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This is a pre print version of the following article:

Original

Effect of ultrasound treatment, oil addition and storage time on lycopene stability and in vitro bioaccessibility of tomato pulp / Anese, Monica; Bot, Francesca; Panozzo, Agnese; Mirolo, Giorgio; Lippe, Giovanna. - In: FOOD CHEMISTRY. - ISSN 0308-8146. - 172:(2015), pp. 685-691. [10.1016/j.foodchem.2014.09.140]

Availability:

This version is available at: 11381/2919422 since: 2022-03-19T11:18:03Z

Publisher:

Published

DOI:10.1016/j.foodchem.2014.09.140

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1 **Effect of ultrasound treatment, oil addition, and storage time on lycopene stability and *in vitro***
2 **bioaccessibility of tomato pulp**

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17

18 **Abstract**

19 This study was performed to investigate the influence of ultrasound processing on tomato pulp
20 containing no or increasing amounts (i.e. 2.5%, 5% and 10%) of sunflower oil on lycopene
21 concentration and *in vitro* bioaccessibility at time zero and during storage at 5 °C. Results confirmed
22 previous findings in that ultrasonication was responsible for cell breakage and subsequent lycopene
23 release in a highly viscous matrix. Neither ultrasound process nor oil addition affected lycopene
24 concentration. A decrease of approximately 35% lycopene content occurred at storage times higher
25 than 15 days, due to isomerization and oxidation reactions. No differences in lycopene *in vitro*
26 bioaccessibility were found between the untreated and ultrasonically treated samples; this parameter
27 decreased as a consequence of oil addition. Losses of lycopene *in vitro* bioaccessibility ranging
28 between 50% and 80% occurred in the untreated and ultrasonically treated tomato pulps with and
29 without oil during storage, mainly due to carotenoid degradation.

30

31

32 **Keywords:** Lycopene, Tomato pulp, Ultrasound processing, Lycopene *in vitro* bioaccessibility,
33 Storage, Dietary oil

34

35 **1. Introduction**

36 Recent findings have shown that unconventional non-thermal technologies, such as high pressure,
37 ultraviolet light, ultrasounds can be addressed towards the development of a wide range of different
38 and technologically evolved ingredients and intermediate products, able to accomplish desired
39 technological and nutritional functions (Mason, Paniwnyk, & Lorimer, 1996; Soria & Villamiel,
40 2010; Manzocco, Panozzo, & Nicoli, 2012). In particular, ultrasound processing is widely exploited
41 at industrial level for its capability to induce changes of some chemical and physical properties of
42 food constituents (Mason et al., 1996). As far as is known, the ultrasounds mechanism of action lies
43 in the rapidly alternating compression and decompression zones propagating into the material being
44 treated, and the cavitation that these zones cause. Cavitation involves the formation and violent
45 collapse of small bubbles, generating shock waves with associated local extreme temperatures and
46 pressures, inside the collapsing bubbles, that in turn produce highly reactive radicals (Leighton,
47 1994). Depending on ultrasound energy and food type, ultrasound processing was found to induce
48 structural and functional modifications of macromolecules (e.g. proteins and polysaccharides)
49 (Vercet, Oria, Marquina, Crelier, & López-Buesa, 2002; Ashokkumar et al., 2008; Wu, Gamage,
50 Vilku, Simons, & Mawson, 2008). According to these authors, ultrasound-induced changes in inter-
51 and intra-molecular interactions would account for either an increase or decrease in texture and
52 viscosity, antioxidant properties, emulsifying capacity, of a number of polymer-containing systems,
53 including foods matrices such as yoghurt and tomato derivatives.

54 Tomato is a worldwide important crop due to its large consumption and versatility to be used as
55 ingredient in many food recipes, and its high lycopene content. The high degree of conjugation and
56 hydrophobicity confer to lycopene molecule the typical red colour as well as unique biological
57 properties, including strong antioxidant activity (Di Mascio, Kaiser, & Sies, 1989; Shi & Le Maguer,
58 2000). It has been suggested that a lower risk of developing cardiovascular diseases and cancer
59 following a diet rich in this carotenoid might be actually related to lycopene antioxidant properties
60 (Tanaka, Shnimizu, & Moriwaki, 2012). These effects are strictly related to the carotenoid

61 bioaccessibility, i.e. the fraction of a nutrient that is released from the food matrix and incorporated
62 into micelles during digestion before being absorbed by enterocytes (Hedrén, Diaz, & Svanberg,
63 2002). The bioaccessibility of lycopene has been shown to increase in the presence of dietary lipids,
64 that would favour its incorporation into micelles (Stahl & Sies, 1992; Böhm, 2002; Colle, Van
65 Buggenhout, Lemmens, Van Loy, & Hendrickx, 2012). In particular, both the type and the amount
66 of lipids resulted to affect lycopene bioaccessibility, lipids containing a large fraction of long chain
67 tryglicerides (e.g. sunflower oil, olive oil, cocoa butter) being more effective in transferring lycopene
68 from the food matrix (Huo, Ferruzzi, Schwartz, & Failla, 2007; Colle et al. 2012). Besides the
69 physiological conditions (e.g. intestinal pH, bile salts level), co-ingestion of fat, fibre, and other
70 carotenoids, occurring during digestion, as well as the food technological history greatly affects
71 lycopene bioaccessibility (Stahl & Sies, 1992; Shi & Le Maguer, 2000). Although processing (e.g.
72 mechanical crushing, pasteurization and sterilization, formulation) and subsequent storage may be
73 responsible for lycopene degradation in tomato products *via* isomerization and oxidation reactions,
74 processed tomato has been shown to be a more available source of lycopene than raw tomato (Stahl
75 & Sies, 1992; Porrini, Riso, & Testolin, 1998). Heat and mechanical forces have been reported to
76 improve lycopene bioaccessibility by breaking down or softening plant cell walls and chromoplast
77 membrane entrapping lycopene (Stahl & Sies, 1992; Svelander, Tibäck, Ahrné, Langton, Svanberg,
78 & Alminger, 2010; Colle, Lemmens, Van Buggenhout, Van Loy, & Hendrickx, 2010a; Knockaert,
79 Pulissery, Colle, Van Buggenhout, Hendrickx, & Van Loey, 2012). Recently, we investigated the
80 effect of increasing ultrasound energies on tomato pulp microstructure and lycopene *in vitro*
81 bioaccessibility (Anese, Mirolo, Beraldo, & Lippe, 2013). These treatments, while causing loss of
82 tomato cell integrity, induced reorganization of partially depolymerised pectins to form a stronger
83 network where lycopene would be entrapped, being thus less accessible for digestion. Similarly,
84 Colle, Van Buggenhout, Van Loey, & Hendrickx (2010b) and Panozzo, Lemmens, Van Loey,
85 Manzocco, Nicoli, & Hendrickx (2013) demonstrated that high pressure homogenization treatments
86 negatively affected the *in vitro* bioaccessibility of lycopene. Also in this case a negative relationship

