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Effect of Electron-beam Irradiation on Polymethoxylated Flavones Content of *Citrus unshiu* Pomaces

– Research Note –

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Abstract

To determine the effect of electron-beam irradiation on the contents of polymethoxylated flavones (PMFs) extracts from citrus pomaces (CP), CP was irradiated at 0, 1, 2, or 5 kGy. Methanol extract of the irradiated CP were prepared and the PMF (nobiletin, sinensetin, and tangeretin) content of the extract was determined. Nobiletin and sinensetin of CP extract significantly increased with irradiation dose-dependent. However, electron-beam irradiation decreased the amount of tangeretin in the CP extract. These data suggest that irradiation can liberate phenolic compounds such as nobiletin or sinensetin, but tangeretin might have different pathway of conversion by irradiation. Therefore, irradiation can be a tool to change the composition of PMFs in CP.

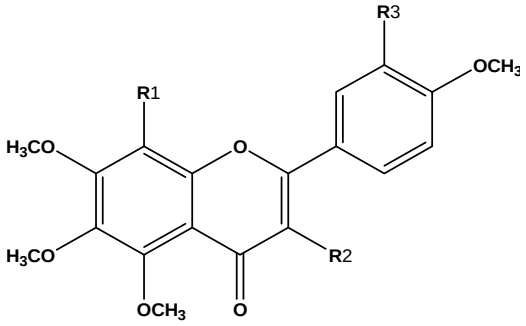
Key words: citrus pomaces, electron-beam irradiation, polymethoxylated flavones

INTRODUCTION

More than four decades of studies have demonstrated that radiation is a safe, effective, and versatile process for food preservation, decontamination, and/or disinfection. Its various applications cover inhibition of sprouting of root crops, insect disinfestations of stored products, fresh and dried food, shelf-life extension of fresh fruits, vegetables, meat and fish, destruction of parasites and pathogenic micro-organisms in food of animal origin, and decontamination of spices and food ingredients, etc. (1).

Electron-beam radiation processing, one form of radiation technology, involves a stream of high energy electrons emitted from an electron gun. Electrons can only penetrate several centimeters into the food, and, for this reason, foods are treated in relatively thin layers. Modest metal shielding of the treatment cell is sufficient to prevent the escape of stray electrons. When not in use, the electron source is turned off by switching off the electric current. No radioactivity is involved (2).

Citrus fruits are a rich source of flavonoids, which are important secondary plant metabolites and are present in plant tissues in relatively high concentrations, mainly as sugar conjugates. Polymethoxylated flavones (PMFs) such as nobiletin, sinensetin, and tangeretin are the major active compounds present in citrus fruits (Fig. 1). Nobiletin and sinensetin are reported as novel promising immunomodulatory and anti-inflammatory drugs (3) and as inhibitors to human mammary cancer cells (4). Tangeretin is a more potent inhibitor of tumor cell growth than free hydroxylated flavonoids, and also possess potent anti-invasive and anti-metastatic activities (5,6).



	R1	R2	R3
Nobiletin	OCH_3	H	OCH_3
Sinensetin	H	H	OCH_3
Tangeretin	OCH_3	H	H

Fig. 1. Structure of main polymethoxylated flavones in citrus fruit.

More than 60 thousand tons of citrus pomaces (CP) are annually produced in South Korea after processing of citrus fruits. CP have been used as a source for molasses, pectin, cold-pressed oils, and limonene (7). CP have also been widely studied because they contain numerous biologically active compounds, including natural antioxidants such as phenolic acids and flavonoids (8,9). Many natural plant phytochemicals exist either in a form

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bound to high-molecular-weight compounds or as part of repeating subunits of high-molecular-weight polymers (10,11).

To obtain natural antioxidants from plants, it is necessary to find an effective processing method to liberate them (12). In our previous study, the electron-beam irradiation could increase phenolic contents and the antioxidant activities of CP (13). The objective of this study was to elucidate the effect of electron-beam irradiation on PMF contents of CP.

MATERIALS AND METHODS

Materials

Citrus pomaces (CP), remained after pressing citrus (*Citrus unshiu*) fruit to make a citrus juice, were kindly supplied by Jeju Samdasoo Citrus Co. (Jeju, Korea). After freeze-drying, CP were finely ground using a blender (Novita MC-811C, Korea) and passed through a 48-mesh sieve. The CP powder was stored at 4°C for further experiments. The standards of sinensetin, nobiletin, and tangeretin were purchased from Extrasynthese Co. (Genay Cedex, France).

