Antioxidant compounds and their bioaccessibility in tomato fruit and puree obtained from a DETIOLATED-1 (DET-1) gene modified genotype

Running title: Antioxidants and bioaccessibility in genetically modified tomato and puree

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Running title: Antioxidant compounds and their bioaccessibility in tomato fruit

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ABSTRACT

The economic value, the ease of cultivation and processing, and the well-known health-promoting properties of tomato fruit, make the tomato an important target for genetic manipulation to increase its nutritional content. A transgenic variety, down regulated in the DETIOLATED-1 (*DET*-1) gene, has been studied in comparison with the parental line, for antioxidant levels in fresh and hot break fruit, as well as the bioaccessibility of antioxidants from puree. Differences in the concentrations of antioxidants between the wild-type and the genetically modified raw tomatoes were confirmed, but antioxidant levels were maintained to a greater extent in the GM puree than that from the parent. The bioaccessibility of the compounds, tested using an *in vitro* digestion model, showed an increase in the genetically modified samples.

Keywords:

Tomato puree, Bioaccessibility, Thermal processing, Genetically modified tomato, Antioxidants.

Highlights

- Antioxidant levels have been studied in fresh fruit and puree of a transgenic variety of tomato.
- Differences in antioxidant concentrations with the wild-type were confirmed.
- Antioxidant levels were maintained to a greater extent in the genetically modified puree.
- The bioaccessibility of the compounds showed an increase in the genetically modified samples.

1. Introduction

Tomato (*Solanum lycopersicum*) is a major worldwide crop, with some 162 metric tonnes produced in 2012, making it the 8th most valuable crop (FAOStat, 2014). Its fruit, whether consumed fresh or processed, is the principal dietary source of lycopene (Shi et al., 2008), containing other antioxidants such as β–carotene, tocopherols, flavonoids and phenylpropanoids. These bioactive compounds have been reported to exhibit many health-promoting activities such as protection against cancer, diabetes, and cardiovascular diseases (Periago et al., 2008).

The majority of the world tomato crop is processed into tomato paste, which is used as an ingredient in products such as soups, sauces and ketchup (Sánchez et al., 2003), whereas raw tomato fruits are mainly consumed in salads, or after home cooking. In general, food processing is thought to decrease the nutritional value in comparison to unprocessed fruits, due to the loss of certain compounds such as vitamins (Klopotek et al., 2005). In contrast, however, it has been reported that food processing increases the bioavailability of lycopene (Shi et al., 2008) and folates (Pérez-Conesa et al., 2009).

Due to its economic importance and health-promoting properties, tomato is an important biotechnological target for enhancing the levels of nutritional and high-value compounds, such as carotenoids and other antioxidants. The genetic modification (GM) of tomato fruit to overproduce metabolites is well established. In most cases, the new GM varieties have been created by pathway engineering (Butelli et al., 2008; Sapir et al., 2008), but also through the manipulation of light perception, which indirectly affects plastid organelle parameters. Thus, during the last decade, the manipulation of light signal transduction components (Davuluri et al., 2005) or photoreceptors (Giliberto et al., 2005) in tomato fruit has facilitated an increase in high-value metabolites, such as carotenoids, phenolics, and tocopherols. These novel varieties, however, have not been

assessed for bioaccessibility of their antioxidants. In this study, a transgenic (GM) variety with elevated antioxidants has been used to investigate bioaccessibility. The GM tomato line was generated using a *cis*genic approach, resulting in the down regulation of the *DETIOLATED-1* (*DET-1*) gene in a fruit-specific manner, using the TFM7 promoter (Conner, 1996). The *DET-1* gene is involved in light perception and its down regulation results in the plant believing it receives a greater quantity of incident light, thus leading to the simultaneous, increased production of antioxidants (Enfissi et al., 2010). The antioxidant concentrations in paste of the wild-type comparator (WT, a T56 processing line) and GM line have been studied and the bioaccessibility of the compounds in puree tested using an *in vitro* digestion model.

2. Material and methods

2.1 Materials

Methanol, acetonitrile, chloroform, *tert*-methyl butyl ether and ethyl acetate were of analytical grade and were purchased from Fisher Chemical (Leicestershire, UK). Formic acid and ammonium acetate, used in the preparation of the chromatographic solvents, were from Sigma-Aldrich (St. Louis, MO), as were chlorogenic acid, ferulic acid, caffeic acid, α-tocopherol, β-carotene and salicylic acid. Rutin was from Extrasynthese (Genay Cedex, France). For *in vitro* digestions, pancreatin from porcine pancreas, bile extract from porcine, and pepsin from porcine gastric mucosa were purchased from Sigma-Aldrich (St. Louis, MO).

