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

*Original*

*Availability:*

This version is available <http://hdl.handle.net/11390/1023950> since 2020-07-16T11:53:11Z

*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  $\mu\text{m}$ , 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/cm<sup>3</sup>. 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  $\mu\text{m}$  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 C<sub>30</sub> column (3  $\mu\text{m}$ ×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  $\mu$ 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 ( $A_{\text{cis}}$ ) 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, Vandebroeck, 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%  $\text{KHPO}_4$ , 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- $\alpha$ -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  $4 \pm 0.05$  with 1 M HCl or 1 M  
161  $\text{NaHCO}_3$  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  $2 \pm 0.05$   
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  $6.9 \pm 0.05$  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%  $\alpha$ -  
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  $\mu\text{m}$  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  $\beta$ -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  $\pm 1$  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%  $\pm 1$  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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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 <sup>a</sup>	2.7 ± 1.0 <sup>a</sup>		465
				468
				469
				470
US treated	8.33 ± 0.02 <sup>a</sup>	13.6 ± 1.7 <sup>b</sup>		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 (C<sub>0</sub>) and bioaccessibility (% B<sub>0</sub>/C<sub>0</sub>) 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 <sub>dm</sub> )		Lycopene bioaccessibility (%) 484	
	Untreated	US treated	Untreated	US treated
0	1.95 ± 0.36 <sup>a</sup>	1.51 ± 0.28 <sup>a</sup>	1.06 ± 0.27 <sup>ab</sup>	1.24 ± 0.36 <sup>a</sup>
2.5	1.44 ± 0.05 <sup>a</sup>	1.64 ± 0.10 <sup>a</sup>	0.99 ± 0.30 <sup>ab</sup>	0.85 ± 0.17 <sup>bd</sup>
5.0	1.42 ± 0.11 <sup>a</sup>	1.47 ± 0.05 <sup>a</sup>	0.33 ± 0.05 <sup>c</sup>	0.84 ± 0.15 <sup>bd</sup>
10.0	1.58 ± 0.12 <sup>a</sup>	1.31 ± 0.08 <sup>a</sup>	0.35 ± 0.07 <sup>cd</sup>	0.65 ± 0.05 <sup>d</sup>

