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

Monica Anese[1*], Francesca Bot[1], Agnese Panozzo[2], Giorgio Mirolo[3], Giovanna Lippe[1]

[1]Dipartimento di Scienze degli Alimenti, The University of Udine, Via Sondrio 2/A, 33100 Udine Italy

[2]Laboratory of Food Technology and Leuven Food Science and Nutrition Research Centre (LeFoRCe), Department of Microbial and Molecular System (M2S), Katholieke Universiteit Leuven, Kasteelpark Arenberg 22, PB 2457, 3001 Leuven, Belgium

[3]Dr. Schär R&D Centre, AREA Science Park, Padriciano, 99, 34149 Trieste, Italy

*Corresponding author: email: monica.anese@uniud.it, tel.:+39 0432 558153, fax: +39 0432 558130

Abstract

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

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

1. Introduction

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

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

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

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

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

2. Materials and methods

2.1. Sample preparation

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

2.2. Ultrasound treatment

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

250 mL capacity (90 mm height, 75 mm diameter) glass vessels. The horn was placed in the centre of the vessel, with an immersion depth in the fluid of 5 mm. In order to minimise water evaporation during sonication, the vessel was closed with a Plexiglas lid fitted with holes allowing horn and thermocouple probes to be placed at the desired positions in the tomato pulp. During the ultrasound treatment, tomato pulp was kept under stirring to allow temperature to equilibrate within the sample. The temperature was recorded as a function of time using a copper-constantan thermocouple probe (Ellab, Denmark), connected to a data-logger (CHY 502A1, Tersid, Milano, Italy). Treatments were performed for 30 min at an ultrasound frequency and amplitude of 24 kHz and 100 μm, respectively. The effective acoustic power applied during sonication, determined calorimetrically by recording the temperature increase against the time of ultrasound application (Raso, Manas, Pagan, & Sala, 1999), was equal to 71 W, bringing forth to a specific acoustic energy of 1462 J/cm^3. The latter was calculated by dividing the acoustic power by the sample volume and multiplying it by the treatment time.

2.3. Lycopene concentration

The extraction of lycopene was performed following the procedure of Sadler, Davis, & Dezman (1990), with minor modifications. The analysis was carried out under subdued light to prevent carotenoid degradation and isomerisation. 0.5 g NaCl and 50 mL extraction solution (pentane:acetone:ethanol, 2:1:1 v/v/v) were added to 2 g of tomato pulp or supernatant containing micelles. The mixture was stirred at room temperature for 20 min. Reagent grade water (15 mL) was added and stirring was continued for 10 min. The apolar phase, containing lycopene, was collected, filtered (Chromafil PET filters, Düren, Germany; 0.20 μm pore size, 25 mm diameter) and transferred to an amber HPLC vial. The HPLC analyses were performed on a Varian Pro Star (model 230, Varian Associates Ldt., Walnut Creek, CA, USA) equipped with a Varian Pro Star photodiode array detector (model 330, Varian Associates Ldt., Walnut Creek, CA, USA), according to Cucu, Huvaere, Van Den Bergh, Vinkx, & Van Loco (2012) with some modifications. Lycopene and its isomers were separated at 35 °C on a reversed phase C_{30} column (3 μm×150 mm×4.6 mm, YMC Europe, Dinslaken,

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

2.4. In vitro *bioaccessibility*

The lycopene *in vitro* bioaccessibility was measured by simulating human digestion in the stomach and small intestine *in vitro*. The procedure described by Moelants, Lemmens, Vandebroeck, Van Buggenhout, Van Loey, & Hendrickx (2012), based on Hedrén et al. (2002), was followed. In particular, 5 g tomato pulp was weighed into a 50 mL capacity opaque falcon tube. The sample was diluted with 5 mL NaCl/ascorbic acid solution (0.9% NaCl, 1% ascorbic acid in water), 5 mL stomach electrolyte solution (0.30% NaCl, 0.11% KCl, 0.15% $CaCl_2 \cdot 2H_2O$, 0.05% $KHPO_4$, 0.07% $MgCl_2 \cdot 6H_2O$ in water) and 10 mL of freshly prepared oil-in-water emulsion. The latter was obtained by suspending 1% (w/v) L-α-phosphatidylcholine from egg yolk (Sigma) in water. 5% (v/v) extra virgin olive oil was then added and the mixture was stirred (Polytron, PT 3000, Cinematica, Littau, Swiss) at 9500 rpm during 10 min. Homogenization was performed at 100 MPa for one cycle using a high pressure homogeniser (Panda PLUS 2000, Gea Niro Soavi, Parma, Italy). To simulate the first phase of gastric digestion, the pH of the mixture was adjusted to 4 ± 0.05 with 1 M HCl or 1 M $NaHCO_3$ and 5 mL pepsin solution (0.52% porcine pepsin, from Sigma, in electrolyte solution) was added. After flushing the headspace of the samples with nitrogen for 10 s, the mixture was incubated at 37 °C for 30 min while shaking end-over-end. The pH of the mixture was then acidified to 2 ± 0.05 to mimic the drop of the gastric pH after the intake of a meal (Tyssandier et al., 2003). The headspace

