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Quantitative Changes in Phenolic Compounds of Safflower (*Carthamus tinctorius* L.) Seeds during Growth and Processing

Eun Ok Kim, Jun Young Lee and Sang Won Choi†

Department of Food Science and Nutrition, Catholic University of Daegu, Gyeongbuk 712-702, Korea

Abstract

Phenolic compounds in safflower seeds were recently found to stimulate bone formation and increase plasma HDL cholesterol levels in estrogen deficient rats, and to inhibit melanin synthesis. Nine phenolic compounds: *N*-feruloylserotonin-5-*O*-β-D-glucoside, 8'-hydroxyarctigenin-4'-*O*-β-D-glucoside, luteolin-7-*O*-β-D-glucoside, *N*-(*p*-coumaroyl)serotonin, *N*-feruloylserotonin, 8'-hydroxy arctigenin (HAG), luteolin (LT), acacetin-7-*O*-β-D-glucuronide (ATG) and acacetin (AT), were quantified by HPLC in safflower (*Carthamus tinctorius* L.) seeds during growth and processing. During growth, levels of the nine phenolic compounds in the seeds increased progressively with increasing growth stages, reached a maximum on July 30 (42nd day after flowering), and then remained relatively constant. During the roasting process, levels of phenolic compounds, except HAG, LT and AT, generally decreased with increased roasting temperature and time, whereas those of HAG, LT and AT increased progressively with increased roasting temperature and time. During the steaming process, levels of other phenolic compounds except HAG and AT generally tended to increase with increased steaming time, whereas those of HAG and AT were scarcely changed. During the microwave treatment, quantitative changes of phenolic compounds were similar to the roasting process, although there were some differences in levels of phenolic compounds between two heat treatments. These results suggest that the steamed safflower seeds after harvesting on late July may be useful as potential dietary supplement source of phenolic compounds for prevention of several pathological disorders, such as atherosclerosis and osteoporosis and aging.

Key words: safflower (*Carthamus tinctorius* L.) seed, phenolic compounds, quantification, HPLC, growth, processing

INTRODUCTION

Safflower seeds are rich in unsaturated fatty acids, proteins, and dietary fibers, which are very important constituents of functional foods (1,2). Particularly, safflower seeds are clinically employed for the treatment of cataclasis, osteoporosis and rheumatoid arthritis in Korea. Our recent studies revealed that phenolic compounds in safflower seeds, such as conjugated serotonins, lignans and flavones, possessed bone protecting effects (3,4), and increased plasma HDL cholesterol level in estrogen deficient rats (5). Additionally, the phenolic compounds have been reported to have a variety of biological actions, such as antioxidation (6-8), antiinflammation (9), anticancer (10) and anti-aging (11-14). Thus, phenolic compounds in safflower seeds are recently receiving much attention as potential therapeutic agents against several pathological diseases. However, few studies on quantification of phenolic compounds during growth and processing of safflower seeds are available.

Recently, we have tried to develop dietary supplements containing phytochemical phenolic compounds in safflower seeds for the prevention of bone diseases. However, utilization of safflower meal as food and feed has been partially limited because some phenolic glycosides in safflower seeds have been found to have bitter taste and cathartic effect (15,16). Additionally, it is very difficult for us to use safflower seed powder as a dietary supplement source because of the hard and non-digestible husk of safflower seeds. Therefore, development of appropriate processing technology is required to prepare for manufacturing high quality safflower seed extract without the above deleterious effects. Many kinds of phytochemical phenolics have been found in plants, and their levels could be affected by cultivar, maturity and processing (17). It was also reported that many naturally occurring phenolics mainly exist as glycosides in plants, but are degraded to aglycones by several processing treatments (18,19). Particularly, such heat pretreatments as roasting, steaming and microwave heating have been reported to play important roles in improving functional constituents and quality of plant seeds (20-24). Therefore,

†Corresponding author. E-mail: swchoi@cu.ac.kr
Phone: +82-53-850-3525, Fax: +82-53-850-3504

systematic study on quantitative changes of phenolic compounds in safflower seed during growth and processing is essential prior to preparation of high quality of safflower seed extract.

