UDC 577.152.193:634.31
ISSN 1330-9862
(FTB-1366)

preliminary communication

Changes in Peroxidase Activity in the Peel of Unshiu Mandarin (*Citrus unshiu* Marc.) Fruit with Different Storage Treatments

Hrvoje Lepeduš[1], Marko Jozić[1], Ivna Štolfa[1], Nikola Pavičić[2], Branimir K. Hackenberger[1] and Vera Cesar[1]*

[1]Department of Biology, University of J. J. Strossmayer, L. Jägera 9, HR-31000 Osijek, Croatia

[2]Department of Pomology, Faculty of Agriculture, University of Zagreb, Svetošimunska 25, HR-10000 Zagreb, Croatia

Received: August 12, 2004
Accepted: February 28, 2005

Summary

The Unshiu mandarin (*Citrus unshiu* Marc.) is the major *Citrus* crop in Croatia. Limiting factors for longer consumption of Unshiu mandarin are low storage performance and the appearance of chilling injuries during storage. Previous studies indicated that oxidative stress might be involved in cold-induced peel damage of harvested *Citrus* fruit. The aim of the present study was to investigate peroxidase distribution, isoenzyme pattern and activity in the peel of Unshiu mandarin fruit. Special goal of our study was to investigate the changes of peroxidase activity in respect to two different hot water dipping (HWD) treatments (3 min at 48 and 52 °C) and two different storage temperatures (1 and 3 °C) combined. Peroxidase activity was detected at the border of oil glands, in the peel surface and in the conducting elements positioned in the inner part of the peel. Electrophoretic analysis revealed the presence of two peroxidase isoenzymes. There were no differences in the electrophoretic pattern after the HWD treatments and cold storage. Lowering of both total and specific peroxidase activity was measured in HWD-treated samples in comparison with the control ones. However, it appeared that significant decrease in total peroxidase activity was influenced by the storage temperatures, while the increase in total soluble protein content was influenced by the HWD pretreatment.

Key words: Citrus unshiu fruit, hot water dip, peroxidase, postharvest cold storage

Introduction

The fruits of different *Citrus* species have a great importance in human nutrition. Besides well known nutritional values, their antioxidative properties also attract attention. The major *Citrus* crop in Croatia is Unshiu mandarin (*Citrus unshiu* Marc.), with the production of approx. 40 000 tons per year. Like many other *Citrus* species, limiting factors for longer consumption of Unshiu mandarin are low storage performance and the appearance of chilling injuries during cold storage. Further, many *Citrus* fruits are susceptible to various biotic stresses, *e.g.* green mold infection as the major postharvest disease. In order to avoid the damage symptoms caused by chilling as well as by fungus infection, different treatments have been applied. These treatments usually involve short-time dipping of the fruits into hot water (HWD). As reported by Porat *et al.* (*1*) such postharvest heat treatments are preferable, since they effectively induce tolerance to cold temperature without impairing

* Corresponding author; E-mail: hlepedus@yahoo.com

any other postharvest qualities. Changes of various biochemical parameters during heat treatments and cold storage have been investigated. It was shown that HWD treatment increased polyamine content in flavedo tissues (outer coloured part of the peel) of late-ripening mandarin (*Citrus reticulata* Blanco) and reduced chilling injury symptoms (*2*). Holland *et al.* (*3*) investigated carbohydrate content changes in flavedo tissue of Fortune mandarin (*C. clementina* hort. ex Tanaka × *C. reticulata* Blanco) after the heat induced chilling tolerance treatment (3 days at 37 °C). It appeared that this high-temperature conditioning treatment avoided the decline in sucrose, but not in glucose and fructose content, occurring during the fruit storage at 2 °C. Investigation of several *Citrus* species and cultivars (*4*) revealed that cold storage of minimally processed segments and juices decreased the ascorbic acid content, which reduced the antioxidant capacity.

Chilling injury of plant cells was supposed to proceed primarily in the biomembranes. Lowering the temperature close to 0 °C tends to decrease the membrane fluidity. Higher portion of unsaturated lipids, which was found in biomembranes of chilling-resistant plants in respect to chilling-sensitive ones, is considered to be the main factor that allows the biomembranes to maintain their function at chilling temperatures (*5*). Besides the changes of biomembrane fluidity, chilling stress was shown to generate the reactive oxygen species (ROS). One of the usually produced ROS is hydrogen peroxide, which can have deleterious effects on plant cells, especially due to biomembrane lipid peroxidation (*6*). The enzymatic scavenging of hydrogen peroxide includes several more or less specific enzymes that can convert ROS to water or maintain the pool of antioxidants in their reduced state (*7*). Most of peroxidases are haem-containing glycoproteins that catalyse the oxidoreduction between hydrogen peroxide and reductants (*8,9*). They usually use various phenolic substrates for H_2O_2 elimination, which makes them very useful as the general indicators for oxidative stress.

