Europ.J.Hort.Sci., **70** (6). S. 271–277, 2005, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

Changes in Total Proteins Related to Chilling Injury of Nectarine

A. Uthairatanakij[1,2], P. Holford[1] and B. McGlasson[1]
([1]Centre for Horticulture, Plant Sciences, University of Western Sydney, Penrith South DC, Australia and [2]King Mongkut's University of Technology Thonburi, Bangkok, Thailand)

Summary

Low-temperature disorders in nectarines (*Prunus persica*) can be delayed by storage in atmospheres containing elevated CO_2; however, this benefit is limited to a few cultivars. The storage of produce at low temperatures itself leads to changes in protein synthesis; therefore, this study examined the applicability of 2-D electrophoresis to assess differences in protein production in cultivars of nectarine that either do ('Arctic Snow') or do not ('Red Gold') respond to elevated CO_2 levels during storage. In addition, the effect of harvest maturity and their response to added ethylene was assessed. Fruit of the two cultivars were either ripened immediately after harvest at 20 °C before protein extraction or stored in air, air + CO_2, air + ethylene or air + CO_2 + ethylene then ripened at 20 °C before protein extraction. 2-D PAGE showed that the expression of approximately 30 proteins varied among cultivars and treatments. Two proteins showed clear differences in expression in both cultivars between fruit ripened immediately after harvest and those that had been stored. A second set of differences in protein expression was found in fruit of 'Red Gold' only and involved 12 proteins whose expression was either reduced or increased by storage: these proteins did not vary in expression in 'Arctic Snow'. A third set of differences in protein expression was observed in 'Arctic Snow' only in atmospheres containing ethylene. Several proteins were down-regulated by ethylene and this down-regulation was negated by the addition of CO_2 to the storage atmosphere. Thus, differences in protein expression can be found between cultivars of nectarines that do or do not respond to the addition of CO_2 to the storage atmosphere and in the cultivar that is known to respond to CO_2, this gas was able to antagonise protein changes mediated by ethylene.

Zusammenfassung

Änderungen der Proteinzusammensetzung in Abhängigkeit von Kälteschäden bei Nektarinen. Bei einigen wenigen Nektarinensorten (*Prunus persica*) können Kälteschäden durch die Zufuhr von CO_2 zur Lageratmosphäre hinausgezögert werden. Die Lagerung der Produkte bei niedrigen Temperaturen bewirkt allein schon Änderungen in der Proteinsynthese. Aus diesem Grund wird hier die Anwendbarkeit der 2-D-Elektrophorese zur Erfassung der Unterschiede in der Proteinsynthese sowohl an Nektarinensorten, die auf die Erhöhung des CO_2-Gehalts während der Lagerung reagieren ('Arctic Snow') als auch an solchen, die nicht reagieren ('Red Gold') untersucht. Zusätzlich wird die Auswirkung der Reifung der Früchte und ihre Reaktion auf Ethylengaben ermittelt. Die Nachreife bei 20 °C vor der Proteinextraktion erfolgte bei Früchten beider Sorten entweder sofort nach der Ernte oder im Anschluss an eine Lagerphase in Normalluft, in Normalluft + CO_2, in Normalluft + Ethylen oder in Normalluft + CO_2 + Ethylen. Die 2-D PAGE zeigte, dass die Expression von annähernd 30 Proteinen in Abhängigkeit von der Sorte und der Behandlung variierte. Zwei Proteine zeigten in beiden Sorten deutliche Expressionsunterschiede in Abhängigkeit davon, ob sie direkt nach der Ernte oder im Anschluss an eine Lagerphase nachreiften. Eine zweite Gruppe wurde nur bei Früchten von 'Red Gold' gefunden und umfasste 12 Proteine, deren Expression durch die Lagerung entweder verringert oder gesteigert wurde: die Expression dieser Proteine variierte bei 'Arctic Snow' nicht. Eine dritte Gruppe mit unterschiedlicher Proteinexpression wurde nur in Früchten von 'Arctic Snow' beobachtet, die in Atmosphäre mit Ethylenzugabe gelagert worden waren. Zahlreiche Proteine wurden durch Ethylen abreguliert und diese Abregulation wurde durch die Zugabe von CO_2 zur Lageratmosphäre wieder aufgehoben. Folglich können Unterschiede in der Proteinexpression zwischen Nektarinensorten, die auf die Zugabe von CO_2 zur Lageratmosphäre reagieren oder nicht, gefunden werden und bei Sorten, deren vorhandene Reaktion auf CO_2 bekannt ist, kann dieses Gas als Antagonist bei Ethylen-verursachten Proteinveränderungen wirken.