Two different tomato genotypes, the T56 wild-type variety as a comparator, and the down regulated *DET-1* line (Davuluri et al., 2004; Enfissi et al., 2010), were used in this study. Four independent plants from each variety were grown in greenhouses under

97 standard conditions of heat, light and day length prior to harvest of fruit (Enfissi et al.,
98 2010). Tomato fruits were harvested at the red ripe state.
99
100 2.*2 Preparation of standards*
101
102 In the analysis of isoprenoid compounds, stock solutions of β-carotene and α-
103 tocopherol (10 μg/μL) were prepared and consecutive dilutions of the working solution
104 (0.1 μg/μL) used to prepare the calibration curves (0.1 μg/μL to 0.006 μg/μL).
105 Lycopene, prolycopene, phytoene and phytofluene standards were extracted from
106 tomato fruit and purified by thin layer chromatography (TLC) using a solvent system of
107 acetone/toluene/water (91:30:7,v/v/v) according to the method of Xu et al. (2003).
108 Their identities were elucidated from their absorption spectra and dose-response curves
109 were prepared from concentrations obtained using the established extinction coefficients
110 (Britton, 1995). In the analysis of flavonoids, a working solution of salicylic acid (0.02
111 μg/μL) was used as internal standard. Standards of chlorogenic acid, ferulic acid,
112 caffeic acid, and rutin were also analysed to determine their retention times and spectra.
113
114 *Preparation of tomato puree*
115
116 Eight fruits, from four independent plants, of the WT genotype and GM
117 genotype were harvested on the same day and scalded at 95 ºC for 10 sec to remove the
118 skin. They were washed in distilled water and seeds and jelly removed. The tomato
119 puree was prepared by removing the tomato fruit skin and using the pericarp tissue after
120 cold blending, and then concentrated by evaporation at 65 °C to half the volume.
121

2.3 Sample analysis

Water activity, soluble solids, moisture content, pH and colour of raw tomato and tomato puree were analysed. The water activity was determined using a dew point sensor (Decagon®, model Aqualab CX2, Decagon Devices, Inc., Pullman, Wash., U.S.A.) at 25 °C. The soluble solids were determined using a refractometer (Atago, NAR T3, Japan) at 20 °C and moisture content by vacuum drying the samples to constant weight at 60 °C (AOAC, 1980). The pH was determined using a pH meter (Crison Instruments GLP31+). The colour was measured through the surface reflectance spectra in a Minolta CM-1000R, where samples were placed in a 10 mm cell, with a white and black background. The reflectance of an infinitely thick layer (R_∞) was determined by applying the Kubelka-Munk theory for multiple scattering to the reflection spectra.

The colour co-ordinates CIE *L*a*b**, chrome and hue of the samples were obtained from R_∞ between 360 and 740 nm for D65 illuminant and 10º observer (Talens et al., 2002).

For the analysis of isoprenoid compounds, small-scale extractions were carried out in 2 mL Eppendorf tubes (Hamburg, Germany). Freeze-dried homogeneous fine powdered tomato (10 mg) was weighed in quadruplicate to represent four technical replicates. Sequentially, methanol (250 μL), chloroform (500 μL) and dH_2O (250 μL) were added to the micro-centrifuge tubes and vortexed. The mixture was incubated on ice for 20 min. A clear partition was formed by centrifugation in an Eppendorf centrifuge 5810R (Hamburg, Germany) at 13,500 g and 4 ºC for 5 min. The non-polar, chloroform phase containing isoprenoids was removed with a pipette and transferred to

147 a new tube. Chloroform (500 μL) was added to the remaining polar aqueous phase and a
148 second extraction by vortex and centrifugation was conducted as described above. Both
149 chloroform extracts were pooled and dried under a stream of nitrogen and the dried
150 residues were stored at -20 ºC until analysis.

151 For the extraction of phenolic compounds, freeze-dried homogeneous fine
152 powdered tomato (20 mg) was weighed into screw capped Pyrex tubes in quadruplicate
153 to represent four technical replicates. To each sample, methanol (2 mL) was added and
154 vortexed. Samples were incubated for 1 h at 90 ºC in a heat block before cooling on ice
155 for 20 min. The methanol supernatant was removed with a pipette, after centrifugation
156 in a Thermo Scientific Heraeus Pico 17 centrifuge (Hampshire, UK) at 4 ºC and 3,000
157 rpm for 10 min, and the extract dried using a GeneVac (Suffolc, UK) evaporator and
158 stored at -20 ºC until analysis.