Investigations on the activity of some antioxidative enzymes in the flavedo of mandarin fruits (*10,11*) indicated that the oxidative stress might be involved in the chilling injury. Recent study done by Sánchez-Ballesta *et al.* (*12*) showed that some genes encoding stress-responsive proteins involved in oxidative damage are altered by heat conditioning of mandarin fruit. The aim of the present study was to investigate peroxidase distribution, isoenzyme pattern and activity in the peel of Unshiu mandarin fruit. Special goal of our study was to investigate the changes of peroxidase activity in respect to two different HWD treatments (48 and 52 °C) and two different storage temperatures (1 and 3 °C) combined. We wanted to discriminate which combination of HWD and cold storage would influence the peroxidase activity to a greater extent.

Materials and Methods

Plant material and treatment descriptions

The materials for study were intact fruits of Unshiu mandarin (*Citrus unshiu* Marc. cv. 'Saigon'). Fruit were harvested on 10 December 2003. Treatments involved dipping the fruits into hot water (HWD) for 3 min, at the temperatures of 48 or 52 °C. The HWD untreated fruits were used as control. Next, the fruits were stored for 8 weeks at the temperatures of 1 or 3 °C. After cold storage, the fruits were placed at the room temperature (18–20 °C) for 7 days (shelf life). Samples were designated as follows: C1 – control samples stored at 1 °C, C3 – control samples stored at 3 °C, S48-1 – samples that were HWD-treated at 48 °C and then stored at 1 °C, S52-1 – samples that were HWD-treated at 52 °C and then stored at 1 °C, S48-3 – samples that were HWD--treated at 48 °C and then stored at 3 °C and S52-3 – samples that were HWD-treated at 52 °C and then stored at 3 °C.

In situ localisation of peroxidase activity

In situ localisation of peroxidase activity was done by immersing tissue pieces in the reaction mixture containing 0.05 % 4-chloro-1-naphtol and 0.01 % H_2O_2 in phosphate buffer (pH=7.5) or 5 mM guaiacol and 5 mM H_2O_2 in phosphate buffer (pH=5.8). The sites of peroxidase activity were detected due to the appearance of dark-blue colouration in the case of 4-chloro-1-naphtol and brown colouration when guaiacol was used as the substrate. Localisation of the conducting elements in the peel was done on the semi-thick tissue sections. Plant material was fixed in 0.05 M phosphate buffer containing 6 % of glutaraldehyde (pH=6.8) at 4 °C. Dehydration was done in 2-methoxyethanol, ethanol, *n*-propanol and *n*-butanol (two changes in each). Then, specimens were embedded in methacrylate resin (Historesin, Leica) according to manufacturer instructions. Sections of 3 μm thickness were obtained using the Leica RM 2155 rotary microtome with a glass knife. Tissue sections were stained with Toluidine blue O in benzoate buffer (pH=4.4) (*13*).

Soluble protein extraction and tissue dry weight determination

Five fruits from each sample category were used for protein extraction. Each fruit was processed separately. Prior to the extraction of soluble proteins the peel was cut into small pieces and macerated in liquid nitrogen. One part of the obtained fine powder was used for the extraction of proteins and the other for the determination of dry weight. The extraction was done in ice-cold 0.1 M Tris-HCl buffer, pH=8.0, with the addition of polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 20 000 x *g* and 4 °C, for 20 min. The obtained supernatant was used for protein quantification, peroxidase activity determination as well as for electrophoretic analysis. The amount of the tissue dry weight was determined by drying on 105 °C for 24 h.

