

Induction of phenolic compounds biosynthesis with light irradiation in the flesh of red and yellow apples

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Abstract

Effect of light irradiation on the accumulation of phenolic compounds was investigated in the flesh slices of three apple (*Malus domestica* Borkh.) cultivars. 'Fuji' and 'Jonathan' with red skin and 'Orin' a yellow-green one were used in this study. The irradiation was carried out at 10, 17, 24 and 30°C for 96 hours, using a mixture of white plus ultraviolet fluorescents. Phenolic acids, anthocyanin and flavonols were the phenolics that increased rapidly by irradiation whereas flavanols, procyanidins and dihydrochalcones did not change in either mature or in ripe fruits of all the three cultivars. There was a positive correlation between anthocyanins, phenolic acids and flavonols in examined cultivars both at the mature and ripe stages. Optimum temperature for the synthesis of phenolic acids, anthocyanins and flavonols was 24°C regardless to the maturity stage and variety. Total phenolic content of 'Fuji' increased through ripening but it decreased in 'Jonathan' and 'Orin'. Therefore, the irradiation to the flesh might be a very useful method for the study of the regulation mechanism of the phenolic compounds accumulation.

Key words: Apple, light irradiation on flesh, ultraviolet-B, flavonoids, phenolic acids, high performance liquid chromatography

Introduction

Apple fruit is known to contain large amounts of phenolics such as chlorogenic acid, flavanols, flavonols, procyanidins and anthocyanins (Awad and de Jager, 2002; Awad *et al.*, 2000). The studies using animals and *in vitro* work have been conducted to define mechanisms by which apples may help in preventing chronic disease caused by lipid and DNA oxidation (Boyer and Liu, 2004). The antioxidative potential of apple, however, is known to depend on the concentration and composition of phenolics (Kondo *et al.*, 2002), which is influenced by many physiological and environmental factors (Lancaster, 1992). Moreover, distribution of these metabolites differs within a fruit (Awad *et al.*, 2000).

The ability for anthocyanin accumulation in apple skin varies within varieties. Red-skinned apples accumulate anthocyanin, while yellow-green ones do not and most of the commercial cultivars with red skin accumulate anthocyanin in the skin but not in the flesh. Although the biosynthesis mechanisms of anthocyanin and other phenolics have been widely studied in the skin, limited information about the flesh is available. Stimulative effect of irradiation on the anthocyanin accumulation, influenced by bagging, temperature and maturity has been reported, recently. These studies show that UV-B stimulates anthocyanin synthesis in the apple skin (Arakawa *et al.*, 1985; Arakawa, 1988; Arakawa, 1991; Wang *et al.*, 2000).

Because of lower content of phenolics, specifically flavonols, in the flesh compared to the skin, pulp may have lower bioactivity than the peel. Many researches have been conducted on the regulation of phenolics accumulation in the skin, and little is known about the flesh. The aim of this study was to investigate the regulation mechanism of anthocyanin and other phenolic compounds accumulation in the flesh, with special attention given

to light and temperature as two key regulatory factors. Mature and ripe fruits of three cultivars with varied genetic background were studied.

Materials and methods

Fruits of 'Jonathan' and 'Fuji' (red cultivars) and 'Orin' (yellow-green) were harvested from 15-year-old trees grafted onto *Malus prunifolia* Borkh. var. *ringo* Asami rootstocks. The trees were grown at the experimental orchard of the Faculty of Agriculture and Life Science, Hirosaki University. Sampling dates for mature and ripe stages of each cultivar were: 'Jonathan'; 123 and 145 days after full bloom (DAFB), 'Fuji'; 134 and 165 DAFB and 'Orin'; 131 and 170 DAFB, respectively. Three fruits of each cultivar were equatorially cut and immediately dipped in a solution of Polyclar SB 100 (20 g L⁻¹), in order to protect the tissues from browning, and then sterilized with 5 percent ethanol for 1 minute. Three sectors of these samples were put into petri dishes and covered with clear polyethylene sheet to maintain the moisture of the tissues during irradiation. The other three halves were wrapped in aluminum foil, to maintain dark conditions during irradiation, as controls. The irradiation was carried out at 10, 17, 24 and 30°C using a mixture of a white fluorescent lamp (FL20-S W, Toshiba, Tokyo; 4 W m⁻²) and a UV-B fluorescent (FL20-S. E., Toshiba Tokyo; 1.3 W m⁻²) with a filter eliminating radiation below 290 nm. The irradiation was carried out for 96 hours, continuously, and some water was daily sprayed on the samples during the irradiation period. The irradiated surface (almost one millimeter in thickness) was then separated and frozen in liquid nitrogen then kept at -80°C until analysis. Phenolic extraction was carried out with 15 percent acetic acid in methanol added to 1 g of finely powdered tissue and then kept in 4°C for 2 hours. These samples were centrifuged at 10,000 g for 15 minutes at 0°C (Himac CR 15, HITACHI, Japan). The

