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Tomato-antioxidants enhance viability of *L. reuteri* under gastrointestinal conditions while the probiotic negatively affects bioaccessibility of lycopene and phenols

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ABSTRACT

Changes undergone by tomato-antioxidants during gastrointestinal digestion of raw and fried tomato, with or without presence of the probiotic *Lactobacillus reuteri ATCC 55730*, were studied.

Frying process enhanced the extractability of antioxidant compounds, being their content higher in fried than in raw tomato. *In vitro* digestion led to a significant loss of antioxidant activity (65 and 75 % losses for raw and fried tomato, respectively), and total lycopene (60 and 50 % losses for raw and fried tomato, respectively); and promoted *trans-cis* lycopene isomerization initiated during frying.

Bioaccessibility of the antioxidant compounds was within 10 % and 30 %, being higher for phenolic compounds in raw tomato but lower for total lycopene. Finally, although the presence of *Lactobacillus reuteri ATCC 55730* reduced the bioaccessibility of antioxidant compounds, the results suggests that the tomato's antioxidant compounds could have a protective effect against the loss of viability of the probiotic.

Key words: tomato-lycopene, *L.reuteri*, bioaccessibility, viability.

1. Introduction

In the last decade of the twentieth century, the concept of nutrition has changed due to modifications in consumers' lifestyle. Nowadays, there is an increasing interest of consumers towards food with significant benefits for the physiological functions of the body (Tojo-Sierra, Leis-Trabazo, & Tojo-González, 2003). The conception of the nineteenth century, where food was only a safe and adequate supply of energy with macro- and micronutrients, has been left behind. The concept of "healthy food", i.e food that does not represent a health risk and that retains its nutritional activity and freshness (Aggett et al., 1999), has moved towards "functional food", defined as a food that is consumed as a part of a normal eating pattern, which contains natural components in modified or not modified concentrations and that provides, in addition to its nutritional value, a beneficial effect on the body (Aggett et al., 1999). Thus, the medical sciences also see in functional foods a strategy for preventing chronic non-transmissible diseases, which have become the main causes of death worldwide. Dietary recommendations have an impact on the consumption of fruits and vegetables as an ideal way to prevent these diseases. Besides its content in nutrients and fiber, fruits and vegetables have other

bioactive compounds that stand out for their antioxidant, anti-inflammatory or immunoregulatory properties, etc. (Bojórquez, Gallego, & Collado, 2013).

Many epidemiological studies have established a correlation between regular consumption of some components present in fruits and vegetables and the low incidence of suffering from certain chronic diseases (Knekt et al., 2002; S. Liu et al., 2000). Amongst the compounds of a marked antioxidant character in fruits and vegetables, ascorbic acid, tocopherols, carotenoids and polyphenols stand out, which exert their antioxidant and anti-carcinogenic effects acting in an additive and / or synergistic way (R. H. Liu, 2003). Among them, lycopene a carotenoid found almost exclusively in the tomato fruit, has up to twice the antioxidant activity of β-carotene, and has consistently been associated with the prevention of cardiovascular disease and different types of cancer (breast, colon and prostate) (Dewanto, Wu, Adom, & Liu, 2002). The amount of lycopene present in tomato depends on the variety of tomato, its degree of maturity and, above all, on the processing for its transformation into juice sauce, soup, etc. (Álvarez-Cruz & Bague-Serrano, 2011; Story, Kopec, Schwartz, & Harris, 2013). Although processing techniques, and especially those where food is exposed to high temperature, can induce losses of total lycopene by oxidation mechanisms, they can also lead, in turn, to an increase of its bioavailability as a result of the isomerization of the *trans* form into the *cis* one (Dewanto et al., 2002; Giovanelli, Zanoni, Lavelli, & Nani, 2002; Heredia, Peinado, Rosa, & Andrés, 2010; Sahlin, Savage, & Lister, 2004). Furthermore, severe heat treatments can even induce the synthesis of not only lycopene or other carotenoids, but also other compounds with antioxidant character (Heredia, Peinado, Barrera, & Andres, 2009; Heredia et al., 2010).

Studies carried out by Koh, Kim, Hwang, & Lim (2013) and Grajek, Olejnik, & Sip (2005) proved that the tomato, in addition to its antioxidant properties, possess also

prebiotic functions due to other compounds such as fiber, oligosaccharides and polysaccharides, which can act on the intestinal environment. Prebiotics are non-digestible food ingredients, whose bacterial fermentation in the colon promotes the activity and the selective growth of certain bacteria, such as bifidobacteria and lactobacilli, and prevents the growth of pathogens (Roberfroid, 2000). The benefits of a bioactive compound once ended the "industrial process" depend on the transformations experienced during the "digestive process", when food is subjected to further process variables that might trigger important changes and reactions, modifying its final functionality or bioavailability (bioaccessibility, bioabsorption, etc.). The best way to determine the benefits obtained from the intake of a food involves subjecting it to the "*in vivo*" digestive process itself, assessing the changes that it undergoes throughout each of the involved steps. Thus, the coefficient of bioavailability is directly analyzed, being defined as the amount of compound that is capable of being released by the food matrix after being transformed into the digestive process in a more soluble form (bioavailability) and crosses the intestinal barrier (biosorption) in order to be then used by the body (Parada & Aguilera, 2007). However, *in vivo* tests are expensive and require long times, particularly in human samples, involving also medical and ethical implications. Therefore, "*in vitro*" models are of great interest, since the results are more reproducible and allow mechanizing studies with various parameters under control. There are scientific evidences that positively support the alternative of using enzymatic methods that reproduce the optimal metabolic conditions of stomach digestion and subsequent absorption in the intestine, compared to *in vivo* assays (During & Harrison, 2005; Ménard et al., 2014).

In the specific case of liposoluble compounds, such as lycopene, they need to form micelles to pass through the intestinal barrier. Therefore, bioavailability and later

absorption of liposoluble compounds is much lower than for the water-soluble ones. A study conducted by During & Harrison, (2005) on intestinal absorption of carotenoids showed a very low absorption of lycopene (3%) compared to other carotenoids such as β-carotene (11%), as well as an increase on its absorption after the addition of retinol.

