Chlorophylls and carotenoids of kiwifruit puree are affected similarly or less by microwave than by conventional heat processing and storage

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\textbf{A B S T R A C T}

The impact of microwave (1000 W – 340 s) and conventional heat (97 °C – 30 s) pasteurisation and storage (4, 10, 22 °C for up to 63 d) on total and individual carotenoids and chlorophylls in kiwifruit puree was evaluated. Bioaccessibility of carotenoids, before and after pasteurisation and storage, was also studied. Microwaves and conventional heating led to marked changes in the chlorophyll (42–100% losses) and carotenoid (62–91% losses) content. First- and second-order kinetics appropriately explained the degradation of total carotenoids and chlorophylls over time, respectively. Pasteurised samples showed significantly ($p < 0.05$) enhanced stability of these pigments, with microwaves ($k = 0.007–0.031$ 100 g mg$^{-1}$ day$^{-1}$ at 4–22 °C) promoting chlorophyll stability to a greater extent than conventional heating ($k = 0.0015–0.034$ 100 g mg$^{-1}$ day$^{-1}$ at 4–22 °C). Bioaccessibility of carotenoids remained ($p < 0.05$) unaffected by processing and storage. These results highlighted that the pigment composition of microwaved kiwifruit was more similar to that of the fresh fruit and better preserved during storage.

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1. Introduction

Fruits have been natural components of the human diet throughout history. Although their consumption seems to have been promoted more in recent times because of their well-known nutritional value and additional associated health benefits such as chronic disease prevention (Antunes, Dandlen, Cavaco, & Miguel, 2010), they have traditionally been perceived as appetising food products, given their wide variety of inviting colours and flavours, mostly conveyed by their pigment composition (Khoo, Prasad, Kong, Jiang, & Ismail, 2011).

In the particular case of kiwifruit (Actinidia deliciosa), a comparatively low-calorie (57 kcal/100 g), nutritious fruit rich in vitamin C, potassium, folate and fibre (Drummond, 2013), chlorophylls and carotenoids are the main pigments that contribute to the characteristic bright green colour of its flesh (Nishiyama, Fukuda, & Oota, 2005). The potential beneficial health properties of carotenoids, in particular, such as anti-inflammatory and anti-oxidant effects (Kaulmann & Bohn, 2014; Khoo et al., 2011), have been widely recognised and have long been considered an interesting study target. Although most investigations have traditionally focused on evaluating food carotenoid content, it should be kept in mind that the positive effect of these secondary plant compounds or any other functional compounds depends not only on their content but also on the extent to which they are bioaccessible and available for absorption after ingestion and digestion (Biehler, Hoffmann, Krause, & Bohn, 2011).

On the other hand, although kiwifruit has been reported to possess great potential for industrial exploitation (Barboni, Cannac, & Chiaromonti, 2010), few processed kiwifruit products are available on the international market nowadays. During processing and storage, dramatic changes are often observed in the pigment pattern of this fruit, resulting in degradation of chlorophylls into pheophytins, pyropheophytins, chlorophyllides and pheophorbides (Cano & Marín, 1992), and cis–trans isomerisation of carotenoids and formation of epoxides, furanoids and other degradation products of these compounds (Khoo et al., 2011). Consequently, the typical bright green colour turns to a yellowish-brown tone (Cano & Marín, 1992), and a product with an appearance very different from that of the raw kiwifruit is obtained (Cano, 1991). Given that colour is a highly important attribute in fruit quality assessment and has a considerable influence on consumer acceptance, these undesirable changes in pigment patterns of processed kiwifruit products may represent an important limitation for their marketing.
Consequently, development and applicability studies on different processing technologies that can guarantee safety and stability while offering superior quality foods may be the key to minimising the aforementioned potential problems, and to addressing consumer expectations regarding the increased demand for ready-to-eat foods with fresh-like characteristics (Picouet, Landl, Abadias, Castellari, & Viñas, 2009). In this respect, microwave heating is considered an interesting alternative to conventional heating methods to extend fruit shelf-life. Given the particular way in which heating takes place during microwave processing, when compared to conventional thermal treatments, microwaves lead to a faster heating rate, approaching the benefits of high-temperature, short-time processing, reducing thermal degradation and maintaining the sensory, nutritional and functional properties of the product (De Ancos, Cano, Hernández, & Monreal, 1999).

