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Basic nutritional investigation

Bioaccessibility of pistachio polyphenols, xanthophylls, and tocopherols during simulated human digestion

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ABSTRACT

Objective: The bioaccessibility of bioactives from pistachios has not been previously evaluated. In the present study we quantified the release of polyphenols, xanthophylls (lutein), and tocopherols from pistachios (raw pistachios, roasted salted pistachios, and muffins made with raw pistachios) during simulated human digestion.
Methods: A dynamic gastric model of digestion that provides a realistic and predictive simulation of the physical and chemical processing and accurately mimics the residence time and the luminal environment within the human stomach was used for the digestion studies.
Results: More than 90% of the polyphenols were released in the gastric compartment, with virtually total release in the duodenal phase. No significant differences were observed between raw shelled and roasted salted pistachio. The presence of a food matrix (muffin) decreased the bioaccessibility of protocatechuic acid (78%) and luteolin (36%). Almost 100% bioaccessibility of lutein and tocopherols was found after duodenal digestion, with no difference among the three samples.
Conclusion: The rapid release of the assayed bioactives in the stomach maximizes the potential for absorption in the duodenum and contributes to the beneficial relation between pistachio consumption and health-related outcomes.

Introduction

Epidemiologic and clinical studies have demonstrated that nut consumption decreases the risk of cardiovascular disease: when subjects consumed test diets including mixed nuts, a 25% greater cholesterol-lowering response was found and this effect was attributed to the large proportion of unsaturated fatty acids present in nuts [1,2]. The results of three almond (50–100 g/d), two peanut (35–68 g/d), one pecan nut (72 g/d), and four walnut (40–84 g/d) studies have demonstrated a decrease in total cholesterol (2–16%) and low-density lipoprotein (LDL) cholesterol (2–19%) compared with control subjects [3]. For pistachios in particular, recent publications have shown beneficial effects on cardiovascular disease risk factors, lipid parameters, endothelial function, inflammation, and oxidative status [4,5]. In a randomized cross-over controlled feeding study, the inclusion of pistachios decreased total cholesterol, LDL cholesterol, non–high-density lipoprotein cholesterol, and plasma stearoyl-coenzyme A desaturase activity in a dose-dependent manner [4]. When pistachios were given to 32 normolipidemic healthy young men for 4 wk, significant decreases in blood glucose, total cholesterol, and serum interleukin-6 were observed, with improved endothelium vasodilation and total antioxidant status [5]. The consumption of pistachio nuts has been shown to significantly decrease oxidative stress, improving total cholesterol and its LDL levels in healthy volunteers [6]. Li et al. [7] showed pistachio consumption decreased plasma triacylglycerols and body weight when compared with a carbohydrate snack in obese subjects. Compared with other tree nuts, pistachios are very rich in phytosterols, potassium, vitamin B6, carotenoids, and tocopherols [8,9] and have been ranked among the 50 foods

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highest in antioxidants [10]. Extensive in vivo and in vitro experiments on the effect of phenolic compounds have shown beneficial health activities as protective agents against cancer and cardiovascular, inflammatory, and aging disorders, and human pathogens [11–13]. Catechins have been shown to be particularly effective in cardiovascular disease prevention and in decreasing the oxidation of LDL [14].

A major challenge in evaluating the role of individual health-promoting components in the pistachio is the lack of information on their behavior in the gastrointestinal (GI) tract and in particular on factors that influence their bioavailability. A component of bioavailability is bioaccessibility, which is defined as the relative amounts of nutrients or phytochemicals "released" from a complex food matrix in the lumen of the GI tract, and therefore could potentially be available for absorption into the body [15]. This process is highly dependent on the physical and chemical properties of the food matrix and the way these change during digestion [16]. The choice of food matrix is therefore crucial for testing the bioaccessibility of several compounds under simulated GI digestion. We recently showed that cell walls play a crucial role in regulating nutrient bioaccessibility from almonds and that polyphenols from almond skin are potentially available for absorption during digestion [15,17].

In this study, we characterized the polyphenols, carotenoids, and tocopherols in raw pistachios and roasted, salted pistachios and investigated their release during simulated human digestion in the gut. A dynamic gastric model of digestion that provides a realistic and predictive simulation of the physical and chemical processing and accurately mimics the transit time and the luminal environment within the human stomach was used for the digestion studies [18,19], together with a duodenal simulated digestion.

To assess the effect of the food matrix, muffins containing raw pistachios were used to investigate the bioaccessibility of polyphenols, xanthophylls, and tocopherols.

Materials and methods

Pistachios

Natural, raw, shelled pistachios (NPs; *Pistacia vera* L.) and roasted salted pistachio (RP) kernels (*P. vera* L.) from California were kindly provided by the American Pistachio Growers (Fresno, CA, USA). Home-made muffins containing NPs were prepared using the following ingredients: sugar (sucrose 178 g), butter at room temperature (118 g), eggs (two standard eggs), full-fat plain yogurt (240 g), white flour (470 g), baking soda (5 g), salt (1.2 g), and NPs (112 g). Each cooked muffin weighed 70 g and contained 12 g of NPs (whole nuts). Control muffins without pistachios were also prepared using the same ingredients.

Chemicals and enzymes

Egg L-α-phosphatidylcholine (lecithin grade 1, 99% purity) was obtained from Lipid Products (South Nutfield, Surrey, UK). Porcine gastric mucosa pepsin, bovine α-chymotrypsin, pancreatic α-amylase, porcine trypsin, porcine colipase, porcine pancreatic lipase, and bile salts were obtained from Sigma (Poole, Dorset, UK). Lipase for the gastric phase of digestion was a gastric lipase analogue of fungal origin from Amano Enzyme, Inc. (Nagoya, Japan).

