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(Poly)phenolic composition of tomatoes from different growing locations and their absorption in rats: A comparative study

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ABSTRACT

The aim of this work was to address whether the growing location of tomato could generate a different (poly)phenol profile able to affect both *in vivo* absorption and (poly)phenol metabolite pattern upon tomato consumption. uHPLC-MS^n analyses allowed to obtain a detailed (poly)phenol profile of tomatoes from two locations in Spain, quantifying 57 (poly)phenolic compounds. However, local and non-local tomatoes showed a different concentration of their native (poly)phenols, which could be attributed to diverse cultivation origin. Rat serum was analysed after an acute tomato feeding. Seven phenolic metabolites were quantified through uHPLC-MS^n. Pharmacokinetic parameters were further evaluated, revealing different serum concentrations of (poly)phenolic metabolites between tomatoes. The maximum peak serum concentrations, reached mainly after 2 h after ingestion, led to suppose that serum metabolites were mostly derived from absorption in the upper gastrointestinal tract. The growing location of tomatoes affected both the content of native (poly)phenols and their *in vivo* absorption.

1. Introduction

There is considerable epidemiological evidence indicating that the consumption of diets rich in fruits and vegetables is associated with a reduction of chronic diseases, including cardiovascular diseases, neurodegeneration, and some types of cancer (Del Rio et al., 2013). In this sense, it is well known that part of the health benefits of the Mediterranean diet could be attributed to the high content of fruits and vegetables rich in bioactive compounds (Cruz-Carrión et al., 2020; Martínez-Huélamo et al., 2015). Within this framework, tomato (*Solanum lycopersicum*) represents an important part of the Mediterranean diet and its regular consumption has been consistently associated with a lower risk of several types of cancer and coronary heart disease (Martínez-Huélamo et al., 2015; Minoggio et al., 2003). Indeed, tomato is a rich source of nutrients and phytochemicals that are widely studied for their potential health properties, including fibre, minerals, vitamins C and E, carotenoids, chlorophylls, (poly)phenols, glycoalkaloids, and organic acids (Asensio et al., 2019; Martínez-Huélamo et al., 2016). The tomato (poly)phenolic composition is genotype-dependent but it is also modulated by many agronomic, geographical and seasonal factors (Cruz-Carrión et al., 2021; Martínez-Valverde et al., 2002). A recent study carried out with the variety of tomatoes "*Rosa de Barbastro*" showed that the location of cultivation influenced the concentration of (poly)phenols. Specifically, higher concentrations of caffeic acid, *p*-coumaric acid, ferulic acid and total phenolic content were found in tomatoes grown in an area while those grown in another area exhibited substantially higher concentrations of chlorogenic acid (Asensio et al., 2019). Furthermore, there is a vast literature on the role of (poly)phenols in the prevention of chronic diseases (Del Rio et al., 2013; Manach et al., 2004). But it is essential that to fulfil this role, these bioactive compounds have to reach the target tissues in an effective concentration to exert their beneficial health effect (Martínez-Huélamo et al., 2016).

Tomatoes contain quercetin, naringenin, rutin and chlorogenic acid as the main phenolic compounds. It is important to keep in mind that the most polyphenols in the human diet are not necessarily the most active within the body, either because they have lower intrinsic activity or

Abbreviations: AUC, area-under-the-curve; C_{max}, maximum serum concentration; LT, local tomato; MRT, mean residence time; n.d., not detected; n.q., not quantified; NLT, non-local tomato; T_{max}, time of peak serum concentration.

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because they are poorly absorbed in the intestine, are highly metabolized, or are rapidly eliminated. In addition, metabolites found in the blood and target organs that result from digestive or hepatic activity may differ from native substances in terms of biological activity (Manach et al., 2004).

After ingestion, (poly)phenols are absorbed, distributed, and extensively metabolised. Absorption of some compounds into the circulatory system takes place in the small intestine (Del Rio et al., 2013). In the course of absorption and before passing to the bloodstream, (poly)phenols are conjugated in the small intestine and subsequently in the liver, *i.e.,* the aglycones undergo some degree of phase II metabolism forming sulfate, glucuronide and/or methylated metabolites (Del Rio et al., 2013; Manach et al., 2004). Recycling back to the small intestine *via* biliary excretion may occur due to enterohepatic recirculation (Del Rio et al., 2013). The unabsorbed phenolic fraction reaches the colon undergoing an extensive catabolism by the resident bacteria (Del Rio et al., 2013). In fact, (poly)phenol metabolites rather than their native forms are those who have been attributed the health benefits (Iglesias-Carres et al., 2019). Therefore, a thorough knowledge of the bioavailability of (poly)phenolic compounds in tomatoes is essential to understand their health effects. In this context, characterization of (poly)phenolic compounds present in tomatoes is of great interest. Thus, the present study aimed at investigating the (poly)phenol content of tomatoes cv. Ekstasis from two geographical origins and evaluating both (poly)phenol absorption and the circulating metabolites through an acute rat feeding study.

2. Materials and methods

2.1. Chemicals and reagents

All solvents and reagents purchased from VWR International (Milan, Italy) were LC grade or LC-MS grade.

Salicylic, *p*-coumaric, caffeic, dihydrocaffeic, 3-caffeoylquinic. 4-caffeoylquinic, 5-caffeoylquinic acids, rutin and quercetin-3-glucuronide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Vitexin was purchased from Extrasynthese (Genay Cedex, France). Caftaric acid was from PhytoLab GmbH & Co. (Vestenbergsgreuth, Germany). Vanillic acid-4-*β*-D-glucoside was from Cayman Chemical (Ann Arbor, MI, USA). Quercetin-3′-sulfate was kindly provided by Professor Alan Crozier (University of Glasgow, United Kingdom).

The following standard compounds were from Toronto Research Chemicals (Toronto, ON, Canada); they are named according to the nomenclature proposed by Kay et al. (Kay et al., 2020) while the commercial names and catalogue number are provided brackets. 3′-hydroxycinnamic acid-4′-glucuronide (caffeic acid 4-β-D-glucuronide, Catalogue N° C080020); 3′-methoxycinnamic acid-4′-sulfate (ferulic acid 4-*O*-sulfate, F308920); 3′-methoxycinnamic acid-4′-glucuronide (ferulic acid 4-*O*-β-D-glucuronide, 308910); 3-(4′-hydroxyphenyl)propanoic acid-3′-glucuronide (dihydro caffeic acid 3-*O*-β-D-glucuronide, D448705); 3-(4′-hydroxyphenyl)propanoic acid-3′-sulfate (dihydro caffeic acid 3-*O*-sulfate, D448710); 3-(3′-methoxyphenyl)propanoic acid-4′-glucuronide (dihydro ferulic acid 4-*O*-β-D-glucuronide, D448315); 3-(4′-methoxyphenyl)propanoic acid-3′-glucuronide (dihydro isoferulic acid 3-*O*-β-D-glucuronide, D448940); 3-(3′-methoxyphenyl)propanoic acid-4′-sulfate (dihydro ferulic acid 4-*O*-sulfate, D448915).

2.2. Tomato sampling and proximate composition analysis

Tomatoes fruits (*Solanum lycopersicum* cv. Ekstasis) conventionally grown in two locations in Spain: in the northeast, Tarragona (41°4′29.24′’ N 1°3′8.78′’ E; local tomatoes, LT) and in the southeast, Almería (36°50′17.3′’ N 2°27.584′ O; non-local tomatoes, NLT), were obtained from a local market in Tarragona at maturity. Whole fruits were frozen in liquid nitrogen and grounded. The homogenates were then freeze-dried for one week at − 55 °C using a Telstar LyoQuest lyophilizer (Thermo Fisher Scientific, Madrid, Spain). The tomatoes powder was kept dry and protected from humidity and light exposure until use. The dietary components of LT and NLT used in this study are detailed in Supplemental Table 1.

