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CHLOROPHYLLS AND CAROTENOIDS OF KIWIFRUIT PUREE ARE INDIFFERENTIALLY OR LESS AFFECTED BY MICROWAVE THAN CONVENTIONAL HEAT PROCESSING AND STORAGE

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Running title: Pigments in microwave vs. conventionally heated kiwi

Abstract: The impact of microwave (1000 W-340 s) and conventional heat (97 ºC-30 s) pasteurisation and storage (4, 10, 22 ºC) on the total and individual content of carotenoids and chlorophylls in kiwifruit puree were 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, microwaves ($k= 0.007-0.031$ $100g \cdot mg^{-1} \cdot day^{-1}$ at 4-22ºC) promoting chlorophylls stability to a greater extent than conventional heating ($k= 0.0015-0.034$ $100g \cdot mg^{-1} \cdot day^{-1}$ at 4-22ºC). Bioaccessibility of carotenoids remained significantly ($p<0.05$) unaffected by processing and storage. These results highlighted that pigment composition of microwaved kiwifruit was more similar to that of the fresh fruit and better preserved during storage.

Keywords: microwave heating, conventional heating, pheophytin, lutein, bioaccessibility, degradation kinetics.

1. INTRODUCTION

Fruits have been natural components of the human diet throughout history. Although their consumption seems to be more recently promoted due to their well-known nutritional value and additional associated health benefits such as chronic disease prevention (Antunes, Dandlen, Cavaco & Miguel, 2011), they have been traditionally 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 (*Actinida deliciosa*), a comparatively caloric (57 kcal/100g) and nutritious fruit rich in vitamin C, potassium, folate and fibre (Drummond, 2013), chlorophylls and carotenoids are the main pigments contributing to the characteristic bright green colour of its flesh (Nishiyama, Fukuda & Oota, 2005). Especially carotenoids have been extensively recognised for their potential health beneficial properties such as anti-inflammatory and anti-oxidant effects (Kaulmann & Bohn, 2014; Khoo et al., 2011) and have been long considered as an interesting study target. Although most investigations have traditionally focused on evaluating carotenoid food content, it should be kept in mind that the positive effect of these secondary plant compounds or any other functional compounds does not only depend on their content, but rather 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, despite kiwifruit having been reported to possess a great potential for industrial exploitation (Barboni, Cannac & Chiaramonti, 2010), few processed kiwifruit products are nowadays finding use on international markets. 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* isomerization of carotenoids and formation of epoxides, furanoids and other degradation products of these compounds (Khoo et al., 2011). Accordingly, 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). Taking into account that colour is a highly relevant attribute in fruit quality assessment that has a considerable influence on the consumers' 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 but offering superior quality foods may be key to minimising these aforementioned potential problems, as well as to address the consumers' expectations regarding the increased demand for ready-to-eat foods with attributed freshness characteristics (Picouet, Landl, Abadias, Castellari & Viñas, 2009). In this respect, microwave heating is considered as an interesting alternative to conventional heating methods to extend fruit shelf-life. Given the particular way of heating taking place during microwave processing, when compared to conventional thermal treatments, microwaves lead to faster heating rate, approaching the benefits of high temperature-short time processing, reducing thermal degradation of the sensorial, 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 pigment of kiwifruit puree compared to a conventional heat treatment, (ii) to study the stability of

these pigments during successive storage of the product (iii) and to assess the impact of both heat processing and storage on the bioaccessibility of carotenoids.

2. MATERIAL AND METHODS

2.1. Chemicals and standards

Unless otherwise stated, all chemicals employed were of analytical quality or superior. Carotenoids standars (lutein, β-carotene, 96% purity) were purchased from CaroteNature (Lupsingen, Switzerland). All other chemicals were from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Kiwifruit preparation and processing

Eight kg of kiwifruit (*Actinida deliciosa* var. Hayward) were 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 ºBrix) were peeled with a knife, washed with distilled water (50 mL per fruit), cut into ca. 10 mm thin slices and homogenized with a Thermomix (TM 21, Vorwerk, Spain) using the fourth power level for one minute.