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

2.5. Viscosity

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

2.6. Total solids content

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

2.7. Colour

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

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

2.8. Peroxide value

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

2.9. Microscopy analysis

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

2.10. Data analysis

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

3. Results and discussion

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

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

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

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

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

carotenoid is tightly entrapped (Table 1).

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

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

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

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

4. Conclusion

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

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

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

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442

Figure captions

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

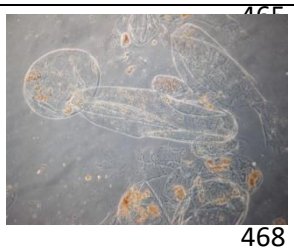
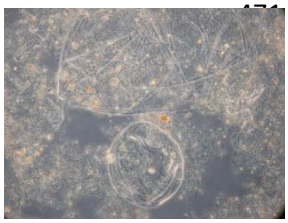
Fig. 2. Changes in lycopene *in vitro* bioaccessibility (% B_t/B_0) of untreated and ultrasonically (US) treated tomato pulps containing no or 10% sunflower oil during storage at 5 °C

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

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

Table 1

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)
Untreated	8.04 ± 0.03[a]	2.7 ± 1.0[a']	
US treated	8.33 ± 0.02[a]	13.6 ± 1.7[b']	

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

481 **Table 2**

482 All-*trans* lycopene concentration (C_0) and bioaccessibility (% B_0/C_0) of untreated and ultrasonically

483 (US) treated tomato pulps containing no or increasing amounts of sunflower oil

Oil (% w/w)	All-*trans* 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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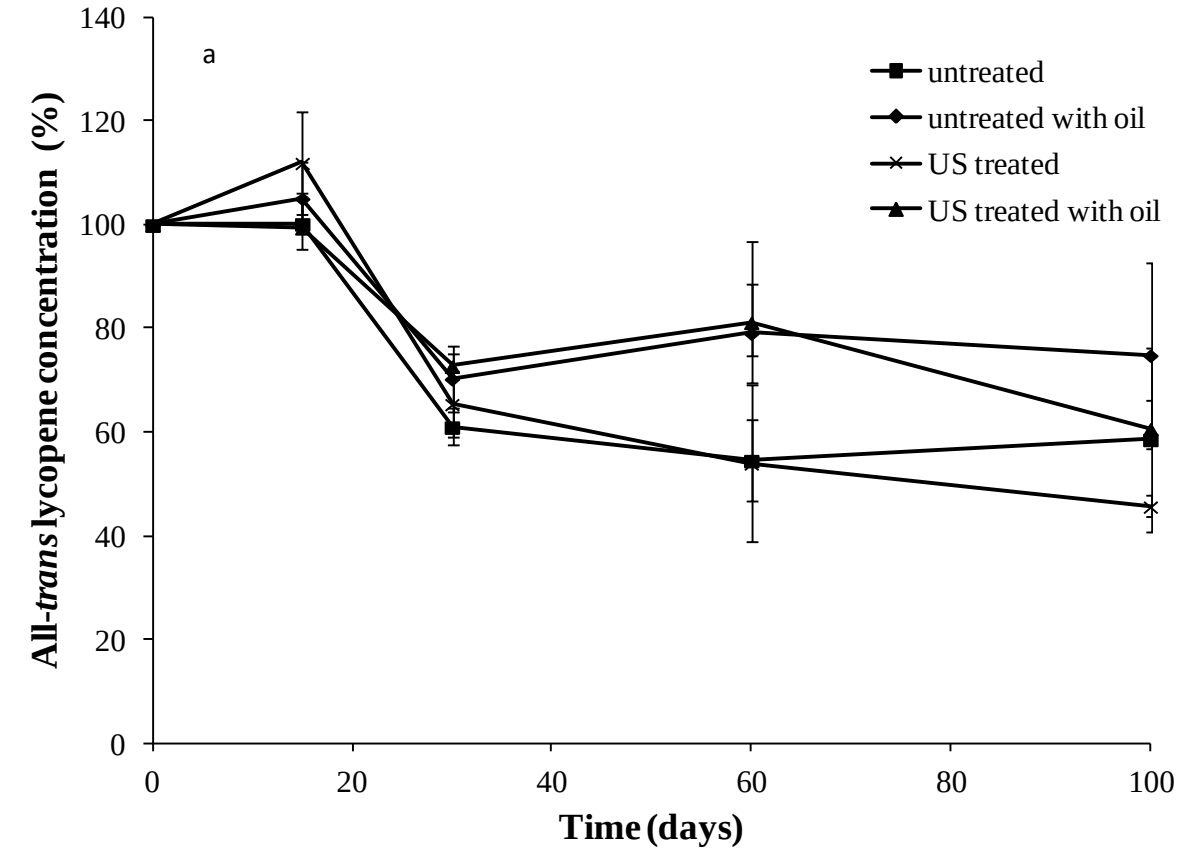

