

Dietary fibre from vegetable products as source of functional ingredients

Rocío Rodríguez*,
Ana Jiménez, Juan Fernández-
Bolaños, Rafael Guillén and
Antonia Heredia

Instituto de la Grasa, CSIC. Avenida Padre García
Tejero, 4, 41012 Sevilla, Spain (Tel.: +34
954691054; fax: +34 954691262;
e-mail: rrodri@cica.es)

The importance of food fibres has led to the development of a large and potential market for fibre-rich products and ingredients and nowadays there is a trend to find new sources of dietary fibre (DF), such as agronomic by-products that have traditionally been undervalued. Although there have been great achievements in this research field, further investigations are needed for designing 'new food systems' that consider the precise functionality of DF from both technological and physiological points of view.

Present knowledge about different aspects of DF and future potential applications of fibres and/or its components as functional foods or ingredients will be the focus of this report.

Fibre through the years

Dietary fibre (DF) has been known and investigated for a 'very long time' and its precise delimitation has been the subject of much discussion and controversy (Asp, 2004), from being considered as waste to being described as a

'universal remedy' that improves any physiological problem within human organism. Neither the first nor the second view is completely true; however it is well known that DF plays an important role in many physiological processes and in the prevention of diseases from different etymology. Also, during the last years DF has acquired an additional importance related to its use as functional ingredient.

At present, there are still many aspects about DF properties and functions that remain unclear. Botanists define fibre as a part of the plant organs, chemical analysts as a group of chemical compounds, consumer as a substance with beneficial effects on human health, and for the dietetic and chemical industries DF is a subject of marketing. This controversy is related to the fact that fibre is not a simple and well defined chemical compound but a combination of chemical substances of distinct composition and structure, such as cellulose, hemicelluloses, lignin, etc. (Heredia, Jiménez, Fernández-Bolaños, Bejarano & Rodríguez, 2002; Thebaudin, Harrington, & Bourgeois, 1997). So, there are an increasing number of studies about DF, not only related to its dietetic aspects but also about its industrial recovery. This is resulting in a better knowledge of its chemical, nutritional and functional characteristics, which contributes to understanding the role that it plays in several physiological processes (Cho & Prosky, 1999; Gheyas, Blankenship, Young, & McFeeters, 1997), as well as the association between low ingest of DF and gastric related diseases (Brillouet & Mercier, 1982).

Although in 400 BC Hippocrates already mentioned the beneficial effects of DF, more recently its utility has been questioned, so while Kellogg (1923) promoted its positive action, McCance and Lawrence (1929) considered DF to be a non-digestible portion of plant foods that irritated the intestine. Lately, Cleave (1956) related certain diseases with 'deficiency of fibre syndrome' and Walker (1947) proposed that DF determined in great extension the digestive tract function.

Burkit (1969) studied the effects of a diet rich in fibre on African people. This author observed that population did not suffer certain diseases, such as colon cancer, that are very common among more developed occidental societies, where fibre consumption was much lower. Walker (1974) carried out a comparative study of the diet of black and white people from South Africa, and found that the black population, which consumed non-refined maize flour with a high content of fibre, had less risk of suffering diseases,

* Corresponding author.

such as atherosclerosis, haemorrhoids and colon cancer, which had a high incidence among white population. Similar results have been reported by Kritchersky (1990); Trowell (1976). Other investigations that have contributed to evidence of the significance of DF in our diet are those from Southgate (1969, 1982), Van Soest (1963a,b; 1973), Van Soest and McQueen (1973), Van Soest and Wine (1967a,b, 1968) and more recently the works from Prosky (Prosky, Asp, Furda, DeVries, Schweizer, & Marland, 1985; Prosky, Schweizer, DeVries, & Furda, 1988; Prosky, Asp, Scheizer, DeVries, & Furda, 1992a; Prosky, Asp, Schweizer, DeVries, Furda, & Lee, 1992b); Asp (Asp, Furda, DeVries, Schweizer, & Prosky, 1988; Asp, Johansson, Hallmer, & Siljeström, 1983); Englyst and Cummings (1986, 1988), Johnson and Southgate (1994); Deharveng (Deharveng, Charrondiere, Slimani, Southgate, & Riboli, 1999), etc.

