ELSEVIER

Postharvest Biology and Technology 8 (1996) 129–141

Postharvest Biology and Technology

The effects of short heat-treatments on the induction of chilling injury in avocado fruit (*Persea americana* Mill)

P. Florissen [a,c], J.S. Ekman [a], C. Blumenthal [b], W.B. McGlasson [a], J. Conroy [a], P. Holford [a,*]

[a] *School of Horticulture, University of Western Sydney, Bourke Street, Richmond, N.S.W. 2753, Australia*
[b] *CSIRO, Grain Quality Laboratory, Division of Plant Industry, Sydney, N.S.W., Australia*
[c] *NSW Agriculture, Dareton, N.S.W., Australia*

Accepted 26 October 1995

Abstract

The ripening of avocado fruit and the development of chilling injury in relation to short heat-treatments has been studied in the cv. 'Hass'. The minimal conditions needed to induce maximal production of heat shock proteins (HSP) in samples of mesocarp tissue were an exposure to 38°C for 4 h. Short heat-treatments applied during the ripening process reduced the maximum rate of ethylene production during the climacteric period, but this was not correlated with lower levels of 1-aminocyclopropane-1-carboxylic acid (ACC). Heat-treatment also hastened the occurrence of the climacteric in fruit treated in the early pre-climacteric period. A similar regime applied to fruit immediately prior to this event delayed the onset of the climacteric. The use of short heat-treatments to overcome the effects of chilling injury was investigated by subjecting the fruit to 38°C for 0, 6, 12, 24, 36 or 48 h prior to transferring them to 0°C for 7, 14 or 21 days. Heating for 6–12 h provided a significant degree of protection from chilling injury and therefore may have potential for extending the period of cold storage.

Keywords: Persea americana; Avocado; Chilling injury; Ripening; Heat shock protein

1. Introduction

Avocado production areas around the world are typically remote from their overseas markets. Hence, there is often a significant delay between harvesting and the arrival of the fruit at the point of consumption, during which time fruit may ripen. Fruit for domestic markets may also be stored during times of over-production. Thus, there is a need to develop an appropriate storage technology to

* Corresponding author. Fax: +61 (4)5 783979.

SSDI 0925-5214(96)00063-1

delay ripening and provide quality produce for both local and export markets. In general, the storage life of produce is inversely related to the respiration rate (Wills et al., 1989). The respiration rate can be reduced, and storage period extended, by the use of low temperatures.

Although storage time and shelf-life of avocados can be lengthened by lowering their temperature, chilling injury is induced below 7°C and this is a major factor influencing the quality of stored avocados (Eaks, 1976; Zauberman et al., 1977). The symptoms of chilling injury in avocado consist of mesocarp discolouration, hardening of vascular strands and 'off flavours'. These symptoms do not appear during cold storage but only develop after fruit are subsequently kept at room temperature (Zauberman et al., 1977). Chilling injury does not occur above 7°C. However, these higher temperatures are not a solution as they permit commercially mature fruit to ripen during storage (Chaplin et al., 1982).

In recent years there has been growing interest in heat-treatments as a method of reducing chilling injury in horticultural crops, thus permitting extended storage times (Hatton, 1990; Wang, 1990). The effects of postharvest heat-treatments on fruits are varied. Elevated temperatures alter the firmness of fruit such as plums, tomatoes and avocados (Eaks, 1978; Tsuji et al., 1984; Biggs et al., 1988), their chemical composition (Klein and Lurie, 1990, 1992; Klein et al., 1990) and colour (Klein et al., 1990; Klein and Lurie, 1992), as well as both respiration (Kerbel et al., 1985; Lurie and Klein, 1990, 1991) and ethylene production (Biggs et al., 1988; Klein, 1989). To date most of the work concerning the effects of postharvest heat-treatments of fruit has concentrated on long periods at elevated temperatures, typically from 12 h to four days (Klein and Lurie, 1991).

Amongst the changes caused by heat-treatments is the induction of heat shock proteins (HSP), which are implicated in protecting plants from heat injury. However, the term HSP is a misnomer as they can be induced by anaerobiosis, chemical stresses and even by chilling. In this study we have determined the minimal conditions required for the maximum induction of HSP in avocado. We then determined if such treatments provide protection from chilling injury.

2. Methods

Plant material

Mature avocado fruit, cv. 'Hass', were obtained from growers in N.S.W. and were used for studies within 24 h of harvest. Avocado fruit for trial 1 were obtained from Alstonville, N.S.W. and for trial 2 from Tolga, Qld. The fruit used in both trials were of a similar size (average size, 242 g) and quality. Prior to treatment all fruit were dipped for 1 min in 2.3 mg ml^{-1} (a.i.) *Prochloraz* fungicide (Schering) and air dried before use.