The purpose of this study was to investigate quantitative changes of phenolic compounds in safflower seeds during growth and processing.

MATERIALS AND METHODS

Material and chemicals

Safflower (*Carthamus tinctorius* L.) seeds were directly harvested from early July to early August at the New Natural Products Institute experimental station farm, Uisong, Gyeongbuk, Korea. The fresh seeds (100 g) for each experiment were obtained from the designated ten plants every week. The samples were freeze-dried immediately after harvesting and stored at -18°C until HPLC analysis. Samples were sun-dried for one week after harvesting and stored at cooling room before heat pretreatments. HPLC solvents were obtained from Merck (Darmstadt, Germany). All other reagents used for this study were analytical grade.

Sample preparation

Dried safflower seeds (100 g) were roasted in an electric roaster (Dongkwang, Korea) with constant stirring at 200, 225, and 250°C for 10 min, and 225°C for 10, 20 and 30 min. Each sample (100 g) was then steamed at 90～100°C in a domestic stainless steel steamer [dimensions 260 (W)×200 mm (H)] for 60, 120 and 180 min. Finally, the sample (100 g) was placed in a rotating glass container (dimensions 290 mm i.d.) in the center of a domestic microwave (MW) oven (Samsung RE-C200T, Suweon, Korea) which was operated at 2,450 MHz frequency with a pulsed variable MW power output from 90 to 700 W which was controlled by a timer, and 21.8 L inner volume, and heated for 1, 3 and 5 min. The three heat pretreated safflower seeds were dried for 6 hr in a drying oven at 50°C before analysis of phytochemical phenolics.

Isolation and identification of phenolic compounds

Nine phenolic compounds: *N*-feruloylserotonin-5-*O*-β-D-glucoside (FSG), 8'-hydroxy-arctigenin-4'-*O*-β-D-glucoside (HAGG), luteolin-7-*O*-β-D-glucoside (LTG), *N*-(*p*-coumaroyl)serotonin (CS), *N*-feruloylserotonin (FS), 8'-hydroxyarctigenin (HAG), luteolin (LT), acacetin-7-*O*-β-D-glucuronide (ATG) and acacetin (AT) were isolated and identified from safflower seeds, as previously described (10,25).

HPLC analysis of phenolic compounds

Ground safflower seeds (20 g) were refluxed twice with aq. 80% EtOH (200 mL) for 2 hr, then filtered and evaporated under reduced pressure. The EtOH extract was redissolved in 80% aq. MeOH (100 mL) and washed with *n*-hexane (300 mL) to remove lipids and pigments. The lower layer was evaporated and then solubilized with 80% aq. MeOH (50 mL). The solution was allowed to stand overnight, then filtered and brought to a 100 mL volume with 80% MeOH solution. The MeOH solution (4 mL) was passed through Sep-Pak C_{18} cartridges (Waters Co., Milford, MA, USA) which were preconditioned with MeOH and H_2O. The first 3 mL was discarded and next 1 mL was used for HPLC analysis. HPLC analysis was performed on an HPLC system (Gilson 506B, USA) equipped with a 170 UV-vis detector, Gilson Unipoint™ 3.0 software and 231 XL autosampler with a 10 μL loop, using a YMC-Pack Pro C_{18} column (5 μm, 4.6 I.D.×250 mm, YMC Inc., USA) at a flow rate of 0.8 mL/min with a UV detector at 270 nm. The column was eluted gradiently with solvent A (0.05% H_3PO_4 in 20% aq. MeOH) to solvent B (80% aq. MeOH) for 60 min. 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. The column was returned to the initial condition for 10 min before the next injection. Duplicate analyses were conducted on duplicate samples.

Statistical analysis

Statistical analysis was performed using ANOVA with Duncan's multiple range test at $p<0.05$.