The aim of this study was to analyze the functional properties of tomato, both raw and fryed, after an *in vitro* gastrointestinal simulation. Specifically, the changes suffered by the antioxidant compounds present in tomato (total phenols, lycopene), total antioxidant activity, through the gastric and intestinal stages and the bioavailability of each compound have been evaluated. Additionally, the possible protective character of tomato on the probiotic *Lactobacillus reuteri* ATCC 55730 (*L. reuteri*) as it passes through the stomach and small intestine (duodenum) has been studied.

2. Materials and Methods

2.1 Reagents

Sodium carbonate, ammonium bicarbonate, potassium dihydrogen phosphate, porcine pepsin (3,200-4,500 U / mg), pancreatin from porcine pancreas (8 × USP) and bovine bile extract, were from Sigma-Aldrich (Deisenhofen, Germany). The Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox (>95 %), Gallic acid (≥ 95 %), lycopene standard (≥ 99 %) were also from from Sigma-Aldrich (Deisenhofen, Germany). Sodium carbonate hydrogen was purchased from Scharlau (Barcelona, Spain). All solvents used for the determination of lycopene were HPLC grade and all other, analytical grade. Bidistilled water was used for chromatographic analysis (Milli-Q, Millipore Corp., Bedford, MA). Lycopene solutions (1 mg / mL) were prepared daily from stock solutions (100 mg / mL in hexane). Standard solutions were stored at -20 ° C.

2.2. Raw materials

The pear type tomato (*Solanum lycopersicum L.*) was chosen to carry out this study, because it is a variety with a high amount of lycopene, widely used in the food industry to obtain processed tomato, due to its high pulp / weight ratio. Its intense red colour and its shape like a pear, clearly identify this type of tomato. It has a thin skin, a mild flavour and a fleshy texture.

2.3. Experimental methodology

2.3.1. Raw and fried tomato preparation

Whole tomatoes (without removing the skin) were adequately washed, cut into quarters and homogenized at 16,380 g-force for 40 seconds in a Thermomix mod. TM31. A homogenate with a fine texture and without any lump was obtained, and part of it was separated for the frying process. For this, olive oil was added to the crushed tomatoes (10 % w/w), and the mix was fried for 10 minutes in a conventional pan provided with a lid. The temperature at the centre of the pan was monitored along the frying process by a THERMOPAR temperature probe, and it remained at 102 ± 1 ° C. Both raw and fried tomatoes were stored in a hermetic and sterile container, in the absence of light, until gastrointestinal simulation.

2.3.2. Selection and culture of probiotic strain

The strain *Lactobacillus reuteri* ATCC 55730 was selected as the probiotic microorganism for the study (Reuter, 2001). *L. reuteri*, besides being a heterofermentative probiotic residing in the gastrointestinal system of humans, is considered one of the few true and autochthonous lactobacilli present in man (Casas & Walter, 2000). This collection strain was plated on MRS agar (Scharlau) at 37 °C for 48 h under anaerobic conditions (AnaeroGen (Oxoid)).

From the pure culture, the microorganism was inoculated into several flasks containing 100 mL of MRS BROTH. From each flask, a plate count on MRS agar plate was performed. Decimal serial dilutions of these flasks in sterile water were prepared and counts were done in duplicate in depth. After the initial count, flasks were incubated at 37 °C for 48 hours under anaerobic conditions.

2.3.3. *In vitro* gastrointestinal digestion

An *in vitro* simulation of the gastric and intestinal stages in sterile conditions was performed, according to the protocol published by García-Hernández, Moreno, Chuan, & Hernández (2012) with modifications. Concretely, *in vitro* gastrointestinal digestions of five different food systems were carried out: raw tomato, fried tomato or *L.reuteri* as simple systems and raw or fried tomato with *L.reuteri* as binary systems. *L.reuteri* concentration was about 10^8 ufc/mL in food systems with presence of the probiotic strain. For the gastric stage simulation, porcine pepsin (Sigma Chemicals) (3.6 g/ L) was re-suspended in sterile saline solution (0.5% w/ v) and the pH was adjusted to 2.0 with HCl 0.5 N. Then, a dilution of the food system to pepsin solution (1:1 (v/v)) was performed and the mixture kept in constant agitation at 224 G-force and 37 °C for 120 min. Sampling for the different analysis was performed at different times of gastric digestion (1, 10, 60 and 120 min).

For intestinal simulation, pancreatin (Sigma Chemicals) (2.5 g/ L) and bile bovine (Sigma Chemicals) were re-suspended in sterile saline (0.5% w/ v) and the pH was adjusted to 8.0 with NaOH 0.1 N. An aliquot of the previous gastric digested sample was mixed with pancreatin solution in a ratio of dilution of 1:1 (v/v) and the mixture kept under constant stirring of 112 G-force at 37º C for 240 min. Sampling was performed after 1, 30, 60, 120 and 240 min of intestinal stage, being the total time of gastrointestinal process 360 min.

Both steps were carried out in a thermostatic chamber with automatic temperature control and orbital agitation (COMECTA WY-100) and in absence of light. The samples collected for the different analyses were stored at -80 ºC for subsequent analysis of the compounds with antioxidant character (phenols, lycopene and total antioxidant activity). *L. reuteri* count was performed on the same day of simulation.

To assess the bioaccessibility of the different antioxidant compounds, a separation by decantation of the supernatant was carried out after 16 hours of repose of the thawed samples at room temperature (Granado-Lorencio et al., 2007). It was only performed with the samples collected at the end of the intestinal simulation stage. This way, it was possible to evaluate the proportion of compound in the soluble form after overcoming the digestive process and, therefore, susceptible to cross the intestinal barrier. In the case of lycopene, it would only be the proportion transferred to micelles (Hedrén, Mulokozi, & Svanberg, 2002).

2.4. Analytical determinations

All analytical determinations were performed in triplicate at each of the sampling times previously specified.

2.4.1. Plate count of *L. reuteri*

Being facultative anaerobes, the culture was carried out in the selective medium MRS BROTH. 1 mL aliquot of the simulation medium was taken at each of the sampling times, placed at the bottom of the Petri dish and quickly mixed with the agar MRS BROTH in a liquid form, in sterility. After cooling the plates, they were taken to the heater, placing them face down in an anaerobic jar (Oxoid). Counts were performed after incubation at 37 ° C for 24 hours.