Determination of conditions for HSP induction

To determine the optimum conditions for HSP production, samples of mesocarp tissue, taken from around the equator of the fruit, were subjected to temperatures of 38°C for 30–240 min or to varying temperatures (22–44°C) for 4 h.

Heat and chilling treatments prior to assessment of chilling injury

For each trial a total of 198 fruit were subjected to heat-treatments. The fruit were divided into six groups of 33 and heat-treated at 38°C for the following periods: 0, 6, 12, 24, 36 and 48 h. Thermocouples were placed into avocado fruit next to the seed and were used to monitor temperature changes during the heat-treatments. To prevent water loss during the heat-treatments fruit were either individually shrink-wrapped in Cryovac MD700 plastic film (trial 1) or were sealed in perforated polyethylene bags (trial 2). Thirty fruit from each of the six groups were placed in storage at 0°C for the following periods of 7, 14 or 21 days. After treatment the fruit were allowed to ripen at 20°C. The remaining three fruit from each treatment were used to monitor respiration and ethylene production. The fruit used for trial 1 were treated before the onset of their climacteric and those used for trial 2 at the onset of their climacteric.

In vivo labelling and extraction of proteins

Cubes of mesocarp tissue were sampled from the equator of the avocado fruit. Sub-samples (150 mg) were incubated with L-[^{35}S] methionine (100 μCi/150 mg tissue) in sterile distilled water under control (22°C) or heat shock conditions detailed above. After incubation, the tissue was washed, frozen and ground in liquid nitrogen and the resulting powder suspended in 1.5% (w/v) SDS, 10% (w/v) β-mercaptoethanol, 0.5 M Tris-HCl, pH 7.5. This suspension was boiled for 3 min, centrifuged at 12,000 g and the supernatant used for further analysis. To determine the percentage incorporation of label into protein, aliquots of the supernatant were spotted onto nitrocellulose filter paper, washed with 10% TCA and their radioactivity determined by scintillation counting.

Gel electrophoresis and fluorography (after Laemmli, 1970)

Samples of approximately 25,000 cpm were separated by SDS-PAGE using a 6% (w/v) polyacrylamide stacking gel and a 15% (w/v) separating gel. Molecular weight standards, in the range of 14–94 kDa (Amersham Int.), were also run. The gels were developed overnight at 40 V using a glycine running buffer [380 mM glycine, 50 mM Tris, 0.1% (w/v) SDS]. Subsequently, the gels were fixed, incubated in Amplify (Amersham Int.), dried and exposed to pre-flashed film at −70°C for 14 days.

Assessment of ripening and flesh injury

The degree of ripeness of each fruit was determined using a subjective assessment of softness determined by hand. Once ripe, the plastic wrapping was removed, the fruit cut in half longitudinally and examined for symptoms of chilling injury. Severity of chilling injury was determined subjectively using a rating scale of 0 to 5, with 0 indicating no visible chilling injury symptoms and 5 indicating severe injury (Chaplin et al., 1982).

Measurement of respiration and ethylene production

The heat-treated fruit used to monitor respiration were individually enclosed in plastic containers which were ventilated with humidified air at approximately 8 l h^{-1}

at a constant temperature of 20°C. Ethylene concentrations in the air stream leaving each container were assayed by gas chromatography using an activated alumina column and flame ionisation detector. Measurements were continued until the fruit began to show visible signs of senescence and decay. The minimum rate of ethylene that could be measured was 0.8 nl kg^{-1} h^{-1}.

Measurement of 1-aminocyclopropane-1-carboxylic acid (ACC)

After storage at 0°C for seven days, a 2-g equatorial slice of mesocarp tissue was taken at the onset of ripening (as determined by commencement of ethylene production) from the ten replicate fruit used for each heat-treatment in trial 1. The tissue samples were frozen in liquid nitrogen and homogenised using a pre-chilled coffee grinder giving a bulk sample for each heat-treatment. ACC levels were determined using the procedure of Lizada and Yang (1979) on three 1-g aliquots of each bulk sample.

Statistical analysis

Analyses of variance were used to determine levels of statistical significance between the time to the commencement of ripening (as indicated by the initial rise in ethylene production), differences in the magnitude of climacteric ethylene production, ACC level and chilling injury. The bars shown on Figs. 1–4 represent the standard error of the relevant means.