RESULTS AND DISCUSSION

Quantification of phenolic compounds by HPLC

Safflower seeds are known to contain several phenolic compounds, such as serotonin derivatives, lignans and flavones (26). However, no reports were found on HPLC analysis for the simultaneous determination of phenolic compounds in safflower seed. So, we performed several HPLC analyses to achieve optimal separation using different composition of two mobile phases, methanol and phosphoric acid, with a linear gradient elution, as previously described. As a result, two solvent systems comprising 0.05% phosphoric acid in 20% MeOH and 80% MeOH gave a better separation of phenolic compounds. The typical HPLC spectra of the defatted EtOH extract from safflower seeds were shown in Fig. 1. Individual phenolics were identified by a comparison of their retention time with those of the nine standard phenolics.

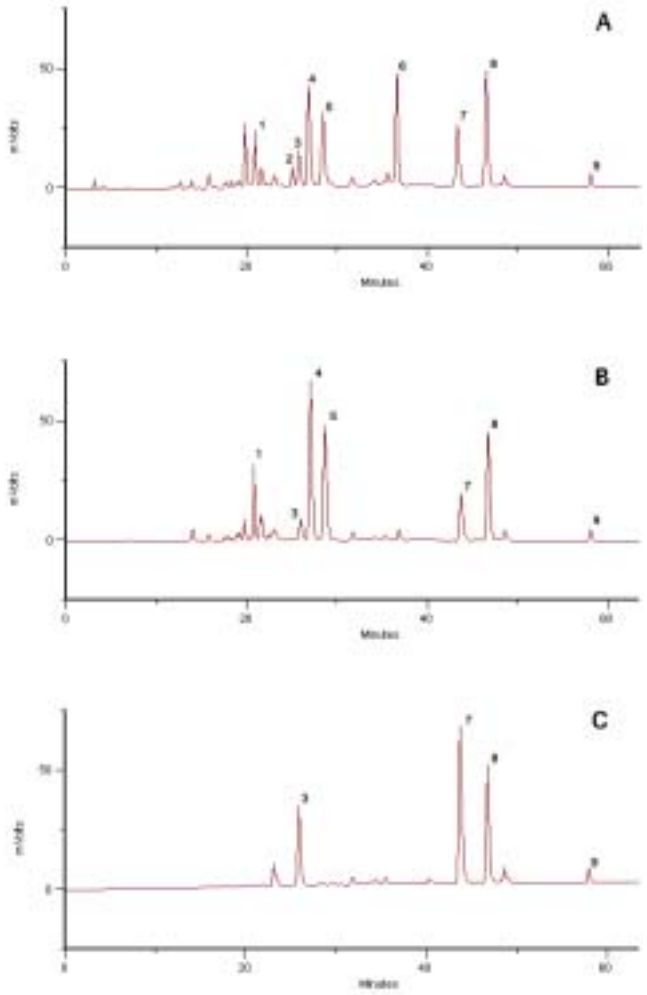

Fig. 1. HPLC chromatograms of the defatted ethanol extract of safflower seeds. A: 270 nm, B: 310 nm, C: 340 nm. 1: *N*-Feruloylserotonin-5-*O*-β-D-glucoside, 2: 8'-hydroxyarctigenin-4'-*O*-β-D-glucoside, 3: luteolin-7-*O*-β-D-glucoside, 4: *N*-(*p*-coumaroyl)serotonin, 5: *N*-feruloylserotonin, 6: 8'-hydroxyarctigenin, 7: luteolin, 8: acacetin 7-*O*-β-D-glucuronide, 9: acacetin. HPLC analysis was performed as described in Materials and Method.

Peaks were identified by co-chromatography with authentic samples isolated previously. Retention time (min) of each phenolic compound was as follows; FSG (20.92), HAGG (25.06), LTG (25.78), CS (26.85), FS (28.43), HAG (36.62), LT (43.32), ATG (46.51), AT (58.10). Each of four different concentrations (10～100 μg/mL for serotonin derivatives and 10～150 μg/mL for lignans and flavones) of standard solutions were injected three times into the HPLC system. All the compounds showed good linearity ($\gamma \geq 0.997$) and recovery rate (92%, 90% and 87% for serotonin derivatives, lignans and flavones, respectively). The calibration lines of nine compounds ($y=2.4251x-1.8236$ for *N*-feruloylserotonin-5-*O*-β-D-glucoside, $y=0.284x-1.173$ for 8-hydroxyarctigenin-4'-*O*-β-D-glucoside, $y=1.5723x-4.2356$ for luteolin-7-*O*-β-D-glucoside, $y=2.3217x-1.9432$ for *N*-(*p*-coumaroyl) serotonin, $y=1.9782x-2.9116$ for *N*-feruloylserotonin, $y=1.5585x-6.4882$ for luteolin, $y=2.8453x-4.562$ for acacetin-7-*O*-β-D-glucuronide, and $y=2.9487x-3.602$ for acacetin) were determined by a least squares regression method. The concentration of phenolics were determined by calibration curves of nine standard phenolics, and expressed as mg% of dried weight.

