Effect of γ-Irradiation on Phenolic Compounds and Phenylalanine Ammonia-Lyase Activity during Storage in Relation to Peel Injury from Peel of *Citrus clementina* Hort. Ex. Tanaka

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The influence of γ-irradiation on the content of phenolic compounds was evaluated on Moroccan *Citrus* fruits (*Citrus clementina* Hort. ex. Tanaka) treated at a mean dose of 0.3 kGy and stored for 49 days at 3 °C. The results show that irradiation has enhanced the synthesis of total phenolic compounds and is correlated with phenylalanine ammonia-lyase activity (PAL) during storage. Accumulation of phenolic compounds in cells is demonstrated and may be explained by the enhancement of PAL activity. HPLC/UV (diode array detector) analysis demonstrated that hesperidin was the major flavanone and nobiletin and heptamethoxyflavone were the major polymethoxylated flavones. Hesperidin is also the major phenolic compound in clementines. Irradiation stimulates the biosynthesis of hesperidin after 14 days of storage, corresponding to the maximum of PAL activity. *p*-Coumaric acid was also identified, and its content was particularly high in irradiated fruits after 49 days of storage. Accumulation of flavonoids and *p*-coumaric acid could be related to a better resistance. The percentage of losses due to peel injury "pitting" during storage was between 1 and 5% after 49 days of storage. The connections between irradiation, enzyme activity, phenolic content, and peel injury are briefly discussed.

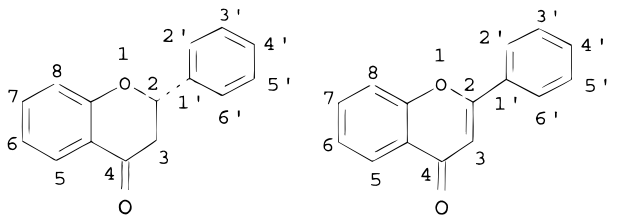
Keywords: *γ-Radiation; Citrus clementina fruit; PAL activity; total phenolic compounds; hesperidin; p-coumaric acid*

INTRODUCTION

Flavonoids present in *Citrus* [flavanones (F) and polymethoxylated flavones (PMF)] have been isolated from the peel and juice of some *Citrus* species (Wilfried et al., 1994a,b). The chemical structure of these compounds is represented in Chart 1. PMF in *Citrus* fruits have been extracted from peel *Citrus* oils and separated by HPLC (Gaydou et al., 1987; Sendra et al., 1988). Specific distributions of these flavonoids have been used for taxonomic studies (Kamiya et al., 1979; Rouseff et al., 1987). In addition, PMF have been studied for their antimicrobial and antiviral activities (Huet, 1982; Laks and Pruner, 1989). Some flavonoids (mainly isoflavonoids) have been previously shown to have a role in plant defense mechanisms as phytoalexins in response to infection (Kodama et al., 1992).

Irradiation is of interest to preserve the quality of foods by decreasing microbial and insect infestation and by extending shelf life (IAEA, 1982; Josephson and Peterson, 1982). Optimal radiation doses for pasteurizing (0.75−2.50 kGy) can extend storage life by 2−6 weeks at 0−5 °C (Angel et al., 1986; Poole et al., 1994). However, the effectiveness of irradiation may differ considerably according to species (IAEA, 1982; Emerson et al., 1966).

Chart 1. Chemical Structures of Flavanone Glycosides and Polymethoxylated Flavones



Flavanone Skeleton **Flavone Skeleton**

Many researchers have reported γ-radiation as a potential treatment for extending the postharvest life of fresh fruits and vegetables (Kader, 1986; Urbain, 1986; Thomas, 1988). However, irradiation with dosages to accomplish the intended purposes has resulted in softening and browning of many fruits and vegetables (Kader, 1986; Urbain, 1986; Maxie et al., 1971). Pitting of the peel can also occur during storage of irradiated citrus fruits. The severity of the peel damage increases with dose of irradiation, storage time, and storage temperature (Urbain, 1986). Development of browning pigments and pitting has been associated with changes in some enzymatic activities, for example, polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), and peroxidase (POD), and in total phenolic compounds

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content (Tan and Lam, 1985; Monselise and Kahan, 1968; Cunha et al., 1993) and with the alteration of the cellular membranes permeability or damage cells (Beaulieu et al., 1999; Riov, 1975).

