LWT - Food Science and Technology 56 (2014) 284–289

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

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

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ARTICLE INFO

Article history:
Received 26 July 2013
Received in revised form
10 October 2013
Accepted 29 November 2013

keywords:
Dried fruits
Nuts
Codigestion
Phenolics
Antioxidant capacity

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.

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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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0023-6438/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.lwt.2013.11.040

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).

3. Results and discussion

3.1. Total phenolic content (TP)

The impact of GI digestion on TP is shown in Table 2. Initial results showed that among 15 samples, walnuts contained the highest amount of TP, which was approximately 30-fold higher than almonds, which had the lowest TP. After the gastric digestion (PG), there was an increase in TP (0.4–10 fold) for all samples besides walnuts, raisins and raisin–nut mixtures. This increase can be attributed to acidic hydrolysis of the phenolic glycosides to their aglycons during simulated gastric digestion. Our findings are in accordance with the results reported by Wootton-Beard et al. (2011) who also obtained an increase in TP of vegetables juices after gastric digestion. When the amount of TP after intestinal digestion was analyzed, 7–69% of the initial values of dried fruits and mixtures were determined in the IN fraction. Previously, results within this range were obtained for pomegranate juice (29%) (Perez-Vicente, Gil-Izquierdo, & Garcia-Viguera, 2002), sweet cherries (27–29%) (Fazzari et al., 2008) and apples (45–63%) (Bouayed, Hoffmann, & Bohn, 2011). Compared to initial and PG values, increases in OUT fractions were observed for raisins, almonds and hazelnuts. This could be explained by the additional time of extraction (plus 2 h) and/or the effect of intestinal digestive enzyme (pancreatin) on the complex food matrix, facilitating the release of phenolics bound to the matrix (Bouayed et al., 2011). As shown in Table 2, consumption of almonds together with all dried fruits significantly decreased the TP in IN fraction ($p < 0.05$). A similar reduction was also observed when dried apricots and hazelnuts are consumed together. For all samples, codigestion of dried fruits with nuts reduced the TP in IN fraction (22–77%) (Fig. 1). Another study also reported a similar reduction in TP in the IN fraction after codigestion of raspberry with ice cream and shredded wheat (McDougall et al., 2005). Furthermore, it has been stated that TP of black tea has been reduced when consumed together with milk, due to either covalent or non-covalent interactions between plant phenolics and proteins (Ozdal, Capanoglu, & Altay, 2013). The same mechanism could be valid in our case, where we observed a decrease in TP after codigestion of dried fruits with nuts. Moreover, fruits are known to contain substantial amount of dietary fiber, which have been suggested to decrease some plant antioxidants bioaccessibility by acting as an entrapping matrix and restricting the diffusion of the enzymes to their substrates. Thereby most of the polyphenols bound to dietary fiber may end up in the large intestine (Palafox-Carlos, Ayala-Zavala, & Gonzalez-Aguilar, 2011). Folin–Ciocalteu assay is highly subject to interferences especially from amino acids and purines that are released from proteins and nucleic acids by the actions of enzymes used for the *in vitro* digestion procedure (Tagliazucchi, Verzelloni, Bertolini, & Conte, 2010). Therefore, this assay alone is insufficient to reflect the changes in phenolics during codigestion. In order to obtain more accurate results, HPLC analysis of individual phenolic compounds should also be performed.

Table 2
Changes in the total phenolic content (TP) during *in vitro* gastrointestinal digestion.*