159

160 *2.4 Chromatographic analysis of isoprenoid compounds*

161

162 Dried isoprenoid extracts were dissolved in ethyl acetate (30 μl). Solutions were
163 centrifuged in an Eppendorf 5810R centrifuge (Hamburg, Germany) at 4 ºC and 13,500
164 g for 5 min to remove possible insoluble particles, and then stored at 4 ºC until
165 injection. The separation of isoprenoids was performed on a Waters Alliance HPLC
166 system (Manchester, UK), equipped with photodiode array detector, using a C_{30}
167 reversed-phase column (250 x 4.6 mm) from YMC (YMC, Inc. Wilmington, NC) at 25
168 ºC. A partial loop mode was used to inject the sample (10 μL). The temperature of the
169 samples was kept at 4 ºC during chromatography. The mobile phases used were: solvent
170 A, methanol; solvent B, water/methanol (20:80, v/v), containing 0.2% of ammonium
171 acetate; and solvent C, *tert*-methyl butyl ether. The separation conditions were isocratic

during the first 6 min (95% A:5% B), and then stepped to 80% A:5% B:15% C from which a linear gradient to 30% A:5% B:65% C for 50 min, at a flow rate of 1 mL/min. The PDA was used in the range of 220 - 600 nm and the separation monitored at 280, 350, and 450 nm.

2.5 Chromatographic analysis of phenolic compounds

A solution (200 μL) containing salicylic acid (internal standard, 0.02 mg/mL) in methanol was used to dissolve the dried extract. Vortexing and a brief sonication were used to aid dissolving the extracts. After centrifugation at maximum speed in an Eppendorf centrifuge 5810R (Hamburg, Germany), the extracts were filtered using 0.2 μm cellulose nitrate filters. Chromatography was performed with a HPLC Agilent 1100 series system (Agilent Technologies, Palo Alto, CA), equipped with a quaternary pump (G1311A), an autosampler (G1313A) and a vacuum degasser (G1379A). Ultraviolet detection was achieved with a G1315B diode array detector, in the range 195 - 300 nm. Each sample (20 μL) was injected onto the HPLC system. The chromatographic separation was developed using a reversed-phase C_{18} column (250 x 4.6 mm; 5 μm) from Hichrom (Berkshire, UK), at room temperature. Mobile phases comprised solvent A, containing water/methanol (98:2, v/v) and 0.05 % formic acid, and solvent B, containing acetonitrile. The solvents were filtered through a 0.22 μm membrane filter and degassed prior to use. The separation conditions were a linear gradient from 5 to 60% of solvent B for 55 min, at a flow rate of 1 mL/min. The separation was monitored at 280, 320 and 550 nm. The column was equilibrated for 8 min under the initial conditions before each injection. The phenolic compounds were identified using standards, and quantification was carried out by comparison with the internal standard.

197

198 *2.6 In vitro gastrointestinal digestion*

199

200 The *in vitro* digestion method was based on previously described methods
201 (Svelander et al., 2010; Anese et al., 2013), with some modifications. Deionized water
202 (90 mL) was added to dry tomato powder (0.5 g). The pH of the solution was adjusted
203 to 4.0 with 1M NaOH. Then, pepsin solution freshly prepared (1g of pepsin in 10 mL
204 0.1 M HCl) was added to provide 0.01 g of pepsin / 5 g of dry tomato. The sample was
205 incubated in a shaking water bath at 37 ºC for 30 min. Previous to the intestinal
206 digestion step, the pH of the gastric digests was raised to pH 6 by addition of 1 M
207 $NaHCO_3$. Then, the pancreatic-bile extract mixture (0.2 g of pancreatin and 1.25 g of
208 bile extract in 50 mL of 0.1 M $NaHCO_3$) was added to provide 0.0025 g of pancreatin
209 and 0.015 g of bile extract per 5 g of dry tomato, and the incubation at 37ºC continued
210 for an additional 60 min. The digests were centrifuged at 5,000 g in a Sorvall centrifuge
211 (Thermo Scientific, Hampshire, UK) for 15 min at 4 ºC. The supernatant was freeze-
212 dried on a Lyophil Lyovac GT2 (Gea Process Engineering, Inc., Columbia, MD) before
213 the extraction and analysis of isoprenoid and phenolic compounds. Concentrations were
214 calculated as μg of antioxidant compound per g of dry tomato before digestion, so that
215 all values were corrected for the weight losses that occurred after centrifugation. In
216 order to enable the comparison of results with literature values, relative bioaccessibility
217 was calculated as the amount of antioxidant compound released during digestion
218 divided by the total content in the initial sample (Granado-Lorencio et al., 2007;
219 Svelander et al., 2010).