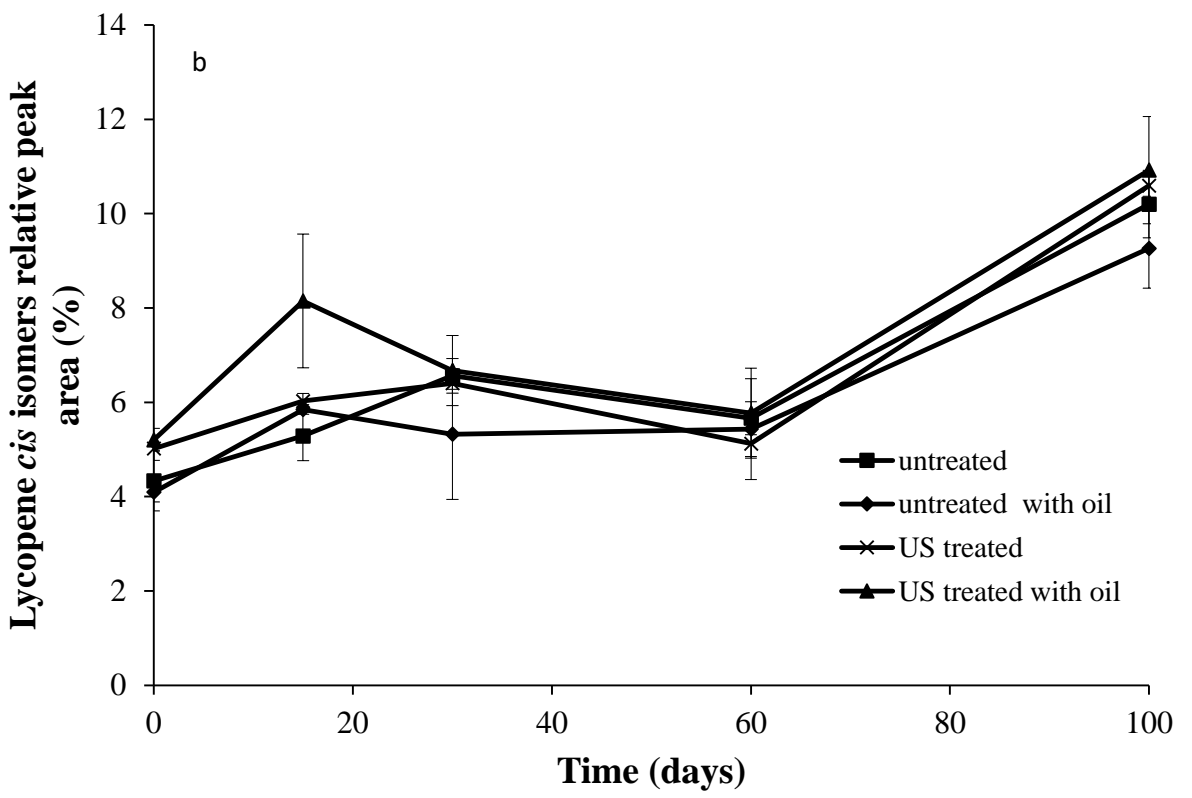
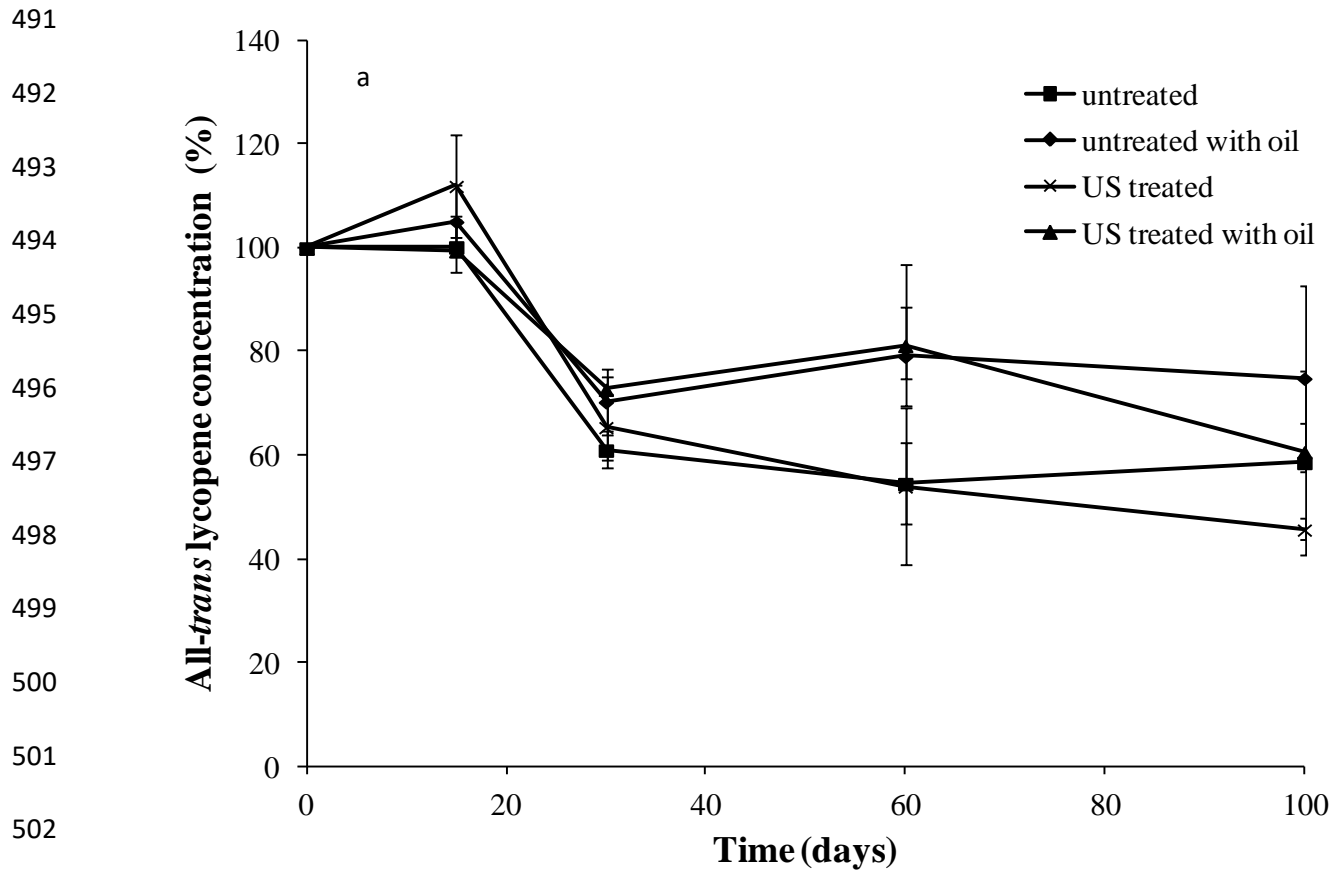
485 Data are the mean of 3 replications ± standard deviation. Significant difference is indicated by  
 486 different letters (P<0.05)

