ELSEVIER

Environmental and Experimental Botany 38 (1997) 1–6

Environmental and Experimental Botany

Fourier transform infrared spectra of zucchini squash stored at chilling or non-chilling temperatures

J. George Buta,* Ling Qi,† Chien Yi Wang

Horticultural Crops Quality Laboratory, PSI, ARS, U.S. Department of Agriculture, 10300 Baltimore Avenue, Beltsville, MD 20705-2350, USA

Received 15 November 1995; revised 15 November 1996

Abstract

Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that might be suitable.

Buta J.G., Qi L. and Wang, C.Y. *Fourier transform infrared spectra of zucchini squash stored at chilling or non-chilling temperatures.* Environmental and Experimental Botany **38,** 1–6, 1997.—Fourier transform infrared spectroscopy (FTIR) was used to investigate spectral changes in the epidermis of zucchini squash resulting from low temperature storage-chilling (5°C) or non-chilling (15°C). Increased levels of fluid (water) were found in the tissue after 2 days of 5°C storage. This increase was reversed to harvest levels when chilled squash were warmed to room temperature for 1 day. After 3 days at 5°C and 1 day at room temperature, a further increase in fluid levels was found in the epidermis. Squash chilled for 3 days were apparently beyond recovery as indicated by spectral changes, although visual symptoms of chilling injury were not apparent until another 3 days of exposure to 5°C. Spectra of epidermis tissues from squash stored at 15°C indicated a pattern of increased non-reversible fluid accumulation when storage was prolonged. These results suggest that FTIR spectroscopy may be a rapid way to detect changes in chilled tissues before the eventual appearance of visible symptoms. © 1997 Elsevier Science B.V.

Keywords: *Cucurbita pepo*; chilling injury; Fourier transform infrared spectroscopy; zucchini squash

1. Introduction

Infrared (IR) spectroscopy has been used recently to investigate biochemical changes in viable plant cells as well as obtain quantitative information about the functional groups of chemical compounds in plant cells [5]. Fourier transform IR (FTIR) investigations have been made of structural changes of proteins and membranes in intact cells [8]. Fourier transform IR allowed the whole IR spectral range to be obtained simultaneously providing rapid and accurate measurements of biological samples [2].

FTIR had been used earlier to investigate

*J.G. Buta Hort Crops Qual. Lab., Bldg 002, BARC-W 10300 Baltimore Ave., Beltsville, MD 20705, USA. Tel: 301 504 5598; fax: 301 504 5107.

†Visiting Scientist from Tianjin Academy of Agricultural Sciences, Tianjin, China

PII S0098 - 8472 (96) 01059 - 3

whether any spectral changes in zucchini squash tissue could be detected after the squash had been stored at low temperatures [1]. Zucchini squash is one among many fruits and vegetables that are susceptible to chilling injury (CI) caused by storage at low temperature such as 5°C. The damage was visually apparent after several days of storage at the chilling temperature or after subsequent warming to ambient temperatures. Visual symptoms of CI were characterized by a water-soaked surface appearance, browning and tissue collapse [7]. Earlier effects of CI prior to the appearance of the visual symptoms were found to be increased membrane permeability, ion and solute leakage, and exudation of fluid into intracellular spaces [4]. Increased fluid (water) leakage from chilled zucchini cortex tissue was found to occur after 5°C storage when measured by FTIR in an earlier study [1]. Little fluid leakage occurred from cortex tissue stored at a low but non-chilling temperature (15°C). The present study was done to investigate whether a similar pattern of IR spectral differences could be detected associated with chemical or sructural changes in the components of the epidermal tissues of zucchini squash after storage for 1 to 6 days at 5 or 15°C with subsequent warming to room temperature. The epidermis was chosen for study since the most apparent CI symptoms were found there. With the use of these storage conditions, the hypothesis was tested that FTIR could be used to investigate whether IR spectral changes were related to detection of when irreversible damage occurred during storage at chilling temperatures.

2. Materials and methods

2.1. Source and handling of plant materials

Zucchini squash (*Cucurbita pepo* L., cv. 'Elite') were harvested at a local farm and fruit were selected for uniformity (16–22 cm length). The initial samples of epidermis tissue were taken from the middle region of the fruit within 4 h of harvest. The remaining fruit were placed in storage at 5, 15 or 20°C after which some fruit were kept at room temperature (ca 25°C) for 24 h. Three squash were taken from each group and sampled after the extent of chilling injury was evaluated, based on the extent of surface pitting.

