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# EVALUATION OF HYDROPHILIC AND LIPOPHILIC ANTIOXIDANT CAPACITY IN SPANISH TOMATO PASTE. USEFULNESS OF FRONT-FACE TOTAL FLUORESCENCE SIGNAL COMBINED WITH PARAFAC

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**Abstract**

The hydrophilic and lipophilic antioxidant activities due to the main bioactive components present in Spanish tomato paste samples were studied, using standardized and fluorescent methods. After extraction, phenolic antioxidants (Folin-Ciocalteu method) and total antioxidant activity (TEAC assay) were evaluated, examining differences between hydrophilic and lipophilic extracts corresponding to different samples. Total fluorescence spectra of extracts (excitation-emission matrices, EEMs) were recorded in the front-face mode at two different ranges: 210-300 nm/ 310-390 nm, and 295-350 nm/380-480 nm, for excitation and emission, respectively, in the hydrophilic extracts. In the lipophilic extracts, the first range was 230-283 nm/290-340 nm, while the second range was 315-383 nm/390-500 nm for excitation and emission, respectively. EEMs from a set of 22 samples were analyzed by the second-order multivariate technique Parallel Factor Analysis (PARAFAC). Tentative assignation of the different components to the various fluorophores of tomato was tried, based on literature. Correlation between the antioxidant activity and score values retrieved for different components in PARAFAC model was obtained. The possibility of using EEMs-PARAFAC to evaluate antioxidant activity of hydrophilic and lipophilic compounds in these samples was examined, obtaining good results in accordance with the Folin-Ciocalteu and TEAC assays.

**Keywords:** tomatoes; lipophilic and hydrophilic antioxidant activities; Folin-Ciocalteu and TEAC assays; front-face fluorescence; excitation-emission matrices-PARAFAC.

# Introduction

As part of Mediterranean diet, tomato is one of the most consumed vegetables in the world, as fresh fruits in salads, various culinary preparations, juices, or processed in the form of purees, concentrates, condiments, and sauces. As been demonstrated in a great number of studies, Mediterranean diet presents health benefits (Sofi et al. 2010; Trichopoulou et al. 2014).

According to the FAO: "tomato is the second most important vegetable crop next to potato." According to the data recorded by this organization, the world production tomatoes has been 182.256.458 tons in 2018 (http://www.fao.org/faostat/en/#data/QC/visualize), being Spain the seventh producer with 4768595.

The consideration of tomato as a functional food has been examined (Canene-Adams et al. 2005). Tomatoes are basically water and have a low caloric power given their low fat and dry matter content, sugars constitute the bulk of soluble solids. However, many tomato products are good sources of potassium and folate, similarly with other popular vegetables, and tomato products are a superior source of α-tocopherol and vitamin C, whereas only carrots, between the other regularly consumed vegetables, are a better source of vitamin A than tomato-based foods. Also, tomatoes contain valuable phytochemicals, including carotenoids, mainly lycopene, β-carotene, phytoene, and phytofluene, and polyphenols as the conjugated forms of quercetin and kaempferol. Health effects derived from tomato components could also be due not only to these bioactive compounds but also to their metabolic products.

The antioxidant capacity of tomatoes can be mainly attributed to some of these nutrients, such us, lycopene, ascorbic acid, and phenolic compounds (Sahlin et al. 2004). These antioxidants compounds can be classified as hydrophilic or lipophilic, being differentiated the lipophilic (LAA) and hydrophilic antioxidant activity (HAA). Carotenoids, especially lycopene and β-carotene as well as vitamin E (α- and γ-tocopherol) are the main lipophilic antioxidants, whereas in the hydrophilic fraction, polyphenolics (flavonoids – quercetin, kaempferol and naringenin, and phenolic acid –

26 caffeic, chlorogenic, ferulic and p-coumaric acids), together with ascorbic acid can be found
27 (Savatović et al. 2012).

28 Some of the factors influencing in the total amount of the antioxidant of tomato activities, such us,
29 the different fractions of skin, pulp or seeds (Toor and Savage 2005), genotype of tomatoes (George
30 et al. 2004), production and processing stages (Capanoglu et al. 2010; Gümüşay et al. 2015; Wu et
31 al. 2004) and so on, have been examined in the case of processed foods from this vegetable. This
32 way, different studies have been performed on the influence of the different stages of production of
33 tomato paste over its content in some antioxidants (Capanoglu et al. 2008; Koh et al. 2012)

34 Due the great interest in these results, it is easily understood the needing for quick and easy analytical
35 methods that allow the determination of each antioxidant compound, a set of them or the evaluation
36 of HAA and LAA.

37 In the last case, different assays have been proposed based on different action modes: hydrogen atom
38 transfer (HAT) and single electron transfer (SET) assays (Moharram and Youssef 2014). Thus, the
39 modified method (Prior et al. 2003) using the ABTS (2,2´-azino bis (3-ethylbenzothiazoline-6-
40 sulfonic acid) diammonium salt) radical decolorization assay (Miller and Rice-Evans 1997) was used
41 to separate the hydrophilic and lipophilic extracts of different finely ground freeze-dried fractions of
42 tomatoes. In the assay for lipophilic and hydrophilic antioxidant capacities using the oxygen radical
43 absorbance capacity ($ORAC_{FL}$) with fluorescein as the fluorescent probe and 2,2´-azobis(2-
44 amidinopropane) dihydrochloride as a peroxyl radical generator (Prior et al. 2003) on over 100
45 different kinds of foods, including fruits, vegetables (as tomatoes), nuts, dried fruits, spices, cereals,
46 infant, and other foods, samples were initially extracted with 1:1 hexane/dichloromethane followed
47 by acetone/water/acetic acid (70:29.5:0.5).

48 In another electron transfer based method (Zanfini et al. 2017) fresh tomato sample was extracted
49 with $CH_2Cl_2$ for the determination of lipophilic antioxidant activity (LAA). The residue was extracted
50 with 60% methanol in water. In the assay proposed by (García-Alonso et al. 2015), tomato lipo- and
51 hydrophilic extracts from a commercially available tomato concentrate were prepared extracting with

52 hexane/water (25/2) or with water, respectively. In a revision of the methods available for the
53 measurement of antioxidant capacity in foods and dietary supplements (Prior et al. 2005), a
54 comparison of methods based upon factors as simplicity, instrumentation required, whether the assay
55 is adaptable to measure HAA and LAA, between others, is included. The authors found that the
56 ORAC method, based on HAT mechanism, and the Trolox equivalent antioxidant capacity (TEAC)
57 assay, based on SET mechanism are the more adaptable to measure lipophilic and hydrophilic
58 antioxidants

59 On the other hand, it must be highlighted that hydrophilic AA measured by $ORAC_{FL}$ method has been
60 found to be around ten times higher than lipophilic AA (Wu et al. 2004) and some compounds
61 included in the hydrophilic extract are fluorescent.

62 These traditional assays are not the only utilized, but methods of antioxidant capacity evaluation
63 include spectroscopy, chromatography and electrochemical techniques (Pisoschi et al. 2016; Pisoschi
64 and Negulescu 2012). These alternative assays try to reduce the consumption of solvent and standards
65 compared to the traditional assays, which are expensive, time-consuming, and laborious.

66 Nowadays, fluorescence spectroscopy is being of great interest for scientific community. Some
67 reviews found in the literature show the use of fluorescence techniques in different kinds of foods
68 (Hassoun et al. 2019; Lei and Sun 2019; Shaikh and O'Donnell 2017).

69 In the case of tomatoes samples, there are not many studies in the literature about the use of excitation-
70 emission fluorescence matrices (EEMs) in combination with multivariate modeling to extract relevant
71 information. The study performed by Orzel et al. focused in the use of excitation–emission
72 fluorescence obtained from tomato pastes and water extracts of them for the evaluation of their
73 hydrophilic antioxidant properties (Orzel et al. 2015). These signals, as well as IR spectra, were
74 analyzed with chemometrics tools, as partial least-squares regression (PLSR) and its N-way variant,
75 to predict the total antioxidant capacity (TAC) or total phenolic content (TPC) of the samples,
76 estimated by ORAC assay and the Folin–Ciocalteu (F-C) reagent, respectively. A PLSR model was
77 built using a set of a few new variables that maximize the covariance between the dependent variable

78 (TAC or TPC) and the explanatory variables (e.g., a collection of spectra). These explanatory
79 variables can be arranged in a matrix form whether they represent a simple IR or UV–vis spectra or
80 unfolded EEMs. The N-way partial least-squares regression can be regarded as an extension of two-
81 way PLSR to model three-or higher-way data.