Key words. *Prunus persica* – postharvest – storage atmosphere – chilling injury

Introduction

WADE (1981) showed that low-temperature disorders in 'JH Hale' peach and 'Fantasia' nectarine fruit can be delayed if the fruit are stored in atmospheres containing elevated CO_2 levels. However, subsequent research has shown that these benefits are limited to a few cultivars; hence, there are no general recommendations for the composition of the storage atmosphere for peaches and nectarines. There are many studies on the action of elevated CO_2 in stored fruit that have suggested different mechanisms by which CO_2 has its effect and MATHOOKO (1996) found that elevated CO_2 can prolong the storage life of produce by either stimulating or inhibiting ethylene production. However, its effects depend on the commodity and its cultivar, physiological age and initial quality, and on the temperature and duration of storage (KADER 1986; KUBO et al. 1990; PESIS et al. 1994).

Low temperatures themselves lead to the synthesis of new proteins and a decrease in the levels of others (CATTIVELLI and BARTELS 1992). For example, MONTERO et al. (1995) reported a reduction in the accumulation of certain polypeptides associated with the ripening process during low-temperature storage of cherimoya fruit. ZAMORANO and MERODIO (1992) demonstrated that the accumulation of certain polypeptides in avocado was delayed by removal of ethylene during low-temperature storage. High CO_2 induced changes in several enzymes in pear and apple fruit (TIAN et al. 1994) and cherimoya (ASSIS et al. 2001) and induced two genes encoding stress-related proteins in tomato fruit (ROTHAN et al. 1997). Whatever the mechanism, the effects of high CO_2 during storage are likely to be related to changes in protein synthesis. However, most of the studies have concentrated on particular proteins associated with ethylene biosynthesis and on enzymes associated with cell wall changes. Those that have looked for more general changes in protein expression have used one-dimensional protein separations. In addition to CO_2, ethylene added continuously during storage can delay the development of low temperature disorders in some cultivars (DONG et al. 2001; PALOU et al. 2003). Therefore, the aim of this study was to examine the applicability of two-dimensional (2-D) electrophoresis to assess differences in protein production in cultivars of nectarine (*Prunus persica* (L.) Batsch) that either do or do not respond to elevated CO_2 levels during cool storage and their response to added ethylene.

Materials and Methods

Source of fruit and storage treatments

Fruit of the nectarine cultivars, 'Red Gold' and 'Arctic Snow', were harvested from commercial orchards at Glenorie and Picton, NSW, Australia, and transported to the Postharvest Laboratory, Centre for Horticulture and Plant Science, University of Western Sydney, Richmond. 'Red Gold' was harvested at early maturity; 'Arctic Snow' was harvested at early and mid stages of maturity. Assessment of maturity was based on skin ground colour and flesh firmness. Five treatments were applied. In the first, fruit were allowed to ripen normally at 20 °C for 5 days for mid maturity fruit and 8 days for early maturity fruit. In the others, all fruit were placed in open mesh bags, then enclosed for three weeks in steel drums continuously ventilated at 0 °C with humidified air, air + 15 % CO_2, air + 15 μL L^{-1} ethylene, or air + 15 % CO_2 + 15 μL L^{-1} ethylene. Fruit were then allowed to ripen at 20 °C for 5 or 8 days. Tissue samples were taken from the nine fruit in each treatment, combined and then frozen in liquid N_2 and stored in plastic bags at –80 °C pending protein analysis.

Protein extraction, assay and separation

The proteins from 5.0 g of frozen nectarine tissue were extracted in a phenol buffer using the method of ABDI et al. (1998) and protein concentrations in extracts assayed following RAMAGLI (1999). Two extractions and separations were made for each treatment.

First-dimension isoelectric focusing (IEF)

First-dimension IEF was carried out using a Protean IEF Cell (Bio-Rad) in 170 mm immobilised pH-gradient gel (IPG) strips (Bio-Rad) rehydrated in 400–450 μL rehydration buffer (8 M urea, 0.5 % (v/v), Biolyte 3/10 (Bio-Rad), 2 % (w/v) 3-3-cholamidopropyl dimethylammonio-l-propane sulfate, 1 % (w/v) dithiothreitol, bromophenol blue (trace) and sample (~200 μg). Rehydration proceeded overnight (~12 h) under active (50 V) conditions at 20 °C. Paper electrode wicks soaked in deionized water were applied to the strips just before focusing and the proteins were then separated and focused using the following program: 300 V, 3 h; 1,000 V, 2–4 h; 10,000 V up to 60,000 V h. The strips were kept in the rehydration tray at –20 °C before equilibration and the second-dimension separation.