Quantitative changes of phenolic compounds in safflower seed during growth and processing

We previously isolated and identified three different types of phenolic compounds from safflower seeds (10,25). Among them, CS, FS and AT, together with matairesinol (MR) were recently found to have anti-osteoporotic and antihyperlipidemic activities as phytoestrogens (3-5). Therefore, it is meaningful to investigate quantitative changes of phenolic compounds in the seeds during maturity and processing prior to preparation of high quality of safflower phenolic extract as dietary supplement source for treatment of bone diseases. Herein, we quantified the nine phenolic compounds by HPLC during growth and processing of safflower seeds.

Quantitative changes of the nine phenolic compounds during growth of the safflower seeds are shown in Table 1. At the early stage of growth (7/2), most phenolic compounds, except two serotonin aglycones (CS and FS), were present as glycosides, and especially, 8'-hydroxyarctigenin (HAG) and acacetin (AT) were not detected throughout the growth stage. Levels of all phenolics increased progressively with increased development stage of the seeds, reached a maximum (11.75～238.59 mg%) on July 30 (42nd day after flowering), and then, remained relatively constant. Two serotonin algycones (CS & FS) and acacetin glycoside (ATG) constituted about 75.9% of the dry weight of the seeds of which 53.4% and 22.5% was the two serotonin aglycones and acacetin glycoside, respectively. In contrast, other phenolic constituents also accounted for about 24.1% of the dry weight of the seeds. Thus, levels of the nine phenolic compounds in the safflower seeds had a tendency to increase during growth; similar trends were also observed in safflower (19), persimmon (27) and green tea (28) leaves.

Quantitative changes in levels of the nine phenolic compounds in safflower seeds during the roasting process are presented in Table 2 and Table 3. We first investigated quantitative changes in phenolic compounds during roasting at five different temperatures (200, 225, 250, 275, 300°C) for 5 min. As shown in Table 2, levels of most of the phenolic constituents, except three phenolic

Table 1. Quantitative changes of nine phenolic compounds in safflower seed during growth (mg%, dry base)

Phenolic[1)]	Growth period					
	July 2 (14th)[2)]	July 9 (21th)	July 16 (28th)	July 23 (35th)	July 30 (42th)	August 6 (49th)
1	2.54±0.12[3)]	12.53±1.03	30.54±2.01	43.84±2.34	51.06±1.37[a4)]	50.12±1.45[a]
2	2.65±0.20	11.45±1.92	22.96±1.03	31.67±1.83	37.54±3.13[a]	35.92±3.27[a]
3	0.92±0.10	4.78±1.23	27.94±1.27	67.52±2.43	86.60±4.73[a]	85.92±4.62[a]
4	21.53±2.94	56.02±4.91	125.27±2.94	194.65±6.82	238.59±5.62[a]	233.82±5.73[a]
5	3.65±1.02	42.63±2.93	84.03±2.38	127.67±4.29	176.01±3.73[a]	173.91±2.82[a]
6	ND[5)]	ND	ND	ND	ND	ND
7	ND	6.32±0.12	10.22±0.51	11.64±0.28[a]	11.75±0.60[a]	11.73±0.34[a]
8	8.93±1.83	71.75±2.64	102.45±1.93	150.56±3.64	174.62±7.03[a]	173.73±7.28[a]
9	ND	ND	ND	ND	ND	ND
Total	40.22±1.04	205.48±2.11	403.41±1.72	627.55±3.09	776.17±3.74	765.15±3.64