220

221 *2.7 Statistical analysis*

Statgraphics Centurion XV v15.2 (Statpoint Technologies, Inc., Warrenton, VA, USA) and Simca-P+ 13.0 (Umetrics AB, Sweden) software were used for the statistical treatment of the samples. ANOVA was used to determine significant differences in composition between the T56 and TFM7 genotypes. PCA was performed in raw tomato and tomato puree of both genotypes before and after *in vitro* digestion. The number of statistical replicates is shown in the corresponding tables or figures, and the normality of data was tested by using the Goodness-of-Fit tests Kolmogorov-Smirnov D and Cramer Von Mises W^2 in Statgraphics software, before application of the statistical procedure.

The workflow of the experiments is shown in Fig. 1.

Results and discussion

No significant differences were observed in ºBrix, water content, pH and water activity (a_w) parameters between the parent and GM genotypes, in both raw and processed tomato samples (Table 1). The concentration of soluble solids of the processed tomato samples was between 11.6-11.7. According to the Codex Alimentarius (Codex Stan 57-1981), values between 7 and 24 ºBrix in processed tomato fruit correspond to tomato puree. Therefore, the increases in carotenoid and phenolic levels in whole *DET-1* fruit (Enfissi et al., 2010) and the skinless preparations used in the present study (Tables 2 and 3) do not alter these four values, suggesting that tomato products from the GM line would have the same mouthfeel and taste as the parental counterpart. In fact, it has been widely described that particularly the a_w of tomato fruit

influences its textural properties, as well as its bacterial growth potential (Pose et al., 2010). The obtained a_w values are in accordance with previously published studies, where this parameter was analysed as being considered a major factor in shelf life for both quality and food safety (Schmidt & Fontana, 2007).

Although no compositional differences were found between both tomato genotypes in raw and processed tomatoes, some differences in the color were detected using surface reflectance spectra. Fig. 2 shows the *a**-L* and *a**-*b** color planes, where the location of fresh and processed samples are indicated. An isohue-line was plotted in *a**-*b** chromatic plane, with the value of the raw tomato WT_R (33.3 ± 0.2º) as reference (Fig. 2B). While all samples showed similar clarity (around 32 - 33 L*), significant differences in hue and chrome were observed between raw and puree tomatoes in both genotypes. In comparison to the WT, chrome and hue slightly increased in GM samples, confirming that GM line has a higher content of pigments than WT genotype. Tomato puree samples showed higher chrome values than raw samples, probably because water loss caused by thermal heating leads to an increase in pigment concentration. Lycopene, which is the major tomato fruit carotenoid, imparts the red color to the tomato, whereas β-carotene, which is ~7% of the total carotenoid, contributes to the yellow-orange-red color, particularly in the case of immature or orange pigmented tomatoes (Lewinsohn et al., 2005). Therefore, the highest values of red hue are shown in ripe GM fruit (GM_R), whereas similar values were observed with wild type ripe (WT_R) and GM puree (GM_P), and the lowest red hue value in WT puree (WT_P). These results agree with those shown in Table 2, with respect to the concentrations of lycopene and β-carotene. No-significant differences in the concentration of lycopene were detected between samples, whereas increasing concentrations of β-carotene were observed in GM_R > GM_P > WT_R > WT_P, in

accord with hue values (Fig. 2B). Thus, the higher values in red hue and chrome detected in GM samples, in comparison to WT, are due to their similar content of lycopene but higher amount of β-carotene.

Carotenoids and α-tocopherol have been analysed and quantified in raw and processed tomato genotypes (Table 2**)**. The β-carotene content in WT_R samples was similar to that described previously (Abushita et al., 2000; Pérez-Conesa et al., 2009). However, the lycopene concentration was lower than that previously published (Periago et al., 2001; Xianquan et al., 2005), probably due to the use of a de-skinned fruit in order to mimic that used commercially. Lycopene is present in the pericarp cells that are attached to the skin, which has been removed in this study. In comparison to its wild type background (WT_R), the raw transgenic tomato fruit, GM_R, showed significant differences (p<0.05) of α-tocopherol, phytoene, phytofluene, lutein and β-carotene and similar content of lycopene. The enhancement of these bioactive compounds in the GM samples is attributed to the manipulation of the *DET*-1 gene (Azari et al., 2010; Enfissi et al., 2010).