Gel electrophoresis

Isoperoxidases were separated under denaturing conditions by means of discontinuous vertical polyacrylamide gel electrophoresis (PAGE). For PAGE the 1-mm stacking (2.5 %) and separating (10 %) polyacrylamide gels were prepared, according to the procedure of Laemmli (*14*). The protein samples were prepared as described by Hou *et al.* (*15*). A volume of 100 μL of protein sample was mixed with 25 μL of 60 mM Tris buffer

(pH=6.8), containing SDS 2 %, glycerol 25 %, 14 mM 2-mercaptoethanol and bromophenol blue 0.1 % and then incubated overnight at the room temperature. The electrophoresis was carried out at 4 °C for 5 h (2 h at 20 mA of constant current followed by 3 h at 150 V of constant voltage). Prior to the peroxidase detection, gel was renaturated. The removal of SDS from the gel was carried out by incubating it twice in 10 mM Tris buffer containing isopropanol 25 % (pH=7.9) for 10 min (*15*). Visualisation of the peroxidase isoenzymes was done by immersing the gel in the reaction mixtures containing either guaiacol or 4-chloro-1-naphtol as the substrates.

Guaiacol peroxidase assay and soluble protein content determination

The activity of guaiacol peroxidases in supernatant was determined spectrophotometrically by measuring the absorbance increase at 470 nm. Reaction mixture contained 5 mM guaiacol and 5 mM H_2O_2 in 0.2 M phosphate buffer, pH=5.8 (*16*). The enzymatic reaction was started by adding 200 μL of the extract to 800 μL of reaction mixture. The protein content of the extracts was determined according to Bradford (*17*), using bovine albumin serum (BSA) as a standard.

Statistical analysis

A factorial analysis of variance was performed to analyse statistical significance, followed by Duncan's test. P-values <0.05 were considered to be significant.

Results and Discussion

In situ localisation of peroxidase activity

The *in situ* localisation of peroxidase activity in the peel of Unshiu mandarin fruit was performed on fresh tissue. Results are shown in Fig. 1. All investigated samples revealed the same distribution of peroxidase activity within the tissue, independently of the treatment type. A dominant feature of the mandarin peel were numerous oil glands positioned near the fruit surface (Fig. 1A). Such glands are formed as the lysigenous spaces by dissolution of the cells. Oil substances are released into the lysigenous secretory cavity upon the breakdown of the cells (*18*). The majority of peroxidase activity was detected at the border of oil cavities and in the peel surface (Fig. 1B). Small areas of the peroxidase activity were detected in the inner parts of the peel (Fig. 2A). Such areas coincided with the localisation of the conducting elements (Fig. 2B) and are most probably involved in lignifications of the cell walls. Releasing of the oil from the gland was known to have phytotoxic effect on the peel tissues. As reported by Knight *et al*. (*19*) oil application on the surface of the Washington navel orange (*C. sinensis* L. Osbeck) caused degeneration of the content of peel cells as well as the loss of biomembrane integrity. The same authors also discussed the possibilities of the prevention of oil releasing laterally from the gland to the surrounding cortical tissue. They believed that flattened layers of thick-walled boundary cells that enclose the gland might have protective function against *in situ* tissue damage. Localisation of the peroxidase activity that we have observed (Fig. 1B) corresponds to the position of the thick-walled cells surrounding the gland. Taking this into consideration, as well as the fact that the peroxidases are able to oxidise various phenolic sub-