The obtained kiwifruit puree was aliquoted, kept below 4ºC in darkness, and then rapidly (5 min) pasteurised by means of microwave technology and conventional heating as described below. Processing conditions were chosen based on preliminary experiments to simulate equivalent pasteurisation treatments in terms of the degree of enzyme and microbial inactivation they achieved (Benlloch-Tinoco, Igual, 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 of 500 g was tempered to an initial temperature of 25 ºC in a thermostatic water bath (Precisterm, Selecta, Spain) set at 30 ºC for 3 min and then heated in the microwave oven in a standard size glass beaker (9 cm inner diameter and 12 cm length) (BKL3-1K0-006O, Labbox, Barcelona, Spain) at 1000W 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 ºC and 94 ºC in the coldest and the hottest spot, respectively. They were immediately cooled in ice-water for 3 min, until the puree reached 35 ºC before they were further aliquoted.

2.2.2. *Conventional thermal treatment*

The conventional thermal treatment consisted of heating the sample to 97 ºC for 30 s in a circulating thermostatic water bath (Precisterm, Selecta). After the kiwifruit was 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 ºC in a thermostatic water bath (Precisterm, Selecta) (30 ºC 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 ºC before further aliquotation thermostatic water bath (Precisterm, Selecta).

150 **2.3. Storage study**

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

158

159 **2.4. Analytical procedure**

160 The MW and C samples as well as a the F samples, which were used as control, were
161 analysed in triplicate as described below, at day 0 and at regular time intervals for each
162 storage temperature tested. Bioaccessibility of carotenoids in the F, MW and C purees
163 was evaluated in triplicate at day 0 and after 63 days of storage at 10ºC as described in
164 the following. Additionally, a physicochemical characterization of F, MW and C purees
165 at day 0 was carried out as described below. Analyses were run in triplicate.

166

167 *2.4.1. Physicochemical properties*

168 Water content (x_w) was measured by drying the sample to constant weight at 60 °C in
169 a vacuum oven (Vaciotem, J.P. Selecta, Barcelona, Spain) following the AOAC 934.06
170 method (2000). Soluble solids were determined by measuring the °Brix in a previously
171 homogenised sample with a portable digital refractometer (Refracto 3PX (Metler Toledo,
172 Buchs, Switzerland) at 20 °C and pH using a digital pH-meter (Basic 2, Crison,
173 Barcelona, Spain).

174

175 *2.4.2. Extraction of pigments*

176 2.4.2.1. Chemical extraction

177 Chlorophylls and carotenoids of the kiwifruit puree were extracted as described by
178 Biehler, Mayer, Hoffmann, Krause and Bohn (2010), with some modifications. In brief,
179 4 g of frozen kiwifruit were weighed into a 15 mL centrifuge tube (BD Biosciences, San
180 Jose, CA, USA) and 6 mL of methanol were added in presence of 0.25 g of sodium
181 carbonate to prevent rapid conversion of chlorophylls to the respective pheophytins. After
182 mixing, sonication and incubation for 5 min on ice, samples were centrifuged (Harrier
183 18/80 refrigerated centrifuge, MSE, London, UK) for 5 min at 2,500 × g at 4ºC. The
184 supernatant was decanted into a 50 mL centrifuge tube, extraction was repeated twice
185 with 9 mL of a mixture of hexane : acetone (1: 1, v/v) and organic fractions were
186 combined. To the combined extracts, 10 mL of saturated aqueous sodium chloride
187 solution was added and the mixture shaken. The supernatant hexane phase was transferred
188 into a 50 mL centrifuge tube, and the lower aqueous phase was re-extracted with 15 mL
189 of hexane and combined with the first extract. Hexane extracts were weighed exactly for
190 volume determination. A 10 mL aliquot was then pipetted from the combined extracts
191 into a 15 mL centrifuge tube, evaporated to dryness under a stream of nitrogen in a
192 TurboVapLVR apparatus (Caliper Life Sciences Benelux, Teralfene, Belgium) and stored
193 at -80ºC until analysis.

