

# Dietary fibre composition, antioxidant capacity and physico-chemical properties of a fibre-rich product from cocoa (*Theobroma cacao* L.)

Elena Lecumberri<sup>a</sup>, Raquel Mateos<sup>a</sup>, María Izquierdo-Pulido<sup>b</sup>, Pilar Rupérez<sup>a</sup>,  
Luis Goya<sup>a</sup>, Laura Bravo<sup>a,\*</sup>

<sup>a</sup> Department of Metabolism and Nutrition, Instituto del Frío (CSIC), José Antonio Novais 10, Ciudad Universitaria, 28040 Madrid, Spain

<sup>b</sup> Department of Nutrition and Food Science, Facultad de Farmacia, Universidad de Barcelona. Av. Joan XXIII s/n, 08028 Barcelona, Spain

Received 7 July 2006; received in revised form 26 December 2006; accepted 28 December 2006

## Abstract

The proximate composition and dietary fibre (DF) content of a fibre-rich product obtained from cocoa were studied. This product contained 60.54% (dry matter basis) of DF, made of mainly insoluble fibre although with appreciable amounts of soluble dietary fibre (10.09% d.m.). The presence of associated polyphenolic compounds (1.32% and 4.46% of soluble polyphenols and condensed tannins, respectively) provides this fibre material with intrinsic antioxidant capacity as determined by the FRAP and TEAC methods. Hydration properties (swelling and water holding capacity) and the glucose retardation index of cocoa fibre were similar to other natural commercial insoluble fibres. The antioxidant capacity of this fibre-rich cocoa powder and its physico-chemical properties render it a suitable product to be used in the preparation of low-calorie, high-fibre foods.

© 2007 Elsevier Ltd. All rights reserved.

**Keywords:** Cocoa; Dietary fibre; Antioxidant capacity; Nutritional properties; Functional food

## 1. Introduction

Cocoa beans are the fruit from the plant *Theobroma cacao* L., a plant tree originated in the rain forests of America which culture has extended to equatorial areas of Africa and Asia. Foods prepared from cocoa beans have been consumed for over 2600 years, initially by early meso-Americans, now constituting one of the most widely consumed processed foods, chocolate. Milk chocolate drinks or solid bars of chocolate contain varying percentages of cocoa liquor, cocoa butter, cocoa solids, sugar and milk; cocoa powder is also used as ingredient in baked chocolate goods such as cookies, chocolate cakes, etc.

Consumed by its flavour and textural properties, chocolate is often considered as a confectionery food rich in fat and calories, but devoid of essential nutrients. However,

this general view is changing and chocolate is receiving much attention from nutritionists and food scientists in recent years due to the high polyphenolic content of cocoa beans. Reports on the polyphenolic content of cocoa products vary greatly in the literature, with values ranging from 3.3 to up to 65 mg/g in cocoa powder or 1.7 to 36.5 mg/g total polyphenols in dark chocolate (Adamson et al., 1999; Vinson, Proch, & Zubik, 1999), being mainly flavan-3-ols (monomeric epicatechin and catechin, as well as their oligomers from dimers to decamers, the procyanidins), with small amounts of anthocyanins (mainly cyanidin glycosides) and flavonols (quercetin glycosides) (Adamson et al., 1999; Hammerstone, Lazarus, Mitchell, Rucker, & Schmitz, 1999). However, although polyphenolic compounds usually accumulate in the outer parts of plants such as shells, skins, etc. (Bravo, 1998) there is scarce information on the polyphenolic content of cocoa husks.

Cocoa polyphenols have been suggested to positively influence cardiovascular health through inhibition of lipid

\* Corresponding author. Tel.: +34 915445607; fax: +34 915493627.

E-mail address: [lbravo@if.csic.es](mailto:lbravo@if.csic.es) (L. Bravo).

peroxidation, platelet activation or cyclo-oxygenase and lipoxygenase activities, and enhancing levels of the endothelial-derived relaxing factor, nitric oxide (Karim, McCormick, & Kappagoda, 2000; Rein et al., 2000; Schewe, Kühn, & Sies, 2002; Steinberg, Bearden, & Keen, 2003; Wan et al., 2001; Wiswedel et al., 2004). Also, cocoa polyphenols have shown to have antimutagenic activity (Yamagishi et al., 2000), and decreased levels of 8-hydroxy-2'-deoxyguanosine, a biomarker of oxidative damage to DNA, have been reported in rats after consumption of cocoa suggesting a potential role in cancer (Orozco, Wang, & Keen, 2003).

Besides flavonoids, cocoa is rich in other component of remarkable nutritional interest such as dietary fibre (DF). According to the updated definition of DF put forward by the American Association of Cereal Chemists Expert Committee on dietary fibre (De Vries, 2003), this consists of plant cell wall polysaccharides, lignin and associated substances resistant to hydrolysis by the digestive enzymes of humans. DF has well-documented beneficial effects on human health and body function; thus, a high consumption of DF is associated with a reduced incidence of disorders and diseases common in developed societies such as chronic bowel disorders, obesity, diabetes, cardiovascular disease and cancer (Bessesen, 2001; Johnson, 2004; Kris-Etherton et al., 2002; Sungsoo Cho & Dreher, 2001).

