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Pigment and colour stability of frozen kiwi-fruit slices during prolonged storage

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Haltbarkeit von Pigment und Farbe gefrorener Kiwifrucht-Scheiben während langer Lagerung

Zusammenfassung. Es wurden die Veränderungen der meisten Pigmentbestandteile gefrorener Kiwifrucht-Scheiben während langer Lagerzeit bei −18 °C und die Korrelation mit Farbmessungen studiert. Kiwifrüchte der Sorten Hayward, Bruno, Monty und Abbot wurden ohne vorherige Behandlung unter Vakuum direkt nach dem Gefrierprozeß verpackt. Mit HPLC wurde quantitativ gemessen und die drei Pigmentbestandteile Xanthophyll, Chlorophyll und β-Carotin identifiziert. Die Farbe der frischen, gefrorenen Scheiben (Hunter-Farb-Werte) korrelierte mit jedem der Pigmentbestandteile. Ein Verlust, scheinbar erster Ordnung, wurde für Chlorophyll und Xantophyll bei den Sorten Hayward und Bruno beobachtet, jedoch sind sie für das Gefrieren bei langer Lagerzeit trotz Farbverlust geeignet.

Abstract. Changes in major pigment constituents of frozen kiwi-fruit slices during prolonged storage at −18° C and correlation with colour measurements (Hunter Lab parameters) were studied. Kiwi-fruit cultivars (Hayward, Bruno, Monty and Abbot) were processed without previous treatment and vacuum packed after freezing. HPLC using a diode array detector was used to individually quantify and identify the three major pigment components (xanthophylls, chlorophylls and derivatives and β-carotene). The colour of fresh and frozen slices by Hunter Color values were correlated with each class of pigment compounds. An apparent first order degradation rate was found for total chlorophylls and xanthophylls. Hayward and Bruno were more suitable for prolonged freezing preservation in terms of colour deterioration.

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Introduction

Chinese gooseberry (*Actinidia chinensis* Planch), the kiwi fruit, is perhaps the most nutritious fruit known among the group of soft berry fruits [1]. It was first introduced from China into New Zealand in 1906, and the USA began its cultivation in 1960. At present, it also grows successfully in Europe, mainly in Italy and Spain.

Fruit that does not meet export standards and is not sold on domestic fresh fruit markets is processed into various products, primarily canned slices in syrup, frozen pulp and slices, juices and wines. Changes in product quality after processing and prolonged storage of these commercial products have been suggested as being responsible for some of the marketing difficulties of kiwi fruit.

As long as the quantity of kiwi fruit available for processing rises, it becomes increasingly important to have a comprehensive knowledge of the composition of the fruit and the changes that it undergoes during processing. Changes in the composition and organoleptic properties on canning and freezing of kiwi fruit were reported by Beutel et al. [2]. Simmons [3] investigated the drying of kiwi fruit with particular reference to colour retention, and gave a qualitative assessment of the candied products.

The biochemical, sensory, and quality changes that occur during processing and prolonged frozen storage of kiwi-fruit pulp were studied by Venning et al. [4]. This work indicated that the temperature and processing time during pulp manufacture and freezing are critical factors in determining the percentage total chlorophyll content of the product.

Robertson [5] reported that even at −18° C, chlorophyll degradation occurs in frozen kiwi-fruit pulp; within 36 days of storage, chlorophyll concentrations had been reduced to less than one-third of their initiatl concentrations. In this study chlorophylls and pheophytins were measured by the spectrophotometric method of Vernon [6], but no work had reported the changes of the complete

and individual pigment pattern of this fruit in slices due to processing and storage.

Kiwi-fruit cultivar selection for processing could be one of the most important aspects for this kind of study. Cano et al. [7] have recently reported the chemical aspects of freezing preservation of four kiwi-fruit cultivars harvested in Spain, in order to obtain information on the compositional differences between cultivars. In this work only chlorophyll a and b changes were reported using a traditional spectrophotometric method for their quantitative evaluation.