82 The aim of this work was to explore the possibilities of using total fluorescence signals to evaluate
83 the antioxidant activity of tomato paste, as an alternative to the established methods which are, in
84 general, tedious and, time and reagents consuming. Specifically, the use of excitation-emission
85 fluorescence matrices (EEMs) to examine different extracts from these samples, which correspond to
86 hydrophilic and lipophilic antioxidant activity. This would allow us to investigate the nature of
87 fluorescent compounds presents in these extracts of tomato paste, by previously constructing a
88 parallel factor analysis (PARAFAC) model to distinguish between the possible components in these
89 signals, and the analysis of the extracts using standardized methods, as the Folin-Ciocalteu and TEAC
90 assays.

91 # Materials and methods

92 **Chemicals and standards**

93 Acetone, acetic acid, sodium carbonate anhydrous, Folin Ciocalteu reagent and ethanol were
94 purchased from Panreac (Barcelona, Spain), while isohexane was provided by VWR Chemicals
95 (Barcelona, Spain) and potassium persulfate from Probus (Barcelona, Spain). Gallic acid, ABTS (2´2-
96 azino-bis [3-ethylbenzothiazoline-6-sulfonic] acid) and Trolox (6 hydroxy-2,5,7,8-trimethyl-
97 chroman-2-carboxylic acid) were obtained from Sigma-Aldrich Química (Madrid, Spain). $ABTS^{\bullet+}$
98 radical was prepared by adding of $K_2S_2O_8$ (88µL) to ABTS solution (7mM, 25mL), storing at low
99 temperature in the dark. For all preparations Milli-Q water, obtained by MilliQ-Water system
100 (Millipore S.A.S, Francia), was used.

101 **Samples**

102 Samples of tomato paste (a total of 22) were obtained from *Centro Tecnológico Nacional*
103 *Agroalimentario "Extremadura" – CTAEX.* These were prepared from tomatoes from different
104 producers in Extremadura, Spain (characteristics in Table S1), submitted to different treatments until
105 obtaining the tomato paste, as seen in the preparation process shown in Fig. 1. These tomatoes were
106 subjected to "Hot-Break" enzymatic inactivation after previous processes of washing, selection and
107 cutting of the raw material. Skins and seeds were removed by sifter and refiners to finally obtain the
108 tomato concentrate after the evaporation and pasteurization processes. These tomato pastes were
109 stored frozen until preparation of the extracts to prevent their degradation.

110 **Extraction process for separation of hydrophilic and lipophilic extracts**

111 All the samples were subjected to a pre-treatment with the aim to separate the hydrophilic and
112 lipophilic components present in the tomato paste. A modified extraction method from Toor and
113 Savage (2005) was used to separate the hydrophilic and lipophilic fractions of the tomato paste. In
114 brief, accurately weighed aliquots of 0.5 g of previously defrosted tomato paste were extracted twice
115 with 10.0 mL of isohexane by shaking each time for 10 min in a vortex, followed by centrifugation
116 at 3000 rpm for 10 min. The extracts were pooled, mixed well, and stored in 2 aliquots of 10.0 mL at
117 low temperature.

118 Once the lipophilic fraction was separated, the solid residue was used for the extraction of the
119 hydrophilic compounds, after drying under nitrogen flow to eliminate the remaining isohexane
120 present. This residue was extracted with 10.0 mL of a mixture of acetone:water:acetic acid,
121 (70:29.5:0.5) by shaking in a vortex and sonicated for 10 min to completely dissolve the hydrophilic
122 components, followed by centrifugation at 3000 rpm for 10 min. The supernatant (hydrophilic extract)
123 was then transferred to two tubes in 2 aliquots of 5.0 mL for their conservation at low temperature.
124 In both extracts, the determination of polyphenolic compounds was carried out by the Folin-Ciocalteu
125 method, and the antioxidant activity was studied by the TEAC assay.

126 On the other hand, the hydrophilic and lipophilic antioxidant activity of the different extracts from
127 tomato paste was evaluated by front-face total fluorescence signal, obtaining the excitation-emission
128 matrices (EEMs). The lipophilic EEMs were obtained directly from the same tomato lipophilic extract
129 already obtained, without previous treatment of them. However, recording EEMs directly in
130 hydrophilic gave bad results, due to the acetone absorbs all the incident radiation on the sample. For
131 this reason, other hydrophilic extracts were prepared using Milli-Q water as extracting agent
132 according to the slightly modified García-Alonso et al. method (2015), as follow: magnetic stirring
133 of 1 g tomato paste in 10.0 mL distilled water for 7 min. Then, the extract was filtered 0.2 μm pore
134 size syringe filter and stored at -4 ºC until analyzed.

135 **Folin-Ciocalteu method**

136 Total antioxidant activity of the polyphenols in the hydrophilic and lipophilic extracts of the tomato
137 fractions were measured by the method, adapted from Toor and Savage (2005), based on a redox
138 reaction between polyphenols and a mixture of Mo(VI) and W(VI) in which lower oxidation states
139 of these metals are obtained. Gallic acid was used as a standard, and the antioxidant activity were
140 expressed as gallic acid equivalents (GAE) per 100 g of tomato paste.

141 The influence of gallic acid concentration was examined between 1.0 mg/L and 25.0 mg/L to find the
142 linear interval of the calibration plot, and then standards between 2.52 mg/L and 15.20 mg/L were
143 utilized to adjust the calibration parameters. The standards were prepared, in triplicate, in 25 mL
144 flasks, adding the corresponding volumes of gallic acid stock solution (100.0 or 1000.0 mg/L). These
145 volumes were diluted with 10 mL of $H_2O$ Milli-Q in 25.0 mL volumetric flask and then treated with
146 0.25 mL of the Folin-Ciocalteu reagent. These solutions were kept in the dark at room temperature
147 for 10 minutes, the time required to complete the oxidation reaction. Subsequently, the mixture was
148 neutralized by adding 2.5 mL of $Na_2CO_3$ (7.5% w/v) and diluted with $H_2O$ Milli-Q to the mark. The
149 analytical signal (absorbance signal at 662 nm) was taken 9 hours after preparation of the samples.

150 When the Folin-Ciocalteu method was applied to hydrophilic extract, 1.5 mL of this was appropriately
151 diluted with 10 mL of $H_2O$ Milli-Q in 25.0 mL volumetric flask, following as described above.

152 On the other hand, the lipophilic extract was prepared by drying a known volume (1.5 mL) of the
153 isohexane extract under nitrogen flow directly in the flask. Subsequently, 2.0 mL of acetone and $H_2O$
154 are added until a volume of 10.0 mL to continue with the same procedure as in the hydrophilic
155 extracts.

156 **TEAC assay (Trolox equivalent antioxidant capacity)**

157 The antioxidant activity of the hydrophilic and lipophilic extracts of the tomato fractions was
158 measured using ABTS (2´2-azino-bis [3-ethylbenzothiazoline-6-sulfonic] acid) radical
159 decolorization assay (Ramírez Anaya 2013). This method consists of an electron transfer reaction
160 (SET), in which the ability of the sample to capture free radicals is measured by the
161 spectrophotometric monitoring at 749 nm of the $ABTS^{\bullet+}$ radical discoloration. Therefore, it is based
162 on the ability of an antioxidant to stabilize the $ABTS^{\bullet+}$ colored cation radical, inhibiting the chain
163 reaction that leads to oxidation. The antioxidant activity was expressed as equivalents of Trolox
164 (μmol Trolox/g of sample).

165 The validation by the TEAC method, using Trolox as internal standard, was carried out by preparing
166 in triplicate seven Trolox standards with concentrations between 0.025 and 0.50 mM. These standards
167 were prepared in 10.0 mL volumetric flasks, adding the corresponding volumes of the standard
168 solution (5.00 mM), and following the above-mentioned procedure. In brief, volumes of 150 μL of
169 the corresponding standard solution were mixed with 3 mL of the diluted $ABTS^{\bullet+}$ solution and the
170 absorption spectra (300 - 900 nm) of each of the standard solutions was recorded at the beginning and
171 30 min after starting the reaction, when the equilibrium state is reached, using ultrapure water to
172 obtain the baseline. The absorbance value at 749 nm was measured at the beginning ($A_0$) and after
173 reaching the equilibrium ($A_1$), and the $ABTS^{\bullet+}$ radical elimination was obtained according to

174 $$\boldsymbol{ABTS^{\bullet+} radical\ elimination = (A_0 - A_1)/A_0}$$

175 The lipophilic and hydrophilic extracts of the tomato paste samples were also analyzed separately. In
176 brief, 500 μL of the liquid extract (mixture of acetone:water:acetic acid (70:29.5:0.5)) of the

177 hydrophilic samples were dried under nitrogen flow to eliminate completely the acetone, and,
178 subsequently, 100.0 μL of ethanol and 3 mL of the diluted ethanol solution of the $ABTS^{•+}$ radical
179 (5:100) were added to the aliquots of 50.0 μL of the different samples. The discoloration due to the
180 cation reduction reaction by the antioxidants in the sample was measured 30 minutes after the start.
181 All assays have been carried out with ethanol, as the $ABTS^{•+}$ radical and the polar antioxidants are
182 soluble in this solvent (Romero et al. 2002).