87 between carotenoid bioaccessibility and product viscosity was found. By contrast, Knockaert et al.
88 (2012) observed that high pressure homogenization of tomato puree improved the lycopene *in vitro*
89 bioaccessibility, especially in the presence of 5% olive oil. Finally, Gupta, Kopec, Schwartz, &
90 Balasubramaniam (2011) found that high pressure homogenization increased lycopene
91 bioaccessibility when applied prior to heating of tomato juice, probably because the already damaged
92 cellular tissues by the high pressure process were further disrupted by heat.

93 The aim of the present study was to investigate the effect of ultrasound processing on tomato pulp
94 added or not added with a lipid phase on lycopene concentration and *in vitro* bioaccessibility at time
95 zero and during storage under refrigerated conditions. Data were compared with those of analogous
96 samples that were not subjected to ultrasound treatment. Contextually, the changes of viscosity,
97 tomato colour and oxidative status of the lipid fraction of the control and ultrasonically processed
98 samples were studied. To our knowledge, no data on the influence of ultrasound processing on
99 lycopene stability and *in vitro* bioaccessibility during storage of tomato derivatives have been
100 reported yet.

101

102 **2. Materials and methods**

103 *2.1. Sample preparation*

104 Commercial pasteurized tomato pulp was sieved to separate seeds and coarse particles, and submitted
105 to ultrasound treatment. Tomato pulp not subjected to ultrasound treatment (untreated sample) was
106 taken as a control. Aliquots of the unprocessed and processed tomato pulps were added with
107 increasing amounts (i.e. 0%, 2.5%, 5% and 10% w/w) of commercial sunflower oil. Samples were
108 then stored at 5 °C for up to 100 days. To inhibit microbial growth during storage, 1.5 g/L potassium
109 sorbate and sodium benzoate (Carlo Erba, Milano, Italy) were added to samples.

110 *2.2. Ultrasound treatment*

111 An ultrasonic processor (Hieschler Ultrasonics GmbH, mod. UP400S, Teltow, Germany) with a
112 titanium horn tip diameter of 22 mm was used. Aliquots of 60 g of tomato pulp were introduced into

113 250 mL capacity (90 mm height, 75 mm diameter) glass vessels. The horn was placed in the centre
114 of the vessel, with an immersion depth in the fluid of 5 mm. In order to minimise water evaporation
115 during sonication, the vessel was closed with a Plexiglas lid fitted with holes allowing horn and
116 thermocouple probes to be placed at the desired positions in the tomato pulp. During the ultrasound
117 treatment, tomato pulp was kept under stirring to allow temperature to equilibrate within the sample.
118 The temperature was recorded as a function of time using a copper-constantan thermocouple probe
119 (Ellab, Denmark), connected to a data-logger (CHY 502A1, Tersid, Milano, Italy). Treatments were
120 performed for 30 min at an ultrasound frequency and amplitude of 24 kHz and 100 μm , respectively.
121 The effective acoustic power applied during sonication, determined calorimetrically by recording the
122 temperature increase against the time of ultrasound application (Raso, Manas, Pagan, & Sala, 1999),
123 was equal to 71 W, bringing forth to a specific acoustic energy of 1462 J/cm³. The latter was
124 calculated by dividing the acoustic power by the sample volume and multiplying it by the treatment
125 time.

126 *2.3. Lycopene concentration*

127 The extraction of lycopene was performed following the procedure of Sadler, Davis, & Dezman
128 (1990), with minor modifications. The analysis was carried out under subdued light to prevent
129 carotenoid degradation and isomerisation. 0.5 g NaCl and 50 mL extraction solution
130 (pentane:acetone:ethanol, 2:1:1 v/v/v) were added to 2 g of tomato pulp or supernatant containing
131 micelles. The mixture was stirred at room temperature for 20 min. Reagent grade water (15 mL) was
132 added and stirring was continued for 10 min. The apolar phase, containing lycopene, was collected,
133 filtered (Chromafil PET filters, Düren, Germany; 0.20 μm pore size, 25 mm diameter) and transferred
134 to an amber HPLC vial. The HPLC analyses were performed on a Varian Pro Star (model 230, Varian
135 Associates Ltd., Walnut Creek, CA, USA) equipped with a Varian Pro Star photodiode array detector
136 (model 330, Varian Associates Ltd., Walnut Creek, CA, USA), according to Cucu, Huvaere, Van Den
137 Bergh, Vinkx, & Van Loco (2012) with some modifications. Lycopene and its isomers were separated
138 at 35 °C on a reversed phase C₃₀ column (3 μm ×150 mm×4.6 mm, YMC Europe, Dinslaken,

139 Germany) with methanol/2-propanol/tetrahydrofuran (4:3:3 v/v/v) containing 0.05% triethylamine as
140 mobile phase. The flow rate was 1 mL/min and the injection volume 20 μ L. Lycopene and its isomers
141 were detected at 472 nm. Retention time and absorption spectra of pure standard (Sigma-Aldrich,
142 Milan, Italy) were used to identify and quantify all-*trans* lycopene. All-*trans* lycopene concentration
143 was expressed as mg/g tomato pulp dry matter. Changes in all-*trans* lycopene concentration during
144 storage were expressed as the percentage ratio between the concentration of the all-*trans* lycopene at
145 the time of analysis (C_t) and the concentration of the all-*trans* lycopene at time zero (C_0). Changes in
146 unidentified lycopene *cis* isomers relative peak area were expressed as the percentage of the all-*trans*
147 lycopene ($A_{\text{all-trans}}$) and *cis* isomers (A_{cis}) total peak area.

