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Evaluating the *in vitro* bioaccessibility of phenolics and antioxidant activity during consumption of dried fruits with nuts

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ABSTRACT

Dried fruits and nuts are considered as healthy snacks and they are often consumed together in the Turkish diet. In order to investigate the effect of codigestion of dried fruits (figs, apricots, raisins) together with nuts (almonds, walnuts, hazelnuts), total phenolics (TP) and total antioxidant capacity (TAC) have been evaluated spectrophotometrically at different phases of simulated gastrointestinal (GI) digestion, using an *in vitro* model. TP, ABTS and CUPRAC results revealed that for all fruit–nut mixtures, the amount recovered in the dialyzed fraction was lower than the recovery of fruits or nuts alone, indicating an antagonistic effect. On the other hand, DPPH results showed that for fig–walnut, fig–hazelnut and apricot–hazelnut mixtures the quantity recovered in the dialyzed fractions were 35–107% higher than the recovery of fruits or nuts alone, representing a synergistic effect. Similarly, FRAP results also demonstrated a synergistic effect in case of fig–walnut, apricot–walnut and apricot–hazelnut mixtures (10–74% higher recovery). Current study provides valuable insights into the changes taking place during *in vitro* GI digestion of dried fruits and nuts.

1. Introduction

Epidemiological studies have shown that the high intake of fruits and vegetables has been associated with a reduced the risk of chronic diseases, such as cardiovascular diseases, diabetes and cancer. These potential beneficial effects have been attributed to the presence of bioactive compounds, such as phenolics, that show antioxidant properties by acting as free radical scavengers or metal chelators, reducing the reactions that produce reactive oxygen species (Lemos, Siqueira, Arruda, & Zambiazi, 2012).

According to FAO statistics 2011, Turkey is the world's leading producer of many fruits and nuts including figs, apricots and hazelnuts (Anonymous, 2011). Fruits are often preserved in dried form since the stability of dried fruits has made it possible to transport them widely and to prolong their shelf life by preventing the development of some microorganisms responsible for deterioration of fresh foods (Santos & Silva, 2008). Moreover, as compared to fresh fruits, polyphenol content and antioxidant activity of dried fruits are expected to be higher due to their low moisture content and thus increased shelf life (Reddy, Sreeramulu, & Raghunath, 2010).

Different methodologies have been employed to evaluate the *in vitro* antioxidant capacity of fruits. Assays such as 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and cupric ion reducing antioxidant capacity (CUPRAC) are simple, cost effective, easily interpreted and display either reduction capacity (FRAP, CUPRAC) or direct free radical inhibition (DPPH and ABTS) (Wootton-Beard, Moran, & Ryan, 2011). It is recommended that at least two (or even all) of these assays should be combined to provide a reliable picture of the total antioxidant capacity of a foodstuff (Contreras-Calderon, Calderoon-Jaimes, Guerra-Hernaandez, & Garcia-Villanova, 2011).

In vitro digestion and dialysis methods for simulating the gastrointestinal (GI) condition are being extensively used since they are rapid, safe, and do not have the same ethical restrictions as *in vivo* methods (Liang et al., 2012). The impact of *in vitro* GI digestion on the stability of polyphenols has already been tested in many fruits (Bermudez-Soto, Tomas-Barberan, & Garcia-Conesa, 2007; Chiang, Kadouh, & Zhou, 2013; Kamiloglu & Capanoglu, 2013; Liang et al., 2012; McDougall, Dobson, Smith, Blake, & Stewart, 2005). The potential availability of antioxidants after

Abbreviations: ABTS, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; ANOVA, analysis of variance; CUPRAC, cupric ion reducing antioxidant capacity; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FAO, food and agriculture organization of the united nations; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalent; GI, gastrointestinal; HPLC, high performance liquid chromatography; IN, dialyzed fraction after intestinal digestion; OUT, non-dialyzed fraction after intestinal digestion; PG, compounds remaining after gastric digestion; TAC, total antioxidant capacity; TE, trolox equivalent; TP, total phenolic content.

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digestion is important as many studies have stated that the bioavailability of certain antioxidants is poor, which would in turn have a limited effect on health. Although it may be helpful to know the antioxidant capacity of fruits before digestion for comparative purposes, still this is not a true reflection of the potential health effects (Ryan & Prescott, 2010). Therefore, the biological properties of antioxidants may depend on their release from the food matrix during the digestion process (bioaccessibility) and may differ quantitatively and qualitatively from those produced by the chemical extraction employed in most studies (Serrano, Goni, & Saura-Calixto, 2007).

Dried fruits and nuts are considered as healthy snacks and they are often consumed together in the Turkish diet. To our knowledge, no previous study evaluated the impact of consumption of dried fruits together with nuts on bioaccessibility of polyphenols using an *in vitro* digestion procedure. Given the above, the aim of this study was to investigate the effect of codigestion of commonly consumed dried fruits together with nuts and to monitor the stability and recovery of total phenolics and antioxidant capacity using the ABTS, DPPH, FRAP and CUPRAC assays.