Equilibration for SDS-PAGE

Equilibration buffers were prepared using 6.0 M urea, 30 % (v/v) glycerol, 2.0 % (w/v) sodium dodecylsulfate (SDS), 0.375 M Tris·base (pH 8.8). To this stock solution, 200 mg of dithiothreitol or 300 mg of iodoacetamide per 10 mL of equilibration buffer were added just before use to form Equilibration Buffer I, and II, respectively. IPG gel strips were placed face up in the rehydration tray gel, which contained 5 mL Equilibration Buffer I and then gently shaken for 15–20 min. The buffer was replaced with Equilibration Buffer II and shaken for a further 15–20 min. After the second equilibration, the IPG gel strips were rinsed with upper buffer (see below) and placed on filter paper on one edge for a few minutes to drain off excess liquid. After equilibration, the IPG gel strips were immediately loaded on to second-dimension gels.

Second-dimension SDS-PAGE

SDS-PAGE gels were prepared according to LAEMMLI (1970), but no stacking gels were used. The separation was performed in 1.5 mm thick slab gels (12 % acrylamide/bis (37.5:1), 0.375 M Tris-HCl (pH 8.8), 1 % so-

dium dodecylsulfate. These gels were overlaid with the IPG strips and sealed with 0.5 % low melting point agarose containing a trace of bromophenol blue. The separations were performed in a Protean II xi Multi-Cell electrophoresis tank (Bio-Rad). For the separation, a 10-times solution of Tris·glycine–SDS electrode buffer was prepared from 25 mM Tris·base, 192 mM glycine and 0.1 % (w/v) SDS. A 1-times concentration of this solution was used as the lower (cathode) buffer and a 2-times concentration was used as the upper (anode) buffer. Proteins were separated using constant current in a 2-step process: 15 mA per gel for 30 min, followed by 40 mA per gel. Electrophoresis was performed at 12 °C and continued until the dye front had reached the bottom line of the gel (~6 h).

Protein visualisation and assessment of expression

The 2-D gels were fixed in 40 % (v/v) 95 % ethanol and 10 % (v/v) acetic acid and shaken for 60–90 min. The gels were then rinsed with distilled water for 5 min, placed in 250 mL 30 % (v/v) 95 % ethanol, 6.8 % sodium acetate, 0.2 % sodium thiosulfate and 1 % glutaraldehyde 25 % and incubated overnight. The next day, the incubation solution was removed and the gels were washed for 3 times 1 h with distilled water. Each gel was stained with 250 mL of 0.2 % (w/v) silver nitrate and 0.02 % (v/v) 37 % formaldehyde for 45 min. After staining, the gels were washed with distilled water for 3-times 15 min and then soaked in 3 % (w/v) sodium carbonate, 0.05 % (v/v) 37 % formaldehyde and 0.002 % (w/v) sodium thiosulfate for 15–20 min to visualise the proteins. The reaction was stopped by adding 1.5 % acetic acid solution for 10 min, the gels were washed with distilled water, sealed in plastic bags and stored at 4 °C.

Gels were photographed and the photographs were inspected by eye to detect proteins whose expression changed. Expression was assessed as '–' = no expression, '+' = weak expression, '++' = strong expression, '–/+' = no expression on one gel and weak expression on the other. Two gels were assessed for each combination of cultivar, harvest maturity, and treatment.

Results

Two major differences in protein expression were detected among fruit ripened immediately after harvest and those ripened after cold storage. Protein A was only expressed in fruit that had been stored (Fig. 1 and Table 1) and was expressed in all stored samples of fruit of both cultivars no matter which treatment they had been exposed to; however, the degree of expression varied between samples. The pattern of expression of protein L1 was the opposite to that of A. In general, L1 was expressed only in fruit ripened immediately after harvest and not in fruit that had been chilled. Exceptions to this were observed in fruit of 'Red Gold' stored in CO_2, where the protein was strongly expressed, and in mid-maturity fruit of 'Arctic Snow' stored in ethylene, where 1 out of the 2 samples weakly expressed this protein.

The second set of differences in protein expression concerns fruit of 'Red Gold' only, in which there were again differences in expression pattern between fruit ripened immediately after harvest and stored fruit. Proteins falling into this category were B2, D1, E2, G, H, I0, I1, I2, I3, K, N, and P2 (Fig. 1 and Table 1). These proteins could be separated into 2 subgroups: (i) those whose expression was reduced by storage (B2, E2, I0, and K) and (ii) those whose expression was increased by storage (D1, G, H, I1, I2, I3, and N). F1 may belong to the former subgroup as it was highly expressed in fruit ripened without storage, it was not expressed in fruit stored in air, it was poorly expressed in fruit stored in CO_2 or ethylene, but it was highly expressed in fruit stored in CO_2 + ethylene.