Despite the unquestionable advances in this research field, no international consensus has been reached on the definition of dietary fibre (Fischer, 2004), nor a unique and precise methodology for its determination. However, most scientists investigating fibre from different scientific sub disciplines have reached an agreement about including fibre among the important ingredients of the diet, and the convenience of a significant fibre intake is addressed in official dietary guidelines (Schaafsma, 2004). It has been established that the human diet must be rich in cell wall material, from fruits, vegetables and cereals, which contains most DF (Cummings, Hudson, Quigley, & Englyst, 1995; Johnson, 1999; Mongeau, Scott, & Brassard, 1999).

What is fibre?

This concept has been associated to several meanings along the years, which has resulted in an international discussion based on the advances in analytical techniques, the new nutritional and physiological information and also the private interests from the food industry (Champ, Langkilde, Brounds, Kettlitz, & Collet, 2003). Initially, the residue that remains after the extraction of plant tissues with diluted acid or basic solutions was called total fibre (Williams & Olmstead, 1935), although it was soon observed that this measurement was not representative of the 'accurate fibre content'; Hipsley (1953) defined it as the sum of cellulose, hemicelluloses and lignin from the diet, but a more rational definition would describe it as a chemical mixture of cell wall polysaccharides and lignin.

The most consistent definition that is now accepted is that from Trowell: "Dietary fibre consists of remnants of the plant cells resistant to hydrolysis (digestion) by the alimentary enzymes of man" (Trowell, 1974, 1976; Trowell, Southgate, Wolever, Leeds, Gassull, & Jenkins, 1976; Trowell, Burkitt, & Heaton, 1985), whose components are hemicelluloses, cellulose, lignin, oligosaccharides, pectins, gums and waxes. However, alternative definitions for fibre continue being proposed every day. Asp and Johansson (1984), as well as Selvendran and Robertson (1994) define

fibre as the group of non-starch polysaccharides and lignin, which includes several indigestible polysaccharides in addition to the main components of the cell wall; Cummings and Englyst (1991) consider that DF is only the non-starch polysaccharide (NSPs); Mongeau *et al.* (1999) have reported that a plant material can be defined as DF if it comes from the cell walls of plant food tissues, keeps intact its structure and can be determined by using official methods of DF analysis. The American Association of Cereal Chemists (2001) adopted this definition for DF: the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine.

Meyer (2004) proposed that the fibres are an integral part of the foodstuffs we consume daily; the main sources in which these fibres occur are plants, vegetables, cereal grains, woody plants, fruits, legumes, leguminous plants, etc. Based on their simulated intestinal solubility, dietary fibres are either classified as insoluble or soluble fibre. Insoluble fibres include lignin, cellulose, and hemicelluloses; soluble fibres include pectins, beta-glucans, galactomanan gums, and a large range of nondigestible oligosaccharides including inulin.

Effects of dietary fibre on human health and diseases

According to well-documented studies, it is now accepted that dietary fibre plays a significant role in the prevention of several diseases, and that diets with a high content of fibre, such as those rich in cereals, fruits and vegetables, have a positive effect on health since its consumption has been related to a decreased incidence of several types of cancer (Beecher, 1999; Jiménez-Escribano, Rincón, Pulido, & Saura-Calixto, 2001).

Despite most components of DF being indigestible, as they cannot be degraded by the enzymes present in the large intestine, these are exposed to bacterial enzymatic activities that can partially degrade them. The extent of this degradation depends on the type of bacterial flora (Heredia *et al.* 2002). Other factors of interest are the time of transit through the colon that determines the duration of the contact with the bacterial enzymes, and the DF components that limit the extent of its decomposition (30–90% polysaccharides, mainly hemicelluloses and pectins) (Kay, 1982). That degradation starts by an extra cellular hydrolysis that converts polysaccharides in mono- and disaccharides, followed by an intracellular anaerobic glycolysis that releases acetate, propionate and butyrate as final products (German & Watkins 2004; Larrauri, Goñi, Martín-Carrón, Rupérez, & Saura-Calixto, 1996). The main effect in the small intestine is associated with the viscous polysaccharides, such as pectins and gums, which decrease the assimilation of nutrients, while the insoluble components do not affect in great extent. The bacterial mass that is formed from the high-fermentable substances (pectins), the residues of the less fermentable polymers (cellulose and hemicelluloses) and the water retained by them, are

responsible for the increase of the faecal bulk (Madar & Odes, 1990; Lefebvre & Thébaudin, 2002).

