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Changes in the Chlorophyll and Pheophytin Concentrations of Kiwifruit during Processing and Storage

Gordon L. Robertson

Department of Food Technology, Massey University, Palmerston North, New Zealand

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ABSTRACT

The chlorophylls and pheophytins in fresh, canned and frozen kiwifruit products were measured. The importance of adjusting the kiwifruit samples to pH 7·5 prior to analysis, in order to minimise chlorophyll degradation, was demonstrated. The concentration of chlorophylls in fresh kiwifruit from commercial orchards varied by a factor of two. In canned kiwifruit slices, all of the chlorophylls had been degraded after heating at 100°C for 5 min. The chlorophylls in frozen kiwifruit purée stored at −18°C were reduced to less than one-third of their initial concentrations within 36 days.

INTRODUCTION

In a previous paper (Robertson & Swinburne, 1981) changes in the chlorophyll and pheophytin contents of kiwifruit during storage and canning were reported. More recently, Matsumoto *et al.* (1983) measured the chlorophyll contents of kiwifruit during ripening. Apparent discrepancies between the results led to a fuller investigation into the methods used to measure chlorophyll and pheophytin in kiwifruit.

Robertson & Swinburne (1981) used the method of Vernon (1960) which utilises specific absorptivities and changes in specific absorptivity for the four components (chlorophyll *a*, chlorophyll *b*, pheophytin *a* and pheophytin *b*) at appropriate wavelengths in 80% acetone. In his paper,

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Vernon (1960) presented data on the chlorophyll *a* and *b* contents of fresh, frozen and canned vegetables obtained using his method. One set of equations he presented is only applicable to non-heated, neutral preparations in which there would be a small conversion of the chlorophylls to the pheophytins. It is unclear whether or not the other set of equations is applicable to heated, non-neutral preparations but it is obviously important to establish that they are if the method is to be applied to acid chlorophyll-containing fruit such as kiwifruit (typically pH 3·2).

Matsumoto *et al.* (1983) used the AOAC (1980) spectrophotometric method for the determination of chlorophyll which utilises the equations of Comar & Zscheile (1942). It permits the determination of chlorophylls *a* and *b* at appropriate wavelengths in an ether solution but is not applicable to a four-component system such as would be the case when pheophytins were present.

In this paper, the two methods are compared and evaluated as to their suitability for measuring the chlorophyll content of kiwifruit.

MATERIALS AND METHODS

Kiwifruit

Fresh kiwifruit (*Actinidia chinensis* Planch) of the Hayward variety were obtained from various commercial orchards in New Zealand and were always hand-peeled immediately prior to analysis. Kiwifruit purée was prepared by hand-peeling the fruit, followed by blending for 3 min in a Kenwood Blender. The purée was then filled into plastic pots, sealed and frozen immediately to $-18\,^{\circ}C$. The frozen purée was stored at $-18\,^{\circ}C$ until required for analysis when it was removed from the freezer and allowed to thaw overnight at room temperature.

Chlorophyll

The spectrophotometric methods of Vernon (1960) and the AOAC (1980) were used to measure chlorophylls.

RESULTS AND DISCUSSION

The chlorophylls and pheophytins in fresh kiwifruit were determined (1) using the method of Vernon (1960) and (2) using the method of Vernon

(1980) but adjusting the puréed kiwifruit to pH 7·5 with 1N NaOH prior to blending with acetone. The results are shown in Table 1 where the most outstanding features are the significantly greater standard deviations and the very much higher concentrations of pheophytins in the kiwifruit analysed without pH adjustment.

The effect of pH on the rate of conversion of chlorophylls to pheophytins has been well documented (Sweeney & Martin, 1961; Jones

TABLE 1
Chlorophylls and Pheophytins (mg/kg) in Fresh Kiwifruit Determined by the Method of Vernon (1960)[a]

Pigment	*No pH adjustment*		*pH adjusted to 7·5*	
	Mean	*Standard deviation*	*Mean*	*Standard deviation*
Chlorophyll *a*	4·2	1·3	6·6	0·4
Chlorophyll *b*	2·5	0·4	2·9	0·5
Total chlorophylls	6·7	1·2	9·5	0·8
Pheophytin *a*	3·5	0·9	0·7	0·5
Pheophytin *b*	8·3	1·4	3·6	0·7
Total pheophytins	11·8	1·5	4·2	1·0