The third set of differences in protein expression was observed in atmospheres containing ethylene, at least to some extent. These differences occurred in 'Arctic Snow' only. Several proteins were down-regulated by ethylene (G, I0, I1, I2 and I3) (Fig. 2 and Table 1). Down-regulation of this group of proteins was negated by the addition of CO_2 to the storage atmosphere in

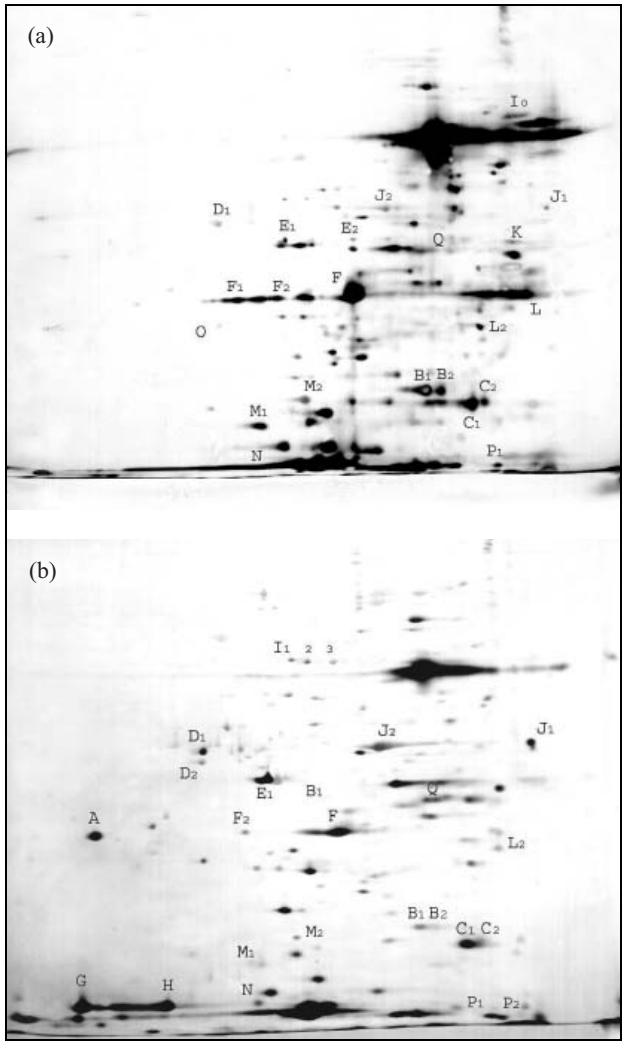


Fig. 1. Separation of proteins from 'Red Gold' nectarines harvested at early maturity. The fruits were either (a) ripened immediately after harvest at 20 °C for 8 days or (b) were stored in air for 3 weeks at 0 °C and ripened at 20 °C for 8 days before proteins were extracted.

Table 1. Protein expression in two cvs of nectarines ripened at 20 °C immediately after harvest (1) or after storage at 0 °C for 3 weeks in atmospheres containing air (2), air + 15 % CO_2 (3), air + C_2H_4 (4) or air + CO_2+ C_2H_4 (5). Fruits of 'Red Gold' were harvested at early maturity (RG-E) and those of 'Arctic Snow' at early and mid maturities (AS-E, AS-M). The data were assessed using the following scale: '–' = no expression, '+' = weak expression, '++' = strong expression, '–/+' = no expression on one gel and weak expression on the other.