2.2. FTIR spectroscopy

Excised sections of undamaged zucchini epidermis tissue (2 mg) were ground immediately with 48 mg of potassium bromide in a mortar. Pellets (3 mm) were prepared with a KWIK Handi-Press. Epidermal tissue was also lyophilyzed before being ground and incorporated into potassium bromide pellets also at 4% (w/w). Protein was isolated by grinding the epidermis tissue with 10 mM phosphate buffer (pH 7.6). After centrifugation protein was precipitated by addition of acetone and the precipitate was incorporated into a potassium bromide pellet (4%). Protein determinations were done using a commercially-available modified micro-Lowry method (Bio-Rad protein assay, Bio-Rad Laboratories, Richmond, CA).

FTIR spectra were obtained using a Nicolet 60 SX FTIR spectrometer (Nicolet Instruments, Madison WI) with a microbeam attachment and a MCT-A detector. Resolution was 4 cm^{-1} and 32 interferograms were acquired within <1 min and transformed. At least three tissue samples per treatment were analyzed and the spectra presented after background and reference subtractions were done. The variability in the magnitude of absorption of spectra was attributed to sample preparation error and variable response of the individual fruit to the storage conditions.

3. Results

Investigation of changes in the IR spectra of zucchini epidermis tissue sections could be done successfully by dispersion of the tissue into the potassium bromide matrix prior to pellet formation; opaqueness of the plant material interfered with other methods of acquiring spectra. Absorption peaks in the spectra of the tissue samples stored at the different temperatures were found at 3400–3300 cm^{-1} corresponding to OH and/or NH bond stretching vibrations, 2900–2600 cm^{-1} for CH_2 and CH_3 stretching, ca 1650 cm^{-1} for Amide I carbonyl stretching, ca 1450 cm^{-1} for Amide II NH bending,

and 1100–1060 cm^{-1} for CO stretching of alcohols [6]. Only one region of the IR spectra, that of the 3400–3300 OH/NH peak, was found to have a pattern of change that could be associated with the different storage and temperature treatments.

In the initial tissue samples (freshly-harvested), absorption maxima were found at 3315–3300 cm^{-1} (Figure 1A). Little change in band shape or numerical assignment of the absorption maxima was found in the spectra (Table 1) obtained from epidermal tissue from squash stored for 1 day at 5°C. However a change occurred in band shape and relative intensities of spectral maxima from squash stored for 2 or 3 days at 5°C (Figure 1B) where increased absorbance and and a spectral shift of the maxima to 3400 cm^{-1} was found for two of the three tissue samples (Table 1). After 6 days at 5°C, approximately the same absorbance maxima at 3400 cm^{-1} were found in spectra of the three squash sampled. Visual symptoms of chilling injury were noted then. The spectral shift of the maxima to 3400 cm^{-1} was more reliable than the absolute absorbance change which initially increased (max at 2 days at 5°C) and then declined.

Other zucchini squash were stored at 5°C and then kept at room temperature (ca 25°C) for 24 h before samples were taken to examine the effect of warming on recovery. Warming did not affect FTIR spectra of the zucchini epidermis stored at 5°C for 1 day [i.e. similar spectra were obtained for squash kept 1 day at 5°C with or without warming (Table 1)]. Recovery was demonstrated after 2 days at 5°C with subsequent 24 h rewarming by a significant decrease in absorbance of the 3400 cm^{-1} peak to the freshly harvested level (Figure 1C and Table 1). A major increase in the the same absorbance peak (OH/NH stretch band) occurred after warming of the zucchini squash stored at 5°C for 3 days (Figure 1D) indicating no recovery. The visible symptoms of chilling injury in the squash became more evident in the rewarmed squash after 3 days of 5°C storage.

When FTIR spectra were obtained of pericarp tissue from squash stored for 15°C for 1, 2 or 3 days, an increase in absorbance at the 3400–3300 cm^{-1} maxima was found to be related to length of storage in comparison to the spectra of the freshly harvested tissue samples (Table 2). A shift in the maxima from 3300 to 3400 cm^{-1} also occurred for the majority of the fruit. Spectra obtained after subsequent 24 h storage of these squash at room temperature exhibited a large increase in the 3400 cm^{-1} absorbance after 2 and 3 days at 15°C (Table 2). When squash were stored at 20°C for 1, 2 or 3 days in another experiment, similar increases in the absorbance (0.8–1.0) of the 3400 cm^{-1} absorption peak were found. No visible symptoms of chilling injury developed during the 6 days of storage at the low but non-chilling temperatures.

