Food Chemistry 130 (2012) 581–590

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Oil matrix effects on plasma exposure and urinary excretion of phenolic compounds from tomato sauces: Evidence from a human pilot study

Sara Tulipani [a], Miriam Martinez Huelamo [a], Maria Rotches Ribalta [a,e], Ramon Estruch [b,c], Elvira Escribano Ferrer [d], Cristina Andres-Lacueva [a,e], Montserrat Illan [a], Rosa M. Lamuela-Raventós [a,c,*]

[a] Department of Nutrition and Food Science-CeRTA, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain
[b] Department of Internal Medecine, Clinical Hospital, Institute of Biomedical Investigation August Pi i Sunyer (IDIBAPS), University of Barcelona, Spain
[c] CIBER 06/003 Physiopathology of Obesity and Nutrition (CIBEROBN), and RETICS RD06/0045/0003, Institute of Health Carlos III, Spain
[d] Department of Pharmacy and Pharmaceutical Technology, Biopharmaceutics and Pharmacokinetics Unit, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain
[e] Ingenio CONSOLIDER Program, FUN-C-FOOD (CSD2007-063), Barcelona, Spain

ARTICLE INFO

Article history:
Received 20 September 2010
Received in revised form 21 May 2011
Accepted 20 July 2011
Available online 27 July 2011

Keywords:
Matrix effects
Tomato sauce
Polyphenols
Pharmacokinetics
Phase II metabolites

ABSTRACT

The health-promoting effects attributable to dietary phenolic compounds strongly depend on their bioaccesibility from the food matrix and their consequent bioavailability. We carried out a pilot randomized controlled cross-over study to evaluate the effect of addition of an oil matrix during tomato sauce processing, on the bioavailability of tomato phenolics. Healthy subjects consumed a single dose of tomato sauce elaborated without oil (OO-F) and with the addition of 5% virgin olive oil (VOO-E) or refined olive oil (ROO-E). Plasma and urine samples were subjected to solid-phase extraction, followed by HPLC–MS/MS analysis. Six phenolic compounds, three aglycones (naringenin, ferulic and caffeic acids) and their corresponding glucuronide metabolites, were identified and quantified in urine after the ingestion of the tomato sauces. Two of the six phenolic urinary metabolites were also quantified in plasma samples. Only after ingestion of the oil-enriched tomato sauces, did the glucuronide metabolites of naringenin show a bi-phasic profile of absorption in plasma, suggesting that the lipid matrix added to the sauce may stimulate the occurrence of re-absorption events by enterohepatic circulation, potentially enhancing the apparent plasma half-life of the flavanone prior to excretion. The interindividual response variability observed underlies the need for further large-scale investigations.

1. Introduction

The Mediterranen diet is an example of a dietary regime associated with a reported low incidence of cancers, CVD, and an overall reduction in mortality (Chrysohoou, Panagiotakos, Pitsavos, Das, & Stefanadis, 2004; Estruch et al., 2006; Knoops et al., 2006). The health-promoting effects of this diet have been mainly attributed to the high consumption of the typical fruit and vegetables of the Mediterranean basin (i.e. leafy green vegetables and tomatoes). Tomato (*Lycopersicon esculentum* Mill., Solanaceae) and tomato processed products (sauce, paste, puree and juice) are typical components, besides being a dietary staple for humans in many other parts of the world. Another major characteristic of the Mediterranean diet is a high consumption of olive oil as the main source of monounsaturated fatty acids, which has long been reported to exhibit numerous biological functions beneficial for the state of health (Cicerale, Lucas, & Keast, 2010). The combination of tomatoes with olive oil, in food preparation, is a typical daily habit, and tomato sauce, the most

