

Free radical scavenging and ferrous ion chelating activities of citrus fruits derived from induced mutations with gamma irradiation

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Abstract: The antioxidant properties of citrus mutant fruits peel and pulp extracts derived from gamma irradiation were determined for free radical scavenging (DPPH, H₂O₂, NO) and ferrous ion chelation inhibition assays. Citrus mutant fruit peels exhibited significantly higher activities on H₂O₂ scavenging and metal chelating activities compared with those of citrus wild-type.

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1. Introduction

Gamma rays are often used on plants in developing varieties that are agriculturally and economically important and have high productivity potential (Naito et al., 2005; Predieri, 2001; Sarto et al., 2006). Research on the antioxidant effects of gamma irradiation on food sources and plants has been done (Bermejo et al., 2011; Camargo et al, 2012), with little information to date available on mutant fruits derived from irradiated shoots.

The aim of the present work was to evaluate the differences in the antioxidant activity in peel and pulp of citrus obtained by non-irradiated and irradiated shoots.

2. Material and Methods

2.1. Irradiation and sample preparation

Gamma irradiation and maintenance were performed as described previously (Kim et al., 2012). Both citrus (*Citrus unshiu* Marc. cv. Miyagawa) wild-type (WT) and mutants (Mut) obtained from same citrus tree. Samples were taken to determine antioxidant activity of citrus fruits in November, 2011 and grouped by morphological characteristics as follows: Mut I, citrus mutants with red color; Mut II, citrus mutants with the large sized, rough peel shape and comparatively high sugar/acid ratio; Mut III, citrus mutants with the pinnate leaves, large sized, rough peel shape and comparatively low sugar/acid ratio; Mut IV, citrus mutants with rough peel shape. Each WT, citrus derived from non-irradiated shoots, was correspond to each Mut. The methanolic extracts of citrus peel and pulp were prepared as described in our previous study (Kim et al., 2012).

2.2. Free radical scavenging assays

1,1-diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide and nitric oxide radical scavenging activities of citrus WT and Mut methanolic extracts were determined as described in our recently published paper (Kim et al., 2012). A dose response curve was plotted to determine the IC₅₀ values which are defined as the concentration sufficient to obtain 50 % of a maximum scavenging capacity. All tests were performed in triplicate.

2.3. Ferrous ion chelating assay

Chelating ability was determined according to the method of Senevirathne et al. (2010) with slight modifications. An aliquot of 250 µL of each methanolic extract was mixed with 5 µL of 2 mM ferrous chloride (FeCl₂). The reaction was initiated by the addition of 10 µL of 5 mM ferrozine. After 10 min at room temperature, the Abs was determined at 562 nm using a Spectra MR microplate reader (Dynex Technologies, Inc., Chantilly, VA, US). Three replicates were made for each test sample to calculate IC₅₀ values.

2.4. Statistical analysis

Comparisons of all results between the Mut sample and WT sample were made by using a nonparametric test (Mann-Whitney U test) with $P < 0.05$ (SPSS, ver. 12.0; SPSS Inc., Chicago, IL, US). For each measurement, three replicate samples were tested.

3. Results and Discussion

The DPPH assay is often used to evaluate the ability of antioxidants to scavenge free radicals which are known to be a major factor in biological damages caused by oxidative stress (Huang et al., 2005).

Table 1. IC₅₀ value in free radical scavenging and ferrous ion chelating properties of peel and pulp extracts of citrus derived from non-irradiated (WT) and irradiated (Mut) shoots