		Treatment							Treatment				
Fruit-Maturity	Protein	(1)	(2)	(3)	(4)	(5)	Fruit-Maturity	Protein	(1)	(2)	(3)	(4)	(5)
RG-E	A	–	++	++	++	++	RG-E	I1	–	++	++	++	–
AS-E		–	+	+	++	++	AS-E		–	+	+	–	++
AS-M		–	++	+	+	++	AS-M		++	++	++	–/+	–
RG-E	B1	++	+	++	++	+	RG-E	I2	–	++	++	++	–
AS-E		+	+	+	+	+	AS-E		–	+	+	–	++
AS-M		++	+	++	+	+	AS-M		++	++	++	–/+	–
RG-E	B2	++	+	+	+	+	RG-E	I3	–	++	++	++	–
AS-E		+	+	+	–	+	AS-E		–	+	+	–	++
AS-M		+	+	+	+	+	AS-M		++	++	++	–/+	–
RG-E	C1	++	++	++	++	++	RG-E	J1	++	++	++	++	++
AS-E		+	++	+	++	++	AS-E		+	+	+	+	+
AS-M		++	++	++	++	+	AS-M		+	+	+	++	++
RG-E	C2	++	+	++	+	+	RG-E	J2	++	++	++	++	+
AS-E		+	+	+	++	–	AS-E		+	+	+	+	++
AS-M		+	+	+	+	++	AS-M		++	+	+	–/+	+
RG-E	D1	–	++	++	++	++	RG-E	K	++	–	–	–	–
AS-E		+	+	+	+	++	AS-E		–	–	–	–	–
AS-M		++	++	++	+	–	AS-M		–	–	–	–	–
RG-E	D2	++	+	+	+	+	RG-E	L1	++	–	++	–	–
AS-E		+	+	+	+	++	AS-E		+	–	–	–	–
AS-M		++	++	++	+	–	AS-M		+	–	–	–/+	–
RG-E	E1	++	++	++	++	++	RG-E	L2	++	++	++	++	–
AS-E		++	++	++	++	++	AS-E		+	++	+	++	++
AS-M		++	++	++	++	+	AS-M		++	+	++	++	++
RG-E	E2	++	–	–	–	–	RG-E	M1	++	+	+	+	+
AS-E		+	+	+	+	+	AS-E		+	++	+	+	++
AS-M		+	+	+	+	+	AS-M		+	++	+	+	+
RG-E	F	++	++	++	++	++	RG-E	M2	+	+	++	+	+
AS-E		+	++	++	++	++	AS-E		+	+	+	++	+
AS-M		++	++	++	++	++	AS-M		++	+	++	+	+
RG-E	F1	++	–	+	+	++	RG-E	N	–	++	++	++	+
AS-E		++	–	–	++	–	AS-E		–	–	–	–	–
AS-M		++	++	++	++	++	AS-M		–	–	–	–	++
RG-E	F2	++	++	++	++	++	RG-E	O	++	++	++	++	++
AS-E		++	++	++	++	++	AS-E		+	+	–	+	+
AS-M		++	+	++	++	++	AS-M		++	++	++	–/+	–
RG-E	G	–	++	++	++	++	RG-E	P1	++	++	++	++	++
AS-E		++	++	++	–	++	AS-E		+	+	+	++	++
AS-M		++	++	++	–/+	–	AS-M		++	++	++	++	++
RG-E	H	–	++	++	++	++	RG-E	P2	–	++	++	++	++
AS-E		+	+	+	–	–	AS-E		+	+	++	++	–
AS-M		++	–	++	–/+	–	AS-M		+	+	++	++	++
RG-E	I0	++	–	–	–	++	RG-E	Q	+	+	++	+	+
AS-E		+	–	++	–	–	AS-E		+	+	+	+	++
AS-M		++	–	++	++	++	AS-M		++	++	+	+	–