Abbreviations: ALA, aldehydic form of ligstroside aglycone; ALA ox, aldehydic form of ligstroside aglycone oxidized; AOA, aldehydic form of oleuropein aglycone; AOA ox, aldehydic form of oleuropein aglycone oxidized; AUC_{last}, area under the plasma concentration-*versus*-time curve from time 0 until the last detectable concentration; CVD, cardiovascular disease; CAD, collision-activated dissociation; CE, collision energy; DP, declustering potential; 3,4-DHPEA, hydroxytyrosol or 3,4-dihydroxyphenylethanol; 3,4-DHPEA-Ac, hydroxytyrosol-acetate; 3,4-DHPEA-EDA, hydroxytyrosol-elenolic acid di-aldehyde; DLA, dialdehydic form of ligstroside aglycone; DOA, dialdehydic form of oleuropein aglycone linked to 3,4-DHPEA-EDA oxidized; FP, focusing potential; FS, full scan; *p*-HPEA, tyrosol or *p*-hydroxyphenylethanol; *p*-HPEA-EDA, tyrosol–elenolic acid di-aldehyde; LOD, limit of detection; C_{max}, maximum plasma concentration; MRT, mean residence time; MI, molecular ion; NL, neutral loss; OO-F, olive oil-free tomato sauce; PFD, polyphenol-free diet; PrIS, precursor ion scan; PIS, product ion scan; ROO-E, refined olive oil-enriched; SPE, solid-phase extraction; SEM, standard error; T_{max}, time to reach the maximum plasma concentration; TFD, tomato-free diet; Q_{∞}, the maximum excreted amount in the 24 h urine collection; VOO-E, virgin olive oil-enriched.

* Corresponding author at: Department of Nutrition and Food Science-CeRTA, Faculty of Pharmacy, University of Barcelona, Av. Joan XXIII s/n, 08028 Barcelona, Spain. Tel.: +34 934024508; fax: +34 934035931.
E-mail address: lamuela@ub.edu (R.M. Lamuela-Raventós).

0308-8146/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2011.07.078

extensively consumed tomato product worldwide, is commonly elaborated and commercialized, both as an oil-free and oil-enriched product. The protective effects of a regular consumption of raw tomatoes and tomato sauce (Giovannucci, 1999) have long been attributed to the most pronounced bioactives in tomato, such as lycopene and other carotenoids (Agarwal & Rao, 2000). Recently, the high content of phenolic compounds such as flavonoids and hydroxycinnamic acids in tomato has been gaining interest because of the multiple biological effects they seem to express, ranging from free radical-scavenging, metal chelation, inhibition of cellular proliferation, and modulation of enzymatic activity and signal transduction pathways (Crozier, Jaganath, & Clifford, 2009). The flavanone, naringenin (4′,5,7-trihydroxyflavanone) and the hydroxycinnamate, chlorogenic acid (5-caffeoylquinic acid) (Slimestad, Fossen, & Verheul, 2008; Vallverdú-Queralt, Jáuregui, Medina-Remón, Andrés-Lacueva, & Lamuela-Raventós, 2010) are among the most abundant phenolics in tomato, and the most extensively studied.

It is known that the extent of the protective effect of the phenolic compounds *in vivo* strongly depends on their bioaccesibility and their consequent exposition in the organism, on their intestinal absorption and presystemic metabolism, up to their systemic distribution and potential interaction with target tissues. In turn, the food matrix, in which the phenolic compounds are contained, is one of the most important factors governing the *in vivo* bioavailability of these compounds. It plays a crucial role in determining their accessibility and extractability from food, and thus their absorption, metabolism and their final biological action in the human body (Ortuño et al., 2010).

Despite growing knowledge of the effects of the thermic and mechanical treatments involved in tomato sauce processing on carotenoid and vitamin bioavailability (Graziani et al., 2003; Unlu et al., 2007), little is known about the changes in the polyphenol/flavonoid content of tomatoes after their processing to tomato sauce (Bugianesi et al., 2004; Vallverdú-Queralt, Medina-Remón, Andrés-Lacueva, & Lamuela-Raventós, 2011). Furthermore, as the combination of tomatoes and tomato products with a lipid matrix has been reported to favour the extractability and bioaccessibility of tomato carotenoids (Fielding, Rowley, Cooper & O'Dea, 2005; Graziani et al., 2003), recent investigations have also shown that the fat content in a meal may enhance the bioavailability of the flavonol quercetin, due to a better solubility of the relatively lipophilic flavonoid in the intestinal tract in the presence of fat (Lesser, Cermak, & Wolffram, 2004). The typical addition of oil, during tomato sauce processing, may influence the bioavailability of the phenolics contained in tomato by modifying their bioaccessibility from the food matrix, modulating the gastric emptying and/or the intestinal and hepatic metabolism of the absorbed phenolic compounds. However, few studies have yet investigated the absorption and excretion of phenolic compounds from raw tomatoes and tomato sauces, and even less information is currently available on the impact of tomato–olive oil combination, during processing, on the bioaccessibility and human absorption of the phenolic compounds contained in the sauce (Capanoglu, Beekwilder, Boyacioglu, Hall, & de Vos, 2008; Graziani et al., 2003).