Qualitative and quantitative differences in the pigment pattern between kiwi-fruit cultivars (Hayward, Abbot, Bruno and Monty) have been studied by Cano [8]. This author established fifteen major pigment compounds as responsible for the colour of fresh kiwi fruits. These compounds can be classified as xanthophylls (oxygenated carotenoids), chlorophylls and their derivatives, and only one hydrocarbon carotenoid, β-carotene. The pattern was very similar to that observed in frozen kiwi fruit except for the appearance of antheraxanthin, a structurally related isomer of lutein epoxide, and pheophytin b, which were not observed in fresh fruit extracts [9].

The present study was undertaken to:

1. Investigate the effects of frozen storage on pigment composition by HPLC and objective colour measurements in kiwi-fruit slices of the four most important cultivars of this soft fruit.
2. Study the correlation between the chemical constituents responsible for product colour and Hunter Lab parameters during frozen storage to establish which classes of pigments are most closely related to the observed changes in appearance after prolonged storage periods.

Materials and methods

Plant material and cold storage. Kiwi fruits (cvs Hayward, Bruno, Monty and Abbot) were harvested from Villaviciosa Research Orchard (Asturias, Spain) in November 1990. Immature kiwi fruit were placed in a refrigerated cabin at 0–1° C and 90–95% relative humidity [10], until the right maturity for processing was reached, approximately 20 days. The fruit characteristics at the time of processing are given in Table 1.

Reagents. The reference samples of chlorophyll a and b (Sigma-Aldrich) were used without further purification. Lutein, zeaxanthin, and all-*trans*-β-carotene were provided by Hoffman-La Roche (Basel, Switzerland). HPLC-grade solvents, methanol and ethyl acetate (Promochem,, Germany), were used without further purification. Pheophytins a and b were prepared by acidifying ether solutions of the chlorophylls with 13% HCl. The acid was removed by washing the ether layer twice with an equal volume of 5% Na_2SO_2. The pheophytin/ether mixture was dried over anhydrous Na_2SO_4 and evaporated under a stream of N_2.

Processing and sample preparation. Kiwi fruits were hand-peeled, sliced (6–8 mm), and frozen at −40° C and 5.5 m/s air rate in an air blast freezer until the temperature of the product was reaised to −20° C. Frozen slices were packed in polyethylene plastic bags, Polyeskin X, (250 g fruit slices), vacuum sealed, and stored at −18° C for a 10-month period.

Determination of colour data. Triplicate samples (approx. 50 g) were thawed under controlled conditious (2 h at 4° C), seeds and cores removed, pureed and placed in a 5-cm-diameter plastic dish to a depth of not less than 2 cm. The sample dish was placed on the light port of a Hunter Lab Model D25-9 colorimeter. The instrument was standardized on a white plate, and accuracy was checked with standard green (Y=43.2, X=36.4, Z=44.1). The sample dish was covered to avoid stray light. The L, a, and b values were recorded, and derived functions for hue (H), saturation (C), and total colour differences (ΔE) were calculated: $h = \arctan(b/a)$; $C = (a^2 + b^2)^{1/2}$; $\Delta E = [(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2]^{1/2}$.

Pigment analysis. Duplicates of all samples were extracted and analysed for prigment composition by using HPLC as follows.

Apparatus. A Hewlett-Packard Model 1040 quaternary solvent delivery system equipped with a Hewlett-Packard 1040A rapid-scanning UV/visible (vis) photodiode array detector was employed. The data were stored and processed by means of a Hewlett-Packard Model 9000/300 computing system and Color Pro plotter. The absorption spectra of the pigments were recorded between 300 and 600 nm at the rate of 12 spectra/min. The HP-9000 computer with a built-in integration programme was used to evaluate the peak area and peak height. Absorption spectra of isolated components in various solvents were recorded on a Perkin-Elmer Lambda 15 UV-VIS spectrophotometer.

Column. Separations were performed on a stainless-steel (10 cm × 4.6 mm i.d.) Hypersil ODS (5-µm spherical particles) column (Hewlett-Packard), which was protected with a Hibard guard cartridge (3 cm length × 4.6 mm i.d.) packed with Spherishorp-C18 (5-µm particle size).

