Carotenoid transfer to oil during thermal processing of low fat carrot and tomato particle based suspensions

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Abstract

Carotenoid solubilization in the oil phase is a prerequisite for carotenoid bioaccessibility during digestion. However, the level of bioencapsulation and the hydrophobicity of carotenoids were proven to strongly affect their transfer to oil during *in vitro* digestion. Therefore, thermal processing (95-110 °C) was exploited to favor carotenoid transfer from tomato- and carrot-based fractions to the oil before digestion. Initially, the total (all-*trans*+*cis*) carotenoid content in the oil increased quickly, thereafter, depending on the temperature applied, either a drop or a plateau was reached at longer treatment times. Treatment conditions of >100 °C for 10 min significantly favoured carotenoid transfer to oil (≥75 %). The rates of transfer to oil were as follows: *β*-carotene≈*α*-carotene>lycopene. The results revealed that the cell wall hinders carotenoid transfer to oil during thermal processing. Overall, the results indicate that typical high temperature short time thermal processing can be sufficient to achieve maximal carotenoid transfer to oil with minimal degradation in real food systems/food emulsions and this can be crucial to improve the nutritional quality of carrot and tomato based products.

Keywords: carotenoids*, structural barriers, thermal processing, carotenoid transfer, kinetics.*

1. Introduction

The evidence for the association between adequate intake of carotenoid-rich fruit and vegetable-based foods and the reduced risk of certain degenerative diseases continues to grow. Carotenoids are a group of lipid soluble pigments present in a wide variety of fruits and vegetables. Their physiological functions are linked to their antioxidant properties and/or pro vitamin A activity (Fernández-García et al., 2012). Carotenoids are polyisoprenoid compounds and can be categorized as either carotenes (composed of carbon and hydrogen atoms) or xanthophylls (oxygenated hydrocarbon derivatives that contain at least one oxygen function such as hydroxyl, keto, epoxy, methoxy, or carboxylic acid groups) (Britton, 1995). Lycopene, α-carotene and β-carotene are the predominant members of the carotenes (Stahl & Sies, 2005). The major biochemical functions of carotenoids are determined by the extended system of conjugated double bonds which is also responsible for their color (Britton, 1995).

In fruit and vegetable tissues, carotenoids are located inside the chromoplast organelles in a specific sub-structure of crystalline, membranous or globular nature, embedded in a cellular structure (Jeffery, Holzenburg, & King, 2012). This natural localization of carotenoids has consequences for their release and stability during processing, storage, and digestion. In this context, release from the matrix, by matrix disruption (during processing or digestion) followed by solubilization into an oil phase, which can be achieved by processing fruits and vegetables in the presence of lipids (Mutsokoti et al., 2015) or during digestion in the presence of lipids (Palmero et al., 2014) is necessary before carotenoids can be incorporated into mixed micelles during digestion (Castenmiller et al., 1999; Fernández-García et al., 2012). However, the level of bioencapsulation and the hydrophobicity of carotenoids were proven to strongly affect their transfer to oil during *in vitro* digestion procedures (Palmero et al., 2013, 2014; Verrijssen et al., 2014). To this regard, thermal processing can be exploited to favor carotenoid transfer to the oil before digestion. In fact, previous investigations have mentioned that thermal treatments can lead to matrix structure disruption (De Belie, Herppich, & De Baerdemaeker, 2000; Sila, et al.,2005)

54 which can facilitate the release of carotenoids from the matrix. In fact, *in vivo* studies established that
55 absorption of lycopene and β-carotene from fresh and unheated carrot and tomato juices is less
56 compared with processed tomatoes and carrot (Agarwal, Shen, Agarwal, & Rao, 2001; Fröhlich,
57 Kaufmann, Bitsch, & Böhm, 2007; Hof et al., 2000; Tydeman et al., 2010). Moreover, Schubert and Ax,
58 (2003) observed an increase in lycopene and asthaxanthin solubility in palm oil with increasing oil
59 temperature, with temperatures of at least 100 °C required to achieve considerable (3 g/L^{-1})
60 concentrations in the oil. This implies that thermal processing of fruit and vegetable matrices in the
61 presence of oil can be a valuable tool to facilitate carotenoid release and their subsequent solubilization
62 into the oil phase. This can be an alternative approach to elaborate new functional foods.
112 However, due to their highly unsaturated structure, carotenoids are prone to isomerization and
113 degradation during thermal processing (Achir et al., 2010). Oxidation is reported to be by far the major
114 cause of carotenoid degradation and has been postulated to be a free radical process (Chen et al., 2009;
115 Xu et al., 2013). Previous investigations have shown that carotenoid degradation reactions are more
116 pronounced in the presence of oil (Colle et al., 2011; Knockaert , et al., 2012). Moreover, Colle et al.
117 (2013) reported that lycopene degradation in an olive oil/tomato emulsion primarily takes place in the
118 oil phase. It is also known that the thermal degradation and isomerization products of carotenoids that
119 are formed in food products can result in an alteration of the nutritional (e.g. reduction or loss of
120 provitamin A and antioxidant activity) and sensory quality (e.g. color changes). Therefore, in the context
121 of improving the nutritional quality of tomato and carrot based products, by facilitating the carotenoid
122 transfer to oil during processing, thermal processing conditions should be carefully selected to maximize
123 carotenoid transfer to the oil phase and at the same time minimize carotenoid degradation.
124 In the past decade, in the context of predicting carotenoid changes during thermal processing, many
125 studies have been conducted in which lycopene and *β*-carotene degradation, assuming first order
126 kinetics in both model and real food systems was described. However, the transport kinetics of

carotenoids from the food matrix to the oil phase and their degradation therein during thermal processing has so far not been described in the literature. Therefore, the aim of the present work was to evaluate the main factors governing the transfer to oil of *α*-carotene and *β*-carotene from carrot and lycopene and *β*-carotene from tomato particle based suspensions during thermal processing. In this study, materials with different levels of carotenoid bioencapsulation were included. In addition, the kinetics of carotenoid transfer to oil and degradation during thermal processing were considered in order to determine the relevant temperature/time conditions required to maximize carotenoid transfer to oil and this can be important for process design and optimization.

2. Materials and Methods

2.1 Materials

All chemicals and reagents used were of analytical or HPLC-grade. All-*trans* lycopene, all-*trans β*-carotene, all-*trans α*-carotene (≥90 %, ≥95 %, ≥95 %, purity by HPLC assay, respectively) and L-*α*-phosphatidylcholine were purchased from Sigma-Aldrich (Borne, Belgium). 5-*cis* lycopene, 9-*cis*, 13-*cis* and 15-*cis β*-carotene (96.8 %, 99 %, 96 %, 96 %, purity by HPLC assay, respectively) were purchased from CaroteNature (Lupsingen, Switzerland). Olive oil (extra virgin) was kindly donated by Vandemoortele (Ghent, Belgium). Red tomatoes (*Lycopersicon esculentum* cv Prunus) and orange carrots (*Daucus carota cv* Nerac) were obtained fresh from a local shop in Belgium and stored at 4 °C for 1 day prior to use.

