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Changes in Flavonoid Contents of Safflower Leaf during Growth and Processing

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Abstract

Eight flavonoids, apigenin-6-*C*-β-D-glucopyranosyl-8-*C*-β-D-glucopyranoside (AGG), quercetin 7-*O*-β-D-glucopyranoside (QG), luteolin 7-*O*-β-D-glucopyranoside (LG), quercetin 7-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside (QAG), luteolin 7-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside (LAG), quercetin (Q), luteolin (L) and acacetin 7-*O*-β-D-glucuronide (AG) were determined by HPLC in the safflower (*Carthamus tinctorius* L.) leaf during growth and processing. During growth, levels of five flavonoid glycosides (AGG, QG, LG, QAG, & LAG) in the leaf increased progressively at over time according to growth stages, reached a maximum before June 11, and then decreased sharply, while those of three flavonoid aglycones (Q, L, & AG) increased greatly at the early stage of growth, reached a peak before May 28, and then decreased rapidly. During the steaming process, contents of five flavonoid glycosides increased rapidly with increased steaming time, reached a maximum after 5 min of steaming, and then decreased, whereas those of flavonoid aglycones except for AG decreased sharply with increased steaming time. During the roasting process, contents of three flavonoid glycosides decreased rapidly with increased roasting time, whereas those of two acetylflavonoid glycosides (QAG & LAG) and three flavonoid aglycones increased progressively with increased roasting time, reached a maximum after 3 min of roasting, and then decreased. These results suggest that appropriate steamed and roasted safflower leaves are a rich source of flavonoids, and may be a good source of bioactive components as a functional leaf tea.

Key words: safflower leaf, flavonoids, HPLC, growth, roasting and steaming process

INTRODUCTION

Flowers of safflower (*Carthamus tinctorius* L. Compositae) are widely used in folk medicine as antithrombotic, analgesic and hepatoprotective agents (1,2), and in the food industry as a coloring and flavoring agent (3). Safflower seeds are clinically employed for the treatment of cataclasis, osteoporosis and rheumatoid arthritis in Korea (4). Recent studies revealed that safflower seeds have been found to improve lipid metabolism in high fat and cholesterol-fed rats (5-7), and to protect against bone fracture and loss (8-11). Several serotonin derivatives and phenolic compounds have been isolated and identified from safflower seeds and flowers, as major principles for several biological actions such as antioxidation, antiinflammation and anticarcinogenesis (12-18). Thus, although many extensive studies have reported on the chemistry and physiological activity of safflower seeds and flowers, few phytochemical studies of safflower leaves, which are currently used as wild edible greens, are available (19).

Flavonoids, naturally occurring phenolics which are widely distributed in plants, have been found to possess a variety of biological effects including antioxidative, antiinflammatory, and estrogenic activity (20,21). Recently, increasing evidence has indicated that a high flavonoid intake is frequently associated with low coronary heart disease and cancer risks (22,23). Flavonoids have been shown to inhibit several key enzymes in the arachidonic cascades, such as prostaglandin synthase, lipoxygenase and cyclooxygenase, which are closely related to tumorigenesis, inflammation, and carcinogenesis (24,25). Thus, dietary flavonoids in plants have received considerable attention as therapeutic agents against several pathological conditions. To date, many kinds of flavonoids have been reported in plants, and their types and contents vary with cultivar, maturation and processing (26,27). Most of flavonoids are present as glycoside conjugates, with sugars bound typically at the C_3 and C_7 positions, and are degraded to aglycones by human intestinal flora after ingestion (28).

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In a previous report, we found that the safflower leaf had several kinds of antioxidative flavonoids such as quercetin, luteolin and apigenin and their glycosides, and were especially rich in two antioxidative acetylflavonoids: quercetin 7-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside and luteolin 7-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside, which are rarely found in plants (29). In addition, it was found that the antioxidative activity of the methanol extract from safflower leaf was affected by steaming and roasting processes (30). This prompted us to quantify flavonoids in the safflower leaf during growth and processing.

The purpose of this study was to investigate changes in flavonoid contents in the safflower leaf during growth and processing.