183 The same procedure has been followed for the lipophilic extracts but, in this case, given the lower
184 concentration of antioxidants, volumes of 3 mL of the diluted solution of $ABTS^{•+}$ radical were added
185 to 150 μL of the extracts, continuing as described above.

## 186 Instrumentation and software

187 To obtain fluorescence EEMs, a Fluorescence Spectrophotometer Varian Model Cary connected to a
188 PC microcomputer via an IEEE 488 (GPIB) serial interface Eclipse was employed, and the Cary
189 Eclipse 1.0 software was used for data acquisitions. A 1.0-cm quartz cell was used to carry out the
190 measurements at front-face fluorescence mode, utilizing a variable-angle front-face accessory,
191 looking for reflected light, scattered radiation, and depolarization phenomena were minimized. Angle
192 of incidence, defined as the angle between the excitation beam and the perpendicular to the cell
193 surface, was set at 34º. The slits of excitation and emission monochromators were set at 5 nm. EEMs
194 were collected obtaining successive emission spectra (with a resolution of 1 nm), varying the
195 excitation wavelength (with a resolution of 3 nm). Two different ranges were recorded (Table 1).

196

197 The data were arranged in 3D array with dimensions MxNxP (samples x number of wavelengths
198 emission x number of wavelengths excitation) in order to apply Parallel Factor Analysis (PARAFAC)
199 (Bro, 1997). PARAFAC was applied in Matlab (Matlab R2007b, version 7.5.0.342), using MVC2, a
200 graphic interface available at http://www.iquir-conicet.gov.ar/descargas/mvc2.rar (Olivieri et al.
201 2009; Olivieri and Escandar 2014). To model the set of fluorescence data by PARAFAC, different
202 number of components must be assayed and the optimum selected. Given that concentrations and

203 spectral values are always positive, non-negative constraints for the resolved profiles for all modes
204 were applied. ACOC program was used to obtain the figures of merit. (Espinosa-Mansilla et al. 2005)

# 205 Results and discussion

206 As mentioned in the previous section, samples of tomato paste were obtained from tomatoes of
207 different producers, and they were stored frozen until preparation of the extract to prevent their
208 deterioration. All the samples were subjected to a pre-treatment with the aim to separate the
209 hydrophilic and lipophilic components present in the tomato paste. In brief, hydrophilic and lipophilic
210 extracts from 22 tomato paste samples were analysed, after validation of the spectrophotometric
211 methods used.

## 212 Measurement of the antioxidant activity in hydrophilic and lipophilic extracts of tomato 213 paste samples.

214 For the determination of the antioxidant capacity in the different extracts of the tomato paste samples,
215 gallic acid was used as standard for obtaining the calibration plot in the F-C method and a
216 hydrosoluble analogue of vitamin C, Trolox, to carry out the TEAC assay (Pérez-Jiménez et al.,
217 2008).

### 218 Analysis of samples using the Folin-Ciocalteu method

219 Calibration results for the Folin-Ciocalteau method used in this study are shown in the supplementary
220 information (Table S2). Reagents need to be added in the order mentioned in Materials and methods,
221 for the redox reaction takes place with a color change from yellow to blue when the pH changes to
222 basic medium. The absorption spectrum (400 - 800 nm) of each of the standard solutions was
223 recorded, showing a shift of $\lambda_{max}$ to lower values as the gallic acid concentration increases
224 (hypsochromic shift), although the absorption band is so broad that this does not implies error. Finally,
225 the absorbance was measured at 662 nm. The stability of the signal was examined, during 48 hours
226 in which samples were kept in darkness, concluding that it can be taken 9 hours after preparation of
227 the samples.

228 The 22 samples of hydrophilic and lipophilic extracts were analyzed following this procedure. These
229 results were expressed in mg GAE/100 g of tomato paste and are shown in Table S4 for the
230 hydrophilic extracts and in Table S5 for the lipophilic ones.

231 The results obtained for hydrophilic and lipophilic extracts from tomato paste showed no very
232 different values among the samples. Fig. 2A and 2B show the total polyphenol for hydrophilic and
233 lipophilic extracts, respectively. In hydrophilic extracts (Fig. 2A) ranges were from 273.9 to 173.4
234 mg GAE/100 g, being the sample T.85 with the highest level of total polyphenols and T.78 the lowest.
235 However, in lipophilic samples (Fig. 2B) the value ranges between 76.8 and 38.2 mg GAE/100 g,
236 being the maximum value for sample T.76 and the minimum for T.103. No correlation has been found
237 between polyphenols content in the hydrophilic and lipophilic extracts of the different samples. The
238 values of total polyphenols by the Folin-Ciocalteu method are much higher in hydrophilic extracts
239 than in lipophilic extracts, due to the higher solubility of polyphenolic compounds in a polar
240 environment (acetone: water: acetic acid) as compared with non-polar one (isohexane). On the other
241 hand, carotenoid compounds were found mainly in lipophilic extracts. Other authors studied the
242 content of total polyphenols without considering the different hydrophilic and lipophilic extracts,
243 obtaining very low values of the amount of total polyphenols (Vallverdú-Queralt et al. 2011; Wu et
244 al. 2004). Toor and Savage (2005), studied both fractions classifying the content according to the
245 different parts present in tomato, showing lower values of the amount of total polyphenols than those
246 obtained in tomato paste sample. The main difference between both types of samples is the amount
247 of water present, with a lower amount in the tomato paste, which implies a higher concentration of
248 the rest of the components.

249 **Analysis of samples using the TEAC assay**

250 Calibration results for this method are shown in the Table S3. The 22 tomato pastes were analyzed
251 following the procedure described in the Materials and methods section. The results of antioxidant
252 activity were calculated through the Trolox calibration plot, using the absorbance as analytical signal
253 expressed as parts per unit of $ABTS^{\bullet+}$ radical elimination. These results are presented in the Fig. 3A

254 and 3B for the hydrophilic and lipophilic extracts, respectively. The antioxidant activity in
255 hydrophilic extracts ranges from 61.1 to 13.7 µmol Trolox/g, showing the highest antioxidant activity
256 for the sample T.124 and the lowest for T.108 (Table S6). However, in lipophilic samples (Fig. 3B)
257 the value ranges between 97.00 and 9.30 µmol Trolox/g, being the maximum value for the sample
258 T.126 and T.77 the minimum (Table S7). It is remarkable that these results show greater dispersion
259 that those of polyphenols content. Also, it can be highlighted that, in some samples, the antioxidant
260 activity is higher in lipophilic extracts from tomato paste samples. These results did not correspond
261 to those observed by other authors, who determined the antioxidant capacity of different varieties of
262 (Martínez-Valverde et al. 2002), being the pear tomato one of the most studied. Zanfini et al. (2017)
263 studied the antioxidant activity of total hydrophilic (HAA) and lipophilic (LAA) of different pear
264 tomatoes (red, yellow, pale yellow and black tomato fruits), observing that HAA was higher than
265 LAA and that the Shiren type tomatoes (red), with a high carotenoid and total phenolic contents,
266 showed the highest antioxidant activity. Vallverdú-Queralt et al. (2011) only analyzed the antioxidant
267 activity in hydrophilic extracts of crushed tomato samples. Toor and Savage (2005) determined such
268 activity in both extracts for the different parts of the fruit (seed, pulp and skin), ranging in hydrophilic
269 extracts from 0.82 to 1.14 µmol Trolox/g and from 0.07 to 0.19 mg µmol Trolox/g for lipophilic
270 extracts. Also, different studies have been performed on tomato paste samples. Hence, Capanoglu et
271 al. (2008) applied different assays to evaluate hydrophilic and lipophilic antioxidant activities in
272 samples taken from various tomato processing steps, and they found that the TEAC method gives
273 considerably higher values of antioxidant activity in hydrophilic than in lipophilic extract. Koh et al.
274 (2012) also examined the influence of processing on the content of the different antioxidants and
275 found that, in general, this diminish when fresh tomatoes are processed to tomato pastes, being
276 flavonoids contents lower than lipophilic antioxidants (carotene and lycopene) in these last, although
277 ascorbic acid continues being the most abundant of the examined antioxidants. Our results could
278 indicate that the contribution of ascorbic acid to the antioxidant activity of the hydrophilic extracts
279 obtained as described, calculated by the TEAC method applied according to the procedure above
280 detailed, could be low. In these cases, the antioxidant activities of lipophilic extracts, due to
281 carotenoids could be higher than HAA, due to polyphenols, antioxidants mainly present in the

282 hydrophilic extracts (Martí 2018). Nevertheless, the influence of different other factors, such as the
283 preparation of the sample, as well as the origin and variety of the fruit, have to be also in consideration
284 (Lenucci et al. 2006). For example, Jacob et al (2010) found that the effects of thermal processing on
285 the nutritional value of tomato paste differ according to the extension of heating, leading to an
286 enhancement of the phenolic antioxidants of tomatoes, which are responsible for maintaining the
287 antioxidant capacity of processed products after losses of ascorbic acid.