2.2 Sample preparation

2.2.1 Oil-in-water emulsion

Oil-in-water emulsion was prepared by mixing 5% olive oil to 1% L-*α*-phosphatidylcholine in deionized water at 9500 rpm for 10 min (UltraTurrax, IKA-Werke GMBH & CO.KG, Staufen, Germany). The mixture was then immediately homogenized at 100 MPa for one cycle (Panda 2 K, Gea Niro Soavi, Parma, Italy).

2.2.2 Chromoplast fraction

151 The chromoplast enriched fraction from tomatoes or carrots was obtained according to the method
152 described by Palmero et al. (2013). The vegetables were first washed in deionized water. Tomatoes or
153 peeled carrots were cut into pieces and mixed (Waring Commercial, Torrington, CT, USA) at low speed
154 for 5 s with 50% 0.05 M ethylenediaminetetraacetic acid (EDTA) solution. The obtained purees were
155 gently filtrated using cheesecloth and further centrifuged (Beckman, J2-HS Centrifuge, Palo Alto, CA,
156 USA) at 27200 *g* for 30 min at 4 °C. The pellet was re-suspended in 100 ml deionized water and
157 represented the chromoplasts enriched fraction.
158 2.2.3 Cell cluster fraction
159 The cell cluster fraction was prepared according to the procedure described by Palmero et al. (2013).
160 First, tomato or carrot purees were obtained. In the case of tomatoes, the pieces were mixed (Büchi B-
161 400 mixer, Flawil, Switzerland) three times for 5 s and sieved (1 mm) to remove seeds. Carrot puree was
162 obtained by mixing (Waring Commercial, Torrington, CT, USA) the carrot pieces with 50% deionized
163 water for 1 min. The obtained purees were then sieved using wet sieving equipment (Retsch AS200,
164 Haan, Germany). The fractions between 40–250 and 160–500 µm were collected representing the carrot
165 and tomato cell cluster fractions, respectively (Lemmens et al., 2009; Palmero et al., 2013).
166 **2.3 Thermal treatments**
167 Thermal treatments were performed by means of a microwave heating system (start E, Milestone S.r.l,
168 Sorisole, Italy). In a first step, the relevant temperature range for the actual kinetic study was
169 determined by screening carotenoid transfer to the oil phase (Lemmens et al., 2010). Hereto, cell
170 clusters and chromoplast enriched fractions from carrots and tomatoes were separately mixed with the
171 oil-in-water emulsion (1.5 g isolated fraction: 15 ml emulsion). The samples were then poured into
172 reactor tubes, each containing a thermowell, closed with a 5 bar valve and incubated in a water bath at
173 40 °C for 4 min. Four other vessels were filled with 16 g of tylose solution (1.5% w/v in deionized water)
174 to serve as thermal load. Thereafter, samples were thermally treated for 20 min in the microwave

heating system at 80, 90, 110 and 120 °C. In one of the samples, the temperature was measured using a fiber optic sensor (ATC-FO, Milestone S.r.l.). The power necessary to reach the treatment (E1, Watt) within 2 min and to maintain it for a defined treatment time, (E2, Watt) was predetermined for each temperature investigated, (Table A1 and Fig A. 1). Follow-up of the power assured the dynamic heating phase was limited to 1.5 min and temperature fluctuations were restricted within ± 1.5 °C. During heating, the samples were stirred using magnetic stirrers to facilitate heat transfer within the samples. In a second step, the actual kinetic experiment was performed. Samples were thermally treated at temperatures ranging from 95 to 110 °C for time intervals ranging from 0 min to 40 min. An example of a time temperature profile is shown in Fig. 1 for each process temperature. For both the screening phase and the kinetic experiment, four reactor tubes per sample were treated and the treatment repeated twice. Immediately after the treatments, samples were immediately cooled down in an ice bath. Thereafter, the content of the four reactor tubes was transferred into a durum flask which was also kept in ice. Next to this, a control sample was prepared, as described above, to which no thermal treatment was applied. The control and treated samples were kept for maximally 3 hours at 4 °C until centrifugation (Beckman Optima XPN-100 Ultracentrifuge, Brea, CA, USA) at 65 000 g for 1 hour at 4 °C was performed to recover the oil. Immediately after, carotenoids were analysed for the recovered oil.

2.4 Carotenoid concentration

Carotenoids were extracted following the procedure described by Sadler et al. (1990) with some modifications. The procedure was performed by mixing 1 ml (chromoplast enriched fraction) or 1 g (cell cluster fraction) with 25 ml of the extraction solution [hexane/acetone/ethanol (50:25:25 v/v/v) containing 0.1% of butylated hydroxytoluene (BHT)] and 1 g of NaCl. Afterwards, the mixture was stirred for 20 min at 4 °C, followed by the addition of 7.5 ml of reagent grade water (18.2 MΩ·cm). For the recovered oil, 0.5 g oil was mixed with 10 ml of the extraction solution and 0.1 g NaCl, stirred for 20 min at 4 °C, followed by addition of 3 ml reagent grade water. The samples were mixed for 10 more minutes

199 at 4 °C and then placed in separation funnels (or glass tubes in case of recovered oil) to collect the
200 organic phase. The isolated organic phase was filtered (Chromafil PET filters, 0.2 µm pore size–25 mm
201 diameter) and transferred into a dark vial for HPLC analysis. The identification and quantification of
202 carotenoids were performed using a HPLC system equipped with a C_{30}-column (3µm×150mm×4.6mm,
203 YMC Europe, Dinslaken, Belgium) and a diode array detector (DAD) (Agilent Technologies 1200 Series,
204 Dinslaken, Belgium). The temperature of the column was kept constant at 25 °C during the analyses. A
205 linear gradient, using methanol (A), methyl-t-butyl-ether (B) and reagent grade water (18.2MΩ·cm) (C),
206 was applied. The starting conditions were 81% A, 15% B and 4% C and the final conditions corresponded
207 to 16% A, 80% B and 4% C. The flow rate was set at 1 ml/min and the gradient was built up in 44 min for
208 all-*trans* lycopene analysis and 25 min for all-*trans α*- and all-*trans β*-carotene analysis. Carotenoid
209 identification was performed at 472 nm for all-*trans* lycopene and the *cis* isomers and at 450 nm for all-
210 *trans β*-carotene and the *cis* isomers, as well as all-*trans α*-carotene on the basis of retention times and
211 spectral characteristics of pure standards as described by Colle et al. (2010a) and Lemmens et al. (2009).
212 Carotenoids were quantified with the use of the corresponding calibration curves of pure standards. The
213 concentration of 9-*cis* and 13-*cis* lycopene where standard solution were not available, was determined
214 from the calibration curve of the all-*trans* lycopene.

215 **2.5 Particle size measurements**

216 The particle size distribution was measured using a laser diffraction particle size analyzer (Beckman
217 Coulter LS 13 320, Miami, FL, USA). The instrument measures particle sizes in the range of 0.04 to 2000
218 µm. The sample was poured into a stirred tank, filled with deionized water until a laser obscuration of
219 40 % was achieved , and pumped into the measurement cell. The intensity profile of the scattered light
220 was used to calculate the volumetric particle size distributions (PSD)s using the Fraunhofer optical
221 model by use of the instrument software (Jamsazzadeh et al., 2015).

