and the concentration was presented as grams of the dry matter per litre.

#### Statistical analysis

The tests in the present paper were carried out in two replicates for each sample and the mean values of the two tests were presented. Data analysis was carried out on software of SAS 8.0 for Windows. The result with Box-Behnken design was showed in Tables 2 and 4.

#### Results and discussion

##### Effect of extraction methods on extractability and chemical composition of extracts

The extractability, i.e. the extracted solid yield (ESY), is important for the production of CM extracts because it affects the utilization efficiency of CM material and the production cost. Table 2 shows that extractability ranges from 397mg g $^{-1}$  to 507 mg g $^{-1}$ . The yield of extraction rose steeply with rise of the extraction temperature. The ESY extracted at 100 $^{\circ}$ C was more than the other temperature combination treatments, and the ESY extracted at 40 $^{\circ}$ C was less than the other temperature combination treatments. From the ESY point of view, extraction at

**Table 1.** Box-Behnken design with three factors and three levels each factors

Treatment number	X1 Temperature ( $^{\circ}$ C)	X2 Time (min)	X3 Flower/ water ratio (w/v)
1	40	10	1:30
2	40	30	1:30
3	100	10	1:30
4	100	30	1:30
5	70	10	1:40
6	70	10	1:20
7	70	30	1:40
8	70	30	1:20
9	40	20	1:40
10	100	20	1:40
11	40	20	1:20
12	100	20	1:20
13	70	20	1:30
14	70	20	1:30
15	70	20	1:30

**Table 2.** Analysis of content of extractability, Luteolin-7-O- $\beta$ -D-glucoside, Luteolin

	Temperature (°C)	Time (min)	Flower/water ratio (w/v)	solids concentration (mg/g)	Luteolin-7-O- $\beta$ -D-glucoside (mg/g)	Luteolin (mg/g)
1	40	10	1:30	397	1.57	0.05
2	40	30	1:30	415	1.74	0.05
3	100	10	1:30	481	2.59	0.11
4	100	30	1:30	507	3.23	0.12
5	70	10	1:40	459	1.99	0.08
6	70	10	1:20	429	1.85	0.06
7	70	30	1:40	436	2.19	0.10
8	70	30	1:20	470	1.98	0.08
9	40	20	1:40	420	1.79	0.05
10	100	20	1:40	497	2.76	0.12
11	40	20	1:20	423	1.78	0.06
12	100	20	1:20	487	2.59	0.10
13	70	20	1:30	462	2.16	0.09
14	70	20	1:30	461	2.14	0.09
15	70	20	1:30	464	2.22	0.10

**Table 3.** Analysis of variables of extractability, luteolin-7-O- $\beta$ -D-glucoside, luteolin in the RSM model <sup>a</sup>

Source <sup>a</sup>	solids concentration	Luteolin-7-O- $\beta$ -D-glucoside	Luteolin
		<i>P</i>	
X1	0.001**	0.001**	0.0005 **
X2	0.027*	0.039*	0.208
X3	0.920	0.251	0.102

<sup>a</sup>: X1=temperature (°C), X2= time (min), X3=flower/ water ratio (w/v). \*  $p < 0.05$ ; \*\*  $p < 0.01$

100°C was ideal. Results of extraction at various conditions also demonstrate that the extraction temperature and extraction time has important effect on extractability (Table 3).

The contents of luteolin and luteolin-7-O- $\beta$ -D-glucoside were the main chemical components to determine the taste and the color qualities of CM beverage. There are 74 peaks on the HPLC profile (Figure 1). Peaks 53 and 68 were identified as luteolin-7-O- $\beta$ -D-glucoside and luteolin, respectively. The other peaks were not identified because of lack of reference compounds. As described in Table 2, the content of luteolin-7-O- $\beta$ -D-glucoside ranged from 1.57 mg g<sup>-1</sup> to 3.23 mg g<sup>-1</sup> and content of luteolin ranged from 0.051 mg g<sup>-1</sup> to 0.116 mg g<sup>-1</sup>. Both luteolin-7-O- $\beta$ -D-glucoside and luteolin rose steeply with the rise of the extraction temperature. From the luteolin-7-O- $\beta$ -D-glucoside point of view, extraction at 100°C was ideal. Extraction time also affected the chemical composition of CM infusion. The content of luteolin-7-O- $\beta$ -D-glucoside rose with the increase of the extraction time, showing the same trend as extraction temperature (Table 3).