3. Results

Induction of HSP

The proteins produced in control and heat-shocked avocado fruit were separated by SDS-PAGE and the resulting banding patterns were analysed by fluorography followed by densitometry. The optimum temperature for the induction of HSP was determined by subjecting mesocarp tissue to a 4-h heat shock at temperatures in the range of 22–44°C (Table 1). Heat shocks at temperatures above 26°C induced a series of proteins with approximate molecular weights of 92, 82, 74, 55, 42.5 and 20 kDa in the mesocarp. Fainter bands were also produced in the 25–28 kDa range. The synthesis of these proteins increased with temperatures up to 38°C after which their production fell abruptly with essentially no proteins being synthesised above 44°C. Therefore, temperatures in the range of 34–38°C appear optimal for HSP induction. Proteins with molecular weights 82, 74, 55 and 20 were synthesised only in tissue subjected to the heat shock treatments. To determine the shortest time required to induce maximum HSP production, tissue was heat shocked at 38°C for 30–240 min (Table 2). The level of HSP expression increased from 30 to 120 min, but little difference could be seen between tissue samples treated for 120 or 240 min.

Effect of heat-treatment on time to commence ripening

Thermocouples placed into the flesh next to the seed in whole fruit showed that the inner part of the mesocarp required a 2-h period to reach 38°C. The time

Table 1
Relative intensities of proteins produced in avocado tissue after treatment for 4 h at varying temperatures and separated by SDS-PAGE. The resulting banding patterns were analysed by fluorography followed by densitometry. All values are adjusted to give their value relative to the most intense band (74 kDa at 38°C)

Molecular weight (kDa)	Temperature of heat shock (°C)									
	22	26	30	32	34	36	38	40	42	44
92	19	19	19	27	50	38	50	23	<10	<10
82	12	23	23	31	54	38	60	27	19	<10
74	<10	19	27	35	92	65	100	38	<10	<10
55	<10	12	12	27	19	12	12	12	12	<10
42.5	<10	12	12	23	12	12	12	12	<10	<10
20	<10	<10	<10	<10	12	12	19	12	<10	<10
Average intensity	–	15	16	26	40	30	42	21	<10	<10

Table 2
Relative intensities of HSP induced in avocado tissue by heat-treatments at 38°C for varying periods of time. All values are adjusted to give their value relative to the most intense band (74 kDa for 120 min). The molecular weights of the proteins are in kDa

Molecular weight (kDa)	Duration of heat shock (min)				
	30	60	90	120	240
92	<10	26	43	58	50
82	17	50	53	58	60
74	34	69	81	100	100
55	34	50	52	62	67
42.5	12	29	31	38	33
20	<10	15.5	19	31	31
Average intensity	–	40	47	58	57

between harvesting and the commencement of ripening (determined by the onset of the climacteric rise in ethylene production) for both trials is presented in Fig. 1. Data from the controls show that the fruit in trial 1 were harvested well before the onset of the climacteric and in trial 2 the climacteric rise occurred within 24 h of commencing the experiment. For both trials, heat-treatments caused significant changes in time to commence ripening ($P < 0.05$). Pre-climacteric fruit (trial 1), subjected to a heat-treatment longer than 6 h showed a reduction in time to ripening (Fig. 1a). Fruit heat-treated for 6 h showed no difference from the control fruit and began to ripen approximately 3.5 days after treatment. This effect of the heat-treatments was not seen in the fruit which had started to ripen (trial 2) when the experiment had commenced. In this second trial, all heat-treatments caused a significant delay in the onset of ripening with a trend for increased time to ripening with increasing length of heat-treatment.

