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Intake and bioaccessibility of total polyphenols in a whole diet

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Abstract

The knowledge of dietary intake of polyphenols and their bioaccessibility in the human gut are key factors in assessing their significance in human health. The aim of this work was to estimate the amount of total polyphenols consumed in a whole diet (Spanish Mediterranean diet) and their intestinal bioaccessibility. Total polyphenols were determined, as the sum of the polyphenols present in methanol:acetone:water extracts (extractable polyphenols) of plant foods, and condensed tannins and hydrolysable polyphenols (non-extractable polyphenols) in the corresponding residues. The polyphenols intestinal bioaccessibility was estimated by an in vitro gastrointestinal model where food polyphenols are released by enzyme digestion and colonic fermentation. The mean daily intake of polyphenols in the Spanish diet was estimated between 2590 and 3016 mg/person/day. The amount of non-extractable polyphenols was almost double that of extractable polyphenols. It was estimated that about 48% of dietary polyphenols are bioaccessible in the small intestine, while 42% become bioaccessible in the large intestine.

Keywords: Polyphenols; Colon; Small intestine; Bioaccessibility; Condensed tannins; Hydrolysable polyphenols

1. Introduction

Polyphenols are the most abundant antioxidants in our diet (Manach, Scalbert, Morand, Remesy, & Jimenez, 2004). In addition to their antioxidant properties, polyphenols may have other specific biological activities. affecting gene expression (Yuan, Gong, & Young, 2005), cell signalling (Wheeler et al., 2004) and cell adhesion (Williams, Sutherland, Whelan, McCormick, & de Jong, 2004). There is growing scientific interest in the biological properties of polyphenols in the prevention of age-related diseases, including cardiovascular disease and cancer (Williamson & Manach, 2005). To elucidate the significance of polyphenols in human health, it is essential to know the amount of polyphenols consumed in the diet and their bioavailability.

Literature data on the content and composition of food polyphenols are partial and insufficient to determine dietary intakes. Certain studies have provided individual data concerning the intake of some types of polyphenols such as flavonols (Crozier et al., 2000), flavanones (Manach, Morand, Gil-Izquierdo, Bouteloup-Demange, & Remesy, 2003), catechins (Higdon & Frei, 2003), phenolic acids (Scalbert & Williamson, 2000) and flavan-3-ols (Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2000), but there is a lack of comprehensive data on total polyphenol intake. Moreover, food polyphenol data usually correspond to polyphenols analyzed in aqueous organic extracts of foods (extractable polyphenols), while significant amounts of potentially bioactive polyphenols that remain in the residues (non-extractable polyphenols) are ignored. The presence of important amounts of non-extractable polyphenols has been reported in specific foods and vegetables (Bravo, Abia, & Saura-Calixto, 1994; Bravo, Mañas, & Saura-Calixto, 1993). Non-extractable polyphenols are

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high molecular weight proanthocyanidins and phenolics associated with dietary fibre and indigestible compounds that are not taken into account in chemical and biological studies.

The authors recently reported the total polyphenol consumption in the Spanish diet, derived from beverages and extractable food polyphenols (Pulido, Hernández-Garcıa, & Saura-Calixto, 2003; Saura-Calixto & Goñi, 2006). Here we seek to determine the total polyphenol intake in a whole diet, including both extractable and non-extractable polyphenols.

To exert their biological properties, polyphenols have to be available to some extent in the target tissue. Therefore, the biological properties of dietary polyphenols may depend on their absorption in the gut and their bioavailability.

Bioaccessibility is defined as the amount of a food constituent that is present in the gut, as a consequence of the release of this constituent from the solid food matrix, and may be able to pass through the intestinal barrier. Only polyphenols released from the food matrix by the action of digestive enzymes (small intestine) and bacterial microflora (large intestine) are bioaccessible in the gut and therefore potentially bioavailable. The amount of bioaccessible food polyphenols may differ quantitatively and qualitatively from polyphenols included in food databases. The bioaccessibility is not taken into account in studies regarding the bioavailability of polyphenols. Moreover, most studies on polyphenol bioavailability use mainly pure single molecules (isolated from food or chemically synthesized) although their bioavailability from whole foods may be substantially different. Some beverages and single foods have also been used in these studies (Williamson & Manach, 2005), but the significance of the results for health may be limited because a specific food may contribute little to the total polyphenol intake in the diet.

An in vitro gastrointestinal model that includes a digestive enzyme treatment to isolate food indigestible compounds (Saura-Calixto, Garcia-Alonso, Goñi, & Bravo, 2000) and in vitro colonic fermentation (Goñi & Martin-Carrón, 2000), was used to estimate the bioaccessibility of dietary polyphenols. This model was previously used in different food sources such as cereals (Perez-Jimenez & Saura-Calixto, 2005) and green leafy vegetables (Serrano, Goñi, & Saura-Calixto, 2005), to analyze polyphenols and carotenoids bioaccessibility, respectively.