[1)]1: *N*-Feruloylserotonin-5-*O*-β-D-glucoside, 2: 8'-hydroxyarctigenin-4'-*O*-β-D-glucoside, 3: luteolin-7-*O*-β-D-glucoside, 4: *N*-(*p*-coumaroyl)serotonin, 5: *N*-feruloylserotonin, 6: 8'-hydroxyarctigenin, 7: luteolin, 8: acacetin-7-*O*-β-D-glucuronide, 9: acacetin.
[2)]Days of growth stage after flowering.
[3)]Values are mean±SD of duplicate analyses.
[4)]Values with the same superscript letters in a row are not significantly different at $p<0.05$.
[5)]Not detected.

Table 2. Quantitative changes of nine phenolic compounds in safflower seeds according to five different roasting temperatures for 5 min (mg%, dry base)

Phenolic[1)]	Roasting temperature					
	Control	200°C	225°C	250°C	275°C	300°C
1	51.06±1.37[2)a3)]	50.38±1.64[a]	48.98±0.87[a]	48.68±1.27[a]	34.87±0.84[b]	32.15±1.02[b]
2	37.54±3.13[a]	37.02±2.11[a]	36.18±2.15[a]	37.83±1.98[a]	32.96±1.43[b]	30.63±1.51[b]
3	86.60±4.73[a]	85.57±2.35[a]	83.87±3.52[a]	82.11±3.72[a]	76.96±1.25[b]	70.53±1.04[c]
4	238.59±5.62[a]	237.45±6.30[a]	235.43±6.05[a]	218.17±3.49[b]	187.92±4.54[c]	185.85±4.02[c]
5	176.01±3.73[a]	175.65±3.40[a]	172.62±4.35[a]	170.90±6.27[a]	148.81±3.18[b]	139.75±2.72[c]
6	ND[4)]	ND	TR[5)]	TR	10.75±0.86	15.82±0.91
7	11.75±0.60[c]	12.26±1.59[c]	13.98±1.62[c]	14.75±1.76[c]	45.24±2.31[b]	75.92±3.02[a]
8	174.62±7.03[a]	172.49±6.79[a]	162.48±5.15[b]	102.88±4.27[c]	48.88±2.04[d]	8.45±0.42[e]
9	ND	TR	12.36±1.02	23.78±1.40	88.28±2.75	128.18±3.82
Total	776.17±3.74	770.82±3.45	765.87±3.09	699.10±3.02	674.67±2.14	687.28±2.05

[1)]Numbers are the same as in Table 1.
[2)]Values are mean±SD of duplicate analyses.
[3)]Values with the same superscript letters in a row are not significantly different at $p<0.05$.
[4)]Not detected. [5)]Trace (<1 mg%).

aglycones (HAG, LT, AT), decreased progressively up to 250°C and then, decreased sharply. Particularly, serotonin derivatives, predominantly phenolic components in safflower seeds, were very susceptible to high temperatures, and their contents greatly decreased with roasting temperatures above 250°C. In contrast, levels of three phenolic aglycones, HAG, LT and AT, increased progressively up to 250°C roasting temperature and then increased sharply. Thus, these results indicate that the roasting process degrades some phenolic compounds, especially serotonin derivatives, and partially converts phenolic glycosides into their corresponding aglycones. Several scientists (6,8,25) previously isolated and identified antioxidant phenolic aglycones, such as CS, FS, MR, and AT, from roasted safflower seeds, and safflower seeds are known to contain several phenolic glycosides with bitter taste and cathartic effect (15,16). On the base of these results, we found that a roasting process could strongly induce antioxidant activity of safflower seed extract and also mitigate to some extent the bitter taste and cathartic effects of the safflower seeds. Thus, the roasting process is a key step for making safflower seed oil condiments and improving the quality characteristics of safflower seed, although some phytochemical phenolic constituents decreased during the roasting process. As shown in Table 3, levels of most of phenolic compounds except HAG, LT and AT decreased sharply with increasing roasting time, and especially two serotonin aglycones, CS and FS, greatly decreased with increasing roasting time. However, HAG level increased slowly up to 20 min and then greatly increased at 30 min, while LT and AT levels increased up to 5 min and 20 min,