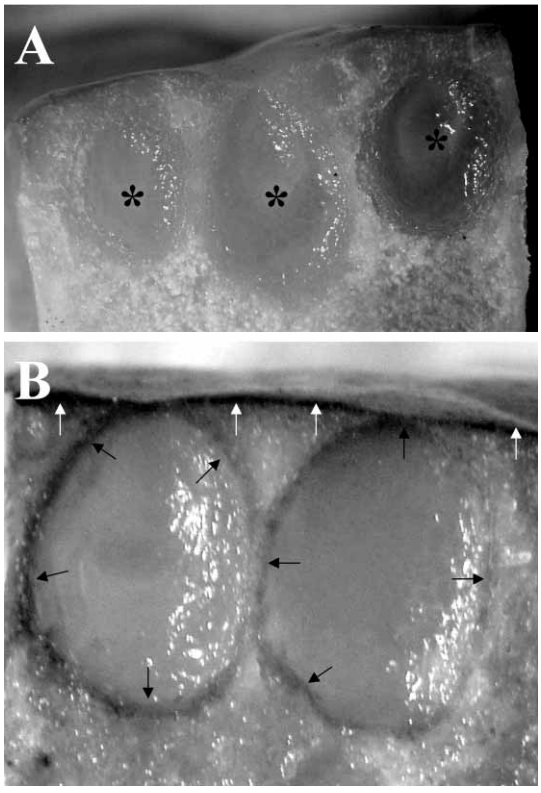

Fig. 1. Localisation of peroxidase activity in the outer part of the Unshiu mandarin (*Citrus unshiu* Marc. cv. 'Saigon') peel of samples C1. The same distribution of the peroxidase activity was detected in all other investigated samples. (**A**) Unstained tissue with numerous oil glands (asterisks) positioned near the fruit surface. (**B**) Tissue stained with 4-chloro-1-naphtol. Peroxidase activity was detected at the border of oil glands (black arrows) and in the peel surface (white arrows). The same distribution of the peroxidase activity was observed when guaiacol was used as the substrate (picture not shown)

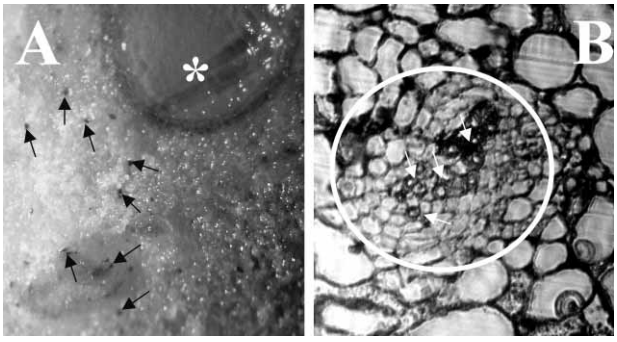

Fig. 2. Localisation of peroxidase activity and conducting elements in the inner part of the Unshiu mandarin (*Citrus unshiu* Marc. cv. 'Saigon') peel of samples C1. The same distribution of the peroxidase activity was detected in all other investigated samples. (**A**) Peroxidase activity detected with 4-chloro-1-naphtol as dotted spots (black arrows) below the oil gland (asterisk). (**B**) Conducting tissue in the inner part of the peel (encircled area) with the lignified xylem cells (white arrows) coincided with the localisation of the peroxidase activity

strates (*8,9*), we believe that besides mechanical barrier, these cells should be considered as the sites of very intensive detoxification processes.

Electrophoretic pattern of isoperoxidase enzymes

Electrophoretic analysis showed the presence of two peroxidase isoenzymes in the peel of Unshiu mandarin (Fig. 3). The higher molecular weight isoperoxidase appeared as a wider activity zone in the gel when compared to the lower molecular weight isoenzyme. There were no differences in the isoperoxidase electrophoretic pattern concerning different HWD treatments nor between different storage temperatures. Also, incubation of the gels containing the same set of samples with two different substrates, 4-chloro-1-naphtol and guaiacol, revealed that both isoenzymes reacted with both substrates.

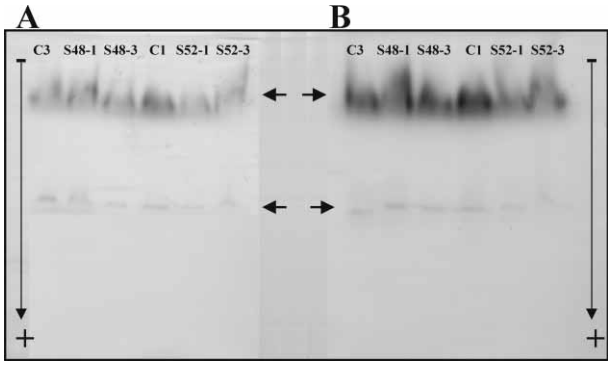

Fig. 3. The electrophoretic pattern of peroxidase isoenzymes from the Unshiu mandarin (*Citrus unshiu* Marc. cv. 'Saigon') peel of the investigated samples, detected with 4-chloro-1-naphtol (**A**) and guaiacol (**B**). The positions of two peroxidase isoenzymes are designated by arrows