Group	Sample	TP (mg GAE/100 g sample)			
		Initial	PG	IN	OUT
1	F	123 ± 7.8^{a}	575 ± 56^{ab}	85 ± 8.8^{b}	369 ± 45^{b}
	Al	45 ± 6.2^{b}	503 ± 13^{b}	190 ± 28^{a}	952 ± 184^{a}
	F–Al	122 ± 13^{a}	592 ± 28^{a}	32 ± 13^{c}	458 ± 20^{b}
2	F	123 ± 7.8^{c}	575 ± 56^{b}	85 ± 8.8^{b}	369 ± 45^{c}
	W	1419 ± 46^{a}	848 ± 118^{a}	220 ± 11^{a}	1332 ± 233^{a}
	F–W	620 ± 116^{b}	1031 ± 104^{a}	78 ± 8.7^{b}	892 ± 31^{b}
3	F	123 ± 7.8^{c}	575 ± 56^{a}	85 ± 8.8^{b}	369 ± 45^{b}
	H	226 ± 2.6^{a}	558 ± 16^{a}	126 ± 14^{a}	1296 ± 412^{a}
	F–H	150 ± 13^{b}	636 ± 108^{a}	76 ± 11^{b}	482 ± 38^{b}
4	Ap	211 ± 9.4^{a}	694 ± 50^{a}	133 ± 8.9^{b}	619 ± 15^{b}
	Al	45 ± 6.2^{c}	503 ± 13^{b}	190 ± 28^{a}	952 ± 184^{a}
	Ap–Al	137 ± 5.2^{b}	637 ± 31^{a}	76 ± 20^{c}	514 ± 20^{b}
5	Ap	211 ± 9.4^{c}	694 ± 50^{c}	133 ± 8.9^{b}	619 ± 15^{c}
	W	1419 ± 46^{a}	848 ± 118^{b}	220 ± 11^{a}	1332 ± 233^{a}
	Ap–W	744 ± 141^{b}	1014 ± 34^{a}	126 ± 11^{b}	952 ± 53^{b}
6	Ap	211 ± 9.4^{a}	694 ± 50^{a}	133 ± 8.9^{a}	619 ± 15^{b}
	H	226 ± 2.6^{a}	558 ± 16^{b}	126 ± 14^{a}	1296 ± 412^{a}
	Ap–H	186 ± 12^{b}	740 ± 81^{a}	90 ± 26^{b}	508 ± 42^{b}
7	R	1322 ± 59^{a}	1294 ± 95^{a}	104 ± 4.4^{b}	1355 ± 129^{a}
	Al	45 ± 6.2^{c}	503 ± 13^{b}	190 ± 28^{a}	952 ± 184^{b}
	R–Al	513 ± 50^{b}	348 ± 25^{c}	57 ± 21^{c}	396 ± 22^{c}
8	R	1322 ± 59^{a}	1294 ± 95^{a}	104 ± 4.4^{b}	1355 ± 129^{a}
	W	1419 ± 46^{a}	848 ± 118^{b}	220 ± 11^{a}	1332 ± 233^{a}
	R–W	1177 ± 69^{b}	711 ± 21^{b}	86 ± 18^{b}	722 ± 82^{b}
9	R	1322 ± 59^{a}	1294 ± 95^{a}	104 ± 4.4^{b}	1355 ± 129^{a}
	H	226 ± 2.6^{c}	558 ± 16^{b}	126 ± 14^{a}	1296 ± 412^{a}
	R–H	749 ± 12^{b}	448 ± 38^{b}	89 ± 7.4^{b}	529 ± 102^{b}

*The data presented in this table consist of average quantities ± SD of three independent samples. Different letters in the columns within each group (1–9) represent statistically significant differences ($p < 0.05$). The terms represent; Initial, as initially determined from sample matrix using 75% aqueous-methanol containing 0.1% formic acid; PG, compounds remaining after gastric digestion; IN, dialyzed fraction after intestinal digestion; OUT, non-dialyzed fraction after intestinal digestion. Abbreviations used for samples are expressed in Table 1.

3.2. Total antioxidant capacity (TAC)

The influence of GI digestion on free radical scavenging activity determined by ABTS assay is shown in Table 3. Similar to TP, highest initial value was obtained for walnuts, whereas almonds had lowest initial amount (43-fold difference). On the other hand, unlike TP, for most of the samples PG values were lower than initials (6–81%), indicating an incomplete release or degradation. The IN fraction represented 3–205% of the initial values. In addition, especially in case of nuts and dried fruit–nut mixtures, TAC of OUT fractions were higher than initial and PG values. It is well known from the literature that the radical scavenger activity of polyphenols is strongly pH-dependent with higher pH values, which significantly increase this capacity. This increase in the radical scavenger activity has been attributed to the deprotonation of the hydroxyl moieties present on the aromatic rings of the phenolic compounds (Tagliazucchi et al., 2010). Moreover, consumption of walnuts together with figs and apricots as well as hazelnuts with figs and raisins significantly increased the TAC of IN fraction ($p < 0.05$) (Table 3). Similar to the results obtained for TP, for all samples, codigestion of dried fruits with nuts reduced the TAC in IN fraction (5–54%) (Fig. 1).