In order to investigate pigment behaviour following pasteurisation and storage of a ready-to-eat kiwifruit puree, the objectives of the present research were (i) to evaluate the effect of applying a microwave heating process on carotenoid and chlorophyll pigments of kiwifruit puree compared with a conventional heat treatment, (ii) to study the stability of these pigments during subsequent storage of the product, and (iii) to assess the impact of both heat processing and storage on the bioaccessibility of carotenoids.

2. Materials and methods

2.1. Chemicals and standards

Unless otherwise stated, all chemicals employed were of analytical or superior quality. Carotenoid standards (lutein, β-carotene, 96% purity) were purchased from CaroTeNature (Lupisingen, Switzerland). All other chemicals were from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Kiwifruit preparation and processing

Eight kg of kiwifruit (A. delicosa var. Hayward) was purchased from a local supermarket in Spain (Mercadona S.A., Valencia, Spain) in June 2013. Fruit pieces selected on the basis of a similar soluble solids content (13–15°Brix) were peeled with a knife, washed with distilled water (50 mL per fruit), cut into slices ca. 10 mm thick and homogenised with a Thermomix (TM 21, Vorwerk, Spain) using the fourth power level for one minute.

The kiwifruit puree obtained was aliquoted, kept below 4°C in darkness, and then rapidly (5 min) pasteurised by means of microwave technology and conventional heating as described below. Processing conditions were chosen on the basis of preliminary experiments to simulate equivalent pasteurisation treatments in terms of the degree of enzyme and microbial inactivation they achieved (Benlloch-Tinoco, Iguá, Rodrigo, & Martínez-Navarrete, 2015).

2.2.1. Microwave treatment

A microwave oven (3038GC, NORM, China) provided with a glass turntable plate was used to treat the kiwifruit puree. A sample weighing 500 g was tempered to an initial temperature of 25°C in a thermostatic water bath (Precisterm, Selecta, Spain) set at 30°C for 3 min and then heated in the microwave oven in a standard-size glass beaker (9 cm inner diameter and 12 cm height) (BKL3-1K0-0060, Labbox, Barcelona, Spain) at 1000 W for 340 s. The temperature of the sample in the coldest and hottest spots, previously identified (data not shown), was continuously recorded by means of a fibre-optic probe (CR/JP/11/11671, Optcom, Dresden, Germany) which was connected to a temperature datalogger (FOTEMP1-OEM, Optcom). The treated samples, termed MW, showed a final temperature of 72 and 94°C in the coldest and the hottest spot, respectively. They were immediately cooled in ice-water for 3 min until the puree reached 35°C, before they were further aliquoted.

2.2.2. Conventional thermal treatment

The conventional thermal treatment consisted of heating the sample to 97°C for 30 s in a circulating thermostatic water bath (Precisterm, Selecta). After the kiwifruit had been mashed, 20 g of puree was placed in TDT stainless steel tubes (1.3 cm inner diameter and 15 cm length) and closed with a screw stopper. A thermocouple, connected to a datalogger, was inserted through the sealed screw top in order to record the time–temperature history of the sample during the treatment. Prior to this heating step, the samples were preheated to 25°C in a thermostatic water bath (Precisterm, Selecta) (30°C for 30 s) to shorten and standardize the come-up time (150 s). The treated samples, termed C, were immediately cooled in ice-water for 45 s until the puree reached 35°C, before further aliquoting.

2.3. Storage study

The heat-treated (MW, C) and the non-treated (F) kiwifruit purees were packaged into clean, sterile plastic tubes (1.7 cm inner diameter and 11.8 cm length) (Ref. 525-0153, VWR, Spain) and then stored in darkness in heat-adjustable incubators at 4, 10 and 22°C for 7, 14, 21, 35 and 63 days. The purpose of the storage at 10 and 22°C was to observe the changes that may take place in the samples in the case of a partial, or total, rupture of the cold chain, respectively, during the shelf-life of the product. Following the storage trials, all samples were stored at –80°C until analysis.