It has been suggested that cocoa hull may be a good source of dietary fibre, with reported values ranging from 38% to 44% of total dietary fibre as non-starch polysaccharides plus Klason lignin (Martín-Cabrejas, Valiente, Esteban, Mollá, & Waldron, 1994; Serra-Bonvehí & Aragay-Benería, 1998). Considering the health benefits associated to the consumption of dietary fibre and polyphenols in the diet, the presence of both bioactive components in cocoa bean husks could highlight the interest of such a product as a potential ingredient for the functional food industry.

In the present work we studied the composition of a fibre-rich product obtained from cocoa bean husks, as well as some properties related to its nutritional quality, such as its polyphenolic content, antioxidant capacity and physico-chemical properties.

## 2. Experimental

### 2.1. Samples and reagents

The dietary fibre rich cocoa product was supplied as a fine powder by Nutrexpa S.A. (Barcelona, Spain). This product was obtained as follows: clean cocoa beans were cracked to loose the shells from the nibs. After this breaking step, the fractions obtained go to the winnowing cabinets where the husks are air-separated from the kernels. Then, husks go through a sterilization process and they are finally ground to a particle size of 75 µm. Carob pod fibre (*Ceratonia siliqua* L.) was obtained from Compañía General del Algarrobo de España S.A. (Valencia, Spain). Guar gum,

cellulose, citrus pectins and apple pectins were all from Sigma Chemical Co. (Madrid, Spain), as were all enzymes and standards, except for the carob pod condensed tannins standard that was supplied by Nestlé S.A. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water soluble analogue of vitamin E, was from Aldrich Chemicals Co. (Guillingam, Dorset, UK). ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid, diammonium salt) and TPTZ (2,4,6-tripyridyl-*s*-triazine) were from Fluka Chemicals (Madrid, Spain). All other reagents were of chromatographic or analytical quality.

### 2.2. Analytical procedures

Dietary fibre was analysed in defatted fibre-rich cocoa samples by the AOAC method modified in our laboratory (Mañas, Bravo, & Saura-Calixto, 1994). Briefly, samples were treated with heat-stable  $\alpha$ -amylase (Sigma, A-3306), protease (P-3910) and amyloglucosidase (A-9913), followed by centrifugation (15 min, 3000g) instead of filtration to separate the soluble and insoluble fractions obtained after enzymatic hydrolysis of digestible compounds. Supernatants were quantitatively collected and pellets were washed twice with 10 mL of distilled water, centrifuged and all supernatants combined. These were transferred into dialysis tubes (12000–14000 MWCO, Dialysis Tubing Visking, Medicell International Ltd., London, UK), and dialysed against water for 48 h at 25 °C (water flow 7 L/h in a 43 L reservoir). Dialysates (soluble dietary fibre, SDF) were hydrolysed with 1 M sulphuric acid at 100 °C, 90 min and non-starch polysaccharides (NSP) determined in the hydrolysate. The residues obtained after enzymatic hydrolysis of samples (insoluble dietary fibre, IDF) were dried overnight at room temperature, and hydrolysed with sulphuric acid (12 M H<sub>2</sub>SO<sub>4</sub>, 1 h, 30 °C, then diluted to 1 M H<sub>2</sub>SO<sub>4</sub> 100 °C, 90 min with shaking). After acid hydrolysis, samples were centrifuged (15 min, 3000g), pellets washed twice with distilled water, and combined supernatants collected for NSP determination. Residues were dried at 105 °C overnight and gravimetrically quantified as Klason lignin (KL).

Uronic acids (UA) in hydrolysates from both SDF and IDF were quantified spectrophotometrically by the Scott (1979) method using galacturonic acid as standard. Neutral sugars (NS) were analysed chromatographically by GLC as alditol acetates (Englyst, Quigley, Hudson, & Cummings, 1992), using inositol as internal standard. A Shimadzu GC-14A gas chromatograph (Shimadzu Co., Kyoto, Japan) fitted with a flame ionization detector was used. Separation of sugars was achieved in a SP-2330 capillary column (30 m × 0.32 mm i.d., Cat No. 2-4073, Supelco, PA, USA). Column temperature was set at 240 °C (isothermal), and injector and detector temperature was 270 °C. Nitrogen was used as carrier gas. Data were collected and processed with an Agilent ChemStation software system (Agilent Technologies, Waldrom, Germany). Total dietary fibre (TDF) was calculated as IDF + SDF; IDF

was calculated as NSP + KL, and SDF as NSP (NSP = UA + NS).

*Soluble polyphenols* were extracted by sequentially washing 1 g of sample with 40 mL of 16 mM hydrochloric acid in 50% aqueous methanol (50:50, v/v, 1 h at room temperature, constant shaking) and 40 mL of acetone–water (70:30, v/v, 1 h room temperature, constant shaking) in 50 mL centrifuge tubes (Bravo & Saura-Calixto, 1998). After centrifugation (15 min, 3000g), supernatants from each extraction step were combined and made up to 100 mL. Polyphenols in the extracts were analysed spectrophotometrically as total polyphenols by using the Folin-Ciocalteu's reagent and gallic acid as standard; also, the soluble tannins fraction in these extracts was quantified by treating the samples for 3 h at 100 °C with 5% HCl–butanol (v/v) (Reed, McDowell, Van Soest, & Horvath, 1982). This same method was used to determine insoluble condensed tannins in the residues obtained after extraction of soluble polyphenols. The proanthocyanidin solutions thus obtained were quantified spectrophotometrically at 553 nm, using carob pod condensed tannins as standard.

