Food Chemistry 110 (2008) 891–896

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Changes in fatty acid composition and electrolyte leakage of 'Hayward' kiwifruit during storage at different temperatures

Maria Dulce C. Antunes [a,*], Evangellos M. Sfakiotakis [b]

[a] Universidade do Algarve, CDCTPV/FERN, Campus de Gambelas, 8005-139 FARO, Portugal
[b] Laboratory of Pomology, Aristotle University, GR 540 06 Thessaloniki, Greece

ARTICLE INFO

Article history:
Received 19 October 2007
Received in revised form 17 December 2007
Accepted 27 February 2008

Keywords:
Kiwifruit
Temperature
Postharvest
Electrolyte leakage
Fatty acids

ABSTRACT

Exposure to low storage temperature induces changes in electrolyte leakage and fatty acids composition, in a way depending on the plant tissue. Those changes alter the response of the fruit to storage conditions. The influence of storage temperature on ripening, fatty acids composition and electrolyte leakage of 'Hayward' kiwifruit were investigated. Harvested fruit were stored at 0, 5, 10, 15 and 20 °C for 5, 12 and 17 days. Measurements of SSC, firmness, flesh colour, fatty acid composition and electrolyte leakage were performed during the experiment. Kiwifruit did not fully ripen during the 17 days storage at any temperature. The major fatty acid component in 'Hayward' kiwifruit consisted of linolenic, followed by oleic, palmitic, linoleic and stearic acid. Membrane permeability and unsaturated/saturated fatty acid ratio increased during storage in all treatments. The highest increase was during the first 5 days and at the lowest temperatures. The increase in unsaturated/saturated fatty acid ratio was caused mainly by a decrease in palmitic and an increase in oleic acids. Stearic, linoleic and linolenic acids had insignificant changes during storage. The main increase in electrolyte leakage and unsaturated/saturated fatty acid ratio occurred during the first storage days and at lower temperatures, probably as a response of the tissue to an adaptation to the new stress storage conditions.

1. Introduction

Temperature plays a key role in the metabolism of fruits and vegetables (Marangoni, Palma, & Stanley, 1996). Stresses such as chilling injury lead to membrane degradation, such as hydrolysis of membrane phospholipids to constituent free fatty acids, and peroxidation of constituent polyunsaturated fatty acids with a corresponding production of free radicals (Palma, Marangoni, & Stanley, 1995; Todd, Paliyath, & Thompson, 1992). These free radicals catalyse further reactions which induce autocatalytic ethylene production, ripening and senescence (Marangoni et al., 1996; Pooter & Schamp, 1989). Antunes and Sfakiotakis (2002) reported that exposing kiwifruit to chilling temperatures (0–10 °C) for 12 days advanced ethylene biosynthesis and ripening upon rewarming of the fruit compared to fruit held at 20 °C.

Current knowledge strongly suggests that membranes of chilling sensitive produce undergo alterations in biophysical properties related to their composition that can alter functionality, while chilling tolerant membranes maintain their liquid-crystalline state (Marangoni et al., 1996). Changes in membrane structure and composition are considered as the primary events of chilling injury and lead to a loss of permeability control and metabolic dysfunction (Marangoni et al., 1996; Valdenegro, Flores, Romojaro, Ramirez, & Martinez-Madrid, 2005). The composition of constituent membrane lipids can affect the fluidity of membranes, with unsaturated fatty acid containing lipids being more fluid than saturated lipids (Marangoni et al., 1996).

A common feature accompanying senescence is increased membrane permeability, expressed as increasing leakage of ions which is associated to chilling of sensitive tissue and/or senescence (Marangoni et al., 1996; Saltveit, 2002). Electrolyte leakage is a parameter that has often been used to indicate physical damage to the plasmalemma resulting from low-temperature stress (Parkin & Kuo, 1989). Saltveit (2002) reported for tomato a progressive increase in electrolyte leakage over a few days of chilling, while kiwifruit showed an increase up to 15% when exposed at −2 °C for 40 h (Gerasopoulos, Chlioumis, & Sfakiotakis, 2006). Low-temperature breakdown, a disorder which causes considerable quality losses during prolonged cold storage in kiwifruit, appears to be related to factors affecting membrane function (Gerasopoulos et al., 2006; Sfakiotakis, Chlioumis, & Gerasopoulos, 2005).