Eggum (1992), after investigating the effects of DF on food components digestibility in animals (rats and pigs) and humans, reported that it had a negative influence on the digestion and assimilation of proteins. This effect was more pronounced while the ingested fibre was less purified. The results of this work were more precise and reproducible in experimental animals than in humans, but a general conclusion could be established that the influence of DF on protein metabolism depends to a great extent on the structure and chemical composition of the fibre.

It has been also reported that fibre polysaccharides affect the absorption of lipids, as they can be strong inhibitors of the pancreatic lipase that participates in the lipid metabolism (Dunaif & Schneeman, 1981). On the other hand, DF contributes to decrease the levels of total cholesterol and low-density lipoproteins in plasma, which is associated to a greater dilution and excretion of bile acids (Gallaher, Locket, & Gallaher, 1992).

Regarding food carbohydrates, it has been established that DF can influence their bioavailability in the intestinal tract. This effect has been confirmed in diabetic patients, whose levels of glucose in blood decreased by having diets rich in fibre.

Fibre from plant food controls the intestinal transit by their action on faecal volume and produces an effect on carbohydrate and lipid metabolism that is usually linked to other minor components that are beneficial for human health, such as flavonoids and carotenoids (Heredia *et al.*, 2002; Lefebvre & Thebaudin, 2002).

DF also acts as protective agent against cardiovascular diseases, diverticulosis, constipation, irritable colon, colon cancer and diabetes (Tavaini & LaVecchi, 1995; Pietinen, 2001). The insoluble fraction of the fibre (IF) seems to be related to the intestinal regulation, whereas the soluble fibre (SF) is associated to the decrease of cholesterol levels and the adsorption of intestinal glucose (Schneeman, 1987).

Foods rich in fibre have also the capacity of binding bile acids, metabolites of cholesterol, which plays an important role in the digestion and absorption of lipids in the small intestine. The primary bile acids known as cholic and queno-deoxycholic acids are dehydrolyzed and converted to the secondary bile acids called deoxycholic and lithocholic acids respectively. These compounds play a decisive role in the etiology of the colon cancer (Nagengast, 1996). The degree of absorption of common bile acids, lithocholic, deoxycholic and cholic acids and cholesterol by fibre from plant food depends on the kind of raw material, conditions of processing and type of bile acids (Gorecka, Korczak, Balcerowski, & Decyk, 2002). Relationships between DF and colon cancer, despite its complexity, are quite well documented, and allow confirmation that there is an inverse relation between the fibre intake and the incidence of that pathology. An explanation could be that low fibre content causes the formation of very compact faeces that may promote

oncogenesis derived from a large time of exposure of the intestinal mucosa to cancer-risk agents (Armstrong & Doll, 1975).

Foods rich in fibre contain a broad spectrum of compounds that may prevent different types of cancer. Also, several fibres have demonstrated, *in vitro*, and *in vivo*, their capacity for adsorbing carcinogenic agents, so it is recommended to consume plant foods with lignified or suberized cell walls that are the most effective for linking hydrophobic carcinogenic agents (Steinmetz & Potter, 1991; Slavin, 2001).

Although there have been many advances about DF properties, there are still many aspects that remain unclear, mainly those related to the relationships between DF and specific pathologies. Further investigations are needed to establish the precise functions of DF components on human health and nutrition.

Methods of analysis

As a consequence of the importance that DF has acquired in recent years, (although there has not been a consensus reached about a unique definition of fibre) numerous methods have been developed for its determination. Several are very specific and precise for the identification and quantification of the different DF components. Many consist of the use of highly purified enzymes that selectively release oligo- and polysaccharides that constitute DF; of special interest are those enzymes that hydrolyze fructans, galactans, mannans, arabinans and β -glucans (Kamp, Asp, Miller, & Schaafsma, 2004).