*Antioxidant capacity* of the fibre-rich cocoa powder was evaluated in the soluble polyphenols extract by two different methods: the FRAP assay as an evaluation of the reducing power of the sample, and the TEAC method to assess its free radical scavenging capacity.

*FRAP assay*: the ferric reducing/antioxidant power (FRAP) as described in Pulido, Bravo, and Saura-Calixto (2000) was used. Briefly, FRAP reagent (containing 10 mM TPTZ in 40 mM HCl, 20 mM FeCl<sub>3</sub> · 6H<sub>2</sub>O and 0.3 M acetate buffer, pH 3.6), prepared daily and kept at 37 °C, was mixed with test samples, water or Trolox. Absorbance readings at 595 nm were taken after 30 min reaction using a Beckman DU640 spectrophotometer (Beckman Instruments Inc., Fullerton, CA) equipped with a thermostated auto-cell holder set at 37 °C. Increases in absorbance are due to Fe<sup>3+</sup> reduction by antioxidants and the subsequent formation of a coloured TPTZ–Fe<sup>2+</sup> complex with an absorbance maximum at 595 nm. A Trolox reference curve (0.1–0.8 mM) was used as a standard and the results were expressed as μmol of Trolox per gram of dry matter.

*TEAC assay*: the capacity of samples to scavenge the free radical cation ABTS<sup>+</sup> was assessed using the method of Re et al. (1999). ABTS<sup>+</sup> was preformed by the reaction of 7 mM ABTS with 2.54 mM potassium persulphate during 12–16 h at room temperature in the dark. Decrease of ABTS<sup>+</sup> absorbance at 658 nm (initially adjusted by dilution in ethanol to an absorbance of 0.7 ± 0.02) in the presence of the polyphenol extract was monitored during 6 min. Trolox (0–0.8 mM) was used for calibration and results expressed as μmol of Trolox per gram of dry matter.

*Glucose retardation index* (GRI) was determined by the procedure of Jenkins, Jenkins, Wolever, Taylor, and Ghafari (1986) slightly modified (Bravo, 1999). Prior to the assay, samples were extracted with 85% ethanol to ensure removal of soluble sugars. For the GRI assay, dialysis bags

(12000–14000 MWCO, Dialysis Tubing Visking, Medicell International Ltd., London, UK) were loaded with 7.5 mL of distilled water (control) or 500 mg of sample plus 7.5 mL of distilled water, and hydrated in a chamber at 100% relative humidity for 60 min. After this time, a glucose solution was added into the bags to a final concentration of 2 mg glucose mL<sup>-1</sup>, thoroughly mixed with the solid samples, and the bags transferred into reservoirs containing 400 mL distilled water and held in a thermostatic water bath at 37 °C for 1 h with constant shaking. At 15 min intervals, 0.5 mL of dialysate was collected and glucose concentration determined spectrophotometrically using a glucose oxidase–peroxidase method (Peridochrom Glucose, GOD-POP, Böehringer Mannheim, Germany). The retardation of glucose diffusion from the dialysis bag into the dialysate was calculated as follows:

$$\text{GRI} = 100 - \left[ \frac{\text{Total glucose diffused from fibre sample}}{\text{Total glucose diffused from control sample}} \times 100 \right]$$

*Swelling*: sample was accurately weighed (200 mg) and transferred into a calibrated cylinder (1.5 cm diameter), and 10 mL of distilled water containing 0.02% sodium azide as a bacteriostat were added. After thoroughly mixing, cylinders were let to stand undisturbed for 18 h at room temperature. Then, the bed volume was recorded and swelling was calculated as mL per gram of dry sample (Robertson et al., 2000).

*Water holding capacity* (WHC): sample (250 mg) was accurately weighed in 50 mL centrifuge tubes, and 25 mL of distilled water containing 0.02% sodium azide were added, thoroughly mixed and let to stand at room temperature for 60 min. After this time, samples were centrifuged (15 min, 3000g) and the supernatant carefully removed. Sample fresh weight was recorded and WHC expressed as the amount of water retained per gram of dry sample (g g<sup>-1</sup> dry matter) (Robertson et al., 2000).

### 2.3. Other analyses

Protein was quantified using an automated nitrogen analyser (LECO FP-2000, LECO Corp., Michigan, USA). Protein was calculated as  $N \times 5.75$ . Fat was quantified after extraction with light petroleum in a Soxtec System HT (Soxtec Extraction Unit 1043 and Service Unit 1046, Tecator, Höganäs, Sweden). Ash content was determined gravimetrically after calcination in a muffle furnace at 550 °C for 16 h. Soluble sugars were measured spectrophotometrically in the extracts obtained after washing samples with 85% ethanol using anthrone/thiourea as reagent and glucose as standard following the conditions described elsewhere (Mañas et al., 1994).