MATERIALS AND METHODS

Material and chemicals

Safflower (*Carthamus tinctorius* L.) leaves were directly harvested from May 2 to June 20 at an Experiment Station farm of New Natural Products Institute, Uisong, Gyeongbuk, Korea. Samples consisting of 5～10 leaves for each experiment were obtained from the designated ten trees every week. The samples were freeze-dried immediately after picking and stored at -18°C until analysis of flavonoids by HPLC. HPLC solvents were obtained from Merck (Darmstadt, Germany). All other reagents used for this study were analytical grade.

Sample preparation

Freeze-dried safflower leaves (10 g) were roasted in an electric roaster with constant stirring at 100°C for 1, 2, 3, and 5 min. Steamed safflower leaves (50 g) were steamed in a domestic stainless steel steamer [dimensions 260 (W)×200 mm (H)] for 1, 3, 5, and 10 min, cooled and then freeze-dried. The two heat pretreated safflower leaves were ground with a mechanical mill and flavonoids analyzed by HPLC.

Quantitation of flavonoids

Freeze-dried safflower leaf (1.0 g) was extracted twice with 80% aq. MeOH (100 mL) in an ultrasonic cleaner (Bransonic 5210R-DTH, USA) for 2 hrs, filtered and evaporated under reduced pressure. The MeOH extract was redissolved in 10 mL of 80% aq. MeOH, allowed to stand overnight and then fitered with Whatman No. 2 filter paper. The filtered MeOH solution was further partitioned twice with CH_2Cl_2 (10 mL) to remove lipids and pigments, and then the upper layer was evaporated *in vacuo*. The concentrated MeOH extract was solubilized with 80% aq. MeOH (5 mL) and passed through 0.45 μm membrane filter (Gelman, USA) and finally injected in HPLC for quantification of flavonoids. 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 1.0 mL/min with UV detector at 350 nm. The column was eluted gradiently with solvent A (0.03% H_3PO_4 in 20% aq. MeOH) to solvent B (80% aq. MeOH) for 50 min. Individual flavonoids were identified by a comparison of their retention times with those of standard flavonoids, which were isolated previously (29). Duplicate analyses were conducted on duplicate samples. Linear correlation coefficients were superior to 0.992 for each flavonoid compound. The calibration lines [y=473.04x+0.8108 for apigenin-6-*C*-β-D-glucopyranosyl-8-*C*-β-D-glucopyranoside (AGG), y=834.63x−0.164 for quercetin 7-*O*-β-D-glucopyranoside (QG), y=377.12x−1.1535 for luteolin 7-*O*-β-D-glucopyranoside (*LG*), y=602.34x+0.6441 for quercetin-7-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside (QAG), y=419.56x+2.227 for luteolin-7-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside (LAG), y=1454.5x−1.2992 for quercetin (Q), y=669.49x+1.6662 for luteolin (L) and y=851.14x+0.3173 for acacetin 7-*O*-β-D-glucuronide (AG)] used were determined by a least square regression method. Levels of flavonoids were determined by calibration curves of standard flavonoids, and expressed as mg% of dried safflower leaf. The recovery rates of eight flavonoids were above 92%.

RESULTS AND DISCUSSION

Previously, we isolated and identified the eight flavonoids, AGG, QG, LG, QAG, LAG, Q, L and AG, from safflower leaf (29). Most of the flavonoids in the leaf were found to have strong antioxidative activity, as active oxygen radical scavengers (29). There were two unusual antioxidant flavonoids that were especially interesting, the acetylflavonoids QAG and LAQ, in safflower leaf. In this study, we quantified the eight flavonoids in safflower leaf by HPLC (Fig. 1) during growth and processing.

Changes in the level of the eight flavonoids in the safflower leaf during growth are shown in Table 1. At the early stage of growth (5/14), total flavonoids accounted for about 2.6% of the dry weight of the leaf of which 96% was the five flavonoid glycosides, AGG, QG, LG, QAG, and LAG. The levels of the five flavonoid glycosides increased considerably with increased development stage of leaf, reached a maximum before June 11, and then decreased rapidly at the later stage of growth. In contrast, levels of the three flavonoid aglycones appeared to have reached a peak before May