Chromatographic procedure. The analytical separations were carried out according to the procedure of Cano [8]. A gradient mixture of methanol/water (75:25), eluent A, and ethyl acetate, eluent B, was used, beginning at time zero until time 10 min with a semifinal composition of eluent B (70%). The gradient eluent composition was followed at time 10 min until time 14 min with the final composition of eluent B (100%). The flow rate employed was 1.7 ml/min, and the chromatographic runs were monitored at 430 nm. At the end of the

Table 1. Initial characteristics of ripe kiwi fruits

Characteristic	Cultivar			
	Hayward	Abbot	Bruno	Monty
Fresh weight f.w.; (g)	120.32	102.04	85.42	80.19
Dry weight (g)	17.88	17.53	15.53	17.81
Pressure test (0.794-cm plunger; N)	87.49	70.21	78.48	75.26
pH	3.35	3.22	3.24	3.36
Acidity (% citric acid)	1.54	1.61	1.62	1.56
Soluble solids (Brix at 20° C)	16.00	16.10	14.70	17.10
Chlorophyll content (mg/100 g f.w.)	2.26	1.81	2.09	1.93
Xanthophyll content (mg/100 g f.w.)	0.68	0.74	0.67	0.77
b-Carotene content (mg/100 g f.w.)	0.036	0.065	0.041	0.005

Table 2. Peak identification of HPLC chromatogram obtained from frozen kiwi fruits

Chemical group	Peak	Components
Xanthophylls	1	9'-*cis*-Violanxanthin
	2	9'-*cis*-Neoxanthin
	3	Violaxanthin
	4	Neochrome (*cis*)
	5	Neochrome (*trans*)
	6	Auroxanthin
	7	Lutein epoxide
	8	all-*trans*-Lutein
	9	Neolutein B
	10	Neolutein A
	11	Antheraxanthin
Chlorophylls	12	Chlorophyll b
	13	Chlorophyll a derivative
	14	Chlorophyll a
	15	Pheophytin b
	16	Pheophytin a
Hydrocarbon carotenoids	17	all-*trans*-Carotene

gradient the column was re-equilibrated under the initial conditions by new gradient conditions beginning at time 14 min until time 20 min with a final composition of eluent B (0%) at the same flow rate (1.7 ml/min).

Preparation of fruit samples and extraction. Frozen kiwi fruits were prepared for analysis in the same way as for consumption, i.e. the inedible parts were removed. The frozen samples were thawed in a refrigerator until the pigment extraction (removing seeds and cores). The general method for extraction was described in a previous study on kiwi-fruit pigments [8]. The procedure consists of chilled acetone extraction of pigments from stabilized kiwi-fruit paste (made for homogenization of small pieces of kiwi fruit ajusted to pH 8–9 to prevent conversion of chlorophyll to pheophytin). After rigorous clean-up, the extracts were evaporated under a stream of N_2, and the residue dissolved with 5 ml of chromatographic-grade acetone. Duplicate 20 µl samples of each extract were injected for HPLC analysis.

Identification and quantification of pigments. Identification was based on the chromatographic behaviour with HPLC (Table 2) and TLC, visible absorption spectra, and specific chemical reactions, as was reported previously [8]. The pigments were quantified in a given sample by means of a calibration curve that included all of the chlorophylls and carotenoids to be assessed in that sample using the HP-9000 computer system. The curves were prepared daily by diluting portions of the starting solutions to the appropriate proportions for the samples being analysed.

Data analysis. Statistical analyses were performed using a software programme InPlot (TM) and InStat (TM). Regression analysis were used to determine if each class of pigment concentration was linear or quadratic with respect to storage period and to correlate the objective colour parameters to the pigment concentrations.

Results and discussion

Figure 1 is a representative HPLC chromatogram plotted at 430 nm of an extract of frozen kiwi fruit, cv Bruno, slices stored during 6 months at −18° C. Xanthophylls, chlorophylls and β-carotene were identified as reported by Cano and Marín [9]. Antheraxanthin, a strucutrally related isomer of lutein epoxide, was the only different xanthophyll compound identified in frozen samples. Pheophytin b was the chlorophyll derivative found in these frozen samples after prolonged storage periods. This compound was not observed in chromatograms of fresh and just-frozen kiwi-fruit extracts. Some authors have previously reported that, even at subfreezing temperatures, chlorophyll degradation occurs [5]. Factors likely to have a significant effect on the rate of chlorophyll degradation include pH, temperature, water activity, and time. Changes in quantities of chlorophylls, xanthophylls and β-carotene for the four kiwi-fruit cultivars are reported in Fig. 2. The Hayward cultivar showed the greater amount of chlorophylls at the processing time where as Monty kiwi-fruit slices container the highest levels of xanthophyll compounds at the same date. The concentration of β-carotene was very low in all cultivars, ranking between 0.005 to 0.065 mg/100 g fresh weight (f.w.) in the fresh fruit (see Table 1).