222 **2.6 Data Analysis**

223 The amount of carotenoids transferred to the oil phase upon thermal treatments of the different
224 isolated fractions was expressed as a relative amount, i.e. % carotenoid transfer according to equation
225 1:

226 Transfer (%) = $\frac{\mu g\ carotenoid\ in\ the\ recovered\ oil}{\mu g\ carotenoid\ in\ the\ control}\ x\ 100$ (1)

227 where the recovered oil represents the carotenoid rich oil phase obtained after centrifugation of the
228 thermally treated carrot and tomato based particle suspensions and the control represents the non-
229 treated sample. In order to identify significant differences among carotenoid transfer to oil for the
230 different isolated fractions thermally treated at different temperatures for 20 min, statistical analyses
231 were performed using Tukey's Studentised Range Test (SAS version 9.4, Carry, NC, USA). The level of
232 significance was set at $P<0.05$.
233 In order to describe the changes in carotenoid concentration as a function of treatment time and
234 temperature during the thermal treatment, two concomitant events can be considered to occur: (i)
235 carotenoid transfer to oil, responsible for an increase in carotenoid concentration in the recovered oil
236 and (ii) carotenoid degradation, accounting for a decrease in carotenoid concentration in the recovered
237 oil. Therefore, the overall process could be described as comprising, in a first step, release from the
238 matrix and subsequent transfer of carotenoids into the oil phase, followed by a second step in which a
239 drop in total (all-*trans*+*cis* isomers) *α*-carotene, *β*-carotene and lycopene concentration as a function of
240 treatment time can be expected. In order to describe the drop in carotenoid concentration, first order
241 kinetics can be assumed. In accordance with these mechanisms, the governing differential equations for
242 the time dependent changes in carotenoid concentrations are:

243 $\frac{dC_{avail}}{dt} = -k_{in}\ (C_{avail} - C_{oil})$ (2)

244 $\frac{dC_{oil}}{dt} = k_{in}\ (C_{avail} - C_{oil}) - k_{deg}\ (C_{oil})$ (3)

where C_{oil} represents carotenoid concentration (all-*trans*+*cis* isomers) in the oil, C_{avail} is carotenoid concentration (all-*trans*+*cis* isomers) that is available at any given time in the system for transfer to oil, k_{in} carotenoid transfer rate constant (min^{-1}) and k_{deg} carotenoid degradation rate constant (min^{-1}). Eq. 2 describes the changes in the carotenoid concentration that is available for transfer from the matrix to the oil, while Eq. 3 describes the evolution of carotenoid concentration in the oil phase.

The temperature dependence of the rate constants k_{in} and k_{out} was quantified by the activation energy, Ea_{in} (J/mol) and Ea_{deg} (J/mol), respectively according to Arrhenius equation (Eq. 4), in which k represents the rate constant (k_{in} or k_{deg}) at temperature T (K), k_{ref} is the corresponding rate constant, $k_{in(ref)}$ or $k_{deg(ref)}$, at a reference temperature (T_{ref}) of 375.5 K and R is the universal gas constant (8.314 J/mol·K).

$$k = k_{ref} \exp\left[\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \quad (4)$$

A one-step non-linear regression approach, assuming first order kinetics, was applied in which k values for individual temperature levels and corresponding activation energies were simultaneously estimated. Therefore, the Arrhenius equation (Eq. 4) was substituted into Eq. 2 and Eq. 3. The differential equations were solved by numerical integration (non-linear regression (NLIN) procedure, SAS version 9.6, Carry, NC, USA). The goodness of fit between the experimental and predicted data was evaluated by visual inspection of residual plots and by means of the adjusted coefficient (R^2-adj) (Eq. 5), considering that the higher the R^2–adj, the better the fit:

$$\text{Adjusted} - R^2 = \left[\frac{(m-1)\left(1-\frac{SSQ_{regression}}{SSQ_{total}}\right)}{(m-j)}\right] \quad (5)$$

where m, j and SSQ represents the number of observations, the number of model parameters and the sum of squares, respectively.

3. Results and discussion

The transfer to oil and degradation kinetics of α-carotene and β-carotene from carrot, and lycopene and β-carotene from tomato particle suspensions during thermal processing was investigated. At first the

matrices were decomposed into cell clusters and chromoplast enriched fractions. In this way the effect of different physical barriers, i.e. the cell wall and the chromoplast substructure, that hinder carotenoid release (Jeffery, Holzenburg, & King, 2012; Palmero et al., 2013) and thus, their transfer to oil was considered. Then, the cell cluster and chromoplast enriched fractions from carrot and tomato were thermally treated and carotenoid transfer to oil as well as degradation as a function of treatment temperature and time were studied by using a kinetic model.

3.1 Screening of carotenoid transfer from carrot and tomato matrices to oil during thermal processing

In order to determine the relevant temperature range for the kinetic study, carotenoid transfer to the oil phase was screened. Hereto, the isolated fractions of tomato and carrot were mixed with an oil phase and thermally treated at 80, 90, 110 and 120 °C for 20 min. The intensity of the thermal treatment was chosen based on the temperature ranges that are generally employed during industrial pasteurization and sterilization of fruit and vegetable-based products, being 65-85 and 110-130 °C, respectively (Ramaswamy & Marcotte, 2005). In addition, as carotenoid transfer from the matrix to the oil can depend on the lipid droplet size (Tyssandier et al., 2003), the oil phase was added in the form of a 5% oil-in-water emulsion (fraction/emulsion 1:10, w/v). The average oil droplet size in the emulsion, as measured by laser diffraction analysis, was around 1.207 ± 0.012 μm and the particle size distribution was consistent throughout the experiment (data not shown). The oil was recovered from the treated and control samples by centrifugation and analysed for total (all-*trans*+*cis* isomers) lycopene, *β* carotene and *α*-carotene content. Carotenoid percentage transfer from carrot and tomato cell cluster and chromoplast enriched fractions to the oil phase as a function of treatment temperature was calculated as the ratio between the amount of carotenoid in the recovered oil and the initial amount of carotenoid in the control sample (Eq. 1) (Fig. 2).