The ratio of unsaturated to saturated fatty acids increased with ripening in 'Honey dew' muskmelons coincidentally with the decrease in chilling sensitivity that has been associated with these factors (Forney, 1990). In apple flesh, however, the degree of fatty acids unsaturation decreased as fruit ripened (Lurie & Ben-Arie,

* Corresponding author. Tel.: +351 289800900; fax: +351 289818419.
E-mail address: mantunes@ualg.pt (Maria Dulce C. Antunes).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.02.089

1983). Heureux, Bergevin, Thompson, and Willemot (1993) found increasing electrolyte leakage and fatty acid unsaturation of tomato during storage at 1 °C. Schirra and Sass (1994) found significant changes in fatty acid composition during storage of grapefruit at temperatures from 4 to 12 °C with the proportion of linolenic and palmitoleic acids increasing and palmitic, stearic and oleic acids decreasing.

The purpose of this study was to investigate the effect of storage temperature on fatty acids composition and membrane permeability of 'Hayward' kiwifruit through storage.

2. Material and methods

2.1. Plant material and treatments

Kiwifruit (cv. 'Hayward') were harvested from an orchard in Pieria-North Greece with a mean of 82N firmness and 5.2% °Brix. Fruits were immediately transferred to the postharvest laboratory at the University Farm at Thessaloniki. Fruit of uniform size and free from defects, were placed in 5-l jars through which a continuous, humidified, ethylene-free air stream was passed at a rate of 100 ml min^{-1}. Jars were kept in separate water baths at a constant temperature of 0, 5, 10, 15 or 20 °C. At intervals of 0, 5, 12 and 17 days, 10 fruit per replication were removed from the jars and used for measurements of flesh firmness, SSC, flesh colour, electrolyte leakage and fatty acid analysis.

The experimental design was a two-factor experiment distributed in a complete randomised design with the temperatures as the first factor, the number of days in storage as second and the jars as replications. Each treatment consisted of four replications with 60 fruit per replication. Experiments were repeated in two consecutive years, showing results a similar pattern. Values presented are of the second year experiment. Statistical analyses were made with the SPSS statistical package. Two-way analysis of variance (ANOVA) and least significant difference (LSD) tests ($P < 0.05$) for comparisons between treatments over time were conducted.

2.2. Measurements of firmness, SSC and colour

Flesh firmness was recorded by puncture with a Chatillon pressure tester, John Chatillon & Sons Inc. USA, fitted with a flat 8 mm diameter plunger at a depth of 7 mm. The SSC (% Brix) were measured by a digital refractometer, model PR1-Atago Co. Ltd, Japan, in juice from the equatorial zone of the fruit. A Chroma meter CR-200 series, CE Minolta, Japan, was used to measure flesh colour using the a^* value, in which a change to more positive values indicates fading of the green colour.

2.3. Fatty acids analysis

Lipid extraction procedure and fatty acids analysis were based on the AOAC Official Methods of Analysis (1984), as described previously (Thomai, Sfakiotakis, Diamantidis, & Vasilakakis, 1998), with modifications. Peeled kiwifruit tissue (20 g without seeds) was homogenised in 100 ml of 2:1 chloroform/methanol solvent with a polytron homogeniser. The homogenate was filtered through Whatman #1 filter paper in a separator funnel and the filtered residue was washed with 50 ml of the same solvent. Twenty five ml of 0.58% NaCl were added to the filtrate in the separator funnel which was shaken well and left for 4 h to separate the two phases. The total lipids were obtained in the chloroform phase which was concentrated to oil in a rotary evaporator under reduced pressure at 40 °C. The oil was placed in an oven at 105 °C for 20 min to eliminate any trace of water, dissolved in 2 ml chloroform and transferred to 8.5 ml Teflon lined screw cap test tubes. Tubes were filled to the top with chloroform and stored at −20 °C for further analysis of fatty acids.

