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Human bioavailability and metabolism of phenolic compounds from red wine enriched with free or nano-encapsulated phenolic extract

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ABSTRACT

The impact of nano-encapsulation of a phenol extract from grape pomace on human plasma pharmacokinetic parameters and urine clearance of phenolic metabolites was examined. A dealcoholised red wine was used as the vehicle for enrichment with both non-encapsulated and nano-encapsulated phenol extracts in a randomised cross-over pharmacokinetic study. The analysis of plasma only showed an increase in the concentration of syringic acid sulphate, catechin sulphate and catechin glucuronide, whereas urine data, especially at interval of 0–6 hours, showed an increase in most of the metabolites from stilbenes, flavan-3-ols, phenolic acids and anthocyanins after the intake of phenol-enriched wines compared with the control wine. The nano-encapsulation of the extract slightly enhanced the bioavailability of malvidin-3-*O*-glucoside, as observed in the higher urine excretion of its native form and its microbial metabolites syringic and gallic acids. Metabolic pathways of different phenolic groups were proposed, with special emphasis on novel pathways of hydroxytyrosol generation.

1. Introduction

The concept of the French paradox was first described by Renaud and Lorgeril in 1992. Moderate wine drinking is part of the Mediterranean Diet, together with abundant and variable plant foods, high consumption of cereals, olive oil as the main (added) fat and a low intake of (red) meat. This healthy diet pattern involves a "Mediterranean way of drinking," which is a regular and moderate wine consumption (up to two glasses a day for men and one glass for women). This pattern of drinking increases longevity, reduces the risk of cardiovascular diseases and does not appreciably influence the overall risk of cancer (Giacosa et al., 2013). Moreover, it has been demonstrated recently that moderate consumption of red wine is associated with a lower prevalence of the metabolic syndrome in an elderly Mediterranean population with a high cardiovascular risk (Tresserra-Rimbau et al., 2015).

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Phenolic compounds from wine can be classified into flavonoids and non-flavonoids, their molecular weight ranging from that of phenolic acids to highly polymerised proanthocyanidins. The most abundant flavonoids in red wine include flavan-3-ols (catechin and epicatechin), anthocyanins (malvidin-3-O-glucoside), flavonols (quercetin), as well as lower proportions of flavanonols and flavones. The main non-flavonoids in red wine are phenolic acids, such as hydroxybenzoic acids (*p*-hydroxybenzoic and gallic acids) and hydroxycinnamic acids (caffeic, caftaric and *p*-coumaric acids), as well as other phenolic derivatives, including stilbenes (resveratrol and its glucoside form piceid) (Woraratphoka, Intarapichet, & Indrapichate, 2007). Wine production involves the generation of huge amounts of by-products (Ayoub, de Camargo, & Shahidi, 2016). These are a rich source of phenolic compounds and could be valorised for further use in the production of functional food ingredients or supplements with high nutritional value. Furthermore, this could also reduce the environmental impact of wine making (Makris, Boskou, & Andrikopoulos, 2007; Teixeira et al., 2014).

The sensorial characteristics of wines, including colour, flavour, tastiness and body, as well as bitterness and astringency, are strongly affected by phenolic compounds. High contents of these compounds in food could lead to rejection by consumers. Moreover, due to low water solubility, phenolic compounds often have a poor bioavailability (Munin & Edwards-Lévy, 2011) and are unstable in alkaline conditions, such as those found in some biological fluids (Dube, Ng, Nicolazzo, & Larson, 2010). In this sense, the emerging microencapsulation techniques would allow the stability and bioavailability of the phenolic compounds to be improved and the rate of active agent release to be controlled and could be a way to avoid unpleasant taste.

