Antioxidant Dietary Fiber Product: A New Concept and a Potential Food Ingredient

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The main characteristics of a natural product, antioxidant dietary fiber (ADF), rich in both dietary fiber (DF) and polyphenolic compounds (PP), obtained from red grape pomace is described. Both nonextractable proanthocyanidins (28.6%) and extractable polyphenols (2.0%) are associated with the dietary fiber matrix. The antioxidant capacity of this product was in vitro determined by lipid oxidation inhibition (LOI) and free radical scavenging (FRE) procedures. One gram of the product showed similar LOI and FRE effect than 400 mg and 100 mg of DL-(α-tocopherol, respectively. Extractable PP of grape ADF showed higher antioxidant capacity than red wine PP. The physiological and nutritional significance of ADF is discussed and the requirements of vegetable materials to be considered as ADF is proposed.

Keywords: *Antioxidants; dietary fiber; polyphenols; grape pomace; proanthocyanidins*

INTRODUCTION

Nowadays the importance of dietary fiber (DF) in health is well defined (Kritchevsky and Bonfied, 1995). In the past 10 years both the WHO and many national government bodies have written guidelines to increase the daily intake of DF-rich foods, but the formulation of new high DF products is a challenge for the food industry.

The most widespread, extensively advertised, and consumed DF products are those derived from cereals (breakfast cereals, bakery products, biscuits, etc). However, over the past decade high dietary fiber materials from fruits (citrus, apple, and others) are being introduced in the market. Fruit DF concentrates have in general a better nutritional quality than those from cereals because of the presence of significant amounts of associated bioactive compounds (flavonoids, carotenoids, etc.) and their balanced composition (higher fiber content, soluble/insoluble DF ratio, water and fat holding capacities, lower energy value, and phytic acid content) than cereal materials (Saura Calixto and Larrauri, 1996).

On the other hand, there is a wide developing market for natural food antioxidants based on two aspects: well-documented protective effect against cancer and cardiovascular diseases of these natural antioxidants (Garewall, 1997), and a general rejection of synthetic antioxidants by the consumers. Fruits, vegetables, and beverages are important sources of natural antioxidants, which are extracted using different solvents and expensive processes such as supercritical fluids (Starmans and Nijhuis, 1996).

Flavonoids and other plant phenolics have been reported to have several biological effects such as antioxidant activity, inhibition of platelet aggregation, and antimicrobial and antiinflammatory action (Ho et al., 1992). Also, dietary flavonoids have been associated with a reduced risk of cardiovascular diseases and cancer (Ohigashi et al., 1997). Grapes and wine contain significant amounts of these compounds. Most of the recent related research is focused on the antioxidant capacity and biological effects of red wine polyphenols (Frankel et al., 1995), and very little effort has been devoted to wine manufacturing byproducts.

This Department has performed in the last years several studies on dietary fibers rich in associated polyphenols as well as specific studies on grape pomace composition and physiological properties, including antioxidant capacity (Saura-Calixto et al. 1991; Bravo et al. 1992, 1994a,b; Larrauri et al. 1996 a,b, 1997; Bravo and Saura Calixto, 1998). The objective of this work is to present a new type of natural product: dietary fiber rich in associated polyphenols compounds (antioxidant dietary fiber). This product combines in a single material the physiological effects of both dietary fiber and antioxidants. It is based on the following considerations:

(1) From a nutritional point of view it is preferable to not remove bioactive compounds from vegetables (food materials) rather than isolating and then incorporating them into food. Polyphenolic extraction always implies incomplete recovery as well as losses of biological activity and synergic effects.

(2) Grapes and grape pomace rather than red wine may be more significant in nutrition and health. Red wine certainly contains high amounts of phenolic compounds (1000−1800 mg/L); however, this is only a minor part of the grape polyphenols, which mostly remain in grape pomace after wine making, mainly in skins and seeds.

MATERIALS AND METHODS

Materials. Antioxidant dietary fiber (ADF) powder, Vitis Fibre (El Granero Integral, SL, Madrid, Spain), was obtained from red grape peels, following a patented procedure (CSIC-

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El Granero Integral, SL, 1997). DL-α-Tocopherol was obtained from Sigma Chemical Company, St. Louis, MO, and red wine, from Bodega Los Llanos, Valdepeñas, Spain.

