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The tomato sauce making process affects the bioaccessibility and bioavailability of tomato phenolics: A pharmacokinetic study

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ABSTRACT

Tomato sauce is the most commonly consumed processed tomato product worldwide, but very little is known about how the manufacturing process may affect the phenolic composition and bioavailability after consumption. In a prospective randomised, cross-over intervention study, we analysed the plasma and urinary levels of tomato phenolic compounds and their metabolites after acute consumption of raw tomatoes and tomato sauce, enriched or not with refined olive oil during production.

Respectively, eleven and four phenolic metabolites were found in urine and plasma samples. The plasma concentration and urinary excretion of naringenin glucuronide were both significantly higher after the consumption of tomato sauce than raw tomatoes. The results suggest that the mechanical and thermal treatments during tomato sauce manufacture may help to deliver these potentially bioactive phenolics from the food matrix more effectively than the addition of an oil component, thus increasing their bioavailability.

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1. Introduction

It is generally recognised that part of the health-promoting effects of the Mediterranean diet may be attributed to the high content of phytochemical constituents in fruit and vegetables, which are among the food items at the bottom of the Mediterranean diet pyramid (Estruch et al., 2013). Phytochemical phenolic compounds have recently attracted increasing attention (Scoditti et al., 2012) and epidemiologic data have shown an inverse association between the risk of overall mortality or cardiovascular diseases and the consumption of polyphenol-rich foods (Cassidy et al., 2013).

Several *in vitro* and human intervention studies have been carried out to unveil the mechanisms of action underlying the health-promoting properties of polyphenols, which include the induction of antioxidant defenses (Wan et al., 2001), blood pressure lowering

Abbreviations: AUC_{last}, area under the plasma concentration-*versus*-time curve from time 0 until the last detectable concentration; BMI, body mass index; BW, body weight; CAD, collision-activated dissociation; CE, collision energy; DP, declustering potential; EP, entrance potential; AUMC, first moment curve; FP, focusing potential; FS, full scan; HCl, hydrochloric acid; HPLC–ESI-QqQ-MS/MS, high performance liquid chromatography coupled to tandem mass spectrometry; IS, internal standard; LPD, low polyphenol diet; $Q_{u\infty}$, maximum excreted amount in the 24 h urine collection; C_{max}, maximum plasma concentration; MRT_{last}, mean residence time; MRM, multiple reaction monitoring; NL, neutral loss; N_2, nitrogen; OF, olive oil-free tomato sauce; PFD, polyphenol-free diet; PTFE, polytetrafluoroethylene; PrIS, precursor ion scan; PIS, product ion scan; ROOE, refined olive oil-enriched tomato sauce; CL_{ren}, renal clearance; SPE, solid-phase extraction; SEM, standard error; t_{max}, time to reach the maximum plasma concentration; TFD, tomato-free diet.

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effects (Desch et al., 2010), the improvement of endothelial function (Widlansky et al., 2007), the inhibition of platelet aggregation and LDL oxidation, and the modulation of inflammatory response (Zhu et al., 2013).

Tomatoes (*Solanum lycopersicum*, formerly *Lycopersicum esculentum*) are one of the key components of the Mediterranean diet, and their regular consumption has been consistently associated with a lower risk of several types of cancer and coronary heart disease (Paran, Novack, Nir Engelhard, & Hazan-Halevy, 2009). Tomatoes are widely consumed both as fresh fruit and as processed products. Tomato sauce is the most commonly consumed tomato product worldwide and particularly in Spain, where it represents 40.8% of all sauce consumption.

The processing of raw tomatoes into tomato sauces involves several thermal and mechanical treatments and may include the addition of a lipid matrix (commonly up to 5% of olive oil) during production (oil-enriched *versus* oil-free sauce) (Chanforan, Loonis, Mora, Caris-Veyrat, & Dufour, 2012). All these steps may affect the final phenolic composition of the end product. The addition of a lipid matrix during tomato sauce preparation, as well as the simultaneous consumption of fats and triglycerides, have been reported to favour the extractability and bioavailability of tomato carotenoids (Fielding, Rowley, Cooper, & O'Dea, 2005). The combined intake of tomato and a lipid component may increase the absorption of fat-soluble compounds, since dietary fat is important for micelle formation in the small intestine, thus contributing to the solubilisation of fat-soluble compounds in the bile salts and their incorporation into the micelles (Hornero-Méndez & Mínguez-Mosquera, 2007). However, to date few studies have investigated the absorption and excretion of phenolic compounds from raw tomatoes and tomato sauces (Tulipani et al., 2012), and even less is known about the impact of a tomato-olive oil combination during processing on the phenolic bioavailability and bioefficacy in humans.

