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Preparation of High Quality Safflower (*Carthamus tinctorius* L.) Seed Extract by High-Pressure Extraction Process

– Research Note –

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Abstract

Safflower seed extract was prepared by a high-pressure extraction technology and its quality characteristics were compared to that of other conventional extraction techniques, such ultrasonic and reflux extractions. Safflower seeds were extracted with 80% aqueous ethanol by three above extraction methods, and further fractionated with Diaion HP-20 column chromatography to obtain a partially purified safflower seed extract (PPSSE). Among the three extraction techniques examined, the reflux extraction showed the higher yields of EtOH extract and PPE than the ultrasonic and high-pressure extractions. Levels of most phenolic compounds in the EtOH extract of safflower seed are higher in reflux and ultrasonic extractions than the high pressure extraction, but levels of two serotonin aglycones, *N*-(*p*-coumaroyl)serotonin (CS) and *N*-feruloylserotonin (FS), in PPSSE were higher in the high pressure extraction than the reflux and ultrasonic extractions. In addition, color values (*L* and *a*) of the PPSSE were higher in the high-pressure extraction than the reflux and ultrasonic extractions, although there were no significant differences in pH and UV maxima absorption spectra among three extraction techniques. These results indicate that the high-pressure extraction technology is a simple and effective extraction for preparation of a high quality of safflower seed extract containing CS and FS with anti-wrinkle activity.

Key words: safflower (*Carthamus tinctorius* L.) seed extract, phenolic compounds, color value, reflux, ultrasonic, high-pressure extractions

INTRODUCTION

Extraction is the first important step in the separation, purification and recovery of biologically active compounds from plant materials. Recently, new extraction techniques, such as ultrasonic, microwave, high-pressure, and supercritical fluid extractions, have been developed to extract essential components of medicinal plants. These techniques provide alternatives to several traditional extraction methods, including soxhlet, heat reflux and boiling techniques (1-4). In particular, the high pressure extraction technique is recently receiving much attention as a new extraction method due to its high extraction efficiency and reduced impurities in the extracting solution, and combination of enzymatic treatment (3,5).

Phenolics are known as secondary metabolites occurred ubiquitously in plants and their health benefits are receiving increasing interest from consumers and food manufacturers (6). Phenolic compounds, such as serotonin derivatives, lignans and flavonoids, in safflower (*Carthamus tinctorius* L.) seed have been reported to have a variety of biological actions, including antiosteoporotic (7,8), antilipidemic (9), anticarcinogenic (10), anti-inflammatory (11), and antioxidant (12-15) activities. In particular, two serotonin derivatives, *N*-(*p*-coumaroyl)serotonin (CS) and *N*-feruloylserotonin (FS), of phenolic compounds have recently been found to promote epidermal cell growth (16), inhibit melanin synthesis (17), and attenuate collagen degradation (18,19). For this, the Korean Food & Drug Administration (KFDA) allowed serotonin derivatives as anti-wrinkle agents in cosmetics, and safflower seed extract including serotonin derivatives is currently used as a promising source of anti-aging functional cosmetics (20). However, two serotonin derivatives are easily susceptible to oxidation and degradation in the presence of light during extraction and purification (21). Therefore, development of an improved extraction technique for preparation of safflower seed extract is required. To date, few studies on extraction techniques of phytochemical phenolics from safflower seeds is available, although characterization and quantitative analysis of phenolic compositions in safflower seeds has already been performed.

The objective of this study was to compare the quality characteristics of safflower seed extracts prepared by a high-pressure extraction technology to the conventional extraction techniques of reflux and ultrasonic extractions.

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MATERIALS AND METHODS

Materials

Safflower (*Carthamus tinctorius* L.) seeds (Uisan) were directly harvested on early August 2008 at a farm in Uisong, Gyeongbuk, Korea. The harvested seeds were dried at 50°C in a drying oven, milled to 20 mesh size with a coffee maker, and stored at 4°C at refrigerator until use.