148 2.4. *In vitro* bioaccessibility

149 The lycopene *in vitro* bioaccessibility was measured by simulating human digestion in the stomach
150 and small intestine *in vitro*. The procedure described by Moelants, Lemmens, Vandebroek, Van
151 Buggenhout, Van Loey, & Hendrickx (2012), based on Hedrén et al. (2002), was followed. In
152 particular, 5 g tomato pulp was weighed into a 50 mL capacity opaque falcon tube. The sample was
153 diluted with 5 mL NaCl/ascorbic acid solution (0.9% NaCl, 1% ascorbic acid in water), 5 mL stomach
154 electrolyte solution (0.30% NaCl, 0.11% KCl, 0.15% $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.05% KHPO_4 , 0.07%
155 $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ in water) and 10 mL of freshly prepared oil-in-water emulsion. The latter was obtained
156 by suspending 1% (w/v) L- α -phosphatidylcholine from egg yolk (Sigma) in water. 5% (v/v) extra
157 virgin olive oil was then added and the mixture was stirred (Polytron, PT 3000, Cinematica, Littau,
158 Swiss) at 9500 rpm during 10 min. Homogenization was performed at 100 MPa for one cycle using
159 a high pressure homogeniser (Panda PLUS 2000, Gea Niro Soavi, Parma, Italy). To simulate the first
160 phase of gastric digestion, the pH of the mixture was adjusted to 4 ± 0.05 with 1 M HCl or 1 M
161 NaHCO_3 and 5 mL pepsin solution (0.52% porcine pepsin, from Sigma, in electrolyte solution) was
162 added. After flushing the headspace of the samples with nitrogen for 10 s, the mixture was incubated
163 at 37 $^\circ\text{C}$ for 30 min while shaking end-over-end. The pH of the mixture was then acidified to 2 ± 0.05
164 to mimic the drop of the gastric pH after the intake of a meal (Tyssandier et al., 2003). The headspace

165 of the samples was flushed again with nitrogen for 10 s and the incubation at 37 °C continued for
166 further 30 min. To imitate the passage through the small intestine, the pH of the partially digested
167 tomato product was raised to 6.9 ± 0.05 and 6 mL pancreatin, lipase and bile salts solution (0.4%
168 porcine pancreatin, 0.2% porcine pancreas lipase, 2.5% bile extract, 0.5% pyrogallol, and 1% α -
169 tocopherol, from Sigma, in water) was added. Finally, the headspace of the sample was flushed with
170 nitrogen for 10 s and incubated for 2 h at 37 °C. The digest was centrifuged (XL-70 Ultracentrifuge,
171 Beckman, Palo Alto, CA, USA) at 165000 g during 67 min at 4 °C to separate the micelles. The
172 supernatant was collected, filtered (Chromafil PET filters, Düren, Germany; 0.20 μm pore size, 25
173 mm diameter) and analysed for lycopene content. The lycopene *in vitro* bioaccessibility was defined
174 as the percentage ratio between the all-*trans* lycopene concentration in the micelles at the time of the
175 analysis (B_t) and the all-*trans* lycopene concentration in the sample at time zero (C_0). Changes in all-
176 *trans* lycopene *in vitro* bioaccessibility during storage were expressed as the percentage ratio of
177 lycopene bioaccessibility measured at the different storage times (% B_t/C_0) and at time zero (%
178 B_0/C_0).

179 2.5. Viscosity

180 Oscillatory measurements were carried out in the frequency range of 0.1-10 Hz, at a constant stress
181 amplitude of 0.4 Pa (i.e. in the linear viscoelastic region of the material) and 20 °C, by using a
182 Stresstech Rheometer (ReoLogica Instruments AB, Lund, Sweden) equipped with a concentric
183 cylinder geometry (C25).

184 2.6. Total solids content

185 The total solids content was measured by gravimetric method (AOAC, 1995).

186 2.7. Colour

187 Colour analysis was carried out using a tristimulus colorimeter equipped with a CR-300 measuring
188 head (Chromameter-2 Reflectance, Minolta, Osaka, Japan). The instrument was standardised against
189 a white tile before measurements. Colour was expressed in L^* , a^* and b^* scale parameters and a^* and

190 b^* were used to compute the hue angle ($\tan^{-1} b^*/a^*$) (Clydesdale, 1978). An increase of this colour
191 parameter was used as an index of redness loss.

192 2.8. Peroxide value

193 The peroxide value (PV) of the samples was assessed according to the European Official Methods of
194 Analysis (1991).

195 2.9. Microscopy analysis

196 Tomato pulps microstructure was analyzed using an optical microscope (Leica DM 2000, Leica
197 Microsystems, Heerburg, Switzerland). The pictures were taken by a digital camera (Leica EC3,
198 Leica Microsystems, Heerburg, Switzerland), using the software Leica Suite LAS EZ (Leica
199 Microsystems, Heerburg, Switzerland).

200 2.10. Data analysis

201 Results obtained are expressed as mean of three replicates \pm standard deviation. One-way analysis of
202 variance was carried out and differences among means were assessed by using the Tukey's multiple
203 comparison test (STATISTICA for Windows, 5.1, Statsoft Inc., Cary, NC, USA). Means were
204 considered significantly different at $P < 0.05$. Correlation analysis was carried out by using Microsoft
205 Office Excel 2007.