For the fatty acids analysis, the oil/chloroform extract was transferred to evaporation tubes and the chloroform phase was evaporated with a rotary evaporator at 40 °C. Concentrated oil was placed in an oven at 60 °C for 30 min for further drying. Four milliliters of 0.5 N methanolic NaOH solution was added to the flasks which were then attached to a condenser and the mixture was boiled by placing the tubes in a boiling water-bath until fat globules disappeared (5–10 min); 5 ml of BF_3/methanol were added with a pipette through the condenser and boiling was continued for another 2 min. Two milliliters heptane were added through the condenser and boiling was further continued for 1 min. The condenser was cooled and 5 ml of saturated NaCl solution were added to float the heptane phase to the neck of the flask. The upper heptane layer was transferred to glass test tubes. Two microliters of the heptane solution were injected into a Varian 3700 gas chromatograph for methyl esters fatty acids identification. The gas chromatograph was equipped with a 4 mm × 2 m column packed with 10% DEGS on 80/100 mesh Chromosorb WAW at 190 °C, an injector at 220 °C and a flame-ionisation detector at 250 °C. The carrier gas was N_2 at a flow rate of 20 ml min^{-1}.

A mixture of fatty acids was used as an external standard. Individual fatty acids were identified and measured by comparing their retention times and peak areas to standards. The unsaturated/saturated fatty acid ratio was calculated by the formula: (18:1 + 18:2 + 18:3)/(16:0 + 18:0) where 16:0 = Palmitic acid; 18:0 = Stearic acid; 18:1 = Oleic acid; 18:2 = Linoleic acid; 18:3 = Linolenic acid.

2.4. Electrolyte leakage

Electrolyte leakage measurements were made as described by Gerasopoulos et al. (2006) with small modifications. Initial conductivity measurements were taken after 6 h incubation. The flasks were put in an oven at 100 °C for 1 h and cooled to room temperature. Then, conductivity was measured again with a WTW conductivity meter LF91 (WTW, Wiss.Techn. Werkstatten, Weilheim, Germany) and taken as total electrolyte leakage.

3. Results

3.1. Effect of chilling on fruit ripening

Kiwifruit ripening parameters (firmness and SSC) did not significantly change during 17 days at 0 and 5 °C (data not shown). Fruit at 10, 15 and 20 °C had significant reduction in firmness and increase in SSC after 12 and 17 days storage but values were still high (64–70 N) and low (7.7–8.2% Brix), respectively, compared to eating ripe kiwifruit.

Flesh colour did not significantly change through storage at any temperature. The a^* values were from −16.9 to −18.3.

3.2. Effect of chilling on fatty acid composition

Figs. 1 and 2 show that, generally, the major fatty acid component in 'Hayward' kiwifruit consisted of linolenic acid (35–40%), followed by oleic acid (26–36%), palmitic acid (15–22%), linoleic acid (9–11%) and stearic acid (2–5%).

Palmitic acid levels had an insignificant decrease during 17 days storage at 20 °C, while in the other treatments the decrease was significant and occurred mostly in the first 5 days (Fig. 1A). Values were always higher at 20 °C followed by 15, 10, 5 and 0 °C. Differences were not significant between 20 and 15 °C or among 10, 5 and 0 °C.