Extraction and fractionation of safflower seeds

Two safflower seed extracts were prepared by three extraction techniques: reflux, ultrasonic and high pressure extractions. Ground safflower seed powder (100 g) was refluxed twice with 80% aq. EtOH (1 L) for 3 hr, filtered and evaporated under reduced pressure. The crude EtOH extract was again solubilized in 80% EtOH and left to stand overnight at refrigerator. The aqueous layer was filtered and evaporated to a small volume, and then subjected onto a Diaion HP-20 column (6×20 cm), which was previously equilibrated with 40% EtOH. The column was eluted first with 40% EtOH (2 L) to remove phenolic glycosides, and then eluted with 80% EtOH (3 L) to isolate two serotonin aglycones. The 80% EtOH fraction was finally concentrated *in vacuo* to yield a partially purified safflower seed extract (PPSSE). Meanwhile, safflower seed powder (100 g) was twice extracted with 80% EtOH in an ultrasonic cleaning bath (Power Sonic 420, 50/60 Hz, 700 W, Hwashin Tech, Korea) for 3 hr, and further fractionated with Diaion HP-20 by the same procedure above and then obtained PPSSE. Finally, safflower seed powder (100 g) was placed into an extraction bag and extracted with 80% EtOH in a high pressure extractor (Armfield FT110 rapid extractor, Tecnolab, Spello, Italy) adjusted to 5～10 bar for 1, 3, and 6 hr. The crude 80% EtOH extract and PPSSE were also obtained by the same procedure above.

Quantification of phenolic compound by HPLC analysis

Quantitative analysis of phenolic compounds in safflower seed in relation to three different extraction techniques was performed by HPLC according to the method previously reported (22). The EtOH extract of the safflower seed and PPSSE, as obtained above, was solubilized in 80% EtOH, passed through 0.45 μm membrane filter (Gelman, USA) and then injected in HPLC. HPLC was performed on a Gilson 506B HPLC System coupled with Gilson 170 UV-vis detector, and Gilson 231 XL autosampler with a 10 μL loop. HPLC analysis was carried out using a YMC-Pack Pro C_{18} column (46 mm i.d. ×250 mm, YMC Inc., USA) with a Guard-Pak C_{18} precolumn insert. The separation was conducted using a linear gradient from 0.05% v/v H_3PO_4 in 20% MeOH (solvent A) to 80% MeOH (solvent B) for 60 min at a flow rate of 0.8 mL/min with UV detection at 270, 310 and 350 nm. The elution profile was as follows: 0～2 min, 100% A, 0% B; 5～10 min, 80% A, 20% B; 15～20 min, 60% A, 40% B; 25～30 min, 40% A, 60% B; 35～40 min, 0% A, 100% B. Duplicate analyses were conducted on duplicate samples. The concentration of phenolics were determined by calibration curves of ten standard phenolics obtained previously (22), and expressed as mg% of dried weight.

pH, UV absorption spectra and colorimetry

pH and UV λ_{max} (nm) of safflower seed extracts were measured with a pH meter (Mettler Toledo, InLab 413, Switzerland) and UV-vis spectrophotometer (S-3100 Sinco, Korea), respectively, after solubilizing with 80% ethanol in 0.1% concentration. Color of safflower seed extract solution was determined by a Minolta colorimeter (CR-200, Minolta Co., Japan). The color was expressed as *L* (lightness), *a* (redness) and *b* (yellowness) values, as control of white ceramic plate was used for calibrating the instrument (*L*=97.78, *a*=-0.39, *b*=2.05).

Statistical analysis

Data presented are means±standard deviation of triplicate determinations. Statistical analysis was performed by using Duncan's multiple range test at $p<0.05$.