Spectra of some pericarp components were obtained as models to examine the spectral changes that had occurred in the whole tissue of squash during storage. FTIR spectra of total protein isolated from the squash epidermis had maxima at 3400 cm^{-1} with absorbances of 0.4–0.5 and were essentially identical to the spectra of the epidermal tissue obtained from the squash stored under the different conditions (not shown). The one area of difference was in the 1000 cm^{-1} band not considered here. If the total protein was then freeze-dried, absorbances of 1.2 and maxima of 3320–3340 cm^{-1} were found similar to the maxima of the samples from the freshly harvested squash (not shown). The maxima were shifted back to 3400 cm^{-1} with the

Table 1
FTIR absorbance of epidermis from zucchini squash stored at a chilling temperature (5°C)

Treatment	Absorbance*	Absorbance maxima†	
		3300 cm^{-1}	3400 cm^{-1}
Initial	0.72 a	3	-
1 day/5°C	0.72 a	2	1
1 day/5°C + 1 day/RT‡	0.82 b	2	1
2 days/5°C	1.17 e	1	2
2 days/5°C + 1 day/RT	0.80 ab	1	2
3 days/5°C	1.00 cd	1	2
3 days/5°C + 1 day/RT	1.25 e	1	2
6 days/5°C	0.90 bc	-	3
6 days/5°C + 1 day/RT	1.10 de	-	3

*Average of three samples—4% w/w in KBr. Means separation within column by Duncan's multiple range test. Measurements followed by different letters are significantly different at $P=0.05$.

†Numbers of spectra having either maxima, indicating change resulting from temperature and duration of storage.

‡RT: room temperature (ca 25°C).

