Changes in Aurantio-Obtusin and Glucoaurantio-Obtusin Content in Cassiae Semen via Treatment with a Crude Enzyme Extract from Aspergillus usamii

HUR, JONG-MOON1, SOON-HO KWON1, JAE-HYUN SO1, MIRA JUN2, YOUNG-HWA KANG1, YU-MI LEE3, KYUNG-BOK LEE4, IN-KOO RHEE1, MOON-SOON LEE5, AND KYUNG-SIK SONG1

1School of Applied Biosciences, College of Agriculture & Life Sciences, Kyungpook National University, Daegu 702-701, Korea
2Division of Food Science, Dong-A University, Busan 604-714, Korea
3Department of Genetic Engineering, College of Natural Sciences, Kyungpook National University, Daegu 702-701, Korea
4College of Medicine, Konkuk University, Daejeon 302-718, Korea
5Chemical Safety & Accidental Prevention Division, Chemical Assessment Department, National Institute of Environmental Research, Incheon 404-708, Korea

Received: March 28, 2007 Accepted: April 29, 2007

Abstract Cassiae Semen (seeds of Cassia tora) showed a remarkably different HPLC chromatogram after being treated with a crude enzyme extract from Aspergillus usamii. Increased and decreased compounds were identified as aurantio-obtusin and glucoaurantio-obtusin, respectively. The aurantio-obtusin content reached its maximum level (133.58±0.39 μg/mg extract) after being incubated for 50 min at 37°C, whereas the inactivated crude enzyme-treated control remained unchanged (54.13±1.33 μg/mg). On the other hand, the glucoaurantio-obtusin content decreased by less than one-third (51.09±1.63 μg/mg) of the untreated control (143.19±2.12 μg/mg/mg), suggesting that an increase in aurantio-obtusin content originated from the enzymatic cleavage of its glucoside glucoaurantio-obtusin.

Keywords: Cassiae Semen, aurantio-obtusin, glucoaurantio-obtusin, Aspergillus usamii, processes, enzyme treatment

We have recently found that simple food processing techniques such as roasting, extrusion, and enzyme treatment (fermentation) could change the contents of active components in Oriental drugs. For example, the amount of liquiritigenin in fermented licorice increased up to 400 times over that of the control [10]. Moreover, the content of paonol in Moutan Cortex and 5-hydroxymethyl furfural in Asparagis Tuber dramatically increased after the roasting process [7, 11].

Regarding chemical changes in Oriental drugs after processing, fifty popularly used medicinal plants were investigated. As a result, dramatic changes were found in the enzyme-treated Cassiae Semen (via HPLC chromatogram).

*Corresponding author
Phone: 82-53-950-5715; Fax: 82-53-956-5715;
E-mail: kssong@kmu.ac.kr

C. Semen purchased from an herbal medicine supplier in Daegu, Korea, was identified by Dr. Jong Hwan Kwak of Sung Kyun Kwan University, Suwon, Korea. Crude enzyme extract was prepared from Aspergillus usamii according to the previous report [10]. The inactivated enzyme was prepared by autoclaving crude enzyme solution. One g of lyophilized 95% EtOH extract of C. Semen was treated with about 260 U (1 U is defined as the enzyme activity needed to produce 1 mmol p-nitrobenzene from p-nitrophenyl-β-D-glucopyranoside per min) of a crude enzyme solution for 60 min at 37°C. Inactivated crude enzyme treated, crude enzyme treated, and untreated C. Semen extracts were analyzed by HPLC. In the crude enzyme treated extract, one peak was dramatically increased (Fig. 1).

To isolate an increased compound, the C. Semen (13 kg) was extracted with 95% EtOH (2×, 3 h) and the extract was evaporated to dryness. The residue (100 g) was then mixed with the 26,000 U of crude enzyme and incubated for 120 min at 37°C. The reaction mixture was suspended in 11 of water and partitioned with an equivalent volume of CH₂Cl₂ (2×). The organic layer was dried with anhydrous Na₂SO₄ and evaporated so as to obtain a CH₂Cl₂ soluble fraction (32.7 g). This fraction was chromatographed onto a silica gel column (Merck Art. 7734, 5.5×40.0 cm, hexane-acetone=100:1-0:100). The eluates were combined into eleven fractions based on their TLC (Merck Art. 5715 and 1.15685) patterns. The fraction 7 (16.2 g), which contained the increased compound, was further purified by silica gel open column chromatography (Merck Art. 7734, 3.5×30.0 cm, hexane-acetone=20:1-8:1). Five fractions (Fr. 7-1 to 7-5) were obtained and the repeated silica gel chromatography (Merck Art. 7734; 1st, 2.0×30.0 cm, hexane-acetone=15:1-5:1; 2nd, 1.4×28.0 cm, hexane-acetone
-15:1-10:1) of Fr. 7-4 afforded compound 1 (381.0 mg). To isolate a decreased compound, untreated C. Semen (1 kg) was extracted with 95% EtOH (2×, 3 h) and the extract (19.2 g) was further purified with MPLC (ODS-S-50A, 26×300 mm, 50%, 100% MeOH). The purification of Fr. 2 (155.6 mg) using MPLC (ODS-S-50A, 11×300 mm, 20%, 25%, 35% MeOH) gave compound 1 (31.2 mg).