Table 3. Quantitative changes of nine phenolic compounds in safflower seeds according to three different roasting times at 250°C (mg%, dry base)

Phenolic[1)]	Roasting time (min)				
	Control	5	10	20	30
1	51.06±1.37[2)a3)]	48.68±1.27[b]	45.78±2.97[c]	24.85±1.55[d]	17.43±1.43[e]
2	37.54±3.13[a]	37.03±1.98[a]	34.63±2.27[b]	12.72±1.02[c]	TR[5)]
3	86.60±4.73[a]	82.11±3.72[a]	66.56±2.87[b]	28.54±1.92[c]	TR
4	238.59±5.62[a]	218.17±3.49[b]	187.63±4.24[c]	134.87±3.88[d]	69.25±2.67[e]
5	176.01±3.73[a]	170.90±6.27[a]	153.28±3.85[b]	117.98±2.15[c]	48.76±2.61[d]
6	ND[4)]	TR	10.54±0.29	15.97±1.39	63.83±1.31 (MR[6)]:6.93)
7	11.75±0.60[b]	14.75±1.76[a]	13.98±1.62[a]	11.80±0.68[b]	8.89±0.11[c]
8	174.62±7.03[a]	102.88±4.27[b]	86.73±3.43[c]	47.81±1.90[d]	5.79±0.57[e]
9	ND	23.78±1.40[a]	52.56±2.02[c]	106.36±3.86[a]	70.84±2.41[b]
Total	775.67±3.74	698.30±3.02	651.69±2.62	500.90±2.03	284.97±1.59

[1)]Numbers are the same as in Table 1.
[2)]Values are mean±SD of duplicate analyses.
[3)]Values with the same superscript letters in a row are not significantly different at $p<0.05$.
[4)]Not detected. [5)]Trace (<1 mg%). [6)]Matairesinol.

Table 4. Quantitative changes of nine phenolic compounds in safflower seeds according to four different steaming times (mg%, dry base)

Phenolic[1)]	Steaming time				
	Control	10 min	30 min	60 min	120 min
1	51.06±1.37[2)NS3)]	52.87±2.80	52.74±2.36	53.58±3.44	51.84±2.84
2	37.54±3.13[b4)]	37.98±1.29[b]	39.87±2.02[ab]	43.83±1.73[a]	43.97±1.92[a]
3	86.60±4.73[d]	87.76±1.23[d]	93.64±1.89[c]	97.73±2.95[b]	105.34±3.03[a]
4	238.59±5.62[a]	239.85±3.20[a]	240.76±4.24[a]	233.76±2.23[a]	217.45±5.04[b]
5	176.01±3.73[NS]	178.65±3.04	179.05±2.95	175.76±4.61	167.63±3.71
6	ND[5)]	ND	ND	TR[6)]	3.13±0.29
7	11.75±0.60[NS]	11.90±1.27	11.99±1.71	13.86±1.26	13.96±1.41
8	174.62±7.03[NS]	178.76±9.23	190.65±9.86	218.37±4.90	208.65±4.95
9	ND	ND	ND	ND	ND
Total	776.17±3.74	787.68±3.15	808.70±3.58	836.89±3.02	811.97±2.90

[1)]Numbers are the same as in Table 1.
[2)]Values are mean±SD of duplicate analyses.
[3)]NS: Not significant.
[4)]Values with the same superscript letters in a row are not significantly different at $p<0.05$.
[5)]Not detected. [6)]Trace (<1 mg%).

respectively, and then decreased sharply. Particularly, it should be noted that matairesinol (MR), a well-known as phytoestrogen, first appeared at 30 min of roasting time (data not shown). From these results, the roasting process at above 250°C for 5 min was found to cause degradation of phytochemical phenolic constituents in safflower seed, although some beneficial effects, decreasing levels of phenolic glycosides with bitter taste and cathartic effects, were achieved by the roasting process.

