レモンおよびリンゴ果皮の抗酸化成分・機能に及ぼす光の影響

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SHORT COMMUNICATION

Effects of Shading on the Levels and Activities of Antioxidative Compounds in the Skin of Lemons and Apples

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Summary

The effects of shading conditions on antioxidative activities of ascorbic acid, β-cryptoxanthin and polyphenols in lemon and apple fruit were studied. The IC_{50} values of superoxide (O_2^-)- and 1-diphenyl-2-picrylhydrazyl (DPPH)-radical-scavenging activities in lemon skin from entirely shaded trees (TS) were high compared to the untreated control (UC). Their IC_{50} values in apple skin were also higher in fruit shaded by paper bags (FS) and in fruit from TS compared to fruit from UC. Ascorbic acid and β-cryptoxanthin concentrations in lemon skin of TS were lower than those of UC. Polyphenolics in apple skins from FS and TS also decreased more than those from UC. These results suggest that shading may regulate the antioxidative components in the fruit, and as a result, may influence antioxidative activity.

Key Words: antioxidant, ascorbic acid, β-cryptoxanthin, polyphenolics.

Introduction

Our previous report (Kondo et al., 2002a, b) showed that the skins of *Citrus* and apple fruit have stronger antioxidative activity than the flesh. Apples contain high levels of polyphenolics (Kondo et al., 2002b), whereas *Citrus* fruits contain high levels of ascorbic acid and carotenoids (Mozafar, 1993). It has been postulated that these components contribute to antioxidative activity.

Environmental factors, such as shading, greatly influence fruit physiology and quality. For instance, fruit grown under optimum lighting conditions accumulate high levels of sugars and anthocyanins (Mozafar, 1993). Few studies on environmental conditions that may influence antioxidative function and antioxidative components, have been published. In this study, relationships between antioxidative activity [superoxide (O_2^-)- and 1-diphenyl-2-picrylhydrazyl (DPPH)-radical-scavenging activity] as well as the levels of antioxidative components, e. g., polyphenolics, ascorbic acid and β-cryptoxanthin, and shading were investigated in lemons and apples.

Materials and Methods

'Lisbon' lemons (*Citrus limon* Burm. f.) (from 20 to 100 fruit, depending on the developmental stage) were sampled in 2001 and 2002 at random from four 21- or 22-year-old trees grafted onto trifoliate orange (*Poncirus trifoliate* Raf.) rootstocks, growing in an orchard at the Hiroshima Prefectural Agriculture Research Center. Four trees were divided into two pairs; and the first two were covered with cheesecloth (75% shading, TS) from 45 days after full bloom (DAFB) until 240 DAFB (harvest). The second pair was left uncovered (untreated control, UC). 'Fuji' apples (*Malus pumila* Mill. var *domestica* Schneid) (from 20 to 100 fruit, depending on the developmental stage) were sampled in 2001 from six randomly selected 13-year-old trees grafted onto M.26 rootstocks, also growing in an orchard of the Hiroshima Prefectural University. Six 'Fuji' trees were divided into three pairs; the first pair was covered with cheesecloth (50% shading, TS) from 45 DAFB until 180 DAFB (harvest). Preliminary trials showed that shading in excess of 50%, i. e., 75% induces apple fruit to abscise (Kondo et al., 1987). In the second pair, only the fruit were shielded in paper bags (75% shading, FS) from 45 DAFB until 180 DAFB; the third pair was left uncovered (UC).

To measure O_2^--scavenging and DPPH-radical-scavenging activities, skin samples (1-10 g FW in triplicate taken from five to twenty fruit selected randomly) were homogenized in 13.7 M ethanol. The homogenate was filtered, and the filtrate evaporated to the aqueous phase. The residue was diluted to 10 ml with distilled water. The radical-scavenging activities and IC_{50} of the antioxidant sample were calculated as previously reported (Kondo et al., 2002a). IC was determined from zero to full inhibition; the point of 50% inhibition of reaction occurs in the mixture containing the sample was recorded as IC_{50}.