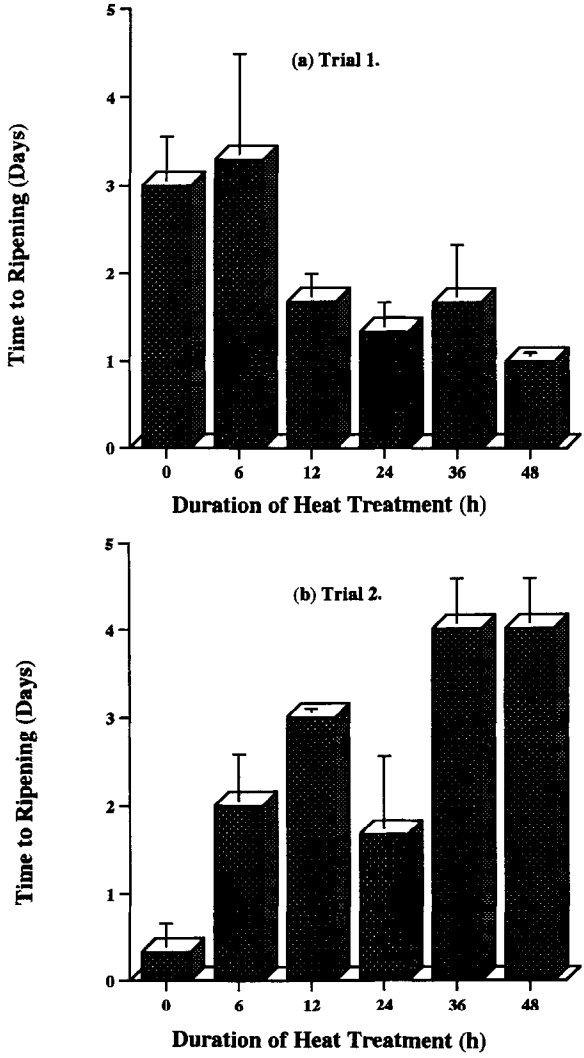

Fig. 1. The effect of varying durations of heat-treatment at 38°C on the time to commencement of ripening (average of three fruit), as indicated by initial ethylene production, for (a) pre-climacteric avocado (trial 1); and (b) fruit treated during the onset of their climacteric (trial 2). The bars represent the standard error of the relevant means.

Effect of heat-treatment on ethylene and ACC production

Heat-treatments also had a significant effect on the magnitude of climacteric ethylene production (Fig. 2a, b). In general, there was a decrease in the peak value of climacteric ethylene with longer heat-treatment times. In order to learn more about the connections between heat-treatment and ethylene production, ACC levels were measured at the onset of ripening in a matching sample of fruit from trial 1

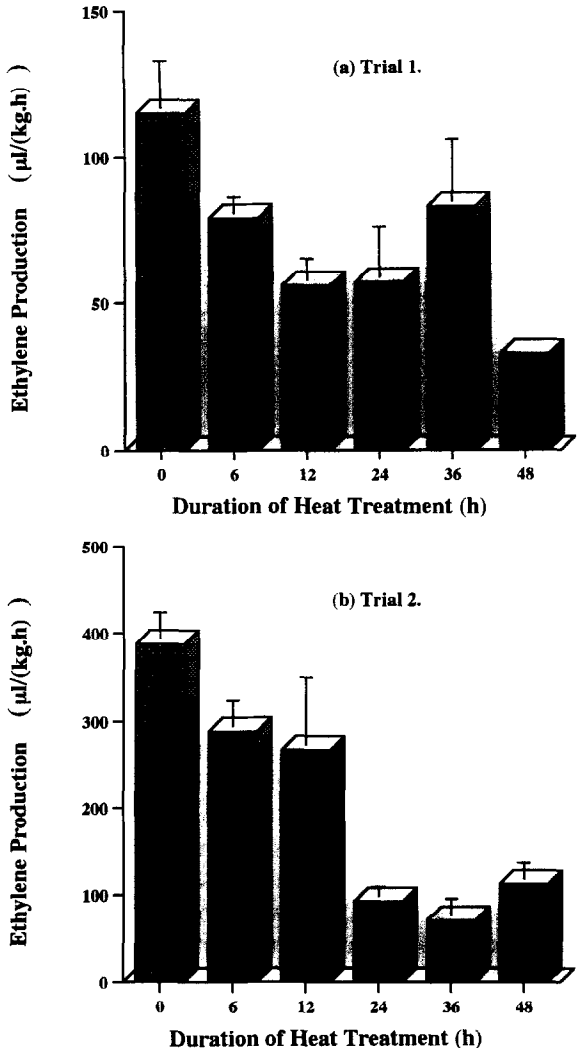

Fig. 2. The effect of varying durations of a 38°C heat-treatment on the peak ethylene production (average of three fruit) during the climacteric. The fruit were treated either (a) before the onset of their climacteric (trial 1); or (b) during the onset of the climacteric (trial 2). The bars represent the standard error of the relevant means.

only (Fig. 3). Those heat-treated for either 6 or 12 h showed a significant increase ($P < 0.05$) in ACC concentration over the control with the fruit treated for 12 h producing over twice the control level. Fruit subjected to longer heat-treatments (24, 36 or 48 h) all produced approximately 0.5 nM g^{-1} of ACC which is significantly ($P < 0.05$) below the control level.

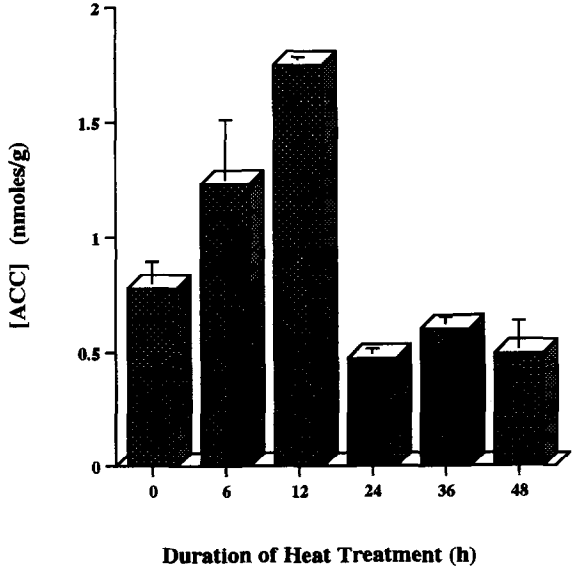

Fig. 3. Levels of ACC in avocado fruit from trial 1 at the onset of their climacteric after treatment for varying durations at 38°C. The fruit were treated prior to their climacteric. The bars represent the standard error of the relevant means.