Free radical scavenging activity initially present, as determined in methanolic extracts by the DPPH assay, was lower compared to PG fractions for almost all samples (walnuts, hazelnuts and raisin–nut mixtures are exceptions) (Table 3). Studies carried out on mulberry (Liang et al., 2012) and gooseberry (Chiang et al., 2013) also revealed that digestion enhances the availability of antioxidants in fruits, where digested fruits show higher TAC. Initial results were in accordance with the results obtained for TP and ABTS, demonstrating that walnuts had the highest TAC, which was approximately 91-fold higher than almonds, which had the lowest TAC. IN fraction of the all samples except for almonds, stand for 2–40% of the initial TAC, whereas IN value of almond was 74% of the initial amount, suggesting that almonds may be a greater source of bioaccessible antioxidants than dried fruits or other nuts investigated in this study. A significant increase in IN fraction of fig–hazelnut and fig–walnut mixtures was observed (1.3 and 2 fold, respectively) when compared to figs alone ($p < 0.05$) (Table 3). On the other hand, TAC of IN fractions of raisin–almond and raisin–hazelnut mixtures were found to be significantly lower than

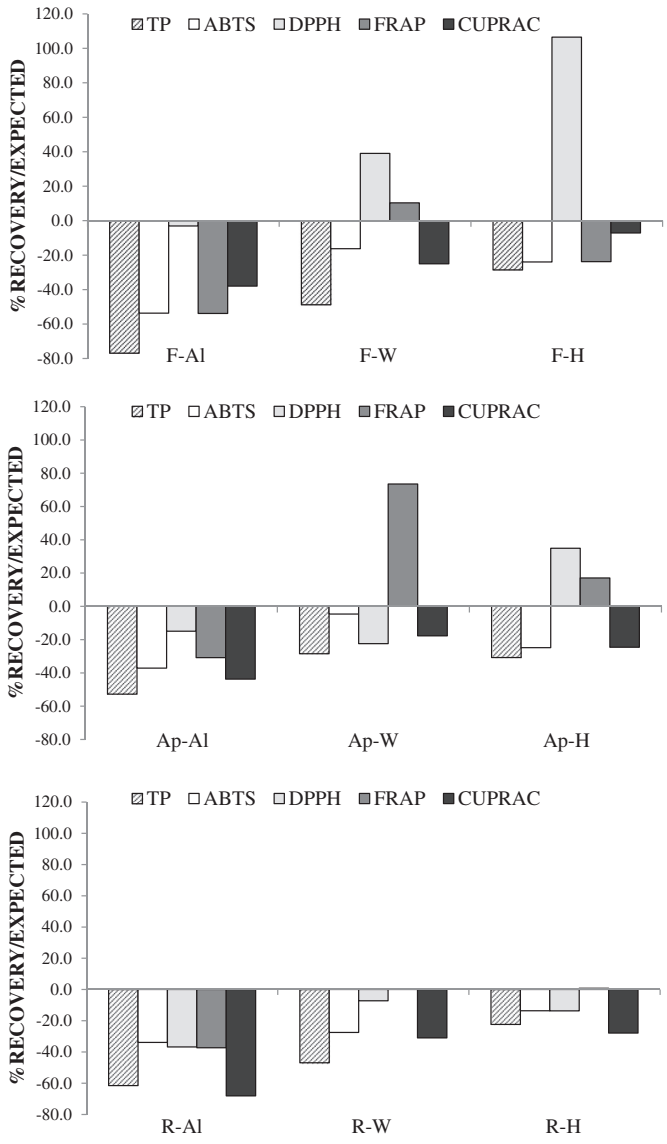


Fig. 1. Recovery of total phenolics (TP) and total antioxidant capacity (TAC) in dialyzed fraction (IN) from codigestion experiments. Figures illustrate the percentage difference between the observed TP and TAC of the mixtures (dried fruit + nut) and the expected value = dried fruit alone + nut alone experiments. Data represent average values of three independent samples. Abbreviations used for samples are expressed in Table 1.

raisins ($p < 0.05$). Codigestion experiments showed that consumption of apricot–hazelnut, fig–walnut and fig–hazelnut mixtures instead of consuming them alone resulted in 35, 39 and 107% higher TAC recoveries in the dialyzed fraction (IN), respectively (Fig. 1). On the other hand, besides these three mixtures, codigestion of other dried fruits and nuts resulted in lower TAC in IN fraction (3–37%).