The freezing process per se produced a slight loss of chlorophylls that was most important in Abbot kiwi-fruit slices (27%). Other varieties showed different changes in pigment content, Hayward slices being the sample least affected by the process (11%). Bruno kiwi-fruit slices suffered a greater loss of xanthophylls whereas the losses of

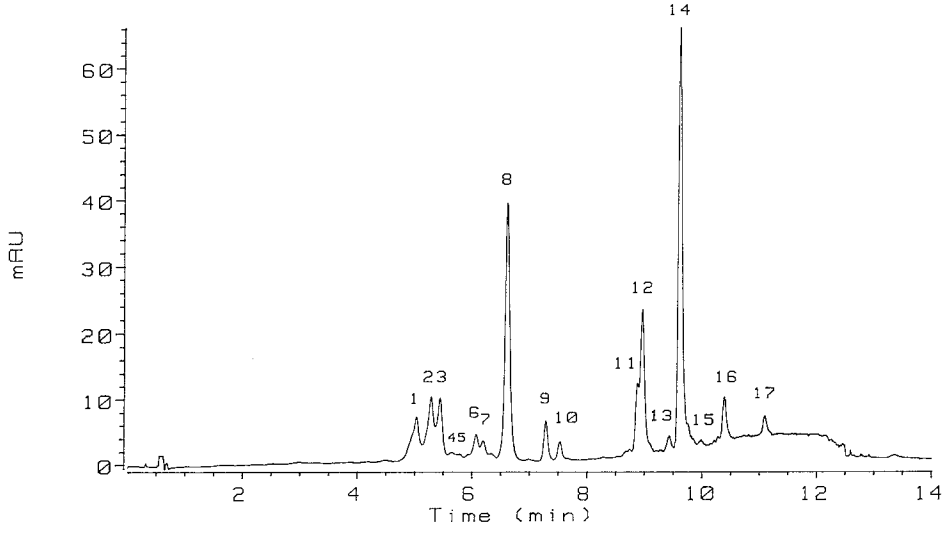

Fig. 1. HPLC chromatogram of extract obtained from frozen kiwi-fruit slices, cv Bruno, stored for 6 months at −18 °C. See Table 2 for peak identification

