

1 **Supercritical CO₂ extraction of tomato pomace: evaluation of the solubility of**
2 **lycopene in tomato oil as limiting factor of the process performance**

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24 **ABSTRACT**

25 This work considered lycopene (lyc) amount and (all-*E*)-lyc:Z-lyc (*E*:Z) ratio as driving parameters of
26 the tomato pomace (TP) supercritical CO₂ extraction (SFE_CO₂) performance. By testing lyc
27 concentrations solubilization in tomato seed oil and *E*:Z ratios of 75:25, 59:39 and 25:75, full and
28 partial equations (SE) were calculated. The application of mass balances to experimental
29 TP_SFE_CO₂ highlighted an extraction yield of 84.6 % TP lyc, although the recovery into the extract
30 was 48.4 % of the extracted lyc (lyc=1339 µg g⁻¹ oil). The SE application to TP_SFE_CO₂ data
31 confirmed that partial solubilization mainly depended on oil availability vs. lyc amount. Thus an
32 improved TP_SFE_CO₂ was designed in which 703 g of exogenous tomato oil will be fluxed from
33 the co-solvent tank: the new process will produce 884 g kg⁻¹ d.m. of extract with an expected
34 recovery of 99.3 % of the extractable lyc (lyc=502 µg g⁻¹ oil).

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47 *Keywords:* Carotenoids; *Solanum lycopersicum*; *E*:Z-isomerization; solubility model; co-solvent; aggregates

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51 **1. Introduction**

52 Lycopene (lyc) is the carotenoid with the highest antioxidant activity and is widely demanded by
53 the cosmetic, food and pharmaceutical industries. Thanks to its singlet molecular oxygen
54 quenching ability lyc is, in fact, one of the ten most marketed carotenoids, with a global economic
55 value estimated at 1.5 USD billion in 2017 and an annual growth rate (CAGR) between 2017 and
56 2022 of 2.3% (BCC Research, 2018). In addition, its 11 conjugated double bonds confer to lyc the
57 possibility of isomerizing into a vast array of Z- isomers. Lyc in tomatoes is predominantly stored in
58 the peel of the fruit in its (all-*E*)- isomeric form, assuming crystal-like structures in the chromoplast
59 with concentrations in a range between 50 and 1000 mg kg⁻¹ fresh tomato peel wet weight (w.w.)
60 (Ilahy et al., 2016).

61 Conventional extraction with organic solvents from tomato fruit represents the current method of
62 production of lyc. However, the agronomic cost, the consumption of land, the competition with
63 food and the use of organic solvents make this production system no longer sustainable;
64 therefore, alternative feedstocks and extraction systems are being investigated.

65 The tomato canning industry is a widely developed business that, during the processing of the
66 fruit, produces pomace (TP) as the major solid waste, which is comprised of peels and seeds
67 (Benítez et al., 2018). This fraction represents about 4 % w.w. of processed tomatoes, that
68 corresponded to more than 1 million t y⁻¹ of TP around the world in 2018 (Benítez et al., 2018).
69 Since lyc is predominantly stored in the peel of the fruit (Machmudah, Winardi, Sasaki, Goto,
70 Kusumoto & Hayakawa, 2012), TP has the potential to become an alternative lyc feedstock.
71 Temperatures adopted during industrial processes can cause the partial conversion of (all-*E*)-lyc to
72 its Z- isomers and its supramolecular structure can switch from needle-shaped crystals to an
73 amorphous state, characterized by a higher solubility and bioavailability (Böhm, Puspitasari-
74 Nienaber, Ferruzzi & Schwartz, 2002; Murakami et al., 2017).

75 Supercritical CO₂ extraction (SFE_CO₂) is a greener alternative to organic solvent extraction, and
76 has already been applied in the food industry to produce solvent-free extracts and reusable
77 exhaust biomasses (Scaglia et al., 2020).

78 TP_SFE_CO₂ main operating parameters (pressure, temperature and CO₂ flow) have been
79 optimized to reach very high lyc recoveries (Vági et al., 2007; Huang, Li, Niu, Li & Zhang, 2008;
80 Ciurlia, Bleve & Rescio, 2009; Nobre, Palavra, Pessoa & Mendes, 2009; Machmudah et al., 2012).

81 The addition of co-solvents was also tested to enhance total yields and carotenoids extraction
82 (Machmudah et al., 2012). The employment of ethanol gave significantly higher lyc extraction
83 yields (Baysal, Ersus & Starmans, 2000; Huang et al., 2008). However, lyc is only partially soluble in
84 ethanol and very different solubilization capabilities were reported for *E*- and *Z*- isomers: in a
85 conventional ethanol extraction from tomato paste, a 93.9:6.1 *E*:*Z* ratio resulted in an extraction
86 yield of 4.3 % of the whole lyc, while the same feedstock but with a *E*:*Z* ratio of 43.8:56.2 produced
87 a much higher yield (59.7 % of the whole lyc content) (Honda et al., 2017). This evidence can be
88 explained by the fact that amorphous aggregates, the form that *Z*-lyc tends to assume at high
89 concentrations, are highly soluble while (all-*E*)- crystals, which are 5 to 80 µm long and 1 to 5 µm
90 thick, tend to diffuse in oils as suspensions rather than solubilizing (Murakami et al., 2017). An
91 increased total-lyc solubilization can also be attained changing the co-solvent physical nature
92 through nano- or micro-emulsifiers (Santos, Martìn, Meireles & Cocero, 2012). Lecithin-based olive
93 oil microemulsions, for example, greatly enhance the efficiency of lyc conventional extractions
94 (Amiri-Rigi, Abbasi & Scanlon, 2016; Amiri-Rigi & Abbasi, 2017; Amiri-Rigi & Abbasi, 2019).

95 SFE_CO₂ extraction of tomato pulp and peel using vegetal oils (i.e. canola, hazelnut and olive) as
96 co-solvent gave better result than ethanol (Gunstone, 1996; Bada, Leòn-Camacho, Prieto &
97 Alonso, 2004; Saldaña, Temelli, Guigard, Tomberli & Gray, 2010; Boudour-Benranchou, Plard,
98 Pinatel, Artaud & Dupuy, 2017; Watanabe et al., 2018).

99 In addition to pulp and peel, TP has an “endogenous” source of oil, which is co-extracted from the
100 seeds during TP_SFE_CO₂ (Scaglia et al., 2020). Tomato seeds account for 20-50 % of the dry
101 matter (d.m.) of TP, that corresponds to 12 %-30 % d.m.⁻¹ of extractable oil (Giannelos, Sxizas, Lois,
102 Zannikos & Anastopoulos, 2005). Its nutritional composition and antioxidant activity are very
103 appreciable due to the presence of unsaturated fatty acids, of which linoleic acid is the most
104 abundant, bioactive phytosterols like cycloartenol, sitosterol and stigmasterol, and tocopherols
105 such as the α - and γ -isomers (Giuffrè & Capocasale, 2016; Durante et al., 2017).

106 By using oil as exogenous or endogenous co-solvent the SFE_CO₂ extracts have a higher added
107 value, due to the presence of a broad spectrum of bioactive compounds, and meet the latest
108 trends in the field of health-related products by virtue of the synergistic effects of these molecules
109 (Friedman, 2013; Durante et al., 2017).

110 In some SFE_CO₂ + co-solvent processes, only a fraction of lyc was present into the extract while a
111 relevant amount of crystals was found into the apparatus and successively recovered by organic
112 solvents rinsing (Ciurlia et al., 2009; Lenucci et al., 2010). Preliminary evidences suggested that the
113 amount of co-solvent and the solubility of lyc greatly affect the final concentration of the extract,
114 therefore becoming the limiting factor when all other parameters are already optimized (Vági et
115 al., 2007; Ciurlia et al., 2009; Nobre et al., 2009).

116 The difficulty in verifying whether the co-solvent had an actual influence on SFE_CO₂ extraction
117 depends on several aspects, including peel vs. seed ratio into TP, oil amount and extractability and
118 lyc content; additionally, it is further complicated by the different solubility of Z- and (all-E)-
119 isomers in oil, as well as by the possibility that their ratio might change during SFE_CO₂ extraction
120 due to the thermal isomerization (Nobre et al., 2009; Yi, Shi, Xue, Jiang & Li, 2009).

121 The solubility of lyc is a parameter that could provide important information about the optimal
122 amount of oil to employ and, consequently, about the ideal amount and size of tomato seeds
123 required to guarantee an optimal extraction efficiency. There has recently been an increase in the
124 interest in the solubility of total-lyc, and Z-lyc vs. (all-*E*)-lyc were also investigated (Sabio et al.,
125 2003; Vasapollo, Longo, Rescio & Ciurlia, 2004; Vági et al., 2007; Ciurlia et al., 2009; Nobre et al.,
126 2009; Machmudah et al., 2012; Honda et al., 2017; Watanabe et al., 2018); however, to the best of
127 our knowledge, there are no reports on lyc solubility in tomato seed oil.

128 Moreover, the isomeric compositions of lyc have never been considered in the determination of
129 the overall solubility. The aim of this work was to study the solubility of different *E:Z* lyc isomers
130 mixtures into tomato oil from a mathematical point of view. To verify if lyc solubility is the limiting
131 factor of lyc recovery in SFE_CO₂ + endogenous co-solvent oil processes, TP extractions were
132 carried out on a SFE_CO₂ pilot scale plant and the data was discussed.

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134 **2. Materials and methods**

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136 *2.1. Materials*

137 TP derived from tomato industrial processing was collected from a tomato sauce canning plant
138 located in northern Italy (OPOE Gruppo Cavicchi Scarl, Dodici Morelli FE); about 100 kg of w.w.
139 biomass was brought to the laboratory for analytical characterizations and extractions.

140 A portion of the TP was accurately separated into peels (P) and seeds (S) in water by exploiting
141 their different densities; all the resulting fractions were then dried in a vacuum oven at 40°C to
142 reach an average intermediate moisture content of 60 g kg⁻¹ w.w.