288 **Evaluation of hydrophilic and lipophilic antioxidants of tomato paste by total fluorescence**
289 **combined with PARAFAC**

290 To explore the possibility of using fluorescence spectroscopy as tool to evaluate phenolic antioxidants
291 and total activity, different experiments were assayed. Firstly, front-face fluorescence was selected to
292 collect the excitation – emission matrices (EEMs) due to the inner-filter effect decreases as compared
293 with conventional fluorescence. Also, the best ranges for each kind of extract were selected and they
294 are shown in Table 1.

295 After that, tomato lipophilic extracts were evaluated. Samples were prepared as detailed in the
296 Materials and methods section. and EEMs were obtained in the two different ranges. Fig. 4 shows
297 contour plots corresponding to the EEMs for one lipophilic tomato paste extract. As observed, both
298 regions are quite different. Range 1 shows a wide band and maxima signal at wide band from 280 to
299 315 nm for emission and from 250 to 280 nm for excitation. This region might be related with the
300 anthocyanins and other polyphenols compounds (Lai et al. 2007). Range 2 shows maxima better
301 defined and the fluorescent intensity for this range is higher as well. In this case, maxima for
302 excitation at 350 and 370 for excitation and maxima at 400, 425 and 450 nm for emission were found.
303 These regions might be also related with flavonoids. In both cases, the EEMs suggest a mix of
304 compounds. Although the presence of carotenoids is not ruled out, from studies by Lai et al. (2007)
305 for tomato skin pigment extracts in methanol, no evidences were found of any lycopene fluorescence
306 peak in the recorded EEMs. Other authors were also unable to find any lycopene fluorescence peaks
307 (Konagaya et al. 2020), even when compared a lycopene standard with tomato extract (Adília Lemos
308 et al. 2015).

309 When PARAFAC was applied to the samples in the different ranges, first step was to select the
310 optimal number of components to explain the main variance of data. To select the optimal number of
311 components the core consistency criteria was used (Bro and Kiers 2003). Hence, the core value is
312 evaluated when the number of components increases until, at a certain point, the core consistency
313 value decreases suddenly below 50%, indicating that the optimal component number is the
314 immediately before the one that causes this change. In this case, the optimal number of components
315 was found to be three. The loadings and scores for the different components were obtained and
316 loadings are shown in Fig.5. The color intensity is proportional to score value and different for each
317 of the components, as shown in the legend to the right of each image. As observed, there are not huge
318 differences when the decomposition of samples was performed. In both ranges, first component
319 presents mainly the same shape that the original EEMs.

320 Scores obtained for each component and a combination of them were related with total polyphenols
321 (mg GAE/100g) and antioxidant activity (µmolTrolox/g). Regarding to polyphenols content, better
322 correlation was found in the case of first range, where the sum of scores and total polyphenols,
323 measured as mg GAE/100 g tomato paste, offered a correlation (r) of 0.826. Also, good correlation
324 was found in the second range between the sum of scores and total polyphenols (r = 0.727). These
325 results are in accordance with expected since these signals were attributed mainly to polyphenols
326 content. In accordance with previous studies by other authors, the fluorescence profiles of these
327 components might correspond with the presence of flavonoids (quercetin, catechin, epicatechin…)
328 and anthocyanins (pelargonidine chloride) (Lai et al. 2007; Orzel et al. 2015).

329 In the case of antioxidant activity, only a good correlation was found between score of the second
330 component in the range 2 and the µmol Trolox/g tomato paste (r =0.80). However, this correlation is
331 a bit uncertain due to the large peaks observed for this component. The lipophilic extract is mainly
332 formed by carotenoid compounds (β-carotene, γ-carotene…) (Jurado Capel 2012; Lai et al. 2007),
333 which are more soluble in organic solvents, however, carotenoids do not exhibit intense fluorescence
334 signal. This might explain the low correlation in this range.

335 Otherwise, tomato hydrophilic extracts were evaluated. Samples were prepared as described in the
336 Materials and methods section, and EEMs were collected in two different ranges shown in Fig.6. As
337 observed, in the first range, the main fluorescence signals appear at 220 and 280 nm for excitation
338 and 360 nm for emission. This region might be related with polyphenols as gallic acid among others.
339 This region also presents more intense signal compared with second range. Second range exhibits a
340 maximum signal non-well-defined, as in the first range, around 325/430 nm for excitation/emission,
341 respectively.

342 In this case, PARAFAC was also applied, and the optimal number of components was three in both
343 ranges. Loadings for components in each range are shown in the Fig. 7. Scores were correlated with
344 total polyphenols and antioxidant activity. As for lipophilic extracts, better correlations were found
345 in the case of first range, where the sum of scores for component 1 and 3 and total polyphenols
346 measured as mg GAE/100 g tomato paste offered a correlation (r) of 0.731 while the scores for
347 component 3 and total polyphenols offered a correlation of 0.744. In the second range, also good
348 correlation was found for total polyphenols and scores for first component (r = 0.790). This
349 component presents a similar shape described for flavonoids by other authors (Lai et al. 2007). As
350 expected, these ranges are attributed to total polyphenols, mainly extracted in the hydrophilic extracts.
351 However, in the case of Trolox content (µmol Trolox/g tomato paste), poor correlations were found
352 for all combination of scores values assayed.

353 The obtained results point to the polyphenolic compounds as the main antioxidant compounds
354 responsible of fluorescent signals in both the hydrophilic and lipophilic extracts of tomato paste. It
355 would be interesting to perform a comparative study with the raw tomato utilized to check if there is
356 a loss of antioxidant compounds during preparation of tomato paste samples. Other possibility is that
357 some of the lipophilic antioxidants that could exhibit fluorescence be in a conjugate non-fluorescent
358 form.

## 359 Conclusions

Fluorescence signals to evaluate the hydrophilic and lipophilic antioxidant activity in different extracts of tomato paste, as an alternative to other established methods, were proposed. Good signals from the EEMs of different extracts from paste samples of Spanish tomatoes were obtained with a simple and fast procedure. The evaluation of hydrophilic and lipophilic compounds in tomato samples by front-face fluorescence combined with PARAFAC was performed obtaining good results in accordance with the Folin-Ciocalteu and TEAC assays analysis. The values of phenolic antioxidants were much higher in hydrophilic extracts than in lipophilic extracts, while the antioxidant activity is slightly greater in these last. No correlation was found, in both polyphenols content and antioxidant activity, between the hydrophilic and lipophilic extracts of the different samples. Some antioxidant compound families were tentatively identified considering the literature data, which could be responsible from the signals in the EEMs as shown the correlation between score values of some components and the hydrophilic and lipophilic antioxidant activity measured by the spectrophotometric assays.

**Compliance with Ethical Standards**

**Ethical Approval**

This article does not contain any studies with human participants or animals performed by any of the authors.

**Conflict of Interest**[2], [1], Olga Monago-Maraña[3], [4], [4] and Teresa Galeano-Díaz*[1,2]

Rosario Pardo-Botello declares that she has no conflict of interest.

Fátima Chamizo-Calero declares that she has no conflict of interest.

Olga Monago-Maraña declares that she has no conflict of interest.

Raquel Rodríguez-Corchado declares that she has no conflict of interest.

Rosa de la Torre-Carreras declares that she has no conflict of interest.

Teresa Galeano-Díaz declares that she has no conflict of interest.

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388 **Informed Consent**

389 Not applicable.

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**Table 1.** Instrumental conditions utilized in the recording EEMS

| **Hydrophilic extracts** | | | | |
|---|---|---|---|---|
| | **Excitation (nm) (3 nm steps)** | **Emission (nm) (1 nm steps)** | **Slit (nm)** | **Voltage (V)** |
| **Range 1** | 210 - 300 | 310 - 390 | 5 | 630 |
| **Range 2** | 295 - 350 | 380 - 480 | 5 | 630 |
| **Lipophilic extracts** | | | | |
| | **Excitation (nm) (3 nm steps)** | **Emission (nm) (1 nm steps)** | **Slit (nm)** | **Voltage (V)** |
| **Range 1** | 230 - 283 | 290 – 340 | 5 | 630 |
| **Range 2** | 315 - 385 | 390 - 500 | 5 | 630 |

## Figure Captions

Fig. 1: Scheme for tomato paste preparation process.

Fig. 2. Total polyphenols content for each sample, expressed in mg GAE/100 g of tomato paste in the hydrophilic (A) and lipophilic (B) extracts.

Fig. 3. Antioxidant activity for each sample (TEAC assay), expressed in μmol Trolox/g of tomato paste in the hydrophilic (A) and lipophilic (B) extracts.