As shown in Table 4, results determined using FRAP assay once again revealed that walnuts had the highest initial TAC value, whereas almonds had the lowest (84-fold difference). Similar to the results obtained for TP and DPPH, TAC was increased after the gastric digestion (7–210%) with some exceptions (walnuts, hazelnuts and raisin mixtures). 7–46% of the initial values were obtained in the IN fraction. Bermudez-Soto et al. (2007), also found an increase in a number of polyphenols after the gastric phase of the *in vitro* digestion process. However after the pancreatic digestion phase these antioxidants were degraded by the alkaline pH, thus giving an overall loss in antioxidant capacity after *in vitro* digestion, as in our study. There was a significant increase in IN fraction of fig–walnut and apricot–walnut mixtures compared to fruits alone ($p < 0.05$) (Table 4). On the contrary, TAC of other mixtures excluding apricot–hazelnut and raisin–hazelnut were found to be significantly lower than fruits alone ($p < 0.05$). Codigestion experiments revealed that consumption of fig–walnut, apricot–hazelnut and apricot–walnut mixtures instead of consuming them alone resulted in 10, 17 and 74% higher TAC recoveries in the dialyzed fraction (IN), respectively (Fig. 1). On the other hand, besides these three mixtures, codigestion of other dried fruits and nuts resulted in lower TAC in IN fraction (24–54%).

Confirming the results obtained for TP and other TAC assays (ABTS, DPPH and FRAP), CUPRAC assay also showed that initially the highest level of TAC was found in walnuts, while the lowest was in almonds with 27-fold difference (Table 4). The trend of PG values varied among the 15 samples, showing both increases and decreases compared to initial values. When the amount of TAC after intestinal digestion was analyzed, 4–143% of the initial values of dried fruits and mixtures were determined in the IN fraction. Similar to ABTS results, TAC was increased in the IN fraction for almonds, indicating a possible formation of new compounds with higher TAC than initial almond value. As in TP, for nuts, increases in OUT fractions were observed compared to initial and PG values. As shown in Table 4, consumption of almonds together with all dried fruits significantly decreased the TAC in IN fraction ($p < 0.05$). A similar condition was also monitored when dried apricots and raisins are consumed together with hazelnuts. Codigestion of dried fruits with nuts reduced the TAC in IN fraction (7–68%) for all samples (Fig. 1).

The correlation coefficients (R^2) between TP and TAC were also calculated. Before the *in vitro* digestion, generally a highly linear relationship was obtained for TP and TAC, with ABTS being the highest with $R^2 = 0.9024$. The correlation between the antioxidant activity assays was also quite high, especially correlation between ABTS and DPPH ($R^2 = 0.9744$). After the gastric phase of digestion, correlation between TP and TAC was quite weak (data not shown) except the correlation between DPPH and CUPRAC ($R^2 = 0.9028$). After the intestinal phase of digestion, the correlations were poor for both IN ($R^2 = 0.0001$–0.6462) and OUT values ($R^2 = 0.1739$–0.8025).

Differences were observed between the two radical scavenging assays (ABTS and DPPH). Theoretically, alterations in the structure of antioxidants following digestion may affect their reactivity with the formation of nitrogen radical which is biologically less relevant in the DPPH assay, leading to the underestimation of TAC (Wootton-Beard et al., 2011). Furthermore, considering the pH condition of antioxidant activity tests performed in this study, it can be presumed that the FRAP test (conducted at a buffered pH = 3.6, to maintain iron solubility) could be more suitable to assess antioxidant activity in the gastric digesta, rather than intestinal digesta. However, the CUPRAC assay could be more appropriate to evaluate the intestinal digesta with a working pH of 7.0. In addition to the dependency of phenolic activity on pH, chemical structure of phenolics also plays an important role in the antioxidant activity, and in this sense it is well known that aglycones display a higher antioxidant activity than their glycosides. Moreover, interaction of phenolics with other dietary compounds released during digestion, e.g. dietary fiber or proteins, is known to affect the polyphenol solubility and availability, and thus the antioxidant potential (Bouayed et al., 2011). Eventually, the measurement of antioxidant activities, especially in the case of multifunctional or complex multiphase systems, cannot be evaluated satisfactorily by a single method. Therefore, several assays should be performed as was done in this study.