Peroxidase activity

Changes in guaiacol peroxidase activity are shown in Fig. 4. The mean value of total guaiacol peroxidase activity was highest in the HWD untreated control fruits stored at 1 °C (sample designated as C1) (0.014 ΔA_{470} min^{-1} g^{-1}) (Fig. 4A). Lowering of the enzyme total activity was recorded in the samples stored at the same temperature but differently HWD-treated. Both samples (S48-1 and S52-1) had the same mean values of total activity (0.012 ΔA_{470} min^{-1} g^{-1}) (Fig. 4A). Mean values of the total peroxidase activity were generally lower in the samples stored at 3 than at 1 °C (Fig. 4A): 0.009 in the control sample (C-3), 0.008 in S48-3 and 0.007 ΔA_{470} min^{-1} g^{-1} in S52-3. Factorial analysis of variance (Table 1) of the obtained data revealed that HWD pre-treatment had no effect on the values of total peroxidase activity. On the other hand the storage temperature was shown to have significant effect on the total peroxidase activity in the investigated samples (Table 1). Mean separation done by Duncan's multiple range test (Table 2) revealed that differences between control samples stored at 1 and 3 °C were significant, while the differences between HWD pretreated samples and control (HWD untreated) ones were not significant. This confirmed that a change

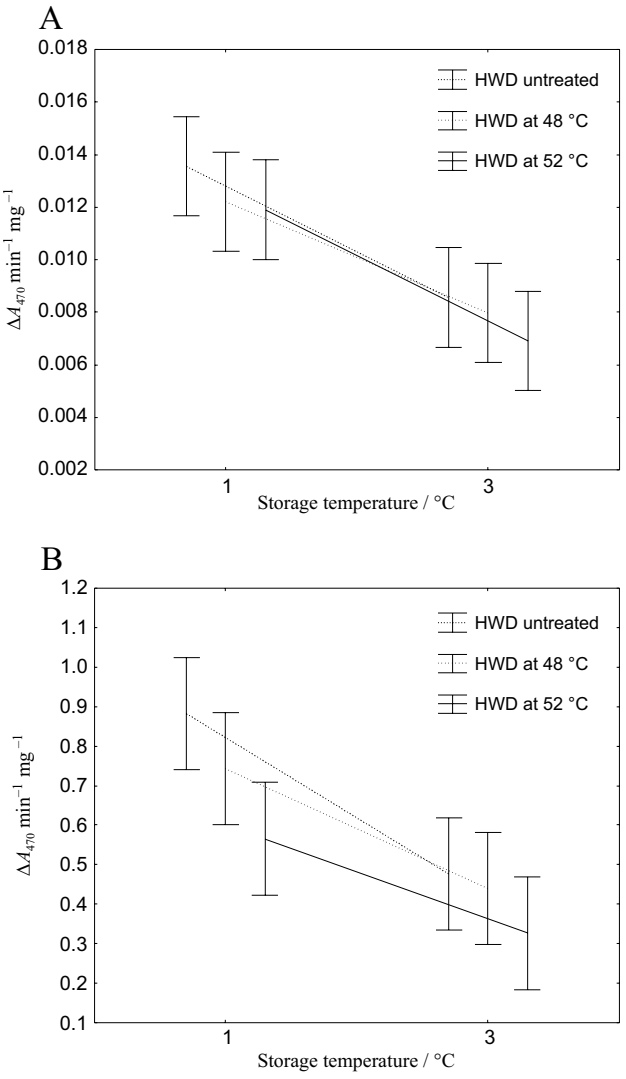

Fig. 4. The mean values of peroxidase activity in the Unshiu mandarin (*Citrus unshiu* Marc. cv. 'Saigon') peel of investigated samples. (**A**) Peroxidase total activity (ΔA_{470} min^{-1}g^{-1}). (**B**) Peroxidase specific activity (ΔA_{470} min^{-1}mg^{-1}). Bars represent 0.95 CI (confidence interval)

in total peroxidase activity was influenced by storage temperature but not by HWD pretreatments.

The mean values for specific activity of guaiacol peroxidases in the investigated samples are shown in Fig. 4B. The highest activity (0.883 ΔA_{470} min^{-1} mg^{-1}) was measured in the HWD untreated control fruits stored at 1 °C (C1). Mean values of specific activity in HWD-treated samples stored at 1 °C (S48-1 and S52-1) decreased in comparison with the control (0.774 in S48-1 and 0.565 ΔA_{470} min^{-1} mg^{-1} in S52-1). Specific peroxidase activity revealed similar changes in the samples stored at 3 °C (Fig. 4B). Mean values in HWD-treated samples (0.439 in S48-3 and 0.326 ΔA_{470} min^{-1} mg^{-1} in S52-3) were lower than in the control (C3) sample (0.476 ΔA_{470} min^{-1} mg^{-1}). Factorial analysis of variance (Table 1) showed that both parameters (HWD pre-treatment and storage temperature) had strong significant effect on the peroxidase specific activity in the investigated samples. However, the mean separation analysis revealed that HWD pre-treat-