The first reported method is that from Weende, developed in the Experimental Station of Weende, Gottinger, Germany, which consists of a sequential extraction with diluted acid and alkali solutions, and was adopted by the 'Association of Official Analytical Chemists' (AOAC) for determining fibre until the 1960s. Later on it was proposed the isolation of fibre by digestion of the samples with trichloroacetic, acetic and nitric acids that did not solubilize cellulose but only lignin. (Van Kamer, 1949).

Van Soest used cationic and anionic detergents that solubilize fats, nitrogen related compounds, simple sugars and soluble starch, while lignin is not altered. These methods are known as 'acid detergent fibre' (ADF) and 'neutral detergent fibre' (NDF) complementary systems. In the first (ADF), hexadecyltrimethyl-ammonium bromide in acidic solution is used to solubilize all the fibre components except cellulose, lignin and ashes. NDF method consists of the treatment of the samples with sodium dodecyl sulphate that solubilizes all the components distinct of cellulose, hemicelluloses, lignin and ashes. Cellulose is then quantified from ADF fraction, after oxidizing lignin by treatment with potassium permanganate, and hemicelluloses are determined by difference between NDF and ADF (Van Soest, 1963a,b, Van Soest, 1965; Van Soest & Wine, 1968). From these original procedures, there have been developed many different analytical methods that are described below.

According to Asp (Asp, Schweizer, Southgate, & Theander, 1992) the methods of fibre analysis are classified in two main groups.

The first is methods that determine unavailable polysaccharides, with the exception of starch, and lignin. These are known as enzymatic-gravimetric methods and consist of quantifying fibre as the residue that remains after the treatment of the samples with specific enzymes that degrade starch and proteins (Asp *et al.*, 1983; Helendoorn, Noordhoff, & Slagman, 1975; Lee, Prosky, & DeVries, 1992; Proski *et al.*, 1984, 1985, 1988). An important advance for these determinations has consisted of the developing of new techniques that separate the insoluble from the soluble fibre, being this last one obtained by precipitation with ethanol (Furda, 1977; Furda, 1981; Schweizer & Würsch, 1979, 1981; Asp & Johansson, 1981). These procedures allow quantifying total fibre (TF) as the sum of soluble and insoluble fibre, as well as determining the chemical and physiological properties of each (Asp *et al.*, 1992).

More recently, the enzymatic-gravimetric methods have been simplified in order to get a unique enzymatic treatment with amyloglucosidase (Li, 1995; Li & Zhao, 1997), or with a mixture of bile and pancreatin without previous thermal treatment (Cranker, Philips, Gonzales, & Stewart, 1997a,b). The enzymatic treatments must be standardized, and highly purified enzymes must be used for avoiding not very reproducible results when determining DF from foods that contain significant amounts of β -glucans, resistant starch and fructans (McCleary, 2000). Despite the above mentioned achievements, inter-laboratories studies are still needed for validating these 'new' methods taking as reference those officially accepted.

The second group is methods that determine non-starch polysaccharides without considering their physiological properties. They are enzymatic-gravimetric methods based on the isolation and fractionation of non-cellulosic polysaccharides, cellulose and lignin, followed by the hydrolysis of each fraction and quantification of their sugar composition by GC analysis (Englyst & Cummings, 1984, 1988; Southgate, 1969; Southgate, 1981; Theander & Westerlung, 1986; Theander, Aman, Westerlund, & Graham, 1990), with or without previous acetylation of the released sugars in each fraction; HPLC techniques that no required the derivatization of the sugars can also be used. There have also developed colorimetric methods that consist of determining the sugar content from the coloured compound that is formed by the reaction between the hydrolyzed sugars and the *p*-aminohydroxybenzoic-acid hydrazide (Faulks & Timms, 1985).

In addition to the above-mentioned methods, other quick-methods exist whose results are faithful and comparable to the traditional procedures, and so their use has been generalized during the last years. These include the use of near infrared spectroscopy (NIR) for determining fibre from different sources (Barton, Akin, Morrison, Ulrich, & Archibald, 2002; Font, R  o, Fern  ndez, & Haro, 2003),

and the quantification of those components of the fibre that may act as prebiotics, mainly oligofructose, inuline and polidextrose, which can be used as functional ingredients in different type of foods, including aqueous matrix (Windham, Kays, & Barton, 1997), as well as those rich in lipids and sugars (Kays, Barton, & Windlam, 1997; 1999; Kays, Windlam, & Barton, 1998).