2.3. Extraction of (poly)phenolic compounds in freeze-dried tomatoes

The (poly)phenolic compounds from LT and NLT were extracted according to Mena et al. (Mena et al., 2016) with modifications. Briefly, 50 mg of each lyophilized tomato were added to 1 mL of 80% aqueous methanol acidified with 0.1% formic acid. Then, mixtures were strongly shaken for 1 min and sonicated for 10 min in an ultrasonic bath and vortexed again. After, samples were centrifuged at 16,600 × *g* for 10 min at 5 °C and the supernatants were collected. The residues were re-extracted twice more with 1 mL of the same solvent, following the protocol indicated above. Finally, supernatants were diluted (1:4 *v/v)* in water acidified with 0.1% (*v/v*) formic acid, vortexed and centrifuged at 16,600 × *g* for 10 min at 5 °C before uHPLC-MS^n analyses.

2.4. Animal experimental study

Male Wistar rats were housed at 22 °C with light/cycle of 12 h and were fed *ad libitum* with a standard chow diet (AO4, Panlab, Barcelona, Spain). The animals were randomly separated into two groups: the LT-administered ($n = 6$) and the NLT-administered ($n = 5$). After an 8-hour fasting period, rats were acutely administered, by intragastric intubation, a dose of 3 g of LT or NLT per kg body weight (bw). Blood samples were obtained from the saphenous vein using nonheparinized vials (Sarstedt, Barcelona, Spain) before (0 h) and 2, 4, 7, 24 and 48 h after tomatoes administration. After, blood samples were centrifuged (2000 × *g*, 15 min, 4 °C) to collect the serum and then stored at – 80 °C until use. Samples were pooled (LT, $n = 6$ and NLT, $n = 5$) to acquire sufficient volumes for chromatographic analysis. Furthermore, according to Margalef et al. (Margalef et al., 2015), pooling of biological samples increases homogeneity and sensitivity and, consequently, this allows the detection of all potential metabolites. This experiment was conducted in strict compliance with the institutional guidelines for the care and use of laboratory animals and was approved by the Ethical Committee for Animal Experimentation of the Universitat Rovira i Virgili (reference number 9495). Finally, the animals used in this study were sacrificed by decapitation after procedure.

2.5. Extraction of tomato-derived (poly)phenolic metabolites in rat serum

Tomato-derived (poly)phenolic metabolites in serum were extracted as previously described by Ardid-Ruiz et al. (Ardid-Ruiz et al., 2018).

2.6. uHPLC-MS^n analyses of tomato-derived (poly)phenols and their (poly)phenolic metabolites in rat serum

The samples were directly analysed by ultra-high performance liquid chromatography (uHPLC) coupled with mass spectrometry (MS), using an Accela uHPLC 1250 apparatus equipped with a linear ion trap MS (LIT-MS) (LTQ XL, Thermo Fisher Scientific Inc., San Jose, CA, USA), fitted with a heated-electrospray ionization (H-ESI-II) probe (Thermo Fisher Scientific Inc.). Analyte separation was performed using an Acquity UPLC HSS T3 (2.1 × 100 mm, 1.8 μm particle size) column coupled with a precolumn Acquity UPLC HSS T3 VanGuard (2.1 × 5 mm, 1.8 μm particle size) (Waters, Milford, MA, USA). All analyses were carried out in negative ionization mode.

Tomato-derived (poly)phenols present in the tomato samples were analysed using full-scan negative ionization mode with a data-dependent MS^3 method scanning from *m/z* 100 to 2000. The mobile phase consisted of (A) 0.1% (*v/v*) formic acid in acetonitrile and (B) 0.1% (*v/v*) formic acid in water. The chromatographic and mass spectrometer conditions were the same reported by Ricci et al. (Ricci et al.,

2019), except for sweep gas that was set to 5 (arb. units). The LC-MS characteristics are reported in the Supplemental Table 2. The quantification of (poly)phenolic compounds in freeze-dried tomatoes was performed using calibration curves of each standard compound or by using the most structurally related compound as reported in Supplemental Table 2. Stock solutions of standard compounds used for quantification of tomato-derived (poly)phenols were prepared in methanol or dimethyl sulfoxide and adequately diluted with water acidified with 0.1% (*v/v*) formic acid to build the calibration curves.

On the other hand, serum (poly)phenolic metabolites were analysed by reducing the formic acid concentration in mobile phase to increase the sensitivity for phenolic acid metabolites. In detail, the mobile phase consisted of (A) 0.025% (*v/v*) formic acid in acetonitrile and (B) 0.025% (*v/v*) formic acid in water by applying the same LC gradient used for analysis of (poly)phenolic compounds in tomato powders. The MS parameters were the same reported by Calani et al. (Calani et al., 2014), except the Collision Induced Dissociation (CID) used that was set to 35 for all metabolites. Serum metabolite profiling after tomato (poly) phenol intake was evaluated through target full MS/MS analyses by monitoring the specific deprotonate molecule (Supplemental Table 3), and then quantified by setting the MS in the Selected Reaction Monitoring (SRM) mode. Where possible, (poly)phenolic metabolites were quantified using a calibration curve prepared with a reference compound. When such standards were not available, metabolites were quantified using a structurally related compound (Supplemental Table 3). Stock solutions of standard compounds used for quantification of tomato-derived (poly)phenolic metabolites were prepared in methanol or dimethyl sulfoxide and adequately diluted with 50% (*v/v*) aqueous methanol acidified with 0.1% (*v/v*) formic acid to build the calibration curves. The limit of detection (LOD) and quantification (LOQ) for all used standards were evaluated. LODs and LOQs were calculated based on the minimal accepted values of the signal-to-noise (S/N) ratio of 3 and 10, respectively.

Helium gas was used for MS/MS experiments. For the quantification of (poly)phenolic metabolites, any compound present at the 0 h timepoint was subtracted from the serum concentration at all other timepoints. All instrumental data were acquired using Xcalibur software 2.1 (Thermo Fisher Scientific Inc.).

2.7. Pharmacokinetic parameters of tomato-derived (poly)phenolic metabolites

Maximum serum concentration of tomato-derived (poly)phenolic metabolites from 0 to 48 h post dose was defined as C_{max}, with T_{max} being the time at which C_{max} was reached. The area-under-the-curve ($AUC_{0\text{-}48}$) serum concentration–time at a 48-hour interval, representing the exposure of the organism to tomato (poly)phenols; and the mean residence time from the time of dosing to the time of the final quantifiable concentration ($MRT_{0\text{-}48}$) were also determined. The calculations of all kinetics parameters were performed by non-compartmental analysis using PKSolver, an add-in program in Microsoft Excel (Zhang et al., 2010).

2.8. Statistical analysis

Student's *t*-test (SPSS, SPSS Inc., Chicago, IL, USA) was used to estimate any differences in the (poly)phenolic composition of LT and NLT, and any differences in tomato-derived (poly)phenolic metabolites and kinetic parameters. Differences at $p < 0.05$ were considered statistically significant.

3. Results

3.1. Tomato-derived (poly)phenolic compounds

The native (poly)phenolic compounds present in lyophilized tomatoes is shown in Table 1. Fifty-seven (poly)phenolic compounds were quantified in LT or NLT. The predominance of phenolic acids was noted, reaching 73% of the total. Specifically, phenolics belonging to the hydroxycinnamic acids were the most abundant phenolic acids, while hydroxybenzoic acids were the least numerous.