[a] All results are the average of ten determinations.

et al., 1962; Haisman & Clarke, 1975), the rate being proportional to the square root of the hydrogen ion concentration. It would appear that, in the unadjusted extracts, the natural acidity of the kiwifruit (typically pH 3·2) was sufficient to convert much of the chlorophyll present at the commencement of the analysis to pheophytin. In the adjusted extracts, on the other hand, it would seem unlikely that the brief blending period (less than 30 s), prior to adjustment of the pH with NaOH resulted in significant conversion of the chlorophylls to pheophytins. Vernon (1960) detected some pheophytins in fresh green beans, although Jones *et al.* (1962) found no pheophytins in fresh cucumbers. However, whether the pheophytins detected in the adjusted fruit were present *ab initio*, or were produced during blending and extraction procedures, cannot be answered at the present time.

In the light of the results in Table 1, it would seem prudent, when determining the chlorophylls and pheophytins in acidic plant material

such as kiwifruit, to adjust the material to pH 7·5 prior to extraction of the pigments into acetone.

In order to further compare methods for determining chlorophylls, fresh kiwifruit were halved. One set of halves was analysed using the AOAC (1980) method while the other set was analysed using the method of Vernon (1960), the latter fruit being adjusted to pH 7·5 prior to extraction with acetone. In one case the whole peeled fruit was analysed, in another the pips and central white core were removed prior to analysis and, in the third case, the unadjusted puréed fruit was heated to convert all the chlorophylls to pheophytins by boiling the purée for 5 min.

The results are presented in Table 2 and indicate that, in fruit where the pips and cores were removed, both methods agreed within experimental error. However, with whole fruit the total chlorophylls determined by the AOAC (1980) method were 20% higher than those determined by the method of Vernon (1960). That this could be due to interference from pheophytins is suggested by the results for boiled fruit where the AOAC method gave a total chlorophyll concentration of 8·2 mg/kg while Vernon's method detected no chlorophylls but did detect 23·6 mg/kg of pheophytins. From its visual appearance (a dull yellow–brown colour), it would be surprising if there were any chlorophylls remaining in the boiled purée and it is difficult to accept that half of the chlorophylls present in the fresh kiwifruit were still undegraded after boiling for 5 min.

TABLE 2

Chlorophylls and Pheophytins in Fresh Kiwifruit Determined by the methods of Vernon (1960) with pH Adjustment and the AOAC (1980) without pH Adjustment

Material		*Chlorophylls (mg/kg) (AOAC, 1980)*	*Chlorophylls (mg/kg) (Vernon, 1960)*	*Pheophytins (mg/kg) (Vernon, 1960)*
Whole, peeled fruit	Total	14·0	11·5	6·5
	a	10·5	8·30	1·7
	b	3·5	3·2	4·8
Pips and cores removed	Total	17·6	17·5	3·8
	a	13·1	11·9	0·9
	b	4·5	5·6	2·9
Puréed fruit after boiling for 5 min	Total	8·2	0	23·6
	a	8·3	0	10·2
	b	−0·1	0	13·4

In their paper on changes in the chemical constituents of kiwifruit during post-harvest ripening, Matsumoto *et al.* (1983) hand-peeled the kiwifruit, removed the seeds and cores, blended the fruit for 3 min and then froze and stored the purée at −26 °C until it was analysed for chlorophylls. No indication was given in the paper about the length of time the frozen purée was stored prior to analysis. However, because of the acidic nature of kiwifruit, blending to disrupt the cells will release the fruit acids and degradation of the chlorophylls will commence immediately.

The rate of chlorophyll degradation at freezer temperatures was investigated by determining the chlorophylls and pheophytins in frozen puréed kiwifruit which had been stored for varying lengths of time at −18 °C. The results are presented in Table 3.