PAL (EC 4.3.1.5) catalyzes in plants the first committed step in the biosynthesis of a diverse range of phenylpropanoid-derived secondary products such as flavonoids and isoflavonoids, coumarins, and lignins (Hanson and Havir, 1979; Jones, 1984).

PAL activity was found to vary greatly with the stage of plant development and with cell and tissue differentiation (Hahlbrock and Scheel, 1989). Various stresses, such as irradiation, wounding, nutrient deficiencies, herbicide treatment, and viral, fungi, and insect attacks, were shown to increase either PAL synthesis or activity in a variety of plants (Chalker-Scott and Fuchigami, 1989).

Accumulation of phenolic compounds varies strongly in relation to the physiological state of the fruit and is a result of an equilibrium between biosynthesis and further metabolism including turnover and catabolism. Acclimation of apple trees to cold climates was found to be associated with a seasonal accumulation of chlorogenic acid (Chalker-Scott and Fuchigami, 1989). In mango fruit, irradiation enhanced some phenolic acids and flavonoids (Lacroix et al., 1990).

The purpose of this study is to investigate the effect of irradiation and subsequent storage at 4 °C on total phenolic compounds and some important flavonoids [FG and PMF; O−Rt means a rutinoside and O−Me a methoxyl (Horowitz and Gentilli, 1977)], PAL activity, and peel injury in clementine fruits.

MATERIALS AND METHODS

Plant Material. Clementines (*Citrus clementina* Hort. ex. Tanaka var. Nour) used in this study were collected from the Agricultural and Development Society (SODEA) of Rabat, Morocco. Selected fruits with the absence of bruises or other quality defects were packed in 10 kg paperboard flats with lids. The day after, 20 fruits boxes were sent by Royal Air Maroc cargo shipment to Mirabel International Airport, Canada. Upon arrival at Mirabel, the fruits were on the same day trucked to the Canadian Irradiation Center (CIC), in Laval. Upon arrival at CIC, the fruits were randomly distributed into two groups: unirradiated and irradiated.

Irradiation Treatment. Fruits were irradiated with a dose of 0.3 kGy at a dose rate of ~6 kGy h^{-1} in the ^{60}Co source carrier type industrial irradiator (MDS Nordion International Inc., Kanata, Canada) at the CIC (Laval, PQ). Optichromic and Gammachrome dosimeters were used to validate the dose distribution throughout the cartons. Upon completion of the irradiation at room temperature, all dosimeters were collected and dosimetry data recorded.

Conditions and Time of Storage. After completion of the irradiation, 10 cartons of each unirradiated [control (C)] and irradiated (I) fruits were stored under refrigeration (3−4 °C), at 84−86% relative humidity (RH). The analysis started on day of arrival at the CIC. Analyses were performed at each week for 49 days.

PAL Activity. PAL activity was determined according to the procedures described by Tan and Lam (1985). Four hundred freeze-dried samples were dissolved in 20 mL of 0.1 M tetraborate buffer, pH 8.8, containing 2.5% polyvinylpolypyrrolidone (PVPP) and 0.2 M $CaCl_2$, under continuous agitation for 10 min. The extracts were centrifuged (25000*g*, 10 min, 0−4 °C). The enzyme unit was defined as the conversion of L-phenylalanine to *trans*-cinnamic acid by absorbance at 280 nm. Results were expressed in katals (mol/s).

Total Phenolic Content. Six hundred and fifty milligrams of freeze-dried peel tissue was shaken twice for 30 min in ethanol/water (80:20 v/v), and the filtrates were pooled. Preconditioned PVPP was use to bind phenolic compounds by adsorption (Doner et al., 1993; McMurrough et al., 1995); 250 mg was sufficient to eliminate >95% of the total phenolic content. According to Marigo (1973), total phenols (TPP) were determined by difference of absorbance before and after adsorption by Folin−Ciocalteu reagent. All tests were carried out on three identical samples of fruit.