Polyphenolics in the skin of apples were analyzed

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with Folin-Ciocalteu reagent according to Kondo et al. (2002b). The concentration of β-cryptoxanthin in lemon skins was determined according to Pupin et al. (1999) using Sudan I as the internal standard in a high performance liquid chromatography (HPLC) as modified by Sumida et al. (1999). The HPLC (Shimadzu LC-VP system) was equipped with a carotenoid C_{30} column (Waters, Milford, USA; 4.6 mm i.d. × 25 cm). Ascorbic acid concentration was analyzed by 2,4-dinitrophenylhydrazine method as previously reported (Kondo et al., 2002a).

Results and Discussion

In the first experiment, the antioxidative function and components in the skin were analyzed because the skin yields consistant results. When the IC_{50} value was high, more skin samples were needed to eliminate 50% of the radial activity.

IC_{50} values of O_2^--and DPPH-radical-scavenging activities in lemon skins were high throughout fruit development in TS, compared with UC (Fig. 1).

Likewise, IC_{50} values of both radical-scavenging activities in FS apples and those from TS were higher than those of UC (Fig. 2). Although ascorbic acid and β-cryptoxanthin concentrations in lemon skins did not significantly differ between TS and UC at 60 DAFB, these concentrations were lower in TS than in UC at 180 and 240 DAFB (Table 1). At 150 and 180 DAFB, polyphenolics in apple skins were highest in UC and lowest in FS (Table 2). Anthocyanin was detected in the skin at 180 DAFB, but the concentration was not significant compared to 150 DAFB (data not shown), which may have resulted in less total polyphenolics than those at 150 DAFB. Ascorbic acid and β-cryptoxanthin in *Citrus* fruit (Yano, 1999) and polyphenolics in apple fruit (Kondo et al., 2002b) are the primary antioxidative components. In this study, these components in the skin decreased proportionately to the degree of shading. Hence, our results support the findings by Mozafar

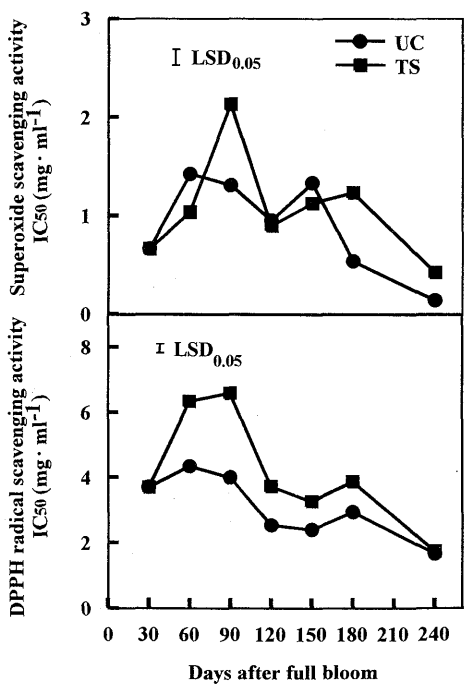

Fig. 1. Changes of O_2^- and DPPH-radical-scavenging activity in 'Lisbon' lemon skin from entirely shaded trees (TS) and untreated control (UC). Data are the means of three replications. IC_{50} value shows the concentration of the sample (mg FW) in 1 ml reaction mixture.

Table 1. Effects of shading on ascorbic acid and β-cryptoxanthin concentrations in 'Lisbon' lemon skin. Means of three replications.

Treatment[z]	Ascorbic acid (mg · g^{-1} FW)		β-cryptoxanthin (ng · g^{-1}FW)	
	60 DAFB			
UC	1.368	NS	45.3	NS
TS	1.328		46.7	
	180 DAFB			
UC	0.594	**	380.8	**
TS	0.458		240.1	
	240 DAFB			
UC	0.566	**	1476.4	*
TS	0.487		518.1	

NS,*, ** : Non-significant, significant at $P \leqq 0.05$ or 0.01, respectively.

[z]UC: Untreated control, TS: Entirely shaded trees.