194

195 2.4.2.2. Simulated *in vitro* gastrointestinal (GI) digestion

196 To mimic the GI digestion conditions *in vivo* and to determine the amount of
197 carotenoids potentially available for further uptake, the methodology proposed by
198 Bouayed, Hoffmann and Bohn (2011) was followed, with some modifications. The
199 release of total carotenoids from the kiwifruit samples after digestion, i. e. 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 phases

Two g of kiwifruit puree sample, 1 g of cream milk (10% fat) and 12 mL NaCl (0.15M) 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 HCl 0.1 M). The mixture was incubated for 1 h in a shaking water bath (GFL 1083 from VEL, Leuven, Belgium) at 37 °C and 100 rpm. Next, 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 pancreatin and porcine bile extract (4 mg/mL pancreatin and 24 mg/mL bile extract dissolved in 0.1 M sodium bicarbonate) were added to the digesta. In turn, the pH was increased to 7-7.5 by adding 0.9 mL of sodium bicarbonate (0.1M) and the final volume was adjusted to 25 mL with NaCl (0.15 M). Then, 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. Obtaining bioaccessible fractions

Aliquots from the GI digestion (ca. 12 mL) were centrifuged (164,000 × g, 4ºC, 35 min), the supernatant (4mL) was filtered through a 0.2 µm PVDF syringe filters and extraction of pigments was carried out twice with 4 mL of a mixture of hexane : acetone (1: 1, v/v). The combined hexane phases were transferred into a 15 mL centrifuge tube,

225 evaporated to dryness under a stream of nitrogen in a TurboVapLVR apparatus (Caliper
226 Life Sciences Benelux, Teralfene, Belgium) and stored at -80ºC until analysis.
227
228 *2.4.3. Pigment identification using UPLC*
229 Separation, identification and quantification of carotenoids and chlorophylls was
230 achieved on a Waters UPLC instrument (Milford, MA) including a P580 pump, a Gina
231 50 autosampler change and a UVD340S change photodiode array detector (Dionex
232 Benelux B.V., Amsterdam, The Netherlands), simultaneously set at 409 (detection of
233 pehophytin a), 431 (detection of chlorophyll a), 436 (detection of pheophytin b), 440
234 (detection of neoxanthin and violaxanthin), 450 (detection of β-carotene and lutein) and
235 459 (detection of chlorophyll b) nm. Separation of carotenoids was performed following
236 the procedure reported by Kaulmann, Jonville, Schneider, Hoffmann and Bohn (2014)
237 using an RP-18 column (2.1 x 100 mm, 1.7 µm particle size) at 40 ºC (Waters Inc., Zellik,
238 Belgium). Injection volume was 4 µL. For quantification, external calibration curves
239 based on 7 points were obtained for each compound, with concentrations ranging from
240 0.01 to 25µg/mL.
241
242 **2.5. Kinetic modelling of pigment degradation**
243 To obtain the kinetic parameters explaining the loss of pigments content in the treated
244 and untreated kiwifruit puree during storage, the amount of total carotenoids and total
245 chlorophylls detected in the samples was plotted vs. time at all temperatures studied. Zero,
246 first and second-order kinetics were hypothesized by applying the corresponding reaction
247 rate expression. Then, the order which best fitted experimental data (data not shown) was
248 selected. Following this criterion, first-order (equation 1) and second-order (equation 2)
249 kinetics were used to describe degradation of total carotenoids and total chlorophylls over

time, respectively. The time for the concentration of a compound to fall to half its initial value (half-life, $t_{1/2}$) was also determined (equation 3 and 4 corresponding to first and second-order kinetic models, respectively).

$$\ln \frac{C}{C_0} = -k \cdot t \quad (1)$$

$$\frac{1}{C} - \frac{1}{C_0} = k \cdot t \quad (2)$$

$$t_{\frac{1}{2}} = \frac{\ln 2}{k} \quad (3)$$

$$t_{\frac{1}{2}} = \frac{1}{k \cdot C_0} \quad (4)$$

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

On the other hand, the temperature dependence of the degradation of these attributes was studied by employing the Arrhenius equation (equation 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.) (equation 6), considering that the higher the R^2-ad. value, the better the fit.

$$k = k_0 \cdot e^{\frac{-E_a}{R \cdot T}} \quad (5)$$

$$Adjusted - R^2 = \left[\frac{(m-1)(1 - \frac{SSQ_{REGRESSION}}{SSQ_{TOTAL}})}{(m-j)} \right] \quad (6)$$