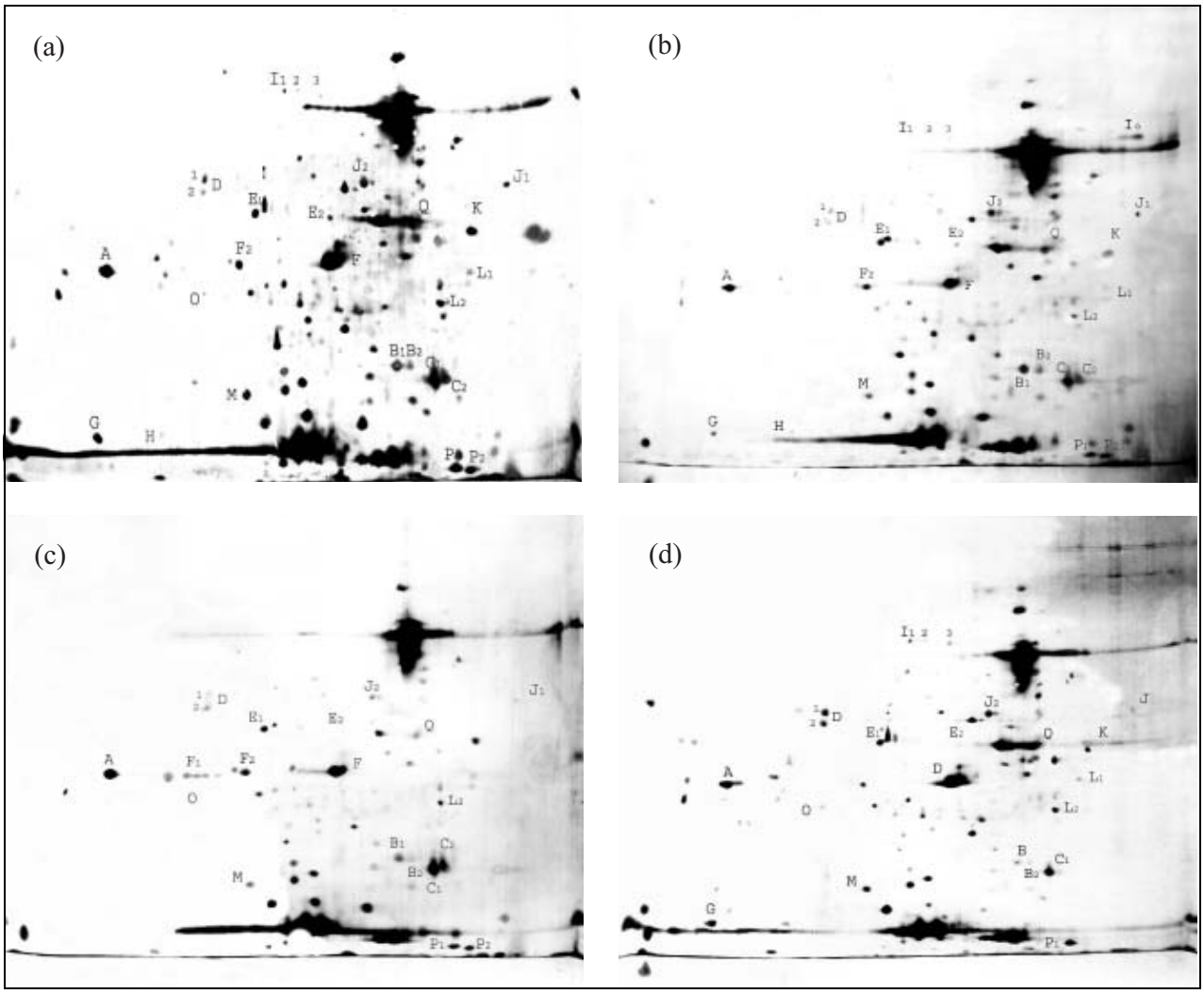


Fig. 2. Separation of proteins from 'Arctic Snow' nectarines harvested at early maturity. The fruits were stored for 3 weeks at 0 °C in (a) humidified air, (b) air + CO_2, (c) air + ethylene or (d) air + CO_2 + ethylene and then ripened at 20 °C for 5 days before protein extraction.

the early maturity fruit, except for H, on which the addition of CO_2 had no effect. Three other proteins (B2, J1 and J2) were also affected by ethylene. B2 was down-regulated by storage in ethylene but only in the early maturity fruit (Table 1). J2 was down-regulated by storage in ethylene but only in mid-maturity fruit. Expression of J1 increased in both ethylene and ethylene + CO_2 in mid maturity fruit but not in early maturity fruit.

One other apparent trend in protein expression was the variation in expression that occurred due to storage in CO_2 + ethylene (Table 1). For a number of proteins (C2, D2, N, O and Q), this treatment induced far more variation in protein expression than the other 4 treatments. Within this treatment, protein expression varied from none to high; however, there was no consistent pattern to the expression among these proteins. For 9 other proteins (C1, C2, D2, E1, F, F2, L2, M1, M2), differences in expression were also detected among the treatments; however, the level of variation was less than for C2, D2, N, O and Q, and there was no clear pattern associated with cultivar or treatment.

Discussion

Several studies have examined changes in protein expression due to postharvest storage practices. However, most have concentrated on particular proteins and those that have looked for more general changes in protein expression have used 1-D protein separation. This study demonstrates the applicability of 2-D electrophoresis for studying changes in protein expression induced in produce by cool storage. The study has detected 30 proteins whose levels of expression varied between stored and unstored fruit. It has also detected differences in protein expression between cultivars of nectarine, between fruit of different maturities and among postharvest treatments.

The expression of proteins A and L1 is clearly altered by cool storage. Changes in the expression of these proteins were found in both cultivars studied and, for 'Arctic Snow', in fruit of both maturities. 2-D PAGE has also identified both the down-regulation and the *de novo* synthesis of proteins in relation to cool storage in cherimoya

fruit (MONTERO et al. 1995). The nature of these proteins and their role in the biology of fruit ripening are not yet determined.

This study has also shown differences in protein expression between the 2 cultivars in response to cool storage and the different storage atmospheres. More proteins changed their expression in 'Red Gold' than in 'Arctic Snow'. The proteins, whose expression changed in 'Red Gold' changed under all storage conditions, and this cultivar was found to be relatively insensitive to the gaseous composition of the atmosphere (UTHAIRATANAKIJ 2004). The relationship between overall protein expression and low-temperature disorders needs further study, and a number of hypotheses need to be tested. Firstly, cultivars that are more plastic in their protein expression may be better able to withstand cool storage. Secondly, and in contrast to the first hypothesis, the higher number of proteins whose expression changes during cool storage may reflect a greater intolerance of cool temperatures and a greater perturbation in their physiology. Lastly, there may be specific proteins whose expression is related to tolerance or intolerance of cool storage. The severity of low temperature disorders in terms of bleeding and juice content were greater in 'Arctic Snow' than in 'Red Gold' (UTHAIRATANAKIJ 2004). This result would seem to discount the second hypothesis. However, a greater number of cultivars need to be studied, and the changes in protein production need to be followed in fruit stored for different durations that do or do not display low-temperature disorders.