supernatant of samples was filtered through a 0.45 μm disposable syringe filter and used for flavonoids quantification by high performance liquid chromatography (HPLC) coupled to a diode array detector with wavelength set at 280, 350 and 530 nm. The column was a 1.5 mm I.D. \times 250 mm (Grand C18-UG 120-5 SE, MASIS, Inc., Aomori, Japan) with a 1.5 mm I.D. \times 35 mm guard column. Elution solvents were (a) 1.5 percent phosphoric acid in water of which the flow was 90 $\mu\text{L min}^{-1}$ and (b) 1.5 percent phosphoric acid, 20 percent formic acid and 25 percent acetonitrile in water with 10 $\mu\text{L min}^{-1}$. Samples (5 μL) were injected on to the column which was maintained at 30°C.

According to standard analysis, identified substances were categorized to six groups; namely, phenolic acids (chlorogenic acid), anthocyanins (cyanidin 3-galactoside), procyanidins (procyanidin B2), flavanols (catechin and epicatechin), flavonols (quercetin glycosides), and dihydrochalcones (phloridzin). Mature and ripe fruit flesh of three cultivars were used for biochemical analysis.

Results

Accumulation of phenolic acids, anthocyanin and flavonols in response to UV-B irradiation: Irradiated flesh of mature and ripe fruits of all three cultivars accumulated phenolic acids, flavonols and anthocyanin (Figs. 1 and 2). 'Fuji' and 'Orin' accumulated the highest flavonols and phenolic acids at 24 and 30°C; whereas for 'Jonathan' 24°C was optimum. However, the concentration of anthocyanin was lower than phenolic acids and flavonols in three varieties. Chlorogenic acid of 'Orin' was the predominant phenolic compound after irradiation.

Total phenolic content of 'Fuji' increased from mature stage to

ripening but in 'Jonathan' and 'Orin' it decreased. Interestingly, total phenolic content of Fuji (before and after irradiation), both at mature and ripe stages, was higher than that of 'Jonathan' followed by 'Orin'. 'Jonathan' accumulated the highest anthocyanin, resulted in redder color, followed by 'Fuji' and 'Orin'. Anthocyanin biosynthesis in the pith of receptacle area was higher than the cortex of receptacle.

Lack of response of flavanols, procyanidins and dihydrochalcones to UV-B irradiation: Irradiation did not stimulate the accumulation of flavanols, procyanidins and dihydrochalcones both in mature and ripe stages in all three cultivars (Figs. 1 and 2). In ripe 'Orin' the flavanols increased slightly at 24°C, but it was not significant. Flavonols increased with fruit ripening regardless to irradiation temperature. Flavanol content of 'Jonathan' was higher than that of 'Fuji' and 'Orin'.

Correlation between the accumulation of anthocyanin and other phenolics: There was not same pattern of correlation between anthocyanin biosynthesis and other phenolics in the examined cultivars (Table 1). In 'Fuji' and 'Jonathan', phenolic acids and flavonols showed a positive correlation with anthocyanin accumulation, which was not affected by ripening. In 'Orin', on the other hand, the correlation coefficient values between anthocyanin versus phenolic acids and flavonols decreased from mature stage to full ripening.

Discussion

Our results showed that the flesh of all three cultivars used in this study had the potential for accumulating anthocyanin as well as many other polyphenolics, although temperature and maturing stage affected it. The flesh of 'Jonathan' accumulated the highest