However, the ratio of flower to water also affected the extractability and the contents of luteolin and luteolin-7-O- $\beta$ -D-glucoside, though they were not as important as

factors of the extraction temperature and extraction time.

#### **Effect of different extracting methods on infusion color**

Ready-to-drink CM production is a new drink, so up to now there is no standard to estimate the relationship between greenness, yellowness and the quality of the CM infusion. But some researches show that yellow pigment of *C. indicum* (one kind of CM) is a species of flavone pigment. So yellowness could be an important indicator to value the quality of CM infusion. Table 4 showed the effects of extracting factors on infusion color. The results showed all of the extraction factors had effect on  $\Delta b$ , an indicator of yellowness. The  $\Delta b$  rose with the rise of the extraction temperature and the ratio of flower to water. Treatments in the ratio of CM to water between 1:20 and 1:40 showed a significant difference. From the total color difference point of view, the ratio of flower to water should be less than 1:40. However, factor of the extraction time did not affect the yellowness of CM infusion.

#### **Effect of different extracting methods on infusion lightness**

Good ready-to-drink CM production needs good lightness. Lightness is an indicator to evaluate the quality of ready-to-drink CM product. The changes in lightness of CM liquors were given in Table 4. The results showed factors of the extraction temperature and the ratio of flower to water had great effect on liquor lightness. "L", the indicator of lightness decreased with the rise of the extraction temperature and the ratio of flower to water. Treatments in ratio of CM to water between 1:20 and 1:40 were different significantly. It suggests the ratio of flower to water should be less than 1:40. However, extraction time showed less affect on the lightness of CM infusion.

The above results showed that the experimental conditions of CM extracting (CME) temperature and the ratio of flower to water were the most important factors which had the effect on quality of CM infusion. It has

**Table 4.** Result of  $\Delta L$ ,  $\Delta a$ ,  $\Delta b$ ,  $\Delta E^a$ 

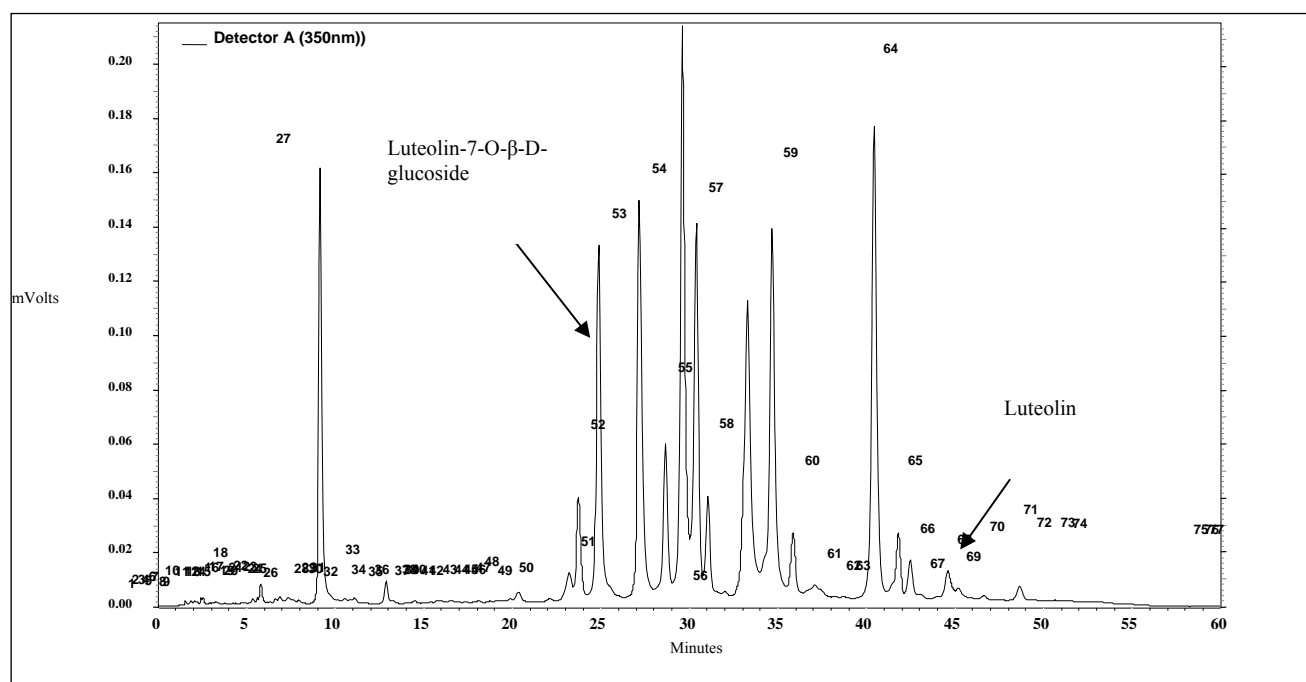
RUN	X1	X2	X3	$\Delta L$	$\Delta a$	$\Delta b$	$\Delta E$
1	40	10	1:30	-4.86	-2.78	15.3	16.3
2	40	30	1:30	-6.09	-2.84	16.3	17.7
3	100	10	1:30	-14.44	-1.70	21.2	25.7
4	100	30	1:30	-10.73	-1.92	21.3	24.0
5	70	10	1:40	-5.47	-2.34	14.5	15.7
6	70	10	1:20	-9.42	-2.28	21.3	23.4
7	70	30	1:40	-9.58	-1.98	17.5	19.7
8	70	30	1:20	-12.88	-1.77	24.6	27.9
9	40	20	1:40	-4.35	-2.43	12.7	13.6
10	100	20	1:40	-10.68	-1.83	18.3	21.3
11	40	20	1:20	-9.20	-2.68	21.3	23.4
12	100	20	1:20	-16.67	-1.55	26.3	31.2
13	70	20	1:30	-8.37	-2.13	19.4	21.2
14	70	20	1:30	-10.16	-1.83	19.9	22.4
15	70	20	1:30	-9.62	-2.00	20.6	22.8