Table 3
The total antioxidant capacity (TAC) determined using ABTS and DPPH assays during *in vitro* gastro-intestinal digestion.*

Group	Sample	ABTS (mg TE/100 g sample)				DPPH (mg TE/100 g sample)			
		Initial	PG	IN	OUT	Initial	PG	IN	OUT
1	F	163 ± 14^{b}	280 ± 9.0^{b}	68 ± 5.3^{b}	340 ± 64^{b}	44 ± 4.4^{b}	247 ± 25^{a}	14 ± 1.7^{a}	81 ± 18^{a}
	Al	91 ± 6.4^{c}	59 ± 1.1^{c}	187 ± 1.5^{a}	1015 ± 124^{a}	26 ± 4.7^{c}	50 ± 6.0^{c}	19 ± 4.1^{a}	41 ± 0.4^{b}
	F–Al	194 ± 15^{a}	351 ± 4.4^{a}	59 ± 9.0^{b}	501 ± 16^{b}	67 ± 2.1^{a}	157 ± 20^{b}	16 ± 1.3^{a}	106 ± 17^{a}
2	F	163 ± 14^{c}	280 ± 9.0^{c}	68 ± 5.3^{c}	340 ± 64^{c}	44 ± 4.4^{c}	247 ± 25^{c}	14 ± 1.7^{b}	81 ± 18^{b}
	W	4002 ± 476^{a}	1354 ± 23^{a}	178 ± 21^{a}	1709 ± 385^{a}	2344 ± 376^{a}	1748 ± 46^{a}	46 ± 11^{a}	837 ± 144^{a}
	F–W	2005 ± 181^{b}	1052 ± 37^{b}	103 ± 15^{b}	810 ± 55^{b}	1184 ± 256^{b}	1284 ± 44^{b}	41 ± 6.5^{a}	662 ± 77^{a}
3	F	163 ± 14^{c}	280 ± 9.0^{b}	68 ± 5.3^{c}	340 ± 64^{b}	44 ± 4.4^{c}	247 ± 25^{a}	14 ± 1.7^{b}	81 ± 18^{a}
	H	561 ± 72^{a}	321 ± 19^{a}	174 ± 6.5^{a}	1109 ± 144^{a}	217 ± 11^{a}	130 ± 3.0^{b}	17 ± 2.6^{b}	92 ± 7.3^{a}
	F–H	284 ± 19^{b}	303 ± 6.6^{ab}	92 ± 12^{b}	423 ± 13^{b}	125 ± 15^{b}	224 ± 5.1^{a}	32 ± 2.3^{a}	76 ± 16^{a}
4	Ap	238 ± 4.4^{a}	291 ± 34^{a}	77 ± 4.3^{b}	380 ± 13^{c}	82 ± 3.8^{a}	281 ± 48^{a}	33 ± 2.4^{a}	123 ± 10^{a}
	Al	91 ± 6.4^{c}	59 ± 1.1^{c}	187 ± 1.5^{a}	1015 ± 124^{a}	26 ± 4.7^{c}	50 ± 6.0^{c}	19 ± 4.1^{b}	41 ± 0.4^{b}
	Ap–Al	210 ± 6.5^{b}	219 ± 3.6^{b}	83 ± 3.9^{b}	559 ± 23^{b}	66 ± 5.2^{b}	155 ± .46^{b}	22 ± 3.9^{b}	124 ± 5.9^{a}
5	Ap	238 ± 4.4^{c}	291 ± 34^{c}	77 ± 4.3^{c}	380 ± 13^{c}	82 ± 3.8^{c}	281 ± 48^{c}	33 ± 2.4^{a}	123 ± 10^{b}
	W	4002 ± 476^{a}	1354 ± 23^{a}	178 ± 21^{a}	1709 ± 385^{a}	2344 ± 376^{a}	1748 ± 46^{a}	46 ± 11^{a}	837 ± 144^{a}
	Ap–W	2113 ± 481^{b}	992 ± 30^{b}	122 ± 8.1^{b}	852 ± 26^{b}	1130 ± 121^{b}	1334 ± 23^{b}	31 ± 7.4^{a}	856 ± 109^{a}
6	Ap	238 ± 4.4^{c}	291 ± 34^{a}	77 ± 4.3^{b}	380 ± 13^{b}	82 ± 3.8^{c}	281 ± 48^{a}	33 ± 2.4^{a}	123 ± 10^{a}
	H	561 ± 72^{a}	321 ± 19^{a}	174 ± 6.5^{a}	1109 ± 144^{a}	217 ± 11^{a}	130 ± 3.0^{c}	17 ± 2.6^{b}	92 ± 7.3^{b}
	Ap–H	359 ± 11^{b}	339 ± 14^{a}	94 ± 27^{b}	399 ± 48^{b}	107 ± 0.3^{b}	208 ± 16^{b}	34 ± 10^{a}	136 ± 9.4^{a}
7	R	2262 ± 186^{a}	887 ± 119^{a}	79 ± 2.0^{b}	1071 ± 189^{a}	1204 ± 70^{a}	1818 ± 50^{a}	54 ± 2.5^{a}	2132 ± 314^{a}
	Al	91 ± 6.4^{c}	59 ± 1.1^{c}	187 ± 1.5^{a}	1015 ± 124^{a}	26 ± 4.7^{c}	50 ± 6.0^{c}	19 ± 4.1^{b}	41 ± 0.4^{b}
	R–Al	1035 ± 71^{b}	400 ± 20^{b}	88 ± 23^{b}	577 ± 102^{b}	596 ± 11^{b}	164 ± 8.5^{b}	23 ± 7.5^{b}	112 ± 11^{b}
8	R	2262 ± 186^{b}	887 ± 119^{b}	79 ± 2.0^{b}	1071 ± 189^{b}	1204 ± 70^{c}	1818 ± 50^{b}	54 ± 2.5^{a}	2132 ± 314^{a}
	W	4002 ± 476^{a}	1354 ± 23^{a}	178 ± 21^{a}	1709 ± 385^{a}	2344 ± 376^{a}	1748 ± 46^{b}	46 ± 11^{a}	837 ± 144^{b}
	R–W	2743 ± 213^{b}	799 ± 222^{b}	93 ± 22^{b}	665 ± 67^{b}	1646 ± 23^{b}	1993 ± 25^{a}	47 ± 8.0^{a}	911 ± 53^{b}
9	R	2262 ± 186^{a}	887 ± 119^{a}	79 ± 2.0^{c}	1071 ± 189^{a}	1204 ± 70^{a}	1818 ± 50^{a}	54 ± 2.5^{a}	2132 ± 314^{a}
	H	561 ± 72^{c}	321 ± 19^{b}	174 ± 6.5^{a}	1109 ± 144^{a}	217 ± 11^{c}	130 ± 3.0^{c}	17 ± 2.6^{c}	92 ± 7.3^{b}
	R–H	1742 ± 27^{b}	333 ± 32^{b}	109 ± 3.5^{b}	440 ± 20^{b}	1062 ± 8.9^{b}	391 ± 29^{b}	31 ± 1.4^{b}	413 ± 64^{b}