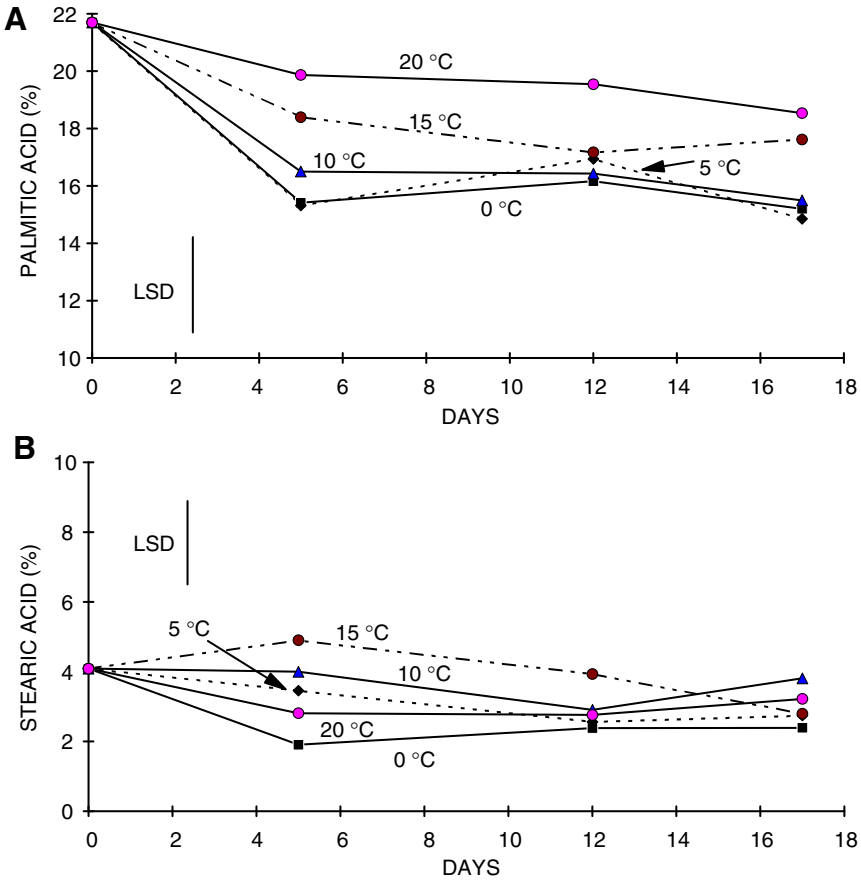

Fig. 1. Palmitic (A) and stearic (B) acid changes of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream at 0, 5, 10, 15 and 20 °C. LSD at $\alpha = 0.05$.

Stearic acid did not change significantly with storage time at any temperature (Fig. 1B). Values were significantly higher at 15 °C than at 0 °C after 5 days, but after 12 and 17 days storage there were not significant differences among treatments.

Oleic acid had a significant increase in the first 5 days storage for all treatments (Fig. 2A). Values were significantly higher at 0, 5 and 10 °C than at 15 and 20 °C. The percentage of oleic acid continued to increase thereafter at 0 and 5 °C while after the other treatments it did not change significantly. After 12 days storage, the percentage of oleic acid was significantly higher at 0 and 5 °C than at 15 and 20 °C. At the end of the experiment, oleic acid was significantly higher at 0 and 5 °C than after the other treatments.

Linoleic acid did not significantly change during storage in any treatment (Fig. 2B). After 5 and 12 days storage values were significantly higher at 20 °C than at 0 °C but after 17 days differences among treatments were not significant.

Linolenic acid did not change or show significant differences among treatments during the experiment (Fig. 2C).

3.3. Effect of chilling on fatty acid unsaturation/saturation ratio

Fruit stored at 0, 5 and 10 °C had a significant increase in the unsaturated/saturated fatty acid ratio after 5 days storage and remained almost constant thereafter (Fig. 3). Unsaturated/saturated fatty acid ratio increased significantly at 15 °C only from 0 to 12 and 0 to 17 days storage, and at 20 °C the increase was insignificant during all experiments.

After 5 days storage, unsaturated/saturated fatty acid ratio was significantly higher at 0 °C followed by 5, 10, 15 and 20 °C although the difference was not significant among the last 3 (Fig. 3). After 12 days storage, values were only significantly higher at 0 than at 15 or 20 °C. After 17 days storage, the unsaturated/saturated fatty acid ratio was significantly higher at 0 and 5 °C than at 10, 15 and 20 °C. During storage, unsaturated/saturated fatty acid ratio was always higher at 0 or 5 °C than at 15 or 20 °C.