143 In order to evaluate the effect of particle size on extraction efficiency, P and S were milled to reach
144 two different maximum sizes of 1.5 mm (milled samples) and 0.2 mm (powdered samples) by
145 using a conventional (cyclotecTM; Oy Cyclotec Ltd, Helsinki, Finland) and ball (Retsch MM200, 2.5
146 min, 30 Hz; Retsch GmbH, Haan, Germany) milling apparatus. The obtained fractions were stored
147 in vacuum-sealed plastic bags at 4 °C in the dark for 3 days before carrying the analyses out.

148 About 2 g of milled and powdered S were extracted with 50 ml of hexane for 8 hours with a
149 Soxhlet extractor to measure the oil content, which was calculated as the difference between the
150 weight of the dried seeds before and after the extraction and used as reference to calculate the oil
151 recovered by TP-SFE_CO₂. Similarly, a Soxhlet extraction on P was performed for lyc quali-
152 quantitative characterization and antioxidant activity assays (see section 2.2.3). All analyses were
153 performed in triplicate.

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155 2.2. *Lycopene characterization*

156 The quantification of *E*- and *Z*-lyc isomers was performed on an Agilent 1260 Infinity HPLC system
157 (Agilent Technologies, Inc. Santa Clara, California, USA) equipped with a C30 Develosil[®] rpaqueous
158 column (5 µm, 250 x 4.6 mm). 20 µl of Soxhlet or SCE extract, diluted in hexane to obtain a final
159 lyc concentration between 0.25 and 250 µg ml⁻¹, was injected at 10 °C with a mobile phase
160 composed of a mixture of Methyl tert-butyl ether (A) and methanol (B) and the following
161 compositional gradient was applied as per literature (Scaglia et al., 2020) with some slight
162 modifications: initial conditions 10% A/90% B, 35 min linear gradient to 45% A, 10-min linear
163 gradient to 60% A, 60 % A/40% B for 11 min, 4 min linear gradient to 10% A. Flow rate was set at
164 1.3 ml min⁻¹. The separation of lyc isomers peaks was monitored with a G1314F Agilent Uv/Vis
165 spectrophotometer at 475 nm. *Z*-lyc isomers tentative identification was based on the literature,

166 by comparing the retention times and the relative retention times of the peaks found (Fig. SM-1)
167 (Urbonaviciene & Viskelis, 2017; Honda, Kageyama, Hibino, Takemura, Goto & Fukaya, 2019).
168 Quantification was based on a (all-*E*)-lyc analytical standard (L9879, Sigma Aldrich, USA).
169 Partially overlapping peaks were considered valid only when their resolution was >1. Resolution
170 values were obtained with the equation $R=(RT_2-RT_1)/0.5*(width_1+width_2)$ where RT was the
171 retention time of the peak 1 and 2. Analyses were conducted in triplicate.

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173 2.3. Antioxidant activity

174 The TEAC (Trolox Equivalent Antioxidant Capacity) assay was conducted to assess the antioxidant
175 capacity of each extract as described by Scaglia et al. (2020) by evaluating the 2,2-azinobis-3-ethyl-
176 benzothiazoline-6-sulfonic acid (ABTS⁺) radical cation decolorization reaction. ABTS⁺ was produced
177 by mixing an aqueous solution of ABTS (7 mM) with potassium persulfate (2.45 mM, final
178 concentration) and by letting the mixture react in the dark at room temperature for 12–16 h.
179 Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard. 100 µl of
180 hexane extract and of properly diluted samples were dissolved in 3.9 ml ABTS⁺ solution, and 1 ml
181 of the resulting solution was read at $\lambda=734$ nm both after 6 minutes and at the end of reaction (in
182 the dark). The antioxidant capacity was expressed as trolox µmol that produce the same
183 decolorization of 1 g of sample ($\mu\text{mol}_{\text{Trolox}} \text{g}^{-1}_{\text{sample}}$) and the calibration curve was linear between
184 0.25 and 12.5 µM trolox (6-point curve, $R^2 = 0.99$). The antioxidant activity of ascorbic acid (A4403,
185 Sigma-Aldrich, USA) was tested as a reference to verify the overall goodness of the procedure. The
186 spectrophotometric analysis was carried out with a UV/visible Varian Cary 60. Analyses were
187 conducted in triplicate.

188 2.4. Lycopene solubility in tomato seed oil

189 *2.4.1 Lycopene enriched tomato oil solution preparation and isomerization*

190 Lyc solubility tests were performed by using a commercial Triple Concentrated Tomato Paste (TCT)
191 (Kata food srl, Belpasso, Catania, Italy). TCT was dried under vacuum at 40°C, grounded to powder
192 size with a ball mill (as described in section 2.1) and successively extracted with a Soxhlet
193 apparatus, using hexane as solvent. The extract obtained from TCT (TCTE) was 63.97 mg g⁻¹ d.m.
194 TCT and had a lyc content of 2031 µg g⁻¹ d.m. TCTE total-lyc was composed by 74.85 % (all-*E*)-lyc
195 and 25.15 % *Z*-lyc isomers.

196 TCTE was successively diluted into ethyl acetate to obtain a stock solution (total-lyc= 126.94 µg ml⁻¹
197 ¹) which was used to perform the successive isomerization and solubilization experiments.

198 Different amounts of stock solution were added to 1 ml of tomato oil to obtain the following total-
199 lyc concentration (LycT): 22, 45, 89, 179, 297, 589, 877, 1440, 2129, 2776, 4021 and 6270 µg ml⁻¹
200 oil, characterized by high (all-*E*)-lyc content (M75t sample series).

201 Chemical isomerization was performed using I₂ as chemical catalyst (326143, Sigma-Aldrich, USA)
202 as described by Meléndez-Martínez, Paulino, Stinco, Mapelli-Brahm & Wang (2014) with some
203 slight modifications.

204 200 µl of tomato seed oil were added to 800 µl of stock solution and successively mixed with 0, 10
205 , 50, 100, 200 and 500 µl of a 10 µg ml⁻¹ solution of I₂ in hexane; the solutions were then exposed
206 to the light of a fluorescence lightbulb for 1h at a 50 cm distance (Meléndez-Martínez et al., 2014).

207 The effects of the isomerization process were evaluated performing lyc quali-quantitative
208 characterization of the treated samples; a significant relationship between *Z*-isomerization effect
209 and I₂ amount was found: *Z*-lyc % increase = 8.36*I₂ - 0.87, R² = 0.99, p<0.001, n=5 where both *Z*-
210 lyc and I₂ added were expressed as % of total-lyc (Fig. SM-2). The equation was successively

211 employed to calculate the I₂ doses needed to obtain an isomers-balanced (M59t) series of
212 solutions and a Z-rich one (M25t).

213 A first cycle of isomerisation was performed adding to M75t sample series a I₂ dose of 1.64 % total-
214 lyc, and the M59t sample series, having a balanced *E:Z* ratio of 59.1:40.9, was obtained. Then, a
215 second isomerization cycle was done, starting from M59t samples and adding I₂ = 5.21% total-lyc:
216 a third-derivative series, M25t, with a *E:Z* ratio of 25.3:74.7 was produced.

217
218 *2.4.2 Lycopene enriched tomato oil solution solubilization experiments.*

219 All samples of M75t, M59t and M25t were vortexed and ethyl acetate was evaporated under
220 vacuum at 40°C overnight. In order to precipitate lyc suspended in the oil, samples were
221 centrifuged at 10000 rpm for 10 minutes and successively 10 µl of oil were carefully collected and
222 dissolved in hexane for the quantification of solubilized total-lyc (lycS) and of its isomers.

223
224 *2.5. TP supercritical CO₂ extraction process*

225 Before carrying the SFE_CO₂ out, 20 kg TP were partially dried under vacuum until they reached
226 intermediate moisture contents of 60 g kg⁻¹ w.w. and were subsequently ground to 1.5 mm and
227 0.2 mm maximum size with a conventional and ball mill as described in section 2.1. Around 1 kg of
228 milled (TPm) and powdered TP (TPp) were extracted at the following operating parameters:
229 pressure 380 bar, temperature 80 °C, CO₂ flow 15 kg h⁻¹. The supercritical apparatus was provided
230 by Separeco S.r.l., Pinerolo (TO) ITALY. The semi-industrial plant used was equipped with a 4.2 l
231 extraction vessel, a 20 kWatt pump with a maximum flow rate of 90 kg CO₂ h⁻¹ and 3 separators

232 (gravimetric, heated cyclonic, cooled cyclonic); the CO₂ flow was monitored with a Coriolis mass
233 flow meter.

234 The extracts were recovered from the gravimetric separator, weighted and stored in the dark at -
235 22 °C in vacuum containers for successive analytical characterizations. The density of the
236 pulverized material had to be adjusted to about 0.25 kg l⁻¹ by adding steel filling bodies to the TP
237 powder before loading the extraction vessel, in order to avoid packing phenomena.

238 The exhaust TP fractions produced (named TP_{m_{exh}} and TP_{p_{exh}} for the TP_m and TP_p respectively)
239 were weighed and chemically characterized as previously described, to perform the TP-SFE_CO₂
240 mass balance (Scaglia et al., 2020). At least 4 cycles of supercritical CO₂ extraction were performed
241 on each TP sample.

242

243 *2.6. Statistical analysis*

244 Every experiment was carried out in triplicate and each collected sample was analysed in
245 triplicate. The mean values, the standard deviations and the linear regressions were calculated
246 using SPSS 25 Software (SPSS Inc., Chicago, USA). Mean values were compared with a one-way
247 Anova test and, for $p < 0.05$, differences were considered statistically significant.

248

249 **3. Results and Discussion**

250

251 *3.1. Tomato pomace chemical characterization and particle size effect on extraction efficiency*

252 The TP was comprised of P and S in the proportion of 627.7 g kg⁻¹ w.w. and 372.2 g kg⁻¹ w.w.
253 respectively; however, due to the very different P and S dry matter (d.m.) content (Table 1), the
254 dry composition of the TP was 520.8 g kg⁻¹ d.m. TP for P and 479.2 g kg⁻¹ d.m. TP for S.