*The data presented in this table consist of average quantities ± SD of three independent samples. Different letters in the columns within each group (1–9) represent statistically significant differences ($p < 0.05$). The terms represent; Initial, as initially determined from sample matrix using 75% aqueous-methanol containing 0.1% formic acid; PG, compounds remaining after gastric digestion; IN, dialyzed fraction after intestinal digestion; OUT, non-dialyzed fraction after intestinal digestion. Abbreviations used for samples are expressed in Table 1.

Table 4
The total antioxidant capacity (TAC) determined using FRAP and CUPRAC assays during *in vitro* gastro-intestinal digestion.*

Group	Sample	FRAP (mg TE/100 g sample)				CUPRAC (mg TE/100 g sample)			
		Initial	PG	IN	OUT	Initial	PG	IN	OUT
1	F	46 ± 4.4^{a}	142 ± 9.6^{a}	21 ± 0.5^{a}	67 ± 0.9^{b}	152 ± 19^{b}	486 ± 42^{a}	124 ± 13^{a}	404 ± 70^{b}
	Al	20 ± 2.9^{b}	49 ± 2.2^{c}	8.3 ± 0.9^{b}	176 ± 44^{a}	95 ± 8.4^{c}	134 ± 8.4^{c}	135 ± 30^{a}	2834 ± 389^{a}
	F–Al	52 ± 4.6^{a}	112 ± 5.5^{b}	6.8 ± 2.5^{b}	75 ± 11^{b}	246 ± 38^{a}	244 ± 7.6^{b}	81 ± 9.4^{b}	308 ± 31^{b}
2	F	46 ± 4.4^{c}	142 ± 9.6^{c}	21 ± 0.5^{c}	67 ± 0.9^{b}	152 ± 19^{c}	486 ± 42^{b}	124 ± 13^{b}	404 ± 70^{c}
	W	1694 ± 198^{a}	520 ± 12^{b}	112 ± 22^{a}	453 ± 38^{a}	2631 ± 152^{a}	1447 ± 293^{a}	299 ± 62^{a}	3204 ± 312^{a}
	F–W	857 ± 194^{b}	1001 ± 30^{a}	74 ± 14^{b}	417 ± 38^{a}	1514 ± 115^{b}	1552 ± 42^{a}	159 ± 2.8^{b}	963 ± 117^{b}
3	F	46 ± 4.4^{c}	142 ± 9.6^{b}	21 ± 0.5^{a}	67 ± 0.9^{b}	152 ± 19^{c}	486 ± 42^{a}	124 ± 13^{a}	404 ± 70^{b}
	H	201 ± 7.9^{a}	89 ± 7.5^{c}	15 ± 1.3^{b}	306 ± 24^{a}	561 ± 17^{a}	188 ± 61^{b}	137 ± 22^{a}	2666 ± 633^{a}
	F–H	82 ± 7.9^{b}	157 ± 4.9^{a}	14 ± 2.8^{b}	98 ± 25^{b}	248 ± 33^{b}	494 ± 39^{a}	121 ± 6.2^{a}	553 ± 56^{b}
4	Ap	108 ± 9.7^{a}	218 ± 50^{a}	36 ± 3.5^{a}	219 ± 34^{a}	374 ± 73^{a}	627 ± 48^{a}	183 ± 35^{a}	680 ± 67^{b}