Effect of heat-treatment on flesh injury

The effect of the duration of heat-treatments on the subsequent levels of damage in fruit chilled for periods at 0°C is presented in Fig. 4. In general, fruit subjected to a short heat-treatment (6 or 12 h) before the onset of their ethylene climacteric (trial 1; Fig. 4a) showed significantly less flesh injury after ripening than either the control fruit or fruit heated for longer times ($P < 0.05$). This occurred for fruit following all periods of storage at 0°C but was most marked in those fruit stored for 21 days, where treated fruit had an injury score of approximately half that of the controls. These results show that there is a 'crossover' point after which the protection from chilling injury is lost and that the heat-treatment has a detrimental effect. In trial 1 this crossover point was between 12 and 24 h at 38°C.

The fruit in trial 2 showed a similar overall trend to those in trial 1 (Fig. 4b). Again there was a crossover point between 12 and 24 h at 38°C after which time heat-treatments exacerbated the flesh injury. However, the protection from chilling injury caused by short periods of heat-treatment was not as marked, with only the fruit stored for 14 days after heat-treatment showing any reduction from control values. The fruit allowed to ripen after 7 or 21 days cold treatment showed no significant differences from the control fruit.

4. Discussion

The molecular weight spectrum of proteins produced by heat-treated avocado was similar to those produced by other species. In maize, Cooper and Ho (1983)

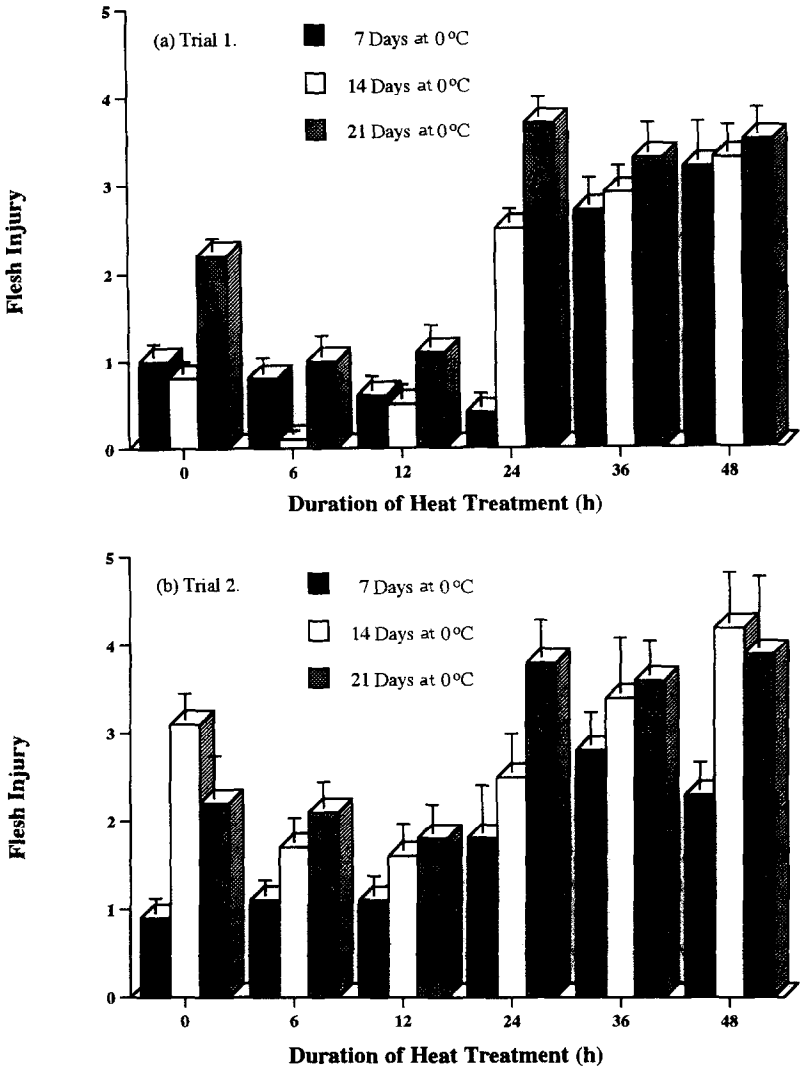

Fig. 4. The effect of varying durations of a 38°C heat-treatment on the level (average of replicate samples) of flesh injury in avocado. The fruit were held at 0°C for intervals of 7, 14 and 21 days before ripening at 20°C. After chilling fruit were held at 20°C and assessments of flesh injury were made when the fruit became noticeably soft. The fruit were treated either (a) before the onset of their climacteric (trial 1); or (b) during the onset of the climacteric (trial 2). The bars represent the standard error of the relevant means.