Table 1. Factorial analysis of variance for total and specific peroxidase activities as well as for the content of soluble proteins in the peel of Unshiu mandarin (*Citrus unshiu* Marc. cv. 'Saigon') fruits

Source of variation	F-value	P-value
Peroxidase total activity		
HWD	1.96	0.11
Storage temperature	60.48	$1.9 \cdot 10^{-6}$
Peroxidase specific activity		
HWD	2.61	0.04
Storage temperature	43.04	$1.2 \cdot 10^{-5}$
Soluble proteins		
HWD	3.43	0.01
Storage temperature	1.37	0.26

ment significantly influenced the specific activity only in the sample S52-1 but not in the samples S52-3, S48-1 and S48-3 in respect to appropriate HWD untreated control samples (C1 or C3) (Table 2). Significance was also detected for both investigated parameters (total and specific peroxidase activity) when HWD untreated control samples stored at different temperatures (C1 and C3) were compared (Table 2).

Table 2. Duncan's multiple range test for total and specific peroxidase activities as well as for soluble protein content in the peel of Unshiu mandarin (*Citrus unshiu* Marc. cv. 'Saigon') fruits

Investigated samples	Peroxidase total activity	Peroxidase specific activity	Soluble proteins
C1 *vs* S48-1	ns	ns	ns
C1 *vs* S52-1	ns	0.005	0.015
C3 *vs* S48-3	ns	ns	ns
C3 *vs* S52-3	ns	ns	ns
C1 *vs* C3	0.001	0.001	ns

ns – not significant

According to Schirra and Cohen (*20*) intermittent warming during storage of Olinda oranges (*Citrus sinensis* L. Obsek), with cycles of 3 weeks at 3 °C followed by 2 weeks at 15 °C, delayed chilling injury development by approximately 10 weeks with respect to storage at 3 °C. This could be correlated with the temperature-dependent changes of antioxidative enzyme activity. The indication that oxidative stress might be involved in cold-induced peel damage of harvested *Citrus* fruit aroused from the results given by Sala (*10*). He reported that the activities of catalase, ascorbate peroxidase and glutathione reductase at low temperature (2.5 °C) were higher in chilling-tolerant than in chilling-sensitive cultivars. It was also shown that HWD (53 °C, 3 min) significantly induced catalase but not ascorbate peroxidase and glutathione reductase activity in fruits of Fortune mandarin (*11*). Further, catalase activity induced by heating rapidly declined when fruits were removed to cold storage. The decrease in guaiacol peroxidase activity in cold-stored HWD-treated fruits that we have observed in our investigation (Figs. 4 and 5) is going along very well with that for catalase activity given by Sala and Lafuente (*11*).

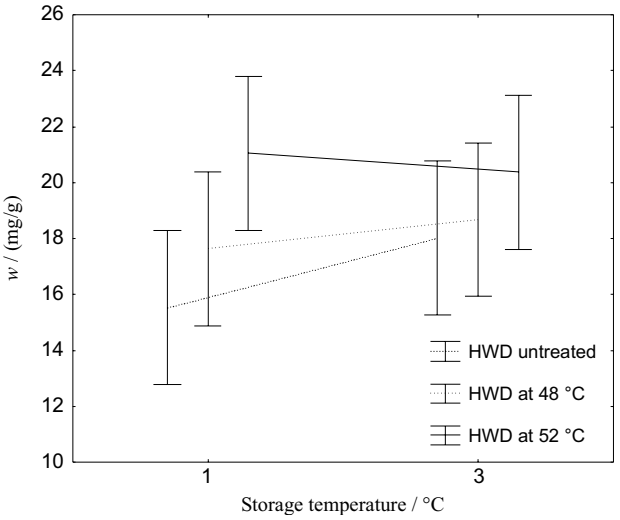

Fig. 5. Total soluble proteins content (mg/g) in the Unshiu mandarin (*Citrus unshiu* Marc. cv. 'Saigon') peel of the investigated samples. Bars represent 0.95 CI (confidence interval)