197 2.4.2. Total phenolic content (TPC)

198 Total phenolic content (TPC) was spectrofometrically determined by Folin-Ciocalteu
199 method (Chang, Lin, Chang, & Liu, 2006). 1 mL of pure methanol was added to 0.5 g of
200 the sample and the mixture was vortexed for 30 seconds. The mixture was brought to a
201 horizontal stirrer for 1 hour at 336 G-Force to favour of polyphenols extraction followed
202 by centrifugation for 5 minutes at 1500 x G-force to favour their separation. Then,
203 distilled water (0.5 ml) and the Folin-Ciocalteu reagent (125 µL) were added to 125 µl of
204 the supernatant. After 6 min, 1.25 ml of sodium carbonate solution (7 % w/v), and 1 mL
205 of distilled water were added. The absorbance was read at 750 nm after 90 min using an
206 UV-Visible emission spectrophotometer (Jasco V-630). Results were compared with a
207 standard curve of Gallic acid and total phenols content expressed as mg equivalents of
208 Gallic acid / g free-fat dry matter.

209 2.4.3. Isomers of lycopene

210 The lycopene in the tomato (raw and fried) as well as in the digesta aliquots samples was
211 extracted following the protocol published by Mayeaux, Xu, King, & Prinyawiwatkul,
212 (2006) with some modifications (Heredia et al., 2010). According to this, sample (0.5 g)
213 was weighed into 15 mL screw-top glass tubes; methanol, acetone, and hexane (6 mL,
214 (1:1:1) (v/v/v)) were added followed by stirring for 30 min. During these 30 min, the
215 tubes were vortexed every 10 min for 1 min in order to encourage even more extraction
216 and obtain a colourless residue. After this, bidistilled water (2 ml) was added to each tube,
217 and these were shaken for 1 min in the vortex in order to separate the hydro soluble and
218 lyposoluble phases adequately. Next, 1 ml of the non-polar phase that contained the
219 lycopene, was transferred to the HPLC vials after being filtered with 0.22 µm nylon
220 filters. Lycopene extractions were carried out in darkness.

Lycopene content was determined by high performance liquid chromatography (HPLC) with a C30 column in an Agilent 1120 Compact system (Agilent Technologies, USA) attached to a UV-spectrophotometric detector equipped with a pump and injector. Solvents methanol, methyl-tert-butyl ether and water were used for the mobile phase in the following proportions, solvent A (v/v/v) (83:15:2) and solvent B (v/v/v) (8:90:2). Gradient elution was carried out as follows: 0–15 min 90 % A, 15.1–25 min from 90 to 5 % A, 25.1–28 min from 5 to 90 % A (initial conditions), at a flow rate of 1 mL/min and column temperature 27.5 ºC ± 3, using a Develosil C_{30} UG-5 (Phenomenex) 250 × 4.6 mm (Phenomenex [phenomenex.com]), and UV detection at 472 nm. Injection volume was 10 µl.

The identification of *trans* lycopene was carried out by comparing its retention time with that of the standard curve and the identification of the *cis* isomers was based on the retention times of these compounds obtained by other authors who worked in similar conditions and according to the Q-ratio appearing for each isomer (Heredia et al., 2010; Lee & Chen, 2001; Qiu, Jiang, Wang, & Gao, 2006). *Trans*-lycopene quantification in samples was achieved by an external calibration curve (from 4.75 to 60 mg/l) obtained with authentic standard of lycopene (all-trans, purity > 99%). Calibration curves on spike samples were used for quantification since matrix effect was observed. Results were expressed as mg of lycopene / g free-fat dry matter.

2.4.2. Antioxidant activity

The antioxidant activity was carried out by the method described by (Peinado, Rosa, Heredia, & Andrés, 2015) with some modifications. According to this method, the violet colour intensity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical dissolution decreases in the presence of antioxidants and this change in absorbance is recorded spectrophotometrically at 515 nm.

Sample (3 g) was diluted in methanol (6 mL, 80 %) and the mixture was shaken at 1200 x g force for 5 minutes. Subsequently, 0.1 ml of the above methanolic extract was added to a DPPH solution (3.9 ml, 0.024 g / L in methanol) and after 30 minutes in absence of light, the absorbance at 515 nm was measured using an UV-Visible emission spectrophotometer (Jasco V-630). The DPPH reduction (%) was calculated as follows:

$$DPPH\ reduction\ (\%) = \left[\frac{Acontrol - Asample}{Acontrol}\right] \times 100 \qquad \text{(eq.1)}$$

Where, $A_{control}$ = initial absorbance of DPPH (without simple addition) and A_{sample} = absorbance after 30 min of sample addition.

The measurement was compared to a standard curve prepared with a solution of the reference antioxidant Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the results were expressed as mg equivalents of trolox / g free-fat dry matter.

2.5. Statistical analysis

Analysis of variance (ANOVA) and the Friedman test (p-value < 0.05) were carried out using Statgraphics centurion to estimate the differences in antioxidants composition of the digested samples. Principal Component Analysis, PCA, (SPSS) was applied to differentiate the tomato samples based on their antioxidant profile.

3. Results and discussion

3.1. Effect of gastrointestinal conditions and tomato antioxidants on the viability of Lactobacillus reuteri ATCC 55730

The *in vitro* method that simulates the gastrointestinal tract is of great interest to find out whether microorganisms can survive through it (García-Hernández et al., 2012). Before subjecting the bacteria to the effects of the gastrointestinal juices, plate counts from the initial dilution were performed in triplicate, being the result 2.80 x 10^9 (5.84 x 10^8) cfu /

ml of *L. reuteri*. **Figure 1** illustrates the count of *L. reuteri* at the beginning (1 min) and at the end of the gastric (120 min) and intestinal digestion (240 min). In general terms, the initial impact of the acidic conditions and the presence of pepsin from the stomach resulted in a decrease of the initial count of the inoculum down to a mean value of 8.06 x 10^8 cfu / mL. This count slightly decreased along the digestion process, although this loss of viability was little affected by time. Regarding the effect of the conditions of the intestinal stage, the effect on survival of *L. reuteri* remained practically identical.