Chemical Analysis. *Extractable polyphenols* (EPP) were extracted from ADF by sequential extractions with methanol/water (50:50, v/v) and acetone/water (70:30, v/v) at room temperature for 60 min in each case. The supernatants were combined and made up to 100 mL with distilled water. The EPP were determined by the Folin-Ciocalteau method using gallic acid as a standard (Larrauri et al., 1997). The residue from this extraction was treated with HCl in 1-butanol (100 °C for 3 h) to determine nonextractable polyphenols (NEPP); the absorbance of the supernatant was measured at 555 nm with carob pod condensed tannins (Nestlé, Ltd.) used as standard (Saura Calixto et al., 1991).

The AOAC enzymatic−gravimetric method (Prosky et al., 1988) modified in our laboratory was used (Mañas and Saura Calixto, 1993) for dietary fiber analysis.

EPP were also determined in supernatants obtained after enzymatic treatments of Vitis Fibre in conditions simulating those of the stomach and small intestine (pepsin, 40, pH = 1.5, 1 h, α-amylase, 37 °C, pH = 6.9, 3 h).

Protein, petroleum ether extract, and ash were determined according to routine laboratory procedures.

Antioxidant Activity Assays. Supernatants containing EPP obtained as described above, were concentrated in a vacuum rotatory evaporator to a final volume of 5 mL. Red wine was used directly from the bottle, and DL-α-tocopherol was dispersed directly in ethanol/water (50:50 v/v).

Inhibition of Lipid Oxidation. The ferric thiocyanate (FTC) method modified in our laboratory was used (Larrauri et al., 1996). In brief, a mixture of sample extract, emulsion of linoleic acid in ethanol, sodium phosphate buffer (pH 7), and distilled water was kept at 40 °C. Absorbances at 500 nm were measured daily until 1 day after the absorbance of the control reached maximum.

The antioxidant activity was plotted against sample concentration in order to determine the concentration required to achieve a 50% inhibition of linoleic acid oxidation.

Free Radical Scavenging Capacity. The DPPH method (Sánchez-Moreno et al., 1998) was used. Briefly, an aliquot of sample extract at different concentration was added to a DPPH• solution, and the absorbances at 515 nm were measured until the reaction reached a plateau. The percentage of remaining DPPH• was plotted against the sample concentration to obtain the amount of the antioxidant necessary to decrease by 50% the initial DPPH• concentration.

RESULTS AND DISCUSSION

Polyphenolic compounds in vegetables are found free or bound to cell walls or to protein. Soluble or extractable polyphenols (EPP) are low or intermediate molecular mass phenolics that are extracted using different solvents, while nonextractable polyphenols (NEPP) are mainly condensed tannins of high molecular mass. EPP appear to be absorbed from the digestive tract and produce systemic effects, while NEPP are quantitatively recovered in feces (Bravo et al. 1994a). ADF contains both EPP and NEPP. The phenolic content of grape antioxidant fiber, and the main physiological characteristics of these compounds are summarized in Table 1 and discussed below.

Dietary Fiber. Antioxidant DF can be obtained from red and white whole grape pomaces originated from wine or grape juice production, as well as from white and red grape skins and seeds (CSIC-El Granero Integral, SL, 1997).

A number of factors such as the grape variety, culture characteristics, or wine-processing procedures (i.e., time of contact between pomace and grape must during fermentation) may influence the composition of the grape antioxidant fibers. Usual ranges of ADF composition in these products are DF (50−75%), EPP (1−9%), and NEPP (15−30%).

Table 1. Polyphenolic Compounds (PP) in Grape Antioxidant Fiber (Vitis Fibre)

	nonextractable PP	extractable PP
total content (% dry matter)	26.9	2.0
solubility in aqueous/organic solvents[a]	not	yes
solubility in digestive fluids,[b] %	not	62
PP bound to DF after enzymatic treatments,[b] %	100	38
fermentability by colonic microflora[c]	not	yes
recovery in feces (% amount ingested)[c]	about 99	about 32
main characteristic[c]	lipid excretion	antioxidant

[a] MeOH/water and acetone/water treatments (see Materials and Methods). [b] Pepsin and amylase treatments (see Materials and Methods). [c] Taken from Matín-Carrón et al. (1997).

Table 2. Major Constituents of Vitis Fibre (Antioxidant DF)

total dietary fiber[a]	64.6 ± 0.5
protein (% dry matter)	14.4 ± 0.2
ether extract (% dry matter)	6.9 ± 0.2
soluble sugars (% dry matter)	2.8 ± 0.06
extractable polyphenols (EPP) (% dry matter)	2.0 ± 0.03
nonextractable polyphenols (NEPP) (% dry matter)	26.9 ± 1.3
ash (% dry matter)	9.2 ± 0.04

[a] Dietary fiber includes the total NEPP and 60% of EPP.