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**Fig. 1**

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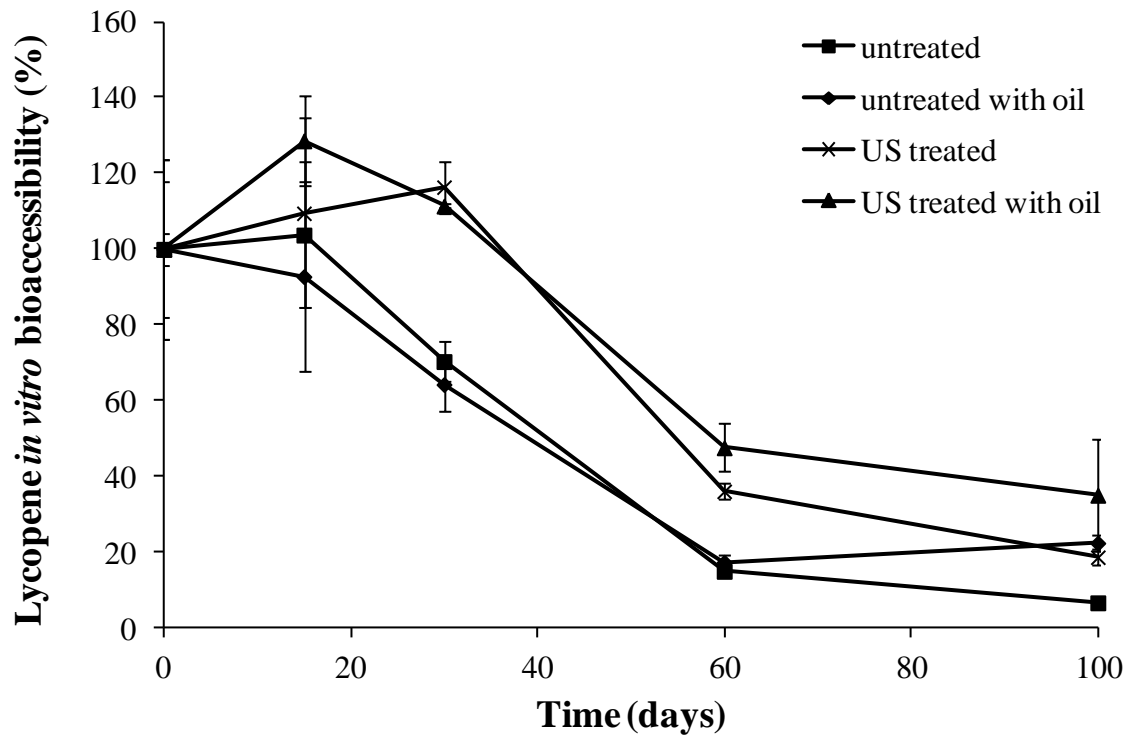
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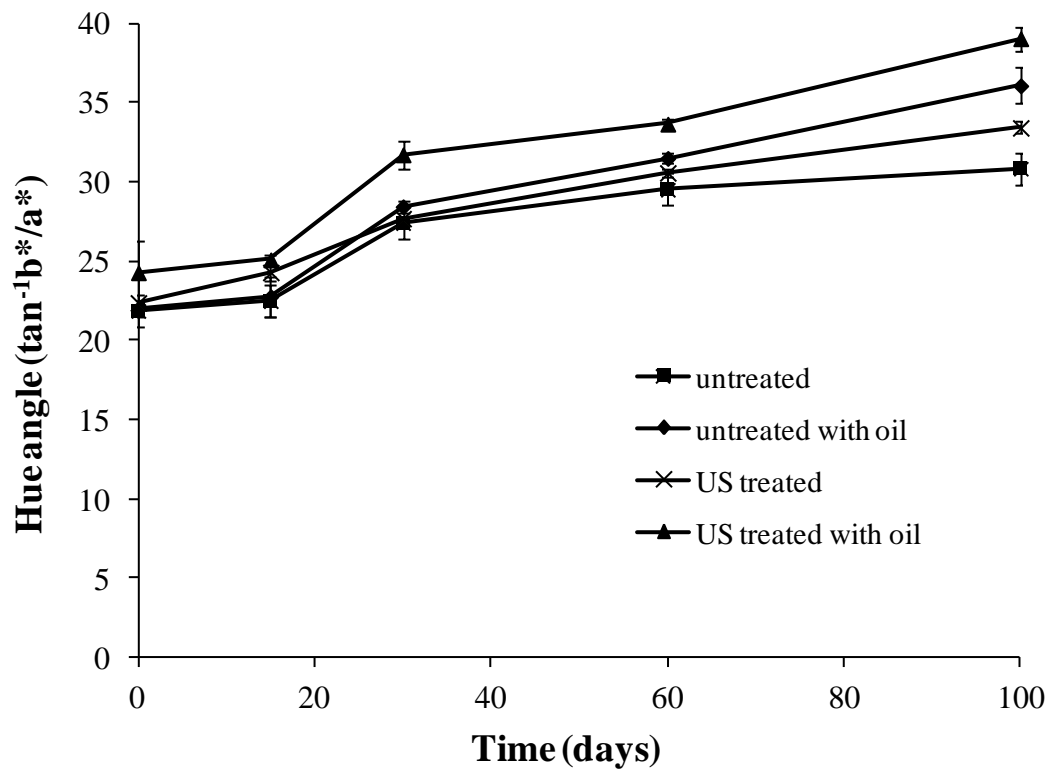
519 **Fig. 2**