2.4. Analytical procedure

The MW and C samples as well as the F samples, which were used as a control, were analysed in triplicate as described below, at day 0 and at regular time intervals for each storage temperature tested. Bioaccessibility of carotenoids in the F, MW and C purees was evaluated in triplicate at day 0 and after 63 days of storage at 10°C as described below. Additionally, a physico-chemical characterisation of F, MW and C purees at day 0 was carried out as described below. Analyses were run in triplicate.

2.4.1. Physico-chemical properties

Water content (xw) was measured by drying the sample to constant weight at 60°C in a vacuum oven (Vaciomet, J.P. Selecta, Barcelona, Spain) following the AOAC (2000). Soluble solids were determined by measuring °Brix in a previously homogenised sample with a portable digital refractometer (Refracto 3PX, Mettler Toledo, Buchs, Switzerland) at 20°C and pH using a digital pH-meter (Basic 2, Crison, Barcelona, Spain).

2.4.2. Extraction of pigments

2.4.2.1. Chemical extraction. Chlorophylls and carotenoids were extracted from the kiwifruit puree as described by Biehler, Mayer, Hoffmann, Krause, and Bohn (2010), with some modifications. In brief, 4 g of frozen kiwifruit was weighed into a 15-mL centrifuge tube (BD Biosciences, San Jose, CA, USA) and 6 mL of methanol was added together with 0.25 g of sodium carbonate to precipitate proteins. The samples were centrifuged (Harrier 18/80 refrigerated centrifuge, MSE, London, UK) for 5 min at 2500g at 4°C. The supernatant was decanted into a 50-mL centrifuge tube, extraction was performed twice with 9 mL of a mixture of hexane and acetone (1:1, v/v) and the organic fractions were combined. Ten milliliters of
saturated aqueous sodium chloride solution was added to the combined extracts and the mixture was shaken. The supernatant hexane phase was transferred to a 50-mL centrifuge tube, and the lower aqueous phase was re-extracted with 15 mL of hexane and combined with the first extract. The hexane extracts were weighed exactly for volume determination. A 10-mL aliquot was then pipetted from the combined extracts into a 15-mL centrifuge tube, evaporated to dryness under a stream of nitrogen in a TurboVapLVR apparatus (Caliper Life Sciences Benelux, Teralifene, Belgium) and stored at −80 °C until analysis.

2.4.2.2. In vitro simulated gastrointestinal (GI) digestion. To mimic in vivo GI digestion conditions and to determine the amount of carotenoids potentially available for further uptake, the methodology proposed by Bouayed, Hoffmann, and Bohn (2011) was followed, with some modifications. The release of total carotenoids from the kiwifruit samples after digestion, i.e. the gastric and small intestinal phases of digestion, was evaluated by analysing aliquots from the GI digesta by UPLC as described below. The percentage of relative bioaccessibility of carotenoids was estimated by calculating the ratio between the mean levels of each carotenoid in the kiwifruit puree samples and after the in vitro digestion process.

2.4.2.2.1. Gastric phase and small intestinal phase. Two g of kiwifruit puree sample, 1 g of cream (10% fat) and 12 mL NaCl (0.15 M) were mixed in a 50-mL plastic centrifuge tube prior to acidification with 0.5 mL HCl (1 M), to achieve a final pH of 3, and the addition of 1 mL of porcine pepsin solution (40 mg/mL in 0.1 M HCl). The mixture was incubated for 1 h in a shaking water bath (GFL 1083 from VEL, Leuven, Belgium) at 37 °C and 100 rpm. After this, the pH was raised to 5–5.5 by adding 0.7 mL of sodium bicarbonate (0.9 M) in order to simulate the transition from the gastric phase to the intestinal phase. Then 4.5 mL of a mixture of pancreatic and porcine bile extract (4 mg/mL pancreatin and 24 mg/mL bile extract dissolved in 0.1 M sodium bicarbonate) was added to the digesta. The pH was increased to 7–7.5 by adding 0.9 mL of sodium bicarbonate (0.1 M) and the final volume was adjusted to 25 mL with NaCl (0.15 M). After this, the samples were incubated in the shaking water bath (100 rpm) at 37 °C for 2 h to complete the intestinal phase of the in vitro digestion process.