255 TP components were subsequently ground and subjected to chemical characterization (Table 1). P
256 extracts and lyc yield did not show significant differences between raw and milled material, while
257 a higher extraction efficiency was obtained from the powdered sample. P lyc was composed of
258 (all-*E*)- and *Z*- isomers (Table 1); (all-*E*)-lyc is usually the predominant form found in raw tomatoes,
259 while the *Z*- fraction derives from isomerisation phenomena that occur when the fruits are
260 subjected to high temperatures, as for example during industrial processing (Shi and Maguer,
261 2000). The higher lyc content of P in the pulverized sample was due to a higher extraction of the
262 (all-*E*)-lyc fraction (Table 1), since a total of 406 µg g⁻¹ more total-lyc was extracted and 302 µg g⁻¹
263 of this increase resulted to be (all-*E*)-lyc; this result, together with the increase of the whole
264 extract amount, suggest that with lower particle sizes higher disruption of the membranes
265 occurred, making lipophilic compounds and “raw” lyc more accessible (Zuorro, Lavecchia, Medici
266 & Piga, 2014).

267 The antioxidant activities of P extracts were comparable to the values found in literature for other
268 tomato extracts (Savatović, Četković, Canadanović-Brunet & Djilas, 2011); although antioxidant
269 power is attributable to the whole antioxidant content (i.e. other carotenoids, lipids, tocopherols),
270 the TEAC values were strictly linked to the lyc amount (Table 1). The grinding effect on the oil
271 release from S was different than the one reported for P, since a higher increase of the yield was
272 obtained from raw to milled size (Table 1). It appears that, while seeds only necessitate a rough
273 milling for the majority of the oil to be extracted, to increase the extraction of lyc from peels lower
274 particle sizes are required; this is probably due to the different nature of the chromoplast- and oil
275 bodies-containing tissues: as a matter of fact the main barrier to the oil bodies is the pericarp

276 (which is broken with only a rough milling of the seeds), since the cell walls of the endosperm cells
277 are prone to weakening and do not represent an impassable barrier to solvents. Chromoplasts, on
278 the other hand, have a thicker barrier on their own, and are localized inside cells whose walls are
279 more impervious to the action of solvents. (Jeffery, Holzenburg & King, 2012; Steinbrecher and
280 Leubner-Metzger, 2018).

281

282 3.2. *TP lycopene solubility in tomato oil*

283 3.2.1. *Solubility test of different (all-E)- and Z-lycopene mixtures*

284 In order to quantify the effect of the different isomers on the overall solubility, the dosed lyc (lyc_T)
285 of M75t, M59t and M25t samples was compared with the solubilized one (lyc_S) for total-, Z- and
286 (all-E)-lyc. Total- lyc_T vs. total- lyc_S relationships were describable by two different linear straight
287 equations (Fig. 1): at lower concentrations, the total- lyc_S were equal to the total- lyc_T values,
288 implying that a full solubilization occurred, while for higher concentrations of total- lyc_T the
289 correspondent total- lyc_S values were progressively lower (Fig. 1). Tomato oil was able to fully
290 solubilize total-lyc until the limit concentration of $460 \mu\text{g g}^{-1}$ oil (as average of M75t, M59t and
291 M25t) was reached. Very low total-lyc concentrations were tested in recent works (total-lyc = 75
292 $\mu\text{g g}^{-1}$ canola oil and total-lyc = $30\text{-}600 \mu\text{g g}^{-1}$ tomato oil), and the evidence that total-lyc was fully
293 solubilized at those concentrations is in agreement with our results (Zuorro et al., 2014). (all-E)-lyc
294 trends were similar to that of total-lyc (Fig. 1, Table 2) but M25t had lower limit concentrations
295 than M75t and M59t, since the solubilization of (all-E)-lyc become boldly unfavourable (Fig. 1).

296 (all-E)- and Z-lyc solubility trends were similar to the total-lyc one, although some exceptions were
297 observed (Fig. 1). (all-E)-lyc limit concentrations were very similar between M75t and M59t, while
298 a reduction occurred for M25t, in which full (all-E)-lyc solubilization became unfavourable due to

299 the very high Z-lyc content (Fig. 1). When the partial solubilization range was considered, (all-*E*)-lyc
300 of M75t showed a great tendency to precipitate for concentrations higher than of 331 $\mu\text{g g}^{-1}$, that
301 corresponded to the intersection point between full and partial straight lines (Table 2). After this
302 point, the entirety of (all-*E*)-lyc added to the solution precipitated and the concentration remained
303 nearly unvaried (average (all-*E*)-lyc_S= 366 \pm 43 $\mu\text{g g}^{-1}$), indicating that the saturation point of the
304 solution had been reached.

305 As expected, Z-lyc had a higher solubility than total-lyc and even more so than the (all-*E*)- fraction.
306 M59t showed a full Z-lyc solubilization at all tested concentrations (Fig. 1, Table 2), while limited
307 solubilization occurred for the M75t and M25t series.

308 Z-lyc partial solubilization is a scarcely investigated phenomenon, and only the recent interest in
309 carotenoids aggregations has provided evidence that isomers composition has a significant effect
310 into relative solubility, due to the formation of H-, J- and amorphous aggregates, originated by the
311 supramolecular interaction of (all-*E*)-, (all-*E*)- + Z- and Z-lyc respectively (Hempel, Schädle, Leptihn,
312 Carlea & Schweiggert, 2016; Murakami et al., 2017; Dong, Zhang, Wang & Wang, 2018).

313 The capability of Z-lyc to form different types of aggregates (J- and amorphous) could explain the
314 reduction of its solubilization when isomers are unbalanced, i.e. in the cases of M75t and M25t
315 samples (Scaglia et al., 2020). An unbalanced mix towards the (all-*E*)- form (i.e. M75t), favors H-
316 aggregates in which needle-like crystals are strongly linked, causing evident (all-*E*)-lyc
317 precipitation; when Z-lyc is more concentrated (i.e. M25t) J-aggregates formation is in turn
318 favored, with Z-lyc positioning itself between (all-*E*)-lyc layers, increasing the total-lyc solubility.

319 Tomato fruit and TP are lyc feedstocks for which very different lyc concentrations were reported
320 (Table 4). Although lower concentrations guaranteed full lyc recovery, the obtainable extracts are

321 less interesting for the nutraceutical and cosmetic fields than those derived from higher starting
322 concentrations, in spite of potential lyc losses.

323 From a qualitative point of view, different isomeric compositions are also expected for tomato
324 fruits and TP (Table 4). To give an idea of how the isomers content influenced the solubilization of
325 total-lyc, a lyc-enriched oil simulating the extract achievable by the TPp extraction was considered
326 (Table 1) ($\text{all-lyc}_{\text{T_TPp}} = 4313 \mu\text{g g}^{-1}$ oil). For the same $\text{all-lyc}_{\text{T}}$, M75t and M59t compositions were
327 simulated, since they represent the *E:Z* ratio in raw tomato and TP, and the total-, all-*E*- and *Z*-lyc_s
328 were calculated (Table 2). The results highlighted that by using TP the achievable lyc concentration
329 would be $3752 \mu\text{g g}^{-1}$ oil, more than twice as high as that from raw tomato fruit ($1587 \mu\text{g g}^{-1}$ oil).

330 In addition to total-lyc concentration, a relevant factor in defining the bioactivity of the extracts is
331 their isomeric composition: in the partial solubilization range a generalized *Z*-increasing
332 phenomenon was described, since at the highest concentrations the *E:Z* ratio of M75t and M59t
333 became 28.6:71.4 and 31.8:68.2 respectively.

334 Current commercial products are mainly composed of (all-*E*)-lyc, that has limited bioavailability
335 due to its low solubility. On the contrary, the amorphous structures formed by *Z*-lyc have a higher
336 solubility into oil and emulsions, properties known to be linked to a higher bioavailability
337 (Murakami et al., 2017).

338 By testing *E:Z* isomer ratios of 95:5 and 55:45, previous studied showed a degree of adsorption in
339 human serum and tissues of about 60% and 99% respectively (Unlu, Bohn, Francis, Nagaraja,
340 Clinton & Schwartz, 2007); the difference was explained by considering the higher chemical
341 stability and bioavailability of a balanced mixture (*E:Z* 55:45) due to the very similar isomer
342 composition that was found in human serum (lyc-serum composition= *E:Z* 37-46:63-54). The

343 decrease in (all-*E*)-lyc was probably due to biological isomerization phenomena or to *Z*-lyc
344 preferred adsorption (Schierle et al., 1997; Unlu et al., 2007; Meroni & Raikos, 2018).

345 Taking into consideration all these factors we can conclude that the ideal isomer ratio for the
346 bioactivity and absorbability of the total-lyc is a balanced one, achievable by using a starting lyc
347 feedstock composition as that applied in this work.

348

349 *3.3. Tomato Pomace supercritical CO₂ extraction*

350 *3.3.1. TP SFE_CO₂ extract yields and lycopene content*

351 TPm and Tpp SFE_CO₂ produced 142.5 and 165.3 g kg⁻¹ d.m. of extract respectively, which were
352 composed of tomato oil and of a small fraction of water (oil:water ratio was of 90:10 as average of
353 TPm and Tpp). Previous works defined TP extracts as an oleoresin of tomato oil in which the
354 lipophilic P fraction was dissolved; recently, the extraction of water during SFE_CO₂ was shown
355 and was linked to the starting TP moisture. With a moisture content of 10% w.w. TP, Scaglia et al.
356 (2020) described a water in oil emulsion extract in which phospholipids acted as emulsifiers.
357 Conventional emulsions improve the lyc adsorption by the human cells but do not influence lyc
358 solubility, contrarily to the evidences found for micro-emulsions (Amiri-Rigi et al., 2016; Amiri-Rigi
359 et al., 2017; Amiri-Rigi et al., 2019).

360 The antioxidant activities of extracts were investigated as well and TPm and Tpp gave similar
361 results (96.86 and 84.33 μmol_{trolox} g⁻¹_{extract}): these values are higher than the ones shown for TP
362 fractions, probably due to the lyc concentrating effect of the extraction process. Nevertheless,
363 they were lower in respect to the expected content (Table 1), supposedly due to either the
364 degradation of thermolabile antioxidant molecules during SFE_CO₂ or to the impossibility of
365 extracting them with CO₂.

366 The total-lyc concentration into the oil was slightly higher for TPp than for TPm, which had a
367 higher (all-*E*)-lyc fraction as well; the experimental data were quantitatively comparable with the
368 theoretical ones but the (all-*E*)-lyc contents were lower by about 5% (Table 1, Table 3).