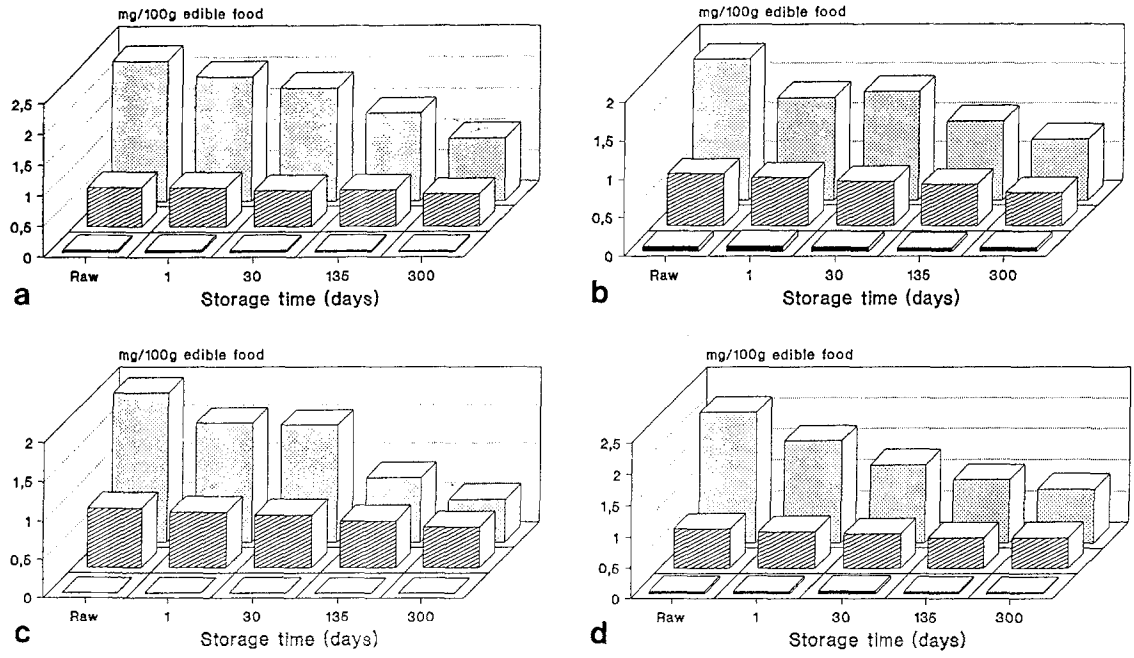

Fig. 2 a–d. Pigment changes in frozen kiwi-fruit slices during storage at −18 °C ■ β-carotene; ▨ xanthophylls; ⬚ chlorophylls. *a* cv. Hayword, *b* cv Abbot; *c* cv Monty; *d* cv Bruno

β-carotene due to tissue freezing were not significant in any cultivar. These loss differences between cultivars could be related to the different tissues damage produced by ice crystal development and distribution during the freezing process. These physical phenomena dependon the freezing rate of the fruit tissue, which is closely connected with the soluble solids of each sample at the processing date. Cellular disruption due these physical process could propitiate the pigment breakdown reactions, especially in chlorophyll compounds, on the thawed product. However, in the HPLC chromatograms of just-frozen kiwi-fruit extracts no chlorophyll derivatives other than those observed in fresh the fruit extract could be identified.

Frozen kiwi-fruit slices, cv Monty, showed the most evident chlorophyll change during storage (Fig. 2). After 10 months, this sample showed a two-thirds reduction of its just-frozen amount, near to 0.531 mg/100 g f.w. The other three cultivars, Hayward, Bruno and Abbot suffered reductions of 40–49% in these pigments. However, the proportions of pheophtins a and b, quantified in the HPLC chromatograms of the extracts obtained from frozen samples stored for 6 months or more prolonged periods, were very low compared with the amounts that could be coming from their correspondent chlorophyll conversion. Therefore, other mechanisms of chlorophyll degradation could have been taking place, probably as a consequence of enzymic reactions that produced an evident bleaching of the fruit tissue. Several authors have reported the regeneration of peroxidase (EC.1.11.1.7.), POD, in some frozen fruit tissues during prolonged storage periods [11]. Studies on the behaviour of POD in frozen kiwi fruits [12] showed that the POD activity strongly increased from 200 days of frozen storage in Abbot and Monty kiwi-fruit slices, whereas this regeneration was slight in Bruno and Hayward samples.

Xanthophyll pigments suffered a lower decrease during storage than chlorophylls in all frozen samples. Reductions oscillated between 34% for Abbot sices to 17% for Bruno and Hayward. This evident reduction in xanthophylls can be observed in the HPLC chromatograms of the respective extracts made from the samples during storage. However, there was no evidence of the transformation or isomerization of these compounds in other ones with a close chemical structure. Only an isomer of lutein epoxide, antheraxanthin (see Fig. 1), could be identified in the extracts made from frozen samples stored for more than 6 months. Therefore, several mecanisms, specially enzymic oxidations, must have been taking place in frozen tissue.

Relating to the effect of freezing and storage in the amount in β-carotene in these samples, Fig. 2 shows the very low proportion of this pigment in kiwi-fruit tissues. Fresh Monty kiwi fruit contained a lower amount of β-carotene; thus, the frozen samples maintained vary low proportions of this hydrocarbon carotenoid until the end of storage. However, the other three kiwi-fruit cultivars, Hayward, Bruno, and Abbot, showed decreases in β-carotene of 41%, 36%, and 29%, respectively.