Irradiation and extract preparation

Approximately 5 g of the CP powder was vacuum-packed in a LDPE (low density polyethylene) bag (5 cm × 5 cm × 4 mm, Nylon/PE/Nylon/PE/Nylon/LLDPE, Cryovac Division, sealed Air Corporation, Duncan, SC, USA). The packaged CP powder was irradiated by an Electron Accelerator (model ELV-4, 1.0 MeV, Main Research Center, EB Tech, Daejeon, Korea) at doses of 0, 1, 2, or 5 kGy. The irradiation machine was operated at room temperature at the condition of 2.7～13.5 mA in accelerating current, 980 mm (length) × 75 mm (width) in beam dimension, and 20 m/min in speed of conveyor belt.

One g of the irradiated CP powder was extracted with 100 mL of methanol in a shaking incubator (100 rpm) for 12 hr at room temperature. Then the extracts were centrifuged at 1,000 × *g* for 15 min, and the supernatants were filtered through a Whatman No. 1 filter paper.

Analyses of PMFs in CP extracts

The amounts of PMFs in the CP methanolic extract were determined using an HPLC system consisting of a Shimadzu LC-10ATVP pumps (Shimadzu Co. Ltd., Kyoto, Japan), a Shimadzu SCL-10 AVP integrated system controller, a Shimadzu SPD-10AVP UV-Vis detector, and a Shimadzu CTO 10 ASVP column oven. The selected column was a Shim-pack VP ODS column (5 μm, 250 × 4.6 mm, Shimadzu Co. Ltd.). Mobile phases were water (solvent A), methanol (solvent B), and 0.1% acetonitrile (v/v) (solvent C). The solvent composition started at 100% solvent A. The gradient was as follows: 10 min, A-86%, B-7%, C-7%; 25 min, A-80%, B-10%, C-10%; 35 min, A-75%, B-10%, C-15%; 42 min, A-0%, B-30%, C-70%; and 50 min, A-0%, B-0%, C-100%. Elution was performed at a solvent flow rate of 0.7 mL/min. Detection was accomplished with a UV-Vis detector, and the chromatograms were recorded at 325 nm. The sample injection volume was 10 μL and the column was maintained at 60°C. The separated peaks were identified by comparing their retention times with the authentic standards.

Total phenolic contents (TPC)

The total phenolic contents of the CP extract were determined according to the method of Gutfinger (14). One mL of the CP extract was mixed with 1 mL of 2% Na_2CO_3. After 3 min, 0.2 mL of 50% Folin-Ciocalteu reagent was added. After 30 min of standing, the mixture was centrifuged at 13,400 × *g* for 5 min. The absorbance of supernatant was measured with a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) at 750 nm. TPC were expressed as tannic acid equivalents.

DPPH radical scavenging activity

The DPPH radical scavenging activity of the CP extract was determined according to the method of Blois (15). After mixing 0.1 mL of CP extract with 0.9 mL of 0.041 mM DPPH in ethanol for 10 min, the absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as percentage according to the following formulation:

$$\%\ \text{DPPH radical scavenging activity} = \left(\frac{1 - \text{sample OD}}{\text{control OD}}\right) \times 100$$

Statistical analyses

All measurements were performed in triplicate, and analyses of variance were conducted by the General Linear Model procedure using SAS software (16). Student-Newman-Keul's multiple range tests were used to test for the significant differences between the mean values for the treatments ($p<0.05$).

RESULTS AND DISCUSSION

Flavonoids are a subset of polyphenols, which are the most bioactive compounds in plants. PMFs are a group of flavonoid compounds that contain several methoxyl groups. The contents of polymethoxylated flavones (PMFs) in CP extract were significantly affected by electron-beam irradiation (Table 1). The amounts of nobiletin and sinensetin of CP extract increased with the increasing of irradiation dose. Even 1 kGy of irradiation

Table 1. Effect of electron-beam irradiation on the polymethoxylated flavones content of methanol extract from citrus pomaces (unit: ppm)

	Irradiation dose (kGy)			
	0	1	2	5
Nobiletin	2802.8±100.1[c]	13401.8±170.4[b]	13510.4±156.9[b]	17280.7±213.6[a]
Sinensetin	272.0±30.3[c]	2391.5±72.4[b]	2428.8±56.9[b]	3259.2±78.5[a]
Tangeretin	13010.3±232.2[a]	6218.2±186.5[c]	6281.7±150.4[c]	7679.7±205.6[b]

[a-c]Different letters within a row are significantly different ($p<0.05$), n=3.

drastically increased the amount of nobiletin in CP extract by almost 5 times compared with the nonirradiated control. The amount of nobiletin increased by more than 6 times by irradiation of 5 kGy, compared with the control.