Extraction and Purification of Flavonoids. For flavanones (F), a freeze-dried sample of peel fruits (500 mg) was homogenized in a 50 mL ethanol/water mixture (80:20 v/v) containing sodium disulfite (0.5%). After stirring at 25 °C for 45 min, the extract was filtered and the residue was treated two more times in the same way. The ethanolic filtrates were collected, and ethanol was evaporated under vacuum. Pigments and lipids were removed by three successive petroleum ether extractions in the presence of ammonium sulfate (40%) and metaphosphoric acid (20%). The flavanones were extracted three times by ethyl acetate in the presence of MeOH (20%). The three ethyl acetate phases were collected, dried on Whatman paper (phase separator silicone treated, catalog no. 2200 185), and evaporated under vacuum. Residue was dissolved in dimethylformamide/water (1:1) and filtered through an Acrodisc filter (0.45 μm) before HPLC analysis.

For polymethoxylated flavones (PMF), the first stage of extraction consists of citrus oil preparation present in 500 mg of freeze-dried peel by 100 mL of methylene chloride. After stirring for 30 min, the extract was filtered and evaporated under vacuum at 35 °C. Citrus oil residue was weighed out for all days of storage and diluted in methylene chloride. Isolation of the PMF in citrus oils was done according to the methods of Sendra et al. (1988) and Rousseff and Ting (1979) using a C_{18} Sep-Pak cartidge. Activation of C_{18} Sep-Pak was done by passing 5 mL of acetonitrile for 2 min two times followed by 5 mL of water (2 min). Then the pretreated citrus oil (10 mL) was percolated through it and then washed with 5 mL of water and 5 mL of acetonitrile. Finally, the PMF were eluted with 10 mL of methanol/methylene chloride (1:1). The solvent was evaporated to dryness, under vacuum at 35 °C. The residue was redissolved in 1 mL of MeOH and filtered through a 0.45 μm filter membrane before HPLC analysis.

Determination of Flavonoid Compounds. At the time of our experiments only three PMF were commercially available: sinensetin, scutellarein, and tangeretin. A solution of each component was prepared separately containing 0.02 mg of sinensetin and scutellarein and 0.04 mg of tangeretin in 1 mL of dimethylformamide/water (1:1) (HPLC grade). For F, the hesperidin and naringin were diluted in dimethylformamide/water (7:3). Because of their low solubility in water, didymin, eriocitrin, poncirin, and neoeriocitrine were diluted in dimethylformamide/water (1:1). The solutions used for HPLC analysis contain 20 mg/L for hesperidin and naringin and 10 mg/L for all other flavanones.

Separation of flavonoid compounds was performed by HPLC using a 5 μm RP C_{18} column (Alltima, 150 mm × 4.5 mm i.d.). The mobile phase (flow rate = 1 mL/min) consisted of acidified distilled water (solvent A) with acetic acid in proportion 96:4 v/v and acetonitrile (solvent B). The best separation was obtained at 35 °C with a column pressure of 86 bar by the following gradient: at 0 min, 17% solvent B; at 25 min, 40% solvent B; at 40 min, 40% solvent B; and at 45 min, 17% solvent B. A diode array detector (Waters 990) was used for characterization of each peak. Identification of F and sinensetin, tangeretin, and scutellarein (PMF) is based on retention times and spectra. The other PMF are identified on the basis of their elution order and spectra obtained from the literature (CEN, 1991; Kirkesey et al., 1988; Sendra et al., 1988). Different samples were analyzed in triplicate, and 20 μL of each sample was injected.

Determination of *p*-Coumaric Acid (PCA). PCA was identified by TLC and quantified by HPLC/UV systems. Detection was achieved with a diode array detector, and chromatograms were recorded at 280 nm.

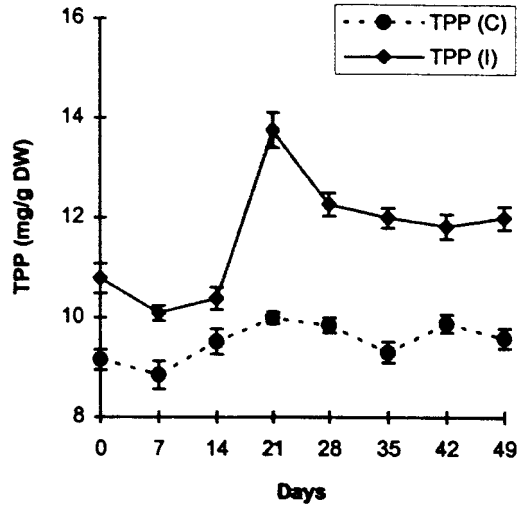

Figure 1. Effect of γ-irradiation on TPP in *C. clementina* peel during storage: c, control; i, irradiated.