2. Materials and methods

2.1. Materials

The different dried fruits and nuts were collected as triplicates from a local market in Istanbul, Turkey. The varieties and origins of the samples are specified in Table 1. All fruits were sun-dried, whereas fruit–nut mixtures were prepared by blending by half. All samples were ground to a fine powder in liquid nitrogen using a pre-cooled grinder (IKA A11, Germany), and stored at −80 °C before analysis.

2.2. Chemicals

In this study, chemicals with analytical purity were used. For simulation of *in vitro* GI system, pepsin, pancreatin, bile salts, dialysis bags (Membra-Cel MD34) and sodium bicarbonate ($NaHCO_3$) were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany).

2.3. Chemical extraction

Three independent extractions for each sample were carried out as described previously by Capanoglu, Beekwilder, Boyacioglu, Hall, and De Vos (2008) with slight modifications. 2 ± 0.01 g of each sample was extracted with 5 ml of 75% aqueous-methanol containing 0.1% formic acid in a cooled ultrasonic bath (VWR ultrasonic cleaner, US) for 15 min. The treated samples were centrifuged (Hettich Zentrifugen Universal 32R, UK) for 10 min at 4 °C and 2700× *g* and the supernatants were collected. Another 5 ml of aqueous-methanol was added to the pellet and this procedure was repeated two more times. All three supernatants were combined and adjusted to a final volume of 15 ml. Prepared extracts were stored at −20 °C until analysis.

Table 1
Dried fruits, nuts and their mixtures examined in this study.

Sample	Scientific name	Variety/Origin	Abbreviation
Fig	*Ficus carica*	Sarilop/Aydin	F
Apricot	*Prunus armeniaca*	Hacihalioglu/Malatya	Ap
Raisin	*Vitis vinifera*	Sultana/Manisa	R
Almond	*Prunus dulcis*	Akbadem/Mugla	Al
Walnut	*Juglans regia*	Sebin/Giresun	W
Hazelnut	*Corylus avellana*	Tombul/Giresun	H
Fig–Almond			F–Al
Fig–Walnut			F–W
Fig–Hazelnut			F–H
Apricot–Almond			Ap–Al
Apricot–Walnut			Ap–W
Apricot–Hazelnut			Ap–H
Raisin–Almond			R–Al
Raisin–Walnut			R–W
Raisin–Hazelnut			R–H

2.4. In vitro gastrointestinal (GI) digestion

The *in vitro* GI digestion model adapted from McDougall et al. (2005) was performed in triplicate for each sample. Briefly, in a 250 ml beaker, 5 or 10 g (in case of mixtures) of samples were mixed with 20 ml distilled water and 1.5 ml pepsin solution. The pH was adjusted to 1.7 with addition of 5 M HCl. The mixture was incubated for 2 h in a Memmert shaking water bath (Nürnberg, Germany) at 37 °C and 100 rpm. After 2 h, 2 ml aliquots of the post-gastric (PG) digestion were collected. 4.5 ml of 4 mg/ml pancreatin and 25 mg/ml bile salt mixtures were added to the remainder in the glass beaker. Segments of dialysis bags were cut and filled with sufficient sodium $NaHCO_3$ to neutralize the sample's titratable acidity (pH:7). Again, after 2 h of incubation in shaking water bath at 37 °C and 100 rpm, the solution in the dialysis tubing was taken as the IN sample representing the material that entered the serum and the solution outside the dialysis bags were taken as the OUT sample representing material that remained in the GI tract. A blank was prepared with identical chemicals but without food matrix, and underwent the same conditions as the samples. PG, IN and OUT samples were stored at −20 °C until further analysis. Prior to analysis, samples were thawed and centrifuged at 23,000× *g* and the supernatants were filtered through a 0.45-μm-membrane filter and assayed for total phenolics and antioxidant capacity using a UV–Vis spectrophotometer (Optima SP-3000 nano, Tokyo, Japan).

2.5. Spectrophotometric assays

The total phenolic content (TP) was determined using Folin–Ciocalteu reagent as described previously by Velioglu, Mazza, Gao, and Oomah (1998). The TP of extracts was expressed as mg of gallic acid equivalent (GAE) per 100 g of sample.

The total antioxidant capacity (TAC) was estimated by four different assays. The 2,2-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazil (DPPH), ferric reducing antioxidant power (FRAP) and cupric ion reducing antioxidant capacity (CUPRAC) assays were performed according to Miller and Rice-Evans (1997), Kumaran and Karunakaran (2006), Benzie and Strain (1996), and Apak, Guclu, Ozyurek, and Karademir (2004), respectively. In all assays, Trolox was used as a standard and TAC of extracts was expressed in terms of mg of Trolox equivalent (TE) per 100 g of sample.

For each assay, samples were analyzed in triplicate and average values were reported.

2.6. Statistical analysis

Data were collected from three independent experiments and reported as mean ± SD. For multiple comparisons, data were subjected to statistical analysis using SPSS software (version 20.0) for the analysis of variance (ANOVA). Duncan's new multiple range test was used to analyze differences between treatments ($p < 0.05$). The correlation coefficients (R^2) for spectrophotometric assays were calculated by using the Microsoft Office Excel 2011 software (Microsoft Corporation, Redmond, WA).