RESULTS AND DISCUSSION

Yield of the EtOH extract of safflower seed and PPSSE

Yield of the EtOH extract and PPSSE obtained by three different extraction techniques, followed by purification step using Diaion HP-20 column chromatography, were given in Table 1. Yields of the EtOH extract of safflower seed in the high pressure extractions for 1, 3, and 6 hr were 3.84%, 5.16%, and 5.27%, respectively. The yields of EtOH extract of safflower seeds in the high pressure extraction were increased with increasing extraction time. The yields of EtOH extract under ultrasonic and reflux extractions were 5.23% and 8.90%, respectively. Meanwhile, yields of the PPSSE under the high pressure extractions for 1, 3, and 6 hr were 0.24%, 0.42%, and 0.43%, respectively. As similar to the EtOH yield, the yields of PPSSE were increased with increasing extraction time. The yields of PPSSE under ultrasonc and reflux extraction were 0.43% and 0.65%, repectively. Thus, the reflux extraction had the most yield of EtOH extract, while no significant differences in

Table 1. Yields of the EtOH extract and the partially purified extract of safflower seed (PPSSE) obtained by the high-pressure, ultrasonic and reflux extractions

Extraction method		Yield (%)	
		80% EtOH extract	PPSSE
High-pressure extraction	1 hr	3.84±0.35[c]	0.24±0.03[c]
	3 hr	5.16±0.40[b]	0.42±0.05[b]
	6 hr	5.27±0.42[b]	0.43±0.05[b]
Ultrasonic extraction		5.23±0.45[b]	0.43±0.05[b]
Reflux extraction		8.90±0.53[a]	0.65±0.11[a]

Data represent mean±SD of triplicate determinations.
Values with different letters within each column are significantly different at $p<0.05$.

yields of the EtOH extract between the high pressure and ultrasonic extractions.

Quantitative changes of phenolic compounds in safflower seed EtOH extract obtained by three different extraction techniques

Quantitative changes of phenolic compounds in safflower seed by three different extraction techniques were presented in Table 2. Four serotonin derivatives [*N*-feruloylserotonin 5-*O*-β-D-glucoside (FSG), *N*-feruloylserotonin (FS), *N*-(*p*-coumaroyl)serotonin 5-*O*-β-D-glucoside (CSG), *N*-(*p*-coumaroyl)serotonin (CS)], four lignans [tracheloside (TCS), trachelogenin (TCG), matairesinoside (MRS) and matairesinol (MR)], and two flavonoids [acacetin 7-*O*-β-D-glucuronide (ACG) and acacetin (AC)] were found in the EtOH extract of safflower seed, as reported previously (22). Here, the major phenolic compounds in safflower seeds, FS and CS, TCG, and ACG, were quantitated by three extraction techniques. In HP-1 (high pressure extraction for 1 hr), levels of CS and FS, TCG, and ACG were 45.73 and 75.38, 8.28, and 59.75 mg%, respectively. In HP-3, levels of CS and FS, TCG, and ACG were 112.54 and 190.45, 16.26, and 92.62 mg%, respectively. In HP-6, levels of CS and FS, TCG, and ACG were 95.37 and 165.93, 57.52, and 107.58 mg%, respectively. Thus, levels of these phenolic compounds were increased with increasing extraction time, but levels of two serotonin aglycones, CS and FS, were increased by 3 hr extraction, and then decreased slightly to 6 hr extraction. In a ultrasonic extraction, the levels of CSG, FSG, MRS, TCS, CS, FS, MR, TCG, and ACG were 109.38, 41.43, 126.93, 82.34, 161.45, 283.06, 31.29, 59.27, and 140.28 mg%, respectively. In a reflux extraction, the levels of CSG, FSG, MRS, TCS, CS, FS, MR, TCG, and ACG were 115.34, 44.73, 127.27, 106.23, 159.43, 289.45, 28.83, 57.27, and 135.37 mg%, respectively. As compared to the high pressure and reflux extraction techniques, the ultrasonic extraction method had the highest content of two serotonin aglycones, CS and FS, while those levels were decreased in the reflux extraction method. Significant differences were not found in levels of other phenolic compounds. Based on these results, the ultrasonic extraction method appears suitable for extraction of two serotonin derivatives (CF and FS), which are widely known as antioxidant, anti-inflammatory and anti-aging agents (11-20). The levels of two serotonin aglycones were decreased with increasing extraction time in the high pressure extraction, indicating that two serotonin aglycones are susceptible to heating during the high-pressure extraction. It is very interesting to note that the levels of most phenolic compounds in safflower seeds were increased by 6 hr extraction of the high pressure, especially level of trachelogenin, a well-known