a
All-trans lycopene concentration (%)
Time (days)
untreated
untreated with oil
US treated
US treated with oil
0
20
40
60
80
100
120
140

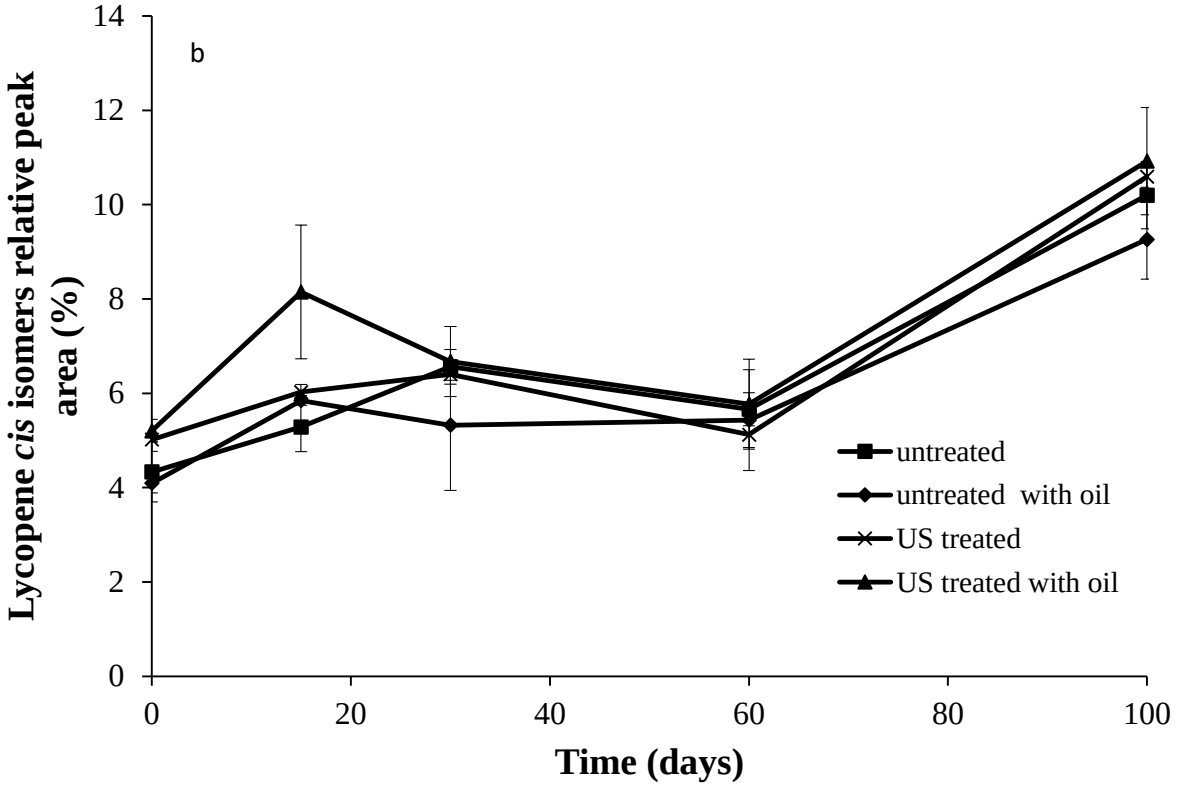

b
Lycopene cis isomers relative peak area (%)
Time (days)
untreated
untreated with oil
US treated
US treated with oil
0
2
4
6
8
10
12
14