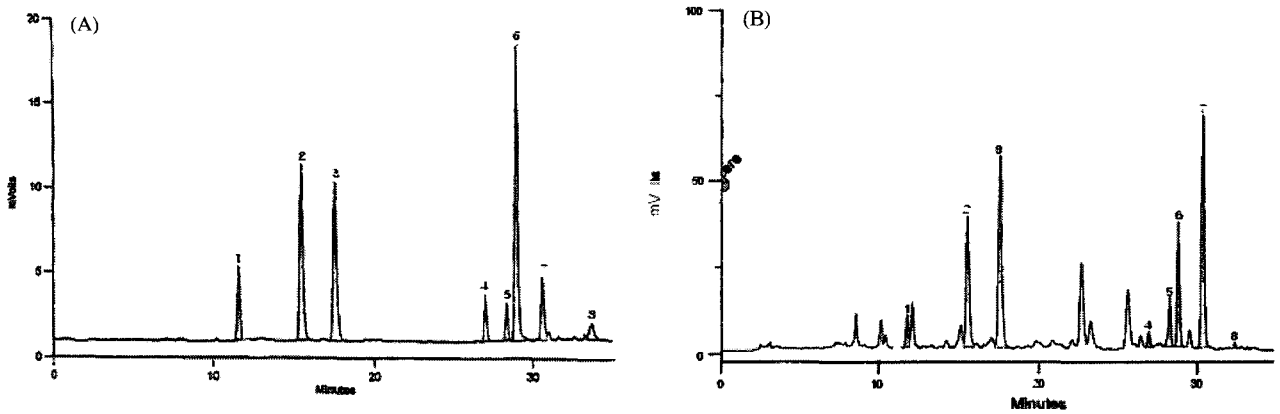

Fig. 1. HPLC chromatograms of eight flavonoid standards (A) and the defatted MeOH extract (B) from safflower leaf. 1: apigenin-6-*C*-β-D-glucopyranosyl-8-*C*-β-D-glucopyranoside, 2: quercetin 7-*O*-β-D-glucopyranoside, 3: luteolin 7-*O*-β-D-glucopyranoside, 4: quercetin-7-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside, 5: luteolin-7-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside, 6: quercetin, 7: luteolin, 8: acacetin 7-*O*-β-D-glucuronide. HPLC conditions are the same as described in Materials and Methods.

Table 1. Changes in concentrations of eight flavonoids in safflower leaf during growth (Unit: mg/100 g, dry base)

Growth period	Content of eight flavonoids[1]							
	1	2	3	4	5	6	7	8
May 14	58.1±2.9[2]	351.3±18.0	1938.4±54.2	14.3±1.1	108.2±6.6	28.8±1.4	62.5±2.0	5.2±0.9
May 21	69.5±4.5	942.5±49.2	2179.2±74.7	20.4±1.4	134.4±4.7	203.7±6.2	348.6±32.2	9.4±1.1
May 28	71.3±7.0	1131.3±51.8	2502.3±34.6	21.3±4.6	139.4±7.1	151.5±4.8	253.3±52.5	10.3±1.2
June 04	74.5±9.2	1290.6±43.6	3143.2±64.6	23.5±1.4	157.5±11.9	76.7±4.2	217.4±27.4	5.5±0.4
June 11	71.3±4.7	1088.3±34.5	2780.6±47.3	24.6±1.5	191.7±5.0	35.4±1.6	111.6±11.5	4.4±0.2
June 18	57.8±2.6	466.5±25.7	1689.4±32.7	12.5±1.1	79.3±1.8	16.6±1.5	24.6±1.2	3.2±0.6
June 26	38.4±1.8	457.3±21.3	1307.6±92.4	14.7±3.2	53.6±1.3	5.3±3.3	7.3±1.1	3.0±1.0

[1]1: apigenin-6-*C*-β-D-glucopyranosyl-8-*C*-β-D-glucopyranoside, 2: quercetin-7-*O*-β-D-glucopyranoside, 3: luteolin-7-*O*-β-D-glucopyranoside, 4: quercetin-7-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside, 5: luteolin-7-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside, 6: quercetin, 7: luteolin, 8: acacetin 7-*O*-β-D-glucuronide.
[2]Values are mean±SD of duplicate analyses.

28, and then decreased drastically at the late of growth stage. Thus, levels of the eight flavonoids in the safflower leaf changed during growth; similar trends were also observed in the levels of catechins in persimmon (31) and green tea (32) leaves during growth.

Changes in levels of the eight flavonoids in safflower leaf during steaming and roasting processes are presented in Table 2 and 3, respectively. During the steaming process, concentrations of the five flavonoid glycosides increased considerably with increased steaming time, especially 1~5 fold increases after 5 min of steaming, as compared to the control, and then decreased. However, contents of the flavonoid aglycones except for AG decreased sharply during growth.