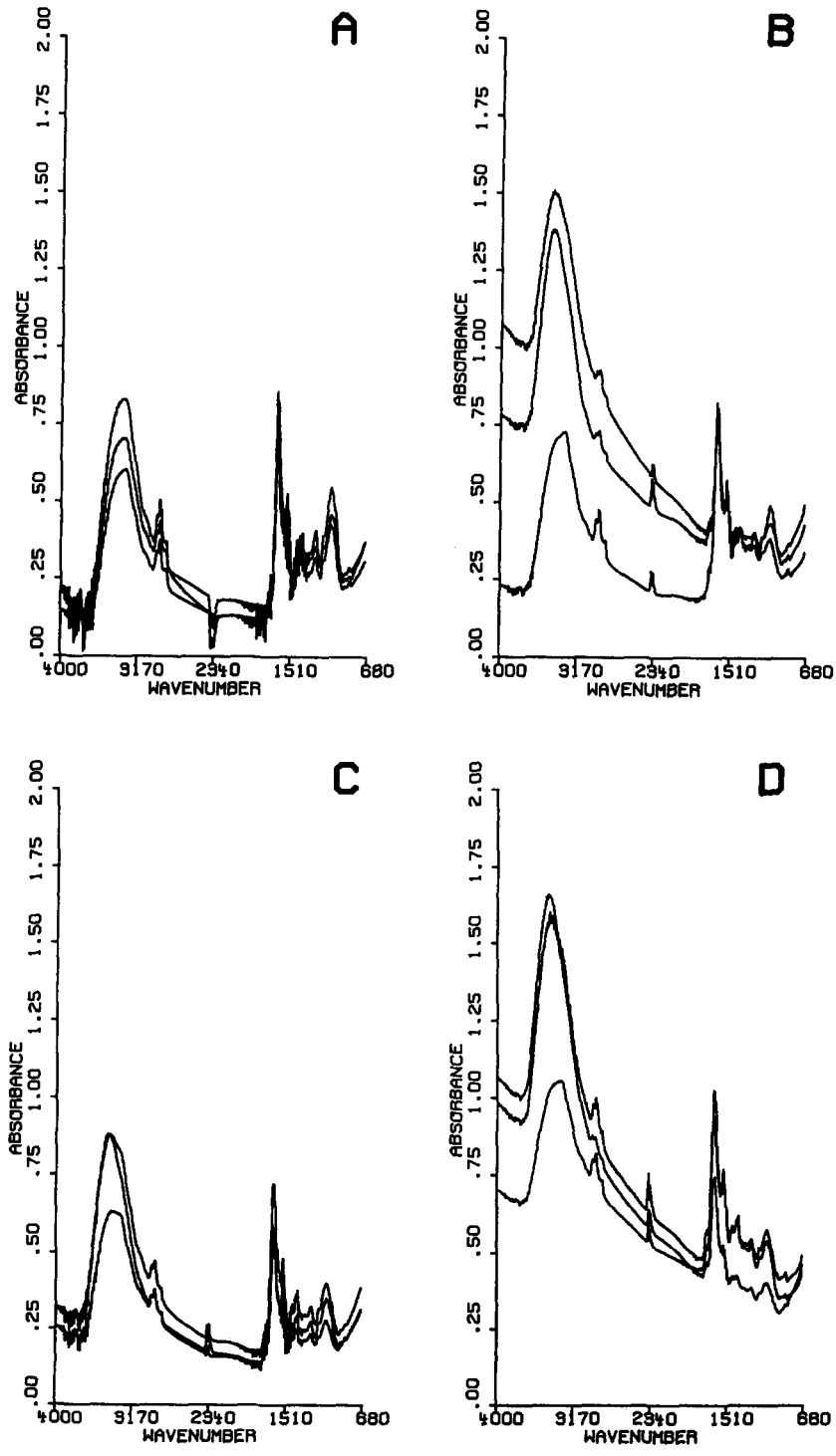

Fig. 1. FTIR spectra of zucchini squash epidermis: (A) Initial harvest; (B) Storage for 2 days at 5°C; (C) Storage for 2 days at 5°C followed by 1 day at room temperature; and (D) Storage for 3 days at 5°C followed by 1 day at room temperature. Samples were dispersed in potassium bromide. Three spectra were overlaid. Statistical variance is indicated in Table 1.

Table 2
FTIR absorbance of epidermis from zucchini squash stored at a non-chilling temperature (15°C).

Treatment	Absorbance*	Absorbance maxima†	
		3300 cm^{-1}	3400 cm^{-1}
1 day/15°C	0.85 a	2	1
1 day/15°C+1 day/RT‡	0.87 a	1	2
2 days/15°C	1.02 ab	1	2
2 days/15°C+1 day/RT	1.42 de	1	2
3 days/15°C	1.17 bc	1	2
3 days/15°C+1 day/RT	1.50 e	-	3
6 days/15°C	0.88 a	-	3
6 days/15°C+1 day/RT	1.28 cd	-	3

*Average of three samples—4% w/w in KBr. Means separation within column by Duncan's multiple range test. Measurements followed by different letters are significantly different at $P=0.05$.

†Numbers of spectra having either maxima, indicating change resulting from temperature and duration of storage.

‡RT: room temperature (ca 25°C).

addition of phosphate buffer to disssolve the precipitate such that the concentration was 1% (w/v). An indication of the quantities of protein in the freshly-harvested epidermis sample was obtained using 96–99% bovine albumin (BSA) as a standard. FTIR maxima (3295 cm^{-1}) with absorbance of 1.0 were found in the spectra of 1 mg BSA protein in the KBR (49 mg). Protein concentrations of ca 2% by weight were found in the freshly-harvested epidermis by the Lowry method.

3. Discussion

Differences between the FTIR spectra of the zucchini epidermis tissue were related to the duration of storage at chilling or non-chilling temperatures. In particular, the time (ca 2 days at 5°C) when apparently irreversible damage to tissue occurred was determined. Changes in absorbance magnitude and shifts of maxima from 3300 to 3400 cm^{-1} occurring after 2 days of chilling were attributed to an increased quantity of fluid (water) being found in the epidermis. These effects were seen in squash stored at both 5° and 15°C for ≥ 2 days and are the result of the altered metabolism of the squash as the low temperature exposure became dominant. These spectral changes were diminished if the squash was warmed to room temperature for 1 day indicating a reversibilty in the chilling-induced accumulation of fluid in the pericarp tissue as expected [9]. Spectra of squash tissue indicated that reversibilty of fluid-accumulation did not occur after 3 days of chilling temperature storage at 5°C (+1 day warming). These irreversible changes due to altered physiology and modification of internal tissue structure would be indicative of the early stages of chilling injury resulting later (after 6 days at 5°C) in visible symptoms such as water-soaking and pitting in localized areas.