206 3. Results and discussion

207 3.1. Effect of ultrasounds and oil incorporation on lycopene concentration and in vitro 208 bioaccessibility

209 Untreated and ultrasonically treated tomato samples were first characterized for their total solids
210 content and viscosity (Table 1). Despite the loss of water as a consequence of the ultrasound treatment
211 was negligible, viscosity greatly increased. The effect of ultrasound processing on the structural
212 properties of tomato pulp has already been investigated (Anese et al., 2013). Ultrasound treatment
213 can cause partial de-esterification of pectin molecules, which may subsequently establish hydrogen
214 bonds and hydrophobic interactions, giving rise to a new network, with increased gel-like properties.

215 No changes in the rheological parameter were found during the storage of tomato pulp (data not
216 shown), indicating that the present experimental conditions caused a permanent viscosity increase.
217 The light microscope images of the untreated and ultrasonically treated tomato pulps (Table 1) clearly
218 show differences in cell integrity. In particular, the unprocessed samples presented intact cells
219 containing lycopene crystals, while broken cells and lycopene distributed in the matrix can be
220 observed in the processed tomato pulp.

221 All-*trans* lycopene concentration of freshly prepared untreated and ultrasonically treated tomato
222 pulps containing no or 2.5%, 5% and 10% sunflower oil are shown in Table 2. Lycopene
223 concentrations were in the range of those reported in the literature data (Tonucci, Holden, Beecher,
224 Khachik, Davis, & Mulokozi, 1995). The addition of oil did not cause any change in the all-*trans*
225 lycopene concentration. Moreover, no significant differences in the carotenoid content were found
226 between untreated and ultrasonically treated samples containing a same amount of oil. These results
227 are in agreement with those already described in the literature for tomato derivatives subjected to
228 ultrasound and high pressure homogenization associated to a temperature increase not exceeding 100
229 °C (Perez-Conesa et al., 2009; Colle et al., 2010b; Knockaert et al., 2012; Anese et al., 2013). It is
230 noteworthy that under the present experimental conditions temperature never exceeded 90 °C.

231 Table 2 also shows the lycopene *in vitro* bioaccessibility at time zero of the untreated and
232 ultrasonically treated tomato pulps containing no or 2.5%, 5% and 10% sunflower oil. Except for the
233 5% oil-containing samples, no significant differences in lycopene *in vitro* bioaccessibility were found
234 between the untreated and ultrasonically processed samples having the same oil content, in contrast
235 with data from the literature (Colle et al., 2010b; Anese et al., 2013; Panozzo et al., 2013). These
236 authors reported a decrease in lycopene *in vitro* bioaccessibility consequently to ultrasound or high
237 pressure homogenization processing of tomato pulp. In fact, despite these processes favoured
238 lycopene release from tomato cells, its uptake into the micelles was hindered by the formation of a
239 strong fibre network entrapping the carotenoid. Further on, the lycopene bioaccessibility values
240 relevant to the samples with no oil added were approximately two to four fold higher than those found

241 by Anese et al. (2013) for tomato pulp subjected to similar processes. These discrepancies can be due
242 to differences in the methods used to assess the carotenoid *in vitro* bioaccessibility. In fact, differently
243 from what reported in the aforementioned papers, the lycopene bioaccessibility in tomato pulps in
244 this study was determined in the presence of an oil-in-water emulsion, added just before the *in vitro*
245 digestion, together with a lipase containing solution (Moelants et al., 2012). The oil-in-water emulsion
246 was added to better mimic the emulsification process in the stomach during lipid digestion (Carey,
247 Small, & Bliss, 1983). By emulsifying, the surface area of the emulsion would increase, thus
248 favouring lycopene extraction mainly from the phospholipid-rich chromoplasts (Lenucci, Serrone, de
249 Caroli, Fraser, Bramley, Piro, & Dalessandro, 2012) and its incorporation into the oil droplets. The
250 lipid droplets are formed by a hydrophobic core containing triglycerides, lycopene and other fat
251 soluble molecules, and surrounded by an amphipathic surface monolayer (Bauer, Jakob, &
252 Mosenthin, 2005). Hydrolysis at the oil droplet surface by lipase would then allow the lycopene to be
253 released and subsequently incorporated into the bile salt micelles (Carey et al., 1983). To confirm this
254 hypothesis, lycopene *in vitro* bioaccessibility was also assessed in untreated and ultrasonically treated
255 tomato pulps in the absence of the oil-in-water emulsion. In both the cases, the lycopene
256 bioaccessibility values were similar to those reported in the previous study (Anese et al., 2013) and
257 approximately 60% lower than those attained for the emulsion-added counterparts. Similar results are
258 reported by Moelants et al. (2012) for β -carotene bioaccessibility measured in carrot-derived
259 suspension without oil addition, with the addition of 2% olive oil as such and with the addition of 2%
260 oil-in-water emulsion at the start of the *in vitro* digestion procedure. The authors found that emulsion
261 addition led to the greatest increase in carotenoid uptake into the micellar phase, followed by the olive
262 oil alone. Overall, the use of the oil-in-water emulsion in the digestion procedure would explain not
263 only the higher lycopene bioaccessibility values we found in this work as compared to the already
264 published ones, but also the almost negligible differences between the untreated and ultrasonically
265 processed tomato pulps. It can be inferred that the use of the oil-in-water emulsion could improve the
266 lycopene transfer into the micelles from the ultrasonically processed matrix, where the dispersed

267 carotenoid is tightly entrapped (Table 1).