The above-mentioned chlorophyll breakdown is illustrated in Fig. 3, where the data points are fitted to the "pseudo" first order equation. Table 3 shows the apparet rate constants for degradation of total chlorophylls and total xanthophylls in four cultivars of kiwi-fruit slices during storage at −18° C. Their levels of significance are $p<0.05$ in all samples. These values indicate that in Monty and Hayward frozen kiwi-fruit slices, chlorophyll a and b degradation was faster than in the other two cultivars. However, the rate constants for xanthophylls appeared to be faster in Monty an Abbot kiwi-fruit samples (Fig. 4). Taking account of these results, the appearance and visual colour of the frozen kiwi-fruit slices after 10

Table 3. Apparent first order degradation rate constants of total chlorophylls and xanthophylls in kiwi-fruit slices during frozen storage at −18° C

Cultivar	Total chlorophylls [k (day)×10^{-3}]	Total xanthophylls [k (day)×10^{-4}]
Bruno	1.77	6.11
Monty	3.89	9.98
Hayward	2.29	5.38
Abbot	1.99	11.99

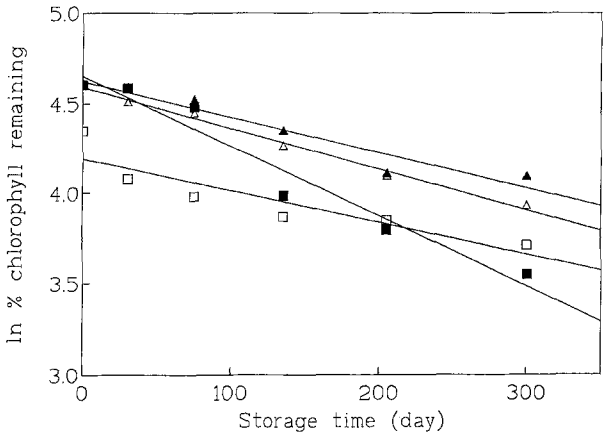

Fig. 3. Apparent first order degradation rate plot of total chlorophylls in frozen kiwi-fruit slices during storage at −18 °C: -0ü, cv Bruno; ■ cv Monty; ▲ cv Monty; △ cv Hoayward; ▲, cv Abbot

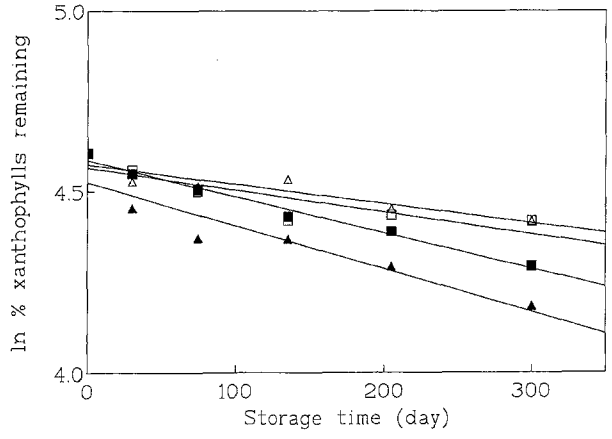

Fig. 4. Apparent first order degradation rate plot of total xanthophylls in frozen kiwi fruit slices during storage at −18 °C: for symbols see Fig. 3 legend

months of storage will depend on the initial concentration of each class of pigments in the fresh product, to the effect of freezing process per se, and to the rate constants of their apparent degradation during storage. Thus it could be important to try to correlate this analytical evidence with objective colour measurements in order to justify the sensorial colour changes observed in the samples.