Soluble protein content

As shown in Fig. 4, greater differences between control and HWD-treated samples were observed in specific activity rather than in total activity of guaiacol peroxidases. This was due to the increased total soluble protein content in HWD-treated samples in comparison with the control ones (Fig. 5). Mean values of the total soluble protein content in the samples stored at 1 °C were: 15.528, 17.633, 21.043 mg/g in C1, S48-1 and S52-1, respectively. In the samples stored at 3 °C the mean values of total soluble proteins were: 18.010, 18.676, 20.366 mg/g in C3, S48-3 and S52-3, respectively. Factorial analysis of variance (Table 1) showed that the concentration of total soluble proteins was significantly influenced by the HWD pretreatments but not by the storage temperature. Mean separation done by Duncan's multiple range test (Table 2) revealed significance only for the sample S52-1 but not for the samples S52-3, S48-1 and S48-3 in respect to the corresponding HWD untreated control samples (C1 or C3). Also, there was no significant difference in total soluble protein content between C1 and C3 samples (Table 2). This confirmed that a change in total soluble proteins was influenced by HWD pretreatments but not by storage temperature. The observed increase of total soluble protein content was expected upon HWD treatments and cold storage. The acclimation of the plant cells on both, high and low temperature stress was achieved through the biosynthesis of the specific sets of proteins (*21,22*). As reviewed by Fink (*23*), cold acclimated plants accumulate such proteins in the epidermis and cell walls that surround the intercellular spaces, while heat-shock proteins accumulate in nuclei, chloroplasts, mitochondria and plasma membranes.

Conclusions

Our study showed that combining the usual postharvest treatments such as HWD petreatment and cold storage influenced the peroxidase activity and the content of total soluble proteins in the peel of Unshiu mandarin in respect to the untreated control samples. Factorial analysis of variance confirmed that the decrease in peroxidase total activity was influenced by the storage temperatures, while the increase in total soluble protein content was influenced by the HWD pretreatment. This is reflected on the peroxidase specific activity, where weak interaction between these two factors was observed (Fig. 4B). However, since no visual symptoms of chilling injury were detected in the investigated fruits, unlike in the previous seasons (unpublished results), we were not able to determine the peroxidase activity and isoenzyme pattern of the injured fruits. This remains to be done in the following seasons. Also, further investigations should include the analysis of freshly harvested fruits as well as the changes of peroxidase activity and protein content in the samples exposed to intermittent warming cycles during storage.