268 Table 2 also shows that the *in vitro* bioaccessibility of lycopene significantly decreased with the
269 increase of the oil content in both the untreated and ultrasonically treated tomato pulps, in agreement
270 with data of Colle et al. (2012). These authors reported that, although lycopene bioaccessibility may
271 be improved by the presence of fat, high levels of lipids containing a large fraction of long chain
272 triglycerides (e.g. olive oil, sunflower oil and fish oil) significantly decreased the lycopene
273 bioaccessibility (Huo et al., 2007). In fact, an increase of the lipid amount could be responsible for an
274 incomplete hydrolysis of triglycerides (Porter et al., 2004). It must be pointed out that, in our
275 experimental conditions, the addition of the oil-in-water emulsion at the start of the *in vitro* digestion
276 procedure contributed to increase the lipid load.

277 3.2. Effect of ultrasounds and oil incorporation on lycopene concentration and *in vitro* 278 bioaccessibility during storage

279 Fig. 1 shows the changes in all-*trans* lycopene concentration and *cis* isomers of untreated and
280 ultrasonically treated tomato pulps containing no or 10% sunflower oil during storage at 5 °C. The
281 highest oil amount was chosen to better show the effect of concentration. No significant differences
282 in the all-*trans* lycopene levels among the samples were found at a same storage time ($P>0.05$).
283 Moreover, lycopene concentration did not vary in the first 15 days of storage, while it significantly
284 decreased up to 30 days ($P<0.05$). By prolonging the storage time, no further decrease in lycopene
285 concentration was observed. Similarly, no significant differences of the relative *cis* isomers peak area
286 values were found among the samples at a same storage time ($P>0.05$). On average, initially only 5%
287 ± 1 of lycopene was present as unidentified *cis* isomers, which is consistent with the thermodynamic
288 stability of the all-*trans* form (Shi & Le Maguer, 2000). The relative peak area of lycopene *cis* isomers
289 increased after 60 days of storage, reaching a mean value of 10% ± 1 at 100 days. These results
290 suggest that the ultrasound treatment as well as the presence of oil slightly affected lycopene
291 isomerization, in agreement with other findings showing that the relative concentration of lycopene

292 *cis* isomers did not vary significantly when tomato is exposed to mild process temperature (Nguyen
293 & Schwartz, 1998).

294 Fig. 2 shows the changes of the lycopene *in vitro* bioaccessibility of untreated and ultrasonically
295 treated tomato pulps containing no or 10% sunflower oil during storage at 5 °C. After an initial lag
296 period, the lycopene *in vitro* bioaccessibility significantly decreased up to 60 days of storage,
297 whereas, by prolonging the time, only slight changes of this parameter occurred. The reduction of
298 lycopene *in vitro* bioaccessibility ranged between 50 and 80%, the untreated tomato pulps showing a
299 greater decrease than the ultrasonically treated ones. A protective effect of the highly viscous matrix
300 of the ultrasonically treated tomato pulp towards lycopene could explain the lower decrease in the *in*
301 *vitro* bioaccessibility of this sample during storage as compared to the unprocessed counterpart.

302 An evidence of lycopene degradation in the untreated and ultrasonically treated tomato pulps
303 containing no or 10% sunflower oil during storage is given by the changes of hue angle values (Fig.
304 3). After a 15 days lag time, the values of this color parameter progressively increased during storage,
305 indicating a redness loss. The non-containing oil samples subjected or not to the ultrasound treatment
306 showed the lowest hue angle values. Bleaching was greater in the ultrasonically treated tomato pulp
307 containing oil, followed by the untreated sample added with oil. These results are consistent with the
308 peroxide values of the lipid fraction of the untreated and ultrasonically treated tomato pulps
309 containing oil (Fig. 4). Initially, a lag phase of about 30 days was observed. It can be inferred that the
310 naturally occurring carotenoids might protect the lipid fraction from oxidative reactions by virtue of
311 their strong antioxidant activity (Anese, Falcone, Fogliano, Nicoli, & Massini, 2002). As known, the
312 protective action exerted by lycopene may result in redness loss. After this time, although a marked
313 increase in peroxide values was observed for both samples, the rate of formation was greater in the
314 ultrasonically processed tomato pulp, plausibly due to the contribution of radical species generated
315 as a consequence of the acoustic cavitation (Ashokkumar et al., 2008). Actually, a good positive
316 correlation was found between the colour and peroxide values data ($R=0.85$, $P<0.01$) of the untreated
317 and ultrasonically treated tomato pulps containing oil. The hue angle parameter correlated well also

318 with the lycopene concentration ($R=0.74$, $P<0.01$) and *in vitro* bioaccessibility ($R=0.74$, $P<0.01$).
319 Overall these results suggest that the losses of lycopene concentration and bioaccessibility occurring
320 during storage may be related to an increase in carotenoid susceptibility to degradation in the presence
321 of unsaturated lipids (i.e. sunflower oil). In fact, carotenoid oxidation reactions are favoured by co-
322 oxidation with lipid hydroperoxides (Rodriguez-Amaya, 2001). However, this may be not the only
323 mechanism for lycopene *in vitro* bioaccessibility reduction. As the decrease of lycopene
324 bioaccessibility during storage was greater than that of lycopene levels, it might be suggested that, in
325 addition to lycopene degradation, other factors, whose nature has to be clarified, could contribute to
326 reduce the lycopene *in vitro* bioaccessibility.

327

328 **4. Conclusion**

329 The results reported here clearly show that ultrasound processing of tomato pulp, while causing a
330 great increase in viscosity, only slightly affected all-*trans* lycopene concentration and *in vitro*
331 bioaccessibility. However, dietary oil incorporation to either the untreated or ultrasonically treated
332 tomato pulp caused a decrease in lycopene bioaccessibility.

333 Upon storage, after an initial lag period, the lycopene *in vitro* bioaccessibility of tomato pulps
334 containing no or 10% oil greatly decreased, mainly due to carotenoid degradation.

335 It can be concluded that ultrasound treatments can be actually applied to steer the structure of tomato
336 derivatives without impairing their stability and functionality. However, these properties can be
337 negatively affected by dietary oil incorporation and storage.