Carotenoids were not detectable (limit of detection and limit of quantification: 0.0005 and 0.002 μg on column, respectively (Lemmens et al., 2011)) in the recovered oil from the control samples for the

292 different isolated fractions of both carrot and tomato. This indicates that, in line with Mutsokoti et al.
293 (2015), unless treatment is applied, the transfer of carotenoids from the matrix to the oil phase is rather
294 limited. A slight increase in percentage lycopene and *β*-carotene transfer to oil was observed for both
295 the cell clusters (Fig. 2A) and chromoplast enriched (Fig. 2B) fraction of tomato when the treatment
296 temperature was increased from 80 to 90 °C, the increase being higher in the chromoplast samples.
297 A similar trend was observed for *α*- and *β*-carotene in the carrot chromoplast enriched fraction (Fig. 2D),
298 while no significant difference ($P<0.05$) in percentage carotenoid transfer in the recovered oil from
299 carrot cell clusters (Fig. 2C) treated at 80 and 90 °C was found. The increase in treatment temperature to
300 110 °C resulted in a marked increase in percentage carotenoid transfer from both cell clusters and
301 chromoplast enriched fractions of carrot and tomato to oil (Fig. 2 A-B). It is noteworthy that such
302 increase varied to a different extent depending on carotenoid type. In fact, percentage carotenoid
303 transfer to oil was higher for *β*-carotene than for lycopene in tomato fractions while, within the carrot
304 fractions, the increase in *α*- and *β*-carotene was similar. This observation maybe expected due to the
305 differences in the chemical structures and consequently in the hydrophobicity of the different
306 carotenoids. Similar results were reported for the transfer of carotenoids to oil during high pressure
307 homogenization (Mutsokoti et al., 2015) and during *in vitro* digestion (Palmero et al., 2014) of tomato
308 and carrot fractions although the transfer during digestion was far less efficient than during processing.
309 Previous investigations have reported that disruption of the cell wall and organelle structure as well as
310 carotenoid-protein complexes can occur under thermal treatment conditions similar to the ones used in
311 the present study (De Belie, Herppich, & De Baerdemaeker, 2000; Hornero-Méndez & Mínguez-
312 Mosquera, 2007; Sila et al., 2005). This can result in increasing the permeability of the cell wall and
313 consequently the release of intracellular carotenoids (Donsì, Ferrari, & Pataro, 2010). Roohinejad et al.
314 (2014), for example, reported an improved extraction of β-carotene from pulsed electric field treated
315 (49.4 min at 52.2 °C) carrot pomace to an oil-in-water microemulsion. Sachindra and Mahendrakar,

316 (2005) reported a similar affect of temperature in increasing the extraction of carotenoids from
317 thermally treated (70 °C, 150 min) shrimp waste in sunflower oil. Therefore, the increase in carotenoid
318 transfer to oil at 110 °C (Fig. 2) can be attributed to the disintegration of cell walls and the chromoplast
319 ultrastructure as a result of the thermal treatment.
320 Data on the solubility of carotenoids in oil as influenced by temperature during thermal processing of
321 fruit and vegetable matrices is not available in literature. Nevertheless, studies on the extraction of
322 carotenoids in supercritical carbon dioxide have shown that increasing the temperature results in an
323 increase in the vapor pressure of lycopene (Egydio, Moraes, & Rosa, 2010; Saldaña et al., 2010; Topal et
324 al., 2006). This in turn leads to an increase in lycopene solubility thus, facilitating its transfer from the
325 matrix to the solvent (Mustapa, Manan, Mohd Azizi, Setianto, & Mohd Omar, 2011). Longo, Leo, and
326 Leone, (2012) reported an increase in lycopene content in the oleoresin obtained from tomato/hazelnut
327 matrix as a result of increasing the treatment temperature from 60 to 100 °C and attributed it to
328 increased lycopene solubility. McKenna et al. (2003) reported a 10 fold increase in lycopene and
329 asthaxanthin solubility at 100 °C compared to room temperature. Analogously, in the present study, the
330 increase in percentage carotenoid transfer to oil at 110 °C can be related to the increase in lycopene and
331 *β*-carotene solubility in the oil phase.
332 The further increase in treatment temperature to 120 °C resulted instead in significantly lower
333 percentage carotenoid transfer than that observed at 110 °C (Fig. 2), suggesting the occurrence of
334 carotenoid degradation (Colle et al., 2010; Lemmens et al., 2010). The latter is mainly attributed to
335 oxidation (Achir, 2010), with the reaction being more pronounced at high temperatures (Rodriguez &
336 Rodriguez-Amaya, 2007). The lower percentage carotenoid transfer observed at 120 °C reflects lower
337 carotenoid concentration in the recovered oil. Considering the mechanism for the oxidative *β*-carotene
338 degradation proposed by Mordi (1993), the lower amount of carotenoids in the recovered oil can be
339 attributed to the cleavage of the carotenoid polyene chain into lower molecular weight compounds that

were not detected by DAD. In view of these results a kinetic study was conducted at temperatures ranging between 95 and 110 °C for increasing time up to 40 minutes.

3.2 Carotenoid transfer to oil and degradation kinetics during thermal processing

The concentration (all-*trans*+*cis* μg/g_{oil}) of *β*-carotene, *α*-carotene and lycopene as a function of time in the oil recovered from thermally treated carrot and tomato chromoplast enriched fractions at the different temperatures applied, is given as single data points in Fig. 3. When the tomato chromoplast fraction was treated at 95 and 100 °C, lycopene (Fig. 3A) and *β*-carotene (Fig. 3B) concentration in the recovered oil increased with increasing treatment time. Conversely, for the carrot chromoplast fraction, *α*-carotene and *β*-carotene concentration in the recovered oil increased up to 7.5 min and levelled off thereafter. The oil recovered from the tomato and carrot chromoplast fraction that was treated at 105 and 110 °C, was characterized by an initial increase in carotenoid concentration within 5 minutes, followed by a decrease in carotenoid concentration at longer treatment times. Overall, carotenoid concentrations in the oil increased with increasing temperature within the first 10 minutes of the treatment (Fig. 3).

The experimental data were modelled by a one-step regression, assuming the proposed model (Eq. 4). The model could fit the experimental data fairly well (Fig. 3) and a good correlation (R^2-adj ≥ 0.89) between experimental and predicted values was found (Table 1). The transfer rate constant of *β*-carotene to the oil, described by the kinetic parameter k_{refin}, was considerably higher than that of lycopene. Moreover, the respective activation energy, Ea_{in}, was lower for *β*-carotene compared to lycopene. In the case of the carrot chromoplast fraction, similar transfer rate constant (k_{refin}) and the same activation energy (Ea_{in}) were found for *α*- and *β*-carotene (Table 1). This once more confirms that transfer to oil depends on carotenoid hydrophobicity which in turn determines carotenoid solubility in the oil. In fact, *α*- and *β*-carotene differ only in the position of the double bond in one of the *β*-ionone rings in their structure (Britton, 1995) and thus, can exhibit similar transfer efficiency to oil. The transfer

rate constants (k_{refin}) of *β*-carotene were also found to be similar for both tomato and carrot chromoplast fraction. This result suggests that the release and subsequent transfer to oil of carotenoids during thermal processing was governed by carotenoid molecular structure, rather than the chromoplast sub-structure. This observation is in agreement with the work of Palmero et al., (2014), where carotenoid transfer to oil was considered during *in vitro* digestion of carrot and tomato fractions. Information on the mass transfer rates of carotenoids into edible oils during thermal processing for comparison purposes is not available.

With regard to carotenoid degradation (k_{refdeg}), in case of the tomato chromoplast based suspension, the *k* value for lycopene was considerably lower than that of *β*-carotene. For the carrot chromoplast based suspension, the k_{refdeg} value of *β*-carotene was only slightly lower than that of *α*-carotene, whereas the respective activation energy values were similar, suggesting *α*- and *β*-carotene degradation rate constants to be similarly temperature sensitive.