Most analyses of prebiotics are based on the use of chromatography techniques, GC, gel filtration and high pH exchange. Analysis by GC is the most complex because a previous step of derivatization of the samples is required for making the compounds volatile, but it presents the advantage that allow the direct determination of the oligosaccharides with low-grade (<10) of polymerization (Joye & Hoebregs, 2000). Methods based on gel filtration involve a previous enzymatic treatment, with inulinase (Dysseler, Hoffem, Fockedey, Quemener, & Thibault, 1994). New advances of molecular biology techniques also make possible the release of non-digestible oligosaccharides from DF with specific prebiotic, physic-chemical, physiological and organoleptic properties (Meyer, 2004). Polidextrose determination also implies the use of enzymes that release these oligosaccharides from the food matrix. In this case the enzymatic mixture consists of fructanase, amyloglucosidase and iso-amylase (Craig, Holden, & Khaled, 2000). These multiple methods of isolation, analysis and quantification of prebiotics that are currently being developed are predicted to be accepted as official methods after being validated.

AOAC Methods

Several of the procedures above mentioned, which are referred to either the DF or some of its components, have already been adopted as official analytical methods within the AOAC (Official Methods of Analysis, 2000):

- Fibre (Crude) Animal Feed and Pet Food. AOAC Official Method 962.09. Crude fibre is loss on ignition of dried residue remaining after digestion of the sample with 1.25% H₂SO₄ and 1.25% NaOH solutions under specific conditions. Method is applicable to grains, meals, flours, feeds, fibre-bearing material, and pet foods from which fat can be extracted to leave workable residue.
- Acid detergent fibre and lignin in feed. AOAC Official Method 973.18. ADF and lignin are measured after acid hydrolysis of the samples by using 72% sulphuric acid solution.
- Fibre (Crude) in Animal Feed and Pet Food. AOAC Official Method 978.10. Principle is the same as in 962.09 except that the sample is exposed to minimum vacuum needed to regulate filtration, and heating of sample solutions prevents gelling or precipitation of possible saturated solutions.
- Total Dietary Fibre in Foods (Enzymatic-Gravimetric Method). AOAC Official Method 985.29. Duplicate samples of dried foods, fat-extracted if containing