In detail, although the total (poly)phenolic compounds did not vary significantly between the two types of tomatoes, 57% of the compounds quantified in tomatoes showed statistical differences. In particular, LT displayed higher quantities of flavonoids than NLT (1.4-fold), while NLT was noted for containing 1.2-fold higher concentrations of phenolic acids. This difference was mainly due to the higher levels of free phenolic acids and hydroxycinnamoylquinic acids, the latter representing 39 to 45% of total phenolic acids. On the contrary, total caffeic and dihydrocaffeic acid derivatives were similar between the two types of tomatoes, while most caffeic acid derivatives compounds varied significantly between LT and NLT. Moreover, total free phenolic acids were more abundant in NLT. Indeed, all free phenolic acids, except *p*-coumaric acid, were significantly higher in NLT; interestingly, one of them, dihydrocaffeic acid, was only detected in NLT although at very low level. Regarding hydroxybenzoic acid derivatives, 2 out of 3 were statistically more abundant in LT, but the total of these phenolic acids did not show significant differences. In addition, although the sum of all the hydroxycinnamic derivatives did not show significant differences, individually, 67% of the forms varied significantly between LT and NLT. In addition, caffeic acid-*O*-hexoside III was the most abundant in LT, while caffeic acid-*O*-hexoside I was the most abundant in NLT. Hydroxycinnamoylquinic acids, which were the predominant tomato-derived phenolics in the current study, were recovered at higher total concentration in NLT than LT, with tricaffeoylquinic acid and dicaffeoylquinic acid III standing out as being significantly 2.2 and 2.1 times higher in NLT, respectively. Finally, the total concentration of phenylpropanoic acid glycosides was similar between both types of tomatoes, in fact, only dihydro ferulic acid-*O*-hexoside and hydroxyphenylpropionic acid-*O*-hexoside varied, the former being higher in LT and the latter in NLT. Overall, 53% of the total (poly)phenol content in LT are represented by phenolics containing caffeoyl groups while in NLT these species reached 62% of the total (poly)phenol fraction.

Regarding flavonoids, they represented only 12% and 8% of the total (poly)phenols in LT and NLT, respectively. Nine of the 15 detected were significantly different between LT and NLT, rutin, the most flavonoid in both tomatoes, being significantly 1.3-times higher in LT. However, other flavonoids, such as kaempferol derivatives, were significantly more abundant in NLT.

3.2. (Poly)phenolic metabolites in rat serum after tomato administration

Serum concentration of tomato (poly)phenol metabolites analysed in rat after acute oral administration of 3 g/kg bw of either LT or NLT are listed in Table 2. In total, 17 compounds corresponding to the most likely detectable metabolites in the samples were monitored (Supplemental Table 3) (Martínez-Huélamo et al., 2016). As a result, a total of 7 (poly)phenolic metabolites were identified and quantified in rat serum, which occurred mainly as sulfate and methyl-sulfate conjugates, except for 4′-hydroxycinnamic acid-3′-glucuronide.

In terms of (poly)phenol intake, LT and NLT provided 10.09 mg and 11.24 mg of (poly)phenols per kg of bw, respectively. The pharmacokinetic profiles of the sum of all detected tomato (poly)phenol metabolites in rat serum presented similar behaviours for LT and NLT treatments (Fig. 1a), and this included a pronounced serum peak at 2 h, decreasing sharply until 7 h and then, in the case of LT, it decreased progressively until 48 h where no metabolite was detected, while in the case of NLT a slight serum peak reappeared at 24 h, detecting only one metabolite at 48 h. However, substantial differences were noted between treatments. The overall serum metabolite concentration varied statistically after ingestion of LT and NLT at each of the time points studied (Table 3). In fact, NLT presented an overall metabolite

Table 1
Concentration of (poly)phenolic compounds in local (LT) and non-local (NLT) Ekstasis tomatoes. The results are expressed as μg/g dw ± SD ($n = 3$).

Compound	LT	NLT
Flavonoids		
Kaempferol-*O*-rutinoside [a]	3.51 ± 0.48	9.27 ± 1.25*
Kaempferol-*O*-rutinoside-*O*-pentoside [a]	4.81 ± 0.58	11.22 ± 2.02*
Luteolin-*O*-hexoside-*C*-hexoside [a]	34.76 ± 3.6	21.2 ± 1.02*
Naringenin	1.64 ± 0.27	2.18 ± 0.47
Naringenin chalcone [b]	1.12 ± 0.20	1.43 ± 0.11
Phloretin 3,5- di-*C*-β-glucopyranoside [c]	107.97 ± 11.5	51.19 ± 4.19*
Quercetin-*O*-dihexoside [a]	5.87 ± 0.76	5.20 ± 0.34
QHRP-*O*-hexoside [a]	1.79 ± 0.43	1.24 ± 0.56
QHRP-coumaric acid [a]	21.16 ± 1.13	10.13 ± 1.57*
QHRP-ferulic acid [a]	10.98 ± 0.4	6.86 ± 0.12*
QHRP-sinapic acid [a]	4.24 ± 0.16	3.66 ± 0.37
QHRP-syringic acid [a]	6.55 ± 0.81	8.28 ± 1.54
Quercetin-*O*-rutinoside-*O*-hexoside [a]	2.52 ± 0.27	0.87 ± 0.13*
Quercetin-*O*-rutinoside-*O*-pentoside [a]	81.03 ± 2.80	64.25 ± 5.82*
Rutin	111.33 ± 1.11	86.38 ± 11.77*
Total, flavonoids	*399.32 ± 16.15*	*283.37 ± 30.38**
Caffeic and dihydrocaffeic acid derivatives		
Caffeic acid derivative I [d]	33.24 ± 1.12	65.55 ± 17.50*
Caffeic acid derivative II [d]	28.10 ± 2.15	14.81 ± 3.34*
Caffeic acid derivative III [d]	13.95 ± 0.68	24.97 ± 6.96
Caffeic acid derivative IV [d]	4.19 ± 0.10	5.37 ± 0.47*
Caffeic acid derivative V [d]	4.79 ± 0.41	4.91 ± 0.98
Caffeoylmalic acid [d]	62.93 ± 3.55	66.80 ± 9.10
Dihydrocaffeic acid derivative [f]	38.14 ± 2.12	32.09 ± 5.61
Total, caffeic and dihydrocaffeic acid derivatives	*185.34 ± 10.14*	*214.49 ± 43.97*
Free phenolic acids		
Caffeic acid	27.30 ± 3.03	40.92 ± 1.25*
Dihydrocaffeic acid	n.d.	8.72 ± 2.73*
p-Coumaric acid	15.57 ± 1.14	16.19 ± 2.02
Salicylic acid	29.04 ± 3.22	54.39 ± 12.34*
Total, free phenolic acids	*71.90 ± 7.39*	*120.22 ± 18.34**
Hydroxybenzoic acid derivatives		
Dihydroxybenzoic acid-*O*-pentoside [g]	24.44 ± 1.46	18.30 ± 1.64*
Hydroxybenzoic acid-*O*-hexoside [g]	40.36 ± 1.28	33.59 ± 3.08*
Syringic acid-*O*-hexoside [g]	37.53 ± 0.93	37.53 ± 5.59
Total, hydroxybenzoic acid derivatives	*102.34 ± 3.66*	*89.42 ± 10.30*
Hydroxycinnamic acid derivatives		
Caffeic acid-*O*-hexoside I [g]	169.42 ± 5.07	235.81 ± 8.06*
Caffeic acid-*O*-hexoside II [g]	67.52 ± 4.03	60.86 ± 5.82
Caffeic acid-*O*-hexoside III [g]	194.60 ± 4.23	159.57 ± 19.72*
Coumaric acid derivative	14.08 ± 1.85	15.00 ± 1.15
Coumaric acid-*O*-hexoside I [g]	87.86 ± 2.09	132.38 ± 1.72*
Coumaric acid-*O*-hexoside II and III [g]	161.79 ± 7.83	113.07 ± 0.57*
Dicaffeoyl-*O*-hexoside [g]	72.09 ± 1.97	109.38 ± 14.05*
Ferulic acid-*O*-hexoside [g]	72.85 ± 2.58	24.30 ± 2.35*
Sinapic acid-*O*-hexoside [g]	27.05 ± 2.16	31.30 ± 3.01
Total, hydroxycinnamic acid derivatives	*867.26 ± 31.81*	*881.67 ± 56.44*
Hydroxycinnamoylquinic acids		
3-*O*-Caffeoylquinic acid	30.09 ± 4.38	44.24 ± 4.39*
4-*O*-Caffeoylquinic acid	223.02 ± 7.43	247.29 ± 7.59*
5-*O*-Caffeoylquinic acid	200.14 ± 33.63	280.73 ± 9.04
Caffeoylquinic acid-*O*-hexoside I [j]	39.64 ± 2.68	37.24 ± 5.62
Caffeoylquinic acid-*O*-hexoside II [j]	52.89 ± 5.94	49.50 ± 5.53
Coumaroylquinic acid [j]	93.38 ± 9.33	96.80 ± 4.70
Dicaffeoylquinic acid I [i]	88.27 ± 3.34	103.29 ± 8.83
Dicaffeoylquinic acid II [h]	39.97 ± 1.91	68.08 ± 1.60*
Dicaffeoylquinic acid III [i]	75.77 ± 2.30	155.79 ± 0.01*
Dicaffeoylquinic acid-*O*-hexoside [h]	44.75 ± 2.45	37.09 ± 1.56*
Feruloylquinic acid [j]	31.76 ± 1.34	31.81 ± 2.03
Tricaffeoylquinic acid [h]	177.11 ± 3.55	390.57 ± 61.65*
Tricaffeoylquinic acid-*O*-hexoside [i]	53.05 ± 4.62	17.93 ± 0.69*
Total, hydroxycinnamoylquinic acids	*1149.83 ± 82.90*	*1560.35 ± 113.23**
Phenylpropanoic acid-glycosides		
Dihydrocaffeic acid-*O*-hexoside I [g]	63.44 ± 3.84	74.47 ± 4.54
Dihydrocaffeic acid-*O*-hexoside II [g]	144.74 ± 4.37	160.71 ± 14.94
Dihydrocaffeoyl-caffeoyl-*O*-hexoside [g]	79.63 ± 4.81	95.00 ± 18.17
Dihydroferulic acid-*O*-hexoside [g]	152.76 ± 9.93	94.48 ± 3.04*
Hydroxyphenylpropionic acid-*O*-hexoside [g]	145.74 ± 6.55	171.36 ± 0.72*
Total, phenylpropanoic acid-glycosides	*586.30 ± 29.49*	*596.02 ± 41.42*
Total, (Poly)phenolic compounds	***3362.30 ± 189.92***	***3745.54 ± 315.02***