Data in Table 2 correspond to an antioxidant dietary fiber product (Vitis Fibre) obtained from red grape skins of a specific grape variety—Cencibel—and wine maker (Bodega Los Llanos, Valdepeñas, Spain).

DF content in ADF, determined by the AOAC method (Prosky et al., 1988) was 64.6%. The extraordinarily high amount of NEPP in this grape antioxidant fiber must also be pointed out. These tannins are proanthocyanidin-insoluble in both aqueous and organic solvents, and they remain in insoluble dietary fiber residues after enzymatic fiber analysis because of their high molecular weight and/or their links to cell walls (Saura Calixto et al. 1991, Bravo and Saura Calixto, 1998). For this reason, NEPP are erroneously identified as polysaccharides or lignin by others authors (Valiente et al., 1995), although their physiological and chemical properties are markedly different.

The effect of fiber on lipid metabolism is related to a reduction in the intestinal absorption of bile acids and dietary lipids. Fibers rich in NEPP may have a significant role in this aspect. Polymeric grape seed tannins significantly increased the fecal excretion of lipids and cholesterol, showing a hypocholesterolemic effect in rats fed high-cholesterol diets (Tebib et al., 1994).

In a previous paper we reported a significant increase of total stool weight, as well as fat, protein, and mineral excretion, in rats fed grape pomace compared to control animals (cellulose diets) (Martin Carron et al., 1997). NEPP were not degraded by digestive enzymes nor fermented by colonic microflora, being recovered quantitatively in feces. Similar results have been reported for other polyphenol-rich fibers such as carob pod and apple pomace. The capacity to enhance lipid excretion is seen to be the main characteristic of dietary fibers rich in NEPP.

Table 3. Amount of Sample Necessary To Reduce by 50% Lipid Oxidation and Free Radical Scavenging

samples	FTC method	DPPH• method
Vitis Fibre	0.7 g (14 mg of EPP)	0.2 g (4.0 mg of EPP)
red wine	23 ml (41.4 mg of EPP)	3.4 ml (6.1 mg of EPP)
DL-α-tocopherol	0.3 g	0.02 g

DF Associated Polyphenols and Antioxidant Capacity. In vitro, in vivo, and epidemiological studies have shown that moderate consumption of red wine reduces the susceptibility of human plasma's LDL to lipid peroxidation and is associated with a reduced risk of coronary heart disease. These effects are mainly attributed to the antioxidant and free radical scavenging capacity of wine polyphenolics.

Grape pomace polyphenols have similar structure and botanical-physiological origins of that of red wine phenolics. In fact, most wine PP are released from grape skins during wine processing. EPP in red grapes, grape skins, and red wine are mainly made of procyanidins, flavonoids, and phenolic acids, compounds widely reported as antioxidants (Escribano-Bailón et al., 1995; Kanner et al., 1994).

The antioxidant capacity of wines has been widely reported, but few papers regarding this property in grapes can be found in the literature (Larrauri et al., 1996b). The antioxidant capacity of ADF, expressed as the amount of sample necessary to reduce by 50% the oxidation of linoleic acid or the scavenging of DPPH• radicals are shown in Table 3.

From these results it may be deduced that EPP exhibit a higher antioxidant capacity in grape pomace than in red wine: 14 mg of EPP from ADF exerted the same effect in the inhibition of lipid oxidation (ferric thiocyanate method) as 41 mg of wine EPP. Similarly, 4 mg of EPP from ADF had the same free radical scavenging capacity as 6.1 mg of wine phenolics.

When compared with a well-known natural antioxidant, 1 g of ADF showed similar inhibition of lipid oxidation and in vitro free radical scavenging capacity than 400 mg and 100 mg, respectively to that of DL-α-tocopherol.

Therefore, ADF showed an excellent antioxidant capacity in vitro. Its potential in vivo effects will depend on the bioavailability of the grape polyphenols. As is described above, data from both animal and human experiments showed that a high proportion of EPP are absorbed in the intestine and detected in plasma (Gugler et al., 1975; Bravo et al.; 1994; Fuhrman et al., 1995; Pellegrini et al., 1996; Martin-Carrón et al., 1997).