338

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442

443 **Figure captions**

444

445 **Fig. 1.** Relative all-*trans* lycopene concentration ($\% C_t/C_0$) (a) and lycopene *cis* isomers relative peak
446 area ($\% A_{cis}/A_{all-trans}$) (b) of untreated and ultrasonically (US) treated tomato pulps containing no or
447 10% sunflower oil during storage at 5 °C

448

449 **Fig. 2.** Changes in lycopene *in vitro* bioaccessibility ($\% B_t/B_0$) of untreated and ultrasonically (US)
450 treated tomato pulps containing no or 10% sunflower oil during storage at 5 °C

451

452 **Fig. 3.** Hue angle of untreated and ultrasonically (US) treated tomato pulps with no or 10% sunflower
453 oil during storage at 5 °C

454

455 **Fig. 4.** Peroxide value of untreated and ultrasonically (US) treated tomato pulps containing 10%
456 sunflower oil during storage at 5 °C

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461

462 **Table 1**

463 Total solids content, viscosity and images of untreated and ultrasonically (US) treated tomato pulps

Sample	Total solids (g/100 g)	Viscosity (Pa s)	Image (200x)	464
Untreated	8.04 ± 0.03 ^a	2.7 ± 1.0 ^a		465
				468
				469
				470
US treated	8.33 ± 0.02 ^a	13.6 ± 1.7 ^b		471
				474
				475
				476
				477

478 Data are the mean of 3 replications ± standard deviation. Means with different letters within the same
 479 column are significantly different (P<0.05)

480

481 **Table 2**

482 All-*trans* lycopene concentration (C₀) and bioaccessibility (% B₀/C₀) of untreated and ultrasonically
 483 (US) treated tomato pulps containing no or increasing amounts of sunflower oil

Oil (% w/w)	All- <i>trans</i> lycopene (mg/g _{dm})		Lycopene bioaccessibility (%) 484	
	Untreated	US treated	Untreated	US treated
0	1.95 ± 0.36 ^a	1.51 ± 0.28 ^a	1.06 ± 0.27 ^{ab}	1.24 ± 0.36 ^a
2.5	1.44 ± 0.05 ^a	1.64 ± 0.10 ^a	0.99 ± 0.30 ^{ab}	0.85 ± 0.17 ^{bd}
5.0	1.42 ± 0.11 ^a	1.47 ± 0.05 ^a	0.33 ± 0.05 ^c	0.84 ± 0.15 ^{bd}
10.0	1.58 ± 0.12 ^a	1.31 ± 0.08 ^a	0.35 ± 0.07 ^{cd}	0.65 ± 0.05 ^d