* Indicates a significant difference ($p < 0.05$) between LT and NLT by the Student's *t*-test. Abbreviations: n.d., not detected; QHRP, Quercetin-*O*-hexoside-*O*-rhamnoside-*O*-pentoside.

concentration equal to 619.5 nM 2 h after administration, whereas LT presented a lower overall serum concentration (367.1 nM). Moreover, the overall metabolite serum concentration was observed at higher levels in NLT in all time points. It is important to note that the overall metabolite concentration at 24 h in NLT was 52.70 nM, which represents a 3.8-fold increase in serum with respect to LT group (14.10 nM). This trend is consistent with the higher intake of phenolics containing caffeoyl groups in NLT rodents than LT ones, especially for caffeoylquinic acids. Indeed, all circulating phenolic metabolites could be derived from caffeic acid metabolism, although native ferulic, dihydrocaffeic and dihydroferulic acids in tomatoes could be subjected to direct phase II metabolism concurring thus to increase the overall phenolic metabolite concentration in bloodstream. Instead, no phase II metabolites of flavonoids have been detected in serum samples, in keeping with the very low amounts of flavonoids provided by both LT and NLT.

3.2.1. Cinnamic acid derivatives

Cinnamic acid derivatives represented the main group of metabolites detected and quantified. Among them, 3′-methoxycinnamic acid-4′-sulfate and hydroxycinnamic acid-sulfate II were the major (poly) phenolic metabolites in both LT and NLT, their levels being much lower after LT administration. These metabolites determined the kinetic profile of this metabolite group in serum. The serum kinetic profile was similar in the first 7 h following administration of LT or NLT to rats, both showed a very noticeable peak at 2 h, after that, LT intake continued to gradually decrease while NLT intake showed another weakly pronounced serum peak at 24 h (Fig. 1b). Both LT and NLT had a lack of detection of these metabolites at 48 h, except for hydroxycinnamic acid-sulfate II, recovered at trace level after NLT intake. Glucuronide conjugates of this group were not detected at any time points after LT and NLT administration, except for 4′-hydroxycinnamic acid-3′-glucuronide, which was quantified during the first 7 h after tomato ingestion.

3.2.2. Phenylpropanoic acid derivatives

The serum kinetic profile of total phenylpropanoic acid derivatives, which corresponds to two metabolites that only occurred as sulfate conjugates, was slightly different from the profile of cinnamic acid derivatives (Fig. 1c). Indeed, concerning LT administration, similar serum metabolite concentrations were observed at 2 and 4 h after administration and then decreased progressively until undetectable at 48 h, while as regards NLT administration, it showed a pronounced peak at 2 h, decreasing until 7 h, then a slight peak at 24 h and a void of detection at 48 h. It is important to note that the circulating metabolites at 4 and 7 h post-consumption of NLT was lower than LT. This differed from the pattern observed in the rest of the time points studied and in cinnamic acid derivatives that were higher for NLT at all time points evaluated.

3.3. Pharmacokinetic parameters after tomato administration

Table 3 shows the pharmacokinetic parameters of the 7 metabolites detected in serum after tomato administration. The C_{max} values of LT-derived metabolites ranged from 10.2 to 143.4 nM, while NLT-derived metabolites ranged from 19.7 to 259.1 nM; being higher in those of NLT than LT, except for 3-(4′-hydroxyphenyl)propanoic acid-3′-sulfate which did not vary significantly between treatments. These C_{max} values were reached for most of the metabolites at 2 h after tomato administration (T_{max}), except for 3-(4′-hydroxyphenyl)propanoic acid-3′-sulfate that reached its maximum concentration at 4 h. These T_{max} values led to suppose an absorption and metabolism mainly in the small intestine rather than colon. Furthermore, like the C_{max} values, the area-under-the-curve ($AUC_{0\text{-}48}$) was greater for all metabolites of NLT than LT, except

Table 2
Concentration of tomato-derived phenolic metabolites in a pool of rat serum at 2, 4, 7, 24, and 48 h after the ingestion of 3 g/kg bw local (LT) or non-local (NLT) Ekstasis tomatoes. Data expressed as mean values ± SD ($tr = 3$).

Metabolite	Intervention	Serum concentration (nM)				
		2 h	4 h	7 h	24 h	48 h
Cinnamic acid derivatives						
3́-methoxycinnamic acid-4́-sulfate	LT	143.42 ± 0.76*	39.05 ± 0.38*	7.64 ± 0.24*	9.67 ± 0.60*	n.d.
	NLT	211.17 ± 3.96	66.83 ± 2.14	11.86 ± 0.39	41.19 ± 0.35	n.d.
4́-hydroxycinnamic acid-3́-glucuronide [a]	LT	29.96 ± 0.80*	4.31 ± 0.22*	0.76 ± 0.00*	n.d.	n.d.
	NLT	54.14 ± 3.30	14.27 ± 1.41	0.60 ± 0.03	n.d.	n.d.
4́-methoxycinnamic acid-3́-sulfate [b]	LT	10.18 ± 0.05*	4.80 ± 0.09*	1.46 ± 0.03*	0.43 ± 0.03*	n.d.
	NLT	19.72 ± 0.54	11.63 ± 0.69	2.49 ± 0.09	0.90 ± 0.07	n.d.
Hydroxycinnamic acid sulfate I [c]	LT	16.64 ± 0.53*	2.98 ± 0.40*	0.73 ± 0.10	0.08 ± 0.08	n.d.
	NLT	31.92 ± 0.17	10.88 ± 0.79	1.16 ± 0.14	0.18 ± 0.01	n.d.
Hydroxycinnamic acid sulfate II [c]	LT	132.31 ± 7.63*	40.02 ± 0.25*	4.76 ± 0.85*	0.43 ± 0.11*	n.d.*
	NLT	259.05 ± 6.23	119.36 ± 6.90	11.98 ± 0.14	1.42 ± 0.07	0.34 ± 0.30
Phenylpropanoic acid derivatives						
3-(3́-methoxyphenyl)propanoic acid-4́-sulfate	LT	12.79 ± 0.22*	8.71 ± 0.57	2.71 ± 0.00	1.84 ± 0.25*	n.d.
	NLT	21.32 ± 0.45	7.62 ± 0.92	1.92 ± 0.37	4.96 ± 0.12	n.d.
3-(4́-hydroxyphenyl)propanoic acid-3́-sulfate	LT	21.81 ± 0.59*	25.71 ± 0.52*	6.44 ± 0.27*	1.66 ± 0.11*	n.d.
	NLT	22.23 ± 0.25	25.96 ± 0.13	4.68 ± 0.09	4.04 ± 0.09	n.d.
Total metabolites	LT	*367.11 ± 10.59**	*125.58 ± 2.43**	*24.49 ± 1.49**	*14.10 ± 1.17**	*0.00 ± 0.00**
	NLT	*619.54 ± 14.90*	*256.56 ± 12.98*	*34.69 ± 1.25*	*52.70 ± 0.71*	*0.34 ± 0.30*