3.4. Effect of chilling on membrane permeability

Membrane permeability of kiwifruit, expressed by the change in electrolyte leakage, significantly increased during the first 5 days storage in all treatments (Fig. 4). Values were higher at 0 °C followed by 5 and 10 °C without significant differences among them. Fruit at 15 and 20 °C showed values of electrolyte leakage significantly lower than the other treatments.

From 5 to 17 days storage, the increase in electrolyte leakage of kiwifruit was higher at 15 and 20 °C than at 0, 5 and 10 °C. However, after 12 days storage, electrolyte leakage was significantly lower at 20 than at 0 °C, while after 17 days storage it was significantly lower at 20 °C than in the other treatments, which did not show significant differences among them.

4. Discussion

Kiwifruit harvested at an early stage of maturity started autocatalysis of ethylene followed by ripening in three more days, after 17–19 days at 20 °C (Antunes, Pateraki, Kanellis, & Sfakiotakis, 2000; Antunes & Sfakiotakis, 2002). In our experiment ripening did not occur during 17 days at temperatures from 0 to 20 °C.

The major fatty acids found in kiwifruit were linolenic, oleic and palmitic and the minor linoleic and stearic acids.

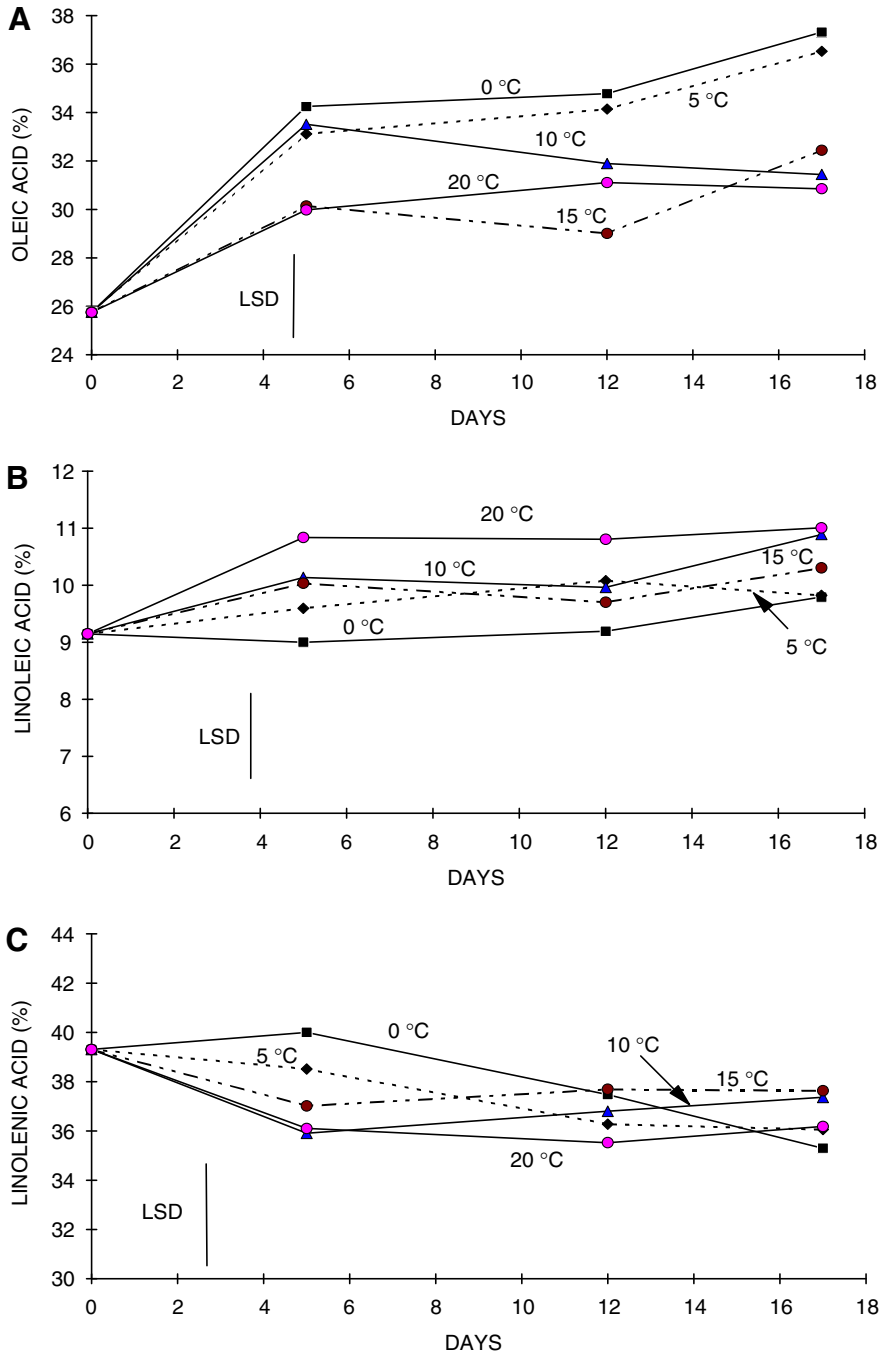

Fig. 2. Oleic (A), linoleic (B) and linolenic (C) acid changes of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream at 0, 5, 10, 15 and 20 °C. LSD at $\alpha = 0.05$.