Storage of the zucchini squash at the low but non-chilling temperature (15°C) led to spectral changes indicating a steady increase in fluid (water) in the epidermal tissue in the 3 day period and no decline in fluid levels on subsequent warming. Spectra of tissue samples from squash stored at 20°C indicated a similar pattern of increase.

In attempting to understand the changes in the fluid content of the epidermis of the intact squash, consideration of changes in the fluid (water) content of the adjoining cortex tissue was useful. The release of fluid from zucchini squash cortex tissue was related to the storage conditions of time and temperature for the squash when investigated by FTIR [1]. Fluid loss from cortex tissue was found to occur as a result of 1 day of storage at 5°C and and a similar amount of loss was found through 3 days of chilling temperature exposure. Significantly less loss of fluid from the cortex occurred after 3 days at 15°C consistent with results for the epidermis. The spectra of the collected fluid was identical to that of water when taken as a thin film.

Nuclear magnetic resonance (NMR) imaging techniques had been used to investigate temperature-related changes in the internal structure of zucchini squash non-destructively [9]. In that study, chilling storage at 2.5°C for 3 days resulted in more intense images than those of intact fruit stored at 12.5°C for 3 days. The higher intensity images were attributed to greater mobility of water in the chilled squash. The most intense portions of the images were localized in the epidermis and the adjoining areas indicating higher water mobility and diffusion in these tissues in chilled fruit after this particular

storage time. Results obtained from FTIR measurements were consistent with the previously reported NMR observations. Early changes detected with both techniques support the concept that increased membrane permeability and possibly breakdown of cellular integrity preceded the appearance of visible CI symptoms.

This study suggests that FTIR could be used as a rapid method to find spectral differences between chilled and non-chilled fruit and that the detection of early but reversible stages of chilling injury appeared possible. Finding reversal of epidermal fluid accumulation after 2 days at 5°C by warming the squash to room temperature supports suggestions made earlier [3] that timely use of ameliorative techniques such as intermittent warming might assist recovery from early onset of chilling injury. Also, FTIR has been used to confirm earlier experiments that indicated longer storage at 5°C followed caused spectral changes associated with fluid accumulation in the epidermis that were not reversible (i.e. recovery from chilling by warming was no longer possible after 3 days). The use of FTIR to show the increased fluid levels in the epidermis of zucchini squash stored at low but non-chilling temperatures such as 15°C provided information on the physical changes associated with tissue softening and subsequent shortening of storage-life reported for squash kept at these temperatures [3].

References

[1] Buta J.G. and Wang C.Y. (1993) Early detection of chilling injury with Fourier transform infrared spectroscopy. *HortScience* **28,** 1043–1044.

[2] Griffiths P.R. and de Haseth J.A. (1986) *Fourier Transform Infrared Spectroscopy*. Wiley-Interscience, New York, NY.

[3] Kramer G.F. and Wang C.Y. (1989) Reduction of chilling injury in zucchini squash by temperature management. *HortScience* **24,** 995–996.

[4] Lyons J.M. (1973) Chilling injury in plants. Ann. Rev. *Plant. Physiol.* **24,** 445–466.

[5] Mantsch H.H. and Casal H.L. (1986) Biological applications of infrared spectrometry. *Fresenius Z. Anal. Chem.* **324,** 655–661.

[6] Nakanishi K. and Solomon P.H. (1977) *Infrared Absorption Spectroscopy*. Holden-Day, San Francisco, CA.

[7] Paull R.E. (1990) Chilling injury of crops of tropical and subtropical origin. Pages 17–36 *in* C.Y. Wang, ed. *Chilling Injury of Horticultural Crops*. CRC Press, Boca Raton, FL.

[8] Sowa S., Connor K.F. and Towill L.E. (1991) Temperature changes in lipid and protein structure measured by Fourier transform infrared spectrometry in intact pollen grains. *Pl. Sci.* **78,** 1–9.

[9] Wang C.Y. and Wang P.C. (1992) Differences in nuclear magnetic resonance images between chilled and non-chilled zucchini squash. *Environ. Exp. Bot.* **32,** 213–219.