2. Materials and methods

2.1. Dietary information

Estimates of plant food intakes in the Spanish diet were based on national consumption data (MAPA, 2001). These data are obtained annually from questionnaires. Six thousand households were surveyed, along with 700 hotels and restaurants and 200 institutions such as schools, hospitals and the armed forces (confidence level 95%; error range 2% in amount of food). Each family daily record the foods present at home. Hotels, restaurants and institutions give information about purchases of foods four times per year. Dietary intakes included in Table 1 correspond to the intake of plant foods in Spain (g/person/day).

2.2. Sample preparation

Two purchases of each individual plant food listed in the National Dietary Survey (MAPA, 2001) (Table 1), were acquired at different local supermarkets. Individual items selected in this study are representative of plant food common in the Spanish diet. The edible portion of the daily amount consumed per capita for each plant food as eaten

Table 1
Intake of plant foods in the Spanish diet

		g Fresh matter/day[c]	g Edible portion/day
Cereals	Rice[a] (7%), white bread (67%), white bread sliced (5%), spaghetti[a] (5%), biscuits (8%), croissants (8%)	221.6	221.6
Vegetables	Potatoes[a] (40%), tomatoes (13%), tomatoes transformed (6%), onions (7%), garlic (1%), cabbage[a] (2%), green beans[a] (2%), cucumber (2%), capsicum (4%), mushrooms (1%), lettuce (7%), asparagus (1%), spinach[a] (1%), chard[a] (1%), others[b] (12%)	330.9	280.8
Nuts	Almonds (12%), peanuts (16%), walnuts (16%), others[b] (56%)	6.8	5.9
Fruits	Oranges (24%), mandarin oranges (6%), bananas (10%), apples (13%), pears (8%), peaches (5%), apricots (1%), strawberries (3%), melon (8%), watermelon (6%), plums (2%), cherries (1%), grapes (3%), kiwi (3%), olives (3%), others[b] (4%)	265.7	200.8
Legumes	Chickpeas[a] (35%),beans[a](31%), lentils[a] (34%)	22.3	22.3
Beverages	Coffee (24%), tea (3%) red wine (10%), white wine (5%), rose wine (3%), beer (30%), fruit juices (9%), cola drinks (16%)	504.9 (ml)	504.9 (ml)
Vegetable oils	Olive (55%), sunflower (37%), others[b] (8%)	56.4 (ml)	56.4 (ml)

[a] Boiled.
[b] Vegetables: artichoke[a], carrot, tender pumpkin[a], celery[a], aubergine[a], turnip[a], leek[a], pumpkin[a], beet root[a], avocado; Nuts: hazelnuts, pistacho; Fruits: pomegranate, mango, pineapple, grapefruit, caqui, chirimoya; Oils: corn, soja.
[c] Confidence level 95%, error range 2% in amount of food.

was weighed and grouped into five duplicate samples, one for each of the five types of plant foods: cereals (total: 221.6 g), vegetables (total: 280.8 g), legumes (22.3 g), nuts (total: 5.9 g) and fruits (total: 200.8 g). These five samples correspond to the total per capita daily intake of solid plant food in the Spanish diet. Each duplicated sample was freeze-dried, ground and stored until analysis.

2.3. Physiological approach: In vitro gastrointestinal model and bioaccessibility of polyphenols

There are two main steps in the methodology proposed to estimate the bioaccessibility of dietary polyphenols: (a) isolation of the indigestible fraction (small intestine bioaccessibility); and (b) colonic fermentation of the indigestible fraction (large intestine bioaccessibility) (Fig. 1, Section A). The indigestible fraction was previously defined as the part of vegetables that is not digested or absorbed in the small intestine and reaches the colon, where it serves as a substrate for fermentative microflora (Saura-Calixto et al., 2000). The indigestible fraction is made up of dietary fibre and other compounds of proven resistance to the actions of enzymes, such as indigestible protein, resistant starch, polyphenols and other bioactive compounds. The indigestible fraction is a physiological alternative to the common dietary fibre concept (Saura-Calixto & Goñi, 2004). Analytical conditions for indigestible fraction determination are close to physiological conditions (pH, temperature, and incubation times). The indigestible fraction is composed of two fractions: a soluble fraction (supernatant of enzymatic digestion) and an insoluble fraction (residue of enzymatic digestion) (Fig. 1, Section A). The estimation of the