369 Chemical isomerization was cited in literature as the main cause of the higher presence of *Z*-
370 isomers in the extracts (Nobre et al., 2009) in comparison with the starting total-lyc composition,
371 since during SFE_CO₂ the high temperature can favor the isomerization of (all-*E*)-lyc; in some
372 cases, even the degradation of the product was reported.

373 This interpretation was used to explain the loss of a part of the starting lyc and was based on the
374 comparison of total-lyc feedstock and extract content, but it overlooked the total-lyc retained in
375 the exhaust biomass. A more correct method was carried out by applying a mass-balance that
376 calculated extract quali-quantitative properties by using feedstock and exhaust biomass data
377 (Scaglia et al., 2020). Based on this approach, at the same SFE_CO₂ condition applied in this work,
378 no lyc degradation occurred and that during longer processes (8 hours) lyc isomerization was a
379 marginal phenomenon that affected at most 21 % of the starting (all-*E*)-lyc (Scaglia et al., 2020).

380 In the present work, the extract amounts experimentally obtained (extract= 142.4 g kg⁻¹ TPm and
381 165.3 g kg⁻¹ TPp) fitted well with that obtained applying the mass balance (extract= 164 g kg⁻¹ TPm
382 and 177.3 g kg⁻¹ TPp) and confirmed the extract composition (Table 1, 3). On the other hand, very
383 different recoveries were obtained between total-lyc derived from the mass balance (recoveries=
384 86.4% and 84.6% total-lyc for TPm and TPp respectively) and from the analyses of the extracts
385 (recoveries= 41.6% and 48.4% total-lyc for TPm and TPp respectively); this difference can be
386 explained by the evidence that a part of the extracted total-lyc was trapped into the SFE_CO₂
387 apparatus (Table 3) and successively came out when the extractor was cleaned with acetone.
388 Several works described a similar behavior (Table 4) but none gave an interpretation for that kind
389 of result, although a deeper data analysis allowed us to find as a common factor a very high co-

390 solvent oil extraction (Vági et al., 2007; Ciurla et al., 2009; Nobre et al., 2009) which was similar to
391 what occurred in this work (84.6% oil and 94.3 % oil for the TPm and TPp respectively) (Table 3).

392 Taking into consideration all previous findings, we supposed that co-solvent amount and total-lyc
393 solubility in the co-solvent became the main limiting factors to total-lyc recovery into the extract.
394 In order to verify this hypothesis, the total-lyc quali-quantitative compositions of the SFE_CO₂
395 extracts (total-lyc_S) were compared with those expected (total-lyc_{S-math}) by using the previously
396 described equations (Table 2) and total-lyc_S was calculated from experimental data when possible
397 (Table 4). The elaboration was possible for three literature works only, in which total-lyc
398 composition had a very high (all-*E*)-lyc content, and M75t equations were applied. Total-lyc_{S-math}
399 resulted to be very similar to the total-lyc_S and allowed us to correctly identify the isomers
400 proportions (Table 4), confirming that total-lyc amount and composition were the main factors
401 that affected lyc yields and recoveries.

402 The same approach was hence applied to the SFE_CO₂ processes of this work (Table 3): mass
403 balances on total-lyc recoveries were used to estimate total-lyc_T (total-lyc_T =2840 µg g⁻¹ extr and
404 total-lyc_T =2671 µg g⁻¹ extr for TPm and TPp respectively), and were subsequently employed to
405 calculate total-lyc_{S-math} (total-lyc_{S-math}=1719 µg g⁻¹ extr TPm and total-lyc_{S-math} =1812 µg g⁻¹ extr TPp)
406 by using the M59t partial solubilization equation (Table 2). Although almost similar, the total-lyc_{S-}
407 _{math} did not fit as well as those previously shown; again, the (all-*E*)-lyc and *Z*-lyc M59t equations
408 application found better correspondence for the (all-*E*)-lyc_{S-math} while *Z*-lyc_{S-math} overestimated *Z*-
409 lyc_S by the same quantity recorded between total-lyc_S and total-lyc_{S-math}. In order to understand
410 the cause, the total-lyc_S composition of the TP extract was compared with that of the M59t dose
411 that had the same total-lyc_S (Table SM-2) and it was observed that the *E*:*Z* composition of the
412 extract was of 51.5:48.5 (average of TPm and TPp) while that of M59t was 63.4:36.6,

413 corresponding, from a quantitative point of view, to a higher Z-lyc content (+245 $\mu\text{g g}^{-1}$) and a
414 lower (all-*E*)-lyc (-181 $\mu\text{g g}^{-1}$) compared to TP extract.

415 M59t composition (Table SM-2) is the outcome of the isomerization process that was carried out
416 by adding I_2 . TE and TP extract are rich in other molecules (i.e. fatty acids, phospholipids) recently
417 described, together with operating temperature, as possible isomerization catalysts (Honda et al.,
418 2017; Yu, Gleize, Zhang, Caris-Veyrat & Renard , 2019). However, the isomerization estimated
419 from previous trials was minimum, thus it was no longer considered in this work (Scaglia et al.,
420 2020).The I_2 isomerization contribution fro M59 was estimated by considering that 181 $\mu\text{g g}^{-1}$ of
421 (all-*E*)-lyc corresponded to the isomerized fraction and the I_2 dose needed to perform the reaction
422 was calculated to be 1.68% of the starting lyc, which was in agreement with the one
423 experimentally employed.

424 With the aim to verify if the TP extract composition depended on the starting total-lyc amount and
425 isomeric ratio, the M59t *E*:*Z* content was thus recalculated not considering the I_2 effect for all
426 doses and the updated equations (total-lyc_S=0.425*total-lyc_T+338, $R^2=0.99$; (all-*E*)-
427 lyc_S=0.146*total-lyc_T+457, $R^2=0.97$, Z-lyc_S=0.76*Z-lyc_T, $R^2=0.99$, and $p<0.01$ for all equations)
428 were employed to recalculate total-, (all-*E*)- and Z-lyc_S of TPm and TPp.

429 By applying the resulting equations, the total-lyc_{S-math} values were 1473 $\mu\text{g g}^{-1}$ extr TPm and 1545
430 $\mu\text{g g}^{-1}$ extr TPp, (all-*E*)-lyc_{S-math} were 742 $\mu\text{g g}^{-1}$ extr TPm and 634 $\mu\text{g g}^{-1}$ extr TPp and Z-lyc_{S-math} were
431 596 $\mu\text{g g}^{-1}$ extr TPm and 678 $\mu\text{g g}^{-1}$ extr TPp: these values fitted very well with the experimental
432 data (Table 3), therefore confirming again the that total-lyc content and isomer composition had a
433 very important role, and that the solubility test data could be used to forecast the concentration
434 of the extracts.

435

436 3.3.2 TP SFE_CO₂ future improvement

437 TP particle size seemed to have had a limited effect in regard to the extract characteristics (Table
438 3); nevertheless, the parameters to apply to the process (Fig. 2) were chosen basing on their
439 influence on the extraction kinetics (Fig. 3a). Process durations were of 7.4 ± 1.8 hours and 2.3 ± 0.09
440 hours for TPm and TPp respectively, and the total CO₂ consumptions were of 95 ± 31 kg CO₂ kg⁻¹
441 TPm and 35.7 ± 1.6 kg CO₂ kg⁻¹ TPp. The feasibility of a process is strictly linked to the cost of
442 manufacturing (COM), that defines its economical sustainability. When SFE_CO₂ is employed, one
443 of the major expenses to be taken into account is the energy required to manage CO₂ compression
444 and de-compression cycles (Boakye-Boaten, Kurkalova, Xiu & Shahbazi, 2017) thus, based on the
445 extract properties and process costs, grinding the TP to powder gave better performances, as
446 confirmed by the literature data (Nobre et al., 2009).

447 The previously described results highlight that the extract obtained by applying the process should
448 be improved to get a full total-lyc recovery, since only 48.4% of the extracted total-lyc was
449 solubilized in the TPp extract.

450 High extract concentrations could be achievable using feedstocks characterized by a high Z-lyc
451 content. In this perspective an alternative feedstock could be tangerine tomatoes, that are
452 characterized by a 94 % Z-lyc content, or its derived by-products (Cooperstone et al., 2015).

453 The isomerization of the feedstocks is also a valid alternative, as demonstrated by the
454 improvement of the solubility obtained in this work when considering M75t and M25t.

455 However, the employment of chemical catalyst as I₂ would not be allowed in the food industry;
456 nevertheless, pre-isomerization steps using heat, food-derived catalysts (i.e. polysulfides) or a
457 combination of the two have already been tested in literature (Honda et al., 2017; Honda et al.,
458 2019; Yu et al., 2019).

459 Alternatively to this approach, an improvement of the SFE_CO₂ extraction yield has been obtained
460 in literature by adding S to the TP, increasing the oil available to solubilize the lyc (Machmudah et
461 al., 2012). In that case an augmentation of the total-lyc recovery of about 20 % was obtained when
462 switching from a 50:50 P:S ratio to a 37:63 one, confirming that lyc solubility vs. oil availability
463 were driving parameters of the extraction process.

464 To verify these aspects for the TPp_SFE_CO₂ the instantaneous extraction kinetics of total-, (all-*E*)-
465 and *Z*-lyc and S oil were analyzed (Fig. 1): initially, the total-lyc_T concentration was lower than the
466 full solubilization limit value and showed the same *E*:*Z* composition of the TPp, clearly indicating
467 that a full solubilization was occurring, while at 12.9 kg CO₂ kg⁻¹ TP the total-lyc_T was higher than
468 the limit concentration and consequently its *Z*-lyc content increased. These are all indicators of the
469 shift to the total-lyc partial solubilization range, because of the S oil depletion that became the
470 limiting factor to a complete total-lyc recovery (Fig. 3b).