	Al	20 ± 2.9^{c}	49 ± 2.2^{c}	8.3 ± 0.9^{c}	176 ± 44^{ab}	95 ± 8.4^{c}	134 ± 8.4^{c}	135 ± 30^{ab}	2834 ± 389^{a}
	Ap–Al	63 ± 2.7^{b}	137 ± 3.7^{b}	15 ± 3.9^{b}	129 ± 18^{b}	244 ± 46^{b}	320 ± 21^{b}	90 ± 7.0^{b}	392 ± 53^{b}
5	Ap	108 ± 9.7^{c}	218 ± 50^{c}	36 ± 3.5^{b}	219 ± 34^{c}	374 ± 73^{c}	627 ± 48^{b}	183 ± 35^{b}	680 ± 67^{b}
	W	1694 ± 198^{a}	520 ± 12^{b}	112 ± 22^{a}	453 ± 38^{b}	2631 ± 152^{a}	1447 ± 293^{a}	299 ± 62^{a}	3204 ± 312^{a}
	Ap–W	1140 ± 218^{b}	1225 ± 57^{a}	129 ± 34^{a}	716 ± 57^{a}	1872 ± 144^{b}	1603 ± 57^{a}	199 ± 35^{b}	992 ± 39^{b}
6	Ap	108 ± 9.7^{b}	218 ± 50^{a}	36 ± 3.5^{a}	219 ± 34^{b}	374 ± 73^{b}	627 ± 48^{a}	183 ± 35^{a}	680 ± 67^{b}
	H	201 ± 7.9^{a}	89 ± 7.5^{b}	15 ± 1.3^{b}	306 ± 24^{a}	561 ± 17^{a}	188 ± 61^{c}	137 ± 22^{ab}	2666 ± 63^{a}
	Ap–H	100 ± 5.9^{b}	192 ± 7.4^{a}	29 ± 5.7^{a}	143 ± 8.8^{c}	264 ± 34^{c}	368 ± 32^{b}	121 ± 22^{b}	583 ± 75^{b}
7	R	646 ± 12^{a}	987 ± 69^{a}	72 ± 3.7^{a}	896 ± 131^{a}	2351 ± 58^{a}	2274 ± 242^{a}	214 ± 7.8^{a}	2723 ± 243^{a}
	Al	20 ± 2.9^{c}	49 ± 2.2^{c}	8.3 ± 0.9^{c}	176 ± 44^{b}	95 ± 8.4^{c}	134 ± 8.4^{b}	135 ± 30^{b}	2834 ± 389^{a}
	R–Al	375 ± 30^{b}	189 ± 24^{b}	25 ± 4.0^{b}	158 ± 22^{b}	958 ± 96^{b}	275 ± 59^{b}	56 ± 6.6^{c}	762 ± 85^{b}
8	R	646 ± 12^{c}	987 ± 69^{a}	72 ± 3.7^{b}	896 ± 131^{a}	2351 ± 58^{b}	2274 ± 242^{a}	214 ± 7.8^{b}	2723 ± 243^{b}
	W	1694 ± 198^{a}	520 ± 12^{b}	112 ± 22^{a}	453 ± 38^{b}	2631 ± 152^{b}	1447 ± 293^{b}	299 ± 62^{a}	3204 ± 312^{a}
	R–W	1332 ± 25^{b}	872 ± 99^{a}	92 ± 11^{ab}	505 ± 19^{b}	4697 ± 254^{a}	1704 ± 151^{b}	177 ± 35^{b}	1064 ± 54^{c}
9	R	646 ± 12^{a}	987 ± 69^{a}	72 ± 3.7^{a}	896 ± 131^{a}	2351 ± 58^{a}	2274 ± 242^{a}	214 ± 7.8^{a}	2723 ± 243^{a}
	H	201 ± 7.9^{b}	89 ± 7.5^{c}	15 ± 1.3^{c}	306 ± 24^{b}	561 ± 17^{c}	188 ± 61^{b}	137 ± 22^{b}	2666 ± 633^{a}
	R–H	143 ± 2.5^{c}	199 ± 12^{b}	44 ± 3.2^{b}	214 ± 43^{b}	1562 ± 139^{b}	435 ± 29^{b}	127 ± 5.3^{b}	724 ± 277^{b}