A previous rat experiment showed that the major part of grape EPP (68%) was digested and therefore potentially antioxidants. On the other hand, we found 62% of the total EPP in supernatants obtained after enzymatic treatments of ADF in conditions simulating those of the stomach and small intestine fluids. This would be the fraction available during digestion. The remaining 38% of EPP might reach the large intestine associated with dietary fiber, being susceptible to absorption after fermentation by the colonic microflora.

Other commercial DF products, such as All Bran Plus (Kellogg España SA), Quaker White Oats (Quaker Trading Ltd, U.K.), an apple, and lemon fibers (Mediterranean Fiber,Indulerida SA, Spain) did not show antioxidant activity, measured by the same experimental procedures used for ADF.

Fruit pulps and especially cereal brans—the most common materials in DF concentrates—have a very low content of PP or other antioxidants as compared to grapes. Moreover, processing of raw materials to obtain DF concentrates may result in important losses of antioxidant capacity.

Ohta et al. (1994) reported antioxidant activity of corn bran hemicellulose fragments. However, this property was measured in hydrolysates of a refined corn bran obtained after oxalic acid (3 h., boiling) and hemicellulase treatments and chromatographic adsorption-elution. These experimental conditions are not physiological and no antioxidant capacity can be expected after ingestion of such as corn bran since digestive fluids do not solubilize nor release PP that are obtained after the referred chemical treatments. In the case of ADF, the antioxidant capacity is derived from PP soluble in digestive fluids without any previous treatment.

In summary, grape pomace is a suitable material to obtain antioxidant dietary fiber (ADF), and Vitis Fibre is an example of this new type of fiber.

ADF could be used as a new food ingredient. In addition to the properties derived from ordinary dietary fibers, a prevention of lipid oxidation in food products can be expected from the presence of antioxidant polyphenols. On the other hand, the potential combined actions of nonextractable proanthocyanidins and bioavailable flavonoids of the ADF are quite promising in nutrition and health.

Further human studies on the biological significance of these types of fibers are needed.

Definition of Antioxidant Dietary Fiber. Finally, to clearly state the concept of antioxidant dietary fiber may be useful to discriminate materials with significant antioxidant capacity from those with negligible action. ADF can be defined as a product containing significant amounts of natural antioxidants associated with the fiber matrix. We propose that a vegetable material should have the following requirements to be considered as an ADF: (1) DF content, measured by the AOAC method (Prosky et al., 1988), should be higher than 50% on a dry matter basis. (2) One gram of ADF should have a capacity to inhibit lipid oxidation equivalent to, at least, 200 mg of vitamin E (measured by the thiocyanate procedure) and a free radical scavenging capacity equivalent to, at least, 50 mg of vitamin E (measured by the DPPH• method). (3) The antioxidant capacity must be an intrinsic property, derived from natural constituents of the material neither by added antioxidants nor by constituents released by previous chemical or enzymatic treatments.

LITERATURE CITED

Bravo, L.; Saura-Calixto, F.; Goñi, I. Effects of dietary fibre and tannins from apple pulp on the composition of faeces in rats. *Br. J. Nutr.* **1992**, *67*, 463−47.

Bravo, L.; Abia, R.; Saura-Calixto, F. Polyphenols as dietary fibre associated compounds. Comparative study on some in vivo and in vitro properties. *J. Agric. Food Chem.* **1994a**, *42*, 1481−1487.

Bravo, L.; Abia, L.; Eastwood, M. A.; Saura-Calixto, F. Degradation of polyphenols (catechin and tannic acid) in the rat intestinal tract. Effect on colonic fermentation and faecal output. *Br. J. Nutr.* **1994b**, *71*, 993−946.

Bravo, L.; Saura-Calixto, F. Characterization of dietary fiber and the in vitro indigestible fraction of grape pomace. *Am. J. Enol. Vitic.* **1998**, *49*, 135−141.

CSIC-El Granero Integral. Concentrado de fibra dietética antioxidante natural de uva y su procedimiento de obtención. Patente Española 9702397, **1997**.

Escribano-Bailón, M. T.; Guerra, M. T.; Rivas-Gonzalo, J. C.; Santos-Buelga, C. Proanthocyanidins in skins from different grape varieties. *Z. Lebensm.-Unters.-Forsch.* **1995**, *200*, 221−224.

Frankel, E. N.; Waterhouse, A. L.; Teissedre, P. L. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J. Agric. Food Chem.* **1995**, *43*, 890−894.

Fuhrman, B.; Lavy, A.; Aviram, M. Consumption of red wine with meals reduces the susceptibility of human plasma low-density lipoprotein to lipid peroxidation. *Am. J. Clin. Nutr.* **1995**, *61*, 549−554.