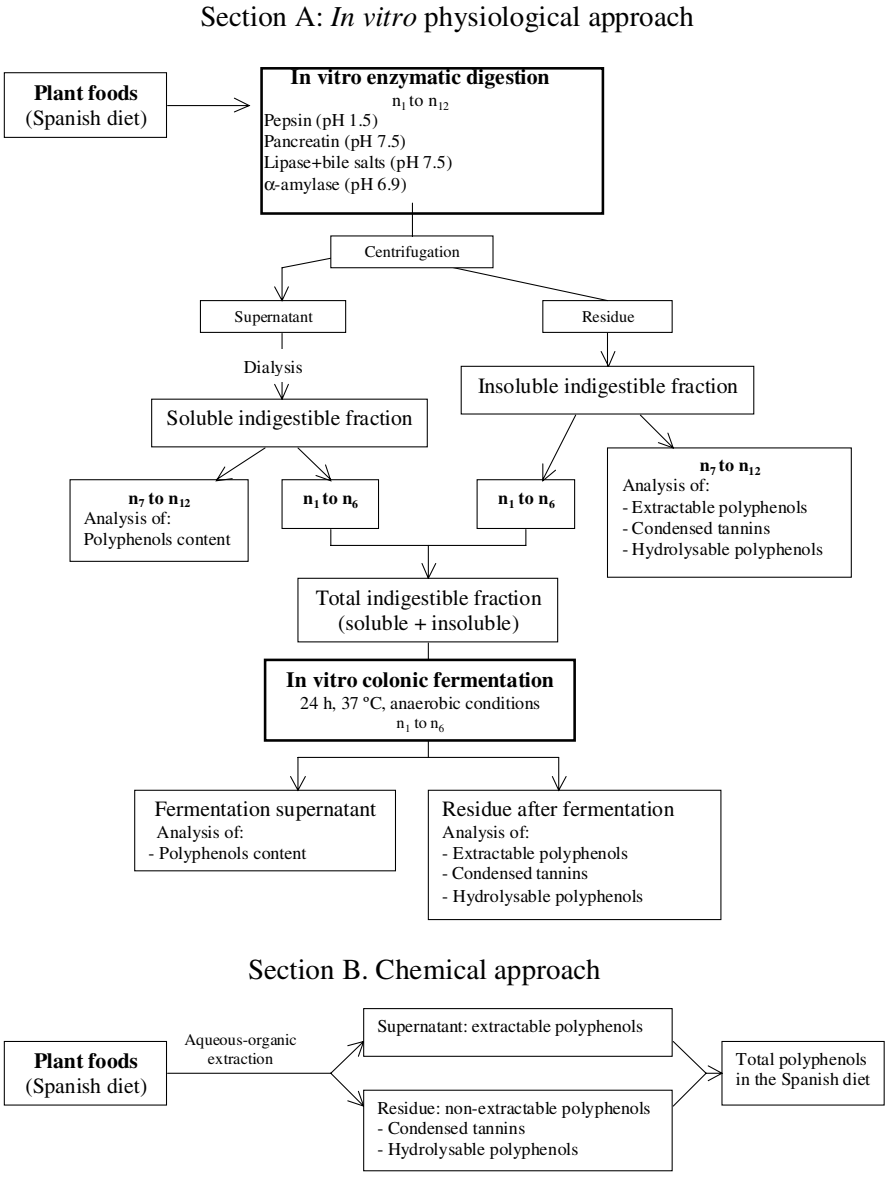

Fig. 1. Schematic of the methodology used to estimate the intake and intestinal bioaccessibility of dietary polyphenols. n = Number of samples for each food group.

bioaccessibility of polyphenols in the small intestine was calculated by the difference between polyphenols content in the original sample and in the residues of enzymatic digestion.

In the in vitro colonic fermentation model, the total indigestible fraction (soluble + insoluble) is fermented in strict anaerobic conditions, using rat caecal content as inoculum. Several compounds are released from the food matrix by the action of bacterial enzymes, while other compounds remains in the food matrix as a part of the residue, after fermentation. The residue after fermentation contains compounds of proven resistance to enzymatic and colonic bacterial degradation, which probably would be excreted in the faeces; these comprise the unaccessible compounds. The difference in polyphenols content between the total indigestible fraction and the residue after the fermentation process allows the estimation of the bioaccessibility of polyphenols in the large intestine.

2.3.1. Determination of indigestible fraction

The procedure to determine the indigestible fraction was described by Saura-Calixto et al. (2000). Twelve samples of each food group (n_1–n_{12}) were successively incubated with digestive enzymes to simulate digestion in the small intestine. Briefly, 300 mg of sample was incubated with pepsin (EC 3.4.23.1, 0.2 ml of a 300 mg/ml solution in HCl-KCl 0.2 M buffer, pH 1.5, 40 °C, 1 h, Merck 7190), pancreatin (1 mL of a 5 mg/mL solution in phosphate buffer 0.1 M; pH 7.5, 37 °C, 6 h, Sigma P-1750), lipase (EC 3.1.1.3, 2 ml of a 7 mg/ml solution in phosphate buffer 0.1 M; pH 7.5, 37 °C, 6 h, Sigma L-3126), bile extract porcine (2 mL of a 17.5 mg/ml solution in phosphate buffer 0.1 M; pH 7.5, 37 °C, 6 h, Sigma B-8631) and α-amylase (EC 3.2.1.1, 1 ml of a 120 mg/ml solution in tris-maleate buffer 0.1 M; pH 6.9, 37 °C, 16 h, Sigma A-3176). Then samples were centrifuged (15 min, 25 °C, 3000*g*) and supernatants removed. Residues were washed twice with 5 ml of distilled water and all supernatants combined. Residues were stored at −18 °C for colonic fermentation (n_1–n_6) and polyphenols analysis (n_7–n_{12}) (polyphenols associated with the insoluble indigestible fraction). Each supernatant was incubated with 100 μl of amyloglucosidase (EC 3.2.1.3, Roche, 102 857) for 45 min at 60 °C, transferred into dialysis tubes (12000–14000 molecular weight cut-off; Dialysis Tubing Visking, Medicell International Ltd., London, UK), and dialyzed against water for 48 h at 25 °C (water flow 7 l/h). Retentates contains soluble dietary fibre and other associate compounds, such as polyphenols and carotenoids. Dialysis retentates (n_7–n_{12}) were stored for the analysis of polyphenols associated with the soluble indigestible fraction, and the other dialysis retentates (n_1–n_6) were concentrated to 5 ml in a R-114 Büchi vacuum rotatory evaporator and then added to their corresponding residue (insoluble indigestible fraction, n_1–n_6, e.g. soluble indigestible fraction n_1 added to insoluble indigestible fraction n_1) and stored at −18 °C for colonic fermentation.