Changes in membrane permeability have been correlated to changes in its lipid composition, either in the sterol level – the ratio of phospholipids to sterols – or the fatty acids composition of the phospholipids (Lurie & Ben-Arie, 1983). In the present study a positive correlation was found between the unsaturated/saturated fatty acid ratio and membrane permeability, while in apples and potato leakage correlated with decreased degree of fatty acid unsaturation (Knowles & Knowles, 1989; Lurie, Sonego, & Ben-Arie, 1987).

The increase in fatty acid unsaturation and membrane permeability were higher at lower temperatures as a response to chilling stress in order to prevent chilling injury as reported for muskmelons (Forney, 1990). The increase in unsaturation may be the cause of the increase on membrane permeability. These results are confirmed by Heureux et al. (1993) in studies on tomatoes. However, Spychalla and Desborough (1990) reported that higher rates of fatty acid unsaturation of potato were related to lower membrane permeability. Whitaker (1994) stated that fatty acid unsaturation increased slightly during chilling in tomato fruit, while Parkin and Kuo (1989) found an increase only upon rewarming of cucumber fruit.

Changes in membrane lipids have been associated with solute leakage increases during ripening of many fruits (Lester & Stein, 1993; Wade, 1995). The same authors reported that the increase in electrolyte leakage was associated to an increase in linolenic and a decrease in linoleic fatty acids for muskmelon and banana.

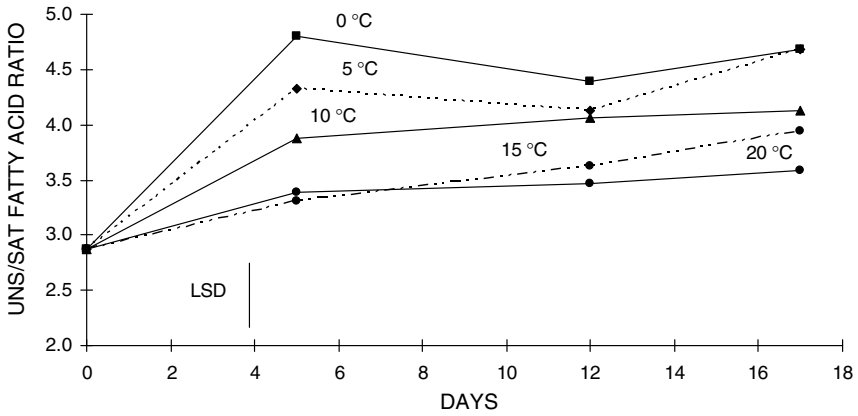

Fig. 3. Unsaturated/saturated fatty acid ratio of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream at 0, 5, 10, 15 and 20 °C. LSD at $\alpha = 0.05$.