471 At the same time, the extraction kinetics highlight that, rather than the total amount of oil, the
472 available fraction at the different extraction times should be high enough to obtain a full
473 solubilization of total-lyc, but not too high to avoid an excessive dilution of total-lyc into the
474 extract. The instantaneous extraction kinetic of the oil from S resulted in very different yields
475 during the process that did not guarantee a constant oil availability (Fig. 3b); therefore, just
476 increasing the overall amount of seeds, as was already tested (Machmudah et al., 2012), do not
477 appears to be the best solution to improve the process. The maximum recovery could be
478 obtainable by using an exogenous source of oil, and by dosing it accordingly to the extraction
479 kinetic through the co-solvent tank. To achieve a complete solubilization of the extracted lyc, the
480 oil should be added so that the concentration of the total-lyc stays under 473±83 µg g⁻¹ oil, thus
481 guaranteeing that the extraction is being carried out in the full solubilization range.

482 Supposing that it is possible to use an exogenous tomato oil as co-solvent, for which lyc
483 solubilization capabilities have already been tested, a theoretical attempt based on TPP_SFE_CO₂
484 kinetic data was done to define the quantity and flow of co-solvent to employ for the process
485 improvement (Fig. 3b). To maximize lyc recovery in the extract, the exogenous oil will be dosed
486 accordingly to the updated solubility equation ($\text{total-lyc}_S = 0.425 * \text{total-lyc}_T + 338$, section 3.3.1).
487 Moreover, basing on the punctual extraction kinetic of oil from TPP (Fig. 3b), we can hypothesize
488 that the best way to add co-solvent oil to the extractor would be a variable amount graphically
489 measurable as the difference between the endogenous S oil and the optimized extract amount.
490 Hence, in order to compensate for the lack of co-solvent during the extraction, the amount of
491 exogenous oil added will increase from 5.87 kg CO₂ kg TP⁻¹ (the moment of the extraction at which
492 the total-lyc/co-solvent ratio starts to fall into the partial solubilization range) to the end of the
493 process (Fig. 3b). In this improved extraction configuration, the total amount of tomato oil to be
494 added will be 702 g kg⁻¹ d.m. to obtain 884 g kg⁻¹ d.m. TP of extract at a total-lyc concentration of
495 502 µg g⁻¹ extract. This value corresponds to a total amount of recovered lyc of 444 mg kg⁻¹ d.m.
496 TP., more than double the amount of lyc actually recovered in the tested TPP_SFE_CO₂.

497

498 **4. Conclusions**

499 The extraction of lyc with SFE_CO₂ has both the solubilities of total-, (all-*E*)- and *Z*-lyc and the
500 availability of co-solvent as limiting factors. The implementation of the solubility equations
501 highlighted that different isomers did not affect the maximum concentration of the full
502 solubilization range. However, very different behaviors were detected in the partial solubilization
503 range from a qualitative and quantitative point of view. The most advantageous isomer
504 compositions are the balanced ones such as that of the TP, in which less aggregates form and that
505 are characterized by a solubility which is twice as high as those of lyc from conventional feedstocks

506 (raw tomatoes). The solubility equations application to TP_SFE_CO₂ data confirmed lyc solubility in
507 oil and oil availability as limiting factors. To overcome total-lyc loss due to its partial solubilization,
508 TP_SFE_CO₂ could be optimized by adding a variable amount of exogenous oil as co-solvent, to
509 obtain a larger quantity of extract with a greater recovery of solubilized total-lyc and an optimal
510 *E:Z* composition.

511

512 **5. Conflicts of interest**

513 The authors declare that they have no competing interests.

514

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527

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529

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668 Tables

669 Table 1 Effect of different particle sizes of peels and seeds on extraction efficiency

			raw	Size milled	powder
peels	P-extract	g kg ⁻¹ d.m. peel	13.9±1.1a ^a	13.3±0.6a	33.2±1.3b
	total-lyc	µg g ⁻¹ d.m. peel	956±59a	945±14a	1351±74b
	(all- <i>E</i>)-lyc	% <i>total</i> -lyc	57.8±0.3a	56.55±0.36 a	61.95±0.21b
	TEAC	µmol _{trolox} g ⁻¹ d.m. peel	21.12±1.24a	19.42±1.74 a	30.51±2.98b
seeds	S-extract	g kg ⁻¹ d.m. seed	7.2±0.4a	252.9±2.9b	302.7±1.8c
	TEAC	µmol _{trolox} g ⁻¹ d.m. seed	0.042±0.001a	2.43±0.36b	2.47±0.1b

^a Means followed in the same line by the same letter for each parameter for each treatment are not statistically different ($p < 0.05$) according to Tukey's test.

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677 Table 2 Mathematical relationships between dosed lyc (lyc_t) and solubilized lyc (lyc_s) of total-, (all-*E*)- and Z-lyc in M75t, M59t and M25t mixtures.

Mixture	lyc	Full solubilization equation ^a	Limit Concentration ^b $\mu\text{g g}^{-1}$ oil	Partial solubilization equation	Amount solubilized ^c (% lyc_{T_TPP})
M75t	total	$lyc_s=(1.093\pm 0.013)*lyc_t$	440±86	$lyc_s=(0.188\pm 0.003)*lyc_t+(399\pm 82)$	28
	(all- <i>E</i>)-	$lyc_s=(1.055\pm 0.0012)*lyc_t$	331±17	$lyc_s= 366 \pm 43$	11.8
	Z	$lyc_s=(1.086\pm 0.039)*lyc_t$	232±98	$lyc_s=(0.808\pm 0.014)*lyc_t+(66\pm 33)$	86.7
M59t	total	$lyc_s=(0.967\pm 0.022)*lyc_t$	473±83	$lyc_s=(0.571\pm 0.009)*lyc_t+(194\pm 26)$	61.6
	(all- <i>E</i>)-	$lyc_s=(0.963\pm 0.021)*lyc_t$	281±92	$lyc_s=(0.275\pm 0.021)*lyc_t+(200\pm 42)$	26.7
	Z	$lyc_s=(1.014 \pm 0.014)*lyc_t$	-	-	100
M25t	total	$lyc_s=(0.959\pm 0.031)*lyc_t$	357±161	$lyc_s=(0.737\pm 0.011)*lyc_t+(84\pm 31)$	75.1
	(all- <i>E</i>)-	$lyc_s=(0.964\pm 0.033)*lyc_t$	38±14	$lyc_s=(0.534\pm 0.007)*lyc_t+(17\pm 5)$	54.7
	Z	$lyc_s=(0.967\pm 0.030)*lyc_t$	361±223	$lyc_s=(0.808\pm 0.014)*lyc_t+(61\pm 30)$	82.7

678 ^a All equations are significant for $p < 0.01$, $R^2 > 0.96$ and coefficients of the equation are significant for $p < 0.01$

679 ^b limit concentration values calculated as linear intersections between the full and the partial solubilization equations. Average values \pm standard
680 deviations were obtained by using the lowest and highest parameters values when calculating the equations.

681 ^c simulation of the effect of the different mixture composition considering the same theoretical content (lyc_T) calculated using the TPp lycopene
682 and oil content (Table 1): total- $lyc_{T_TPp} = 4313 \mu g g^{-1}$ oil.

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Table 3 Supercritical CO₂ extraction yields, mass balance and characterization of the extracts

		amount g kg ⁻¹ w.w.	d.m. ^b g kg ⁻¹ w.w.	water g kg ⁻¹ w.w.	oil g kg ⁻¹ d.m.	total-lyc	lyc <i>E:Z</i> composition	TEAC μmol g ⁻¹ extract
	raw	1000	918.4	81.6	172.4	607±9 (μg g ⁻¹ d.m.TP)	56.55:43.45	
TPm	exhaust	839.7	941.3	58.7	33.8	117.9±1.3 (μg g ⁻¹ d.m. TP _{exhaust})	39:61	
	extract	142.5±14 ^a	-	32 ^b	132 ^b	1313±18 (μg g ⁻¹ extract)	48.33:51.67	84.33±1.63
	raw	1000	920.2	79.8	169	609±77 (μg g ⁻¹ d.m.TP)	61.94:38.05	
TPp	exhaust	814	831.1	60.8	11	113.3±1.8 (μg g ⁻¹ d.m. TP _{exhaust})	42.9:57.1	
	extract	165.3±9.2 ^a	-	30.3 ^b	147 ^b	1339±32 (μg g ⁻¹ extract)	55.45:44.55	96.86±1.98

^a weight of the extract

^b amount calculated applying mass balance

Table 4 Review of the SFE_CO₂ extractions of tomato and its byproducts performed with the addition of vegetal oil as co-solvents

Reference	Feedstock	Feedstock (g d.m.)	lyc and oil content in feedstock	Co-solvent	extract yield	total-lyc extract yield	total-lyc _s ^a	total-lyc _{s_math} ^b
Baysal et al., 2000	TP	53	<i>total-lyc</i> : 0.310 g kg ⁻¹ d.m. TP	ethanol (5% w.w. TP)	-	56% extractable <i>total-lyc</i> 158 µg g ⁻¹	-	-
Sabio et al., 2003	TP	40-50	<i>total-lyc</i> : 0.294 g kg ⁻¹ d.m. TP oil 210 g kg ⁻¹ d.m. S	tomato oil from TP	83.7 (g kg ⁻¹ feedstock) 398 (g kg ⁻¹ oil)	80% extractable lyc 0.235 mg g ⁻¹ TP <i>total-lyc_T</i> ^c 3.51 mg g ⁻¹ extr	2808 µg g ⁻¹ extr	-
Vasapollo, et al., 2004	dried tomato	100	-	halzenut oil (10% w.w. feedstock)	-	60% extractable <i>total-lyc</i>	-	-
Vági et al., 2007	TP	1000	<i>total-lyc</i> : 0.229 g kg ⁻¹ d.m. TP ; oil: 33.9 g kg ⁻¹ d.m. TP	tomato oil from TP	30.7 (g kg ⁻¹ feedstock) 905 (g kg ⁻¹ oil)	70.1% extractable lyc extr	4750 µg g ⁻¹ extr	-
Vági et al., 2007 ^d	TP	1000	<i>total-lyc</i> : 0.006 g kg ⁻¹ d.m. TP oil: 153.3 g kg ⁻¹ d.m. TP	tomato oil from TP	150.5 (g kg ⁻¹ feedstock) 982(g kg ⁻¹ oil)	0.005 mg g ⁻¹ TP <i>total-lyc_T</i> [*] 32 µg g ⁻¹ extr	32 µg g ⁻¹ extr	32 µg g ⁻¹ extr
Huang et al., 2008	tomato saponified skins plus seeds	100	<i>total-lyc</i> : 0.305 g kg ⁻¹ d.m. TP	ethanol (16% w.w. TP)	-	93.8% extractable lyc 0.286 mg g ⁻¹ d.m. TP	-	-