In terms of relative viability compared to the initial inoculum count (**Figure 1**), the survival of the probiotic decreased to 28.8 ± 0.2 % for *L. reuteri* digestion, up to 29.1 ± 0.4 % in the case of raw tomato + *L. reuteri* and to 29.2 ± 0.6 % for fried tomato + *L. reuteri* systems at time 1 min of gastric stage, due to the shock produced by gastric juices. Probiotic bacteria, like other bacteria, present certain difficulties in adapting to extreme acidic media, even though the integrity of its cell wall offers them some resistance. However, a loss of viability occurred as they entered into contact with the gastric pH. It is believed that only those that already had its cell wall damaged by external factors died (Kirjavainen, Salminen, & Isolauri, 2003).

After 120 minutes of exposure to pepsin, the final survival was 22 % when *L.reuteri* was digested alone, 23.7 % for raw tomato + *L. reuteri* digestion and 26.3 % for fried tomato + *L. reuteri* digestion, confirming the low impact of the gastric conditions on their viability. In addition, when tomato was added to the system, the survival of the prebiotic to gastric conditions significantly improved ($p < 0.05$). Duodenal conditions (pancreatin and basic pH) had a slight effect on the viability of *L. reuteri* compared to the gastric conditions, this occurring at the beginning of the intestinal stage (**Figure 1**). The mean cumulative viability after the whole gastrointestinal simulation was 16.3 % for *L. reuteri* digestion, 24 % for fried tomato + *L. reuteri* digestion and 26.3 % for fried tomato + *L.*

296 *reuteri* digestion. The possible protective effect of tomato on *L. reuteri* has been
297 previously attributed to the presence of antioxidant compounds and prebiotic fibre in
298 other fruits (Fontana, Antoniolli, & Bottini, 2013; Mrabet et al., 2012). Therefore, the
299 consumption of probiotic products combined with tomato (raw or processed), (i.e. yogurt
300 and toasts with tomato), might increase the probiotic effect of yogurt. Noteworthy, that
301 only the compounds that get over the conditions of the stomach and small intestine are
302 able to reach the large intestine and exert its beneficial effect (Aggett et al., 1999).
303
304 ***3.2. Evolution of total phenolic content (TPC) of tomato along gastrointestinal***
305 ***digestion in presence of L. reuteri***
306 **Figure 2** shows the TPC (mg eq of Gallic acid/ g fat-free dry matter) of raw and fried
307 tomato in presence and absence of the probiotic *L. reuteri* along gastrointestinal digestion.
308 Raw and fried tomato presented a phenolic content of 35.71 ± 1.3 and 41.4 ± 1.2 mg eq.
309 Gallic acid/ g fat-free dry matter, respectively before digestion. The slight increase in
310 TPC after frying may be related to the inactivation of some enzymes, such as polyphenol
311 oxidase and peroxidase, responsible for the conversion of o-diphenols into o-quinones at
312 process temperature above 88 °C (Sellés-Marchart, Casado-Vela, & Bru-Martínez, 2006,
313 2007). Certain phenols could be produced due to reactions between ingredients with the
314 consequent increase in TPC (Boileau, Merchen, Wasson, Atkinson, & Erdman, 1999).
315 As it can be observed, TPC of tomato, significantly decreased ($p < 0.05$) under gastric
316 conditions from the early beginning of this stage. Specifically, a significant decrease of
317 62.88 ± 0.12 %, 73.103 ± 0.103 % took place after 1 min of gastric digestion of raw and
318 fried tomato, respectively; and this loss slight increased until 66.70 ± 0.05 % and 77.7 ±
319 0.09 % in raw and fried tomato when *L. reuteri* was present in the system. According to
320 Kemsawasd et al. (2016), the presence of polyphenols could be able to enhance the

probiotic survivability in dark chocolate protecting them from the oxygen toxicity. This fact occurs along with an oxidation of tomato-TPC, and therefore with an additonal decrease of them. This fact could also be taken place in this system: tomato + L.reuteri.

Although the initial gastric *shock* on TPC was accused, the residence time of the food in stomach, or time of contact between the food and gastric juices seems to be irrelevant. The impact of the intestinal conditions (basic pH and presence of pancreatin and bile salts) on TPC was, in general, minimum with a slight additional decrease of TPC only registered in digested fried tomato with or without *L. reuteri* after 60 min. According to this results, TPC of fried tomato are less stable compared with the TPC of raw tomato; moreover, *L. reuteri* seems to negatively affect TPC stability along digestion, which is in accordance with previous studies (Boileau et al., 1999).

Aditionally, it could be interesting to take into account the changes undergone by the TPC from olive oil because of the contribution to the food products with olive oil addition (Tuck & Hayball, 2002).

3.3. Evolution of lycopene isomers along gastrointestinal digestion in presence of L. reuteri

Total lycopene content was considerably higher in fried than in raw tomato (5.1035 ± 0.0105 and 1.83 ± 0.04 mg/ g of free-fat dry matter, respectively), with above 10 % of *trans-cis* isomerization after frying. This fact, evidences the predominance of the release and solubilisation of lycopene from its crystallized form, versus its oxidation during frying in presence of oil (Mayeaux et al., 2006). The isomeric distribution (% respect the total lycopene content) of *trans*, *5-cis* and *other cis* in raw tomato can be observed in **Figure 3a** being 82.1 ± 0.9, 8.7 ± 0.2 and 9.21 ± 1.02 % compared to 71.8 ± 0.2, 16.3 ± 0.4 and 11.9 ± 0.4 % in fried tomato (**Figure 3b**).