*The data presented in this table consist of average quantities ± SD of three independent samples. Different letters in the columns within each group (1–9) represent statistically significant differences ($p < 0.05$). The terms represent; Initial, as initially determined from sample matrix using 75% aqueous-methanol containing 0.1% formic acid; PG, compounds remaining after gastric digestion; IN, dialyzed fraction after intestinal digestion; OUT, non-dialyzed fraction after intestinal digestion. Abbreviations used for samples are expressed in Table 1.

Comparing *in vitro* studies is difficult since differences may arise from the starting material as well as the *in vitro* digestion procedure used. Dialysis is a complex process affected by several factors such as, volume and composition of the buffer used, sugar concentrations in the sample or ability of certain molecules to bind with the membrane (Bermudez-Soto et al., 2007). For instance, high sugar content of the samples facilitates the diffusion of water from the dialysis tubing to the food phase, thereby decreasing the volume of the dialyzed fraction (Gil-Izquierdo, Zafrilla, & Tomas-Barberan, 2002). All of these parameters can affect the dialyzation of a specific compound, which cannot be truly associated with absorption of the compound *in vivo* or to levels of this compound in serum (Bermudez-Soto et al., 2007).

4. Conclusion

No studies to date have reported the consequences of codigestion of dried fruits and nuts on TP and TAC following the *in vitro* digestion. By measuring the TP and TAC of the fruit–nut mixtures after the *in vitro* digestion, we have provided the biologically relevant data detailing the antioxidant properties of the tested samples quickly and cost-effectively. This study demonstrated that consumption of fig-nut and apricot–walnut/hazelnut mixtures instead of consuming them alone results in higher TAC recoveries in the dialyzed fraction, which denotes the serum bioavailability. *In vitro* GI digestion models can be considered as a novel method and therefore further studies should be carried out in order to support the findings of this study. In further studies, we are planning to focus on bioaccessibility of individual polyphenols rather than total antioxidant capacity, perhaps coupled together with cellular models such as Caco-2 in order to obtain more qualitatively well-correlated results with human studies.

Acknowledgment

This study was financially supported by the EU 7th Frame ATHENA Project (FP7-KBBE-2009-3-245121-ATHENA).

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