Nobre et al., 2009 ^d	TP	1.5	<i>total-lyc</i> : 0.188 g kg ⁻¹ d.m. TP <i>E:Z</i> : 81.6:18.4 oil: 202 g kg ⁻¹ d.m. TP	tomato oil from TP	153 (g kg ⁻¹ feedstock) 757 (g kg ⁻¹ oil)	93% extractable lyc 0.262 mg g ⁻¹ d.m. TP LycT* 1843 µg g ⁻¹ extr	1150 µg g ⁻¹ extr	1246 µg g ⁻¹ extr
Ciurlia et al., 2009 ^d	tomato (pulp+skins)+ roasted hazelnut (1:1)	3000	<i>total-lyc</i> : 5.24 g kg ⁻¹ d.m. tomato <i>E:Z</i> : 92:8 oil: 750 g kg ⁻¹ d.m.	hazelnut oil (35.8 % feedstock)	287 (g kg ⁻¹ feedstock) 800 (g kg ⁻¹ oil)	3.8 mg g ⁻¹ tomato oil 80 % <i>total-lyc</i> _T * 4200 µg g ⁻¹ extr	1290 µg g ⁻¹ extr (<i>E:Z</i> 31.48:68.52)	1189 µg g ⁻¹ extr (<i>E:Z</i> 32.75:67.24)
Saldana et al., 2010	tomato fruit without seed	10	<i>total-lyc</i> : 16.4 g kg ⁻¹ d.m.	canola oil (5% w.w. TP)	-	<i>total-lyc</i> : 0.0571 mg g ⁻¹	-	-
Machmudah et al., 2012	TP (P:S 37:63 w w ⁻¹ dm)	4	<i>total-lyc</i> : 0.3 g kg ⁻¹ d.m. TP oil: 214.2 g kg ⁻¹ d.m. TP	tomato oil from TP	-	<i>total-lyc</i> : 0.170 mg g ⁻¹ TP	-	-
Honda et al., 2017	dried tomato pulp	3	<i>Total-lyc</i> : 7.107 g kg ⁻¹ d.m. <i>E:Z</i> : 91.2 :8.8	olive oil	--	<i>total-lyc</i> : 0.462 mg g ⁻¹ d.m.	-	-
		3	<i>Total-lyc</i> : 7.101 g kg ⁻¹ d.m. <i>E:Z</i> : 69.6:30.4	olive oil	-	<i>total-lyc</i> : 1.101 mg g ⁻¹ d.m.	-	-

		3	<i>Total-lyc</i> : 5.955 g kg ⁻¹ d.m. <i>E:Z</i> : 24.3:75.7	olive oil	-	<i>total-lyc</i> : 1.643 mg g ⁻¹ d.m.	-	-
Watanabe et al., 2018	dried tomato pulp	4	-	hazelnut oil	-	66.4 % lyc recovery		

^a *total-lyc_S*: lyc solubilized in the extract

^b *total-lyc_{S_math}*: expected solubilized lyc calculated by using *total-lyc_T* equation reported in Table 2

^c *total-lyc_T*: *total-lyc* content of the extract calculated considering oil and *total-lyc* recoveries

^d experimental data at which equations (Table 2) of this work were applied

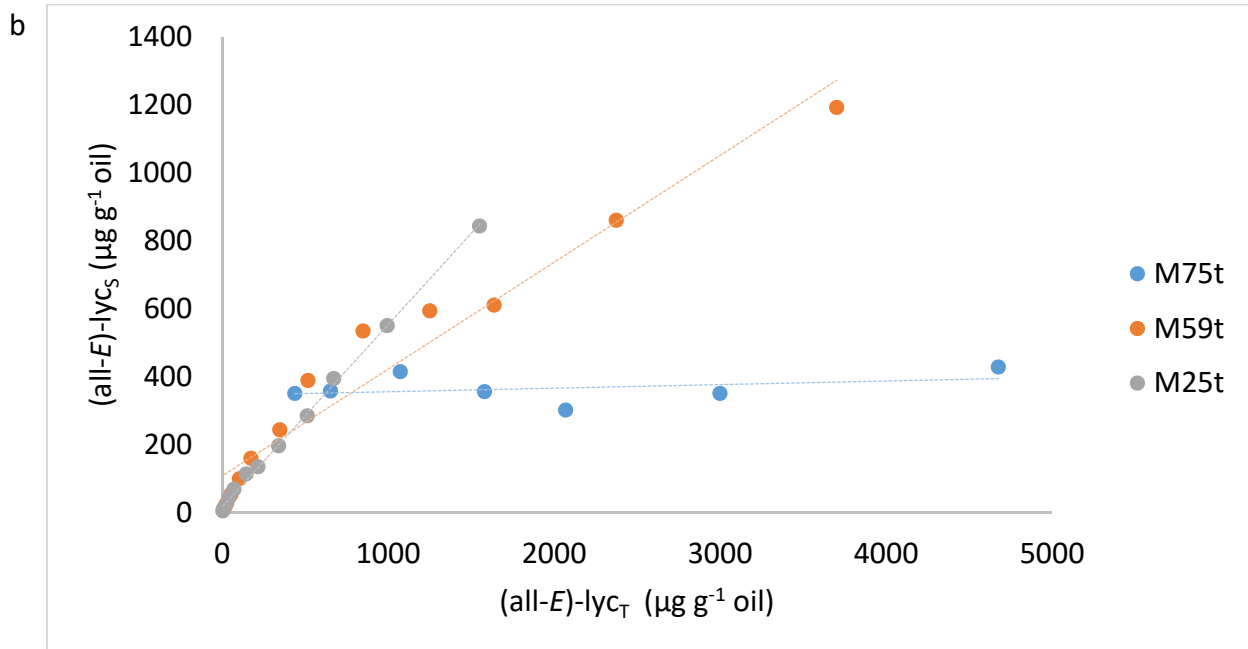
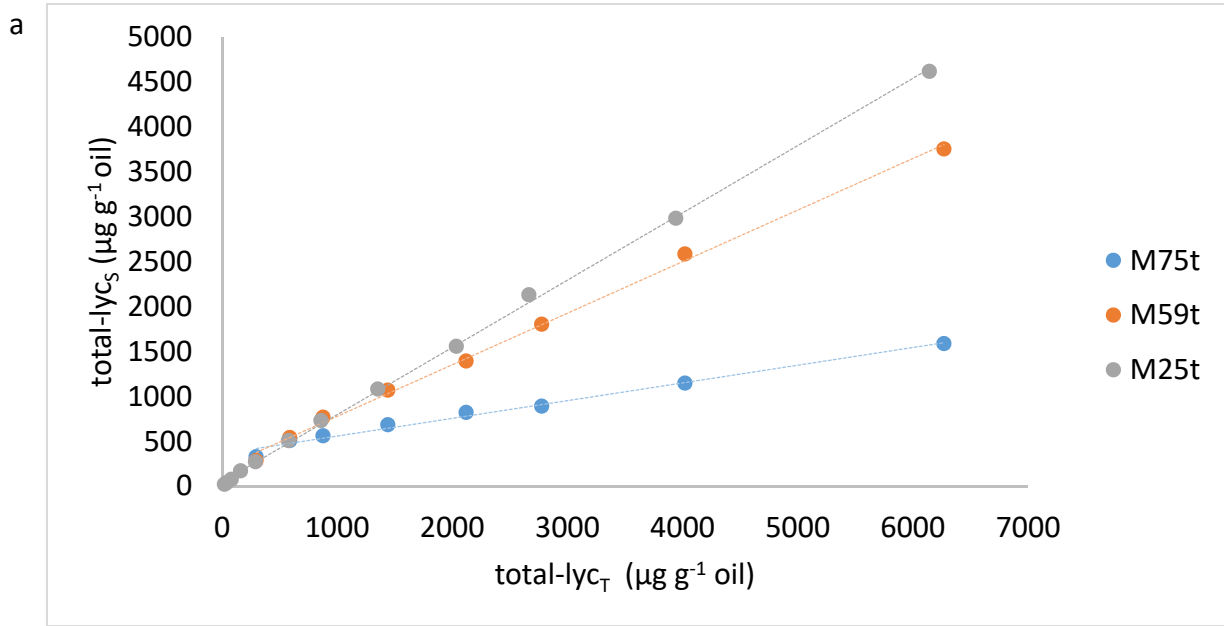
Caption Figure

Fig. 1 Total-lyc (a), (all-*E*)-lyc (b), Z-lyc (c) solubilized (lyc_s) vs. dosed lyc (lyc_T) into tomato oil for trends for M75t, M59t and M25t mixtures.

Fig. 2 Schematic representation of the TP_SFE_CO₂ process

Fig. 3 (a) TPm and TPp SFE-CO₂ extract kinetic trend. Values are average of data from 4 extraction cycles; (b) TPp-SFE-CO₂ extract kinetic trend with exogenous co-solvent oil addition. Error bars were calculated by applying the propagation of error formulae.