During the steaming process (Table 4), levels of most of phenolic compounds except two seortonin aglycones, CS and FS, tended to increase with increased steaming time, especially about 1.1~1.3 fold increases after 60 min of steaming, as compared to the control, and then decreased slightly. However, levels of the two serotonins, CS and FS increased slightly during the early steaming process and then decreased. Thus, contrary to the roasting process, the steaming process was shown to somewhat increase phenolics levels, but did not cause degradation of phenolic glycosides to their corresponding aglycones. These facts supported earlier reports that levels of phenolic constituents in several plants increased or decreased during different roasting and steaming processes (18,20,29). However, it is evident that the steaming and roasting processes lead to the conversion and generation of phenolic compounds in safflower seed, although their precise mechanism is so far poorly understood.

During the microwave treatment (Table 5), levels of most of phenolic compounds, except three phenolic aglycones, HAG, LT and AT, tended to decrease progressively with increased microwave heating time, especially 1~4 fold decrease after 10 min of microwave, as compared to the control. However, levels of the three

Table 5. Quantitative changes of nine phenolic compounds in safflower seeds according to four different microwave treatment times (mg%, dry base)

Phenolic[1)]	Microwave time				
	Control	1 min	3 min	5 min	10 min
1	51.06±1.37[2)a3)]	52.76±2.42[a]	47.56±1.93[b]	30.63±2.59[c]	23.87±0.82[d]
2	37.54±3.13[a]	38.62±1.52[a]	36.97±1.77[ab]	34.86±2.93[b]	19.76±1.65[c]
3	86.60±4.73[a]	87.15±2.34[a]	84.34±3.33[ab]	80.21±1.35[b]	27.54±1.34[c]
4	238.59±5.62[a]	239.87±2.38[a]	222.76±4.13[b]	203.79±2.39[c]	137.86±1.51[d]
5	176.01±3.73[a]	177.76±11.01[a]	170.76±11.69[a]	148.90±3.74[b]	104.87±4.32[c]
6	ND[4)]	ND	ND	TR[5)]	13.53±0.72
7	11.75±0.60[b]	12.97±1.56[ab]	13.63±1.69[ab]	16.90±1.50[b]	54.87±1.2[a]
8	174.62±7.03[a]	175.54±5.40[a]	170.32±5.52[a]	145.60±2.55[b]	46.30±1.39[c]
9	ND	TR	2.72±0.12	14.46±1.01	35.86±1.34
Total	776.17±3.74	771.70±3.80	749.06±3.78	675.37±2.26	464.46±1.60

[1)]Numbers are the same as in Table 1.
[2)]Values are mean±SD of duplicate analyses.
[3)]Values with the same superscript letters in a row are not significantly different at $p<0.05$.
[4)]Not detected. [5)]Trace (<1 mg%).

phenolic aglycones, HAG, LT and AT increased greatly with increased microwave time, although HAG only detected at late microwave time of 10 min. Thus, the microwave process, like the roasting process, was found to decrease phenolics levels, and to cause degradation of phenolic glycosides to their corresponding aglycones, although there are some difference in levels of phenolic compounds between the roasting and the microwave processes. A pleasant aroma or taste developed in the safflower seeds during the roasting process for 10 min at 250°C roasting temperature, and microwave treatment for 5 min, but the roasting and microwave processes for 20 min and 10 min, respectively, resulted in the production of extensive charring, and very low yields of safflower seed oil and extracts (data not shown).

In conclusion, the nine phenolic compounds during growth and processing of safflower seeds were quantified by HPLC. Levels of most of phenolic compounds increased rapidly until late July, and then remained relatively constant afterward, but two phenolic aglycones, HAG and AT, were not detected throughout growth stage. During the roasting and microwave processes, levels of most phenolic compounds, except phenolic aglycones in the seeds decreased progressively with increased roasting and microwave times, while three phenolic aglycones, HAG, LT and AT increased slowly with increased roasting and microwave times. In contrast, during the steaming process, levels of phenolic compounds in the seeds increased slowly with increased steaming time, while two HAG and AT were hardly detected during the steaming process. These results suggest that the steamed safflower seeds harvested in late July may be useful as a source of dietary supplement for prevention of several pathological diseases, such as atherosclerosis and osteoporosis and aging.

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