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 carotenoids and chlorophylls 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 statistical significant differences. Following 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 SPSS Statistics 19 software program (IBM SPSS), based on 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. To this respect, pigment pattern of this fruit, before and after microwave and

conventional heat pasteurisation, was evaluated (Table 1, Figure 1). None of these two treatments significantly affected the physicochemical properties of the product. The average 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, mean value (± standard deviation) of total carotenoid and total chlorophyll content was shown to be 0.53±0.06 mg·100g^{-1} and 2.58±0.08 mg·100g^{-1}, respectively. Among the 5 different carotenoid compounds identified in this fruit, lutein, which was accompanied by two minor *cis*-isomers (neolutein A and B), was the most abundant component (60%), followed by β-carotene, neoxanthin and violaxanthin. The content of chlorophyll a and b in kiwifruit was 1.609±0.003 mg·100g^{-1} and 0.49±0.05 mg·100g^{-1}, respectively. The most common derivatives of chlorophylls, pheophytin a and b, were also detected in the fresh fruit (Figure 1). As it has been previously stated by Cano (1991), the presence of pheophytins in untreated kiwifruit tissues may be due to the 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 & Martín, 1992, De Ancos et al., 1999; McGuie & Ainge, 2002; Montefiori, Mcghie, Hallet & 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 (Figure 1). Thermal degradation, a process promoting the formation of oxidation compounds and the decomposition of pigments into more volatile, low molecular weight and colourless components, appears to be the most likely cause for these losses (Heaton & Marangoni, 1996; Rios, Fernández-García, Mínguez-Mosquera & Pérez-Gálvez, 2008).

315 Carotenoids were about equally affected by microwave and conventional processing,
316 with no statistically significant differences between the two processes overall. In
317 pasteurised puree (MW, C) the total carotenoid content was reduced by 67±7% and it was
318 observed that neoxanthin (91% losses) and lutein (62% losses) were the most and least
319 thermolabile compounds in kiwifruit, respectively (Figure 1). Greater resistance of
320 carotenoids to thermal processing, however, has been observed in other fruit products.
321 According to Lee and Coates (2003) and Gama and Sylos (2007), when Valencia orange
322 juice was heat pasteurised (90ºC-105ºC for 10-30s), losses of carotenoids ranged from
323 20% to 46% and 9% to 38%, respectively. Lee and Coates (1999) did not found significant
324 changes in β-carotene and lycopene content after thermal pasteurisation (91 ºC for 10 s)
325 of red grapefruit juice. Lessin, Catigani and Schwartz (1997) stated that carotenoid
326 content of orange juice decreased up to a 50% during heat pasteurisation (80ºC for 2 min),
327 and losses in carotenoid compounds of canned peaches ranged from 25% to 59%. On the
328 other hand, although provitamin A activity has been reported to be slightly changed
329 during pasteurisation (Gama & Sylos, 2007; Lee & Coates, 2003), in the present study, a
330 considerable loss of β-carotene (86%) was detected in the MW and C samples. Overall,
331 the discrepancy with literature data might be attributed to the great variability of
332 carotenoid stability in different food matrixes (Lee & Coates, 1999).

333 As expected, chlorophylls were shown to be more thermolabile than carotenoids
334 (Cervantes-Paz et al., 2014). The chlorophyll pattern was noticeably changed after
335 processing due to chlorophyll degradation to pheophytins, pheophytin a becoming the
336 predominant compound in the treated samples (Figure 1). The MW puree showed a
337 content of chlorophyll a and b of 0.349±0.014 $mg \cdot 100g^{-1}$ and 0.29±0.04 $mg \cdot 100g^{-1}$,
338 respectively. The content of chlorophyll a in the C puree was shown to be 0.13±0.05
339 $mg \cdot 100g^{-1}$, while chlorophyll b was not detected in this sample, possible more rapidly