Lutein and β-carotene standards were purchased from Extrasynthese (Genay, France). Polyphenol standards were purchased from Sigma-Aldrich (Milan, Italy).

All the solvents used were high-performance liquid chromatographic (HPLC) grade and purchased from Sigma-Aldrich.

Determination of polyphenols

Pistachio polyphenolic extracts were prepared as previously reported [20]. NPs, RPs, or pistachio muffins (10 g) were first homogenized with an Ultra-Turrax (IKA Works, Inc., Wilmington, DE, USA) and then extracted five times with *n*-hexane (100 mL) under constant agitation (2 h) to remove lipids. After filtration, the residues were mixed with 100 mL of methanol/HCl 0.1% (v/v), extracted, and centrifuged. The pellets were extracted four more times. All methanol fractions were combined and evaporated, after which the residues were dissolved in distilled water (40 mL) and extracted five times with ethyl acetate (40 mL). The organic phases were combined, dried with Na_2SO_4 for 20 min, and evaporated under vacuum.

For HPLC separations, an Ascentis Express C18 column (150 × 4.6 mm, 2.7 μm; Ascentis Express, Supelco, Bellefonte, PA, USA) was used. The mobile phase was water/formic acid (99.9:0.1, v/v; solvent A) and acetonitrile/formic acid (99.9:0.1, v/v; solvent B); the linear gradient profile was as follows: 0 min 0% B, 60 min 100% B, 70 min 100% B, and 71 min 0% B. The data were acquired using a photodiode array detector in the range of 190 to 400 nm and chromatograms were extracted at 270 nm and by mass spectrometry. The mass spectrometric acquisition was performed using electrospray ionisation in negative mode.

The results were expressed as milligrams per 100 g.

Carotenoid analysis

Milled NPs, RPs, or pistachio muffins (10 g) were extracted five times in the dark with *n*-hexane (100 mL) under magnetic stirring for 2 h at room temperature. All extracts were combined together and subjected to rotary evaporation to remove the solvent.

For the β-carotene determination, the five hexane portions were combined, filtered, concentrated, and made up to a known volume of dichloromethane to measure the β-carotene spectrophotometrically using a previously prepared calibration curve. The β-carotene was quantified using a Shimadzu UV-2410PC ultraviolet-visible spectrophotometer (Shimadzu Corp., Kyoto, Japan) at 450 nm.

For xanthophyll determinations, the oil obtained (5 g) was re-extracted by dimethylformamide (30 mL) and subsequently treated with five portions (10 mL) of *n*-hexane in a separating funnel. All chlorophylls, chlorophyll derivates, and xanthophylls were retained in the dimethylformamide phase, whereas lipids and carotenes were retained in the *n*-hexane phase. The dimethylformamide phase was treated with a 2% Na_2SO_4 solution at 0°C and transferred to a mixture (100 mL) of *n*-hexane/ethyl ether (1:1, v/v). The aqueous phase was discarded to remove polyphenols and other water-soluble compounds.

The organic phase was evaporated to dryness at 30°C. The dry residue was then dissolved in a mixture of methanol/methyl-*tert*-butyl ether (1:1, v/v) and analyzed by HPLC [21].

For HPLC separations, a YMC 30 (YMC Europe, Schermbeck, Germany) analytical column (250 × 2.1 mm inner diameter, 5-μm particle size) was used. The mobile phase consisted of a binary gradient of methanol (solvent A) and methyl-*tert*-butyl ether (solvent B), with the following gradients: 0 min 5% B, 30 min 45% B, 50 min 95% B, 50 to 55 min 5% B, and 55 to 60 min 5% B. Ultraviolet-visible spectra were acquired in the range of 325 to 750 nm and chromatograms acquired at 450 nm for the xanthophylls and 660 nm for the chlorophylls.

The results were expressed as milligrams per 100 g.

Tocopherol analysis

Weighed and milled NPs, RPs, or pistachio muffins (10 g) were extracted five times with *n*-hexane (100 mL) in the dark under magnetic stirring for 2 h at room temperature. All aliquots were combined and subjected to rotary evaporation to remove the solvent.

For HPLC separations, a microsilica column (Ascentis Supelco SI; 250 × 1.0 mm, 5-μm particle size) was used. The mobile phase consisted of *n*-hexane/isopropanol (99:1), the flow rate was 50 μL/min, and the injection volume was 2 μL.

The method was validated according to the Eurachem guidelines for each component, namely α-tocopherol, γ-tocopherol, and δ-tocopherol [22].

The results were expressed as milligrams per 100 g.

Simulated human digestion

Oral digestion

The aim of this procedure was to simulate the chewing of the pistachio meals in the mouth. This is the initial step in the digestion process and was designed to simulate the salivary amylase activity and mechanical breakdown of the food. The NPs or RPs (50 g) were minced three times using a mincer (Lexen, Grove City, OH, USA) to simulate the mechanical oral breakdown. Simulated salivary fluid (25 mL) at pH 6.9 (0.15 M NaCl, 3 mM urea) and human salivary amylase (900 U) dissolved in simulated salivary fluid (1 mL) were added to the minced pistachios and mixed for 20s. This produced a paste consisting of equal amounts of the solid and aqueous phases as calculated by human chewing (Institute of Food Research, unpublished data). The simulated oral processing of pistachio muffins was performed by mixing each minced muffin (70 g) with simulated salivary fluid (45 mL) and human salivary amylase (1260 U) to produce a paste that could be swallowed.

Gastric digestion

The dynamic gastric model of digestion incorporates the inhomogeneous gastric mixing, antral shearing, and rate of delivery to the duodenum with