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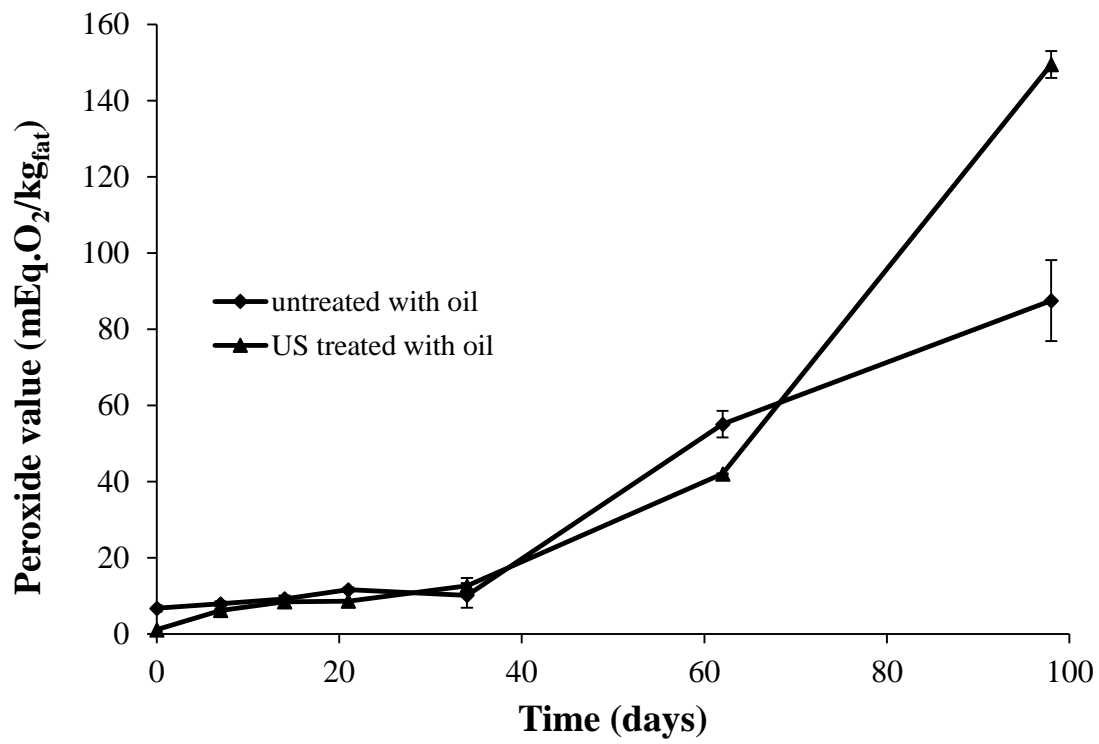




522

523 **Fig. 3**

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527 **Fig. 4**

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