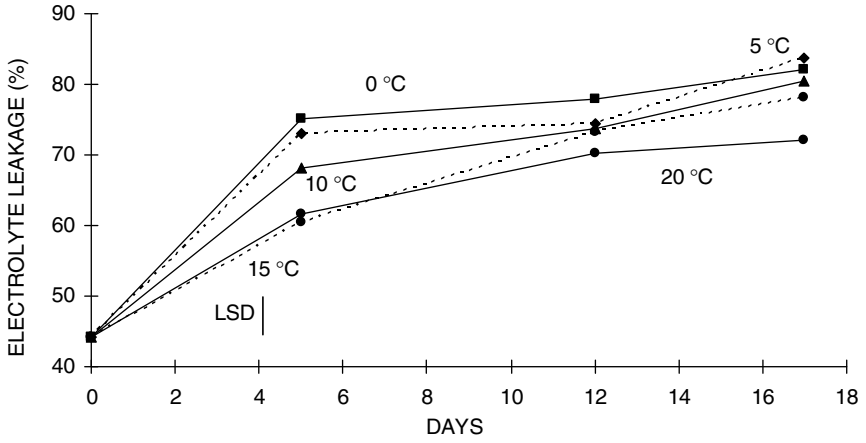

Fig. 4. Electrolyte leakage of harvested 'Hayward' kiwifruit kept in a continuous, humidified, air stream at 0, 5, 10, 15 and 20 °C. LSD at $\alpha = 0.05$.

Marangoni et al. (1996) stated that ion leakage was correlated to losses in linolenic acid, a fatty acid particularly prone to oxidation. In our study, although slight, there was a decrease in linolenic acid probably due to the slight advance in fruit ripening. Linoleic acid slightly increased in kiwifruit. The increase in linoleic acid has been related to the acclimatisation of fruits at low-temperature to prevent chilling injury (Marangoni et al., 1996).

Forney (1990) reported that the increase in unsaturation of fatty acids in muskmelons was primarily due to the increase of palmitoleic and oleic acids since the other fatty acids had only small changes. The results of our study showed that in kiwifruit the increase in unsaturated/saturated fatty acid ratio was mostly due to a decrease in palmitic and an increase in oleic acids. The changes in those fatty acids were more pronounced at lower temperatures.

In the present study, it was observed that the most pronounced increase in the unsaturated/saturated fatty acid ratio and electrolyte leakage occurred in the first 5 days storage, which may be related to primary adaptive change to cold storage of the chilling resistant fruit (Marangoni et al., 1996).

The increase in unsaturated/saturated fatty acid ratio in kiwifruit was caused mainly by a decrease in palmitic and an increase in oleic acids. Stearic, linoleic and linolenic acids had small changes during storage. The unsaturated/saturated fatty acid ratio correlated positively with electrolyte leakage in 'Hayward' kiwifruit. The main increase in electrolyte leakage and unsaturated/saturated fatty acid ratio occurred during the first storage days and at lower temperatures, probably as a response of the tissue to an adaptation to the new stress storage conditions.

References

Antunes, M. D. C., Pateraki, I., Kanellis, A. K., & Sfakiotakis, E. M. (2000). Differential effects of low temperature inhibition on the propylene induced autocatalysis of ethylene production, respiration and ripening of 'Hayward' kiwifruit. *Journal of Horticultural Science & Biotechnology, 75*(5), 575–580.

Antunes, M. D. C., & Sfakiotakis, E. M. (2002). Chilling induced ethylene biosynthesis in 'Hayward' kiwifruit following storage. *Scientia Horticulturae, 92*, 29–39.

Association of Official Analytical Chemists (1984). *Official methods of analysis*. Washington, DC: AOAC.

Forney, C. F. (1990). Ripening and solar exposure alter polar lipid fatty acid composition of 'Honey Dew' muskmelons. *HortScience, 25*, 1260–1264.