### 2.4. Statistics

Samples were analysed in triplicate unless stated otherwise. Results are expressed as mean values ± standard deviations. To assess for differences in the physico-chemical

properties between the cocoa-fibre product and other soluble or insoluble dietary fibre sources, multiple sample comparison was performed using the Statgraphics Plus program version 2.1 (Statistical Graphics Corp., Rockville, MD). Analysis of variance (ANOVA) followed by Duncan's multiple comparison test were used to contrast groups. Cochran's test was performed to test for homogeneity of variances, and to discriminate among means the Fisher's least significant difference procedure was applied. No transformation of raw data was required for swelling and GRI values, although logarithmic transformation of raw data was required in the case of WHC values to ensure variance homogeneity. The level of significance was  $P < 0.05$ .

### 3. Results and discussion

Dietary guidelines recommend a minimum daily intake of DF of 25 g (equivalent to 12.5 g DF per 1000 calories consumed) (Marlett et al., 2002; USDA, 2000), which is considerably higher than the estimated intakes in Western countries (Sungsoo Cho & Dreher, 2001). Therefore, there is a need to increase fibre intake, which has prompted the consumption of dietary supplements or fibre-enriched food products. DF components like pectins, gums, cellulose and others have been used as functional ingredients by the food industry, with an extensive market of food by-products as DF sources. In the present work we report the composition, as well as the antioxidant and some physico-chemical properties of a fibre-rich product obtained from cocoa bean husks, which might be used as an ingredient in the preparation of different food products and/or dietetic, low-calorie high-fibre foods.

Table 1 shows the proximate composition of the dietary fibre-rich cocoa powder. This product had a relatively low fat content in comparison with cocoa beans where fat constitutes over 50% of the dry weight (Valiente, Esteban, Mollá, & López-Andréu, 1994). Martín-Cabrejas et al. (1994) reported a fat content in cocoa hulls of 18.5% (dry matter basis), higher than the values found in the fibre-rich cocoa powder (less than 7%). Also, the content of soluble sugars was negligible (lower than 0.5%), which together with the low fat content represent an advantage of this product, with a reduced caloric value. Protein and ash were higher than those reported by Martín-Cabrejas et al. (1994), as well as total dietary fibre content, which was

Table 1  
Proximal composition of the fibre-rich cocoa product (% dry matter)

	Fibre-rich cocoa product
Protein	16.71 ± 0.18
Fat	6.62 ± 0.38
Ash	11.42 ± 0.04
Soluble sugars	0.35 ± 0.04
Dietary fibre	60.54 ± 0.32
Polyphenols	5.78 ± 0.29

Mean values ± STD ( $n = 3$ ).

close to 60%. Total polyphenols (as the sum of soluble polyphenols plus insoluble condensed tannins) in this cocoa fibre amounted to close to 6% of the dry matter.

Total dietary fibre (TDF), as well as the content of the major constituents of the soluble and insoluble DF fractions (neutral sugars, uronic acids and Klason lignin), are shown in Table 2. TDF content of this cocoa product was extremely high, over 60% of the dry matter. This implies that this product might be of interest for the food industry, considering its potential application as a functional ingredient in confectionery, bakery or in the preparation of low-fat, high-fibre dietetic products. As for the constituent fractions, SDF accounted for less than 17% of the TDF content of the fibre-rich cocoa product, made of mainly pectic substances as shown by the high uronic acids content (7.13% of the dry matter). Quantitatively, IDF was the main component of this cocoa product, accounting for over 80% of the TDF and 50% of the total dry weight. Close to one third of this IDF fraction corresponded to non-starch polysaccharides (18.1% of the dry matter), the remaining being Klason lignin (KL). Serra-Bonvehí and Aragay-Benería (1998) reported a TDF content of 43.9% in cocoa husks, with values for SDF higher than those analysed in the present work. These authors did not mention the KL residue, yet Chung, Iiyama, and Han (2003) reported values of 32% in cocoa hulls, which are similar to the values in this fibre-rich cocoa product.

Table 2  
Content and composition of the soluble and insoluble dietary fibre fractions of the cocoa product (% dry matter)

	Fibre-rich cocoa product	
	(% Dry matter)	% Relative to TDF
Soluble dietary fibre	10.09 ± 0.38	16.67
Rhamnose	0.29 ± 0.04	–
Fucose	n.d.	–
Arabinose	0.29 ± 0.01	–
Xylose	0.09 ± 0.01	–
Mannose	0.51 ± 0.01	–
Galactose	1.36 ± 0.02	–
Glucose	0.41 ± 0.03	–
Neutral sugars <sup>a</sup>	2.96 ± 0.10	4.89
Uronic acids	7.13 ± 0.29	11.78
Insoluble dietary fibre	50.42 ± 0.70	83.32
Rhamnose	0.15 ± 0.01	–
Fucose	0.06 ± 0.02	–
Arabinose	0.94 ± 0.01	–
Xylose	0.97 ± 0.01	–
Mannose	0.96 ± 0.01	–
Galactose	0.91 ± 0.01	–
Glucose	10.53 ± 0.15	–
Neutral sugars <sup>a</sup>	14.53 ± 0.16	24.01
Uronic acids	3.48 ± 0.13	5.75
Klason lignin	32.41 ± 0.40	53.56
Total dietary fibre (TDF)	60.51 ± 0.32	100

n.d.: Not detected; TDF: total dietary fibre.