Fig. 1

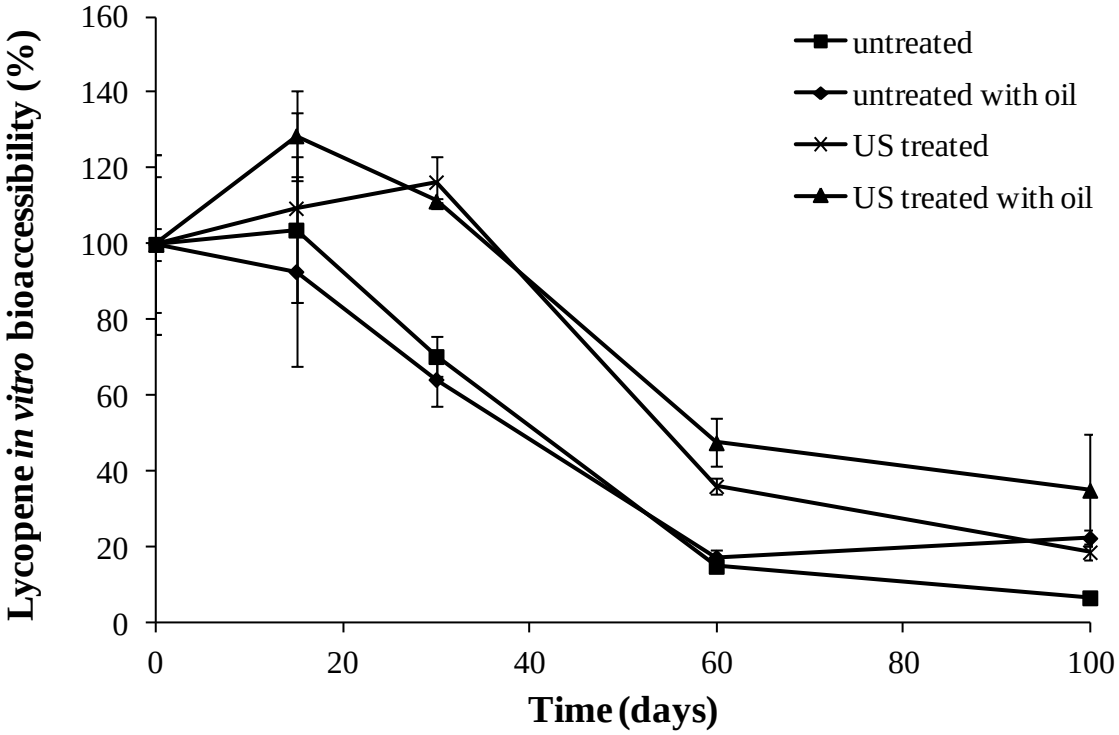
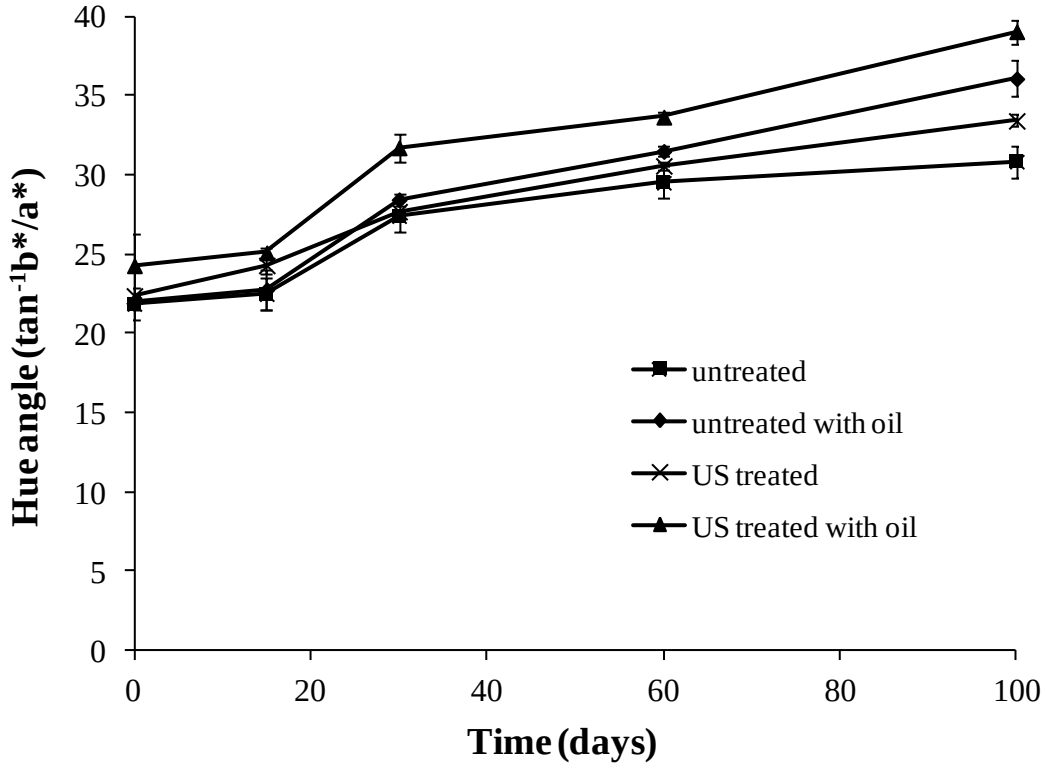