Nano- or micro-encapsulation is the process by which core materials enriched with bioactive compounds, such as antioxidants, enzymes, polyphenols and micronutrients, are packed into wall materials to form capsules to be delivered to the controlled target site and to protect them from an adverse environment (Gouin, 2004; Lee, Mijan, Ganesan, & Kwak, 2013). Capsules ranging from 3 to 800 μm in size are called microcapsules and the technology is called micro-encapsulation (Ahn, Chang, & Kwak, 2010). If the particle size ranges from 10 to 1000 nm, these are called nanospheres and the technology is termed nano-encapsulation (López, Gavara, & Lagaron, 2006). Nano- or micro-encapsulation technology is already well known in the fields of medicinal, pharmaceutical and cosmetic products and it is also emerging in the food industries (Huang, Yu, & Ru, 2010). Over recent years, the main applications of encapsulation in the food industry have been focused on improving the bioavailability of bioactive compounds, such as vitamins A and E, isoflavones, phytosterols, lycopene, and lutein (Gunasekaron & Ko, 2014).

Human bioavailability of flavanols and phenolic acids from enriched cocoa-nut creams (Vitaglione et al., 2013), and curcumin from enriched bread (Vitaglione et al., 2012), with free or encapsulated polyphenols, has previously been reported. In the case of wine, some studies have been performed regarding the encapsulation of grape by-products rich in phenolic compounds, such as grape seeds (Zhang, Mou, & Du, 2007) or grape pomace (Aizpurua-Olaizola et al., 2016). However, the effect of nano-encapsulation on the bioavailability of wine polyphenols has yet to be studied. Moreover, although many studies have focused on the bioavailability of specific phenolic compounds from wine, such as resveratrol or anthocyanins (Bell et al., 2000; Urpi-Sarda et al., 2007; Vitaglione et al., 2005), a complete pharmacokinetic study is needed that describes the plasma and urine parameters, taking into account all the phenolic groups present in grape or wine products.

In the current study, the impact of the nano-encapsulation of a phenol extract obtained from grape pomace on human plasma pharmacokinetic parameters and urine clearance of phenolic metabolites was investigated. For this purpose, a dealcoholised red wine was used as the vehicle for enrichment with both non-encapsulated and nano-encapsulated grape pomace extracts in a randomised cross-over human pharmacokinetic study. Additionally, a detailed study was performed to identify new metabolites and elucidate the individual metabolic pathways of the different phenolic groups in red wine.

2. Materials and methods

2.1. *Chemicals and reagents*

Standards of catechol (as the internal standard (IS)), *p*-hydroxybenzoic acid, protocatechuic acid, gallic acid, caffeic acid, syringic acid, ferulic acid, epicatechin and catechin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Quercetin, *trans*-resveratrol, dimer B_2, cyanidin-3-O-glucoside and malvidin-3-O-glucoside were purchased from Extrasynthese (Genay, France) and *p*-coumaric acid and vanillic acid from Fluka Co. (Buchs, Switzerland). Stock solutions of individual phenolic standard compounds were prepared by dissolving each compound in methanol at a concentration of 1000 mg/L. These were stored in amber glass flasks at 4 °C. Standard stock mixes of the phenolics were prepared weekly at a concentration of 50 mg/L dissolved in Milli-Q water.

Methanol (HPLC grade), acetonitrile (HPLC grade), acetone and acetic acid were purchased from Scharlau Chemie (Sentmenat, Barcelona, Spain). Ortho-phosphoric acid (85%) was acquired from Panreac (Barcelona, Spain). Ultrapure water was obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA).

The zein protein used for nano-encapsulation was supplied by CHEMOS GmbH & Co (Regenstauf, Germany). L-Lysine was from Sigma-Aldrich and maltodextrin (MD) Glucidex® 21D was from Roquette Frères (Lestrem, France). Food grade ethanol was purchased from Panreac.

2.2. *Dealcoholised red wine and phenolic extract*

Dealcoholised red wine, which was used as a matrix for phenolic enrichment, and the phenolic extract, prepared from grape pomace, were from Bodegas Miguel Torres, S.A. (Vilafranca del Penedès, Barcelona, Spain). The phenolic extract was prepared from grape pomace by Bodegas Miguel Torres, S.A., and the nano-encapsulation was performed by the CNTA (Centro Nacional de Tecnología y Seguridad Alimentaria, San Adrián, Navarra, Spain). The phenolic extract was analysed by