Mean values ± STD ( $n = 3$ ).

<sup>a</sup> Sum of constituents sugars (by GLC).

As for the constituent sugars, the IDF fraction was rich in glucose (Table 2), suggesting that cellulose was the major non-starch polysaccharide (NSP) of cocoa fibre (about 10% of the dry matter), which agrees with reports from other authors (Chung et al., 2003; Redgwell et al., 2003; Serra-Bonvehí & Aragay-Benería, 1998). Minor amounts of monosaccharides such as arabinose, xylose, mannose or galactose, together with a significant content of uronic acids were indicative of the presence of hemicelluloses (xyloglucans, arabinoxylans, glucuronoxylans) and pectic substances associated to the cell wall matrix in cocoa bean shells. Together neutral sugars plus uronic acids in the fibre-rich cocoa powder amounted to 18.01% of the dry matter in the IDF fraction (Table 2). As to SDF (10.09% of the dry matter), it showed appreciable amounts of galactose and mannose, which may be suggestive of the presence of minor amounts of galactomannans or else these monosaccharides may be part of pectins, which are the major component of SDF. Pectic substances, both soluble and associated to insoluble cell wall polysaccharides in the IDF fraction and determined as uronic acids, amounted to nearly 10% of the dry cocoa fibre product. This is in accordance with results reported by Redgwell et al. (2003), who found that cellulose and pectins were the major cell wall polysaccharides in cocoa shells.

The NSP fraction of the cocoa fibre powder showed a rather balanced content of both soluble (close to 40% of the total NSP) and insoluble NSP, which can be considered as another appreciable attribute of this fibre product. Many commercial DF sources, usually obtained as by-products of the food industry (e.g. cereal brans, wine by-products), mostly provide IDF and little SDF, a fraction of nutritional and technological interest due to its functional properties. Taken together, NSP in soluble and insoluble DF made up nearly half the TDF content of the cocoa fibre (28.1% dry matter), the other half being Klason lignin (Table 2). This fraction is not considered as a DF constituent by some authors or in countries where the definition of DF is that of indigestible non-starch polysaccharides of plant origin (Englyst & Cummings, 1990), although it is considered as an integral component of DF as officially defined by the AOAC (Prosky et al., 1988) and also as a component of the indigestible fraction of foods (De Vries, 2003; Saura-Calixto, García-Alonso, Goñi, & Bravo, 2000). KL encompasses not only lignin, but also other polyphenols – including condensed tannins –, resistant protein, Maillard reaction products, etc. (Bravo & Saura-Calixto, 1998), being a non-digestible constituent of plant foods that might account for some of the physiological properties of DF (Bravo, Abia, & Saura-Calixto, 1994; Saura-Calixto et al., 2000).

Polyphenols (PP), especially highly polymerized compounds like condensed tannins (CT) and phenols bound to protein and polysaccharides from cell walls, are some of the substances associated to DF and partly quantified in the Klason lignin residue. Some polyphenolic compounds behave as DF constituents, resisting hydrolysis

by digestive enzymes, increasing fecal bulk, and protein and fat excretion (Bravo, 1998; Bravo, Abia et al., 1994; Saura-Calixto et al., 2000). But polyphenols are also well-known bioactive compounds that have shown to be protective against diseases like coronary heart disease, cancer, neurodegenerative disorders, etc., mostly through their antioxidant and free radical scavenging capacities (Bravo, 1998; Wan et al., 2001). The content of PP naturally present in the cocoa fibre product is shown in Table 3, as well as the reducing power and the scavenging capacity against the ABTS<sup>•+</sup> radical cation of polyphenolic extracts obtained from this fibre. The content of both CT and soluble PP was lower than expected. Especially the concentration of soluble PP, with only 1.32% of the dry matter (with nearly 80% of these soluble phenols corresponding to proanthocyanidins), was very low with a value nearly five times smaller than that found in cocoa powder (Vinson et al., 1999). Still, the total polyphenolic content of this cocoa product was higher than that reported in other foods like cereals, legumes, vegetables, nuts or fruits (Saura-Calixto & Goñi, 2006). As a consequence of the low soluble polyphenolic content of the fibre-rich cocoa product, the antioxidant activity of the extracts was also relatively low (Table 3). However, the reducing power of these extracts (FRAP values) was higher than that found in the mentioned groups of plant foods, while the free radical scavenging capacity (determined by the TEAC assay) was comparable to that found in fruits, vegetables and legumes, and higher than in cereal foods (Saura-Calixto & Goñi, 2006). It is worth noting that the antioxidant capacity of the phenolic compounds in foods is usually attributed to the soluble fraction. This soluble, low-molecular weight polyphenolic fraction, in spite of its limited bioavailability (reviewed in Williamson & Manach, 2005), is partly absorbed in the gastrointestinal tract and thus it would contribute to the antioxidant status in vivo, whilst the high-molecular weight condensed tannins would remain in the intestinal tract, therefore limiting their potential biological effects to this location.