In tomato puree (WT_P and GM_P), the α-tocopherol content significantly increased with the heat treatment, probably due to heating disrupting the cell wall and internal membranes, thus increasing the release of the compound from the tomato matrix. Similar results have been observed with tomato sauce, tomato soup, baked tomato slices and tomato juice after a short-term heating treatment (Seybold et al., 2004). In the present study, GM tomato puree (GM_P) showed an increase of 50% in α-tocopherol concentration in comparison with raw GM tomato. The amount of α-tocopherol in WT tomato puree (WT_P) also showed 50% higher values than in GM_P. The concentrations of phytoene and phytofluene decreased significantly (p<0.05) in WT_P samples, whereas they showed a significant increase in GM_P samples, in

297 comparison to their respective raw tomatoes (WT_R and GM_R). This could be due to
298 phytoene and phytofluene being sequestrated in other sub-plastid structures, which
299 would increase their availability after thermal heating. In this context, a recent study on
300 the GM line showed that the increased production of carotenoids caused a higher
301 number of β-carotene and lycopene crystal-like structures in the thylakoid-like
302 membrane fractions of the GM line and phytoene/phytofluene in plastoglobules
303 (Nogueira et al., 2013). The storage of endogenous carotenoids in crystal-like structures
304 was previously reported (Rosso et al., 1967 & 1968) and it seems that this sequestration
305 mechanism has been upregulated in the transgenic lines containing increased
306 carotenoids.

307 The lutein and β-carotene contents showed significant decreases (p<0.05) after
308 the heating in both WT and GM lines, probably because there is a degradation of these
309 compounds after the thermal heating (Seybold et al., 2004). Although heating
310 treatments can promote the availability of lycopene, as it has been observed by several
311 authors (Seybold et al., 2004; Roldán-Gutiérrez & Luque de Castro, 2007), the
312 conditions applied in the present study (constant temperature of 65°C until 11-12 ºBrix
313 were reached) did not lead to an increase of the lycopene extraction. In fact, no
314 significant differences in concentration (p<0.05) were observed for this compound
315 among all samples. Similar results were obtained by others authors working with tomato
316 products when using soft heating treatments (Pérez-Conesa et al., 2009).

317 A range of phenolic compounds were identified in WT and GM raw and puree
318 tomato samples (Table 3). These compounds are generally the main phenolics identified
319 in tomato, although their content varies depending on genetic and environmental
320 factors, as well as cultural practices (Slimestad & Verheul, 2009). Generally, the
321 presence of flavonoids in tomato is very small, as they are confined entirely in the skin.

Among the different flavonoids, rutin has been found to be the main compound in ripened tomatoes (Slimestad et al., 2008). In this study, rutin was identified and quantified in the genetic modified genotype, but not in raw samples, probably due to tomato skin being removed for the study. The presence of rutin in the genetic modified raw and puree samples could be explained if the concentration in the transgenic is so high that the skin is saturated as a site of sequestration, resulting in deposition in the pericarp. However, although some studies suggest the adaptation of cellular structures to facilitate sequestration of the increased carotenoids content in transgenic lines (Nogueira et al., 2013), more studies would be necessary to confirm the mechanisms of how this re-location of compounds occurs in the pericarp.

In comparison to their wild type background (WT_R), the raw transgenic tomato GM_R shows a higher content in all phenolic compounds, with increases of 75, 45, and 91% in the amounts of chlorogenic acid, caffeic acid, and ferulic acid, respectively. These increases were expected, as the genetic modification introduced in the TFM7-*DET*-1 genotype interferes in the normal metabolic routes, elevating the levels of these compounds (Enfissi et al., 2010). Regarding the effect of the thermal processing, no significant differences (p<0.05) were observed between puree samples and the untreated samples. Previous investigations have reported that total phenolic compounds in tomatoes remained unchanged with low intensity thermal processing (Dewanto et al., 2002).