The degradation rate constants and activation energy values of lycopene, *α*- and *β*-carotene, k_{refdeg} and Ea_{deg}, respectively (Table 1) were found to be higher compared to the values reported for conventional (Nguyen & Schwartz, 2001; Chen et al., 2009; Colle et al., 2010b and Knockaert et al., 2012) or microwave heating (Fratianni, Cinquanta & Panfili, 2010; Vikram, Ramesh, & Prapulla, 2005). For example, Fratianni, Cinquanta and Panfili, (2010) found a rate constant of 0.658 min^{-1} and an activation energy of 188 kJ/mol for *β*-carotene degradation in orange juice under microwave heating conditions for a process temperature range of 70-85 °C. A possible reason for this discrepancy lies in the different composition of the food systems, in particular the composition of the oil phase. In the present work, the oil phase was applied as an emulsified system, while Colle et al. (2010b) used olive oil as such. Moreover the particle suspensions used in the present study can be considered as more dilute systems compared to puree/oil systems used by Knockaert et al. (2012).

387 In order to investigate further the influence of the matrix on carotenoid transfer to oil during thermal
388 processing, cell clusters from carrot and tomato, representing matrices with an extra barrier (the cell
389 wall) governing carotenoid release (Palmero et al., 2013), were also considered (Fig. 4).
390 Similarly to the chromoplast based suspensions (Fig. 3), in the tomato and carrot cell cluster based
391 suspensions treated at 95 °C (Fig. 4), the concentration of carotenoids in the recovered oil increased
392 with increasing treatment time. On the other hand, the oil recovered from tomato and carrot cell
393 clusters treated at 100, 105 and 110 °C was characterised by an initial increase followed by a decrease in
394 carotenoid concentration (Fig. 4). Overall, the higher the temperature applied, the shorter the time
395 needed for the carotenoid concentration in the recovered oil to reach its maximum value. In general,
396 the increase in carotenoid concentration in the oil was obtained within 10 min of treatment at all the
397 temperatures applied. Also, the decrease in carotenoid concentration was steeper with increasing
398 temperature and considerably greater for α- and β-carotene compared to lycopene (Fig. 4).
399 The concentration of α- and β-carotene in the oil recovered from carrot, and of lycopene and β-carotene
400 in the oil recovered from tomato cell cluster fractions as a function of treatment temperature and time
401 was also modelled (Eq. 4) and is illustrated with full lines in Fig. 4. A good correlation ($R^2 \geq 0.89$) between
402 the experimental and predicted values was observed (Table 2).
403 The k values reported in Table 2 confirm the effect of temperature on carotenoid transfer to oil and
404 degradation, as observed in the chromoplast fraction (Table 1). In particular, for tomato cell clusters, a
405 higher rate constant (k_{refin}) of β-carotene transfer to oil compared to lycopene was found (Table 2),
406 indicating that the mass transfer rate to oil of β-carotene proceeded much faster than lycopene. With
407 regard to degradation, a higher rate constant (k_{refdeg}) of β-carotene compared to lycopene for tomato
408 cell clusters was found. In carrot cell clusters on the other hand, the degradation rate constant of α- and
409 β-carotene was similar. Comparing β-carotene from the chromoplast (Table 1) and cell clusters (Table 2)
410 based suspensions, higher degradation rate constants (k_{refdeg}) were obtained for chromoplast compared

411 to the cell cluster regardless of the source, carrot or tomato. However, activation energies (Ea_{deg}) were
412 similar in all the fractions from both carrot and tomato. This suggests that while *β*-carotene transfer to
413 oil is influenced by the matrix, its sensitivity to degradation during thermal processing is influenced by
414 its hydrophobicity. This seems logic because the release from the matrix and subsequent mass transfer
415 of the carotenoids into oil is affected by the natural physical barriers while degradation is reported to
416 occur primarily in the oil phase.
417 The maximum concentration attained in the oil depended not only on temperature/time combination,
418 but also on carotenoid type and level of bioencapsulation, being always higher for the chromoplast
419 compared to the cell cluster fractions (Fig. 4). At 110 °C for example, 75.9±2.9 µg lycopene/g oil was
420 found in the oil recovered from tomato cell cluster fraction and this value was reached within 5 min. On
421 the other hand, 117.8±2.1 µg lycopene/g oil was found in the oil recovered from tomato chromoplast
422 fraction after 3 min treatment time. This is also reflected in the higher carotenoid transfer rate
423 constants, k_{refin}, from the chromoplast (Table 1) compared to the cell cluster fractions (Table 2). In
424 particular the estimated k_{refin} values of lycopene and *β*-carotene in the chromoplast were twice as high
425 as the values estimated for the corresponding carotenoids in the cell cluster fraction of tomato. Also,
426 the estimated k_{refin} values of *α*-carotene and *β*-carotene were found to be higher for the chromoplast
427 compared to the cell cluster fractions of carrot. The lower k_{refin} values for the cell cluster fractions are
428 explained by the presence of the cell wall that hinders carotenoid release and then transfer to oil. When
429 carotenoid transfer to oil was considered during *in vitro* digestion, Palmero et al. (2014) reported a
430 similar effect of the cell wall in limiting carotenoid transfer to oil from carrot and tomato fractions.
431 Generally, the estimated rate constants did not seem to be remarkably influenced by the source
432 (tomato vs carrot) but rather by the level of bioencapsulation (cell wall and chromoplast substructure).
433 As explained by Colle et al. (2011), this indicates the influence of the food system on the rate constants.
434 Although the estimated kinetic parameters are apparent, a clear insight in the effect of carotenoid

hydrophobicity and level of bioencapsulation could be observed. All in all, the results indicate that, in order to achieve maximum carotenoid transfer to oil, first, food systems should be disrupted to a level where the cell wall is broken. Second, thermal treatments should then be performed at temperatures above 105 °C but less than 120 °C. Furthermore, while the level of bioencapsulation plays a crucial role in influencing carotenoid transfer to oil, carotenoid chemical structure, and hence hydrophobicity, appears to be the main factor influencing carotenoid transfer to oil and degradation.