Lycopene in vitro bioaccessibility (%)
160
140
120
100
80
60
40
20
0
0
20
40
60
80
100
Time (days)
untreated
untreated with oil
US treated
US treated with oil

Fig. 2



522

523 **Fig. 3**

524

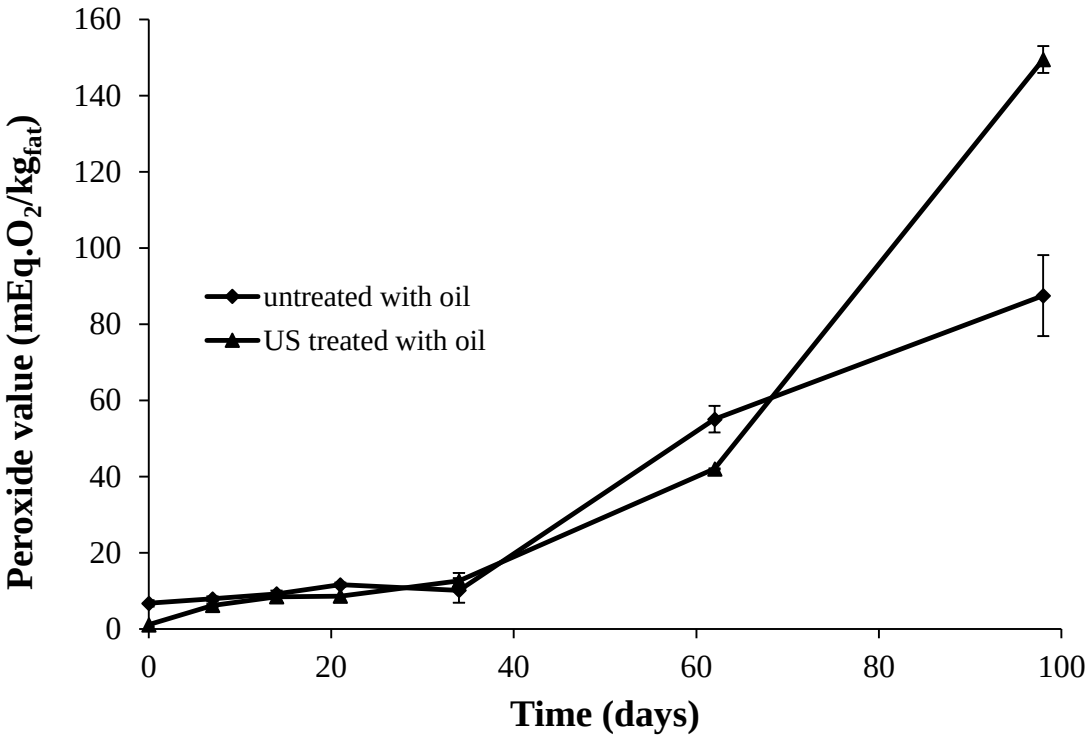

Peroxide value (mEq.O2/kgfat)
160
140
120
100
80
60
40
20
0
0
20
40
60
80
100
Time (days)
untreated with oil
US treated with oil

Fig. 4

532