Principal component analysis (PCA), used to assess the variance among carotenoids and phenolics in the raw and processed tomatoes of the genetically modified tomato fruit with its background variety, is shown in Fig. 1 of Supplementary material. These results are in agreement with previously published proteomic studies where raw tomato proteins from these varieties were analysed, showing a good qualitative

correlation between transcripts and protein levels, and distinguishing between the transgenic and non-transgenic tomatoes in the basis of their proteomes (Mora et al., 2013).

Simulation of gastric and duodenal processes and evaluation of the amounts of isoprenoid and phenolic compounds released from matrix in raw tomato fruit and tomato puree of both genotypes was carried out. The nutrient bioaccessibility, defined as the fraction of an ingested nutrient released from the matrix and available for intestinal absorption (Parada & Aguilera, 2007), is a prerequisite for its bioavailability (Holst & Williamson, 2008) and depends on the nutrient localization in the food matrix and, for some components, constitutes the maximum amount available for consumption. Fig. 2 of Supplementary material shows the variance among carotenoid and phenolic compounds concentration released from matrix identified in raw and processed tomato of the GM tomato fruit with its background variety. The multivariate and pairwise statistical analyses demonstrate significant differences in the concentration of antioxidant compounds between GM and WT. Although non-significant differences were observed in the amount of antioxidants released from matrix in raw and processed WT tomato, significant differences (p<0.5) have been described between raw and processed GM tomato. The concentrations of individual carotenoid and phenolic compounds released from matrix are listed in Table 4. Whereas non-statistical differences were observed in *cis*-lycopene 1 and 2 compounds between samples, *trans*-lycopene showed significant differences (p<0.05) in concentration between WT and GM.

The bioaccessibility of antioxidants released from matrix after *in vitro* digestion is shown in Table 1 in Supplementary material. Despite similar percentages of bioaccessibility for the same compound, absolute values in concentration of

antioxidants available in GM are higher than WT, as the initial concentration was higher in GM for all compounds. In the case of the untreated WT tomato (WT_RD), only 5% of lycopene was released from the vegetable matrix with non-significant differences with the results obtained in WT puree (WT_PD). In this sense, Svelander et al., (2010), studied the impact of different processing methods on *in vitro* bioaccessibility of lycopene in tomato fruit, showing similar lycopene accessibility values when raw and LTLT (low temperature and long time) cutted tomatoes were analysed. The bioaccessibility percentage of phenolic compounds in raw fruits is higher than that observed for isoprenoids. However, regarding digested raw samples, the ferulic acid percentage of bioaccessibility is higher in GM genotype in comparison to WT. Finally, losses in the GM puree are lower than those observed after the digestion in the raw GM. Thus, both isoprenoids and phenolic compounds showed an increase in the bioaccessible concentration when the genetic modified tomato genotype was used in comparison to the wild type.

Conclusion

This study provides a basic understanding of the changes that occur in some isoprenoid and phenolic compounds in a genetic modified tomato from which the gene responsible for the negative regulation of light perception has been down regulated. As a result, the profile of antioxidants in this genotype shows an increase in comparison with the wild type. The changes in the profile have been described in both genotypes after thermal treatment applied to prepare tomato puree, and the bioaccessibility of the identified compounds have been studied using an *in vitro* gastrointestinal model. The higher bioaccessibility described in this study for the compounds analysed in GM samples may be due to at a certain level of expression, these compounds can no more be

located in the corresponding organelles as those are saturated and then take up other cellular structure which make them more available after digestion. In summary, the genetic modified puree showed a higher increase in carotenoids and α-tocopherol compounds after the heating treatment in comparison to the wild type as well as in the studied phenolic compounds. The higher concentrations in bioactive compounds in the GM puree could be utilised in the diet and to improve the efficiency of the industrial processing of tomato derivatives as well as naturally increase the self-life of these products.

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523

Figure 1. Flow diagram showing the experimental design of the study. Different lines indicates (⟶) technological processing flow, sample digestion (-·-·-·-), and (-----) analysis carried out in each sample.