2.3.2. In vitro colonic fermentation

The in vitro fermentation method was described by Barry et al. (1995) and standarised by Goñi and Martin-Carrón (2000). Male Wistar rats (body weight of 200 ± 5 g) fed with standard maintenance diets adjusted to rat dietary requirements (AO4, Panlab, Barcelona, Spain) were supplied by the breeding Centre at the Faculty of Pharmacy (University Complutense of Madrid, Spain). Rats were killed in a carbon dioxide chamber and fresh rat caecal contents were used as inoculum. Caeca were removed through abdominal midline incisions. Rat caecal contents were scraped, weighed and added to a flask containing sterile anaerobic medium to give a 100 g/L inoculum. The anaerobic medium adapted from Goering and Van Soest (1970), contained trypticase, micromineral and macromineral solutions and resazurin as anaerobic redox indicator. The inoculum was mixed (10 min) in a Stomacher 80 Lab Blender (Seward Medical, London, UK) and filtered (1 mm mesh) before use. Total indigestible fractions from enzymatic treatments (n_1–n_6) were mixed with fermentation medium (8 mL, 4 °C, 16 h). Tubes were sealed with rubber caps (No. 407-0-13, Ormacisa, Madrid, Spain). Two ml of inoculum were added and the headspace rinsed with carbon dioxide (1 min). Tubes were placed in a shaking water bath (37 °C, 24 h). Blanks containing no substrate and lactulose (Sigma L-7877) were included in the experiment as zero and completely fermentable substrate, respectively. All the steps were carried out in an oxygen-free CO_2 saturated atmosphere. After incubation time, pH was measured and NaOH (1 M) was used to stop the fermentation process. Samples were centrifuged (2500*g*, 10 min, 25 °C) and the supernatants and residue were collected and stored at −80 °C for polyphenols analysis (residue: polyphenols associated with the residue after fermentation; supernatants: polyphenols available after colonic fermentation). The polyphenols content of supernatants and residue were corrected with blanks of fermentation.

2.4. Chemical approach: Determination of polyphenol content

A chemical approach was employed to determine the total content and intake of polyphenols in the diet (Fig. 1, Section B).

2.4.1. Extractable polyphenols

Original sample was extracted by shaking at room temperature with methanol–water (50:50 v/v, 50 ml/g sample, 60 min, room temperature; constant shaking) and acetone–water (70:30 v/v, 50 ml/g sample, 60 min, room temperature; constant shaking). After centrifugation (15 min, 25 °C, 3000*g*) supernatants were combined and used to determine extractable polyphenols content in the original samples by the Folin–Ciocalteau procedure (Singleton, Orthofer, & Lamuela-Raventos, 1999). The test sample (0.5 ml) was mixed with 0.5 ml of Folin–Ciocalteu reagent and swirled. After 3 min, 10 ml of sodium carbonate

solution (75 g/l) was added and mixed. Additional distilled water was added (14 mL) and mixed thoroughly by inverting the tubes several times. After 1 h, the absorbance at 750 nm was recorded. The results were expressed as gallic acid equivalents.

2.4.2. Non-extractable polyphenols

In the residue of the methanol/acetone/water extraction of polyphenols, condensed tannins-proanthocyanidins and hydrolysable polyphenols were determined separately.

2.4.2.1. Condensed tannins-proanthocyanidins. Residues from the methanol/acetone/water extraction were treated with 5 ml/l HCl–butanol (3 h, 100 °C) (Reed, McDowell, Van Soest, & Horvarth, 1982) for condensed tannins determination. Condensed tannins were calculated from the absorbance at 550 nm of the anthocyanidin solutions. Condensed tannins from Mediterranean carob pod (*Ceratonia siliqua* L) supplied by Nestlé S.A. were treated under the same conditions to obtain standard curves.