The aim of the present study was to investigate whether the absorption and metabolism of the tomato phenolics is influenced by the addition of a lipid matrix during tomato sauce processing, and eventually by the oil typology. For these purposes, we carried out a randomized controlled cross-over study, administering (to human volunteers) a single dose of tomato sauce without oil (OO-F) and tomato sauces elaborated with the addition of virgin olive oil (VOO-E) or refined olive oil (ROO-E) during processing.

2. Materials and methods

2.1. Standards and reagents

Chlorogenic acid, caffeic acid, ferulic acid, isoferulic acid, *p*-hydroxybenzoic acid, protocatechuic acid, *m*- and *p*-coumaric acids, gallic acid, naringenin-7-*O*-glucoside, naringin (naringenin-7-*O*-rhamnoglucoside), quercetin, rutin (quercetin-7-*O*-rutinoside), kaempferol and blank human plasma were purchased from Sigma–Aldrich (St. Louis, MO, USA). Naringenin (4′,5,7-trihydroxyflavanone), the internal standard ethylgallate (IS, added to sample before extraction) and the external standard taxifolin (ES, added to sample after extraction) were purchased from Extrasynthese (Genay, France). HPLC-grade methanol, acetonitrile and formic acid were purchased from Scharlau Chemie S. A. (Barcelona, Spain), and *o*-phosphoric acid (85%) and hydrochloric acid (37%) from Panreac Quimica SA (Barcelona, Spain). Ultrapure water (Milli-Q) was obtained from a Millipore system (Millipore, Bedford, MA, USA).

2.2. Tomato sauce material

A commercial tomato (*L. esculentum* L.) variety, suitable for tomato sauce elaboration, was used for the study. Virgin and refined olive oils were kindly furnished by Juan Ballester Rosés company (S.A., Tortosa, Spain). The OO-F, VOO-E and ROO-E tomato sauces were processed at Torribera campus, University of Barcelona (UB, Barcelona, Spain) by a standardized industrial scale-like making process. Fruits were washed, chopped in a breaker unit and weighed. The VOO and the ROO were rapidly heated up to 110 °C before adding the chopped tomatoes (5% of oil, w/w), and the mixture was cooked at 99 °C for 90 min. The chopped tomatoes were crushed into pulp, which was then separated from seeds and skin, cooled down, aliquotted and stored in vacuum bags at −20 °C until the day of the test. Approximately 1000 g of fresh tomatoes yielded 500 g of tomato sauce. Five percent of water was added to the final OO-F tomato sauce product, in order to standardize the amount of tomato compounds ingested by each intervention.

2.3. Preparation of extracts

For the extraction of phenolic compounds from the starting raw tomatoes and the tomato sauces, 5 ml of 80% ethanol in ultrapure water (v/v), acidified with 0.1% formic acid, were added to 0.5 g of sample. The mixture was vortexed for 1 min and then sonicated for 5 min on ice. After centrifugation at 900*g* for 20 min at 4 °C, the supernatant was collected; a further 5 ml of the acidified 80% ethanol/water solution were added to the pellet, and the extraction procedure repeated. The two supernatants were combined, and the ethanolic component was evaporated to dryness by a sample concentrator (Techne, Duxford, Cambridge, UK) at room temperature under a stream of nitrogen gas. After filtration of the acqueous extracts (~2 ml) with 4 mm, 0.45 μm PTFE syringe filters (Waters Corporation, USA), 50 μl of the resulting filtrate were injected into the LC/MS/MS system, in triplicate.

2.4. Subjects and study design

A total of five healthy men, aged between 25 and 36 (BMI = 25 ± 1.2 kg/m^2), volunteered for this randomised crossover dietary study. The study was explained to subjects through verbal and written instructions, and written informed consent was obtained before participation. Only male subjects were recruited, in order to reduce anthropometric variables and the menstrual cycle phase-related variability in premenopausal women, putatively affecting the absorption, metabolism and excretion of tomato