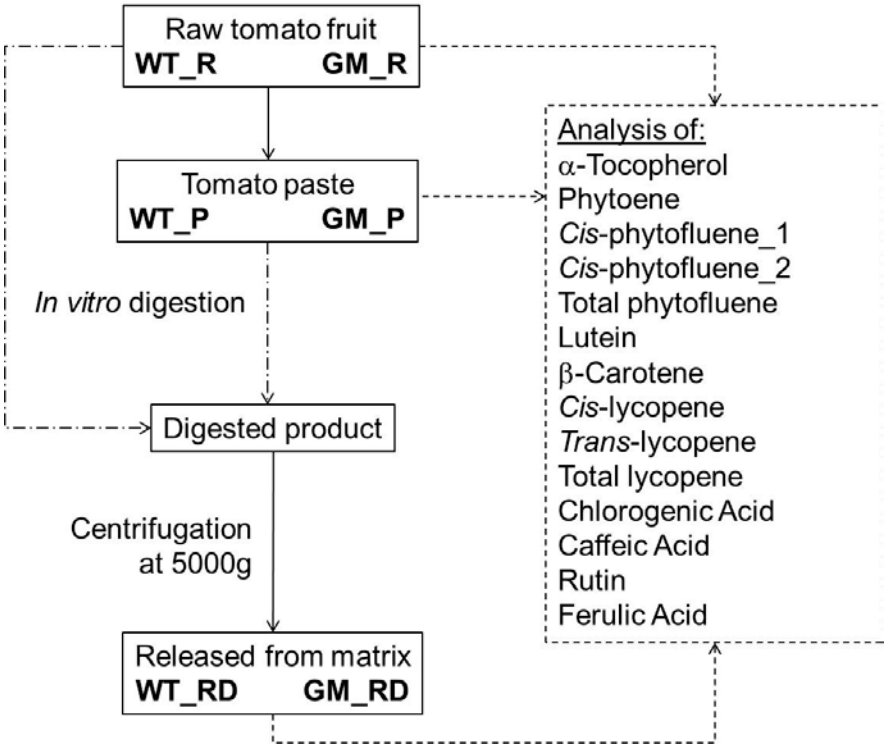

Figure 2. A) a*-L* and **B**) a*-b* color planes with the location of fresh and processed samples. The line included in B) plane is the iso-hue line of the raw tomato WT_R.

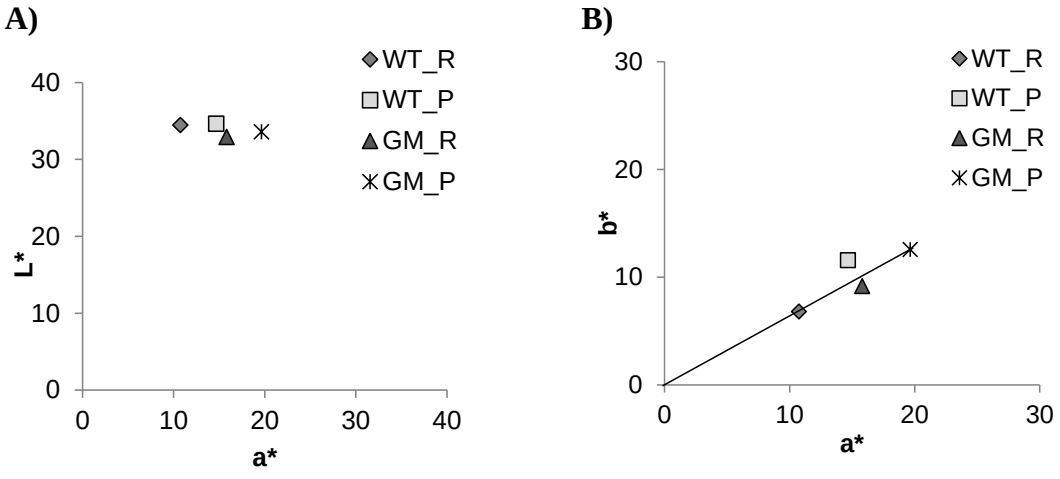

Table 1. Chemical composition (n=3) of raw tomato fruit (R) and tomato puree (P) from wild-type (WT) and genetically modified (GM) genotypes.

Sample	ºBrix	Water content (g/100g raw fruit)	pH	a_w
WT_R	5.6 ± 0.1[a]	93.0 ± 0.1[a]	3.68 ± 0.05[a]	0.991 ± 0.0.003[a]
GM_R	5.5 ± 0.2[a]	92.8 ± 0.3[a]	3.66 ± 0.03[a]	0.992 ± 0.003[a]
WT_P	11.6 ± 0.2[b]	86.4 ± 0.3[b]	3.60 ± 0.02[b]	0.986 ± 0.004[b]
GM_P	11.7 ± 0.2[b]	86.5 ± 0.6[b]	3.62 ± 0.01[b]	0.987 ± 0.002[b]

[a,b] Different letters in the same row indicate significant differences ($p < 0.5$).

a_w, water activity

Table 2. Quantitation of carotenoid compounds and α-tocopherol for WT and GM raw and puree samples.