The working hypothesis of the present study was that domestic or industrial-scale processing in tomato sauce production may produce changes in phenolic extractability due to the disruption of the plant cell wall and thus result in an easier release of bound polyphenolic and flavonoid compounds (van het Hof, West, Weststrate, & Hautvast, 2000). As already hypothesised for tomato carotenoids, the addition of oil during tomato sauce processing may also influence the bioavailability of the relatively lipophilic phenolics in tomato, by modifying their bioaccessibility from the food matrix, and modulating the gastric emptying or hepatic metabolism of the absorbed phenolic compounds. The aim of the present study was to investigate whether the human bioavailability of the phenolic compounds found in tomatoes is influenced by the sauce making process and the addition of refined olive oil during manufacture.

2. Materials and methods

2.1. Standards and reagents

Caffeic acid, chlorogenic acid, dihydrocaffeic acid, ferulic acid, gallic acid, isoferulic acid, kaempferol, *m*-coumaric acid, naringenin-7-*O*-glucoside, *p*-coumaric acid, *p*-hydroxybenzoic acid, protocatechuic acid, quercetin, rutin and human plasma were purchased from Sigma–Aldrich (St. Louis, MO, USA); and naringenin and ethylgallate (internal standard (IS)) from Extrasynthese (Genay, France). HPLC-grade ethanol, acetonitrile, methanol and formic acid were purchased from Scharlau Chemie S.A. (Barcelona, Spain), while hydrochloric acid 37% (HCl) and *o*-phosphoric acid 85% were supplied by Panreac Quimica S.A. (Barcelona, Spain). Ultrapure water (Milli-Q) was obtained from a Millipore system (Millipore, Bedford, MA, USA).

2.2. Tomato and tomato sauce

Commercial tomatoes, "liso rojo rama" variety, used for both the elaboration of the two sauces to the intervention of raw tomato, were purchased from a local market (Barcelona, Spain). Refined olive oil was kindly furnished by Juan Ballester Rosés Company S.A. (Tortosa, Spain). To ensure that the refined olive oil added did not introduce any phenolic compounds to the sauce, a liquid–liquid extraction was used and the extract was analysed by HPLC with a diode array detector (Boselli, Di Lecce, Strabbioli, Pieralisi, & Frega, 2009) obtaining a polyphenol-free profile. Raw tomato sauce ("oil-free": OF) and a refined olive oil-enriched tomato sauce (ROOE) were processed at the Torribera Campus, University of Barcelona (Santa Coloma de Gramanet, Spain) by a standardised industrial scale-like manufacturing process, as previously described by Tulipani et al. (2012). Briefly, tomatoes were cleaned, cleaved, mixed and weighted. For the elaboration of ROOE sauce, 5% of oil was added and the same amount of water was aggregated to obtain the OF sauce. The mixture was cooked for 60 min at 99 °C and finally crushed. The obtained sauces were vacuum-packed and stored in the freezer at −20 °C until the day of the study when were thawed in the refrigerator and administered at room temperature. Considering that 1000 g of fresh tomatoes yielded approximately 500 g of tomato sauce, 3.5 g of sauce per kg of body weight (BW) (3.5 g kg^{-1} BW) and 7 g kg^{-1} BW of raw tomatoes were administered to each volunteer across the three interventions, to standardise the intake.

2.3. Phenolic extraction of the dietary interventions

0.5 g of raw tomato or tomato sauce was weighed and 5 mL of 80% ethanol acidified with 0.1% formic acid was added to the samples. The mixture was vortexed for 1 min and then sonicated for 5 min on ice to prevent the degradation of the phenolic compounds. After centrifugation at 4000 rpm for 20 min at 4 °C, the supernatant was collected and another 5 mL of the acidified 80% ethanol solution was added to the pellet, and the extraction procedure repeated. Both 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. 400 ng mL^{-1} of ethylgallate (IS) was added to the aqueous extracts (2 mL), which were filtered with 4 mm 0.45 μm polytetrafluoroethylene (PTFE) syringe filters (Waters Corporation, USA), and injected (20 μL) in triplicate into the high performance liquid chromatography coupled to tandem mass spectrometry (HPLC–ESI-QqQ-MS/MS) (Vallverdú-Queralt, Jáuregui, Medina-Remón, & Lamuela-Raventós, 2012).

2.4. Subjects and study design

Eight healthy nonsmoking subjects (50% women, 27–46 years, BMI = 23 ± 0.93 kg m^{-2}) with no history of cardiovascular, hepatic or renal disease and no adherence to any special diets at least during the 4 weeks prior to the trial, were recruited for the randomised crossover dietary study. The study was explained to participants through verbal and written instructions, and written informed consent was obtained before participation. To standardise the baseline point, subjects were asked to follow a tomato-free diet (TFD) during the 3 days preceding the dietary intervention, and a low polyphenol diet (LPD) in the 24 h immediately preceding the test. The subjects were provided with a list of acceptable and unacceptable foods, together with two standardised low polyphenol meals for the evening preceding the test, and the day of the test. On the day of each intervention, all the subjects consumed together a standardised lunch, and were asked to fill in a 24-h food

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