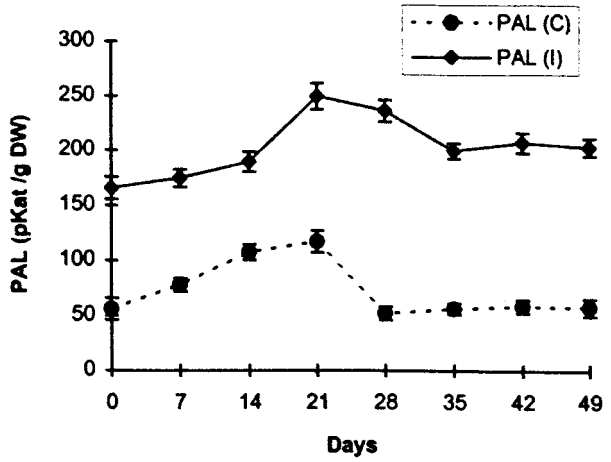

Figure 2. Effect of γ-irradiation on PAL activity of *C. clementina* peel during storage: c, control; i, irradiated.

Peel Injury. Damage consisting of pitting was evaluated by counting the fruits brownish in color for each treatment and expressed as a percentage of the total fruits.

Statistical Analysis. Analysis of variance and Duncan's multiple-range tests with $P \leq 0.05$ were employed to analyze statistically all results. Student's *t* test was utilized at the time of the analysis of variance and paired-comparison with $P \leq 0.05$ (Snedecor and Cochran, 1978). For each measurement, three replicates of three samples were tested.

RESULTS AND DISCUSSION

Figure 1 shows an increase of the TPP content in irradiated fruits. The content remained higher in irradiated fruits during the whole storage. The evolution of the TPP is very similar in control and in irradiated samples with a maximum at 21 days of storage period. This content is related to PAL activity, which also reached a maximum at 21 days of storage (Figure 2). A good correlation was observed between the TPP content and PAL activity ($C_r = 0.80$) (Figure 3). PAL activity was significantivelyhigher ($P \leq 0.05$) in irradiated fruits than in control fruits (Figure 2). Accumulation of phenolic phytoalexins is accompanied by PAL activity. The appearance of 6,7-dimethoxycoumarin (scoparone) in *Citrus*, conferring resistance to *Phytophtora citrophtora*, has been reported by Afek et al. (1986). The 7-hydroxycoumarin from grapefruit flavedo was also found to inhibit spore germination and germ tube elongation of *Penicillium digitatum* (Vernenghi et al., 1987). Indeed, the accumulation of phenolic compounds in irradiated fruits is an important factor in resistance. These compounds in irradiated fruits could be an important factor in resistance.

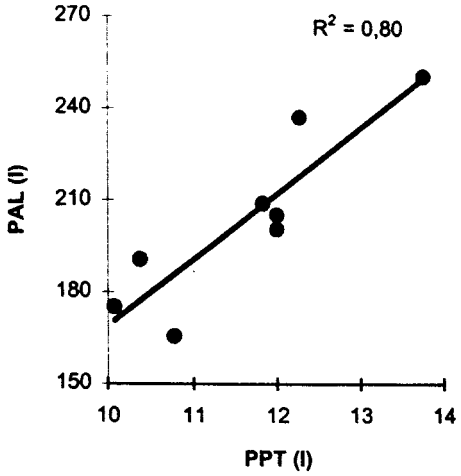

Figure 3. Relationship between phenolics and PAL activity in *C. clementina* peel during storage: c, control; i, irradiated.

In chilled apple fruit, Tan (1980), explained the variation of PAL activity by a decrease of PAL inhibitor protein (protease) in vitro. Riov et al. (1972) observed that the increase in PAL activity in irradiated grapefruit was accompanied by a parallel increase in ethylene production. It seems that irradiation produces an increase in PAL activity through its effects on ethylene production. The authors also observed that when fully ripe grapefruits are irradiated at relatively low doses ($\leq$2 kGy), ethylene production and PAL activity are low after 5 days of storage at room temperature. According to the authors, these observations indicate that the tissue is able to overcome the initial radiation stress. In this study, PAL activity remains high in irradiated and unirradiated fruits during storage at 4 °C and during 49 days of storage. According to Macheix et al. (1990), temperature may be the regulator of PAL activity for stimulation or reduction of PAL activity. Faragher et al. (1983) observed that PAL activity is 2 times higher in apple stored at 10 °C as compared to that in apple stored at 24 °C.