346 *Cis-isomers* of lycopene being slightly shorter and polar than their correspondent *trans*
347 molecules, will be more soluble in bile acidic micelles thus, easily incorporated into
348 intestinal mucosa cells and in the chylomicrons of the lipoproteins (Boileau et al., 1999).
349 **Figure 3** illustrates the changes undergone by lycopene isomers (mg of *trans*, *5-cis* or
350 *other cis*/ g of free-fat dry matter) along the gastrointestinal digestion of raw and fried
351 tomato with or without presence of the probiotic *L.reuteri*. As it can be observed, total
352 lycopene significantly decreased under gastric conditions; acidic pH and pepsin had a
353 stronger effect on lycopene from raw compared to fried tomato (residual lycopene (%)
354 after gastric digestion of raw and fried tomato: 47.6 ± 0.9 and 72.1 ± 1.7). This significant
355 loss of total lycopene occurred in all cases, mainly after 1 min of gastric digestion (Moraru
356 & Lee, 2005) with additional losses taking place along the gastrointestinal digestion of
357 fried tomato; thus resulting in a final residual total lycopene (%) of 46.9 ± 0.3 and 50.4 ±
358 2.2 in raw and fried digested tomatoes, respectively. Off notice, the higher total lycopene
359 content after the gastrointestinal digestion in fried tomato. These *in vitro* results are in
360 agreement with those obtained with *in vivo* studies, where the consumption of tomato
361 sauce cooked with oil increased the concentration of lycopene in blood serum two and
362 three times fold compared to the consumption of fresh tomatoes (Borguini & Ferraz Da
363 Silva Torres, 2009). With regard to the influence of *L.reuteri* on lycopene changes along
364 digestion, results evidenced a negatively impact of the probiotic presence on tomato-
365 lycopene. Total residual lycopene (%) after the *in vitro* digestion of raw and fried tomato
366 with *L.reuteri* resulted in approximately 8 % lower content than without the probiotic.
367 Certain studies suggest that strains with probiotic effect may affect the bioavailability,
368 metabolism and final amount of carotenoids (Fabian & Elmadfa, 2007).
369 Gastrointestinal digestion lead to *trans-cis* isomerization of lycopene from both raw and
370 fried tomato, this isomerization being more accused by lycopene from fried tomato

371 (**Figure 3**). Nevertheless, isomerization phenomenon did not seem to be affected by the
372 probiotic *L.reuteri.* Apparently, in both human and animal tissue, *trans* and *cis* isomers
373 coexist in equilibrium (≈ 50 %) (Boileau et al., 1999; Wilberg & Rodriguez-Amaya,
374 1995). Digestion process seems to favour *trans* into *cis* isomers conversion to get closer
375 to the tissue distribution.
376
377 ***3.4. Changes of the antioxidant activity of raw and fried tomato along digestion in***
378 ***presence of L. reuteri***
379 Raw and fried tomatoes showed an antioxidant activity of 28.2 ± 0.7 and 24.23 ± 1.05 mg
380 eq. of Trolox/ g of free-fat dry matter, respectively. **Figure 4** gathers the antioxidant
381 activity (mg eq. Trolox/ g of free-fat dry matter) of raw tomato and fried tomato along
382 the gastrointestinal digestion in presence or absence of *L.reuteri.* The initial biochemical
383 *shock* of gastric conditions (after 1 min) greatly reduced the antioxidant activity in all
384 cases as for TPC and lycopene. It is well known that acid pH accelerates the loss of
385 functionality of antioxidant compounds (Amorati, Pedulli, Cabrini, Zambonin, & Landi,
386 2006), and hence, their antioxidant activity. On the other hand, and unlike for TPC and
387 lycopene, the antioxidant activity was strongly affected by the initial contact with the
388 intestinal conditions (presence of pancreatin at basic pH); while in no case there was an
389 effect of the residence time in either of the two stages, gastric and duodenal, on this
390 parameter. Finally, it should be noted that at the end of the gastrointestinal digestion, fried
391 tomato presented a slightly higher antioxidant activity than the raw one. Particularly, the
392 residual antioxidant activity (%) for raw, fried, raw + *L. reuteri* and fried + *L.reuteri* was
393 52.1 ±0.1, 57.6 ± 1.3, 31.2 ± 0.1 and 60.8 ± 2.1 at the end of the gastric stage, and 31.3 ±
394 0.1, 37.7 ± 0.6, 23.7 ± 0.2 and 35.3 ± 0.5 at the end of intestinal one.

A PCA was conducted in order to better understand the influence of the gastro intestinal conditions on the antioxidant compounds of raw and fried tomato. **Figure 5** illustrates the two-dimensional plots of the sample scores (raw and fried tomato samples at each gastrointestinal time), and compound loadings (phenol content, antioxidant activity and lycopene content) obtained by the PCA. The first two dimensions explained 97.2 % of the total variance (PC1, 72.7 % and PC2, 24.5 %). As it can be observed**,** the raw and fried tomato samples are grouped together in the plot according to the different heat treatments and intestinal conditions. PC1 clearly differentiates samples depending on their lycopene content, with raw tomato samples at the left side of the plot (green markers) and fried tomato samples at the right side of the plot (red markers); these last having a higher content on lycopene. On the other hand, PC2 groups samples according to their antioxidant activity and phenols content. According to this, samples are divided between the different gastro intestinal stages, with raw and fried tomato before digestion located at the top of the plot (filled markers), samples from the gastric stage in the middle (light filled markers) and samples from the intestinal stage at the bottom (unfilled markers). Furthermore, the PCA also illustrates how the addition of *L.reuteri* (squared markers) seemed to have a negative effect on the antioxidant compounds and lycopene of both, fried and raw tomato.

3.5. Bioaccessibility of total phenols and lycopene and its isomeric distribution in micelles

Bioaccessibility (%) of total phenols and lycopene in the supernatant after 16 hours of resting and separation by decantation (Parada & Aguilera, 2007), is presented in **Table 1**. Results showed a significantly higher bioaccessibility of phenolic compounds when tomato was digested raw than fried as well as a significant negative impact of *L. reuteri* on the bioaccessibility of these compounds. As far as lycopene is concerned, the intake

of fried tomatoes would be advisable compared to that of raw tomatoes, with a twofold times bioaccessibility for fried tomato. Likewise, it is important to point out that the bioaccesible fraction of either raw or fried tomato was richer in lycopene *cis*-isomers than the 240 min-intestinal digested samples. This fact is in agreement with the higher solubility in micelles of *cis*-forms than *trans*. Finally, *L. reuteri* did not present a significant statistically effect neither on lycopene bioaccessibility nor on its isomeric distribution in the bioaccessible fraction.