Although the content of polyphenolic compounds and the antioxidant capacity of the fibre-rich cocoa product were relatively low, it is important to emphasize that these are remarkable attributes of this product, especially in

Table 3  
Phenolic content and antioxidant activity of the fibre-rich cocoa product

	Fibre-rich cocoa product
<i>Soluble polyphenols (% d.m.)</i>	
As total phenols <sup>a</sup>	1.32 ± 0.02
As soluble tannins <sup>b</sup>	1.04 ± 0.06
<i>Condensed tannins (% d.m.)</i>	
FRAP (µmol TE/g d.m.)	72.32 ± 0.67
TEAC (µmol TE/g d.m.)	7.73 ± 0.47

TE: Trolox equivalents.

Mean values ± STD (n = 4).

<sup>a</sup> Analysed by the Folin-Ciocalteu's method.

<sup>b</sup> Analysed by the method of Reed et al. (1982).

Table 4  
Physico-chemical properties of cocoa fibre in comparison with other commercial fibres

	Swelling (mL/g d.m.)	WHC (g/g d.m.)	GRI (%)
Fibre-rich cocoa product	6.51 ± 0.11 <sup>a</sup>	4.76 ± 0.32 <sup>a</sup>	4.40 ± 0.6 <sup>a</sup>
Cellulose	n.d.	0.71 ± 0.13 <sup>b</sup>	n.d.
Carob pod fibre	n.d.	5.53 ± 0.64 <sup>a</sup>	8.23 ± 1.71 <sup>b</sup>
Guar gum	n.d.	63.07 ± 3.69 <sup>c</sup>	25.80 ± 4.10 <sup>c</sup>
Apple pectins	7.42 ± 1.15 <sup>a</sup>	16.51 ± 3.77 <sup>d</sup>	25.79 ± 2.81 <sup>c</sup>
Citrus pectins	10.45 ± 1.21 <sup>b</sup>	28.07 ± 5.37 <sup>c</sup>	27.01 ± 1.40 <sup>c</sup>

WHC: water holding capacity; GRI: glucose retardation index; n.d.: not determined.

Different superscript letters within a column denote statistically significant differences ( $P < 0.05$ ). Mean values ± STD ( $n = 3$ ).

comparison with other sources of dietary fibre like cellulose, wheat bran, etc., commonly used in the food industry, which lack of intrinsic antioxidant capacity. Therefore, this quality of the cocoa fibre product enhances its interest as an alternative source of dietary fibre.

On the other hand, the physico-chemical properties of DF and in consequence its technological functionality and nutritional effects are highly determined by the composition of the soluble and insoluble DF fractions. This fibre-rich cocoa powder, with a high IDF content as seen above, showed physico-chemical properties common to insoluble fibre materials as it can be seen in Table 4, where the hydration properties (swelling and water holding capacities) and glucose retardation index of the cocoa fibre product in comparison with some other sources of DF, both soluble (guar gum, and apple and citrus pectins) and insoluble (cellulose and carob pod fibre) are shown. As it could be expected, the studied physico-chemical properties of the fibre-rich cocoa product were similar to those of fibre materials such as the carob pod fibre, which is a product rich in IDF (about 70%) but with low SDF content (2% d.m.) (Bravo, Grados, & Saura-Calixto, 1994). The water holding capacity (WHC) of the fibre-rich cocoa product was higher than that reported by Serra-Bonvehí and Aragay-Benería (1998) for cocoa husks (4.76 vs. 3.62 g H<sub>2</sub>O g<sup>-1</sup> dry matter) in spite of the higher SDF content found by these authors. Cellulose, as a pure IDF component, showed lower swelling and water retention capacities than both cocoa and carob pod fibres, indicating that non-cellulosic components of both fibres (i.e. hemicellulose and pectic substances) markedly contribute to their hydration properties. However, both IDF-rich materials (cocoa and carob pod fibres) and cellulose had a much lower hydration capacity than the analysed SDF materials, guar gum and fruit pectins, which also showed a high ability to retain glucose in their matrix as compared with the insoluble fibres.

In general, soluble fibres have a high hydration capacity, holding water and swelling to form viscous solutions. They also adsorb and retain other substances like minerals, non-polar molecules (i.e. fats, bile acids), glucose, etc. Insoluble fibres can also adsorb and retain water within their fibrous matrix, yet not forming viscous solutions, presenting the

ability to adsorb other components similarly to SDF. Technologically this results in the use of soluble fibre components (pectins, gums, carrageenans, alginates, etc.) as thickening and gelling agents, foam and emulsion stabilizers, film-forming and fat-mimetic agents, in flavour encapsulation, etc. Insoluble fibres contribute to stabilise food systems, improving product density and minimizing shrinkage, as texturizing agents that contribute to mouthfeel, etc. Fibres are also used as anticaking and antisticking agents, and thanks to their hydration properties they help to retard staling, control moisture and ice crystal formation, reducing syneresis and increasing food stability (Gelroth & Ranhotra, 2001).

All these properties of DF of interest for the food industry are matched by the beneficial physiological effects of fibre. It has been shown that a high consumption of DF is associated with a reduced incidence of disorders and diseases common in developed societies such as chronic bowel disorders, obesity, diabetes, cardiovascular disease and cancer (Bessesen, 2001; Johnson, 2004; Kris-Etherton et al., 2002; Sungsoo Cho & Dreher, 2001). When these benefits associated to DF are further improved by the presence of naturally occurring polyphenols that naturally confer intrinsic antioxidant properties to the cocoa fibre, the potential interest of this product as a food ingredient is notably enhanced.