Table 1 show the names and chemical structures of some important flavonoids (F and PMF) examined in this study. F, PMF, and pCA concentrations in irradiated and nonirradiated clementines during storage have been reported in Table 2. This table shows all flavonoid and *p*-coumaric acid contents corresponding to extracts from control and irradiated clementine peel, respectively, during 28 days of storage.

Flavanones (F). In clementine fruits, only flavanone rutinosides are found (hesperidin, narirutin, eriocitrin, and didymine) (Kanes et al., 1993; Mouly, 1995). The F concentrations from both samples were found to be very different (6−10 mg/g of MS). These results demonstrated that irradiation enhanced the synthesis of these F. In all cases, an improvement of these compounds was observed after 14 days of storage. During the first week of storage, the content of these compounds is significantly lower ($P \leq 0.05$) in irradiated samples. Didymine was the most affected F by irradiation. F concentrations are significantly higher in irradiated samples ($P \leq 0.05$).

Table 1. Citrus Flavonoids Examined in This Study

		substituent linked to flavone and flavanone skeleton						
flavonoid	abbreviation	3	5	6	7	8	3′	4′
eriocitrin	ERI		−OH		−O−Rt		−OH	−OH
narirutin	NAT		−OH		−O−Rt			−OH
hesperidin	HES		−OH		−O−Rt		−OH	−OMe
didymin	DID		−OH		−O−Rt			−OMe
scutelarein	SCU		−OMe	−OMe	−OMe			−OMe
isoscutelarein	ISCU		−OMe		−OMe	−OMe		−OMe
tangeretin	TAN		−OMe	−OMe	−OMe	−OMe		−OMe
sinensetin	SIN		−OMe	−OMe	−OMe		− OMe	−OMe
nobiletin	NOB		−OMe	−OMe	−OMe	−OMe	−OMe	−OMe
quercetogetin	QUE	−OMe	−OMe	−OMe	−OMe	−OMe		−OMe
heptamethoxyflavone	HEP	−OMe	−OMe	−OMe	−OMe	−OMe	−OMe	−OMe

Table 2. Summary of the Average Concentrations and Retention Times (t_r) of Four F, Seven PMF, and PCA Found in *C. clementina* Peel in Control Samples (C) and Samples Irradiated during Storage (I)

	t_r	0 days		7 days		14 days		21 days		28 days	
	(min)	C	I	C	I	C	I	C	I	C	I
F[a]											
ERI[c]	4.277	0.07 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.04 ± 0.01	0.15 ± 0.01	0.19 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
NAT	6.098	0.29 ± 0.02	0.22 ± 0.01	0.34 ± 0.05	0.023 ± 0.10	0.45 ± 0.01	0.33 ± 0.01	0.18 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.29 ± 0.01
HES	7.385	7.80 ± 0.10	6.06 ± 0.10	7.50 ± 0.10	6.00 ± 0.20	9.00 ± 0.13	8.79 ± 0.12	5.80 ± 0.10	8.09 ± 0.10	7.40 ± 0.10	8.47 ± 0.10
DID	13.064	0.09 ± 0.01	0.04 ± 0.01	0.11 ± 0.01	0.05 ± 0.01	0.11 ± 0.01	0.15 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
MF[b]											
SIN	23.429	1.44 ± 0.10	1.14 ± 0.01	1.10 ± 0.01	0.91 ± 0.02	0.80 ± 0.02	0.10 ± 0.01	0.76 ± 0.01	0.96 ± 0.01	0.83 ± 0.01	0.85 ± 0.01
ISCU	24.169	0.85 ± 0.03	0.60 ± 0.01	0.50 ± 0.01	0.44 ± 0.01	0.40 ± 0.03	0.47 ± 0.02	0.38 ± 0.01	0.54 ± 0.01	0.45 ± 0.01	0.48 ± 0.01
QUE	25.475	0.77 ± 0.01	0.57 ± 0.01	0.55 ± 0.01	0.51 ± 0.01	0.40 ± 0.01	0.60 ± 0.01	0.36 ± 0.01	0.60 ± 0.01	0.37 ± 0.01	0.45 ± 0.01
NOB	26.878	5.92 ± 0.12	4.70 ± 0.10	4.00 ± 0.13	3.82 ± 0.13	3.92 ± 0.10	4.80 ± 0.10	3.15 ± 0.10	4.06 ± 0.01	3.12 ± 0.01	4.08 ± 0.01
SCU	27.445	1.41 ± 0.10	1.21 ± 0.01	0.70 ± 0.10	0.64 ± 0.01	0.78 ± 0.01	1.07 ± 0.01	0.73 ± 0.01	0.78 ± 0.01	0.72 ± 0.01	0.85 ± 0.01
HEP	28.977	5.70 ± 0.15	4.57 ± 0.10	3.60 ± 0.13	4.45 ± 0.13	4.40 ± 0.11	5.80 ± 0.11	3.60 ± 0.10	4.00 ± 0.10	3.47 ± 0.10	3.8 ± 0.10
TAN	31.466	2.00 ± 0.10	1.71 ± 0.02	1.62 ± 0.02	1.56 ± 0.10	1.46 ± 0.12	2.15 ± 0.11	1.35 ± 0.10	1.32 ± 0.10	1.43 ± 0.10	1.36 ± 0.10
PCA[a]	3.71	0.80 ± 0.03	0.04 ± 0.01	0.86 ± 0.03	0.06 ± 0.01	1.17 ± 0.13	0.85 ± 0.03	0.04 ± 0.01	0.53 ± 0.01	0.04 ± 0.01	1.24 ± 0.01