showed the synthesis of HSP with molecular weights ranging from 18 to 87 kDa. Burke et al. (1985) observed the induction of 8 HSP with molecular weights between 21 and 100 kDa in field-grown cotton which had been subjected to a temperature of 40°C. In a range of both monocots and dicots, Mansfield and Key (1987) demonstrated that the major HSP produced have molecular weights

of 15–25 kDa; avocado also shows expression in this range. The conditions for maximal HSP production in avocado is also similar to that found for carrot (Hwang and Zimmerman, 1989), soybean (Hsieh et al., 1992) and maize (Cooper and Ho, 1983).

Avocado fruit subjected to a pre-treatment of 38°C before storage at 0°C showed reduced symptoms of flesh injury compared to controls. The symptoms observed in fruit heat-treated for up to 12 h were typical of those ascribed to chilling injury (Chaplin et al., 1982). The optimum treatment period for protection was between 6 and 12 h, since times of greater duration exacerbated the level of flesh injury. There are several possible explanations for the protection against chilling injury arising from short heat-treatments. Firstly, there may be protection by HSP induced during the treatment period. It has been proposed that the ever changing environmental conditions which a plant experiences in the field are continuously exposing it to 'stressful' conditions (Ho and Sachs, 1989). To cope with these effects, stress proteins appear to be synthesised to alleviate detrimental changes in the plant's physiology via effects on protein conformation and degradation (Vierling, 1991). Further, it is suggested that exposure of plant tissues to one stress may protect the plant against another stress (Ho and Sachs, 1989; Klein and Lurie, 1991). In the study reported here, the optimal temperature for the induction of protection in avocado fruit correlates well with the conditions for optimal HSP production. After 12 h a crossover point, where the heat-treatment no longer protected against chilling, was reached after 24 h of heating at 38°C. This may represent the point at which the protection afforded by the production of HSP is offset by damage from the heat-treatment itself.

Another possible reason for the protection against chilling injury is due to changes in the composition of the fatty acids found in the plant's membranes. Plants grown above their optimal temperature typically have reductions in the degree of saturation of fatty acids as well as decreased lipid/protein ratios (Raison, 1985). These changes in saturation have been ascribed to increases in desaturase activity rather than de novo synthesis (Harris and James, 1969; De la Roche and Andrews, 1973). Cheesebrough (1990) showed a peak level of desaturase activity in mung bean pods after a treatment at 35°C for 10 h. This period approximates to the treatment times in this study that resulted in protection from chilling injury.

A last explanation for the effectiveness of heat-treatment against chilling injury is related to the reduction in ethylene production. A number of studies have suggested that increased levels of ethylene are associated with increased levels of chilling injury. Kosiyachinda and Young (1976) reported that 'Hass' avocados were most chilling sensitive during the climacteric rise and at the climacteric peak. The severity of chilling injury in ripened avocados was more severe in fruit treated with low levels of exogenous ethylene than in those stored in an ethylene-free atmosphere (Chaplin et al., 1983; Lee and Young, 1984). Lee and Young also found that ethylene treatment increased the threshold temperature for chilling injury. In the present study all fruit subjected to heat-treatments had lower peak levels of climacteric ethylene. These data, together with those in the present study suggest that increased ethylene levels make the fruit more susceptible to chilling injury.

Hence the inhibition of ethylene production and a reduction in the activity of enzymes involved in browning processes by heat-treatments may in part explain the reduction in chilling injury in heat-treated fruit (Klein and Lurie, 1991; Lurie et al., 1991). The longer the period of the heat-treatment, the lower was the peak in ethylene production. A further study by Lurie and Klein (1992) using tomatoes showed that fruit heat-treated for three days prior to chilling produced higher amounts of ethylene and had lower levels of chilling injury than controls: this result may be due in part to a fungal infection which occurred in these fruit. However, this result suggests that the effects of ethylene on chilling injury would bear closer examination.