Changes in the total colour difference (ΔE) parameter during storage of frozen kiwi-fruit slices at −18 °C is shown in Fig. 5. The total colour difference parameter

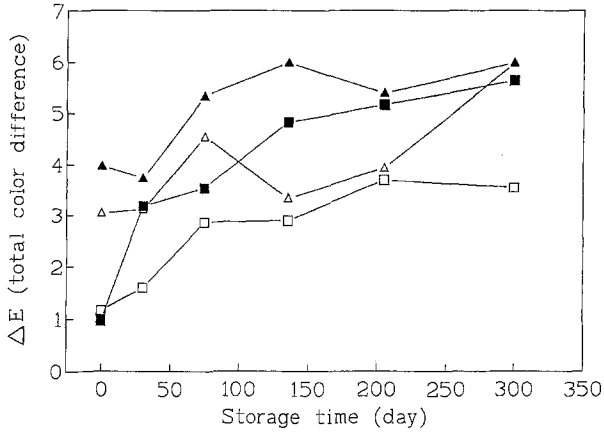

Fig. 5. Changes in total colour difference (ΔE) parameter during storage of frozen kiwi-fruit slices: for symbols see Fig. 3 legend

combines the L (liminosity), a_L (greenness) and b_L (yellowness) parameters in order to integrate these three colour characteristics between fresh fruit slices and frozen ones at each storage period. This difference continously increased during frozen storage, and was the most evident change that took place in Monty kiwi-fruit slices. This sample lost the greater amount of chlorophyll compounds during storage, as described above. In terms of the effect of the freezing process, the Abbot slices showed greater total colour differences relating to the corresponding fresh product; this could be related to the observed loss of chlorophylls (27%) the greatest one between cultivars. However, this cultivar was not that most affected during storage because Hayward and Monty kiwi fruit showed a significantly increase in the total colour difference parameter. In conclusion, the most suitable cultivars of kiwi fruit for freezing preservation in terms of colour deterioration could be CVS Hayward and Bruno. The Hayward cultivar maintained the gratest, chlorophyll and xanthophyll contents during storage, showing relatively low degradation rate constants for both pigment classes. The Bruno kiwi-fruit cultivar was the second most suitable for this process in terms of total colour difference and pigment degradation constants, which were very low in comparison to other cultivars.

The changes in other objective colour parameters such as hue (h = arctan b/a) during storage could explain the losses or breakdown of certain classes of pigments. Correlation studies between this parameter and the two more important pigment classes in this fruit are shown in Figs. 6 and 7. These graphs were constructed with all the data points, both chemical analyses of pigments and hue parameter from each pair of a_L and b_L values obtained at different storage times, and all cultivars of frozen kiwi-fruit samples. Correlation coefficients of −0.7726 and −0.7194 were obtained for total chlorophylls/hue parameter and total xanthophylls/hue parameter, respectively. These results confirm that in the changes in kiwi-fruit appearance, samples tend to be more yellow and luminous [9], and other chemical compounds produced by other bio-chemical mechanisms must be taken into account. However, the correlation values indicated that the most important factor responsible for colour changes is

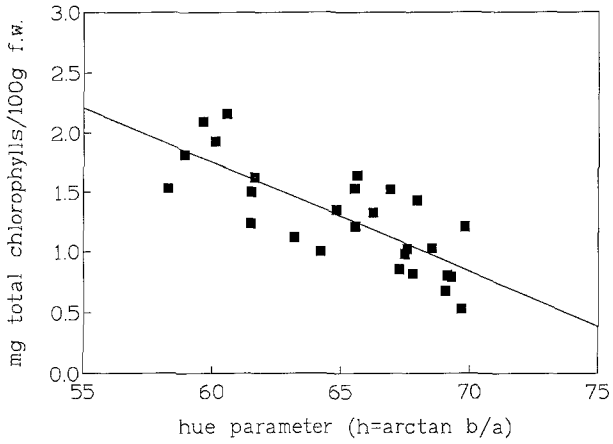

Fig. 6. Correlation plot of total chlorophyll content versus hue parameter (h = arctan b/a) of frozen kiwi-fruit slices: f.w., fresh weight; correlation coefficient (r) = −0.7726

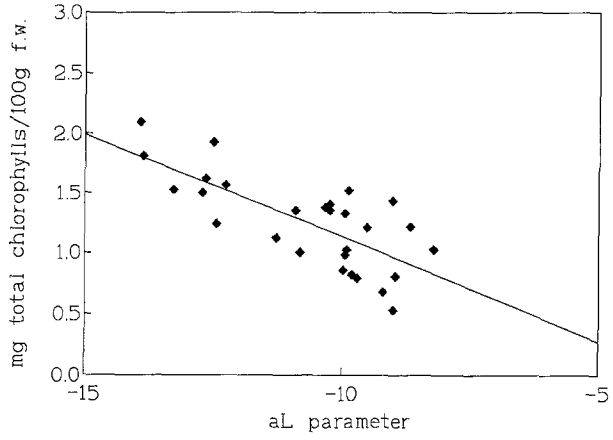

Fig. 8. Correlation plot of total chlorophyll content versus a_L (greenness) parameter of frozen kiwi-fruit slices: $r = -0.7385$