References

1. R. Porat, D. Pavoncello, J. Peretz, S. Ben-Yehoshua, S. Lurie, Effects of various heat treatments on the induction of cold tolerance and on the postharvest qualities of 'Star Ruby' grapefruit, *Postharvest Biol. Technol. 18* (2000) 159–165.
2. G.A. Gonzalez-Aguilar, L. Zacarias, M. Mulas, M.T. Lafuente, Temperature and duration of water dips influence chilling injury, decay and polyamine content in 'Fortune' mandarins, *Postharvest Biol. Technol. 12* (1997) 61–69.
3. N. Holland, H.C. Menezes, M.T. Lafuente, Carbohydrates as related to the heat-induced chilling tolerance and respiratory rate of 'Fortune' mandarin fruit harvested at different maturity stages, *Postharvest Biol. Technol. 25* (2002) 181– 191.
4. A. Del Caro, A. Piga, V. Vacca, M. Agabbio, Changes of flavonoids, vitamin C and antioxidant capacity in minimally processed citrus segments and juices during storage, *Food Chem. 84* (2004) 99–105.
5. L. Taiz, E. Zeiger: *Plant Physiology*, Sinauer Associates, Sunderland, Massachusetts (1998).
6. E. Kuzniak, H. Urbanek, The involvement of hydrogen peroxide in plant responses to stresses, *Acta Physiol. Plant. 22* (2000) 195–203.
7. A. Polle: Defence Against Photooxidative Damage in Plants. In: *Oxidative Stress and the Molecular Biology of Antioxidant Defences*, J.G. Scandalios (Ed.), Cold Spring Harbor Laboratory Press, New York (1997) pp. 623–666.
8. T. Gaspar, C. Penel, D. Hagege, H. Greppin: Peroxidases in Plant Growth: Differentiation and Development Processes. In: *Biochemical, Molecular and Physiological Aspects of Plant Peroxidases*, J. Lobarzewski, H. Greppin, C. Penel, T. Gaspar (Eds.), Lublin (1991) pp. 249–280.
9. S. Hiraga, K. Sasaki, H. Ito, Y. Ohashi, H. Matsui, A large family of class III plant peroxidases, *Plant Cell Physiol. 42* (2001) 462–468.
10. J.M. Sala, Involvement of oxidative stress in chilling injury in cold-stored mandarin fruits, *Postharvest Biol. Technol. 13* (1998) 255–261.
11. J.M. Sala, M.T. Lafuente, Catalase enzyme activity is related to tolerance of mandarin fruits to chilling, *Postharvest Biol. Technol. 20* (2000) 81–89.
12. M.T. Sánchez- Ballesta, Y. Lluch, M.J. Gosalbes, L. Zacarías, A. Granell, M.T. Lafuente, A survey of genes diferentially expressed during long-term heat-induced chilling tolerance in citrus fruit, *Planta, 218* (2003) 65–70.
13. N. Feder, T.P. O'Brien, Plant microtechnique: Some principles and new methods, *Am. J. Bot. 55* (1968) 123–142.
14. U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature, 227* (1970) 680–685.
15. W.-C. Hou, H.-J. Chen, C.-F. Chang, Y.-H. Lin, Purification and properties of fatty acid esterases from yam (*Dioscorea batatas* Decne) tuber, *Bot. Bull. Acad. Sinica. 40* (1999) 305–310.
16. B.Z. Siegel, W. Galston, The peroxidase of *Pisum sativum*, *Physiol. Plant. 42* (1967) 212–226.
17. M.M. Bradford, A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding, *Anal. Biochem. 72* (1976) 248–254.
18. K. Esau: *Anatomy of Seed Plants*, John Wiley & Sons, New York (1977).
19. T.G. Knight, A. Klieber, M. Sedgley, Structural basis of rind disorder oleocellosis in Washington navel orange (*Citrus sinensis* L. Osbeck), *Ann. Bot. 90* (2002) 765–773.
20. M. Schirra, E. Cohen, Long-term storage of 'Olinda' oranges under chilling and intermittent warming temperatures, *Postharvest Biol. Technol. 16* (1999) 63–69.
21. E.T. Palva: Gene Expression Under Low Temperature Stress. In: *Stress-Induced Gene Expression in Plants*, S. Basra (Ed.), Harwood Academic Publishers GmbH, Chur (1993) pp. 103–130.
22. M.A. O'Connel: Heat Shock Proteins and Thermotolerance. In: *Stress-Induced Gene Expression in Plants*, S. Basra (Ed.), Harwood Academic Publishers GmbH, Chur (1993) pp. 163–183.
23. S. Fink: *Pathological and Regenerative Plant Anatomy*, Gebrüder Borntraeger, Berlin (1999).

Promjene aktivnosti peroksidaza u kori ploda mandarine Unshiu (*Citrus unshiu* Marc.) pri različitim uvjetima skladištenja

Sažetak

Mandarina sorte Unshiu (*Citrus unshiu* Marc.) glavna je sadnica agruma u Hrvatskoj. Ograničavajući su faktori duže upotrebe te sorte kratka izdržljivost pri skladištenju i po-

java smrzotina tijekom skladištenja. Prijašnja istraživanja pokazala su da bi oksidativni stres mogao utjecati na pojavu smrzotina na kori ubranih plodova agruma. Svrha je ovog istraživanja bila istražiti distribuciju peroksidaza, vrstu i aktivnost izoenzima u kori sorte Unishiu. Poseban je cilj bio istražiti promjene aktivnosti peroksidaza u uvjetima postignutim kombiniranjem umakanja u vruću vodu (HWD) temperatura od 48 ili 52 °C (3 min) i skladištenjem na dvjema različitim temperaturama (1 i 3 °C). Aktivnost peroksidaza ustanovljena je na granici između uljnih stanica i okolnog tkiva, u površinskom sloju kore i u provodnim elementima koji se nalaze u unutrašnjem dijelu kore. Elektroforetska analiza otkrila je prisutnost dvaju izoenzima peroksidaze. Bez obzira na kombinacije prethodne obrade HWD-om i različitih temperatura skladištenja nisu uočene razlike u strukturi elektroforetskog prikaza pri elektroforetskom mjerenju. Izmjereno je snizivanje kako ukupne tako i specifične aktivnosti peroksidaza uzoraka prethodno obrađenih HWD-om u usporedbi s kontrolnim uzorcima. Pokazalo se, međutim, da je značajno snizivanje ukupne aktivnosti peroksidaza uzrokovano temperaturom skladištenja, a povećanje koncentracije proteina HWD-om.