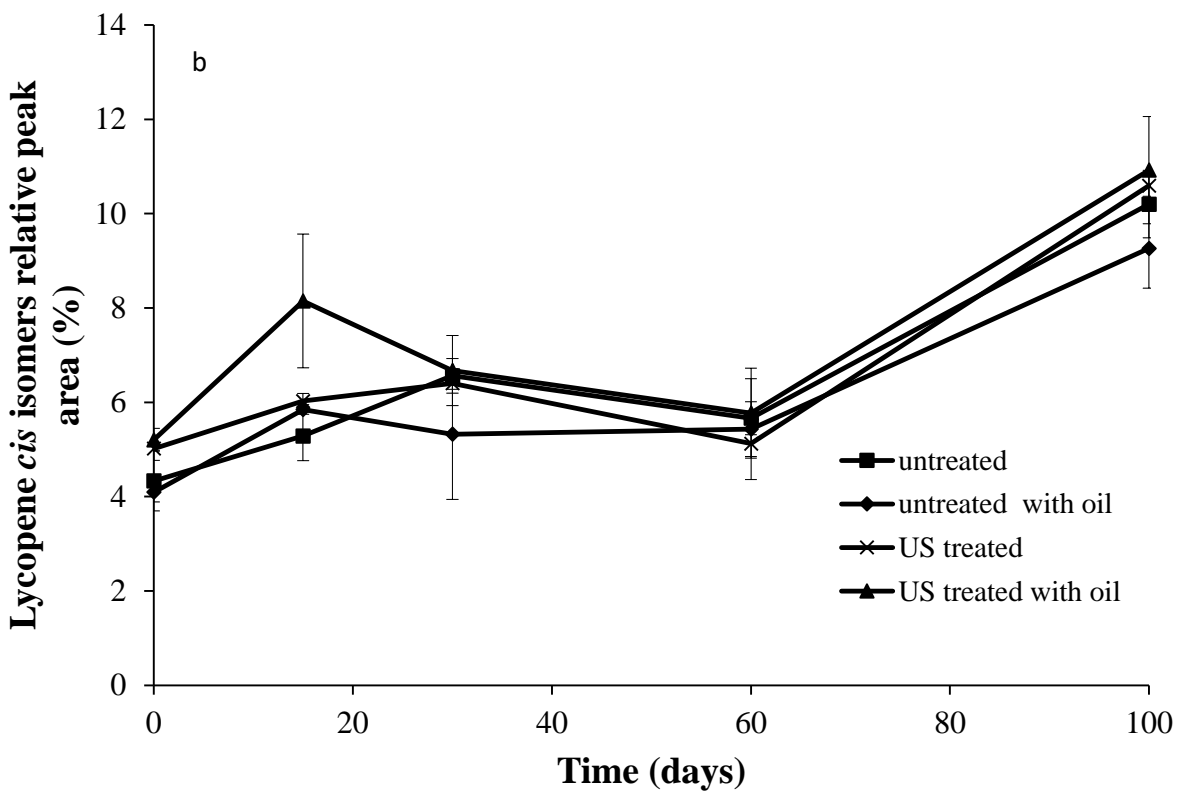
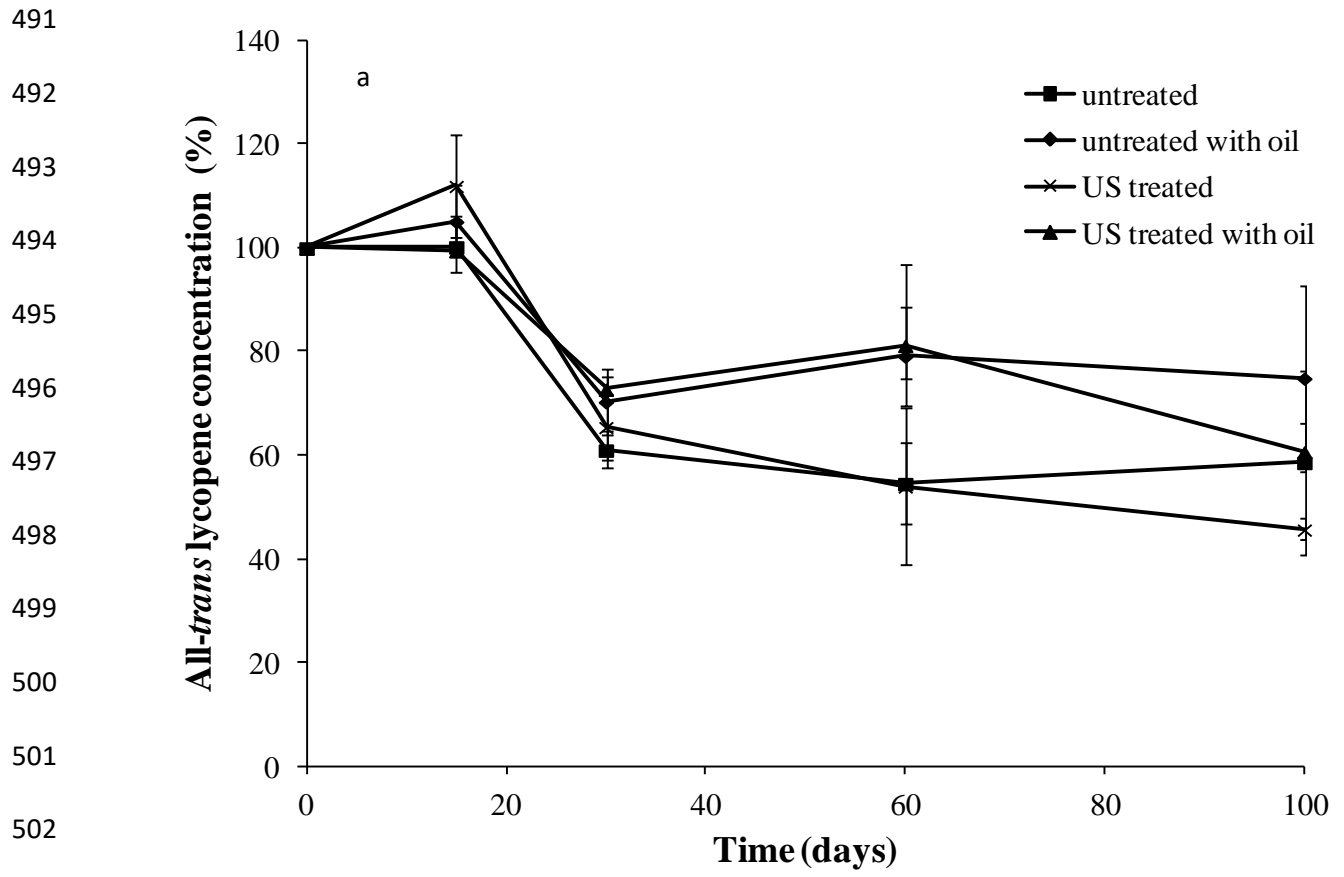
485 Data are the mean of 3 replications ± standard deviation. Significant difference is indicated by
 486 different letters (P<0.05)