2.4.2.2.2. Obtainment of bioaccessible fractions. Aliquots from the GI digestion (ca. 12 mL) were centrifuged (164,000g, 4 °C, 35 min), the supernatant (4 mL) was filtered through 0.2-μm PVDF syringe filters and extraction of pigments was performed twice with 4 mL of a mixture of hexane and acetone (1:1, v/v). The combined hexane phases were transferred to a 15-mL centrifuge tube, evaporated to dryness under a stream of nitrogen in a TurboVapLVR apparatus (Caliper Life Sciences Benelux, Teralifene, Belgium) and stored at −80 °C until analysis.

2.4.3. Pigment identification using UPLC

Separation, identification and quantification of carotenoids and chlorophylls was achieved on a Waters UPLC instrument (Milford, MA) including a P580 pump, a Gina 50 autosampler and a UVD340S photodiode array detector (Dionex Benelux B.V., Amsterdam, The Netherlands), simultaneously set at 409 (detection of pheophytin a), 431 (detection of chlorophyll a), 436 (detection of pheophytin b), 440 (detection of neoxanthin and violaxanthin), 450 (detection of β-carotene and lutein) and 459 (detection of chlorophyll b) nm. Separation of carotenoids was performed following the procedure described by Kaulmann, Jonville, Schneider, Hoffmann, and Bohn (2014) using an RP-18 column (2.1 × 100 mm, 1.7 μm particle size) at 40 °C (Waters Inc., Zellik, Belgium). Injection volume was 4 μL. For quantification, external calibration curves based on 7 points were obtained for each compound, with concentrations ranging from 0.01 to 25 μg/mL.

2.5. Kinetic modelling of pigment degradation

To obtain the kinetic parameters explaining loss of pigment content in the treated and untreated kiwifruit puree during storage, the amount of total carotenoids and total chlorophylls detected in the samples was plotted vs. time at all temperatures studied. Zero-, first- and second-order kinetics were hypothesized by applying the corresponding reaction rate expression. Then the order that best fitted the experimental data (data not shown) was selected. Following this criterion, first-order (Eq. (1)) and second-order (Eq. (2)) kinetics were used to describe degradation of total carotenoids and total chlorophylls, respectively, over time. The time for the concentration of a compound to fall to half its initial value (half-life, \( t_{1/2} \)) was also determined (Eqs. (3) and (4), corresponding to first- and second-order kinetic models, respectively).

\[
\ln \frac{C}{C_0} = -k \cdot t
\]  

(1)

\[
\frac{1}{C} - \frac{1}{C_0} = k \cdot t
\]  

(2)

\[
\frac{t}{2} = \ln 2 \div k
\]  

(3)

\[
\frac{t}{2} = \frac{1}{k \cdot C_0}
\]  

(4)

where \( C \) represents the concentration of the compound at \( t \) (mg 100 g\(^{-1}\)); \( C_0 \) the concentration of each compound at time zero (mg 100 g\(^{-1}\)); \( k \) the first-order (days\(^{-1}\)) or second-order rate constant (100 g mg\(^{-1}\) day\(^{-1}\)); \( t \) the storage time; \( t_{1/2} \) the half-life of the compound (days).

On the other hand, the temperature dependence of the degradation of these attributes was studied by employing the Arrhenius equation (Eq. (5)). In every case, the goodness of the fit between the experimental and predicted data was assessed by means of the adjusted regression coefficient (R\(^2\)-ad.) (Eq. (6)), considering that the higher the R\(^2\)-ad. value, the better the fit.

\[
k = k_0 \cdot e^{\frac{E_a}{R} \cdot \frac{1}{T}}
\]  

(5)

Adjusted-R\(^2\) = \[
\frac{\sum (m - \hat{y}_j)^2}{\sum (y_j - \bar{y})^2 - (m - j)}
\]

(6)

where \( k \) represents the rate constant; \( k_0 \) the pre-exponential factor; \( E_a \) the activation energy (kcal mol\(^{-1}\)); \( R \) the gas constant (1.987 kcal mol\(^{-1}\) K\(^{-1}\)); \( T \) the absolute temperature (K); \( m \) the number of observations; \( j \) the number of model parameters; SSQ the sum of squares.