TABLE 3
Changes in the Chlorophylls and Pheophytins during Storage of Frozen Kiwifruit Purée at −18 °C[a]

Time of storage (Days)	*Chlorophyll* a *(mg/kg)*	*Chlorophyll* b *(mg/kg)*	*Pheophytin* a *(mg/kg)*	*Pheophytin* b *(mg/kg)*
Fresh	3·1	3·7	2·2	0·5
1	2·2	1·7	4·1	5·0
36	1·0	0·9	5·6	6·0
68	0·6	0·3	5·9	6·2

[a] Each result is the average of three determinations.

The lower chlorophyll and pheophytin concentrations in the fresh fruit described in Table 3 (compared with those in Table 2) are not unexpected since the fruit used to make the purée was paler than normal kiwifruit and of a smaller size, being packing house reject material. However, the important conclusion which can be drawn from the results in Table 3 is that, even at −18 °C, chlorophyll degradation occurs. Within 36 days of storage the chlorophyll concentrations had been reduced to less than one-third of their initial concentrations. Factors likely to have a significant effect on the rate of chlorophyll degradation include pH, temperature, water activity and time. Lajolo *et al.* (1971) investigated the storage life of spinach (as measured by conversion of 20% of the chlorophyll to

pheophytin) as a function of water activity and found a linear relationship between water activity (a_w) and the logarithm of time; the lower the a_w, the shorter the time for 20% loss of chlorophyll. The a_w of foods at sub-freezing temperatures is independent of composition and depends only on temperature; at −18 °C the a_w is 0·84 (Fennema, 1981).

Countering the increased rate of degradation as a consequence of the lower a_w is the effect of temperature. Olson & Dietrich (1968) measured the effect of storage at temperatures of −7 °C, −12 °C and −18 °C on the time for a 10% decrease in chlorophyll content in peas, spinach and green beans. While the time required was considerably longer at lower temperatures, the three products exhibited quite different rates. The rate of chlorophyll degradation for chopped spinach was twice that for unchopped spinach. Thus, storing samples of kiwifruit purée at sub-zero temperatures until analysis for chlorophyll content will lead to erroneous results because of the rapid and significant degradation of chlorophyll which occurs.

The per cent conversion of the chlorophylls in kiwifruit slices on heating at 100 °C were reported by Robertson & Swinburne (1981). However, as they used the method of Vernon (1960), without pH adjustment, to measure the chlorophyll changes, their procedure was repeated but with the kiwifruit samples adjusted to pH 7·5 immediately prior to analysis. The results are presented in Table 4 and show that, after heating at 100 °C for 5 min, all the chlorophyll had been converted to pheophytin. As the standard commercial procedure for canning sliced

TABLE 4
Changes in Chlorophylls and Pheophytins of Kiwifruit Slices on Heating at 100 °C[a]

Time of heating at 100 °C (min)	*Total chlorophyll (mg/kg)*	*Total pheophytin (mg/kg)*
0	7·57	2·00
5	0	7·86
10	0	7·29
15	0	10·14
20	0·14	8·29
30	0	8·43

[a] Each result is the average of three determinations.

kiwifruit utilises a processing time of 15 min at 100 °C (often preceded by passage of the cans through an exhaust box for up to 20 min), it is not surprising that canned kiwifruit slices have a yellow–brown appearance. Reducing the processing time will not result in an improvement in the colour since the fruit acids released on slicing, in combination with the elevated temperature, lead to rapid conversion of chlorophylls to pheophytins.

The results presented above indicate that the pH of kiwifruit samples needs to be raised to 7·5 prior to analysing the samples for chlorophylls and pheophytins using the method of Vernon (1960) in order to minimise the degradation of the chlorophylls during analysis. The AOAC (1980) method for chlorophyll determination suffers from interference by pheophytins and can lead to erroneous results. Storing kiwifruit samples in freezers prior to analysis will slow down, but not stop, significant degradation of the chlorophylls to pheophytins. This conclusion has important ramifications for kiwifruit processors since over half of the kiwifruit is processed into frozen products, such as pulp, in New Zealand. The colour of these products will change on storage and, unless cognisance is taken of this fact, disputes could arise between processors and buyers regarding the colour of frozen kiwifruit products. These results also confirm the popular observation that there are considerable variations in the colour of fresh kiwifruit, one batch of fruit analysed in Table 1 having almost twice the chlorophyll content of the batch analysed in Table 3.

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