Fig. 4. EEMs of a lipophilic extract in the two different ranges examined. Range 1 (left): excitation from 230 to 283 nm and emission from 290 to 340 nm and range 2 (right): excitation from 315 to 383 nm and emission from 390 to 500 nm.

Fig. 5. Contour plots of the different components obtained by PARAFAC decomposition for the group of lipophilic extracts.

Fig. 6. EEMs of an hydrophilic extract in the two different ranges studied. Range 1 (left): excitation from 210 to 300 nm and emission from 310 to 390 nm and range 2 (right): excitation from 295 to 350 nm and emission from 380 to 480 nm.

Fig. 7. Contour plots of the different components obtained by PARAFAC decomposition for the group of hydrophilic extracts.

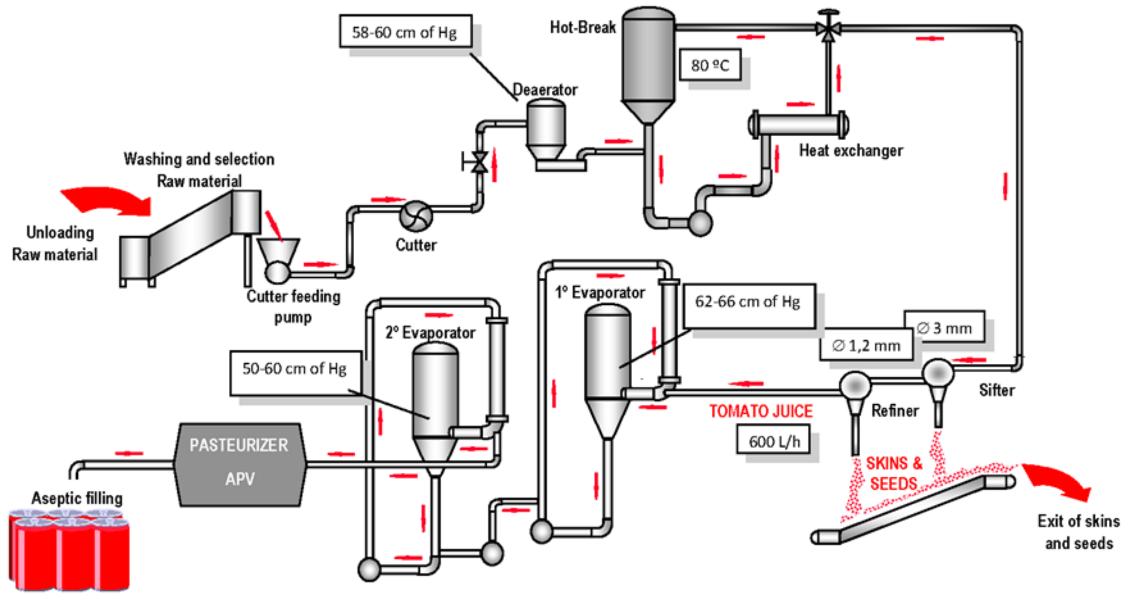


Figure 1

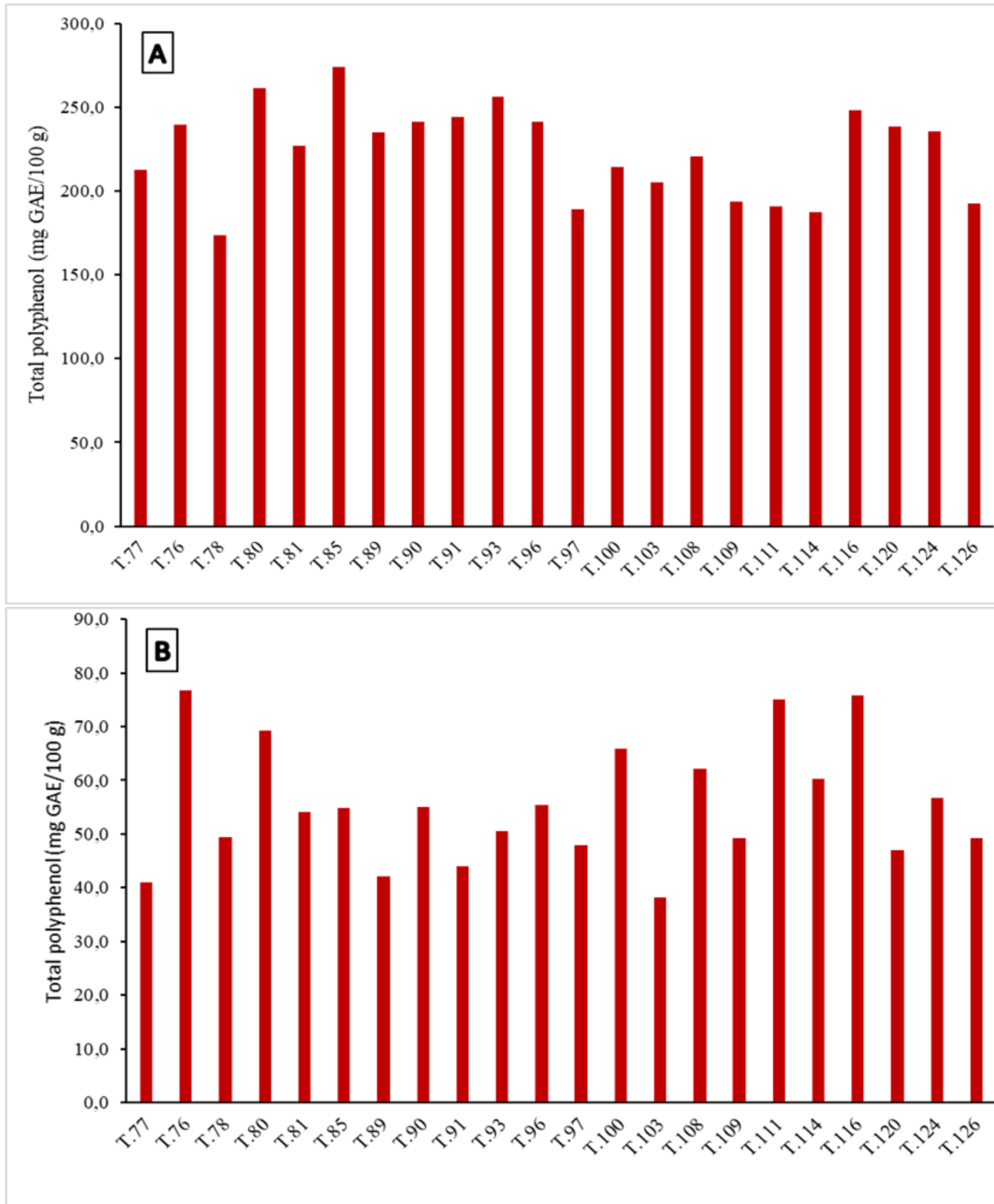


Figure 2

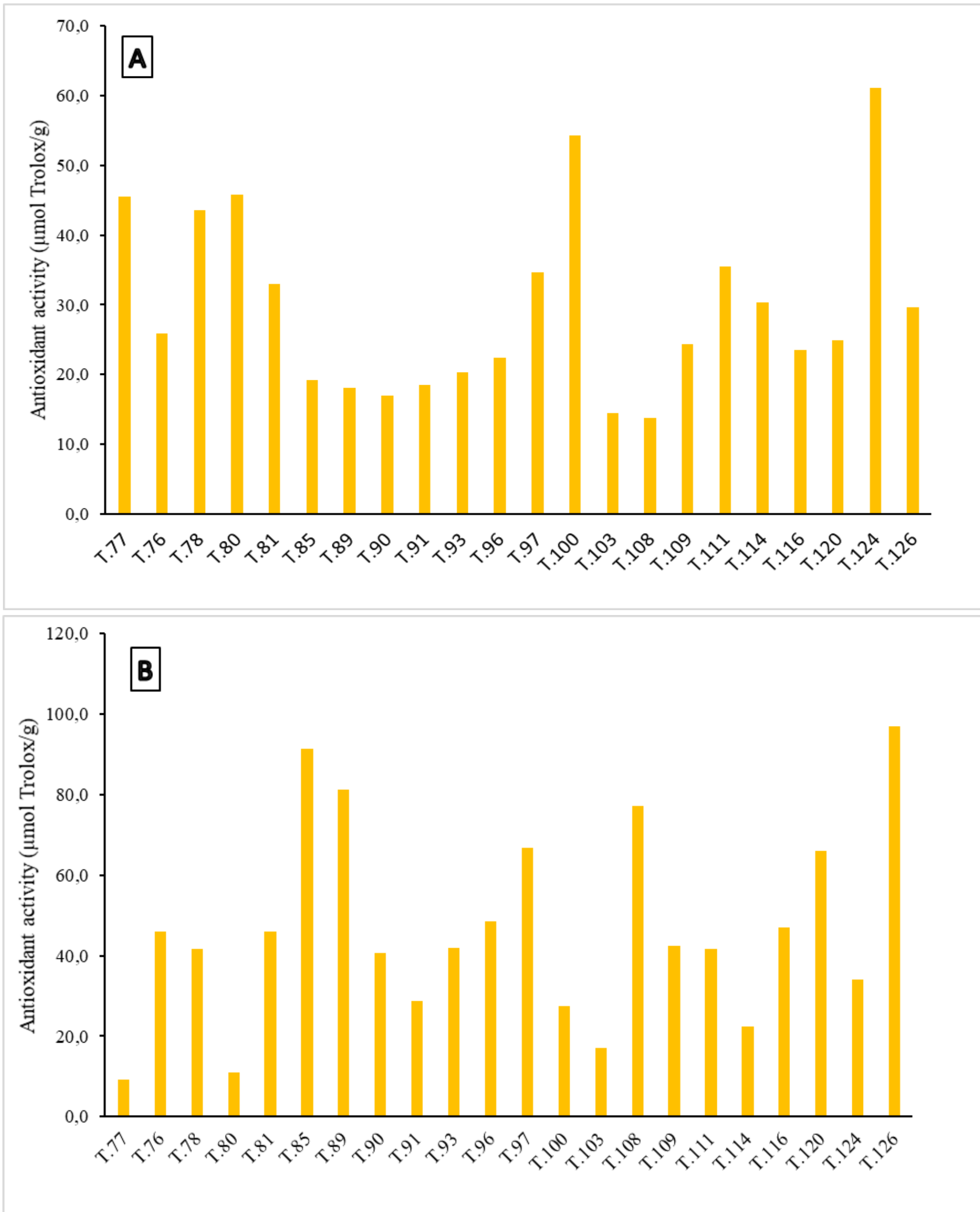


Figure 3

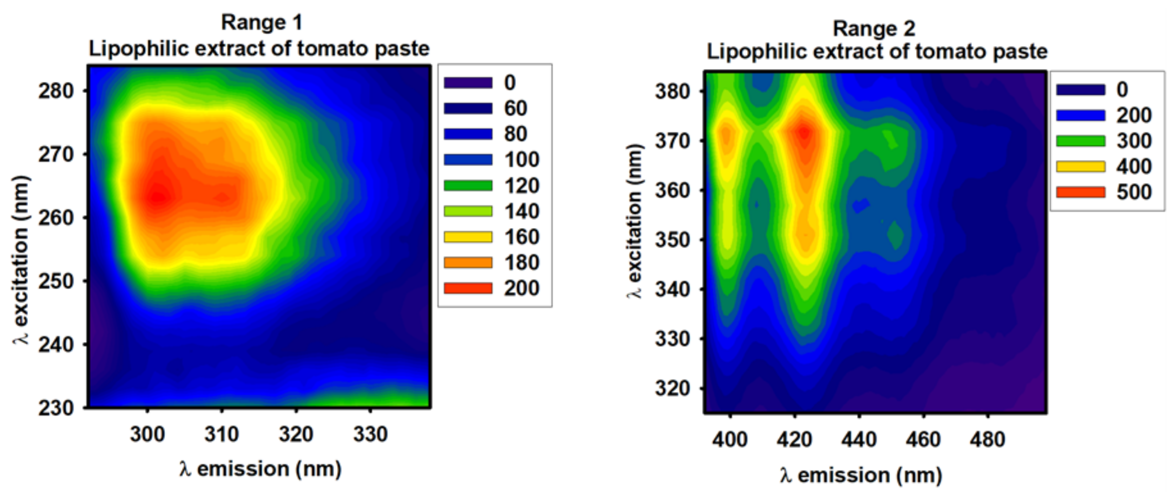


Figure 4

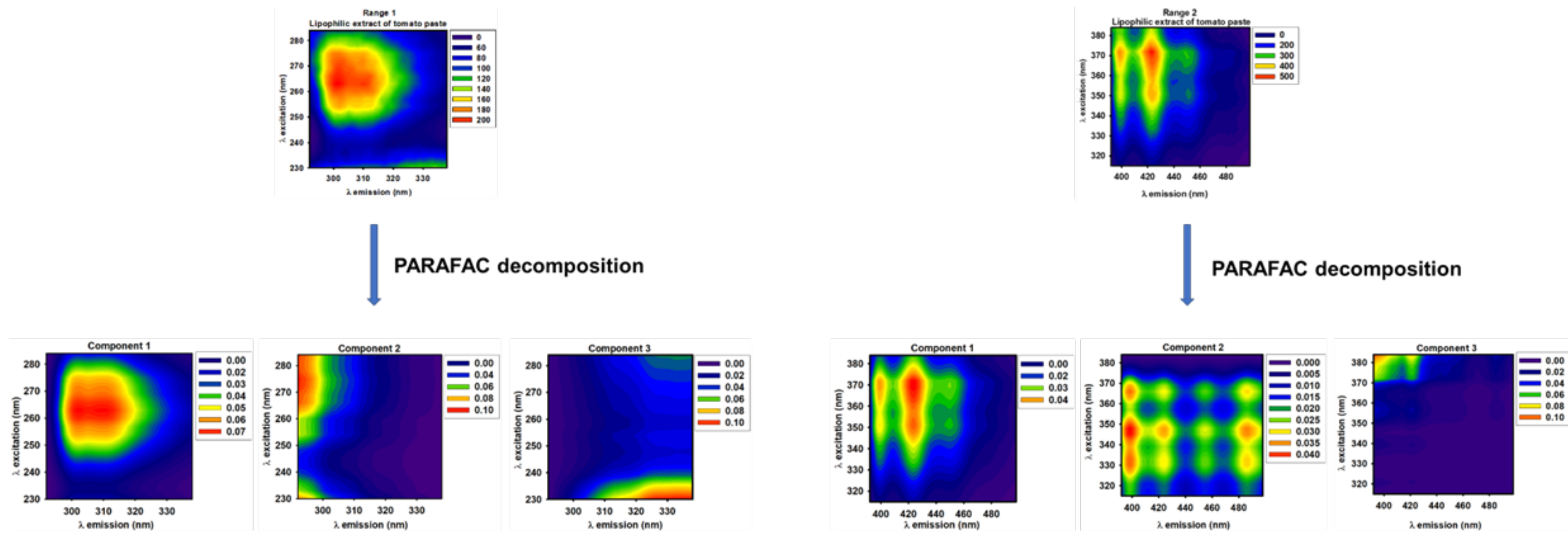


Figure 5

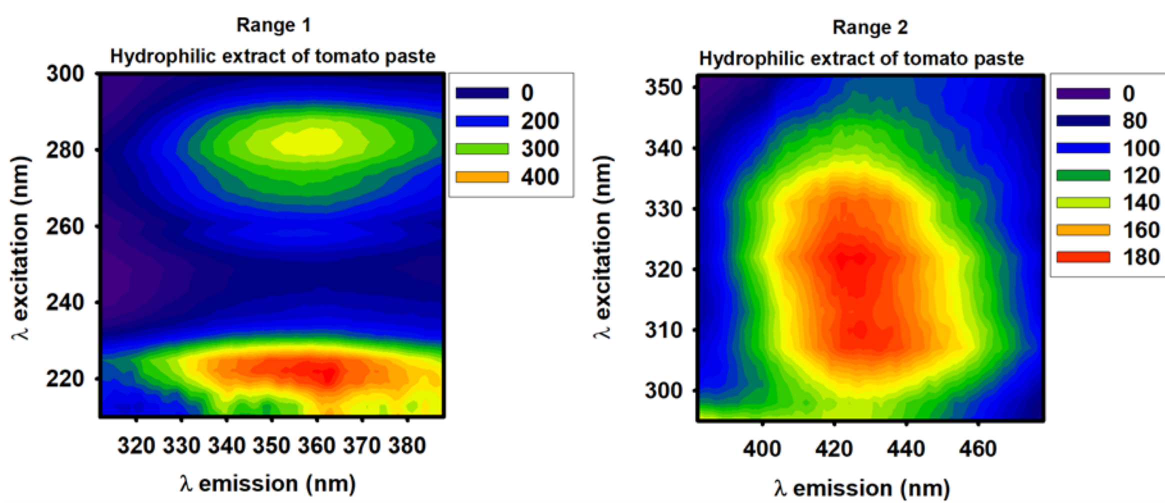


Figure 6

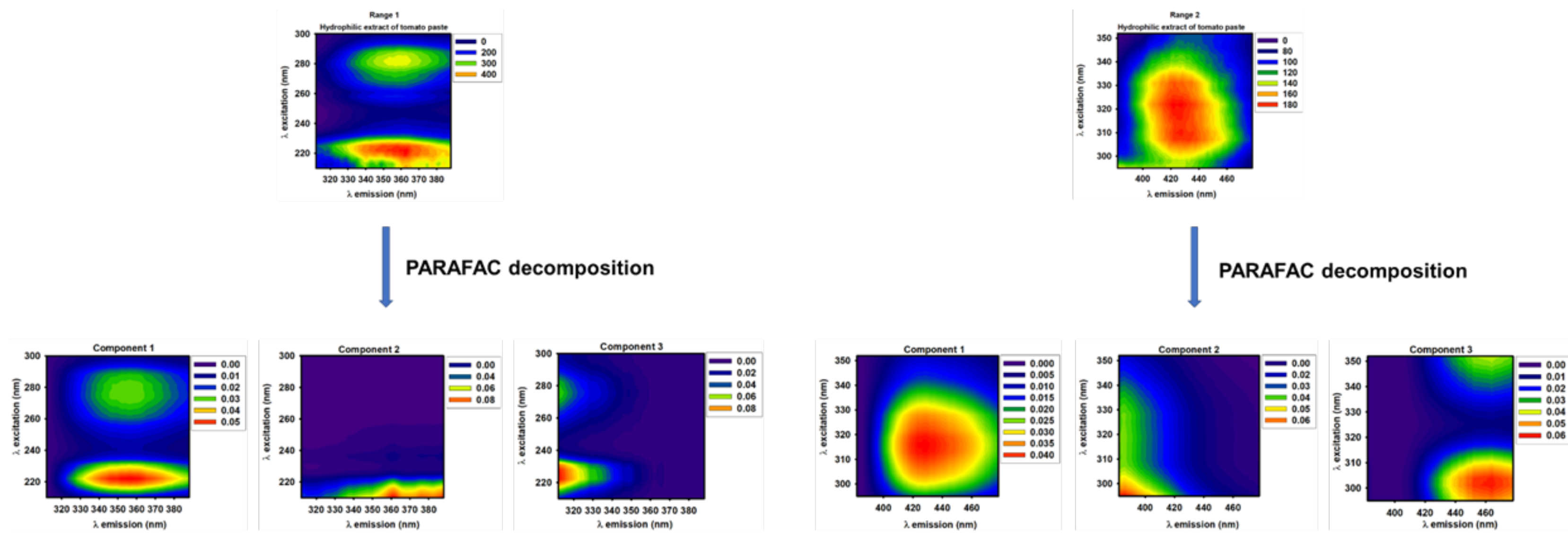


Figure 7

*__Table S1__. Characteristics of tomato paste samples*

| Sample | % water | pH | ºBrix | Acidity (g citric acid/100g) | g NaCl/ 100 g | color | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | L | a | b | a/b |
| 76 | 56.69 | 4.38 | 30.16 | 1.83 | 0.14 | 23.78 | 26.87 | 14.78 | 1.82 |
| 77 | 52.69 | 4.38 | 36.98 | 2.45 | 0.20 | 23.85 | 31.51 | 14.45 | 2.18 |
| 78 | 67.86 | 4.35 | 29.26 | 2.05 | 0.19 | 25.15 | 30.04 | 15.07 | 1.99 |