Fig. 1



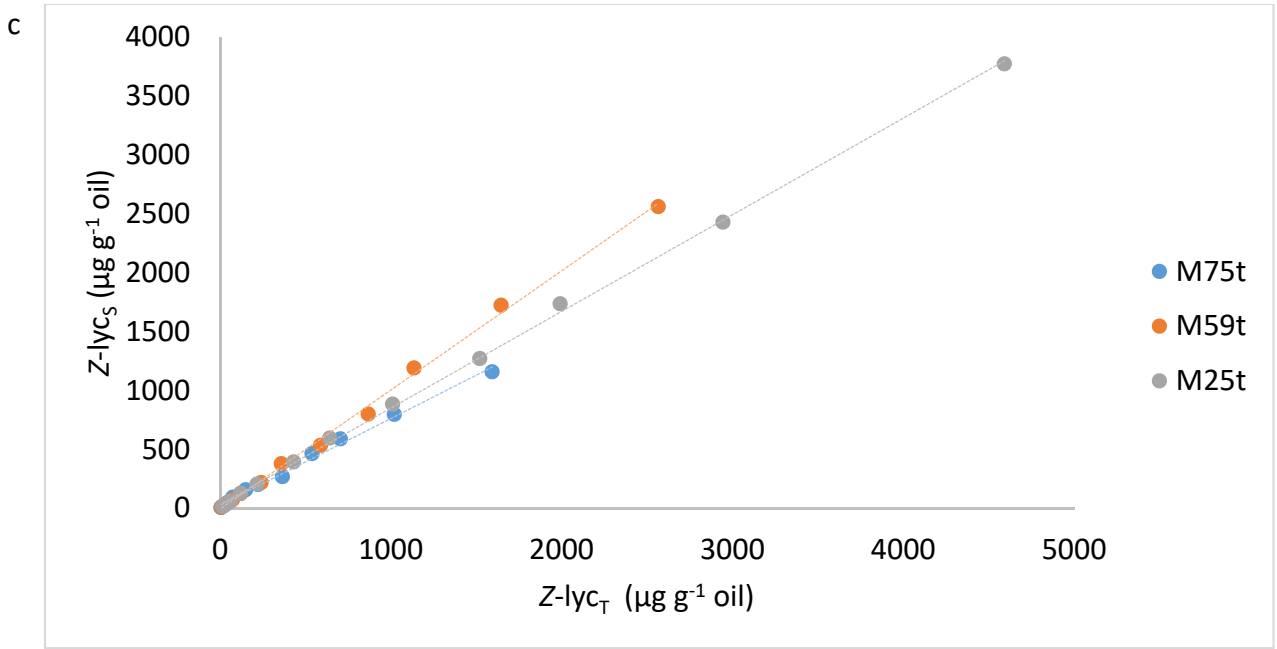


Fig. 2

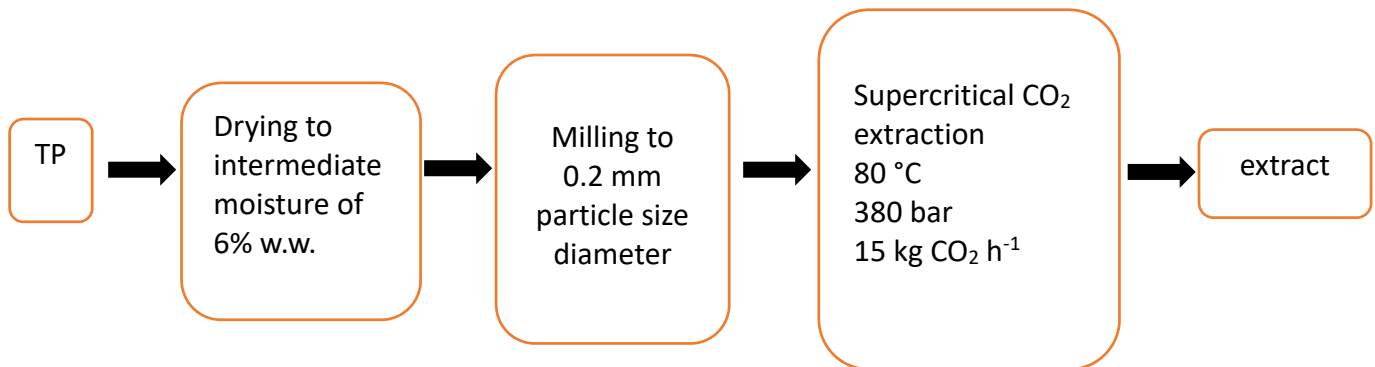
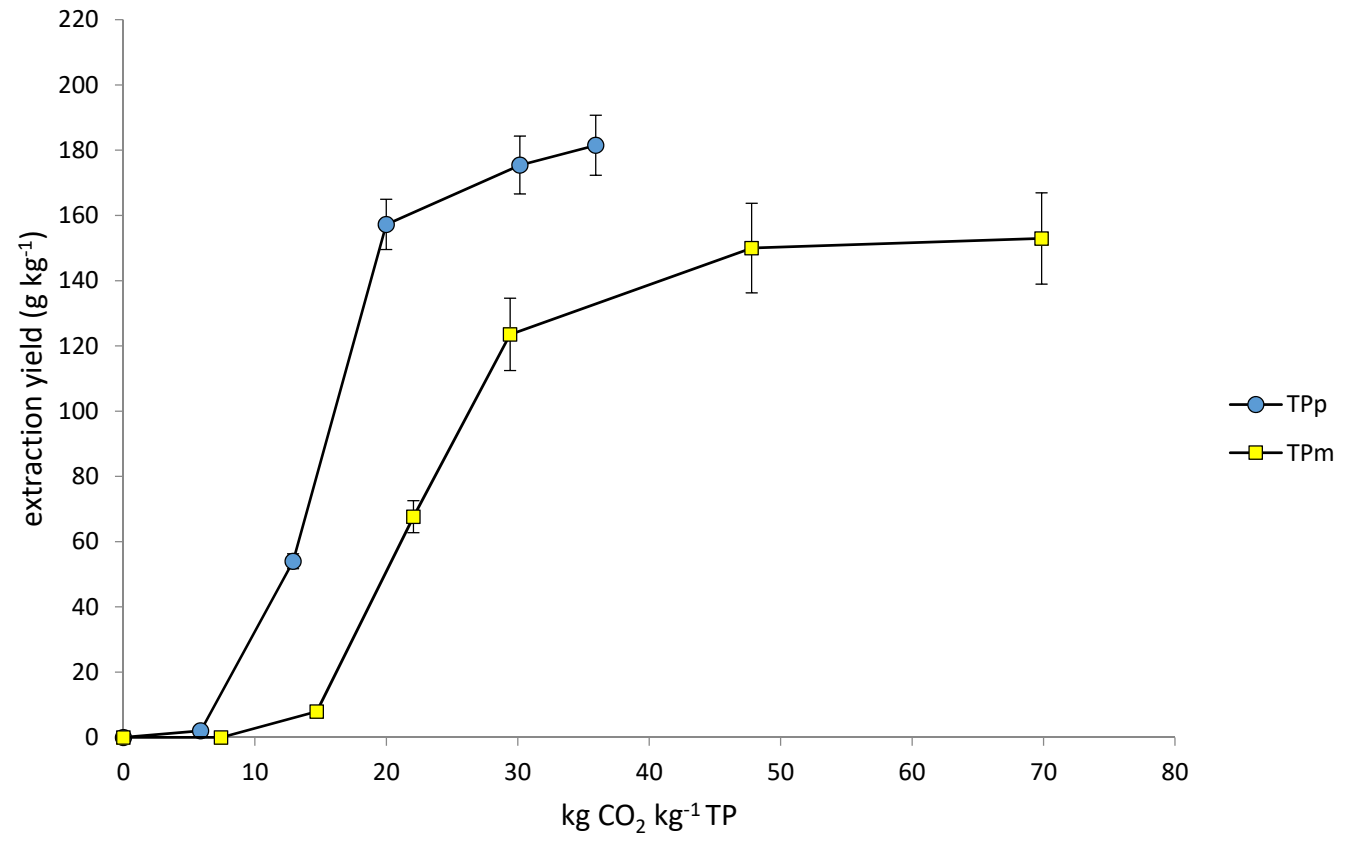
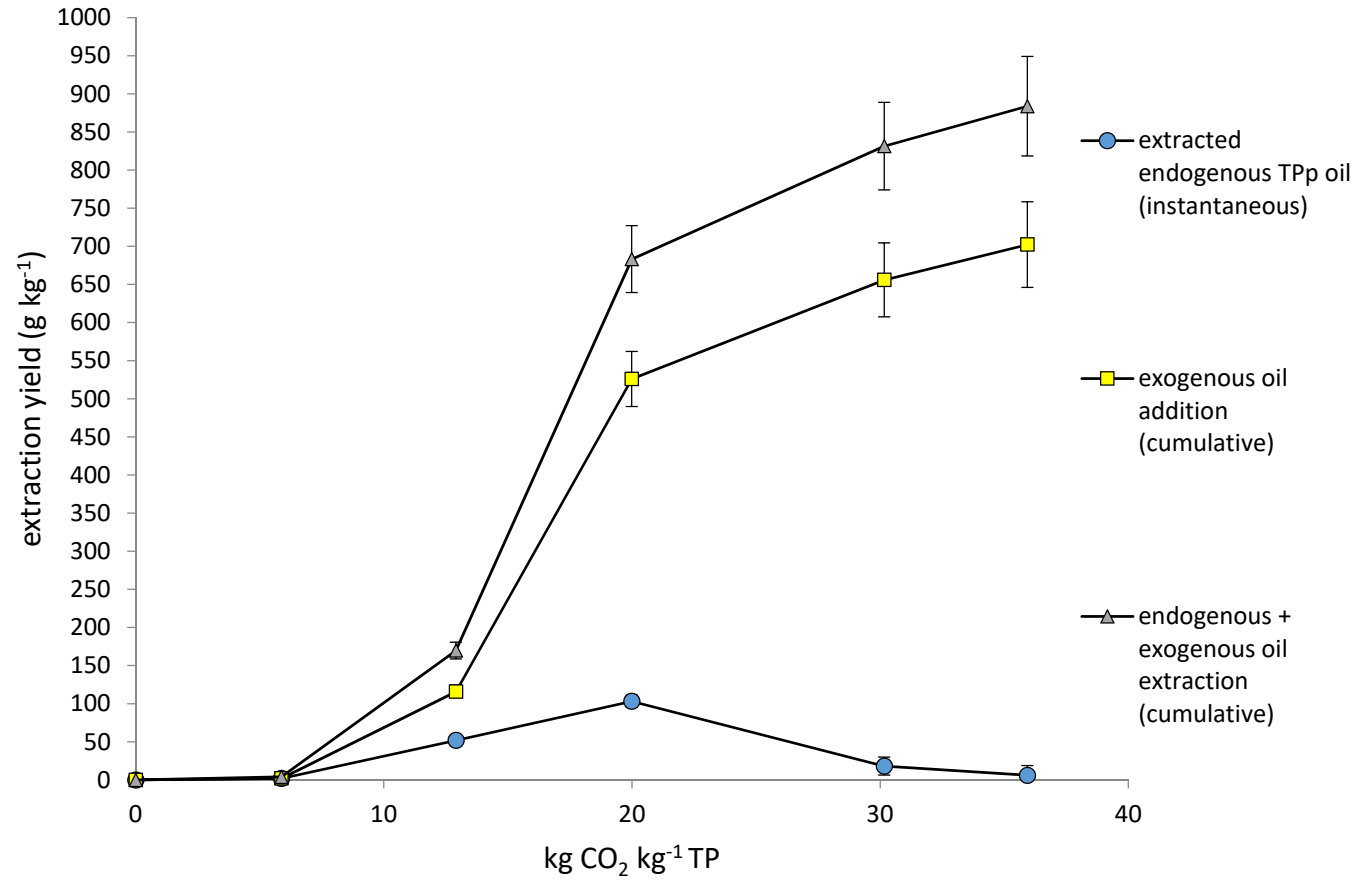