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503
 504
 505 **Fig. 1**

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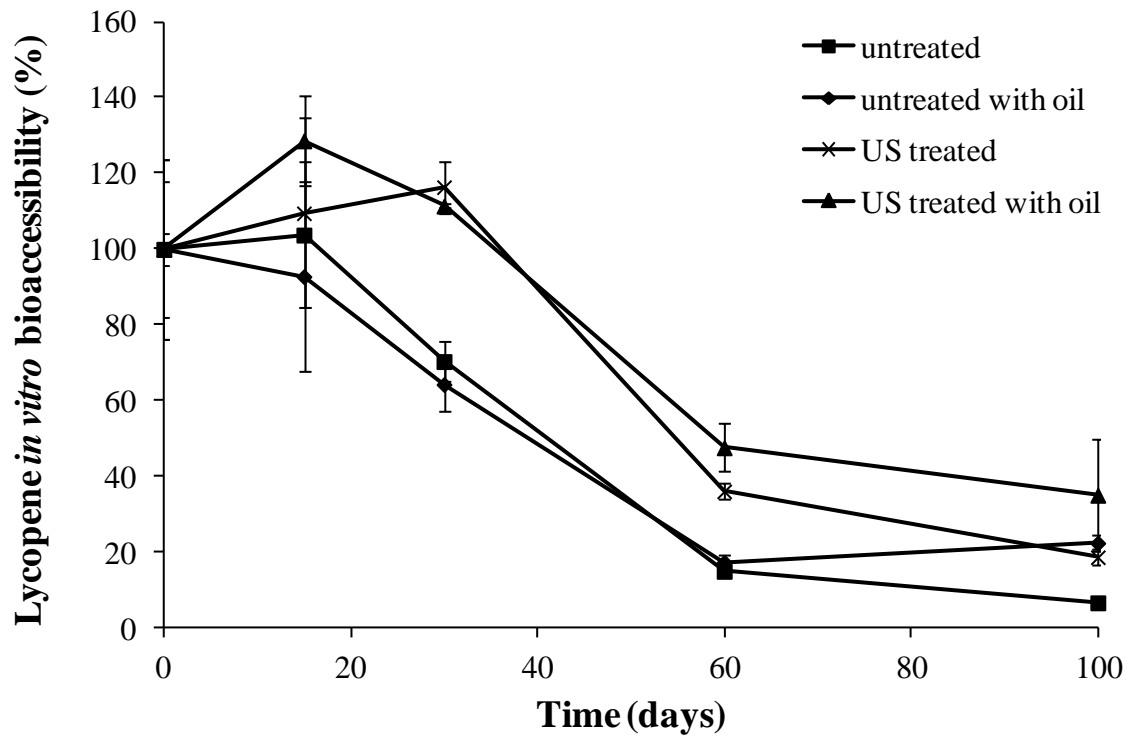
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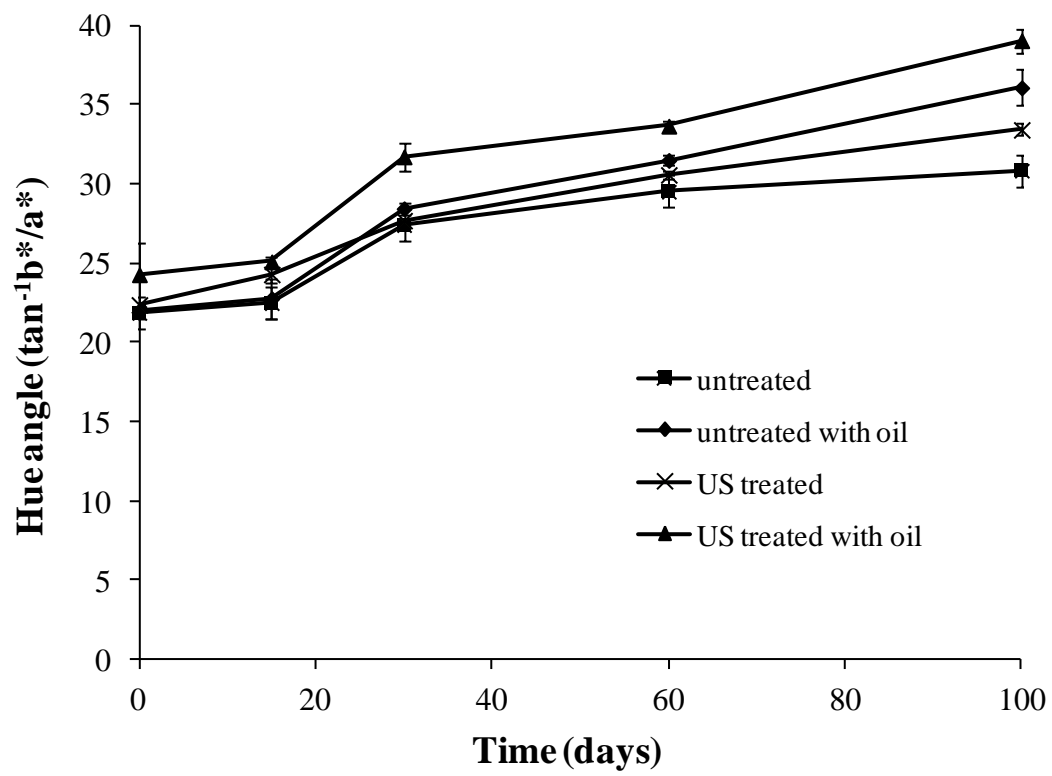
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519 **Fig. 2**

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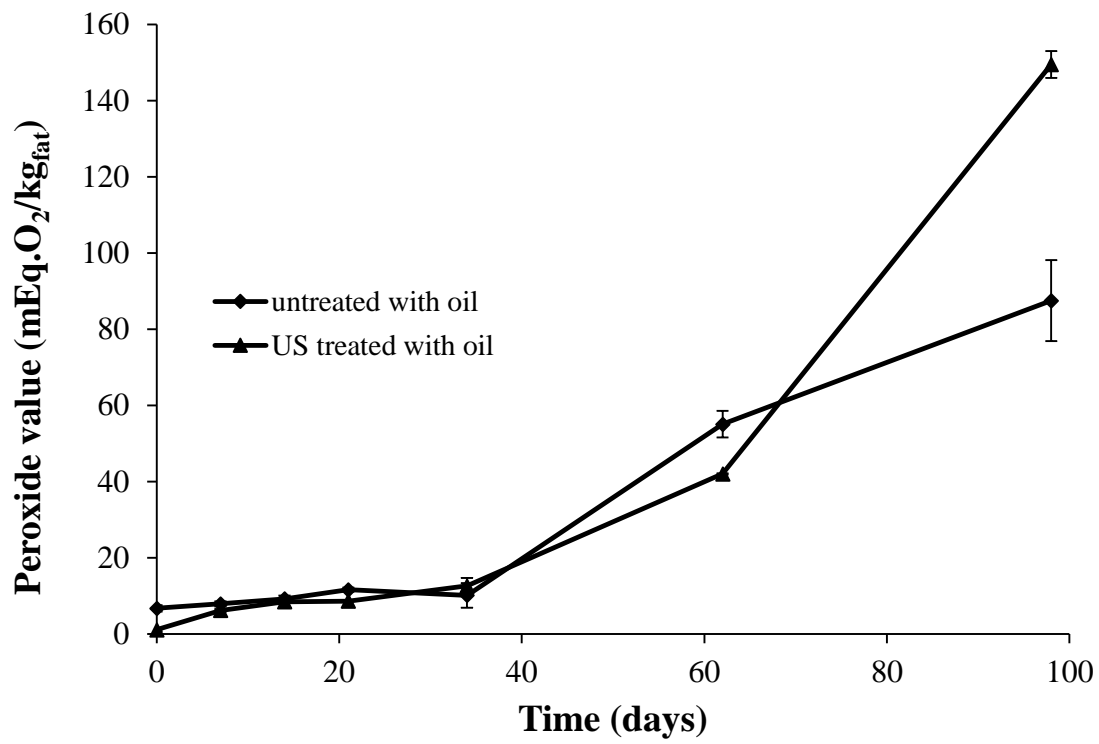




522

523 **Fig. 3**

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527 **Fig. 4**

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