In order to evaluate the selectivity for transfer to oil of the carotenoids within each matrix, carotenoid concentration ratios in the recovered oil from each fraction as a function of treatment temperature were calculated and plotted against the treatment time (Fig. 5). At 95 and 100 °C, the *β*-carotene/lycopene ratios in tomato fractions initially increased [0.45±0.16 to 0.68±0.03 in the cell cluster fraction (Fig. 5A), and 0.28±0.04 to 0.71±0.04, in the chromoplast fraction (Fig. 5B)] within 5 minutes, indicating a preference for the transfer of *β*-carotene to oil. Thereafter, this was followed by a decrease in the ratio *β*-carotene/lycopene. On the other hand, at 105 and 110 °C, the *β*-carotene/lycopene ratio decreased and reached a plateau after 10 minutes. This observation suggests that lycopene might be more strongly bound within the matrix than *β*-carotene resulting in its limited release and subsequent transfer to oil (k_{refin} lycopene < *β*-carotene, Tables 1 & 2). As the thermal treatment intensity increases, this coincides with increasing matrix disruption and consequently enhances lycopene release and its transfer to oil. However, at the same time, degradation reactions also become more pronounced with increasing treatment intensity so that the net effect on carotenoid concentration in the oil is what seems to be a balance between the transfer into oil and degradation. Hence the tendency towards a plateau. Generally, in carrot fractions, (Fig. 5C & 5D), the ratio *α*-carotene/*β*-carotene remained fairly constant (between 0.5 and 0.7) with treatment time and temperature, indicating that there was no preference between *α*- and *β*-carotene for transfer to oil. This makes sense since the estimated kinetic parameters (Tables 1 & 2) revealed that the temperature

sensitivity of the degradation rate constants of the two carotenoids was similar. However, within the first 3 minutes of treatment at 95 and 100 °C, a decrease [0.68±0.10 to 0.48±0.09, (Fig. 5C) and 0.74±0.25 to 0.44±0.12, (Fig. 5B)] in the ratio α-carotene/β-carotene was observed.

4. Conclusions

In this study, the transfer to oil of lycopene and β-carotene from tomato as well as α- and β-carotene from carrot based particle suspensions upon thermal treatments was investigated. The results showed that treatment of the particle suspensions at temperatures above 100 °C for treatment time of 10 minutes was necessary to significantly favour carotenoid transfer to oil. Carotenoid transfer to oil and degradation kinetics gave insight in the effect of the natural structural barriers and carotenoid hydrophobicity in determining carotenoid release and its subsequent transfer to the oil phase during thermal processing. Overall, the results revealed that the major effect of the thermal treatments on carotenoid transfer to oil was governed by the carotenoid bioencapsulation. From a practical point of view, the results suggest that when the aim is to achieve maximum carotenoid transfer to oil high treatment temperatures for short treatment times, such as under typically UHT conditions, are necessary and this can be important to enhance the nutritional quality of carrot and tomato based foods.

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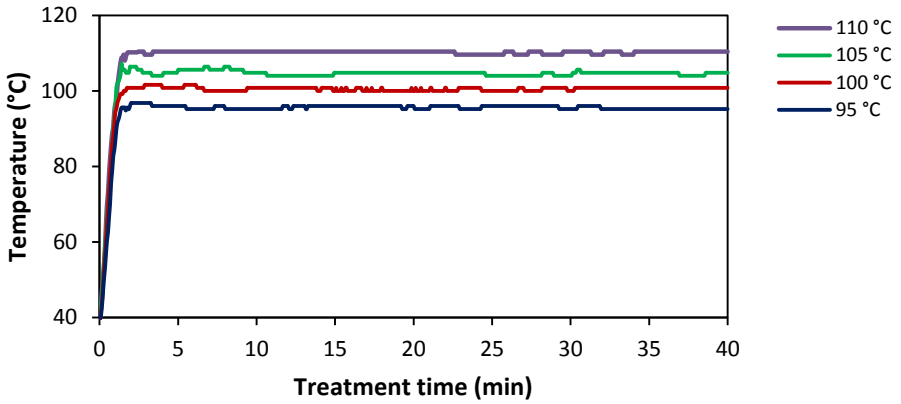

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608 **Fig. 1.** Example of typical time/temperature profiles of samples microwave heated at temperatures
609 between 95 and 110 °C.
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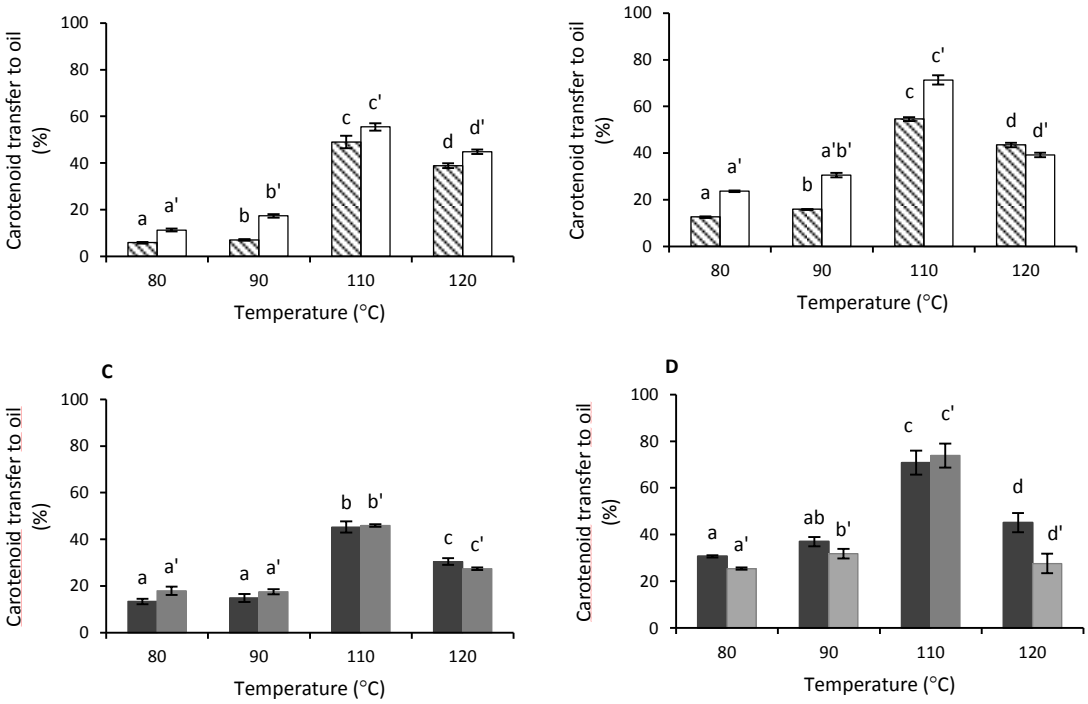