4. Conclusions

The application of a heat treatment, such as frying, promotes the generation and release of phenolic compounds and total lycopene and its isomerization *trans* to *cis*. Nevertheless, the initial *shock* of gastric conditions (acid pH and presence of pepsin) caused significant losses of phenolic compounds, total lycopene, antioxidant activity, as well as probiotic viability during the digestion of raw and fried tomato. *Trans-cis* isomerization in lycopene progressed with an isomeric distribution closed to 50 % at the end of the gastrointestinal digestion. The results indicated a protective effect of tomato, raw or fried, against the loss of viability *L.reuteri* as it passes through the stomach and small intestine; whilst the presence of the probiotic negatively contributed to the antioxidants gastro-resistance giving as a result higher losses during digestion. Finally, the bioaccessibility of the tomato phenols and lycopene ranged between 10 and 30 %. The phenolic compounds presented higher bioaccessibility when coming from the intake of raw tomatoes; while the fried tomato lycopene turned out to be more bioaccessible.

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Figure Captions:

Figure 1. Effect of the gasstrointestinal in vitro simulation variables on the viability of Lactobacillus reuteri ATCC 55730 in the different food systems. Raw tomato and Fried tomato.

Letters (a,b) differentiate between homogeneous groups within each digestion time given by the ANOVA (p-value > 0.05)

Figure 2. Total phenolic content (TPC) evolution expressed as mg eq. Gallic acid/ g of fat-free dry matter along the in vitro gastrointestinal digestion within the different food systems, raw tomato, raw tomato + L.reuteri, fried tomato and fried tomato + L.reuteri.

Letters (a,b,c) differentiate between homogeneous groups within each digestion time given by the ANOVA (p-value > 0.05)

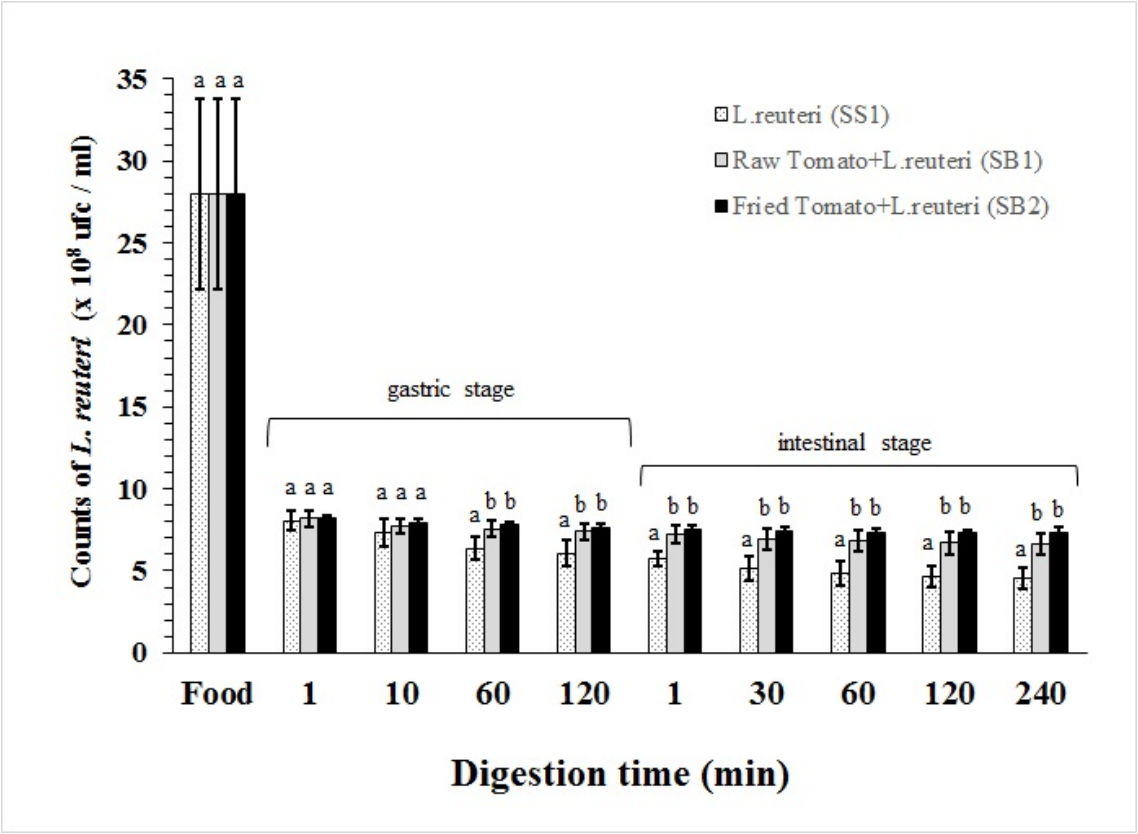
Figure 3. Lycopene content evolution expressed as mg / g of fat-free dry matter (total, all- trans, 5-cis and other cis) along the in vitro gastrointestinal digestion within the different food systems, raw tomato (a) and fried tomato (b). White bars represent the food without the addition of L.reuteri and spotted bars with the addition of L.reuteri.

Letters (a,b,c) differentiate between homogeneous groups within each digestion time and the four studied systems (raw tomato, raw tomato + L.reuteri, fried tomato and fried tomato + L.reuteri) given by the ANOVA (p-value > 0.05)

Figure 4. Antioxidant activity (AA) evolution expressed as mg eq. Trolox/ g of fat-free dry matter along the *in vitro* gastrointestinal digestion within the different food systems, raw tomato, raw tomato + *L.reuteri*, fried tomato and fried tomato + *L.reuteri*.

Letters (a,b) differentiate between homogeneous groups within each digestion time given by the ANOVA (p-value > 0.05)

Figure 5. Biplots for the different scores, samples of raw tomato (green markers) and fried tomato (red markers), at the different times of the *in vitro* gastrointestainl digestion (dark-filled markers correspond with the food systems before digestion, raw and fried tomato; light-filled markers correspond with samples during the gastric stage and unfilled markers correspond with samples during intestinal stage. Round markers correspond to systems without the addition of *L.reuteri*, and squared markers correspond to samples with the addition of *L.reuteri*). Compound loadings: Antioxidant activity (AA), total phenolic content (TPC) and lycopene content (total, all-*trans*, 5-*cis* and other *cis*) obtained by means of the PCA. (PC1, 72.7 % and PC2, 24.5 %).