degraded in the C samples due to chlorophyllase or other enzymatic activity. From these data it can be claimed that microwave technology allowed for a 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 (2008) in kale and spinach after drying (50-75ºC). It is widely accepted that chlorophyll a is more susceptible to heat loss than chlorophyll b (Chen & Chen, 1993). Nevertheless, 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 peas. 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 fruit and vegetables exhibiting dissimilar degradation rate 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, stability of carotenoids and chlorophylls during storage of the pasteurised and fresh puree was investigated. Figures 1 and 2 illustrate the evolution of the total content of these pigments, respectively, in the MW, C and F samples during storage at 22, 10 and 4 ºC. Stability of individual carotenoid and chlorophyll compounds over time was also followed 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 pigments of kiwifruit. The statistical analysis indicated that the storage time, the processing and their interaction brought about significant ($p<0.05$)

differences in the total and individual carotenoid content. However, no significant effect of the storage temperature was detected. Carotenoids tended to be significantly ($p<0.05$) reduced over time, their decrease being ameliorated by pasteurisation (Figure 2). Both the microwave and conventional heat treatments promoted stability of carotenoids during storage compared to the untreated samples (F). Nevertheless, no positive effect of processing was observed for β-carotene and neoxanthin (Table 1), which were gradually degraded over time and started to completely disappear after 35 and 14 days of storage at 4 and 10 ºC or 14 and 4 days at 22ºC, respectively. 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 content of these compounds was 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 was studied. As it has been previously published by several authors investigating different food matrixes, 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 exclusively calculated at 4ºC 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, $k=0.022\pm0.005$ $days^{-1}$; R^2-ad.=0.834, were almost twice as fast as in the microwaved, $k=0.010\pm0.003$ $days^{-1}$; R^2-ad.=0.935, and the conventionally heated samples, $k=0.008\pm0.001$ $days^{-1}$; R^2-ad.=0.943. According to Gama and Sylos (2007), oxidative degradation is the principal cause of carotenoid losses depending on the availability of oxygen and is stimulated by heat, light, enzymes, metals, and co-oxidation with lipid hydroperoxides. Given that, in the present study, the treated

389 and untreated samples were exposed to equal storage conditions in terms of temperature,
390 light, etc., it was considered that the increased stability against enzymatic breakdown
391 provided by pasteurisation, such as via peroxidases (Baldermann, Naim, & Fleischmann,
392 2005; Lessin et al., 1997), may well explain the superior stability of carotenoids found in
393 the MW and C samples over time.

394 On the other hand, all the samples exhibited rapid degradation of chlorophylls (a and
395 b) at all temperatures investigated (22, 10 and 4ºC). These compounds were gradually
396 converted to pheophytins, which significantly ($p<0.05$) increased in concentration during
397 the first few days of storage before gradually decreasing. Similarly, a transient
398 accumulation, prior to a drastic decrease, of pheophytin and chlorophyllide was observed
399 by other authors in stored coleslaw and spinach, respectively, (Heatong & Marangoni,
400 1996; Yamuchi & Watada, 1991). As suggested by Weemaes et al. (1999), after complete
401 pheophytinization of chlorophylls, pheophytins might continue to be further degraded to
402 pheophorbides which may be eventually converted to some colourless components by
403 following different pathways (Heaton & Marangoni, 1996). The evolution of chlorophyll
404 derivative compounds (ChD), pheophytin a and b, was followed during storage. From the
405 statistical analysis it was seen that the total content of chlorophylls and their derivative
406 compounds were significantly ($p<0.05$) affected by the storage time, the processing
407 technique, the storage temperature and their interactions. On the whole, the content of
408 ChD significantly ($p<0.05$) decreased over time in all the samples, though also here
409 pasteurisation seemed to promote a certain stability of these pigments, their degradation
410 over time being slower in MW and C puree (Figure 3). As expected, the higher the storage
411 temperature, the faster the degradation of these pigments over time.

412 In order to further study the impact of processing and storage temperature on the
413 stability of ChD in the kiwifruit puree, their degradation kinetics were analysed by means

414 of a second-order model. The values of the kinetic rate constant (k) and half-destruction
415 time ($t_{1/2}$) for the F, MW and C samples stored at 22, 10 and 4 ºC are presented in Table
416 2. Additionally, to determine the effect of temperature on the studied parameters, the
417 obtained rate constants were fitted to the Arrhenius equation. The obtained activation
418 energies (E_a) are also shown in Table 2. In order to describe the effect of both the
419 treatment and the storage temperature on the rate of decrease of ChD, it was considered
420 that the lower the $t_{1/2}$ and the higher the k values, the faster the degradation of these
421 compounds. Moreover, a higher value of activation energy means a greater dependence
422 of the kinetic rate constant on the storage temperature.