| 80 | 58.79 | 4.36 | 37.15 | 2.28 | 0.15 | 24.34 | 32.18 | 14.80 | 2.17 |
| 81 | 66.83 | 4.44 | 29.12 | 1.79 | 0.08 | 23.28 | 32.56 | 14.67 | 2.22 |
| 85 | 58.34 | 4.42 | 36.90 | 2.30 | 0.13 | 23.63 | 32.04 | 14.57 | 2.20 |
| 89 | 65.68 | 4.42 | 29.22 | 2.31 | 0.15 | 22.54 | 32.14 | 14.25 | 2.26 |
| 90 | 66.12 | 4.42 | 29.13 | 2.13 | 0.11 | 24.93 | 29.68 | 11.57 | 2.57 |
| 91 | 57.01 | 4.42 | 37.42 | 2.53 | 0.11 | 25.32 | 28.86 | 11.83 | 2.44 |
| 93 | 56.85 | 4.43 | 37.33 | 2.90 | 0.07 | 23.52 | 32.04 | 14.73 | 2.18 |
| 96 | 55.38 | 4.47 | 37.25 | 2.60 | 0.09 | 23.75 | 32.20 | 14.76 | 2.18 |
| 97 | 57.67 | 4.45 | 38.62 | 3.28 | 0.13 | 24.85 | 34.04 | 15.42 | 2.21 |
| 100 | 65.15 | 4.52 | 30.04 | 2.05 | 0.17 | 24.38 | 32.38 | 15.08 | 2.15 |
| 103 | 66.06 | 4.51 | 29.38 | 1.86 | 0.03 | 24.62 | 30.09 | 15.14 | 1.99 |
| 108 | 63.89 | 4.55 | 30.09 | 1.70 | 0.15 | 22.72 | 31.74 | 14.24 | 2.23 |
| 109 | 58.26 | 4.35 | 37.32 | 2.38 | 0.11 | 24.63 | 33.82 | 15.23 | 2.22 |
| 111 | 65.18 | 4.36 | 28.68 | 1.82 | 0.07 | 24.78 | 30.04 | 15.04 | 2.00 |
| 114 | 64.82 | 4.36 | 30.77 | 1.94 | 0.09 | 22.80 | 27.70 | 14.17 | 1.96 |
| 116 | 65.36 | 4.44 | 29.74 | 1.17 | 0.22 | 23.19 | 31.63 | 14.63 | 2.16 |
| 120 | 62.29 | 4.35 | 31.33 | 2.02 | 0.13 | 24.63 | 33.79 | 15.19 | 2.22 |
| 124 | 66.57 | 4.53 | 28.72 | 1.48 | 0.12 | 23.19 | 29.90 | 14.32 | 2.09 |
| 126 | 58.19 | 4.36 | 37.25 | 2.80 | 0.20 | 23.09 | 32.97 | 14.49 | 2.28 |

***Table S2***. *Figures of merit obtained for calibration of Folin-Ciocalteu method, using the ACOC program.*

| **Figure of merit** | | |