612

613 **Fig. 2.** Percentage carotenoid transfer to the oil phase during thermal processing. (A) Tomato cell clusters and (B)
614 chromoplast-enriched fractions: (▨) lycopene; (□) *β*-carotene (C) Carrot cell clusters and (D) chromoplast enriched
615 fractions: (■) *α*-carotene; (■) *β*-carotene. Different letters within each fraction for each carotenoid indicates
616 significant differences ($P<0.05$).
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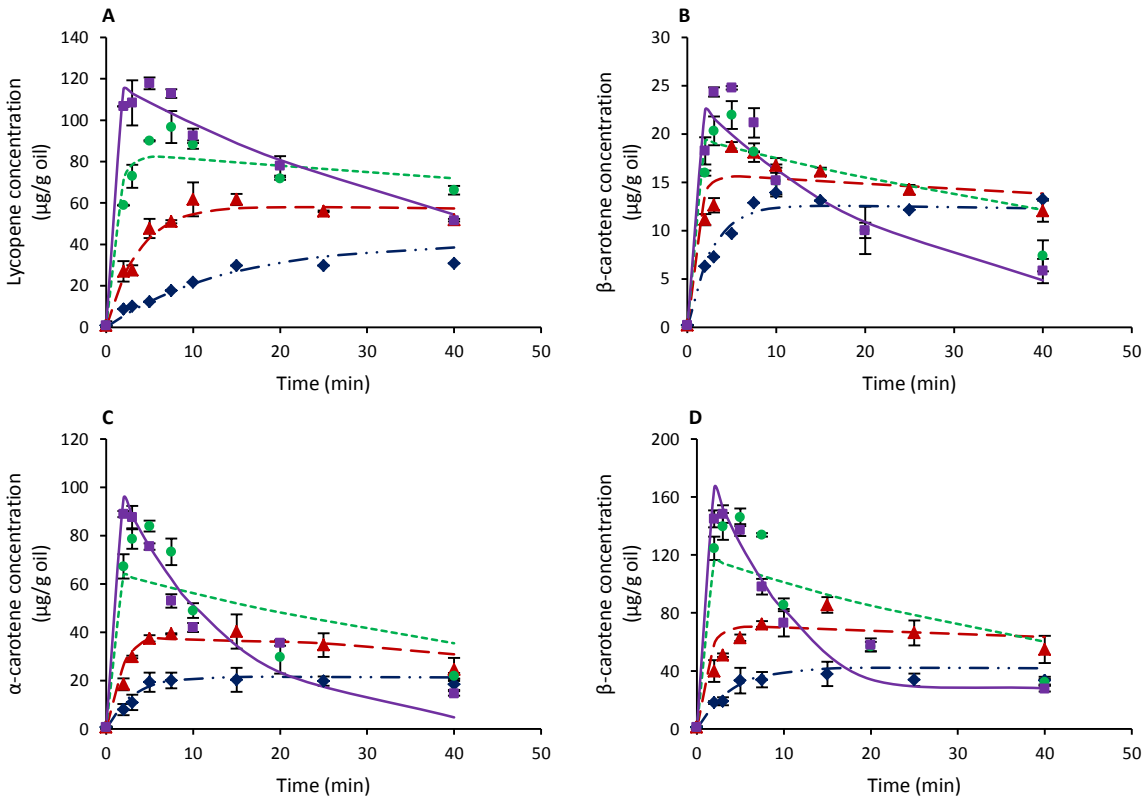

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619 **Fig. 3**. Time course of the concentration (all-*trans*+*cis*) in the oil of (A) Lycopene and (B) *β*-carotene from tomato;
620 (C) *α*-carotene and (D) *β*-carotene from carrot chromoplast-enriched fractions thermally treated at (◆——) 95 °C,
621 (▲– – –) 100 °C, (●·····) 105 °C and (■——) 110 °C. The lines represent the concentration values predicted by the
622 kinetic model, whereas the experimental data are represented by the symbols. Error bars represent standard
623 deviations (n=8).
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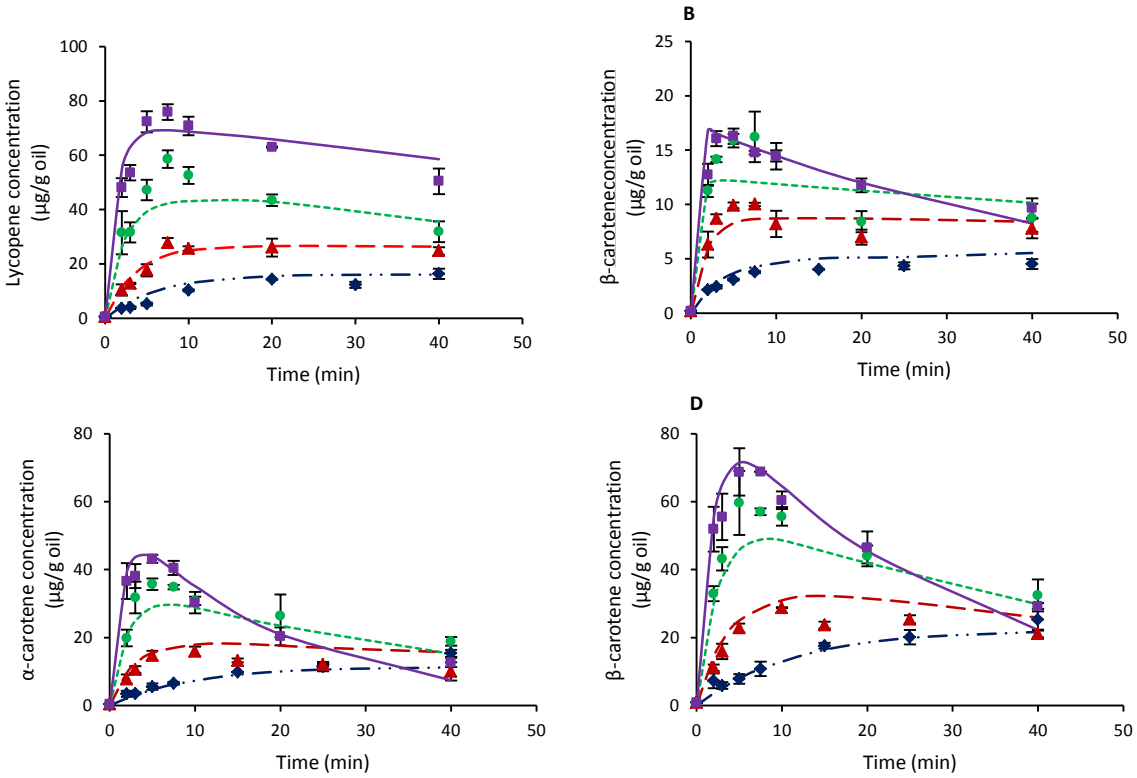
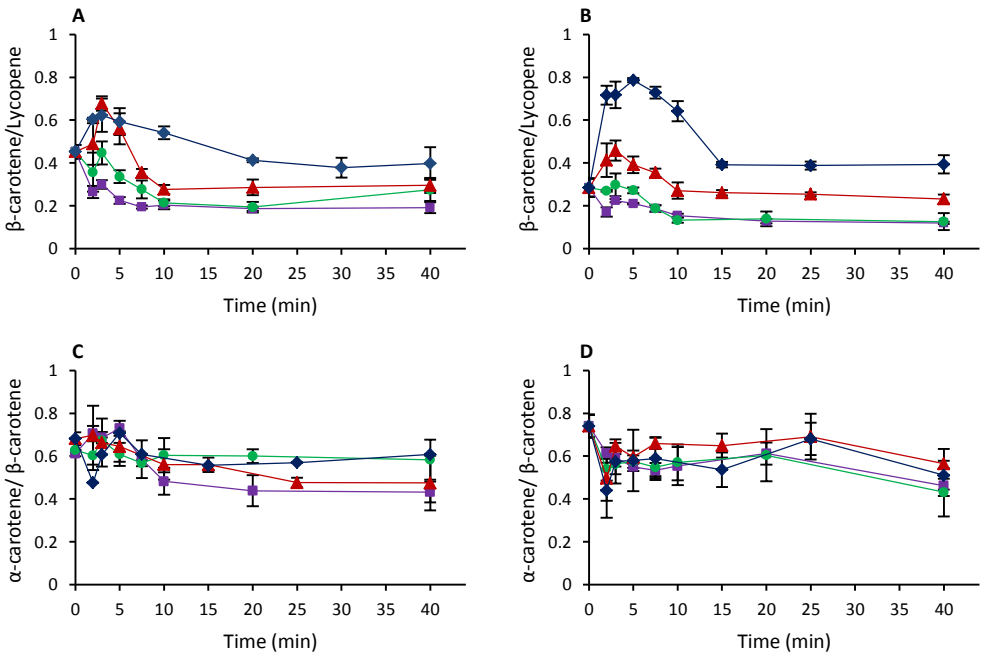