423 From the results obtained, pasteurisation clearly contributed to stabilize the total
424 content of ChD in the product over time, the F sample showing considerably higher
425 degradation rates and lower half-destruction times than the MW and C samples at any of
426 the studied temperatures (Table 2). Microwave technology helped to prevent ChD losses
427 during storage to a greater extent than conventional heating, with differences being
428 particularly noticeable at 4 and 10 ºC. However, as deduced from E_a values, pasteurization
429 treatment led to a greater thermal sensitivity of these pigments, especially when
430 microwaves were used to pasteurize the kiwifruit puree. Degradation of chlorophyll
431 compounds is primarily attributed to enzyme activity (magnesium dechelatase,
432 chlorophyllase, chlorophyll oxidase, peroxidase, etc.) (Heaton & Marangoni, 1996;
433 Yamauchi & Watada, 1991). Accordingly, the higher stability of chlorophylls and
434 derivative compounds exhibited by the treated kiwifruit puree might be associated with
435 greater enzymatic stability brought about processing. In this respect, despite the fact that
436 chlorophylls a and b were completely lost during processing and storage, pasteurising the
437 kiwifruit puree might still help to prevent further degradation of pheophytins to colourless

compounds and the consequent colour change from olive green to a lighter white tone, especially if the product is processed under microwave heating.

Although equal heat degradation and stability of carotenoids was observed in the MW and C samples, pasteurising the kiwifruit puree by applying microwaves may be assumed to be beneficial in order to obtain a processed kiwifruit with a colour more similar to that of the fresh fruit and superiorly maintained over time, given the greater preservation of chlorophylls brought about by this technology. The treatments compared in the present study were selected considering 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 versus conventional heating to preserve the pigment composition of kiwifruit puree during its shelf-life may be assumed.

3.3. Bioaccesibility 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 which extent they become accessible in the GI tract after ingestion (bioaccessibility) represents a key feature in the assessment of the role of different food matrixes as dietary sources of these compounds. In the present investigation, the bioaccesibility of carotenoids detected in kiwifruit was evaluated, before and after pasteurisation and storage. Results are shown in Figure 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

462 accessible compounds in the product, respectively. These results are in line with previous
463 works dealing with the bioaccessibility of carotenoids in different fruit products
464 (O'conncll, Rayan & O'Brien, 2007; Rodríguez-Roque, Rojas-Graü, Elez-Martínez &
465 Martín-Belloso, 2014), being generally lower for the more apolar carotenes than for
466 xanthophylls (Bohn, 2008). However, neither pasteurisation (MW, C) nor storage had a
467 noticeable effect on the bioaccessibility of carotenoids from the kiwifruit matrix, as no
468 significant differences among the studied samples were observed (Figure 4). A plausible
469 explanation for the results obtained in the present study might be: on the one hand, that
470 the severity of the pasteurisation treatments was insufficient to promote structural
471 changes in the kiwifruit matrix and on the other hand, that thermal processing might not
472 produce further destruction of previously homogenised matrixes (e.g. purees), as it was
473 suggested by Hornero-Méndez and Mínguez-Mosquera (2007).

474 In any case, as pointed out by Cilla et al. (2012), the food processing effects on
475 bioaccessibility of carotenoids are more complex than the positive effects that might be
476 expected. Although it has been extensively reported that thermal processing tends to
477 enhance the bioaccessibility and bioavilability of carotenoids and other functional
478 compounds in various vegetable based food matrixes, this fact cannot be taken for
479 granted, since, according to Van Buggenhourt et al. (2010), the data reported by different
480 authors on this topic has not been found, up to date, consistent and may largerly depend
481 on the distribution and original presence of carotenoids in various forms, such as in
482 crystalline form or in form of oil droplets (Schweigert et al., 2012).

483

484 **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 a greater preservation of chlorophylls over 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 on 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 pasteurised and to improve their market acceptance.

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