Fig. 3

a



b



SUPPLEMENTARY MATERIAL

Supercritical CO₂ extraction of tomato pomace: evaluation of the solubility lycopene in tomato oil as limiting factor of the process performance

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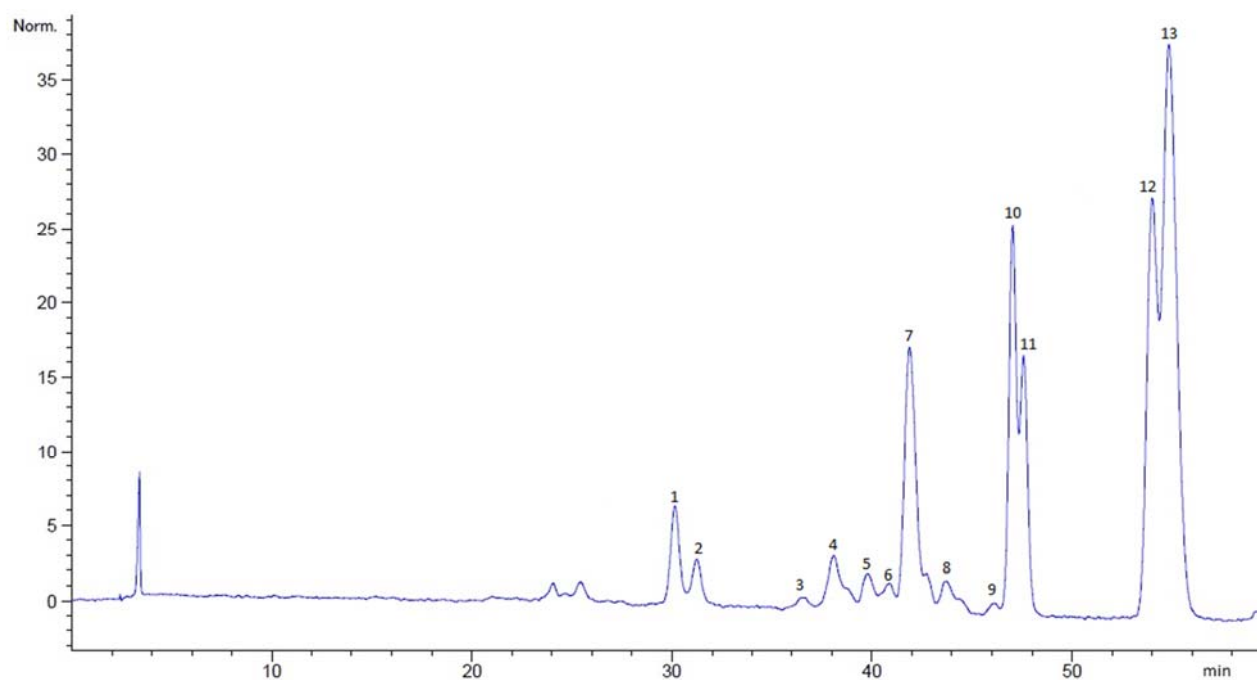


Fig. SM-1. Chromatogram of the M25T 2665 $\mu\text{g g}^{-1}$ oil LycT dose. 1: (9, 13'Z)-lyc; 2: unidentified Z-lyc; 3: (5, 13 Z)-lyc; 4: (15Z)-lyc; 5: unidentified Z-lyc; 6: (13Z)-lyc; 7: unidentified Z-lyc; 8: (5, 13Z)-lyc; 9: (5, 9Z)-lyc; 10: (9Z)-lyc; 11: (7Z)-lyc; 12: (all-E)-lyc; 13: (5Z)-lyc

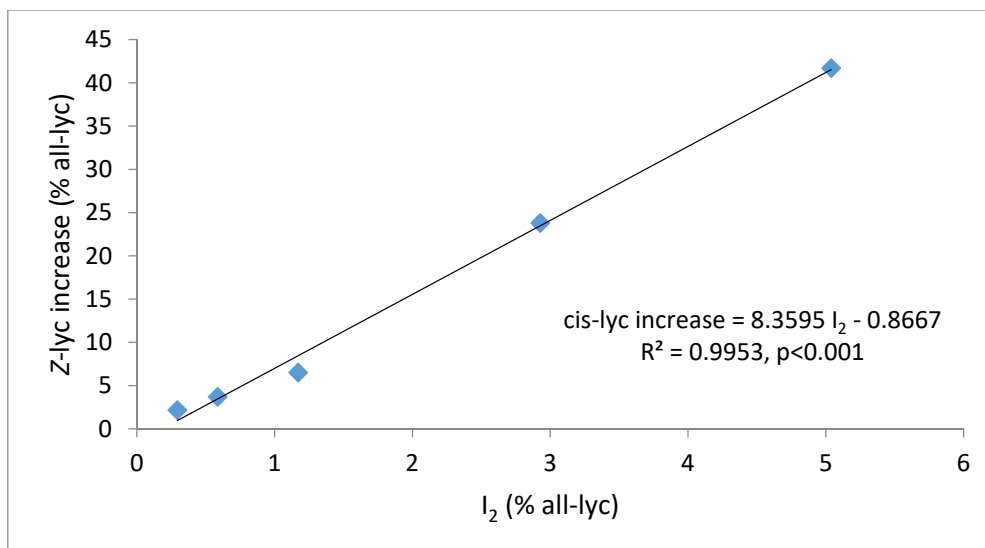


Fig. SM-2. Isomerization effect of I₂ addition to lyc-enriched tomato seed oil (p < 0.05)

Table SM- 1. *Total*-, (*all-E*)-, *Z*-lyc content of the lyc-enriched oil solutions

	M75t		M59t		M25t	
	lycT	lycS	lycT	lycS	lycT	lycS
	22	25±1	22	24±1	21	22±1
	45	47±4	45	44±2	40	38±1
	89	90±3	89	90±3	81	77±1
	179	194±0	179	173±7	161	171±4
	297	328±1	297	285±16	291	273±12
<i>total-lyc</i>	589	508±6	589	484±6	577	507±3
	877	561±1	877	738±22	859	730±24
	1440	683±2	1440	970±10	1353	1081±38
	2120	821±2	2120	1332±7	2035	1556±217
	2776	891±13	2776	1831±9	2665	2129±17
	4021	1146±10	4021	2584±4	3941	2980±2
	6270	1587±17	6270	3752±63	6144	4614±39
	17	18±1	13	14±1	5	5±1
	33	35±4	26	26±1	10	10±1
	67	67±3	53	53±2	20	19±1
	133	144±1	105	100±4	41	43±1
	221	233±1	175	160±6	73	69±3
<i>(all-E)-lyc</i>	439	351±6	348	266±1	146	114±5
	654	358±1	518	359±8	217	135±15
	1074	415±2	850	435±4	342	197±9
	1582	356±2	1252	532±2	514	285±1
	2071	302±13	1640	641±6	673	395±1
	3000	351±10	2375	861±28	995	551±6
	4677	429±11	3703	1193±38	1552	844±5
	6	6±1	9	10±1	15	16±1
	11	12±2	18	18±1	30	28±1
	23	23±1	37	38±1	60	58±1
	45	50±1	73	73±3	120	127±3
	75	95±1	121	125±10	217	204±9
<i>Z-lyc</i>	150	157±1	241	218±6	431	393±5
	223	202±1	359	379±14	642	596±39
	366	268±2	589	535±6	1011	884±46
	539	464±1	868	800±5	1521	1271±215
	705	589±6	1136	1190±14	1992	1735±17
	1021	796±1	1646	1723±32	2945	2428±4
	1593	1158±5	2566	2559±25	4592	3770±45

Table SM- 2. TPm and TPp SFE_CO₂ Z-lyc isomeric composition and comparison with that of M59t obtained performing isomerization with I₂

lyc isomer	TPp extract	TPm extract	TPm and TPp extracts average	M59t dose
			$\mu\text{g g}^{-1}$ oil	
<i>total-lyc</i>	1339±32	1313±18	1326±18	1391
(all- <i>E</i>)-lyc	744±16.2	636±16.4	690±76	509±3
9,13'-Z-lyc	85.8±1	80.4±0.7	83.1±4	68±3
Unidentified Z-lyc 1 (RT=31.3 min)	23.4±1.1	21.5±6.8	22.5±1	17±1
5,13'-Z-lyc	16.6±1.4	34.4±4.3	25.5±13	18±5
15-Z-lyc	21.8±3.6	21.9±5.1	21.9±0	24±0
Unidentified Z-lyc 2 (RT=40.6 min)	17.7±2.3	24.4±1.6	21.1±5	10±1
13-Z-lyc	163±5	170±5	167±5	199±13
Unidentified Z-lyc 3 (RT=43.6 min)	11.3±0.2	19.4±1	15.4±6	9±0
5,13-Z-lyc	11.6±1.3	15.7±0.4	13.6±3	7±0
5,9-Z-lyc	7.4±0.5	5.4±0.5	6.4±1	10±2
9-Z-lyc	103 ±11.2	143±2.4	123±27	134±5
7-Z-lyc	14.2±4.7	21.0±0.6	17.6±5	57±3
5-Z-lyc	119 ±14.1	120±6	120±1	329±7