The increase of PMFs by irradiation was more distinctive in the amounts of sinensetin. Only 1 kGy of irradiation increased the amount of sinensetin by almost 9 times and 5 kGy of irradiation did almost 12-times, compared with the nonirradiated control. This result corresponds with the reports from other research groups (17-21). Breitfellner et al. (18) reported that radiolysis of phenolics (gallic acid, 4-hydroxybenzoic acid, cinnamic acid, *p*-coumaric acid, caffeic acid, etc) in an aqueous solution led to their efficient degradation and to a notable hydroxylation. Fan et al. (19) reported that the free radicals generated during irradiation may act as stress signals and may trigger stress responses in lettuce, resulting in an increased antioxidant synthesis.

Phenolic compounds are the most active antioxidant derivatives in plants (22). Chemically, phenolics can be defined as substances possessing an aromatic ring bearing one or more hydroxyl groups (23). Phenolic compounds are known to act as antioxidants not only because of their ability to donate hydrogen or electrons but also because they form stable radical intermediates (24). The total phenolic contents of CP were significantly increased by electron-beam irradiation (Table 2). The increase of phenolic compounds in CP by irradiation was consistent with the increase of the PMFs such as nobiletin and sinensetin in Table 1. Many natural plant polyphenols exist either in a form bound to high-molecular-weight compounds or as part of repeating subunits of high-molecular-weight polymers (10,11). Several methods such as heat treatment, far-infrared (FIR) radiation, electron-beam-irradiation, fermentation, and protease treatment have been studied to liberate and activate low molecular weight natural antioxidants (10,25,26). Lee et al. (27) also reported that irradiation at 20 kGy increased phenolic contents and antioxidant activity of green tea stem extract. In the by-products of citrus fruit after juice, there are free phenolic compounds rich in flavones and glycosylated flavones, and bound phenolic compounds contain mainly phenolic acids and flavonols (8). Many covalently bound forms such as esters, amides and glycosides of phenolic acids have been detected in citrus fruits (28), and electron-beam irradiation was effective to increase phenolic contents of CP (13).

Electron-beam irradiation, however, decreased the amount of tangeretin in CP extract, suggesting that tangretin may have a different pathway of conversion by irradiation than that of nobiletin or sinensetin. Even though there was a trend to increase the DPPH radical scavenging activity of CP by irradiation, it was not a significant increase (Table 3). Yi et al. (29) reported that IC_{50} of tangeretin and nobiletin against DPPH radical scavenging activity was 39.20 and 40.97 μg/mL, respectively. The results of radical scavenging activity can be attributed to the decrease of tangeretin in irradiated CP extract, despite of the increase of nobiletin and sinensetin. Therefore, it can be assumed that irradiation could liberate certain phenolic compounds resulting in the production of nobiletin and sinensetin. But the tangeretin might have different pathway of conversion by irradiation from that of nobiletin or sinensetin. Gamma irradiation between 0.5 kGy and 5 kGy on soybean also decreased the glycosidic conjugated isoflavones and increased the corresponding aglycons (30). A dose-de-

Table 2. Effect of electron-beam irradiation on total phenolic contents (TPC) of methanol extract from citrus pomaces (unit: μM)

	Irradiation dose (kGy)			
	0	1	2	5
TPC	323.2±2.6[b]	335.1±5.4[a]	334.1±3.9[a]	335.3±5.1[a]

[a,b]Different letters within a row are significantly different ($p<0.05$), n=3.

Table 3. Effect of electron-beam irradiation on DPPH radical scavenging activity (RSA) of methanol extract from citrus pomaces (unit: %)

	Irradiation dose (kGy)			
	0	1	2	5
DPPH RSA	45.2±2.3[a]	46.1±1.8[a]	46.4±0.9[a]	45.7±2.6[a]

[a]Different letters within a row are significantly different ($p<0.05$), n=3.

pendent breakdown of aroma glycosides of nutmeg was also observed with radiation treatment (31).

All the three PMFs in this study are secondary metabolites in citrus, which mainly exist in part of peel. They are related to the first stages of fruit development and decrease during maturation (32). Their existing forms in citrus fruit are uncertain. Thus, it is difficult to understand why electron-beam irradiation increased the contents of nobiletin and sinensetin, while decreased that of tangeretin. There might be differences in their existing forms resulting in the different effect of electron-beam irradiation. The results of this study suggest the need for further investigations of separated single compound of PMFs.

In conclusion, electron-beam radiation of citrus pomaces caused a significant increase in the amount of polymethoxylated flavones such as nobiletin and sinensetin. Tangeretin, a form of polymethoxylated flavones, decreased by irradiation. Therefore, irradiation could liberate phenolic compounds such as nobiletin or sinensetin, while tangeretin could have a different pathway of conversion by irradiation. These data suggest irradiation can be a tool to change the composition of PMFs in CP.

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