2.6. Statistical analyses

Assumptions of normality and equality of variance were tested by normality plots and box plots, respectively. Linear mixed models correlating carotenoid and chlorophyll content (dependent variables) with the type of sample, storage temperature and storage time (fixed factors) were developed using the SPSS Statistics 19 software program (IBM SPSS, Inc., New York, NY, USA). A p-value of 0.05 (2-sided) was assumed to reflect significant statistical differences. After significant Fisher–F tests, post hoc tests (Bonferroni’s) were conducted. Additionally, non-linear and linear regression analyses were carried out in order to estimate the kinetic parameters using the Levenberg–Marquardt estimation method.
3. Results and discussion

3.1. Pigment composition of kiwifruit: processing effects

One of the main goals of the present research was to obtain an understanding of how the pigment composition of kiwifruit is affected by different thermal processing conditions. For this purpose, the pigment pattern of the fruit was evaluated before and after microwave and conventional heat pasteurisation (Table 1). Neither of the two treatments significantly affected the physico-chemical properties of the product. The mean values (±standard deviation) obtained were 84.8 ± 0.4 g water 100 g product⁻¹, 14.1 ± 0.3 g soluble solids 100 g liquid phase in the product⁻¹ and pH = 3.36 ± 0.08. In fresh kiwifruit, the mean value (±standard deviation) of total carotenoid and total chlorophyll content was shown to be 0.53 ± 0.06 and 2.58 ± 0.08 mg (±standard deviation) of total carotenoid and total chlorophyll component (68%), followed by anthin. The content of chlorophyll a and b in kiwifruit was 79 ± 19 g, respectively. Among the 5 different carotenoid compounds identified in this fruit, lutein, which was accompanied by β-carotene, neoxanthin and violaxanthin. The content of chlorophyll a and b in kiwifruit was 1.609 ± 0.003 and 0.49 ± 0.05 mg 100 g⁻¹, respectively. The most common derivatives of chlorophylls, phophytin a and b, were also detected in the fresh fruit (Fig. 1). As previously stated by Cano (1991), the presence of pheophtyins in untreated kiwifruit tissues may be due to rapid conversion of chlorophylls to these derivative compounds under low pH conditions. These results are in good agreement with those published by other authors for the same fruit (Cano, 1991; Cano & Marín, 1992; De Ancos et al., 1999; McGhie & Ainge, 2002; Montefiori, McGhie, Hallett, & Costa, 2009).

The processing step (MW, C) significantly (p < 0.05) affected the quantitative pigment concentration of kiwifruit, both the carotenoid and chlorophyll contents being reduced in the treated puree (Fig. 1). Thermal degradation, a process promoting the formation of oxidation compounds and the decomposition of pigments into more volatile, low molecular weight, colourless components, appears to be the most likely cause for these losses (Heaton & Marangoni, 1996; Rios, Fernández-García, Mínguez-Mosquera, & Pérez-Gálvez, 2008).

Carotenoids were affected more or less equally by microwave and conventional processing, with no statistically significant differences between the two processes overall. In pasteurised puree (MW, C) the total carotenoid content was reduced by 67 ± 7% and it was observed that neoxanthin (loss of 91%) and lutein (loss of 62%) were the most and least thermolabile compounds in the kiwifruit, respectively (Fig. 1). However, greater resistance of carotenoids

<table>
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<th>Sample</th>
<th>T (°C)</th>
<th>Days</th>
<th>Lutein</th>
<th>Neolutein A + B</th>
<th>β-Carotene</th>
<th>Neoxanthin</th>
<th>Violaxanthin</th>
<th>Total carotenoids</th>
<th>Pheophytin a</th>
<th>Phophytin b</th>
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</table>