Gerasopoulos, D., Chlioumis, G., & Sfakiotakis, E. (2006). Non-freezing points below zero induce low-temperature breakdown of kiwifruit at harvest. *Journal of Science of Food and Agriculture, 86*, 886–890.

Heureux, G. P., Bergevin, M., Thompson, J. E., & Willemot, C. (1993). Molecular species profile of membrane lipids of tomato pericarp during chilling. *Acta Horticulturae, 343*, 286–287.

Knowles, N. R., & Knowles, L. O. (1989). Correlations between electrolyte leakage and degree of saturation of polar lipids from aged potato (*Solanum tuberosum* L.) tuber tissue. *Annals of Botany, 65*, 217–223.

Lester, G., & Stein, E. (1993). Plasma membrane physicochemical changes during maturation and postharvest storage of muskmelon fruit. *Journal of the American Society for Horticultural Science, 118*, 223–227.

Lurie, S., & Ben-Arie, R. (1983). Microsomal membrane changes during the ripening of apple fruit. *Plant Physiology, 73*, 636–638.

Lurie, S., Sonego, L., & Ben-Arie, R. (1987). Permeability, microviscosity and chemical changes in the plasma membrane during storage of apple fruit. *Scientia Horticulturae, 32*, 73–83.

Marangoni, A. G., Palma, T., & Stanley, D. W. (1996). Membrane effects in postharvest physiology. *Postharvest Biology and Technology, 7*, 193–217.

Palma, T., Marangoni, A. G., & Stanley, D. W. (1995). Environmental stresses affect tomato microsomal membrane function differently than natural ripening and senescence. *Postharvest Biology and Technology, 6*, 257–273.

Parkin, K. L., & Kuo, S. J. (1989). Chilling-induced lipid degradation in cucumber (*Cucumis sativa* L Cv. Hybrid C) fruit. *Plant Physiology, 90*, 1049–1056.

Pooter, H. L., & Schamp, N. M. (1989). Involvement of lipoxigenase-mediated lipid catabolism in the start of the autocatalytic ethylene production by apples (cv Golden Delicious): A ripening hypothesis. *Acta Horticulturae, 258*, 47–53.

Saltveit, M. E. (2002). The rate of ion leakage from chilling-sensitive tissue does not immediately increase upon exposure to chilling temperatures. *Postharvest Biology and Technology, 26*, 295–304.

Schirra, M., & Sass, P. (1994). Fatty acid profile changes in Marsh Seedless grapefruit in response to storage conditions. *Acta Horticulturae, 368*, 429–434.

Sfakiotakis, E., Chlioumis, G., & Gerasopoulos, D. (2005). Preharvest chilling reduces low temperature breakdown incidence of kiwifruit. *Postharvest Biology and Technology, 38*, 169–174.

Spychalla, J. P., & Desborough, S. L. (1990). Fatty acids, membrane permeability, and sugars of stored potato tubers. *Plant Physiology, 94*, 1207–1213.

Thomai, T., Sfakiotakis, E., Diamantidis, G., & Vasilakakis, M. (1998). Effects of low preharvest temperature on scald susceptibility and biochemical changes in 'Granny Smith' apple peel. *Scientia Horticulturae, 76*, 1–15.

Todd, J. E., Paliyath, G., & Thompson, J. E. (1992). Effect of chilling on the activities of lipid degrading enzymes in tomato fruit microsomal membranes. *Plant Physiology and Biochemistry, 30*, 517–522.

Valdenegro, M., Flores, F. B., Romojaro, F., Ramirez, M., & Martinez-Madrid, M. D. (2005). Susceptibility of Spanish melon fruits to chilling injury during cold storage. *Acta Horticulturae, 682*, 1219–1225.

Wade, N. L. (1995). Membrane lipid composition and tissue leakage of pre- and early-climacteric banana fruit. *Postharvest Biology and Technology, 5*, 139–147.

Whitaker, B. D. (1994). Lipid changes in mature-green tomatoes during ripening, during chilling, and after rewarming subsequent to chilling. *Journal of the American Society for Horticultural Science, 119*, 994–999.