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634 **Figure 1.**

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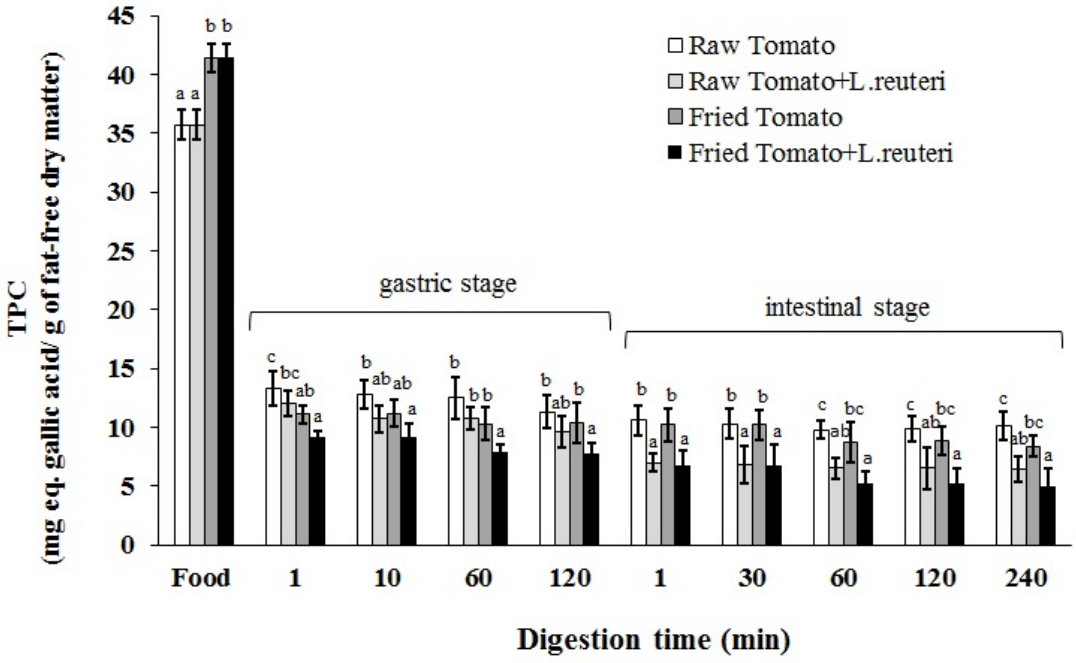

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637 **Figure 2.**

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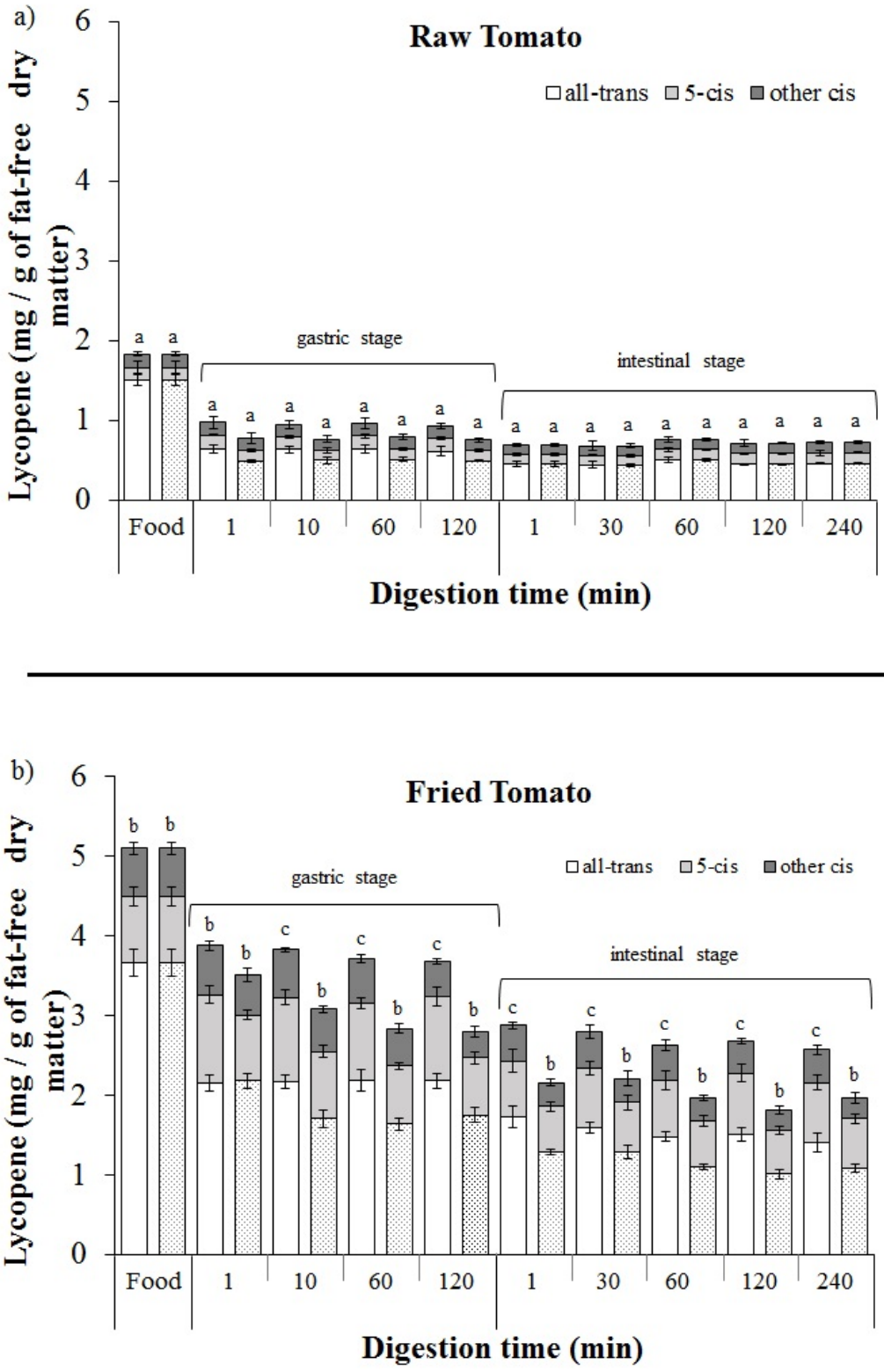