Values in a column with * are significantly different ($p < 0.05$) between LT and NLT ingestion by the Student's *t*-test. Abbreviations: n.d., not detected; tr, technical replicates.

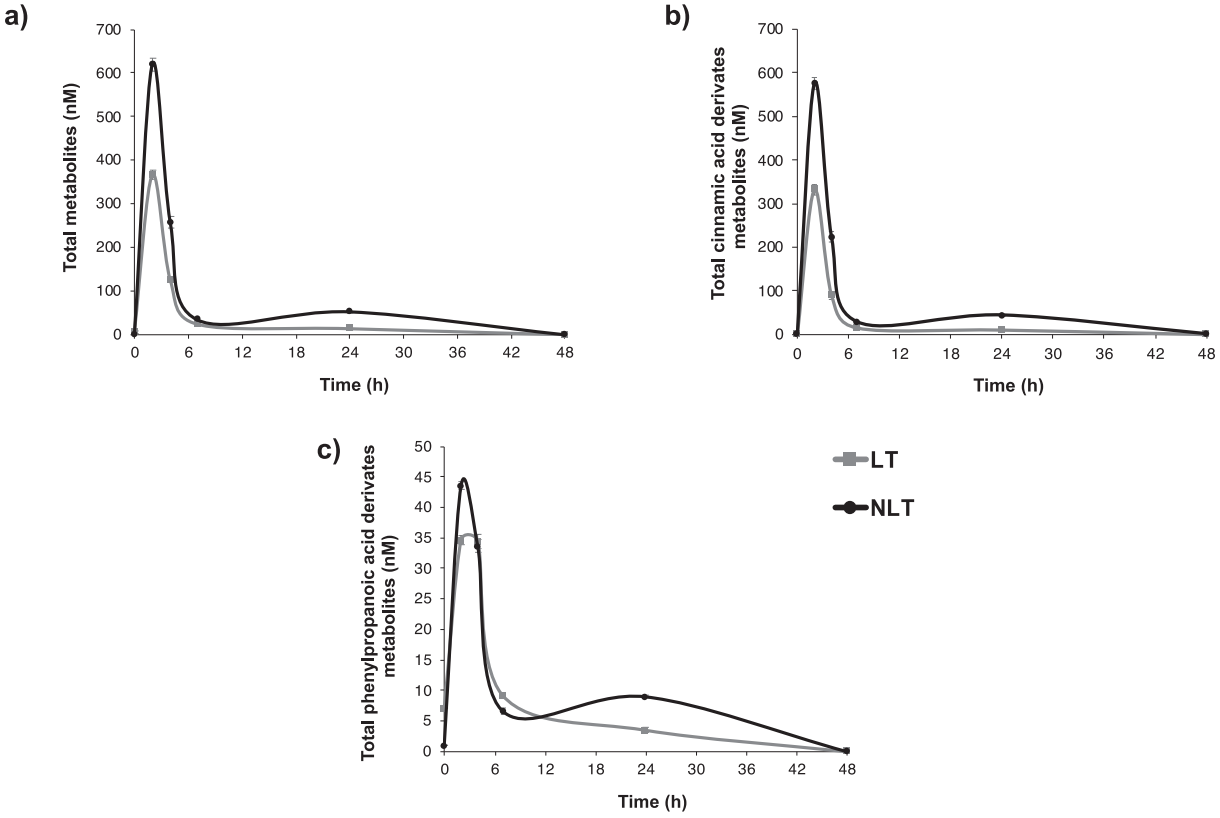

Fig. 1. Serum pharmacokinetic profile of phenolic metabolites after LT and NLT administration: (a) total metabolites; (b) total cinnamic acid metabolites; (c) total phenylpropanoic acid metabolites. Concentrations (nM ± SD) were quantified using a uHPLC-MS^n method in a pool of rat serum at 2, 4, 7, 24 and 48 h after the consumption of 3 g/kg bw of tomatoes.

for 3-(4′-hydroxyphenyl)propanoic acid-3′-sulfate which did not differ. Indeed, the highest $AUC_{0\text{-}48}$ was observed for 3′-methoxycinnamic acid-4′-sulfate, reaching in NLT group a value 1.9 times higher than LT one. Furthermore, 3 of the 7 metabolites differed statistically in terms of mean residence time ($MRT_{0\text{-}48}$) after consuming LT or NLT, with higher MRT values after consuming NLT.

4. Discussion

This work aimed to characterize the (poly)phenol composition of two tomatoes cultivated at different locations and to elucidate whether the geographical origin of tomato cultivation can modulate (poly)phenols bioavailability and metabolism in rats. To ensure that the only independent variable was the cultivation location, Ekstasis tomatoes,

Table 3
Pharmacokinetic parameters of phenolic metabolites in a pool of rat serum after the ingestion of 3 g/kg bw of local (LT) or non-local (NLT) Ekstasis tomatoes. Data expressed as mean values ± SD ($tr = 3$).

Metabolite	Intervention	C_{max} (nM)	T_{max} (h)	$AUC_{0\text{-}48}$ (nM × h)	$MRT_{0\text{-}48}$ (h)
3-(3́-methoxyphenyl) propanoic acid-4́-sulfate	LT	12.79 ± 0.22*	2	90.06 ± 3.97*	13.96 ± 1.19*
	NLT	21.32 ± 0.45	2	122.96 ± 4.06	33.46 ± 3.91
3-(4́-hydroxyphenyl) propanoic acid-3́-sulfate	LT	25.71 ± 0.52	4	186.36 ± 3.75	7.93 ± 0.22*
	NLT	25.96 ± 0.13	4	190.57 ± 1.82	15.75 ± 0.25
3́-methoxycinnamic acid-4́-sulfate	LT	143.42 ± 0.76*	2	543.08 ± 5.20*	11.34 ± 0.54*
	NLT	211.17 ± 3.96	2	1058.20 ± 12.42	30.32 ± 0.26
4́-hydroxycinnamic acid-3́-glucuronide	LT	29.96 ± 0.80*	2	71.84 ± 1.05*	2.47 ± 0.02
	NLT	54.14 ± 3.30	2	144.86 ± 3.03	2.55 ± 0.06
4́-methoxycinnamic acid-3́-sulfate	LT	10.18 ± 0.05*	2	50.53 ± 0.64*	7.13 ± 0.22
	NLT	19.72 ± 0.54	2	100.99 ± 3.15	7.23 ± 0.42
Hydroxycinnamic acid-sulfate I	LT	16.64 ± 0.53*	2	48.71 ± 0.40*	3.72 ± 0.58
	NLT	31.92 ± 0.17	2	104.21 ± 0.36	3.63 ± 0.01
Hydroxycinnamic acid-sulfate II	LT	132.31 ± 7.63*	2	415.88 ± 22.19*	3.36 ± 0.06
	NLT	259.05 ± 6.23	2	969.55 ± 33.37	4.37 ± 0.43