2.4.2.2. Hydrolysable polyphenols. Hydrolysable polyphenols comprise hydrolysable tannins, phenolic acids, and hydroxycinnamic acids that are released from the food matrix by strong acidic hydrolysis. No proanthocyanidins or flavonoids were detected in the hydrolysates of hydrolysable polyphenols analysis. Hydrolysable polyphenols were determined by a methanol/H_2SO_4 90:10 (v/v) hydrolysis of 200 g from the residues of the methanol/acetone/water extraction at 85 °C for 20 h (Hartzfeld, Forkner, Hunter, & Hagerman, 2002) from the original sample, insoluble indigestible fraction and the residues after fermentation. The hydrolysate was recollected for polyphenols analysis with Folin–Ciocalteu reagent.

2.5. Calculations

To determine the polyphenols bioaccessibility the following calculations were used:

- Polyphenols accessible in the small intestine: difference between polyphenols content in the original sample and polyphenols associated with the indigestible fraction.
- Polyphenols accessible in the large intestine: differences between polyphenols associated to the indigestible fraction and polyphenols content in the residue after fermentation.

To estimate the intake of polyphenols, the food intake measure uncertainty is added and the extended uncertainty is calculated for the total intake of polyphenols. The data is presented as a range with a confidence level of 95%.

2.6. Statistical analysis of data

All data were reported as mean ± standard deviation for al least four replicates in each treatment.

3. Results and discussion

3.1. Polyphenol intake

Plant foods and beverages are the main sources of antioxidants in the diet. Dietary antioxidants are complex mixtures of hundreds of compounds and the gastrointestinal tract is the major site of their synergistic action. In addition to vitamins C, E and carotenoids, a plant food contains a complex array of phenolic compounds that may help to increase antioxidant capacity of foods (Prior & Cao, 2000; Richelle, Tavazzi, & Offord, 2001). Polyphenols are quantitatively the main dietary antioxidant and they possess higher in vitro antioxidant capacity than vitamins and carotenoids (Gardner, White, McPhail, & Duthie, 2000). The combination of synergistic effects of dietary antioxidants may contribute to the health benefits conferred by the diet. One of the major difficulties of elucidating the health effects of polyphenols is the large number of phenolic compounds found in foods, yielding different biological activities as shown in several in vitro studies (Lambert, Hong, Yang, Liao, & Yang, 2005). A phenolic molecule is often characteristic of a plant species or even of a particular organ or tissue of the plant. It is therefore difficult to ascertain the precise nature of all of the polyphenols that are ingested.

There is a wealth of articles in the literature dealing with specific types of polyphenols in single foods, but information on consumption of polyphenols in diets refers only to specific types of compounds such as flavonols, flavones, catechins or phenolic acids (Manach, Williamson, Morand, Scalbert, & Remesy, 2005). Studies addressing total polyphenols in a whole diet are scarce.

We previously determined total polyphenol content in all vegetable foods and beverages consumed in the Spanish diet (Saura-Calixto & Goñi, 2006). We chose the Folin–Ciocalteu method because quantification of total food polyphenols was the main objective of this work. Possible interferences between extractable polyphenols and other compounds such as amino acids and sugars were checked at an earlier stage and found to be negligible. Most literature data on food polyphenols concern only compounds dissolved in aqueous organic extracts (extractable polyphenols), but this approach may be limited by the extraction techniques, since some polyphenols, especially polyphenols with a high degree of polymerization and polyphenols associated with high molecular weight compounds (non-extractable polyphenols), may escape the standard extraction methods employed. This food polyphenol fraction may become bioactive in the human gut once it is released from the food matrix by the action of digestive enzymes in the small intestine and bacterial degradation in the large intestine (Jenner, Rafter, & Halliwell, 2005).

The experimental design was carried out in two phases: (a) chemical approach of dietary polyphenols intake and (b) physiological approach of polyphenols bioaccessibility (Fig. 1).

The chemical approach followed here consisted of two main steps (Fig. 1, Section B): (1) determination of extractable polyphenols in methanol–water:acetone–water extracts of all plant foods consumed in the Spanish diet plus total polyphenols in dietary beverages; (2) determination of condensed tannins and hydrolysable polyphenols in the residues of the corresponding extracts.

Table 2 shows the total polyphenol content of plant foods in the Spanish diet. Fruits, legumes and nuts are the solid food groups with the highest polyphenol content (mg/g of original dry sample); beverages and vegetables oils present higher polyphenol contents than solid plant foods. The non-extractable polyphenol content (condensed tannins plus hydrolysable polyphenols) is much higher than the extractable polyphenol content in all food groups. Substantial amounts of condensed tannins were found in fruits, legumes and nuts but were not found in cereals and vegetables or in several specific cereal products (bread, rice, oat and wheat bran) (Perez-Jimenez & Saura-Calixto, 2005). Similar results were found by Gu et al. (2004). Hydrolysable polyphenols were a quantitatively important fraction of polyphenols in all groups.