[a] mg/g of DM. [b] mg /100 g of oils. [c] ERI, eriocitrin; NAT, narirutin; HES, hesperidin; DID, didymin; SIN, sinensetin; ISCU, isoscutelarein; QUE, quercetogetin; NOB, nobiletin; SCU, scutelarein; HEP, heptamethoxyflavone; TAN, tangeretin; PCA, *p*-coumarin.

γ-Irradiation stimulates biosynthesis of F after 14 days of storage. The differentiation observed in this study is in agreement with earlier works (Riov et al., 1972; Lacroix et al., 1990).

Polymethoxylated Flavones (PMF). Seven PMF are identified in citrus clementine peel oils corresponding to sinensetin, isoscutellarein, quercetogetin, nobiletin, scutelarein, tangeretin, and heptamethoxyflavone. The results demonstrated that all PMF are significantly higher ($P \leq 0.05$) in irradiated samples after 14 days of storage (Table 2). The content of total PMF in control samples is significantly lower than in irradiated samples ($P \leq 0.05$). In all cases an improvement of these compounds was observed between days 14 and 21. Nobiletin and heptamethoxyflavone were the compounds most affected by irradiation treatment. A degradation of ≈1 mg % was observed in these compounds as compared with 0.3 mg % in other PMF isolated in the fruits. These results are in agreement with earlier works (Sendra et al., 1988; Gaydou et al., 1987; Berahia, 1993). The differentiation observed is induced with long storage times. Lability of liaison 2−3 that induced a degradation of PMF in irradiated fruits during the first days of storage is well demonstrated in Figure 7. A good correlation was noticed between percent of degradation (log D) and totality of OCH_3 linked to the flavone skeleton. This result showed that degradation is an exponential function coupled to the number of methoxyl groups (OCH_3) with a correlation coefficient (C_r) of 0.83.

***p*-Coumaric Acid (PCA).** Irradiation stimulates biosynthesis of PCA in clementine peel. At day 1, the content of PCA in unirradiated samples was 0.8 mg g^{-1} pf dry weight as compared to 0.04 mg g^{-1} of dry weight in irradiated samples, which can be explained by degradation of PCA during irradiation treatment. However, in unirradiated samples and after 14 days of storage, the content of PCA was completely degraded, whereas in irradiated samples, rapid synthesis of this compound was observed. PCA is a precursor of coumarins: The double-bond must change from the trans to the cis configuration and the aromatic nucleus must undergo oxidation ortho to the side chain to give 2,4-dihydroxycinnamic acid. Riov et al. (1971, 1972) found that irradiation stimulates biosynthesis of the coumarins scopoletin and scopolin in grapefruit peel. Ben-Yehoshua et al. (1992) showed that UV treatment induced antifungal coumarins in relation to resistance of citrus fruit against pathogens. Arimoto et al. (1986) identified antifungal compounds in the peel of unin-