Eaks (1978) also found a negative correlation between increasing time at elevated temperatures and maximum rate of ethylene production. He measured the production of ethylene in fruit ripened either at a constant temperature of 40°C or at 40°C for one or two days and then transferred to 20°C. Fruit held continuously at 40°C failed to produce any climacteric ethylene, whereas fruit heat-treated for one or two days resumed ripening when returned to 20°C. This suggests that there is a reversible inhibition of ethylene production during ripening, at least during the initial stages of the ripening process. Biggs et al. (1988) found that ethylene production in tomatoes was reversibly inhibited above 34°C and was correlated with a rapid decline in ACC synthase activity. This implies that heat may also reversibly denature the enzyme involved in ethylene formation (ACC oxidase). Yang et al. (1990) have also proposed that both ACC synthase and ACC oxidase are inhibited by high temperature stress. In this study there was reduction of climacteric ethylene production for fruit which were heat-treated at both pre-climacteric and climacteric stages, hence it appears that heat-treatment disrupts the mechanisms of ethylene production regardless of the stage of ripening. In contrast, Paull (1990) suggests that reduction of maximum rate of ethylene production with increased heating time implies that heat irreversibly inhibits ethylene synthesis by denaturing the enzymes involved in ethylene biosynthesis.

The difference in amount of chilling injury between the trials may be explained by the influence of the heat-treatments upon ethylene production. In trial 1, heat-treatments of 24 h or more induced heat damage. This may have been caused by the production of wound-induced ethylene which, while being below that of the control level, may still have been at a concentration high enough to bring about the climacteric. In trial 2, heat-treatments possibly affected the enzymes involved in ethylene production. In this case, as the fruit was at the commencement of the climacteric, any wound-induced ethylene would not have caused an advance in the onset of the ethylene climacteric.

5. Conclusion

HSP in avocado mesocarp are maximally expressed after a heat shock at 38°C for 4 h. Further, short heat-treatments of 6–12 h applied to pre-climacteric avocado fruit provides partial protection from chilling injury. However, some of this protection is lost once the climacteric has begun. Consequently, attention must be paid to the

developmental stage of the fruit if heat-treatments are to be applied commercially. It may be preferable to restrict the duration of the heat-treatment to 6 h as this induces the least change in the physiology of the fruit. Further investigation of the crossover point would also be useful to determine the exact treatment time at which the beneficial effects of heat-treatment are outweighed by the detrimental effects. Also, alterations in the treatment regimes may result in further improvements in the degree of protection. The period at elevated temperatures which provides protection from chilling injury correlates well with the minimal conditions required to induce HSP production. This protection may be due to either the production of HSP or by some effect caused by a reduction in ethylene production or sensitivity.

Acknowledgments

Thanks are due to K. Montagu for assistance with computer processing of data, G. Morgan for technical support throughout the project and Prof. Yossi Mizrahi for his criticism of this manuscript.

References

Biggs, M.S., Woodson, W.R. and Handa, A.K., 1988. Biochemical basis of high temperature inhibition of ethylene biosynthesis in ripening tomato fruits. Physiol. Plant., 72: 572–578.

Burke, J.J., Hatfield, J.K., Klein, R.R. and Mullet, J.E., 1985. Accumulation of heat shock proteins in field grown cotton. Plant Physiol., 78: 394–398.

Chaplin, G.R., Wills, R.B.H. and Graham, D., 1982. Objective measurement of chilling injury in the mesocarp of stored avocados. HortScience, 17: 238–239.

Chaplin, G.R., Wills, R.B.H. and Graham, D., 1983. Induction of chilling injury in stored avocados with exogenous ethylene. HortScience, 18: 952–953.

Cheesebrough, T.M., 1990. Decreased growth temperature increases soybean steroyl-acyl carrier protein desaturase activity. Plant Physiol., 93: 555–559.

Cooper, P. and Ho, T.D., 1983. Heat shock proteins in maize. Plant Physiol., 71: 215–222.

De la Roche, I.A. and Andrews, M.K., 1973. Changes in phospholipid composition of a winter wheat cultivar during germination at 2°C and 24°C. Plant Physiol., 51: 468–473.

Eaks, I.L., 1976. Ripening, chilling injury and respiratory response of *Hass* and *Fuerte* avocado fruits at 20°C following chilling. J. Am. Soc. Hortic. Sci., 101: 538–540.

Eaks, I.L., 1978. Ripening, respiration and ethylene production of *Hass* avocado fruits at 20–40°C. J. Am. Soc. Hortic. Sci., 103: 576–578.

Harris, P. and James, A.T., 1969. The effect of low temperatures on fatty acid biosynthesis in plants. Biochem. J., 112: 325–330.

Hatton, T.T., 1990. Reduction in chilling injury with temperature manipulation. In: C.Y. Wang (Editor), Chilling Injury of Horticultural Crops. CRC Press, Boca Raton, Fla., pp. 269–280.

Ho, T.H. and Sachs, M.M., 1989. Stress-induced proteins: characterisation and the regulation of their synthesis. In: M. Marcus (Editor), The Biochemistry of Plants, Vol. 15. Academic Press, New York, N.Y., pp. 347–378.