Although 'Arctic Snow' did not alter its protein expression to the same extent as 'Red Gold', those proteins whose expression did change did so in response to the addition of ethylene to the storage atmosphere. These changes were most pronounced in early maturity fruit. Some studies have shown that the addition of ethylene to the storage atmosphere can reduce the incidence of low-temperature disorders (DONG et al. 2001; ZHOU et al. 2001); however, the effects of this gas have not been consistent among varieties (PALOU et al. 2003). This study shows that ethylene in the storage atmosphere can influence protein expression, and changes in protein expression provide a mechanism by which this gas has its effect. However, the effects of the gas depend on the maturity of the fruit when they are placed in storage.

There was 3 days difference in age between the early and mid-maturity fruit. However, this small difference was enough to result in different patterns of protein expression resulting from exposure to ethylene in 'Arctic Snow'. The differences between the cultivars and maturities identified in this study may explain the discrepancies between other studies in the response to added ethylene in storage in terms of the development of low-temperature disorders. In particular, the proximity of the fruit to their climacteric may determine whether they will respond to the addition of ethylene.

Although 'Arctic Snow' responded to ethylene in terms of changes in protein synthesis, these changes do not seem to be related to the fruit's ability to withstand low temperatures. The addition of ethylene to the storage atmosphere had no significant effect on the development of low-temperature disorders in 'Arctic Snow' (UTHAIRATANAKIJ 2004). Indeed, 'Red Gold' responded the most to the addition of ethylene, as the ethylene-treated fruit maintained their ability to soften better than fruit in the other treatments (UTHAIRATANAKIJ 2004). No changes in protein production could be seen that related to ethylene treatment. Therefore, it is still to be determined whether ethylene has its effect by inducing changes in protein synthesis.

Several studies have shown reductions in protein expression due to the addition of CO_2 to the postharvest atmosphere (GORNY and KADER 1996; DEL CURA et al. 1996; ROTHAN et al. 1997). In this study, the addition of CO_2 to the storage atmosphere had little effect on protein expression. Two proteins (H and I) showed increases in expression due to the addition of CO_2 to the storage atmosphere of 'Arctic Snow'. However, the effect was seen only in the mid maturity fruit and was not apparent in the CO_2 + ethylene treatment. MATHOOKO et al. (1995) reported that CO_2 stress induced ACC synthase and ACC oxidase activity in cucumber. It is possible that the two new proteins relate to CO_2 stress. CO_2 did seem to be able to antagonise protein changes mediated by ethylene. CO_2 may affect metabolic changes through altering intracellular pH or the levels of cofactors or intermediates (KADER 1986; SIRIPHANICH and KADER 1986; LANGE and KADER 1997; HOLCROFT and KADER 1999). Therefore, it needs to be determined whether the changes in protein expression caused by CO_2 are the result of such factors or a direct result of effects on transcription.

References

ABDI, N., W.B. MCGLASSON, P. HOLFORD and M. WILLIAMS 1998: Responses of climacteric and suppressed-climacteric plums to treatment with propylene and 1-methylcyclopropene. Postharvest Biol. Technol. **14**, 29–39.

ASSIS, J.S., R. MALDONADO, T. MUNOZ, M.I. ESCRIBANO and C. MERODIO 2001: Effect of high carbon dioxide concentration on PAL activity and phenolic contents in ripening cherimoya fruit. Postharvest Biol. Technol. **23**, 33–39.

CATTIVELLI, L. and D. BARTELS 1992: Biochemistry and molecular biology of cold-inducible enzymes and proteins in higher plants. In: WRAY, J.L (ed.): Inducible plant proteins. Society for Experimental Biology Seminar Series **49**. Cambridge University Press, pp. 267–288.

DEL CURA, B., M.I. ESCRIBANO, J.P. ZAMORANO and C. MERODIO 1996: High carbon dioxide delays post-harvest changes in RuBPCase and polygalacturonase-related protein in cherimoya peel. J. Am. Soc. Hort. Sci. **121**, 735–739.

DONG, L., H. ZHOU, L. SONEGO, A. LERS and S. LURIE 2001: Ethylene involvement in the cold storage disorder of 'Flavortop' nectarine. Postharvest Biol. Technol. **23**, 105–115.