Values in a column with * are significantly different ($p < 0.05$) between LT and NLT ingestion by the Student's *t*-test. Abbreviations: AUC, area-under-the-curve serum concentration–time; C_{max}, maximum serum concentration; MRT, mean residence time; T_{max}, time of peak serum concentration; tr, technical replicates.

conventionally grown in two locations in Spain: in the northeast (local tomatoes LT) and in the southeast (non-local tomatoes NLT), were used. To our knowledge, the findings of the present study give the most detailed information on tomato (poly)phenol characterization as well as their absorption and metabolism in rats. In fact, in our study, relevance has been given to these less studied components, as Hanson et al. (Hanson et al., 2004) have shown that among tomato antioxidants, such as lycopene, β-carotene, vitamin C and (poly)phenolic compounds, the last ones were the most tightly related to antiradical capacity and inhibition of lipid peroxidation, which suggests that (poly)phenolic compounds may contribute strongly to tomato antioxidant activities.

The (poly)phenolic profiles of both tomatoes were consistent with the major (poly)phenolic subclasses in several tomato varieties (Asensio et al., 2019; Barros et al., 2012; Cruz-Carrión et al., 2021; Martínez-Valverde et al., 2002; Minoggio et al., 2003). In our work, 4-*O*-caffeoylquinic acid was the most abundant compound in LT, while tri-caffeoylquinic acid was the most abundant in NLT, also coinciding with the most abundant compounds found in four tomato varieties in northeastern Portugal homegardens (Barros et al., 2012). Among quantified flavonoids, phloretin 3′,5′-di-*C*-β-glucopyranoside and rutin were the predominant compounds in both tomatoes comprising 49 to 55% of the total flavonoid content, in agreement with these results found by Slimestad et al. (Slimestad et al., 2008) in different tomato types. Total flavonoid content varied significantly between LT and NLT, being higher in the former, which is consistent with the fact that flavonoid content varies according to the origin of the crop (Asensio et al., 2019; Slimestad et al., 2008; Stewart et al., 2000). On the other hand, NLT was characterized by a higher total phenolic acid content compared to its local counterpart, this is in agreement with Asensio et al. (Asensio et al., 2019) who revealed significant effects of location in the concentration of some phenolic acids, *i.e.*, caffeic, chlorogenic, ferulic and *p*-coumaric acid, from Spanish traditional tomato. Similarly, our results are in line with studies conducted by San José et al. (San José et al., 2014) where they found that the environment plays a key role in determining the phenolic composition of eggplant fruits.

The *in vivo* absorption of (poly)phenolics compounds after LT and NLT acute administration was evaluated in Wistar rat serum at different times. It is well documented that dose administration and food matrix are key factors modulating the bioavailability and metabolism of (poly) phenolic compounds (Bohn, 2014; Margalef et al., 2014). In order to assess whether the consumption of tomato fruit from two distinct geographical origins, *i.e.,* LT or NLT, has a differential impact on their (poly)phenolic bioavailability, we administered the same amount of lyophilized LT or NLT (3 g/kg bw) to the animals. Blood collection points were defined on the basis that early collection times (*i.e.*, 2 to 4 h) provide information on the absorption from the small intestine, while later time points (*i.e.,* 7 to 48 h) provide information on their colonic metabolism (Margalef et al., 2016). It should be noted that different studies have evaluated the bioavailability and metabolism of tomato (poly)phenols (Kamiloglu et al., 2013; Kolot et al., 2019; Martínez-Huélamo et al., 2015, 2016; Ohkubo et al., 2017), but the effect of crop origin on tomato (poly)phenol bioavailability, to our knowledge, has not been previously evaluated. In the present study, a total of seven metabolites were quantified in rat serum following consumption of LT or NLT. It could be hypothesized that the synergy of the factors modulating phenolic bioavailability, such as environmental factors, food-related factors, interactions between food components, chemical properties and host-related factors, led to detection of theses metabolites (Bohn, 2014; Del Rio et al., 2013). Cinnamic acid and phenylpropanoic acid metabolites detected in this study were mainly recovered as sulfate and methyl-sulfate conjugates. Actually, when comparing glucuronide and sulfate or methyl-sulfate metabolites, independently of the intervention, both sulfate and methyl-sulfate conjugate forms were clearly more prevalent than the glucuronide ones. Indeed, sulfation is generally a higher-affinity, lower-capacity pathway than glucuronidation, so that when the ingested dose increases, a shift from sulfation toward glucuronidation occurs (Manach et al., 2004). Our results contrasted with the trend observed for the "*liso rojo rama*" tomato variety, where four metabolites of phenolic compounds were identified in plasma and the glucuronide form was clearly more prevalent (Martínez-Huélamo et al., 2015). This fact may be attributed to differences in dosing, metabolites quantified, tomato varieties and the model used (Del Rio et al., 2013). Moreover, nM C_{max} were attained in ≤ 4 h for all metabolites, indicating absorption from the small intestine, most of which had a relative short $MRT_{0\text{-}48}$ as they were rapidly removed from the bloodstream.

In this work, relevant differences in the (poly)phenolic absorption and metabolism in rat serum after LT and NLT acute administration were identified. Pharmacokinetic values give insight into behaviour of (poly) phenolic metabolites that will be circulating due to tomato intake, which will be valuable information to understand possible bioactivities and health effects. Epidemiological studies confirm that the presence of different antioxidant molecules in tomatoes lead cancer-preventive properties by decreasing serum lipid levels and low-density lipoprotein oxidation (Martí et al., 2016). Indeed, anti-cancer properties of chlorogenic and caffeic acids from tomatoes have been evaluated. In one such study by Yang et al. (Yang et al., 2012), the chlorogenic acid was found to be able to induce apoptosis by reducing mitochondrial membrane potential levels and increasing activation of caspase-3-pathways *in vitro* in human U937 Leukemia Cells. In another such study by Rajendra-Prasad et al. (Rajendra Prasad et al., 2011), the caffeic acid was found to be able to inhibit cancer cell proliferation by oxidative mechanism in human HT-1080 fibrosarcoma cell line. Despite the interest of these works, it is well-known that chlorogenic and caffeic acids are not able to reach those cell tissues as they are transformed by gut

microbiota and phase II enzymes. Therefore, the research community should be very cautious when designing future studies and should take into account the physiological context of the metabolism of phenolic compounds. When comparing kinetic parameters, the highest C_{max} values of all metabolites were evidenced after NLT administration. Similarly, the exposure to tomato (poly)phenols (AUC_{0-48}) was markedly higher when animals were administered with NLT. The reason for these varying metabolite profiles can be attributed to the fact that the metabolites identified could be derived from phenolic acids and NLT was characterized as having the highest amount of this phenolic subclass, mainly caffeic acid. It was observed that after ingestion of LT or NLT, total (poly)phenolic metabolites are metabolized and absorbed in a similar, but not identical, manner. In fact, the highest concentrations were reached at 2 h, with a second less pronounced peak at 24 h, always showing a higher concentration after NLT administration, while at 7 h the concentrations were closest (LT 25 nM and NLT 34 nM). The 24-h peak serum could be explained by the action of colon microbiota towards dicaffeoyl- and tricaffeoylquinic acids, that reached higher content in NLT than LT, concurring thus to a slight increase of serum phenolic metabolites in NLT rats than LT ones. The factors influencing variability in the appearance of metabolites in serum, which may be related to other dietary components of tomatoes such as non-digestible carbohydrates, should be explored along with the actions of the absorbed metabolites (Jaganath et al., 2006). In this sense, LT contained a higher protein content, and as is known, (poly)phenols are known to form complexes with proteins, resulting in changes in the structural, functional and nutritional properties, and digestibility of both compounds (Ozdal et al., 2013). Trombley et al. (Trombley et al., 2011) have suggested that bioavailability of plant (poly)phenols may be influenced by the covalent interaction between (poly)phenols and proteins. Indeed, it has been speculated that high amounts of protein may limit the availability and fermentation of (poly)phenols and the formation of metabolites from the microbiota through complexation (Bohn, 2014). This could partially explain the lower metabolite levels found after 24 h of LT tomato consumption. This statement is consistent with other studies in which a decrease in the bioavailability of black tea (poly)phenols was observed due to the effect of protein-phenol interactions (Van Der Burg-Koorevaar et al., 2011). However, the mechanisms of interactions between tomato (poly)phenols and proteins should be investigated.