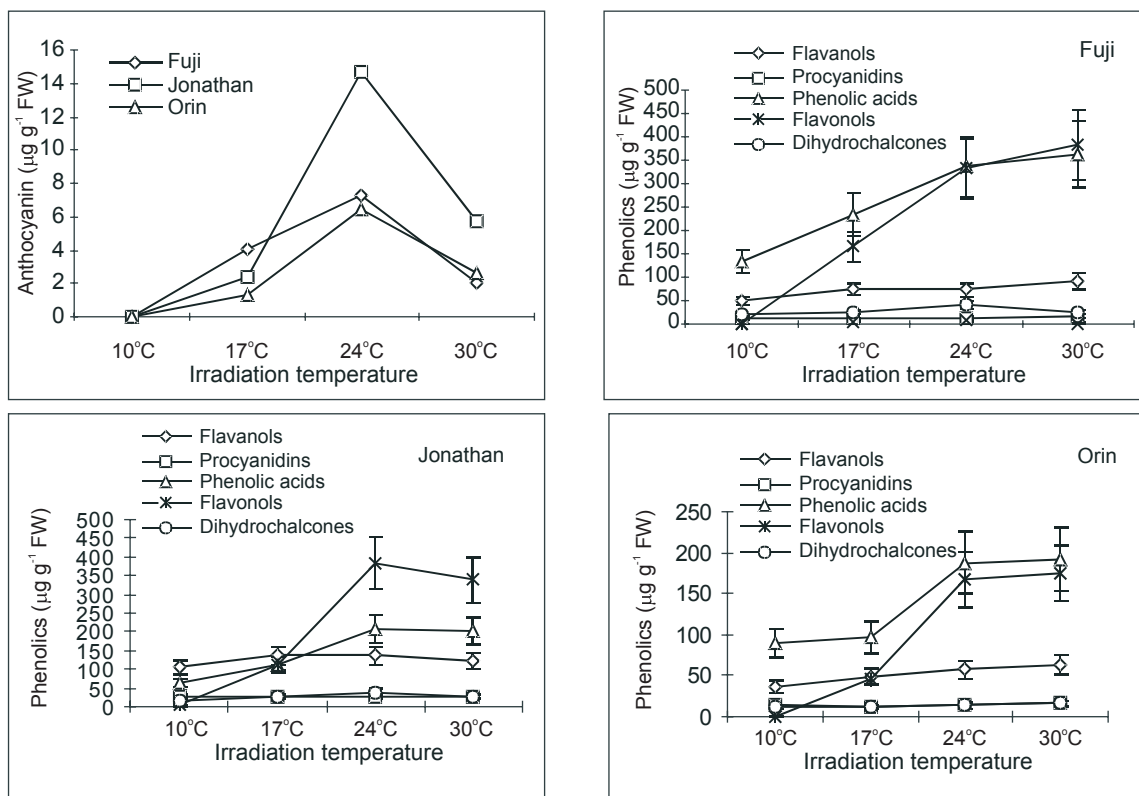


Fig. 1. Effect of UV-B irradiation on the accumulation of anthocyanin and other phenolic compounds in three apple cultivars at mature stage. Phenolic content of the three cultivars ($\mu\text{g g}^{-1}$ FW), irradiated at 10, 17, 24 and 30°C. Vertical bars represent SE.

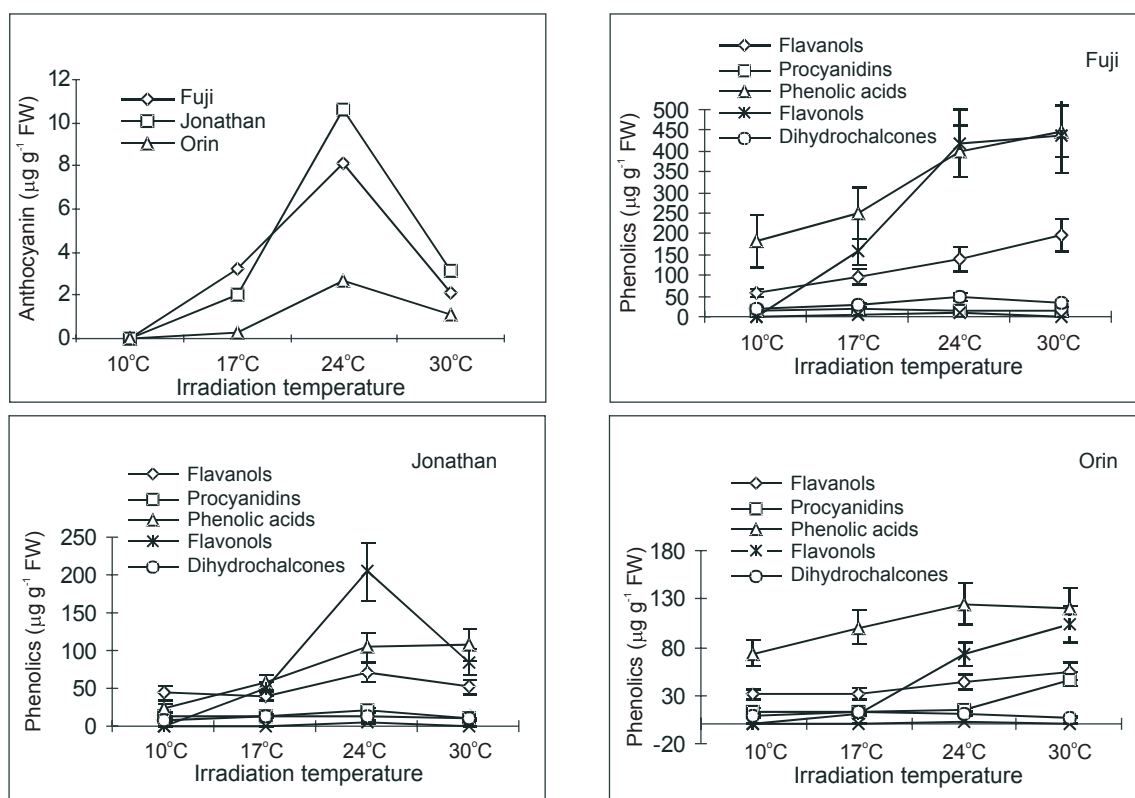


Fig. 2. Effect of UV-B irradiation on the accumulation of anthocyanin and other phenolic compounds in three apple cultivars at ripe stage. Phenolic content of the three cultivars ($\mu\text{g g}^{-1}$ FW), irradiated at 10, 17, 24 and 30°C. Vertical bars represent SE.