Compound	WT_R		WT_P		GM_R		GM_P	
	Average C[1]	SD[2]	Average C[1]	SD[2]	Average C[1]	SD[2]	Average C[1]	SD[2]
α-Tocopherol	151[a]	15	379[b]	17	378[b]	32	751[c]	48
Phytoene	192[a]	7	93[b]	6	302[c]	17	384[d]	18
Cis-phytofluene_1	44[a]	4	22[b]	3	100[c]	8	121[d]	6
Cis-phytofluene_2	32.2[a]	0.9	29.4[a]	0.7	54[b]	2	81[c]	5
Total phytofluene	76[a]	4	51[b]	3	154[c]	10	202[d]	11
Lutein	19.5[a]	0.8	8.5[b]	0.2	50[c]	3	31[d]	2
β-Carotene	111[a]	8	74[b]	5	445[c]	37	389[d]	23
Cis-lycopene	37[a]	3	31[b]	2	39[b]	6	37[b]	5
Trans-lycopene	352[a]	76	313[a]	21	260[a]	9	386[a]	90
Total lycopene	394[a]	77	376[a]	21	337[a]	9	420[a]	87

1.- Concentration in mg/g of dry tomato. Each value represents the mean of four samples.

2.-Standard deviation.

a-d. Different letters in same compound indicate significant differences ($p<0.05$) in concentration.

Table 3. Quantitation of phenolic compounds for WT and GM raw and puree samples in μg/g dry tomato.

Compound	WT_R		WT_P		GM_R		GM_P	
	Average C[1]	SD[2]	Average C[1]	SD[2]	Average C[1]	SD[2]	Average C[1]	SD[2]
Chlorogenic Acid	390[a]	17	360[a]	45	1543[b]	198	1211[b]	279
Caffeic Acid	139[a]	8	137[a]	15	256[b]	33	278[b]	54
Rutin	n.d.	-	n.d.	-	1965[a]	232	1611[a]	309
Ferulic Acid	91[a]	8	74[a]	8	965[b]	67	812[b]	174

1.- Concentration in μg/g of dry tomato. Each value represents the mean of four samples.

2.- Standard deviation.

a-d.- Different letters in same compound indicate significant differences ($p<0.05$) in concentration.

n.d.- non-detected.

Table 4. Quantitation of carotenoid and phenolic compounds released from matrix after *in vitro* digestion of raw tomato fruit and tomato puree.

Compounds	WT_RD		WT_PD		GM_RD		GM_PD	
	Average C[1]	SD[2]	Average C[1]	SD[2]	Average C[1]	SD[2]	Average C[1]	SD[2]
α-Tocopherol	15[a]	2	41[b]	2	59[c]	11	60[c]	16
Phytoene	18[a]	3	7.9[b]	0.2	48[c]	8	24[a]	6
Cis-phytofluene_1	2.8[a]	0.5	2.70[a]	0.08	15[b]	3	9[c]	3
Cis-phytofluene_2	3.2[a]	0.5	2.84[a]	0.14	9.6[b]	1.3	5.3[c]	1.5
Total phytofluene	6.1[a]	1.0	5.5[a]	0.2	25[b]	4	14[c]	4
Lutein	5.0[a]	0.5	4.8[a]	0.5	10[b]	2	7[a]	3
β-Carotene	12[a]	2	10.2[a]	0.5	49[b]	5	29[c]	8
Cis-lycopene 1	19[a]	2	19[a]	2	25[a]	10	27[a]	12
Cis-lycopene 2	19[a]	2	19[a]	2	24[a]	10	25[a]	12
Trans-lycopene	43[a]	7	41[a]	2	64[b]	8	108[c]	27
Total lycopene	82[a]	10	79[a]	5	114[a]	27	160[b]	50
Chlorogenic Acid	216[a]	7	215[a]	29	562[b]	21	786[c]	50
Caffeic Acid	71[a]	4	58[a]	12	165[b]	11	228[c]	24
Rutin	n.d.	-	n.d.	-	764[b]	42	979[c]	105
Ferulic Acid	26[a]	2	16[a]	3	393[b]	34	362[c]	20

1.- Concentration in mg/g of dry tomato. Each value represents the mean of four samples.

2.- Standard deviation.

a-d.- Different letters in same compound indicate significant differences (p<0.05).

n.d.- non-detected.