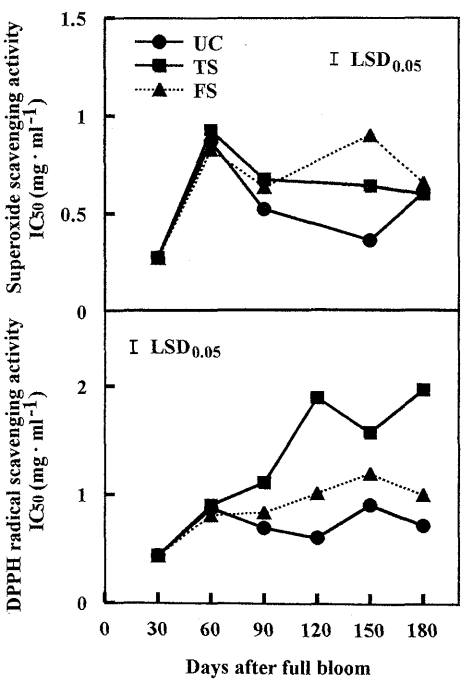

Fig. 2. Changes of O_2^- and DPPH-radical-scavenging activity in 'Fuji' apple skin from entirely shaded trees (TS), in skin shaded by paper bags (FS) and untreated control (UC). Data are the means of three replications. IC_{50} value shows the concentration of the sample (mg FW) in 1 ml reaction mixture.

Table 2. Effects of shading on polyphenolic concentrations in 'Fuji' apple skin. Means of three replications.

Treatment[z]	Polyphenolics ($mg \cdot g^{-1}FW$)
30 DAFB	
UC	8.86
150 DAFB	
UC	4.03a[y]
TS	2.37b
FS	1.15c
180 DAFB	
UC	2.09a
TS	2.12a
FS	1.19b

[z]UC: Untreated control, TS: Entirely shaded trees, FS: Fruit shaded by paper bags.
[y]Different letters indicate significant difference by Newman－Keuls test, $P \leqq 0.05$.

(1993) on oranges that fruit grown on exposed branches have a higher ascorbic acid concentration than those on shaded ones. Flavonoid concentrations in the fruit from the outer canopy of apple trees were likewise higher than those from the inner canopy (Awad et al., 2001).

Flavonoid and carotenoid syntheses are influenced by light through photoreceptors such as chloroplast and phytochrome (Mozafar, 1993). It has been postulated that plants produce polyphenolics to defend themselves against ultra violet (UV) radiation (Strack and Wray, 1989). This suggests that fruit may produce large amounts of polyphenolics in an environment strong in UV radiation (Saure, 1990). In our study, the lower polyphenolic concentrations at FS compared to TS demonstrate that polyphenolics in the skin may be produced primarily *in situ*. The accumulation of β－carotene and ascorbic acid in fruit is also influenced by light intensity (Mozafar, 1993). Therefore, light intensity seems to influence the synthesis of primary antioxidative compounds, such as ascorbic acid and β－cryptoxanthin in lemons and polyphenolics in apples, thus, enhancing their nutrient value.

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摘　要

レモン'リスボン'およびリンゴ'ふじ'果実の抗酸化機能およびアスコルビン酸，β－クリプトキサンチンおよびポリフェノールに及ぼす光条件の影響を検討した．レモン果皮におけるスーパーオキサイドアニオン(O_2^-)ラジカルおよび1,1－ジフェニル－2－ピクリルヒドラジル(DPPH)ラジカル消去活性のIC_{50}値は，無処理区に比べ樹冠遮光区の果皮で高くなった．リンゴ果皮においても，これらのIC_{50}値は無処理区に比べ樹冠遮光区および果実遮光区で高くなった．樹冠遮光区のレモン果皮ではアスコルビン酸およびβ－クリプトキサンチン濃度が無処理区の果皮より低下し，リンゴでも樹冠および果実遮光区の果皮のポリフェノール濃度は低下した．以上の結果は，光が果実中における抗酸化成分を制御し，抗酸化機能に影響することを示唆する．