anthocyanin followed by 'Fuji' and 'Orin', respectively, which is same as the order of the anthocyanin synthesis ability of the skin of these cultivars suggesting that the light threshold of the flesh is same as that of the skin.

While apple flesh contains low level of flavonols as reported by Awad *et al.* (2000), it increased with light irradiation showing that the synthesis of this group of flavonoids is a light-dependent process. A positive correlation between anthocyanins, phenolic acids and flavonols, but not the other phenolics, show that the expression of the genes controlling the synthesis. The quantity and quality of phenolics changed with irradiation, depending on the irradiation temperatures. Optimum temperature for the synthesis of phenolic acids, anthocyanins and flavonols was 24°C, regardless to the maturity stage and variety. The response of flesh to light irradiation is different from that of skin. Our previous study showed that temperature had different effects on phenolic accumulation in skin depending on the maturity stage; mature and ripe fruits accumulated the highest phenolics at 17 and 24°C, respectively (Bakhshi and Arakawa, in press).

Activation of the expression of the flavonoid biosynthetic genes

Table 1. Correlation coefficient value (r) of anthocyanin versus other identified phenolic compounds in the light (white plus UV-B) irradiated flesh of 'Fuji', 'Jonathan' and 'Orin' cultivars

Phenolic classes	Mature			Ripe		
	'Fuji'	'Jonathan'	'Orin'	'Fuji'	'Jonathan'	'Orin'
Flavanols	0.39 ns	0.51 ns	0.55 ns	0.47 ns	0.56 ns	0.38 ns
Procyanidins	0.19 ns	0.22 ns	0.28 ns	0.36 ns	0.58 ns	0.21 ns
Phenolic acids	0.61 *	0.77 **	0.9 **	0.64 *	0.68 *	0.65 *
Flavonols	0.84 **	0.92 **	0.88 **	0.81 **	0.93 **	0.79 **
Dihydrochalcones	0.21 ns	0.34 ns	0.16 ns	0.36 ns	0.28 ns	0.12 ns

*, ** Significant at $P=5\%$ and 1% , respectively; ns- not significant.

by enhanced light has recently been reported in other studies (Lancaster, 1992; Jaakola *et al.*, 2004). All the three cultivars examined in this study accumulated low amounts of phenolics at 10°C which coincides with our former results on the skin. Although low temperatures have been suggested to induce Phenylalanine Ammonia-lyase (PAL) activity (Lancaster, 1992), it seems that low temperature could reduce the UV-B enhancement of quercetins and chlorogenic acid.

Phenolic acids, procyanidins, catechins and dihydrochalcones were the phenolics identified in the flesh among which phenolic acids increased significantly with irradiation. This might be related to the induction of PAL activity by UV-B, which mediates the synthesis of coumaroyl CoA. Coumaroyl CoA then gives rise to phenolic acids and flavonoids. Anthocyanin biosynthesis in the irradiated flesh of 'Orin' that does not naturally accumulate red color might be related to the simultaneous effect of UV-B and wounding on the induction of PAL activity (Lancaster, 1992). However, apple sectors kept in the dark during irradiation did not show any change in their phenolic content showing that only wounding is not enough for phenolic accumulation (data not

shown). Other than anthocyanin and quercetin, phenolic acids increased with irradiation. This coincides with the result of Awad *et al.* (2000) who reported that the level of chlorogenic acid in the shaded side of skin (receiving low light) was lower than that of the sun exposed side.

In the last two decades, interest in using natural ingredients in foods has increased dramatically. The emphasis given to natural antioxidants results from concerns over the toxicity of some synthetic antioxidants and from the research findings that point to a relationship between active dietary ingredients, such as natural antioxidants, and their protection of cells from free radical-induced oxidative stress in the human body (Valenzuela *et al.*, 2003).

Our results show that apple fruit is a potentially good source of phenolic compounds as natural antioxidants. The present study revealed that light and temperature have strong effect on the phenolics accumulation in the apple flesh as well as the skin. This finding offers the exciting possibility of improving the polyphenolic content by optimizing the environmental factors. Light irradiation on the flesh of apple also seems to be a good method for the study on the expression of the genes controlling the accumulation of phenolic compounds.

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