<sup>a</sup>: X1=temperature (°C), X2= time (min), X3=flower/ water ratio (w/v)

**Table 5.** Analysis of variables of  $\Delta L$ ,  $\Delta a$ ,  $\Delta b$ ,  $\Delta E$  in the RSM model <sup>a</sup>

RUN	$\Delta L$	$\Delta a$	$\Delta b$	$\Delta E$
Source <sup>a</sup>			P	
X1	0.003**	0.002**	0.001**	0.001**
X2	0.360	0.372	0.049*	0.137
X3	0.016*	0.640	0.0001**	0.0005**

<sup>a</sup>: X1=temperature (°C), X2= time (min), X3=flower/ water ratio (w/v).

**Figure1.** HPLC profile of CM infusion constituents

remarkable effect on chemical compositions, extractability, lightness and color of CM infusion.

Compared with each experimental condition, it showed that CM extracting (CME) temperature was the most important factor in CM extraction. The solid extraction yield, color differences and important components luteolin-7-O- $\beta$ -D-glucoside and luteolin were significantly different with the changes of CME temperature ( $p < 0.05$ ). Figure 1

showed that there are many unidentified components in the CM infusion. Because of lacking reference compounds, we could not identify what they are. From the experiments, we also found that the flavonoids were not easily dissolved in the water, but they were easily dissolved in the alcohol. The total concentration luteolin-7-O- $\beta$ -D-glucoside extracted using alcohol was about 6g/kg~7 g/kg, which was treble of the CM infusion

extracted by hot water (100°C). At the same time, when the extraction temperature was increased, the concentrations of luteolin-7-O-β-D-glucoside and luteolin increased. The best condition were the CME temperature of 90~100°C, in which more flavonoids were extracted in the CM infusion.

CME time is another important factor. The solid extraction yield, luteolin-7-O-β-D-glucoside and yellowness were significant different with the change of CME time. That means the longer the extraction time, the more solid was extracted. As production efficiency was taken into account, CME time must be controlled. So 20~30 min is recommended. Sample particle size would affect the extraction rate. When the sample was grounded to smaller particle size, the functional chemical components would be extracted in a shorter time.

The ratio of flower to water has a remarkable effect on the infusion color of CM infusion. When the ratio of flower to water decreased, the infusion became lighter and less yellow. In many companies, this CM infusion will be diluted with water or mixed with other extracts, for example, tea extract and other herbal extracts to make functional drinks. That suggests the ratio of flower to water should be determined by the product formulas. If only *Chrysanthemum Morifolium* flower was extracted, the ratio of flower to water 1:40 is recommended. However, the ratios of 1:20~1:40 will be good for those companies to dilute *Chrysanthemum Morifolium* extract with the other herbal extracts during making ready-to-drink CM products.

From above, the best conditions for extracting *Chrysanthemum Morifolium* flower were 90~100 °C for 20~30min at ratio of flower to water between 1:20~1:40.

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