Table 2. Comparison of phenolic levels in safflower (*Carthamus tinctorius*) seed according to the high-pressure, ultrasonic, and reflux extractions

Phenolic compound	Content (mg%, dry base)				
	High pressure extraction			Ultrasonic extraction	Reflux extraction
	1 hr	3 hr	6 hr		
N-(*p*-Coumaroyl)serotonin 7-*O*-β-D-glucoside	82.45±11.87[b]	83.37±11.85[b]	91.26±12.42[a]	109.38±21.46[a]	115.34±22.41[a]
N-Feruloylserotonin 7-*O*-β-D-glucoside	28.94±2.44[c]	28.93±2.35[c]	34.63±2.98[b]	41.43±3.81[a]	44.73±4.61[a]
Matairesinoside	106.33±13.27[b]	108.13±13.51[b]	115.48±13.19[a]	126.93±22.38[a]	127.27±22.48[a]
Tracheloside	50.25±5.23[c]	57.48±5.68[c]	59.25±5.27[c]	82.34±15.64[b]	106.23±20.73[a]
N-(*p*-Coumaroyl)serotonin	45.73±3.27[c]	112.54±13.23[b]	95.37±12.41[b]	161.45±13.22[a]	159.43±12.35[a]
N-Feruloylserotonin	75.38±11.72[d]	190.45±13.72[b]	165.93±12.74[c]	283.06±31.10[a]	289.45±35.20[a]
Matairesinol	16.93±1.25[c]	28.75±2.77[ab]	31.20±3.54[a]	31.29±3.18[a]	28.83±2.40[b]
Trachelogenin	8.28±0.79[c]	16.26±1.30[b]	57.52±4.48[a]	59.27±5.04[a]	57.27±5.19[a]
Acacetin 7-*O*-β-D-glucuronide	59.75±4.10[c]	92.62±12.39[b]	107.58±17.49[b]	140.28±24.93[a]	135.37±23.03[a]
Acacetin	ND	ND	ND	ND	ND

Data represent mean±SD of triplicate determinations.
Values with different letters within each row are significantly different at $p<0.05$.

phytoestrogen (23,24), was increased significantly. Recently, it was also found that trachelogenin in safflower seed increased greatly by germination (25).

Contents of two serotonin aglycones of PPSSE obtained by a Diaion HP-20 column chromatography

Comparison of two serotonin aglycones contents of PPSSE obtained by a Diaion HP-20 column chromatography of the seed EtOH extracts according to three different extractions was shown in Table 3.

Two serotonin aglycones, CS and FS, of the PPSSE obtained by a Diaion HP-20 column chromatography of the EtOH extract of safflower seed were quantitated by HPLC to select proper extraction methods. In a high pressure extraction, levels of CS and FS were 21.32% and 35.53%, respectively, whereas those of CS and FS in ultrasonic and reflux extractions were 18.59%, 32.67% and 16.34%, 29.27%, respectively. The levels of two serotonin aglycones of the high pressure extraction was similar to that of a ultrasonic extraction, but two levels of a reflux extraction were lower than that of the high pressure and ultrasonic extractions. Thus, lowering of two serotonin aglycones (CS and FS) of the PPSSE in the reflux extraction was due to chemical oxidation during the heat extraction, although the specific mechanism of chemical oxidation is not known. These results suggest that the high pressure and ultrasonic extractions of safflower seed were superior to the reflux extraction method because of the oxidation and degradation of two serotonin aglycones during reflux heating extraction.