We would note that most of the literature data on food polyphenols refer exclusively to extractable polyphenols and ignore non-extractable polyphenols. The widely used term "total phenolic content" or "total polyphenol content" may be incomplete since it refers only to extractable polyphenols; the actual total polyphenol content in foods is made up of extractable polyphenols plus non-extractable polyphenols.

We estimated that the mean total intake of polyphenols (extractable + non-extractable) in the Spanish diet ranges from 2590 to 3016 mg/person/day (Table 3). Although cereals present the lowest polyphenol content (mg/g of original sample), because of the high proportion of cereals in the Spanish diet, they are the principal food source of polyphenols in the diet, followed by fruits and beverages.

Even though the estimated non-extractable polyphenol content of the diet is almost double that of extractable polyphenols, their physiological effects are closely associated with their degree of bioaccessibility. It is clear that food polyphenols must be bioavailable in some form to exert biological effects. We believe that enzymatic release from the food matrix is one of the main steps in the bioavailability of polyphenols in the small intestine.

3.2. Bioaccessibility of dietary polyphenols

Polyphenols released from the food matrix during the digestive process (named bioaccessible polyphenols) are potentially bioavailable or susceptible to absorption through the gut barrier. There is abundant data in the literature on the content and composition of readily extractable food polyphenols, but comparative few on the non-extractable polyphenols and their bioaccessibility.

There are two main steps in the methodology used in this work to estimate dietary polyphenol bioaccessibility (Fig. 1, Section A): (1) isolation of the indigestible fraction of foods and (2) colonic fermentation of the indigestible fraction. The estimation of the bioaccessibility of polyphenols in

Table 2
Total polyphenols content of plant foods in the Spanish diet (mg/g original dry sample)

Food group	Extractable polyphenols	Condensed tannins	Hydrolysable polyphenols	Total
Cereals	1.07 ± 0.02	n.d.[b]	4.72 ± 0.46	5.79
Vegetables	2.86 ± 0.13	n.d.[b]	4.56 ± 0.33	7.42
Legumes	1.54 ± 0.20	7.66 ± 0.40	5.93 ± 0.32	17.43
Fruits	5.38 ± 0.20	12.33 ± 2.90	6.97 ± 0.68	28.38
Nuts	8.94 ± 0.48	2.00 ± 0.10	8.61 ± 0.69	20.15
Beverages	754.6 (mg/100 mL)[a]	–	–	754.6
Oils	31.3 (mg/100 mL)[a]	–	–	31.3

[a] From Saura-Calixto and Goñi, 2006.
[b] n.d.: Not detected.

Table 3
Total polyphenols intake of plant foods in the Spanish diet (mg/g original dry sample)

Food group	Group intake[a]	Extractable polyphenols	Condensed tannins	Hydrolysable polyphenols	Total polyphenols intake range
Cereals	221.65 ± 4.43	173.75 ± 3.28	n.d.[c]	766.44 ± 74.70	793–1087
Vegetables	280.19 ± 5.61	98.81 ± 4.50	n.d.[c]	157.54 ± 11.41	230–283
Legumes	22.19 ± 0.44	26.08 ± 3.42	129.74 ± 6.79	100.47 ± 5.44	238–275
Fruits	200.60 ± 4.01	134.47 ± 4.98	308.19 ± 72.49	174.24 ± 16.99	470–763
Nuts	5.96 ± 0.12	51.20 ± 2.75	11.45 ± 0.57	49.32 ± 3.95	102–121
Beverages	504.9 (ml)[b] ± 10.10	613.7 ± 13.64[b]	–	–	580–647
Oils	52.0 (ml)[b] ± 1.04	8.3 ± 1.14[b]	–	–	5–11
Total		1106.31 ± 16.19	449.38 ± 72.81	1248.01 ± 77.74	2591–3016

[a] g Edible portion/person/day.
[b] From Saura-Calixto and Goñi (2006).
[c] n.d.: Not detected.

the small and large intestine was calculated by the analysis of polyphenol content in the supernatants and residues obtained by these methods. This methodology has been used for studies of food polyphenol and carotenoid bioaccessibility (Perez-Jimenez & Saura-Calixto, 2005; Serrano et al., 2005). Here we undertake the determination of bioaccessibility in a whole diet (Spanish diet).

Plant foods contain indigestible compounds (indigestible fraction) that are neither digested nor absorbed in the small intestine. It consists not only of dietary fibre but also of other compounds of proven resistance to the action of digestive enzymes, such as resistant starch, resistant protein, polyphenols, Maillard compounds and other associated compounds (Saura-Calixto et al., 2000). The indigestible fraction passes into the colon, where it provides a substrate for fermentative microflora. The residue of the indigestible fraction after the fermentation is a part of the unavailable matter excreted in faeces (Guillon, Renard, Hospers, Thibault, & Barry, 1995).