(–): not detected. All values on wet weight basis. Three independent replicates were used to calculate each mean value and the corresponding standard deviation. Different letters in columns between the same sample at each temperature indicate significant statistical differences (p < 0.05) according to Bonferroni test.
to thermal processing has been observed in other fruit products. According to Lee and Coates (2003), Gama and de Sylos (2007), when Valencia orange juice was heat pasteurised (90–105 °C for 10–30 s), losses of carotenoids ranged from 20% to 46% and 9–38%, respectively. Lee and Coates (1999) did not find significant changes in β-carotene and lycopene contents after thermal pasteurisation (91 °C for 10 s) of red grapefruit juice. Lessin, Catigani, and Schwartz (1997) stated that carotenoid content of orange juice decreased by up to 50% during heat pasteurisation (80 °C for 2 min), and losses in carotenoid compounds of canned peaches ranged from 25% to 59%. On the other hand, although provitamin A activity has been reported to be slightly changed during pasteurisation (Gama & de Sylos, 2007; Lee & Coates, 2003), in the present study a considerable loss of β-carotene (86%) was detected in the MW and C samples. Overall, the discrepancy with data in the literature might be attributable to the great variability of carotenoid stability in different food matrices (Lee & Coates, 1999).

As expected, chlorophylls were shown to be more thermolabile than carotenoids (Cervantes-Paz et al., 2014). The chlorophyll pattern was noticeably changed after processing owing to chlorophyll degradation to pheophytins, with pheophytin a becoming the predominant compound in the treated samples (Fig. 1). The MW puree showed chlorophyll a and b contents of 0.349 ± 0.014 and 0.29 ± 0.04 mg 100 g⁻¹, respectively. The chlorophyll a content in the C puree was shown to be 0.13 ± 0.05 mg 100 g⁻¹, while chlorophyll b was not detected in this sample, possibly because it was more rapidly degraded in the C samples owing to chlorophyllase or some other enzymatic activity. From these data it can be claimed that microwave technology allowed significantly (p < 0.05) greater preservation of chlorophylls than conventional heating, which, in contrast, led to almost complete degradation of these pigments (92–100%). A similar range of chlorophyll degradation was found by Lefsrud, Kopsell, Sams, Wills, and Both (2008) in kale and spinach after drying (50–75 °C). It is widely accepted that chlorophyll a is more susceptible to heat loss than chlorophyll b (Chen & Chen, 1993). However, the conventionally pasteurised kiwifruit puree presented losses of similar magnitude for both chlorophyll compounds. Similar results were published by Turkmen, Poyrazoglu, Sari, and Sedat Velioglu (2006) for thermally processed peaches. As pointed out by Weemaes, Ooms, Van Loey, and Hendrickx (1999), the food matrix may have a strong impact on resistance of chlorophylls a and b to heat degradation, with different fruits and vegetables exhibiting dissimilar rates of degradation of these pigments.

### 3.2. Effect of storage time on pigment composition of kiwifruit puree

In order to understand the changes in pigment composition of kiwifruit puree throughout the shelf-life of the product, the stability of carotenoids and chlorophylls during storage of the pasteurised and fresh puree was investigated. Figs. 1 and 2, respectively, illustrate the evolution of the total content of these pigments in the MW, C and F samples during storage at 22, 10 and 4 °C. The stability of individual carotenoid and chlorophyll compounds over time was also monitored in all the samples (Table 1).

Linear mixed models were used to evaluate the effect of storage temperature, storage time and type of sample on kiwifruit pigments. The statistical analysis indicated that storage time, processing and their interaction brought about significant (p < 0.05) differences in the total and individual carotenoid contents. However, no significant effect of storage temperature was detected. Carotenoids tended to be significantly (p < 0.05) reduced over time, their decrease being ameliorated by pasteurisation (Fig. 2). Both the microwave and conventional heat treatments promoted stability of carotenoids during storage compared with the untreated samples (F). However, no positive effect of processing was observed for β-carotene and neoxanthin (Table 1): β-carotene gradually degraded over time and started to disappear completely after 35 days of storage at 4 and 10 °C or 14 days at 22 °C, while neoxanthin started to disappear completely after 14 days of storage at 4 and 10 °C or 4 days at 22 °C. In this respect, despite the fact that pasteurisation had a significant (p < 0.05) detrimental effect on carotenoids at onset (Section 3.1), no significant differences in the contents of these compounds were observed among the samples (F, MW, C) after 14 days of storage. In order to further investigate the impact of processing on the stability of carotenoids during storage, the degradation kinetics of total carotenoids were studied. As has been reported previously by various authors investigating different food matrices, total carotenoid degradation was appropriately described by first-order kinetics (Hidalgo & Brandolini, 2008).