During roasting process, the levels of the three flavonoid glycosides, AGG, QG and LG, decreased sharply with increased roasting time. However, levels of the two acetylflavonoid glycosides, QAG and LAG, and of the three flavonoid aglycones, Q, L and AG, increased progressively up to 46 and 3 times, as well as 2~3 times after 3 min of roasting, respectively, as compared to the control, and then decreased. Thus, these results indicate

Table 2. Changes in concentrations of eight flavonoids in safflower leaf during steaming (Unit: mg/100 g, dry base)

Steaming time (min)	Content of eight flavonoids[1]							
	1	2	3	4	5	6	7	8
Control	74.5±9.2[2]	1290.6±43.6	3143.2±64.6	23.5±1.4	157.5±1.9	76.7±6.2	217.4±27.4	5.5±0.4
1	75.4±2.1	1323.4±33.9	3225.6±32.4	38.3±8.7	174.5±2.3	69.5±1.3	125.6±1.4	7.8±1.2
3	77.3±6.4	2204.3±64.7	3372.6±44.7	53.6±5.3	201.5±3.5	56.7±3.5	119.5±4.2	10.3±1.4
5	81.8±2.3	3326.5±53.2	4054.3±57.1	103.1±10.2	276.4±3.3	52.5±4.4	77.8±1.7	21.3±1.5
10	79.4±4.4	2588.7±48.5	3507.6±48.3	83.6±6.4	212.6±4.9	40.6±2.1	72.3±3.3	19.4±1.3

[1]See the legend of Table 1.
[2]Values are mean±SD of duplicate analyses.

Table 3. Changes in concentrations of eight flavonoids in safflower leaf during roasting (Unit: mg/100 g, dry base)

Roasting time (min)	Content of eight flavonoids[1)]							
	1	2	3	4	5	6	7	8
Control	74.5±9.2[2)]	1290.6±43.6	3143.2±64.6	23.5±1.4	157.5±10.9	76.7±6.2	217.4±27.4	5.5±0.4
1	70.2±3.1	983.4±58.1	2328.5±48.6	82.4±11.4	178.7±10.2	85.7±3.3	254.3±10.1	8.3±1.3
2	54.6±6.9	875.3±32.9	2053.4±38.6	531.2±32.5	322.5±22.9	98.4±2.0	318.9±21.0	10.4±1.2
3	46.5±2.7	732.4±53.5	1930.5±59.0	1103.4±30.8	542.7±23.8	132.4±10.8	418.2±23.9	17.7±1.4
5	13.5±2.3	605.6±34.8	1478.6±35.1	1069.6±76.0	487.6±21.1	129.3±12.9	365.7±11.3	11.6±1.1

[1)]See the legend of Table 1.
[2)]Values are mean±SD of duplicate analyses.

that the steaming and roasting processes caused considerable increases or decreases in concentrations of flavonoid glycosides and aglycones. In particular, it is very interesting to note that levels of the two flavonoid acetyl glycosides (QAG and LAG) with strong antioxidative activity, increased during the steaming and roasting processes. This fact support an earlier report that acetylisoflavone glucosides in soybean increased during heating process (33,34). Ko and Lee (35) reported that the levels of tannins in green tea decreased slightly during the steaming and roasting process. In addition, Joung et al. (36) also reported that the chemical compositions of persimmon leaf were changed according to different processing methods. Thus, it is evident that the steaming and roasting processes lead to the conversion of flavonoid, although the precise mechanism of the conversion of flavonoids is so far poorly understood.

It is concluded that we detected eight flavonoids in safflower leaf of which quercetin- and luteolin-glucosides were the major flavonoids. Concentrations of flavonoid glycosides increased rapidly until early June, and then decreased, while flavonoid aglycones decreased from the early of growth stage. During the steaming process, flavonoid glycosides in the leaf increased progressively up to 5 min of steaming, and then decreased, while flavonoid aglycones decreased sharply with increased steaming time. In contrast, during the roasting process, flavonoid glycosides in the leaf decreased slowly with increased roasting time, while flavonoid aglycones increased progressively after 3 min of roasting, and then decreased. These results suggest that safflower leaf harvested during early June, with appropriate steaming and roasting processing, may be a good source of bioactive components as a functional leaf tea.

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