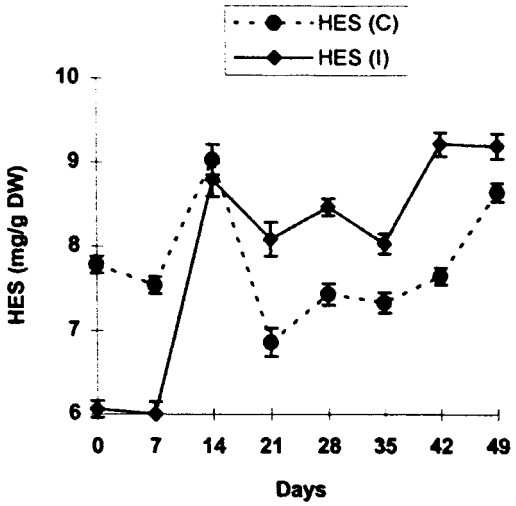

Figure 4. Effect of γ-irradiation on content of hesperidin in *C. clementina* peel during storage: c, control; i, irradiated.

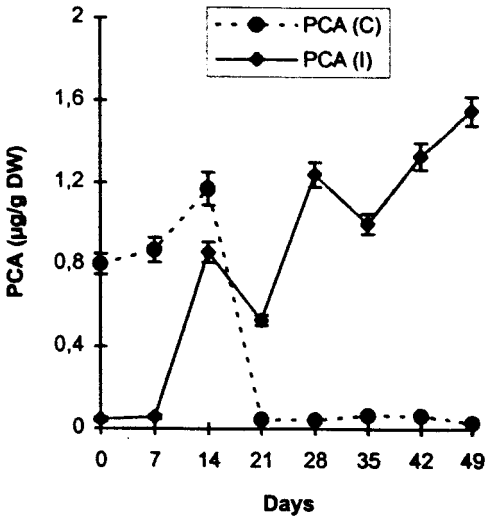

Figure 5. Effect of γ-irradiation on content of PCA in *C. clementina* peel during storage: c, control; i, irradiated.

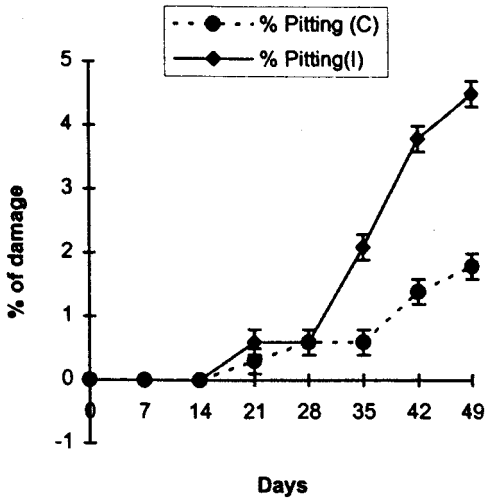

Figure 6. Peel injury (percent pitting) in *C. clementina* fruit during storage: c, control; i, irradiated.

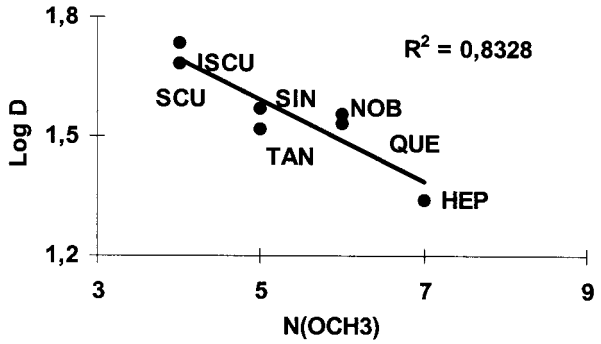

Figure 7. Relationship between PMF induced by irradiation and OCH_3 linked to flavone skeleton.

fected Satsuma mandarin as citrinol, naringin, and hesperidin.