Group	IC ₅₀ value (mg/mL) [†]			
	Scavenging effect on DPPH radical	Scavenging effect on hydrogen peroxide	Scavenging effect on nitric oxide radical	Chelating effect on ferrous ions
Peel				
WT I	0.14 ± 0.007	0.20 ± 0.002	2.77 ± 0.165	2.84 ± 0.091
Mut I	0.16 ± 0.011	0.13 ± 0.003*	2.92 ± 0.072	2.09 ± 0.289
WT II	0.25 ± 0.005	0.17 ± 0.010	2.18 ± 0.082	3.19 ± 0.163
Mut II	0.38 ± 0.031*	0.11 ± 0.002	5.35 ± 0.031*	7.59 ± 1.241*
WT III	0.43 ± 0.007	0.18 ± 0.027	1.81 ± 0.027	6.28 ± 0.796
Mut III	0.56 ± 0.049*	0.11 ± 0.003*	2.03 ± 0.093	5.68 ± 0.331
WT IV	0.37 ± 0.005	0.20 ± 0.003	3.42 ± 0.349	4.73 ± 0.474
Mut IV	0.33 ± 0.047	0.13 ± 0.002*	2.80 ± 0.320	3.98 ± 0.269
Pulp				
WT I	0.37 ± 0.032	0.27 ± 0.012	6.97 ± 2.155	3.21 ± 0.209
Mut I	0.53 ± 0.075*	0.25 ± 0.012	5.60 ± 0.371	2.91 ± 0.160
WT II	0.53 ± 0.075	0.24 ± 0.037	4.54 ± 0.200	2.05 ± 0.269
Mut II	0.36 ± 0.031*	0.32 ± 0.014*	6.31 ± 0.886*	2.19 ± 0.244
WT III	0.35 ± 0.031	0.48 ± 0.029	4.83 ± 0.265	1.35 ± 0.037
Mut III	0.41 ± 0.038	0.28 ± 0.180*	4.25 ± 0.193	2.24 ± 0.319
WT IV	0.63 ± 0.098	0.24 ± 0.015	4.06 ± 0.367	2.23 ± 0.266
Mut IV	0.64 ± 0.083	0.29 ± 0.039	4.56 ± 0.249	2.51 ± 0.144

[†] IC₅₀, the concentration of citrus methanolic extracts that inhibited 50% of radicals. IC₅₀ was obtained by interpolation from linear regression analysis. Each values is expressed as mean ± standard deviation (*n* = 3).

* Values are significantly different from corresponding WTs (*p* < 0.05).

The pulp extracts of Mut II showed a significantly higher (*p* < 0.05) radical scavenging activity (0.36 mg/mL IC₅₀) than that of WT (0.53 mg/mL IC₅₀) while other Mut groups showed similar or lower scavenging activities (Table 1).

The inhibitory abilities of citrus WT and Mut fruits on hydroxyl radical are also shown in Table 1. The pulp extracts of all Mut groups showed lower hydroxyl radical scavenging activity on hydroxyl radical. The IC₅₀ values of Mut groups were 0.11-0.13 mg/mL (Table 1), which was lower than the corresponding WT (0.17-0.20 mg/mL). Hydroxyl radicals are known to be capable of abstracting hydrogen atoms from phospholipid membranes, and thus bring about peroxidic reactions of lipids (Karawita et al., 2005). Therefore, the higher hydroxyl scavenging activity shown in the pulp extracts of citrus mutants tested in this study can be used to minimize the adverse effects from the hydroxyl radical.

The nitric oxide scavenging potential of citrus Mut peel and pulp as compared with WT is

presented in Table 1. The peel extract of citrus Mut IV (2.80 mg/mL IC₅₀) exhibited significant (*p* < 0.05) scavenging effects compared with WT (3.42 mg/mL IC₅₀) (Table 1). Furthermore, scavenging activities of the pulp extract of citrus Mut I (5.60 mg/mL IC₅₀) were slightly higher than the activities of the WT (6.97 mg/mL IC₅₀). The role of nitric oxide in various disease states has attracted the attention of scientists worldwide. The nitric oxide does not interact with bioorganic macromolecules such as DNA or proteins directly. However, under aerobic conditions, the nitric oxide molecule is very unstable and reacts with the oxygen to produce intermediates such as NO₂, N₂O₄ and N₃O₄ and stable products like nitrate and nitrite (Marcocci et al., 1994) and peroxynitrite when reacted with superoxide (Wink et al., 1991) which is highly toxic to humans.

Ferrozine can make complexes with ferrous ions. In the presence of chelating agents, complex (red colored) formation is interrupted and as a result, the red color of the complex is decreased. Thus, the chelating effect of the coexisting chelator

can be determined by measuring the rate of color reduction. The formation of the ferrozine-Fe²⁺ complex is interrupted in the presence of pulp extracts of citrus Mut I, III and IV groups (2.09-5.68 mg/mL IC₅₀) and peel extracts of Mut I (2.91 mg/mL IC₅₀), indicating that they have chelating ability (Table 1). Chelation therapy reduces iron-related complications and thereby improves quality of life and overall survival (Shinar and Rachmilewitz, 1990; Hebbel et al., 1990). The accumulation of toxic quantities of iron causes tissue damage and leads to various complications in human. Therefore, citrus mutants can be observed as a potent ferrous-chelating source worthy of further investigation.

A further research is in progress to characterize the antioxidative mechanism of citrus extracts induced by gamma irradiation.

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