pH, UV absorption and colorimetry of the PPE of safflower seed

The pH, UV absorption and colorimetry of PPSSE of obtained by Diaion HP-20 column chromatography of the EtOH extract of safflower seed were determined and presented in Table 4. pH of three PPSSE ranged from 5.4 to 5.8, and UV absorption maxima ranged 282～285 and 315-317 nm, without significant differences among three extraction methods. Color values of the PPSSE in the high pressure extractions was the highest in *L* value (29.07～31.58) and *b* value (7.82～10.43) and the lowest in *a* value (0.26～2.06), while *L* value (26.83) and *b* value (4.45) of a ultrasonic extraction were lower than those of a high pressure extractions. In the reflux extraction, *L* value (24.98) was the lowest, but *a* value (2.57) was higher compared to other extraction methods. Thus, colors of PPSSE were the lightest in the high pressure extraction, while those of PPSSE were the darkest red in the ultrasonic and reflux extractions. These results suggest that the high pressure extraction is suitable for preparation of safflower seed extract containing serotonin aglycones. The conventional extraction techniques, reflux and ultrasonic extractions, used widely for the extraction of phenolic compounds from plants are usually lengthy and non-selective (3). However, the high-pressure process has become an important alternative to the conventional extraction methods, due to relatively shorter times and greater selectively (26,27). Herewith, these data indicate that the high-pressure extraction technology results in the efficient extraction of phytochemical phe-

Table 3. Comparison of levels of two serotonin aglycones in the partially purified safflower seed extract (PPSSE) obtained by a Diaion HP-20 column chromatography of the seed EtOH according to three different extractions

Phenolic compound	Content (%, PPSSE)		
	High-pressure extraction[1)]	Ultrasonic extraction	Reflux extraction
N-(*p*-Coumaroyl)serotonin	21.32 ± 0.81^{a}	18.59 ± 0.51^{b}	16.34 ± 0.45^{c}
N-Feruloylserotonin	35.53 ± 1.80^{a}	32.67 ± 1.46^{b}	29.27 ± 1.03^{b}

Data represent mean ± SD of triplicate determination.
Values with different letters within each row are significantly different at $p<0.05$.
[1)]A high-pressure extraction at 3 hr.

Table 4. Comparison of pH, UV maximum absorption and color values in the partially purified safflower seed extract (PPSSE) according to three different extractions

Extraction method		pH	UV λ_{max} (nm, EtOH)	Colorimetry		
				L	*a*	*b*
High-pressure extraction	1 hr	5.4 ± 0.2^{NS}	283.5 ± 0.3 & 315.6 ± 0.2^{NS}	31.58 ± 0.20^{a}	0.26 ± 0.13^{e}	10.43 ± 0.19^{a}
	3 hr	5.6 ± 0.3	283.2 ± 0.1 & 315.5 ± 0.3	29.79 ± 0.24^{a}	1.74 ± 0.08^{d}	8.85 ± 0.34^{b}
	6 hr	5.6 ± 0.1	285.2 ± 0.3 & 317.7 ± 0.4	29.07 ± 0.13^{a}	2.06 ± 0.11^{c}	7.82 ± 0.14^{c}
Ultrasonic extraction		5.7 ± 0.3	282.2 ± 0.4 & 314.5 ± 0.3	26.83 ± 0.08^{b}	3.06 ± 0.02^{a}	4.45 ± 0.11^{d}
Reflux extraction		5.8 ± 0.4	284.5 ± 0.2 & 317.3 ± 0.2	24.98 ± 0.04^{c}	2.57 ± 0.11^{b}	1.47 ± 0.06^{e}

Data represent mean ± SD of triplicate determinations.
Values with different letters within each column are significantly different at $p<0.05$.
NS: not significantly different at $p<0.05$.

nolics from safflower seeds.

In conclusion, the high pressure extraction was superior to a ultrasonic and reflux extractions because of higher extraction yield of phenolic compounds including two serotonin aglycones and lighter color of PPSSE, although polyphenolic content in safflower seed extract was higher in the ultrasonic and reflux extractions than the high-pressure extraction. This study is the first report on preparation of high quality safflower seed extract for improving skin-aging in functional cosmetics. Further study is needed to optimize the high-pressure extraction for preparation of safflower seed extract.

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