|---|---|---|
| **Slope (m) (L/mg)** | | 0.0952 |
| **Origin value (b) (A)** | | -0.004 |
| **Standard deviation of slope ($S_m$)** | | 0.001 |
| **Standard deviation of origin ($S_b$)** | | 0.008 |
| **Standard deviation of regression ($S_{y/x}$)** | | 0.019 |
| **Determination coefficient ($R^2$)** | | 0.998 |
| **Analytical sensitivity ($\gamma^{-1}$) (mg/L)** | | 0.248 |
| **Limit of detection (LOD) (mg/L)** | **Long-Winefordner*** | 0.291 |
| | **Clayton**** | 0.498 |
| **Limit of quantification (LOQ) (mg/L)** | **Long-Winefordner** | 1.00 |
| | **Clayton** | 1.67 |

*G. L. Long and J. D. Winefordner, Anal. Chem. 1983, 55, 07, 712A–724A

**C. A. Clayton, J. W. Hines, and P. D. Elkins, Anal. Chem. 1987, 59, 20, 2506-2514

***Table S3.*** *Figures of merit obtained for Trolox calibration, using the ACOC program.*

| Figure of merit | | |
|---|---|---|
| **Slope (m) (L/mg)** | 1.88 | |
| **Origin value (b) (A)** | 0.026 | |
| **Standard deviation of slope ($S_m$)** | 0.030 | |
| **Standard deviation of origin ($S_b$)** | 0.007 | |
| **Standard deviation of regression ($S_{y/x}$)** | 0.023 | |
| **Determination coefficient ($R^2$)** | 0.999 | |
| **Analytical sensitivity ($\gamma^{-1}$) (mg/L)** | 0.012 | |
| **Limit of detection (LOD) (mg/L)** | **Long-Winefordner (mg/L)** | 0.012 |
| | **Clayton (mg/L)** | 0.028 |
| **Limit of quantification (LOQ) (mg/L)** | **Long-Winefordner (mg/L)** | 0.040 |
| | **Clayton (mg/L)** | 0.093 |