At the level of metabolite groups, specifically, with regard to caffeic acid derivatives, *i.e.,* hydroxycinnamic acid sulfate I and II, and 4′-hydroxycinnamic acid-3′-glucuronide, higher concentrations of metabolites were identified after NLT ingestion. The reason could be attributed to the fact that NLT showed higher concentrations of chlorogenic acids, mainly 3-*O*-, 4-*O*- and 5-*O*-caffeoylquinic acids. Literature data regarding the absorption of chlorogenic acid in its intact form are still fragmentary and not exhaustive (Bugianesi et al., 2004). Moreover, the highest serum concentration of cinnamic acid derivatives was found at 2 h after tomato administration, and this was similar for both LT and NLT. In both cases, 3′-methoxycinnamic acid-4′-sulfate accounted for greater than 91% of the cinnamic acids found in serum at 2 h, the serum appearance of 3′-methoxycinnamic acid-4′-sulfate was largely due of caffeic acid conjugations by rat catechol-*O*-methyltransferases and sulfotransferases. Lastly, the content of phenylpropanoic acid derivatives peaked a second time in serum 24 h after tomato administration in both treatment groups, especially in NLT. The higher total phenylpropanoic acid-glycosides content in NLT could contribute to this higher concentration. In particular, regarding the other main phenylpropanoic acid-glycoside catabolites, dihydrocaffeic acid derivatives (3-(3′-hydroxyphenyl)propanoic acid-4′-sulfate and 3-(4′-hydroxyphenyl)propanoic acid-3′-sulfate), it has been suggested that these compounds are able to scavenge intracellular reactive oxygen species (Del Rio et al., 2013), which may contribute to the known antioxidant capacity of tomatoes, among other biological activities. It is important to note that the bioavailability and metabolism of (poly)phenolic compounds are the principal limiting factors of their bioactivity (Bohn, 2014). Therefore, the differences described in this work could be related to relevant variations in the biological effects generated by consuming tomatoes produced in different areas. However, a limitation of this study was that only the circulating levels of (poly)phenolic compounds after tomatoes intake was studied. Therefore, further studies are needed to evaluate their distribution, metabolism and excretion.

In conclusion, this study demonstrated that the differences in the (poly)phenolic and nutritional composition of Ekstasis tomatoes from two geographical origins of cultivation led to different (poly)phenolic kinetic profiles in rat serum. As a result of these differences on absorption and metabolism of tomato (poly)phenols, it is suggested that the health-promoting effects of consuming tomatoes could differ depending on their growing location. Lastly, further human intervention trials are required to corroborate these results and to correlate individual tomato (poly)phenols with its putative health effects.

CRediT authorship contribution statement

Álvaro Cruz-Carrión: Formal analysis, Methodology, Writing – original draft. **Luca Calani:** Formal analysis, Methodology, Writing – review & editing. **Ma. Josefina Ruiz de Azua:** Investigation. **Pedro Mena:** Supervision, Methodology, Funding acquisition, Writing – review & editing. **Daniele Del Rio:** Supervision, Funding acquisition. **Manuel Suárez:** Conceptualization, Funding acquisition, Writing – review & editing. **Anna Arola-Arnal:** Conceptualization, Funding acquisition, Writing – review & editing. All authors critically revised the paper and read an approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.132984.

References

Ardid-Ruiz, A., Ibars, M., Mena, P., Del Rio, D., Muguerza, B., Bladé, C., … Suárez, M. (2018). Potential involvement of peripheral leptin/STAT3 signaling in the effects of resveratrol and its metabolites on reducing body fat accumulation. *Nutrients, 10*(11), 1–16. https://doi.org/10.3390/nu10111757

Asensio, E., Sanvicente, I., Mallor, C., & Menal-Puey, S. (2019). Spanish traditional tomato. Effects of genotype, location and agronomic conditions on the nutritional quality and evaluation of consumer preferences. *Food Chemistry*, *270*(July 2018), 452–458. https://doi.org/10.1016/j.foodchem.2018.07.131.

Barros, L., Dueñas, M., Pinela, J., Carvalho, A. M., Buelga, C. S., & Ferreira, I. C. F. R. (2012). Characterization and Quantification of Phenolic Compounds in Four Tomato (Lycopersicon esculentum L.) Farmers' Varieties in Northeastern Portugal Homegardens. *Plant Foods for Human Nutrition, 67*(3), 229–234. https://doi.org/10.1007/s11130-012-0307-z

Bohn, T. (2014). Dietary factors affecting polyphenol bioavailability. *Nutrition Reviews, 72*(7), 429–452. https://doi.org/10.1111/nure.12114

Bugianesi, R., Salucci, M., Leonardi, C., Ferracane, R., Catasta, G., Azzini, E., & Maiani, G. (2004). Effect of domestic cooking on human bioavailability of naringenin,

chlorogenic acid, lycopene and β-carotene in cherry tomatoes. *European Journal of Nutrition, 43*(6), 360–366. https://doi.org/10.1007/s00394-004-0483-1

Calani, L., Ounnas, F., Salen, P., Demeilliers, C., Bresciani, L., Scazzina, F., … Del Rio, D. (2014). Bioavailability and metabolism of hydroxycinnamates in rats fed with durum wheat aleurone fractions. *Food and Function, 5*(8), 1738–1746. https://doi.org/10.1039/c4fo00328d

Cruz-Carrión, Á., de Azua, M. J. R., Mulero, M., Arola-Arnal, A., Suárez, M., Ruiz de Azua, M. J., … Suárez, M. (2020). Oxidative Stress in Rats is Modulated by Seasonal Consumption of Sweet Cherries from Different Geographical Origins: Local vs. Non-Local. *Nutrients, 12*(9), 2854. https://doi.org/10.3390/nu12092854

Cruz-Carrión, Á., Ruiz de Azua, M. J., Bravo, F. I., Aragonès, G., Muguerza, B., Suárez, M., & Arola-Arnal, A. (2021). Tomatoes consumed in-season prevent oxidative stress in Fischer 344 rats: Impact of geographical origin. *Food & Function*. https://doi.org/10.1039/D1FO00955A

Del Rio, D., Rodriguez-Mateos, A., Spencer, J. P. E., Tognolini, M., Borges, G., & Crozier, A. (2013). Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants & Redox Signaling, 18*(14), 1818–1892. https://doi.org/10.1089/ars.2012.4581

Hanson, P. M., Yang, R. Y., Wu, J., Chen, J. T., Ledesma, D., Tsou, S. C. S., & Lee, T. C. (2004). Variation for antioxidant activity and antioxidants in tomato. *Journal of the American Society for Horticultural Science, 129*(5), 704–711. https://doi.org/10.21273/jashs.129.5.0704

Iglesias-Carres, L., Mas-Capdevila, A., Bravo, F. I., Aragonès, G., Arola-Arnal, A., & Muguerza, B. (2019). A comparative study on the bioavailability of phenolic compounds from organic and nonorganic red grapes. *Food Chemistry, 299*(June). https://doi.org/10.1016/j.foodchem.2019.125092