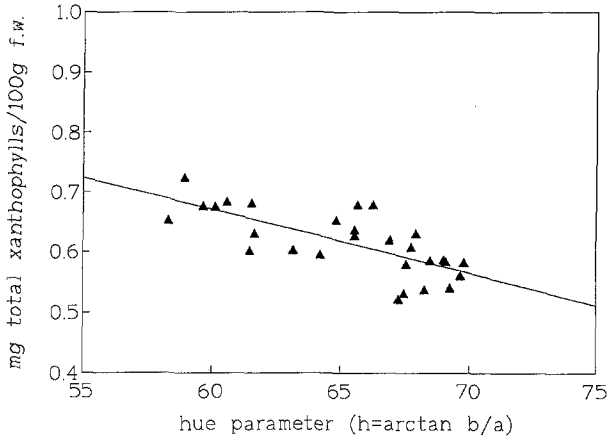

Fig. 7. Correlation plot of total xanthophyll content versus hue parameter (h = arctanb/a) of frozen kiwi-fruit slices: $r = 0.7194$

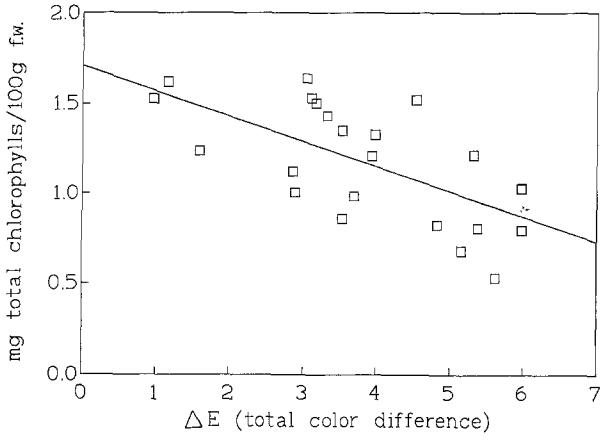

Fig. 9. Correlation plot of total chlorophyll content versus total colour difference of frozen kiwi-fruit slices: $r = -0.6481$

pigment breakdown. If the correlation studies were carried out with the total chlorophyll data and the a_L parameter, a very similar result was obtained $r = -0.7385$ (Fig. 8). This could be explained by the colour characteristic of the a_L parameter defined by the greenness. Previous reports on chlorophyll stability during processing and storage showed that the analyses of degradation products indicated that oxidation during storage was not a dominant factor in chlorophyll conversion and loss of colour [13].

The total chlorophylls/total colour difference (ΔE) correlation coefficient showed a very low value, −0.6481 (Fig. 9). Therefore, not only chlorophyll breakdown was implicated in the colour changes. Venning et al. [4] found only a slight decrease in the −a/b parameter during storage at −18 °C of kiwi-fruit pulp. The considerable differences between their results and the present study may be mainly due to differences relating to the analytical determination of chlorophyll pigments and, especially, to the sample preparation for colour and chlorophyll determination. However, the results from the frozen kiwi-fruit slices agreed with the premise that changes in visual colour were noticelable only after a considerable degradation of chlorophyll compounds had taken place.

In conclusions, these studies show that Hayward and Bruno kiwi-fruit cultivars are more suitable for freezing preservation in slices in terms of colour deterioration. The higher correlation coefficients between the most important chemical class of pigments in this fruit and the Hunter Lab colour parameters were obtained with the ratio total chlorophylls/hue (h = arctan b/a), $r = -0.7726$. Chlorophyll and xanthophyll brakdown was obersved during storage but these changes in pigment content were not the only causative mechanisms of colour deterioration of kiwi-fruit slices at prolonged frozen storage periods.

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