Hsieh, M.-H., Che, J.-T., Jinn, T.-S., Chen, Y.-M. and Lin, C.-Y., 1992. A class of soybean low molecular weight heat shock proteins. Plant Physiol., 99: 1279–1284.

Hwang, C.H. and Zimmerman, J.L., 1989. The heat shock response of carrot: protein variations between cultured cell lines. Plant Physiol., 91: 552–558.

Kerbel, E.L., Mitchel, F.G. and Mayer, G., 1985. Effect of post harvest treatments for insect control on the quality and market life of avocados. HortScience, 22: 92–94.

Klein, J.D., 1989. Ethylene synthesis in heat treated apples. In: M. Clijsters, R. de Proft, R. Marcell and M. van Poucke (Editors), Biochemical and Physiological Aspects of Ethylene Production in Lower and Higher Plants. Kluwer Academic Press, Dordrecht, pp. 184–190.

Klein, J.D. and Lurie, S., 1990. Prestorage heat treatment as a means of improving poststorage quality of apples. J. Am. Soc. Hortic. Sci., 115: 265–269.

Klein, J.D. and Lurie, S., 1991. Postharvest heat treatment and fruit quality. Postharvest News Inf., 2: 15–19.

Klein, J.D. and Lurie, S., 1992. Heat treatments for improved postharvest quality of horticultural crops. Hortic. Tech., 2: 316–320.

Klein, J.D., Lurie, S. and Ben-Arie, R., 1990. Quality and cell wall components of Anna and Granny Smith apples treated with heat, calcium and ethylene. J. Am. Soc. Hortic. Sci., 115: 954–958.

Kosiyachinda, S. and Young, R.E., 1976. Chilling sensitivity of avocado fruit at different stages of the respiratory climacteric. J. Am. Soc. Hortic. Sci., 101: 665–667.

Laemmli, U., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680–685.

Lee, S.K. and Young, R.E., 1984. Temperature sensitivity of avocado fruit in relation to ethylene treatment. J. Am. Soc. Hortic. Sci., 109: 689–692.

Lizada, M.C.C. and Yang, S.F., 1979. A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Anal. Biochem., 100: 140–145.

Lurie, S. and Klein, J.D., 1990. Heat treatment of ripening apples: differential effects on physiology and biochemistry. Physiol. Plant., 78: 181–186.

Lurie, S. and Klein, J.D., 1991. Acquisition of low-temperature tolerance in tomatoes by exposure to high temperature stress. J. Am. Soc. Hortic. Sci., 116: 1007–1012.

Lurie, S. and Klein, J.D., 1992. Ripening characteristics of tomatoes stored at 12°C and 2°C following prestorage heat treatments. Sci. Hortic., 51: 55–64.

Lurie, S., Klein, J.D. and Ben-Arie, R., 1991. Prestorage heat treatment delays development of superficial scald on 'Granny Smith' apples. HortScience, 26: 166–167.

Mansfield, M.A. and Key, J.L., 1987. Synthesis of low molecular weight proteins in plants. Plant Physiol., 84: 1007–1017.

Paull, R.E., 1990. Postharvest treatments and fruit ripening. Postharvest News Inf., 1: 355–363.

Raison, J.K., 1985. Alterations in the physical properties and thermal response of membrane lipids: correlations with acclimation to chilling injury and high temperatures. In: J.B. St. John, E. Berlin and P.C. Jackson (Editors), Beltsville Symposium in Agricultural Research, Vol. 9. Rowman and Allanheld, N.J., pp. 383–401.

Tsuji, M., Harakawa, H. and Komiyama, Y., 1984. Changes in shelf life and quality of plum fruit during storage at high temperatures. J. Jap. Soc. Hortic. Sci., 52: 469–473.

Vierling, E., 1991. The roles of heat shock proteins in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol., 42: 579–620.

Wang, C.Y., 1990. Alleviation of chilling injury of horticultural crops. In: C.Y. Wang (Editor), Chilling Injury of Horticultural Crops. CRC Press, Boca Raton, Fla., pp. 269–280.

Wills, R.B.H., McGlasson, W.B., Graham, D., Lee, T.H. and Hall, E.G., 1989. Postharvest: An Introduction to the Physiology and Handling of Fruit and Vegetables. BSP Professional Books, Oxford, 174 pp.

Yang, R.-F., Cheng, T.-S. and Shewfelt, R.L., 1990. The effect of high temperature and ethylene treatment on the ripening of tomatoes. J. Plant Physiol., 136: 368–372.

Zauberman, G., Schiffmann-Nadel, M. and Yanko, U., 1977. The response of avocado fruits to different storage temperatures. HortScience, 12: 353–354.