In summary, the cocoa product analysed in this study is very rich in DF, mostly insoluble fibre, with appreciable amounts of antioxidant polyphenolic compounds naturally associated to this fibre product. Although rich in IDF, this cocoa product contains nearly 10% of pectic substances, which is higher than the amount of soluble fibre usually supplied by other IDF-rich products like cereal brans commonly used as DF sources. The antioxidant capacity of this cocoa fibre and its physico-chemical properties make it a suitable product to be used in the preparation of low-calorie, high-fibre foods like chocolate cookies, chocolate cakes, dietetic chocolate supplements, etc. where the colour and flavour of this cocoa fibre might be advantageous.

#### Acknowledgements

We want to thank Ms. I Fernandez and Ms. MR Redondo for their technical support. The financial contribution of the Spanish Ministerio de Ciencia y Tecnología (project AGL2000-1314) and Nutrexp SA is acknowledged. E. Lecumberri has an I3P grant from the Spanish Council for Scientific Research (CSIC) and R. Mateos was a postdoctoral fellow of the Spanish Ministerio de Educación y Cultura.

#### References

- Adamson, G. E., Lazarus, S. A., Mitchell, A. E., Prior, R. L., Cao, G., Jacobs, P. H., et al. (1999). HPLC method for the quantification of procyanidins in cocoa and chocolate samples and correlation to total antioxidant capacity. *Journal of Agricultural and Food Chemistry*, 47, 4184–4188.