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640 **Figure 3.**

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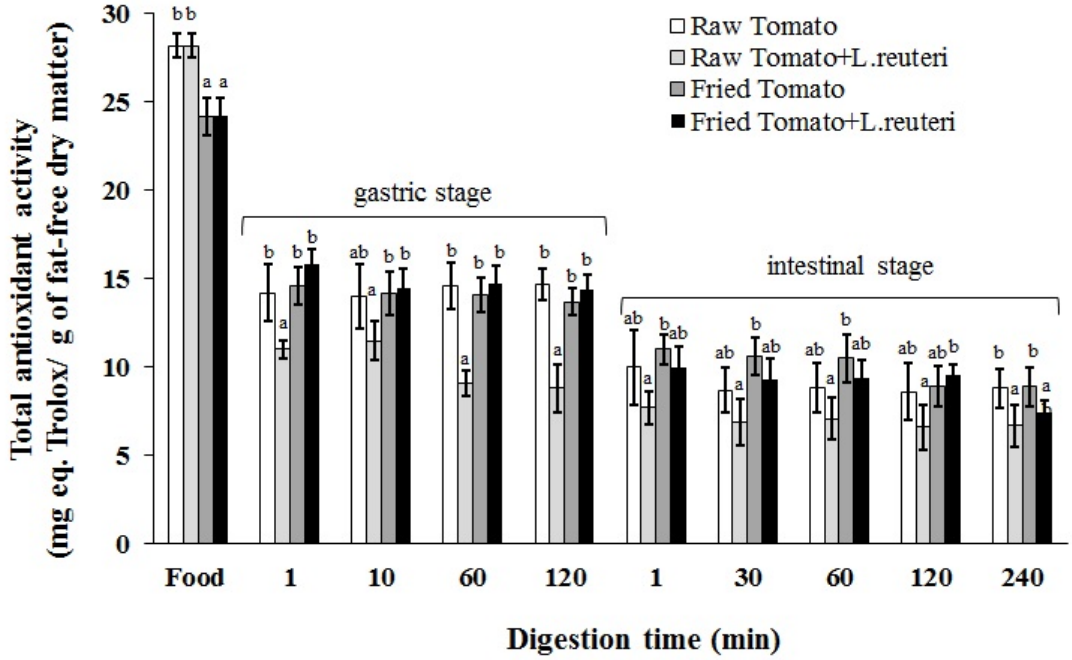

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643 **Figure 4.**

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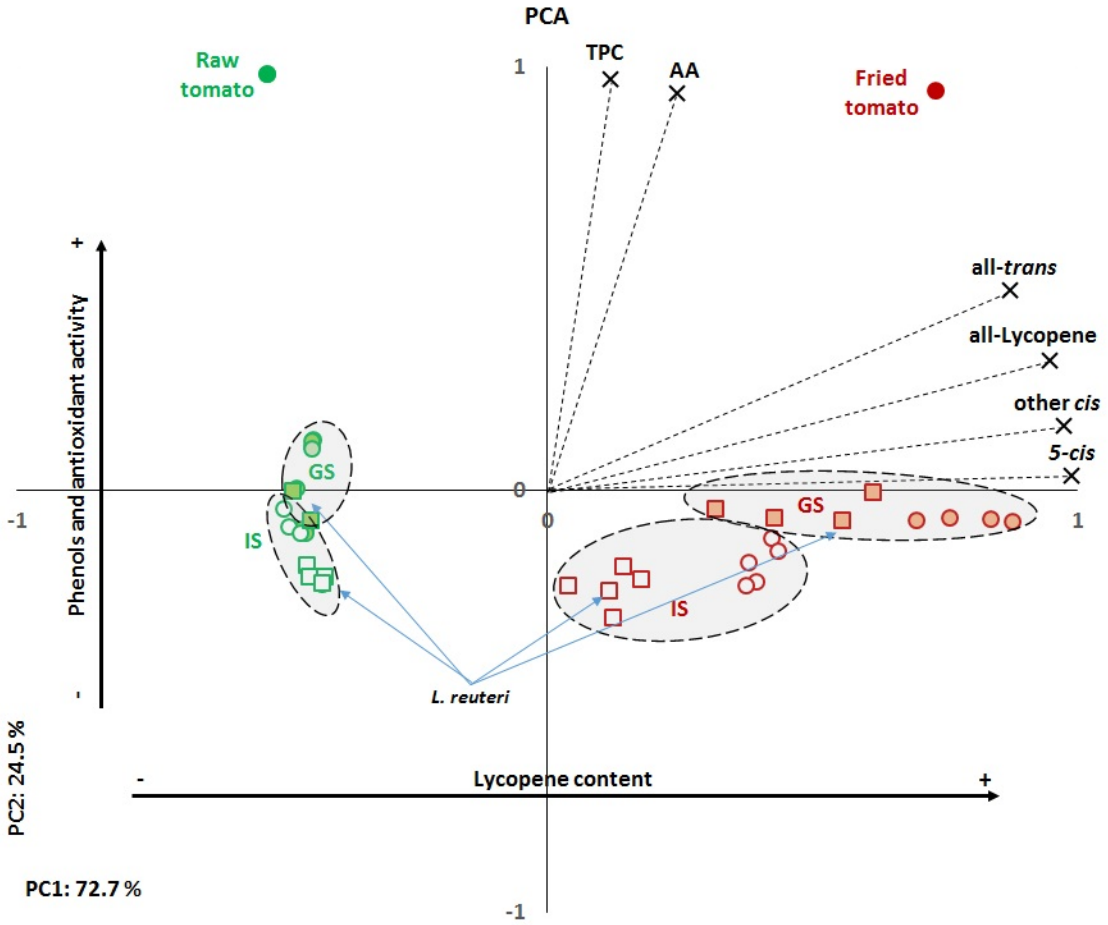

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646 **Figure 5.**