Fig. 4. Time course of the concentration (all-*trans*+*cis*) in the oil of (A) Lycopene and (B) *β*-carotene from tomato; (C) *α*-carotene and (D) *β*-carotene from carrot cell cluster fractions thermally treated at (◆–··–) 95 °C, (▲– – -) 100 °C, (●------) 105 °C and (■——) 110 °C. The lines represent the concentration values predicted by the kinetic model, whereas the experimental data are represented by the symbols. Error bars represent standard deviations (n=8).



636

637 **Fig. 5**. Ratio of the concentration, *β*-carotene/ lycopene in the recovered oil from tomato (A) cell clusters and (B)
638 chromoplast fractions; *α*-carotene/ *β*-carotene in the recovered oil from carrot (C) cell clusters and (D)
639 chromoplast fraction as a function of the treatment time. (◆) 95 °C, (▲) 100 °C, (●) 105 °C and (■)110 °C.
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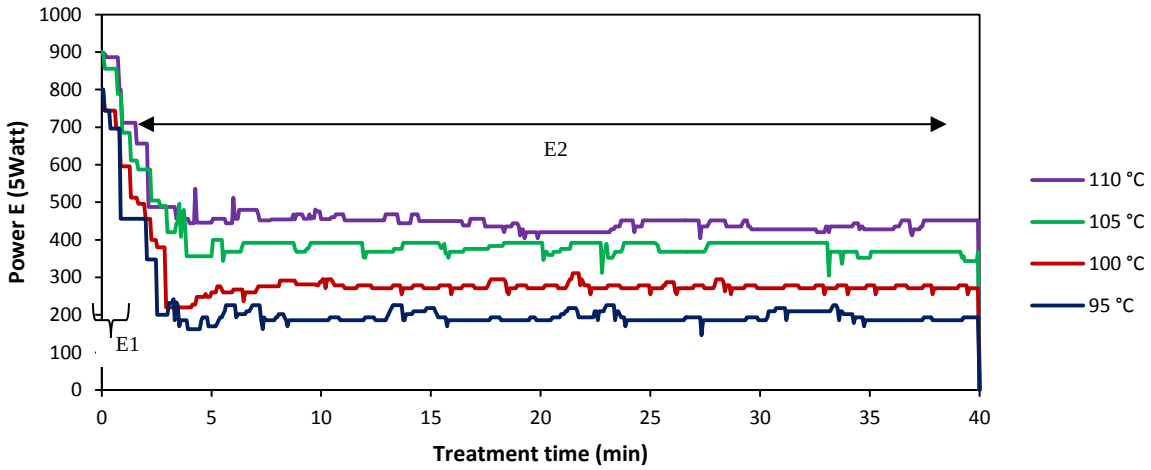

642

643 **Fig. A1.** A typical power/time profile during microwave heating of tomato and carrot based particle suspensions.

644

Table 1. Kinetic parameters for the modelling of the changes in concentration (all-*trans*+*cis*) in the oil of lycopene, *α*- and *β*-carotene during thermal treatments of carrot and tomato chromoplast enriched fractions.

Matrix	Carotenoid	$k_{ref\ in}$ ($\cdot 10^{-2} min^{-1}$)	$k_{ref\ deg}$ (min^{-1})	Ea_{in} ($\cdot 10\ kJ \cdot mol^{-1}$)	Ea_{deg} ($\cdot 10\ kJ \cdot mol^{-1}$)	R^2-adj
Tomato chromoplasts	*β*-carotene	0.66 ± 0.14	1.70 ± 0.28	28.7 ± 2.8	23.7 ± 2.7	0.870
	Lycopene	0.18 ± 0.01	0.49 ± 0.05	38.0 ± 1.7	29.6 ± 1.8	0.971
Carrot chromoplasts	*α* -carotene	0.67 ± 0.20	1.86 ± 0.49	39.1 ± 4.2	26.3 ± 4.3	0.890
	β-carotene	0.75 ± 0.17	1.49 ± 0.34	39.1 ± 3.7	27.1 ± 3.7	0.889

T_{ref} = 102.5 °C

R^2-adj represents the goodness of fit between experimental and predicted data

651 **Table 2**. Kinetic parameters for the modelling of the changes in concentration (all-*trans*+*cis*) in the oil of lycopene,
652 *α*- and *β*-carotene during thermal treatments of carrot and tomato cell cluster fractions.
653

Matrix	Carotenoid	$k_{ref\ in}$ ($\cdot 10^{-2}$ min^{-1})	$k_{ref\ deg}$ (min^{-1})	Ea_{in} ($\cdot 10$ kJ·mol^{-1})	Ea_{deg} ($\cdot 10$ kJ·mol^{-1})	R^2-adj
Tomato cell clusters	*β*-carotene	0.31 ± 0.05	0.89 ± 0.19	30.8 ± 3.7	22.7 ± 3.7	0.890
	Lycopene	0.09 ± 0.01	0.35 ± 0.07	23.5± 3.7	11.9 ± 4.2	0.956
Carrot cell clusters	*α* -carotene	0.30 ± 0.06	0.67 ± 0.15	39.6 ± 3.5	28.6 ± 3.0	0.913
	β-carotene	0.22 ± 0.04	0.50 ± 0.11	35.7± 3.7	26.8 ± 3.9	0.899

654 T_{ref} = 102.5 °C
655 R^2-adj represents the goodness of fit between experimental and predicted data

656
657

Table A1. Predetermined power to achieve the treatment temperature (E1, Watt) and to maintain it (E2, Watt) during microwate heating of tomato and carrot based particle suspensions.

$T_{treatment}$ (°C)	T (min: sec)	E1 (Watt)	E2 (Watt)
80	00:08	900	
	00:01	600	
	00:30	500	
	19:21		300
90	00:15	900	
	00:01	800	
	00:30	750	
	01:45	600	
	17:29		300
95	00:15	1000	
	00:01	800	
	00:30	600	
	01:45	400	
	37:29		250
100	00:15	1000	
	00:01	900	
	00:30	600	
	01:29	500	
	37:45		300
105	00:30	900	
	00:01	800	
	00:30	700	
	00:45	600	
	38:29		350
110	00:45	1000	
	00:01	700	
	01:14	600	
	38:00		400
120	00:45	1000	
	00:01	800	
	00:44	700	
	00:30	600	
	18:00		400