Compound 1, isolated from the crude enzyme treated C. Semen, showed the same retention time as the increased peak in the HPLC. Compound 1 was obtained as a yellow crystalline powder and was visible under UV. Compound 1 gave a deprotonated molecular ion [M-H]⁻ at m/z 329 in the ESI-MS. Characteristic fragmentation ions at m/z 314 (329-15), 299 (314-15), and 284 (299-15) were produced by the consequential loss of a methyl group. In the ¹H-NMR analysis (400 MHz, DMSO-d₆), two characteristic signals at δ 3.80 and 3.84 (s, each 3H) were from two methoxyl groups. The signal at δ 2.29 (3H, s) indicated a methyl group that directly bonded to an aromatic ring. In addition, the signals at δ 7.17 (1H, s) and 7.78 (1H, s) confirmed that compound 1 had an aromatic ring. A total of 17 carbon signals including two methoxy carbon signals (δ 57.86 and 59.07) were detected in the ¹³C-NMR spectrum (100 MHz, DMSO-d₆). By comparing these NMR data with previous reports [8], 1 was identified as aurantio-obtusin.

Compound 2, whose retention time corresponded to the decreased peak in HPLC, was isolated from untreated C. Semen. Compound 2 was also obtained as a yellow crystalline powder and was visible under UV. In the ESI-MS, a protonated molecular ion [M+H]⁺ at m/z 493 implied a molecular weight of 492. The fragmentation ion at m/z 331 [M+H-Glc]⁺ suggested that 2 might contain one glucose unit in the structure. Other spectral data were very similar to those of 1 except for the additional signals of a sugar moiety. By comparing the NMR data with those of the references [20], 2 was identified as glucourantio-obtusin.

One g of ethanolic extract of C. Semen was treated with 260 U of crude enzyme and incubated for 10, 20, 30, 40, 50, 60, 90, and 120 min. The temperature was fixed at 37°C since this is the optimal temperature of the main glucosidase in Aspergillus usamii [4]. An HPLC analysis was performed on a Gemini 5μ C18 (4.6×250 mm) column with a gradient solvent system, by varying the proportion of solvent A (water 99%-acetic acid 1%) to solvent B (acetonitrile 99%-acetic acid 1%). Solvent B the increased to 100% in 50 min and kept at 100% for 10 min at a flow rate of 0.8 ml/min. Detection was carried out under UV 280 nm. The aurantio-obtusin and glucourantio-obtusin isolated in this work (more than 99% under UV 280 nm in HPLC analysis) were used as standards. The calibration curve for aurantio-obtusin was Y=0.297X-0.108 (r²=0.9996) and it was Y=0.0037X-0.0006 (r²=0.9996) for glucourantio-obtusin, where Y represents the peak area and X is the weight in μg.

Aurantio-obtusin reached its maximum level at 50 min after crude enzyme treatment (Fig. 3). Under these conditions, the content of aurantio-obtusin increased about 2.4 times (133.58±0.39 μg/mg extract) that of the control (54.13 ±1.33 μg/mg extract). On the other hand, glucourantio-obtusin content decreased about 2.8 times (51.09±1.63 μg/mg extract) compared with that of the control (143.19±2.12 μg/mg extract).
mg extract). Recently, *A. usamii* has been documented as having strong glucosidase activity [4]; therefore, aurantio-obtusin could be produced via the enzymatic cleavage of its corresponding glucoside glucouraurantio-obtusin.

Food processing techniques have generally focused on promoting nutritional values and/or improving specific flavors or color in foodstuffs. Previous studies have mainly focused on the sensory evaluation of roasted *C. semen* and identification of compounds produced by the roasting process [13, 19, 21]. Recently, as the tertiary functions (physiological characteristics) of food are being emphasized, biological as well as chemical changes after food processing have become a hot topic. The enzyme-mediated synthesis or degradation of glycosides has been widely investigated to enhance the physicochemical properties of biologically important compounds such as ascorbic acid and isoflavone glycosides [5, 12]. In our experiments, no significant changes in biological activity of *C. semen* were recognized (i.e., antioxidative using DPPH [1], antioxidant activity using PEP [14] and β-secretase inhibition [6], anticoagulating activity by APTT (activated partial thromboplastin times) [16], antihypertension by ACE inhibition [3], and cytotoxicity both in tumor (NIH-3T3) [9] and normal (HUVECs) [15] cells, between the before and after treatments of the crude enzyme solution (Table 1). Our results, however, are noteworthy in that aurantio-obtusin has been known to have antimutagenic effect [2], to possess inhibitory activity toward cAMP phosphodiesterase [17], and to direct inhibitory activity on aldose reductase [18]. Only one report was available regarding glucouraurantio-obtusin, describing its antiplatelet aggregation activity [20]. Simple processing techniques are expected to be useful in increasing the content of biologically active substances in Oriental drugs.

**Acknowledgments**

This work was supported by a grant from the “Traditional Technology Innovation Research Lab. Program,” Korea Ministry of Science and Technology. We also extend our gratitude to the Korea Ministry of Commerce, Industry and Energy for their financial support.

**REFERENCES**