Since no significant effect of storage temperature was observed, kinetic data were calculated only at 4 °C for each sample. The results obtained seemed to corroborate the positive effect of pasteurisation on the preservation of carotenoids over time, without revealing noticeable differences between microwave and conventional heating technology. The losses of carotenoids in the fresh kiwifruit puree (k = 0.022 ± 0.005 days⁻¹; R²-ad. = 0.834) were almost twice as fast as in the microwaved samples (k = 0.010 ± 0.003 days⁻¹; R²-ad. = 0.935) and the conventionally heated samples (k = 0.008 ± 0.001 days⁻¹; R²-ad. = 0.943). According to Gama and de Sylos (2007), the principal cause of carotenoid losses is oxidative degradation, which depends on the availability of oxygen and is stimulated by heat, light, enzymes, metals, and co-oxidation with lipid hydroperoxides. Given that the treated and untreated samples in the present study were exposed to equal storage conditions in terms of temperature, light, etc., it was considered that the increased stability against enzymatic breakdown – such as via peroxidases – provided by pasteurisation (Baldermann, Naim, & Fleischmann, 2005; Lessin et al.,
may well explain the superior stability of carotenoids found in the MW and C samples over time.

On the other hand, all the samples exhibited rapid degradation of chlorophylls (a and b) at all temperatures investigated (22, 10 and 4 °C). These compounds were gradually converted to pheophytins, which significantly \( (p < 0.05) \) increased in concentration during the first few days of storage before gradually decreasing. Similarly, a transient accumulation, prior to a drastic decrease, of pheophytin and chlorophyllide was observed by other authors in stored coleslaw and spinach, respectively (Heaton & Marangoni, 1996; Yamauchi & Watada, 1991). As suggested by Weemaes et al. (1999), after complete pheophytinisation of chlorophylls pheophytins might continue to be further degraded to pheophorbides, which may eventually be converted to some colourless components by following different pathways (Heaton & Marangoni, 1996). The evolution of chlorophyll derivative compounds (ChDs), pheophytin a and b, was monitored during storage. From the statistical analysis it was seen that the total contents of chlorophylls and their derivative compounds were significantly \( (p < 0.05) \) affected by storage time, processing technique, storage temperature and their interactions. On the whole, the ChD contents decreased significantly \( (p < 0.05) \) over time in all the samples, although here, too, pasteurisation seemed to promote a certain stability of these pigments, their degradation over time being slower in MW and C purees (Fig. 3). As expected, the higher the storage temperature, the faster the degradation of these pigments over time.

In order to further study the impact of processing and storage temperature on the stability of ChDs in the kiwifruit puree, their degradation kinetics were analysed by means of a second-order model. The values of the kinetic rate constant \( (k) \) and half-life \( (t_{1/2}) \) for the F, MW and C samples stored at 22, 10 and 4 °C are presented in Table 2. Additionally, to determine the effect of temperature on the parameters studied, the rate constants that were obtained were fitted to the Arrhenius equation. The activation energies \( (E_a) \) obtained are also shown in Table 2. In order to describe the effect of both treatment and storage temperature on the rate of decrease of ChDs, it was considered that the lower the \( t_{1/2} \) and the higher the \( k \) values, the faster the degradation of these compounds. Moreover, a higher activation energy value means a greater dependence of the kinetic rate constant on the storage temperature.

From the results obtained, pasteurisation clearly contributed to stabilisation of the total ChD contents in the product over time, with the F sample showing considerably higher degradation rates and lower half-life times than the MW and C samples at any of the temperatures studied (Table 2). Microwave technology helped to prevent ChD losses during storage to a greater extent than conventional heating, with differences being particularly noticeable at 4 and 10 °C. However, as deduced from the \( E_a \) values, pasteurisation treatment led to greater thermal sensitivity of these pigments, especially when microwaves were used to pasteurise the kiwifruit puree. Degradation of chlorophyll compounds is primarily attributed to enzyme activity (magnesium dechelatase, chlorophyllase, chlorophyll oxidase, peroxidase, etc.) (Heaton & Marangoni, 1996; Yamauchi & Watada, 1991). Accordingly, the higher stability of chlorophylls and derivative compounds exhibited by the treated kiwifruit puree might be associated with greater enzymatic stability brought about by processing. In this respect, despite the fact that chlorophylls a and b were completely lost during processing and storage, pasteurising the kiwifruit puree might still help to prevent further degradation of pheophytins to colourless compounds and a consequent colour change from olive green to a lighter whitish colour, especially if the product is processed by microwave heating.