Garewall, A. S., Ed. *Antioxidants and disease prevention*; CRC Press: Boca Raton, FL, 1997.

Gugler, R.; Leschik, M.; Dengler, H. J. Disposition of quercetin in man after single oral and intravenous doses. *Eur. J. Clin. Pharmacol.* **1975**, *9*, 229−234.

Ho, Ch. T.; Lee, Ch. Y.; Huang, M. T. *Phenolic compounds in food and their effects in health*; ACS Syposium Series 507; American Chemical Society: Washington, DC, 1992.

Kanner, J.; Frankel, E.; Grnit, R.; German, B.; Kinsella, J. E. Natural antioxidants in grapes and wines. *J. Agric. Food Chem.* **1994**, *42*, 64−69.

Kritchevsky, D., Bonfield, Ch., Eds. *Dietary fiber in health and disease*; Eagan Press: St. Paul, MN, 1995.

Larrauri, J. A.; Goñi, I.; Martín-Carrón, N.; Rupérez, P.; Saura-Calixto, F. Measurement of health promoting properties in fruit dietary fibres: Antioxidant capacity, fermentability and glucose retardation index. *J. Sci. Food Agric.* **1996a**, *71*, 515−519.

Larrauri, J. A.; Rupérez, P.; Saura Calixto, F. Antioxidant activity of wine pomace. *Am. J. Enol. Vitic.* **1996b**, *47*, 369−372.

Larrauri, J. A.; Rupérez, P.; Saura Calixto, F. Effect of drying temperature on the stabilitity of polyphenols and antioxidant activity of red grape pomace peels. *J. Agric. Food Chem.* **1997**, *45*, 1390−1393.

Mañas, E.; Saura-Calixto, F. Ethanolic precipitation: A source of error in dietary fiber determination. *Food Chem.* **1993**, *47*, 351−355.

Martín-Carrón, N.; García-Alonso, A.; Goñi, I.; Saura-Calixto, F. Nutritional and physiological properties of grape pomace as a potential food ingredient. *Am. J. Enol. Vitic.* **1997**, *48*, 328−332.

Ohigashi, H., Osawa, T., Terao, J., Watanabe, S., Yoshikawa, T., Eds. *Food factors for cancer prevention*; Springer: Tokyo, 1997.

Ohta, T.; Yamasaki, S.; Egashira, Y.; Sanada, H. Antioxidant activity of corn bran hemicellulose fragments. *J. Agric. Food Chem.* **1994**, *42*, 653−656.

Pellegrini, N.; Pareti, F. I.; Stabile, F.; Brusamolino, A.; Simonetti, P. Effect of moderate consumption of red wine on platelet aggregation and haemostatic variables in healthy volunteers. *Eur. J. Clin. Nutr.* **1996**, *50*, 209−213.

Prosky, L.; Asp, N. G.; Schweizer, T. F.; DeVries, J. W.; Furda, I. Determination of insoluble, soluble, and total dietary fibre in foods and food products: Interlaboratory study. *J.*−*Assoc. Off. Anal. Chem.* **1988**, *71*, 1017−23.

Sánchez-Moreno, C.; Larrauri, J. A.; Saura Calixto, F. A procedure to measure the antiradical efficiency of polyphenols. *J. Sci. Food Agric.* **1998**, *76*, 270−276.

Saura Calixto, F.; Goñi, I.; Mañas, E.; Abia, R. Klason Lignin, condensed tannins and resistant protein as dietary fibre constituents: determination in grape pomaces. *Food Chem.* **1991**, 299−309.

Saura-Calixto, Y.; Larrauri, J. A. Nuevos tipos de fibras dietéticas de alta calidad (New type of high quality dietary fibers). *Rev. Alim. Equip. Technol.* **1996**, *1*, 71−74.

Starmans, D. A. J.; Nijhuis, H. H. Extraction of secondary metabolities from plant material: A review. *Trends Food Sci. Technol.* **1996**, *7*, 191−197.

Tebib, K.; Bitri, L.; Besancon, P.; Rouanet, J. M. Polymeric grape seed tannins prevent plasma cholesterol changes in high-cholesterol-fed rats. *Food Chem.* **1994**, *49*, 403−406.

Valiente, C.; Arrigoni, E.; Esteban, R. M.; Amado, R. Grape pomace as a potential food fiber. *J. Food Sci.* **1995**, *60*, 818−820.

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