Jaganath, I. B., Mullen, W., Edwards, C. A., & Crozier, A. (2006). The relative contribution of the small and large intestine to the absorption and metabolism of rutin in man. *Free Radical Research, 40*(10), 1035–1046. https://doi.org/10.1080/10715760600771400

Kamiloglu, S., Boyacioglu, D., & Capanoglu, E. (2013). The effect of food processing on bioavailability of tomato antioxidants. *Journal of Berry Research, 3*(2), 65–77. https://doi.org/10.3233/JBR-130051

Kay, C. D., Clifford, M. N., Mena, P., McDougall, G. J., Andres-Lacueva, C., Cassidy, A., … Crozier, A. (2020). Recommendations for standardizing nomenclature for dietary (poly)phenol catabolites. *American Journal of Clinical Nutrition, 112*(4), 1051–1068. https://doi.org/10.1093/ajcn/nqaa204

Kolot, C., Rodriguez-Mateos, A., Feliciano, R., Bottermann, K., & Stahl, W. (2019). Bioavailability of naringenin chalcone in humans after ingestion of cherry tomatoes. *International Journal for Vitamin and Nutrition Research, 90*(5–6), 411–416. https://doi.org/10.1024/0300-9831/a000574

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. *American Journal of Clinical Nutrition, 79*(5), 727–747. https://doi.org/10.1093/ajcn/79.5.727

Margalef, M., Guerrero, L., Pons, Z., Bravo, F. I., Arola, L., Muguerza, B., & Arola-Arnal, A. (2014). A dose-response study of the bioavailability of grape seed proanthocyanidin in rat and lipid-lowering effects of generated metabolites in HepG2 cells. *Food Research International, 64*, 500–507. https://doi.org/10.1016/j.foodres.2014.07.019

Margalef, M., Iglesias-Carres, L., Pons, Z., Bravo, F. I., Muguerza, B., & Arola-Arnal, A. (2016). Age related differences in the plasma kinetics of flavanols in rats. *Journal of Nutritional Biochemistry, 29*, 90–96. https://doi.org/10.1016/j.jnutbio.2015.11.007

Margalef, M., Pons, Z., Bravo, F. I., Muguerza, B., & Arola-Arnal, A. (2015). Plasma kinetics and microbial biotransformation of grape seed flavanols in rats. *Journal of Functional Foods, 12*, 478–488. https://doi.org/10.1016/j.jff.2014.12.007

Martí, R., Roselló, S., & Cebolla-Cornejo, J. (2016). Tomato as a source of carotenoids and polyphenols targeted to cancer prevention. *Cancers, 8*(6). https://doi.org/10.3390/CANCERS8060058

Martínez-Huélamo, M., Tulipani, S., Estruch, R., Escribano, E., Illán, M., Corella, D., & Lamuela-Raventós, R. M. (2015). The tomato sauce making process affects the bioaccessibility and bioavailability of tomato phenolics: A pharmacokinetic study. *Food Chemistry, 173*, 864–872. https://doi.org/10.1016/j.foodchem.2014.09.156

Martínez-Huélamo, M., Vallverdú-Queralt, A., Di Lecce, G., Valderas-Martínez, P., Tulipani, S., Jáuregui, O., … Lamuela-Raventós, R. M. (2016). Bioavailability of tomato polyphenols is enhanced by processing and fat addition: Evidence from a randomized feeding trial. *Molecular Nutrition and Food Research, 60*(7), 1578–1589. https://doi.org/10.1002/mnfr.201500820

Martínez-Valverde, I., Periago, M. J., Provan, G., Chesson, A., & Provan, G. (2002). Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (Lycopersicum esculentum). *Journal of the Science of Food and Agriculture, 82*(3), 323–330. https://doi.org/10.1002/jsfa.1035

Mena, P., Sánchez-Salcedo, E. M., Tassotti, M., Martínez, J. J., Hernández, F., & Del Rio, D. (2016). Phytochemical evaluation of eight white (Morus alba L.) and black (Morus nigra L.) mulberry clones grown in Spain based on UHPLC-ESI-MSn metabolomic profiles. *Food Research International, 89*, 1116–1122. https://doi.org/10.1016/j.foodres.2016.06.012

Minoggio, M., Bramati, L., Simonetti, P., Gardana, C., Iemoli, L., Santangelo, E., … Pietta, P. G. (2003). Polyphenol pattern and antioxidant activity of different tomato lines and cultivars. *Annals of Nutrition and Metabolism, 47*(2), 64–69. https://doi.org/10.1159/000069277

Ohkubo, A., Chida, T., Kikuchi, H., Tsuda, T., & Sunaga, K. (2017). Effects of tomato juice on the pharmacokinetics of CYP3A4-substrate drugs. *Asian Journal of Pharmaceutical Sciences, 12*(5), 464–469. https://doi.org/10.1016/j.ajps.2017.05.004

Ozdal, T., Capanoglu, E., & Altay, F. (2013). A review on protein-phenolic interactions and associated changes. *Food Research International, 51*(2), 954–970. https://doi.org/10.1016/j.foodres.2013.02.009

Rajendra Prasad, N., Karthikeyan, A., Karthikeyan, S., & Venkata Reddy, B. (2011). Inhibitory effect of caffeic acid on cancer cell proliferation by oxidative mechanism in human HT-1080 fibrosarcoma cell line. *Molecular and Cellular Biochemistry, 349*(1–2), 11–19. https://doi.org/10.1007/S11010-010-0655-7/FIGURES/6

Ricci, A., Cirlini, M., Calani, L., Bernini, V., Neviani, E., Del Rio, D., Galaverna, G., & Lazzi, C. (2019). In vitro metabolism of elderberry juice polyphenols by lactic acid bacteria. *Food Chemistry*, *276*(July 2018), 692–699. https://doi.org/10.1016/j.foodchem.2018.10.046.

San José, R., Sánchez-Mata, M. C., Cámara, M., & Prohens, J. (2014). Eggplant fruit composition as affected by the cultivation environment and genetic constitution. *Journal of the Science of Food and Agriculture, 94*(13), 2774–2784. https://doi.org/10.1002/jsfa.6623

Slimestad, R., Fossen, T., & Verheul, M. J. (2008). The flavonoids of tomatoes. *Journal of Agricultural and Food Chemistry, 56*(7), 2436–2441. https://doi.org/10.1021/jf073434n

Stewart, A. J., Bozonnet, S., Mullen, W., Jenkins, G. I., Lean, M. E. J., & Crozier, A. (2000). Occurrence of flavonols in tomatoes and tomato-based products. *Journal of Agricultural and Food Chemistry, 48*(7), 2663–2669. https://doi.org/10.1021/jf000070p

Trombley, J. D., Loegel, T. N., Danielson, N. D., & Hagerman, A. E. (2011). Capillary electrophoresis methods for the determination of covalent polyphenol-protein complexes. *Analytical and Bioanalytical Chemistry, 401*(5), 1527–1533. https://doi.org/10.1007/s00216-011-4846-1

Van Der Burg-Koorevaar, M. C. D., Miret, S., & Duchateau, G. S. M. J. E. (2011). Effect of milk and brewing method on black tea catechin bioaccessibility. *Journal of Agricultural and Food Chemistry, 59*(14), 7752–7758. https://doi.org/10.1021/jf2015232

Yang, J.-S., Liu, C.-W., Ma, Y.-S., Wwng, S.-W., Tang, N.-Y., Wu, S.-H., Ji, B.-C., Ma, C.-Y., Ko, Y.-C., Funayama, S., & Kuo, C.-L. (2012). Chlorogenic Acid Induces Apoptotic Cell Death in U937 Leukemia Cells through Caspase- and Mitochondria-dependent Pathways. *In Vivo*, *26*(6), 971 LP – 978. http://iv.iiarjournals.org/content/26/6/971.abstract.

Zhang, Y., Huo, M., Zhou, J., & Xie, S. (2010). PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. *Computer Methods and Programs in Biomedicine, 99*(3), 306–314. https://doi.org/10.1016/j.cmpb.2010.01.007