Although equal heat degradation and stability of carotenoids were observed in the MW and C samples, pasteurising the kiwifruit puree by applying microwaves may be assumed to be beneficial in order to obtain processed kiwifruit with a colour more similar to that of the fresh fruit and better maintained over time, given the greater preservation of chlorophylls brought about by this technology. The treatments compared in the present study were selected on the basis...
of the results of previous research, in which it was observed that the possibility of some stability-enhancing effects associated with microwaves might explain their ability to provide equal or superior enzymatic and microbial stability of kiwifruit and to preserve its nutritive and functional value (Benlloch-Tinoco et al., 2015). Taking all these aspects into account, the superiority of microwave technology over conventional heating to preserve the pigment composition of kiwifruit puree during its shelf-life may be assumed.

3.3. Bioaccessibility of carotenoids in kiwifruit puree

Bioactive compounds need to be released from the food matrix and solubilised in order to be available for absorption. Consequently, evaluating to what extent they become accessible in the GI tract after ingestion (bioaccessibility) represents a key feature in the assessment of the role of different food matrices as dietary sources of these compounds. In the present investigation, the bioaccessibility of carotenoids detected in kiwifruit was evaluated before and after pasteurisation and storage. Results are shown in Fig. 4. The carotenoids identified in the kiwifruit puree showed a fractional bioaccessibility that ranged from 29 ± 3% to 47 ± 2%, with β-carotene and lutein being the least and most accessible compounds in the product, respectively. These results are in line with previous works dealing with the bioaccessibility of carotenoids in different fruit products (O’Connell, Ryan, & O’Brien, 2007; Rodríguez-Roque, Rojas-Graü, Elez-Martínez, & Martín-Belloso, 2014), being generally lower for the more apolar carotenes than for xanthophylls (Bohn, 2008). However, neither pasteurisation (MW, C) nor storage had a noticeable effect on the bioaccessibility of carotenoids from the kiwifruit matrix, as no significant differences among the studied samples were observed (Figure 4). A plausible explanation for the results obtained in the present study might be: on the one hand, that the severity of the pasteurisation treatments was insufficient to promote structural changes in the kiwifruit matrix, and, on the other hand, that thermal processing might not produce further destruction of previously homogenised matrices (e.g. purees), as suggested by Hornero-Méndez and Mínguez-Mosquera (2007).

In any case, as pointed out by Cilla et al. (2012), the effects of food processing on the bioaccessibility of carotenoids are more complex than the positive effects that might be expected. Although it has been extensively reported that thermal processing tends to enhance the bioaccessibility and bioavailability of carotenoids and other functional compounds in various vegetable-based food matrices, this cannot be taken for granted, since, according to Van Buggenhout et al. (2010), the data reported so far by different authors on this topic has not been found to be consistent and may largely depend on the distribution and original presence of carotenoids in various forms, such as in crystalline form or in the form of oil droplets (Schweiggert et al., 2014).
4. Conclusions

Both processing conditions and storage time had a strong impact on the pigment composition of kiwifruit, with chlorophylls being affected to a greater extent than carotenoids. Pasteurisation enhanced the stability of pigment compounds in the kiwifruit puree. Microwaves allowed greater preservation of chlorophylls during processing and storage, a finding that might help to palliate the dramatic colour changes typically undergone by kiwifruit-based products under these conditions. Fractional bioaccessibility, however, remained unchanged following processing and storage, suggesting only minor changes in their tissue distribution following processing. Accordingly, microwave technology may be successfully employed as an innovative tool that could aid in maintaining the natural colour of fresh kiwifruit in the pasteurised fruit and improve its market acceptance.

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