GORNY, J.R. and A.A. KADER 1996: Regulation of ethylene biosynthesis in climacteric apple fruit by elevated CO_2 and reduced O_2 atmospheres. Postharvest. Biol. Technol. **9**, 311–323.

HOLCROFT, D.M. and A.A. KADER 1999: Carbon dioxide induced changes in color and anthocyanin synthesis of stored strawberry fruit. HortScience **34**, 1244–1248.

KADER, A.A. 1986: Biochemical and physiological basis for effects of controlled and modified atmospheres on fruit and vegetables. Food Technol. **40**, 99–100.

KUBO, Y., A. INABA and R. NAKAMURA 1990: Respiration and ethylene production in various harvested crops held in CO_2-enriched atmospheres. J. Am. Soc. Hort. Sci. **115**, 975–978.

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

LANGE, L.D. and A.A. KADER 1997: Elevated carbon dioxide exposure alters intercellular pH and energy change in avocado fruit tissue. J. Am. Soc. Hort. Sci. **122**, 253–257.

MATHOOKO, F.M., Y. KUBO, A. INABA and R. NAKAMURA 1995: Characterization of the regulation of ethylene biosynthesis in tomato fruit by carbon dioxide and diazocyclopentadiene. Postharvest Biol. Technol. **5**, 221–233.

MATHOOKO, F.M. 1996: Regulation of ethylene biosynthesis in higher plants by carbon dioxide. Postharvest Biol. Technol. **7**, 1–26.

MONTERO, L.M., M.I. ESCRIBANO, J.L. DE LA PLAZA and C. MERODIO 1995: Chilling temperature storage induces changes in protein patterns and protease activity in cherimoya fruit. Postharvest Biol. Technol. **5**, 251–260.

PALOU L, C.H. CRISOSTO, D. GARNER and L.M. BASINAL 2003: Effect of continuous exposure to exogenous ethylene during cold storage on postharvest decay development and quality attributes of stone fruits and table grapes. Postharvest Biol. Technol.**27**, 243–254.

PESIS, E., C. AMPUNPONG, B. SHUSIRI and E.W. HEWETT 1994: Enhancement of ethylene and CO_2 production in apple fruit following short-term exposure to high CO_2. Postharvest Biol. Technol. **4**, 309–317.

RAMAGLI, L.S. 1999: Quantifying protein in 2-D PAGE solubilization buffers. In: LINK, A.J. (ed). Methods in Molecular Biology Vol. **112**. Humana Press Inc., Totowa, NJ, USA, pp. 99–103.

ROTHAN, C., S. DURET, C. CHEVALIER and P. RAYMOND 1997: Suppression of ripening-associated gene expression in tomato fruits subjected to a high CO_2 concentration. Plant Physiol. **114**, 255–263.

SIRIPHANICH, J. and A.A. KADER 1986: Changes in cytoplasmic and vacuolar pH in harvested lettuce as influenced by CO_2. J. Am. Soc. Hort. Sci. **111**, 73–77.

TIAN, M.S., E.W. HEWETT and R.E. LILL 1994: Effects of carbon dioxide on ethylene-forming enzyme in Japanese pear and apple. Postharvest Biol. Technol. **4**, 1–12.

UTHAIRATANAKIJ, A. 2004: Responses of nectarines to atmospheres containing high carbon dioxide concentrations. PhD thesis, University of Western Sydney, NSW, Australia.

WADE, N.L. 1981: Effects of storage atmosphere, temperature, and calcium on low-temperature injury of peach fruit. Sci. Hort. **15**, 145–154.

ZAMORANO, J.P. and C. MERODIO 1992: Influence of ethylene removal on the protein pattern of avocado fruits at low temperature storage. Acta Hort. **343**, 275–277.

ZHOU, H., L. DONG, R. BEN-ARIE and S. LURIE 2000: The role of ethylene in the prevention of chilling injury in nectarines. J. Plant Physiol. **158**, 55–61.

Received November 2, 2004 / Accepted August 8, 2005

Addresses of authors: Apiradee Uthairatanakij, Centre for Horticulture and Plant Sciences, University of Western Sydney, Locked Bag 1797, Penrith South DC, NSW 1797, Australia and King Mongkut's University of Technology Thonburi (Bangkhuntien), 83 Moo 8 Tientalay 25 Rd., Thakham, Bangkhuntien, Bangkok 10150, Thailand, and Paul Holford (corresponding author) and Barry McGlasson, Centre for Horticulture and Plant Sciences, University of Western Sydney, Locked Bag 1797, Penrith South DC, NSW 1797, Australia, e-mail: p.holford@uws.edu.au.