Experimental conditions for isolation of the indigestible fraction are close to physiological ones. Samples were successively incubated with digestive enzymes (pepsin, lipase, pancreatin, amylase) to simulate digestion in the small intestine. Protease and amylase treatments hydrolyze protein and starch that should favor the release of polyphenols linked or adsorbed to these macronutrients. The indigestible fractions were fermented along with their associated polyphenols. The colonic fermentation procedure used in this work is reported elsewhere (Goñi & Martin-Carrón, 2000) and has been used in bioaccessibility studies (Serrano et al., 2005). Nevertheless, in vivo, the actual amount of polyphenols may differ since the colonic microflora changes in response to gross nutritional shifts (e.g., weaning), progressive change (such as aging) or variations in food intake.

Table 4 shows the total polyphenol content found in the insoluble and soluble indigestible fractions of foods. Major proportions of polyphenols in indigestible fractions are associated with the insoluble indigestible fraction, containing polyphenols that remain in the food matrix. Polyphenols associated with the soluble indigestible fraction will not pass through the intestinal barrier. These polyphenols may have some antioxidant effect on the small intestine since they are soluble in the digesta media. Major parts of food polyphenols are associated with the insoluble indigestible fraction, among them condensed tannins and hydrolysable polyphenols plus an appreciable amount of extractable polyphenols. Fruits and legumes present the highest concentrations of these compounds (Table 4). Moderate quantitative differences were found in polyphenols associated with soluble indigestible fraction (0.88–1.40 mg/g).

The total amount of polyphenols available in the small intestine can be estimated by subtracting the total polyphenol content in the indigestible fraction (Table 4) from the total polyphenol content in the original sample (Table 2). Fruits and nuts presented the highest values. These results suggest that a high proportion of hydrolysable polyphenols (58%) were released by digestive enzyme treatment. Few data on bioavailabilitiy of hydrolysable polyphenols were found in the literature (Kern et al., 2003). On the other hand, condensed tannins presented low bioaccessibility in the small intestine; similar amounts were found in both original sample and insoluble indigestible fraction (Tables 2 and 4). These results are consistent with studies reporting that proanthocyanidins are not absorbed in the small intestine (Rios et al., 2002).

Another important site in the gastrointestinal tract where polyphenols become available is the large intestine. The total polyphenols associated with the indigestible fraction reach the colon, where they become fermentation substrate for bacterial microflora along with the non-digestible food constituents. The abundant microflora in the colon plays a critical role in the metabolism of polyphenols. After microbial enzyme metabolism of any polyphenols that reach the colon, there are two possible routes available, namely absorption of intact polyphenols through the colonic epithelium and passage into the bloodstream or breakdown of the original polyphenol structures into metabolites (Williamson & Manach, 2005). Also, unabsorbed polyphenols that reach the colon may counteract the effects of dietary pro-oxidants in the colon produced during colonic bacterial metabolism.

Table 5 shows the amount of polyphenols found in the fermentation supernatants and in the corresponding residue after fermentation of the indigestible fraction. Polyphenols associated with the residue after fermentation

Table 4
Potential small intestine bioaccessibility of polyphenols of plant foods in the Spanish diet (mg/g dry original sample)

	Cereals	Vegetables	Legumes	Fruits	Nuts
Polyphenols associated to the indigestible fraction					
Soluble indigestible fraction	1.10 ± 0.20	1.20 ± 0.20	1.40 ± 0.60	1.20 ± 0.20	0.88 ± 0.01
Insoluble indigestible fraction					
Extractable	0.60 ± 0.10	0.90 ± 0.10	1.50 ± 0.40	3.20 ± 0.10	4.42 ± 0.26
Condensed tannins	n.d.[a]	n.d.[a]	7.50 ± 0.10	10.70 ± 1.10	1.13 ± 0.22
Hydrolysable	1.83 ± 0.65	3.39 ± 0.43	2.59 ± 0.08	1.71 ± 0.17	2.26 ± 0.07
Total	3.53 ± 0.66	5.49 ± 0.44	12.99 ± 0.42	16.81 ± 1.12	8.69 ± 0.35
Polyphenols bioaccessible in the small intestine[b]	2.26	1.93	4.44	11.57	11.46

[a] n.d.: Not detected.
[b] Polyphenol content in the original sample (see Table 2), minus polyphenols associated with the indigestible fraction (soluble plus insoluble).

Table 5
Potential large intestine bioaccessibility of polyphenols of plant foods in the Spanish diet (mg/g dry original sample)

	Cereals	Vegetables	Legumes	Fruits	Nuts
Polyphenols in the fermentation medium	n.d.[a]	0.60 ± 0.06	1.50 ± 0.30	0.40 ± 0.01	1.90 ± 0.06
Residue after fermentation					
Extractable	0.11 ± 0.06	0.11 ± 0.03	0.37 ± 0.05	0.40 ± 0.02	0.19 ± 0.06
Condensed tannins	n.d.[a]	n.d.[a]	0.36 ± 0.22	0.49 ± 0.04	0.05 ± 0.03
Hydrolysable	0.62 ± 0.02	1.00 ± 0.02	1.35 ± 0.02	2.08 ± 0.03	0.51 ± 0.01
Total	0.73 ± 0.06	1.11 ± 0.07	2.08 ± 0.38	2.97 ± 0.05	0.75 ± 0.09
Polyphenols bioaccessible in the large intestine[b]	2.80	4.38	10.91	13.84	7.94

[a] n.d.: Not detected.
[b] Polyphenols associated to the indigestible fraction (see Table 3), minus polyphenols content in the residue after fermentation.

may be potentially excreted in faeces. Small amounts of condensed tannins were detected in the residue after fermentation, which means that most of the condensed tannins are released from the food matrix and solubilised to the fermentation media by the action of colonic bacteria enzymes. Hydrolysable polyphenols were fermented in smaller proportions than condensed tannins.