As demonstrated previously, hesperidin (hesperetin 7-O-rutinoside) is the major phenolic compound in clementines (Oufedjikh et al., 1996). The content of this compound was significantly lower ($P \leq 0.05$) in unirradiated fruits during the first week of storage. A maximum was reached at day 14 for unirradiated and irradiated samples (Figure 4). However, after 14 days of storage, the content of this compound was significantly higher ($P \leq 0.05$) in irradiated samples. The content of hesperidin was relatively stable between days 14 and 35. However, after day 35, a significant improvement of this compound was observed ($P \leq 0.05$). Irradiation stimulates the biosynthesis of hesperidin after 14 days of storage corresponding to the maximum PAL activity reached at day 21.

Irradiation also stimulates the biosynthesis of PCA in clementines. At day 1, the content of PCA in unirradiated samples was 0.8 mg g^{-1} of dry weight as compared to 0.045 mg g^{-1} of dry weight in irradiated samples, which can be explained by degradation of PCA during irradiation treatment. However, in unirradiated samples, after 14 days of storage, the content of PCA was completely degraded, whereas in the irradiated samples, rapid synthesis of this compound was observed (Figure 5). At day 49, the content of PCA in treated samples was 1.5 mg g^{-1} of dry weight as compared to 0.03 mg g^{-1} of dry weight. Riov et al. (1971, 1972) also reported studies showing that irradiation stimulates the biosynthesis of coumarins (scopoletin and scopolin) in grapefruit peel.

Previous studies (Riov et al., 1972; Monselise and Kahan, 1968) have shown that *Citrus* fruits generally show characteristic pitting of peel after a few days when treated with doses ranging from 50 to 300 kGy. The percentage of fruit developing peel injury increases with higher storage temperatures and longer storage periods (Margana, 1977; Abdellaoui et al., 1995; Dennison and Ahmed, 1972). Previous investigations demonstrated a substantial level of antifungal activity in the flavedo of just-harvested *Citrus* fruit, indicating the presence of preformed antifungal materials (Ben-Yehoshua et al., 1987; Kim et al., 1991; Brown and Barmore, 1983). However, the nature of these substances was not studied. Arimoto et al. (1986) identified antifungal compounds in the peel of uninfected Satsuma mandarin as citrinol, naringin, and hesperidin. Ben-Yehoshua et al. (1987) isolated from flavedo tissues of grapefruit several coumarin-derived preformed antifungal materials: osthol (7-methoxy-8-prenylcoumarin), auraptene (2,3-epoxy-7-methoxy-8-prenylcoumarin), 7-[(6,7-epoxy-3,7-dimethyl-2-octyl)oxy]coumarin, and 7-geranoxycoumarin.

Damage induced by irradiation doses is reported in Figure 6. Similar types of damage have been also seen with other *Citrus* species. However, in our study the percentage of losses remained negligible (1−5%) in comparison with other works in which damage exceeded 20% during the first days of storage (Riov et al., 1972; Abdellaoui et al., 1995; Jobin et al., 1992). At 3 °C, the damage appearred 21−49 days after the irradiation was applied. The extent of damage depended on the state of maturity, or on the color of the flavedo. Damage was also dependent on the dose of irradiation and the temperature of storage. Recently Stevens et al. (1991) and Droby et al. (1991) described certain reduction of green mold decay in UV-treated *Citrus* fruits accompanied with PAL enzyme activation. This enzyme plays an important role in the defensive reactions of plants, including phytoalexin induction and cell-wall reinforcement (Ben-Yehoshua et al., 1992).

Conclusion. The present study suggests that γ-irradiation at the level of irradiation used causes also some physiological and biochemical changes in the fruits and seems to be able to stimulate PAL activity and the synthesis of phenolic compounds, which are important

in extending storage periods. The content of hesperidin and *p*-coumaric acid was significantly ($P \leq 0.05$) higher in irradiated samples after day 14. The improvement of the content of these compounds is also related to an activation of PAL enzyme in irradiated fruits observed after 14 days of storage. Application of γ-irradiation at 0.3 kGy combined with lower temperature is an important development encouraging the possibility of reducing exogenous fungicide residues and may induce the fruit to build its own resistance against pathogens. However, damage is a serious factor that may hinder possible commercial use.

Our work is in progress to separate coumarin compounds of irradiated *Citrus clementina* and to identify the presence of antifungal compounds. Further research aims at optimization of fruit treatment and storage conditions that would enhance positive γ-ray effects and diminish undesirables ones.

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