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Objective Measurement of Chilling Injury in the Mesocarp of Stored Avocados[1]

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Abstract. **A method to measure the severity of chilling injury (CI) in stored avocados *(Persea americana* Mill.) is described that gives better results than visual appraisal. Colored metabolites are extracted from chilled mesocarp and measured in a colorimeter.**

The postharvest life of avocado fruit can be extended by refrigeration. However, in common with many tropical and subtropical species, low temperature (~ 0 to 10°C) storage causes physiological dysfunction (8) known as chilling injury (CI). The presence of CI results in decreased market value and, ultimately, in complete wastage. The chief symptom of CI observed in avocado fruit is a gray or dark-brown discoloration of the mesocarp (edible pulp) (1, 5). This discoloration is due to an accumulation of oxidized phenolic compounds (7).

Certain physiological responses to CI in plants, such as abnormal respiration rates (2) and electrolyte leakage from cells (9) can be measured by laboratory techniques. How ever, these methods are generally impractical for routine CI assessment in stored fruit. In the absence of suitable quantitative methods, subjective assessments of CI severity based on visual rating or arbitrarily defined classifications such as "acceptable" and "not acceptable" have been used (10, 11). Such methods only provide a 2-dimensional assessment of a disorder having a 3-dimensional distribution.

In a study of enzymatic browning in freshly cut avocados, Kahn (6) reported that there was differential susceptibility to browning among avocado cultivars, based on visual appraisal of browning rates. These studies have been extended by Golan and Sadovski (4) who determined browning potential by measuring the rate of color change in prescribed target areas of exposed mesocarp using a color difference meter. This measurement technique was considered unsuitable for adaptation to measurement of CI because of the uneven distribution of symptoms throughout the mesocarp of

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individual fruit (1).

This paper describes a method for assaying CI based on extraction and measurement of the soluble colored metabolites present in the mesocarp of chilled avocado fruit. Some of the characteristics of extracts obtained by the method are described, as well as data showing the severity and distribution of CI symptoms.

Mature 'Fuerte' avocado fruit were harvested and grouped randomly into treatments consisting of 5 fruit which were stored for various times at 5°C, then ripened at 20°. Ripe fruit were bisected longitudinally, inspected visually for mesocarp discoloration, and rated subjectively for CI by several assessors using a scale of 0 to 5, with 0 = no visible injury and 5 = severe injury.

Fruit halves were cut transversely into 4 sections of equal thickness (~25mm), and each mesocarp section, with the skin removed, was mixed by hand to a uniform paste. Mesocarp samples (1 g) were then blended with a solution (5 ml) of 10 chloroform: 10 methanol: 9 water according to a modified Bligh and Dyer technique (3). Homogenates were then centrifuged (at about 700 x *g)* for 15 min. The water-methanol layer containing the colored metabolites was decanted and its absorbance was measured in a simple photometer without a filter (Eel Portable Colorimeter). The values obtained were used as an index of CI severity. The absorbance o extracts in loosely stoppered vials remained stable for at least 2 hours at room temperature.

Table 1. Effect of pH on absorbance of extract from chilled avocado mesocarp (pH adjusted with HC1 and NaOH).

Extract pH	Absorbance
2.02	0.50
6.25	0.72
6.60[z]	0.76
6.90	0.75
7.35	0.74
7.65	0.73
7.80	0.74
11.80	1.30

[z]Unadjusted extract.

Table 2. CI index in extracts from transverse sections of mesocar of avocado stored for different periods at 5°C. Extracts from non-stored control fruit had zero absorbance.

	CI index (absorbance)[z]		
	Storage time (wks)		
Fruit section no.[y]	2	6	8
1	0.10a[x]	2.27c	3.46de
2	0.29a	2.90cd	3.70ef
3	1.35b	4.20f g	5.15h
4	1.41b	4.53gh	5.08h

[z]Absorbance based on a 1:5 (w/v) extraction. Each value is a mean of 5 fruit sections.
[y]Transverse sections about 25 mm thick. Section 1 at the pedicel end and section 4 at the stylar end.
[x]Means separation by Duncan's multiple range test, 5% level.

Extract pH was found to vary depending on the section within individual fruit from which it was derived. Moreover, after their pH was adjusted with NaOH and HCl, absorbance of individual extracts showed pH dependence (Table 1). However, pH of extracts was always within the range 6.25 to 7.80 where absorbance was not markedly affected. Hence, the use of a pH buffer solution in the extraction procedure was considered unnecessary.

Serial dilution of a strongly colored extract showed that absorbance was linearly related to extract concentration (r = 0.995). Likewise, absorbance was proportional to variations in the ratio of sample weight to extractant volume. This colorimetric technique, therefore, determines relative differences in the concentration of soluble colored

metabolites extracted from chilled avocado fruit.

From visual appraisal, the severity of CI symptoms increased from slight (mean visual rating 1.2) after 2 weeks storage to severe (rating 5) after 8 weeks at 5°C. However, as shown previously (1), the symptoms were initially more severe at the stylar end compared to the pedicel end, especially after 2 weeks storage when there was no injury apparent in the pedicel end of the fruit. Because of this variable symptom distribution, a single visual rating value does not provide a realistic measure of CI. The data from the objective measurement of CI (Table 2) clearly quantify the increase of CI index (severity) with storage time and also the gradient of CI within individual fruit. Disadvantages of visual rating methods, additional to those described above, were also evident. For example, cut fruit darken on exposure to air (6) and CI ratings made 1 hour after cutting were about 40% higher than those made immediately after the fruit were cut. This additional darkening was confined to the surface layer and had a negligible effect on absorbance values obtained by the objective method. Also, visual rating values given by various assessors varied by up to 2 rating units in individual fruit.

This objective method overcomes the limitations of visual rating methods and therefore provides a more accurate, sensitive, and reproducible measure of CI in stored avocados. The method may also have application in studies with other fruit which display internal browning responses.

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