Appreciable amounts of phenolics were found in the fermentation media (Table 5). These may represent two kinds of compounds: (1) polyphenols released from the food matrix by the action of bacterial enzymes remaining intact after 24 h of colonic fermentation and (2) fermentation metabolites of polyphenols as phenylacetic, phenylpropionic and phenylvaleric acids produced by the metabolization of condensed tannins (Deprez et al., 2000). Both kinds of compounds are potentially absorbed in the colon; indeed, fermentation by-products of condensed tannins are found in urine after consumption of condensed tannins in rats (Gonthier et al., 2003). Legumes were the food group that showed the highest concentration amount of polyphenols in the fermentation media per gram of original sample, probably due their high condensed tannin content. Nevertheless, other polyphenols fermentation metabolites may not be detected by the Folin–Ciocalteu procedure, such as carbon dioxide (Walle, Walle, & Halushka, 2001). The biological properties of both conjugated derivatives and microbial metabolites have rarely been examined (Scalbert, Morand, Manach, & Remesy, 2002).

Polyphenol availability of the fruit and legume groups in the large intestine was high (Table 6). However, few phenolics from the fruit group were detected in the fermentation supernatants, in comparison with the legume group, indicating that most of the polyphenols released from the food matrix during colonic fermentation in the fruit group were metabolised to other non-phenolic compounds. The large intestine is the largest site of proanthocyanydin bioaccessibility; our results suggest that about 95% of proanthocyanidins were released from the food matrix by the action of bacterial enzymes.

Our results (Tables 3–5) serve to estimate the daily amount of dietary polyphenols bioaccessible in the small and large intestine and the non-bioaccessible polyphenols (Table 6). It is estimated that about 48% of total polyphenols from solid vegetable foods in the diet are bioaccessible

Table 6
Estimation of the intestinal bioaccessibility of polyphenols from plant foods in the Spanish diet (mg/person/day)

Group	Bioaccessibles in the small intestine	Bioaccessibles in the large intestine	Non-bioaccessible polyphenols
Cereals	367	455	119
Vegetables	67	151	38
Legumes	36	185	35
Fruits	197	346	74
Nuts	62	45	4
Beverages	614	–	–
Oils	8	–	–
Total	1351	1182	270

in the small intestine. Polyphenols from beverages are completely bioaccessible because they pass directly into the intestinal fluids, and they are the largest contributors of bioaccessible polyphenols in the small intestine. However, only a small part of small intestine bioaccessible polyphenols can be absorbed through the intestinal mucosa and therefore metabolized. Numerous studies have shown bioavailability of bioaccessible polyphenols in the small intestine to be very low, reporting values between 5% and 10% (Clifford, 2004). Therefore, in addition to polyphenols associated with the indigestible fraction, a major part of the polyphenols bioaccessible in the small intestine may also reach the colon because of their low bioavailability. If we assume that only 5–10% of dietary polyphenols (including beverage polyphenols) are absorbed in the small intestine, this means that between 2669 and 2736 mg/day of polyphenols reach the colon. This amount is made up of polyphenols that are bioaccessible in the small intestine plus polyphenols associated with the indigestible fraction. The significance of the presence of so large an amount of polyphenols in the large intestine for gastrointestinal health remains to be elucidated. Minor parts of dietary polyphenols (10%) were associated with the residue after fermentation, which suggests that 90% of dietary polyphenols may be bioaccessible in the gut as intact molecules and/or as polyphenol fermentation metabolites.

Intakes and bioaccessibility were estimated in this work on the basis of total polyphenol determination. Further research using HPLC analysis is required to determine the different polyphenol groups and individual polyphenols

in the corresponding aqueous organic extracts and digesta media obtained by these methods.

3.3. Conclusions

The mean daily intake of polyphenols in the Spanish diet was estimated between 2590 and 3016 mg/day. This amount includes both extractable and non-extractable polyphenols. Non-extractable polyphenols or polyphenols associated with the indigestible fraction are the majority fraction of dietary polyphenols. It was estimated that about 48% of dietary polyphenols are bioaccessible in the small intestine, while 42% become bioaccessible in the large intestine. Small amounts (10%) were inaccessible and remained in the food matrix after the whole digestion process. These data may be useful for the design and interpretation of epidemiological and intervention studies on the effects of polyphenols and vegetables foods on health. Further research is needed to elucidate which specific compounds out of the general pool of food polyphenols may produce significant biological effects.

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