- Bessesen, D. H. (2001). The role of carbohydrates in insulin resistance. *Journal of Nutrition*, *131*, 2782S–2786S.
- Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews*, *56*, 317–333.
- Bravo, L. (1999). Propiedades y aplicaciones de la fibra de algarroba (*Prosopis pallida* L.). *Alimentaria*, *13*, 67–73.
- Bravo, L., & Saura-Calixto, F. (1998). Characterization of the dietary fiber and the in vitro indigestible fraction of grape pomace. *American Journal of Enology and Viticulture*, *49*, 135–141.
- Bravo, L., Abia, R., & Saura-Calixto, F. (1994). Polyphenols as dietary fiber associated compounds. Comparative study on in vivo and in vitro properties. *Journal of Agricultural and Food Chemistry*, *42*, 1481–1487.
- Bravo, L., Grados, N., & Saura-Calixto, F. (1994). Composition and potential uses of mesquite pods (*Prosopis pallida* L.): comparison with carob pods (*Ceratonia siliqua* L.). *Journal of the Science of Food and Agriculture*, *65*, 303–306.
- Chung, B. Y., Iiyama, K., & Han, K.-W. (2003). Compositional characterization of cacao (*Theobroma cacao* L.) hull. *Agricultural and Chemical Biotechnology*, *46*, 12–16.
- De Vries, J. W. (2003). On defining dietary fibre. *Proceedings of the Nutrition Society*, *62*, 37–43.
- Englyst, H. N., & Cummings, J. H. (1990). Dietary fibre and starch: definition, classification and measurement. In A. R. Leeds (Ed.), *Dietary fibre perspectives: Reviews and bibliography* (pp. 3–26). London: John Libbey.
- Englyst, H. N., Quigley, M. E., Hudson, G. J., & Cummings, J. H. (1992). Determination of dietary fibre as nonstarch polysaccharides with gas-liquid chromatography. *Analyst*, *117*, 1707–1714.
- Gelroth, J., & Ranhotra, G. S. (2001). Food uses of fiber. In S. Sungsoo Cho & M. L. Dreher (Eds.), *Handbook of dietary fiber* (pp. 435–451). New York: Marcel Dekker Inc.
- Hammerstone, J. F., Lazarus, S. A., Mitchell, A. E., Rucker, R., & Schmitz, H. H. (1999). Identification of procyanidins in cocoa (*Theobroma cacao*) and chocolate using high-performance liquid chromatography/mass spectrometry. *Journal of Agricultural and Food Chemistry*, *47*, 490–496.
- Jenkins, D. A. J., Jenkins, M. J. A., Wolever, T. M. S., Taylor, R. H., & Ghafari, H. (1986). Slow release carbohydrate: mechanism of action of viscous fibres. *Journal of Clinical Nutrition and Gastroenterology*, *1*, 237–240.
- Johnson, I. (2004). New approaches to the role of diet in the prevention of cancers of the alimentary tract. *Mutation Research*, *551*, 9–28.
- Karim, M., McCormick, K., & Kappagoda, C. T. (2000). Effects of cocoa extracts on endothelium-dependent relaxation. *Journal of Nutrition*, *130*, 2108S–2119S.
- Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., et al. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *American Journal of Medicine*, *113*, 71S–88S.
- Mañas, E., Bravo, L., & Saura-Calixto, F. (1994). Sources of error in dietary fibre analysis. *Food Chemistry*, *50*, 331–342.
- Marlett, J. M., Yang, E. J., & Slavin, J. L. (2002). Position of the American Dietetic Association: health implications of dietary fiber. *Journal of the American Dietitians Association*, *102*, 993–1000.
- Martín-Cabrejas, M. A., Valiente, C., Esteban, R. M., Mollá, E., & Waldron, K. (1994). Cocoa hull: a potential source of dietary fibre. *Journal of the Science of Food and Agriculture*, *66*, 307–311.
- Orozco, T. J., Wang, J. F., & Keen, C. L. (2003). Chronic consumption of a flavonols- and procyanidin-rich diet is associated with reduced levels of 8-hydroxy-2'-deoxyguanosine in rat testes. *Journal of Nutritional Biochemistry*, *14*, 104–110.
- Prosky, L., Asp, N.-G., Scheweizer, T. F., De Vries, J. W., & Furda, I. (1988). Determination of insoluble, soluble, and total dietary fiber in foods and food products: interlaboratory study. *Journal of the Association of Official Analytical Chemists*, *71*, 1017–1023.
- Pulido, R., Bravo, L., & Saura-Calixto, F. (2000). Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry*, *48*, 3396–3402.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radicals in Biology and Medicine*, *26*, 1231–1237.
- Redgwell, R., Trovato, V., Merinat, S., Curti, D., Hediger, S., & Manez, A. (2003). Dietary fibre in cocoa shell: characterization of component polysaccharides. *Food Chemistry*, *81*, 103–112.
- Reed, J. D., McDowell, R. E., Van Soest, P. J., & Horvath, P. J. (1982). Condensed tannins: a factor limiting the use of cassava forage. *Journal of the Science of Food and Agriculture*, *33*, 213–220.
- Rein, D., Paglieroni, T. G., Wun, T., Pearson, D. A., Schmitz, H. H., Gosselin, R., et al. (2000). Cocoa inhibits platelet activation and function. *American Journal of Clinical Nutrition*, *72*, 30–35.
- Robertson, J. A., de Monredon, F. D., Dysseler, P., Guillon, F., Amadò, R., & Thibault, J.-F. (2000). Hydration properties of dietary fibre and resistant starch: a European collaborative study. *Lebensmittel-Wissenschaft und Technologie*, *33*, 72–79.
- Saura-Calixto, F., & Goñi, I. (2006). Antioxidant capacity of the Spanish Mediterranean diet. *Food Chemistry*, *94*, 442–447.
- Saura-Calixto, F., García-Alonso, A., Goñi, I., & Bravo, L. (2000). In vitro determination of the indigestible fraction in foods: an alternative to dietary fiber analysis. *Journal of Agricultural and Food Chemistry*, *48*, 3342–3347.
- Schewe, T., Kühn, H., & Sies, H. (2002). Flavonoids of cocoa inhibit recombinant human 5-lipoxygenase. *Journal of Nutrition*, *132*, 1825–1829.
- Scott, R. W. (1979). Colorimetric determination of hexuronic acids in plant materials. *Analytical Chemistry*, *51*, 936–941.
- Serra-Bonvehí, J., & Aragay-Benería, M. (1998). Composition of dietary fibre in cocoa husk. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A*, *207*, 105–109.
- Steinberg, F. M., Bearden, M. M., & Keen, C. L. (2003). Cocoa and chocolate flavonoids: implications for cardiovascular health. *Journal of the American Dietetic Association*, *103*, 215–223.
- Sungsoo Cho, S., & Dreher, M. L. (2001). *Handbook of dietary fiber*. New York: Marcel Dekker Inc.
- US Department of Agriculture (2000). *Nutrition and your health: dietary guidelines for Americans*. 5th Ed, Washington DC: Department of Agriculture and Department of Health and Human Services.
- Valiente, C., Esteban, R. M., Mollá, E., & López-Andréu, F. J. (1994). Effect of roasting on dietary fiber cocoa beans. *Journal of Food Science*, *59*, 123–124.
- Vinson, J. A., Proch, J., & Zubik, L. (1999). Phenol antioxidant quantity and quality in foods: cocoa, dark chocolate, and milk chocolate. *Journal of Agricultural and Food Chemistry*, *47*, 4821–4824.
- Wan, Y., Vinson, J. A., Etherton, T. D., Proch, J., Lazarus, S. A., & Kris-Etherton, P. M. (2001). Effects of cocoa powder and dark chocolate on LDL oxidative susceptibility and prostaglandin concentrations in humans. *American Journal of Clinical Nutrition*, *74*, 596–602.
- Williamson, G., & Manach, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *American Journal of Clinical Nutrition*, *81*(suppl.), 243S–255S.
- Wiswedel, I., Hirsch, D., Kropf, S., Gruening, M., Pfister, E., Schewe, T., et al. (2004). Flavanol-rich cocoa drink lowers plasma F<sub>2</sub>-isoprostane concentrations in humans. *Free Radicals in Biology and Medicine*, *37*, 411–421.
- Yamagishi, M., Natsume, M., Magaki, A., Adachi, T., Osakabe, N., Takizawa, T., et al. (2000). Antimutagenic activity of cacao: inhibitory effect of cacao liquor polyphenols on the mutagenic action of heterocyclic amines. *Journal of Agricultural and Food Chemistry*, *48*, 5074–5078.