***Table S4.*** *Experimental data and total polyphenols present in the hydrophilic extracts*

| Sample name | weight (g) | A (662 nm) | C (µg/mL) | Total polyphenols mg GAE/100 g |
|---|---|---|---|---|
| **T.76** | 0.5041 | 0.688 | 7.252 | 239.8 ± 7.1 |
| **T.77** | 0.5036 | 0.610 | 6.435 | 212.9 ± 7.2 |
| **T.78** | 0.5077 | 0.500 | 5.283 | 173.4 ± 7.0 |
| **T.80** | 0.5039 | 0.751 | 7.913 | 261.7 ± 7.2 |
| **T.81** | 0.5077 | 0.655 | 6.909 | 226.8 ± 7.1 |
| **T.85** | 0.5063 | 0.790 | 8.319 | 273.9 ± 7.2 |
| **T.89** | 0.5027 | 0.673 | 7.097 | 235.3 ± 7.2 |
| **T.90** | 0.5068 | 0.696 | 7.335 | 241.2 ± 7.2 |
| **T.91** | 0.5065 | 0.705 | 7.428 | 244.4 ± 7.1 |
| **T.93** | 0.5024 | 0.733 | 7.721 | 256.2 ± 7.1 |
| **T.96** | 0.5319 | 0.731 | 7.703 | 241.4 ± 7.2 |
| **T.97** | 0.4710 | 0.506 | 5.346 | 189.2 ± 6.8 |
| **T.100** | 0.5057 | 0.617 | 6.506 | 214.4 ± 7.6 |
| **T.103** | 0.5003 | 0.584 | 6.164 | 205.4 ± 7.1 |
| **T.108** | 0.4673 | 0.587 | 6.196 | 221.0 ± 7.2 |
| **T.109** | 0.5071 | 0.559 | 5.894 | 193.7 ± 7.7 |
| **T.111** | 0.5094 | 0.552 | 5.826 | 190.6 ± 7.1 |
| **T.114** | 0.5014 | 0.534 | 5.640 | 187.5 ± 7.0 |
| **T.116** | 0.5236 | 0.741 | 7.804 | 248.4 ± 7.1 |
| **T.120** | 0.5035 | 0.683 | 7.199 | 238.3 ± 6.9 |
| **T.124** | 0.5007 | 0.671 | 7.076 | 235.6 ± 7.1 |
| **T.126** | 0.5087 | 0.557 | 5.875 | 192.5 ± 7.2 |

**Table S5.** *Experimental data and total polyphenols present in the lipophilic extracts*

| Sample name | weight (g) | A (662 nm) | C (µg/mL) | Total polyphenols mg GAE/100 g |
|---|---|---|---|---|
| **T.76** | 0.5037 | 0.140 | 1.548 | 76.81 ± 10 |
| **T.77** | 0.5014 | 0.078 | 0.822 | 40.98 ± 10 |
| **T.78** | 0.5026 | 0.099 | 0.992 | 49.32 ± 10 |
| **T.80** | 0.5044 | 0.135 | 1.398 | 69.29 ± 10 |
| **T.81** | 0.5006 | 0.084 | 1.082 | 54.02 ± 10 |
| **T.85** | 0.5025 | 0.098 | 1.103 | 54.86 ± 10 |
| **T.89** | 0.5005 | 0.0767 | 0.842 | 42.07 ± 10 |
| **T.90** | 0.5019 | 0.098 | 1.105 | 55.03 ± 10 |
| **T.91** | 0.5020 | 0.082 | 0.884 | 44.01 ± 10 |
| **T.93** | 0.5036 | 0.104 | 1.020 | 50.63 ± 10 |
| **T.96** | 0.5015 | 0.099 | 1.111 | 55.38 ± 10 |
| **T.97** | 0.5003 | 0.087 | 0.957 | 47.82 ± 10 |
| **T.100** | 0.5038 | 0.118 | 1.328 | 65.89 ± 10 |
| **T.103** | 0.5011 | 0.070 | 0.766 | 38.20 ± 10 |
| **T.108** | 0.5048 | 0.109 | 1.256 | 62.18 ± 10 |
| **T.109** | 0.5049 | 0.093 | 0.995 | 49.26 ± 10 |
| **T.111** | 0.5040 | 0.137 | 1.514 | 75.11 ± 10 |
| **T.114** | 0.5029 | 0.098 | 1.212 | 60.23 ± 10 |
| **T.116** | 0.5034 | 0.157 | 1.528 | 75.87 ± 10 |
| **T.120** | 0.5017 | 0.093 | 0.944 | 47.01 ± 10 |
| **T.124** | 0.5006 | 0.093 | 1.134 | 56.63 ± 10 |
| **T.126** | 0.5049 | 0.125 | 0.995 | 49.26 ± 10 |

***Table S6**. Experimental data and total antioxidant capacity expressed in μmol Trolox/g of tomato paste in hydrophilic extracts.*

| Sample name | Weight (g) | $ABTS^{\bullet+}$ radical elimination (parts per unit) | C (μM) | μmol Trolox/g |
|---|---|---|---|---|
| **T.76** | 0.5041 | 0.42 | 207.2 | 25.89 ± 0.04 |
| **T.77** | 0.5036 | 0.71 | 364.0 | 45.54 ± 0.04 |
| **T.78** | 0.5077 | 0.69 | 350.5 | 43.50 ± 0.04 |
| **T.80** | 0.5039 | 0.72 | 365.8 | 45.74 ± 0.04 |
| **T.81** | 0.5077 | 0.53 | 265.4 | 32.94 ± 0.04 |
| **T.85** | 0.5063 | 0.32 | 153.9 | 19.15 ± 0.04 |
| **T.89** | 0.5027 | 0.30 | 144.6 | 18.12 ± 0.04 |
| **T.90** | 0.5068 | 0.28 | 136.9 | 17.02 ± 0.04 |
| **T.91** | 0.5065 | 0.31 | 149.1 | 18.54 ± 0.04 |
| **T.93** | 0.5024 | 0.33 | 161.5 | 20.26 ± 0.04 |
| **T.96** | 0.5319 | 0.38 | 188.7 | 22.35 ± 0.05 |
| **T.97** | 0.4710 | 0.52 | 259.6 | 34.72 ± 0.04 |
| **T.100** | 0.5057 | 0.85 | 435.9 | 54.31 ± 0.04 |
| **T.103** | 0.5003 | 0.24 | 114.6 | 14.42 ± 0.04 |
| **T.108** | 0.4673 | 0.22 | 101.7 | 13.71 ± 0.04 |
| **T.109** | 0.5071 | 0.40 | 195.7 | 24.32 ± 0.04 |
| **T.111** | 0.5094 | 0.57 | 287.1 | 35.50 ± 0.04 |
| **T.114** | 0.5014 | 0.48 | 241.5 | 30.34 ± 0.04 |
| **T.116** | 0.5236 | 0.40 | 195.7 | 23.54 ± 0.04 |
| **T.120** | 0.5035 | 0.40 | 198.9 | 24.89 ± 0.04 |
| **T.124** | 0.5007 | 0.94 | 485.6 | 61.10 ± 0.04 |
| **T.126** | 0.5087 | 0.48 | 239.5 | 29.66 ± 0.04 |

***Table S7**. Experimental data and total antioxidant capacity expressed in µmol Trolox/g of tomato paste in hydrophilic extracts.*

| Sample name | Weight (g) | $ABTS^{\bullet+}$ radical elimination (parts per unit) | C (µM) | µmol Trolox/ g |
|---|---|---|---|---|
| **T.76** | 0.5037 | 0.13 | 55.07 | 45.92 ± 0.08 |
| **T.77** | 0.5014 | 0.05 | 11.12 | 9.31 ± 0.08 |
| **T.78** | 0.5026 | 0.12 | 49.85 | 41.66 ± 0.08 |
| **T.80** | 0.5044 | 0.05 | 13.29 | 11.07 ± 0.08 |
| **T.81** | 0.5006 | 0.13 | 54.85 | 46.02 ± 0.08 |
| **T.85** | 0.5025 | 0.23 | 109.3 | 91.32 ± 0.08 |
| **T.89** | 0.5005 | 0.21 | 96.78 | 81.21 ± 0.08 |
| **T.90** | 0.5019 | 0.12 | 48.69 | 40.74 ± 0.08 |
| **T.91** | 0.502 | 0.09 | 34.25 | 28.66 ± 0.08 |
| **T.93** | 0.5036 | 0.12 | 50.21 | 41.88 ± 0.08 |
| **T.96** | 0.5015 | 0.14 | 57.83 | 48.43 ± 0.08 |
| **T.97** | 0.5003 | 0.18 | 79.44 | 66.69 ± 0.08 |
| **T.100** | 0.5038 | 0.09 | 32.95 | 27.47 ± 0.08 |
| **T.103** | 0.5011 | 0.07 | 20.33 | 17.04 ± 0.08 |
| **T.108** | 0.5048 | 0.20 | 92.86 | 77.26 ± 0.08 |
| **T.109** | 0.5049 | 0.12 | 51.01 | 42.43 ± 0.08 |
| **T.111** | 0.504 | 0.12 | 50.07 | 41.72 ± 0.08 |
| **T.114** | 0.5029 | 0.08 | 26.86 | 22.43 ± 0.08 |
| **T.116** | 0.5034 | 0.13 | 56.23 | 46.92 ± 0.08 |
| **T.120** | 0.5017 | 0.18 | 78.86 | 66.02 ± 0.08 |
| **T.124** | 0.5006 | 0.10 | 40.49 | 33.97 ± 0.08 |
| **T.126** | 0.5049 | 0.25 | 116.6 | 96.98 ± 0.08 |