- >10% fat, are gelatinized with Termamyl (heat-stable α -amylase), and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. Four volumes of ethyl alcohol are added to precipitate soluble dietary fibre. Total residue is filtered, washed with 78% ethyl alcohol, 95% ethyl alcohol, and acetone. After drying, residue is weighed. One duplicate is analyzed for protein, and other is incinerated at 525°C and ash is determined. Total dietary fibre = weight residue – weight (protein + ash).
- Fibre Acid – Detergent and Protein Crude in Forages. Near infrared Reflectance Spectroscopic Method. AOAC Official Method 989.03. Random portions of prepared sample are loaded into sample holder of NIR spectrometer. Instrument is part of system that has been calibrated using representative samples from population to be tested. Equations selected from calibration statistics, which have been validated, are used to calculate acid-detergent fibre and crude protein content of feed and forage samples.
 - Insoluble Dietary Fibre in Food and Food Products. Enzymatic-Gravimetric Method, Phosphate Buffer. AOAC Official Methods 991.42. Duplicate test portions of dried foods, fat-extracted if they contain >10% fat, are gelatinized with Termamyl (heat-stable α -amylase) and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. Soluble dietary fibre is removed by filtering and washing residue with water. Remaining residue, insoluble dietary fibre (IDF), is washed with 95% ethanol and acetone, dried, and weighed. One duplicate is analyzed for protein, and the other is incinerated at 525°C to determine ash. IDF is weight of residue less weight of protein and ash.
 - Total Soluble and Insoluble Dietary Fibre in Foods. Enzymatic-Gravimetric Method. MES-TRIS Buffer. AOAC Official Methods 991.43. Duplicate samples of dried foods, fat-extracted if containing >10% fat, undergo sequential enzymatic digestion by heat stable α -amylase, protease and amyloglucosidase to remove starch and protein. For total dietary fibre (TDF), enzyme digestate is treated with alcohol to precipitate soluble dietary fibre before filtering, and TDF residue is washed with alcohol and acetone, dried, and weighed. For insoluble and soluble dietary fibre (IDF and SDF), enzyme digestate is filtered, and residue (IDF) is washed with warm water, dried and weighed. For SDF, combined filtrate and washes are precipitated with alcohol, filtered, dried, and weighed. TDF, IDF, and SDF residue values are corrected for protein, ash, and blank.
 - Total Dietary Fibre. Enzymatic-Gravimetric Method. AOAC Official Methods 992.16. Foods samples, dried and ground, are fat extracted if containing >10% fat. A portion of sample is treated in autoclave with heat stable amylase, amyloglucosidase, and protease to remove starch and protein. Enzymatically undigested fibre is precipitated by ethanol and filtered. Residue is dried, weighed, ashed, and reweighed. A second portion of sample is refluxed with neutral detergent and treated with α -amylase from porcine pancreas to remove water soluble carbohydrates and protein. Residue is dried, weighed, ashed, and reweighed. Total dietary fibre is calculated as sum of the 2 residues.
 - Soluble Dietary Fibre in Food and Food Products. Enzymatic-Gravimetric Method (Phosphate Buffer). AOAC Official Methods 993.19. Duplicate test portions of dried foods, fat-extracted if >10% fat, are gelatinized with heat-stable α -amylase and then enzymatically digested with protease and amyloglucosidase to remove protein and starch. IDF is removed by filtering and washing residue with water. SDF in filtrate is precipitated by adding 95% ethanol to filtrate. Precipitate is filtered and washed with 78% ethanol, 95% ethanol, and acetone, dried, and weighed. One duplicate is analyzed for protein, and second is incinerated at 525°C to determine ash. SDF is weight, of residue minus weight of protein and ash.
 - Total Dietary fibre in Foods and Foods Products with $\leq 2\%$ Starch. Non-Enzymatic-Gravimetric Method. AOAC Official Method 993.21. Dried fruit, vegetable, or isolated fibre sources are suspended in water and incubated 90 min at 37°C to solubilize sugars and other water-soluble components. Water-soluble fibre components are then precipitated with ethanol. Residue is washed sequentially with 78% ethanol, 95% ethanol, and acetone and then dried at 105°C. One duplicate is analyzed for crude protein, the other for ash. Total dietary fibre (TDF) is calculated as weight of residue less weight of protein and ash.
 - Total Dietary Fibre determined as Neutral Sugar Residues, Uronic Acid Residues, and Klason Lignin. Gas Chromatographic – Colorimetric – Gravimetric Method. AOAC Official Methods 994.13. Starch is removed from sample in acetate buffer using thermo stable α -amylase and amyloglucosidase. Soluble polymers are precipitated with 80% ethanol. Precipitated and insoluble polysaccharides are hydrolyzed with H₂SO₄. Released neutral sugars are quantified by gas – liquid chromatography as alditol acetates. Uronic acids in acid hydrolysate are determined by colorimetry, and Klason lignin is determined gravimetrically as ash-free acid-insoluble residue. Total dietary fibre is defined as amylase-resistant polysaccharides plus Klason lignin.
 - Fructans in Food Products. Ion Exchange Chromatographic Method. AOAC Official Methods 997.08. Fructans are extracted from the sample with boiling water. Extract aliquot is hydrolyzed using lyophilized amyloglucosidase to remove starch present. Subsequently, part of that hydrolysate is treated with inulinase followed by determination of released sugars. The initial sample, first and second hydrolysates are analyzed using high performance anion exchange chromatography with pulsed amperometric detection

(HPAEC – PAD). In sugar analysis 1, free fructose and sucrose are determined in initial sample. In sugar analysis 2, sum of free glucose and glucose from maltodextrins and starch are determined in the first hydrolysate. In sugar analysis 3, total amount of glucose and total amount of fructose from the hydrolysate plus glucose and fructose from the second hydrolysate are determined. Fructans are calculated from concentrations of glucose and fructose.

- Food povidextrose (ref. 2000.11). This is extracted from the food matrix with hot water and then is centrifuged. The supernatant is filtered through an ultra filtration cell that retains high molecular weight impurities. The filtrate is incubated with an enzymatic mixture (iso-amylase, amyloglucosidase and fructanase) in order to separate other interfering oligosaccharides, mainly malto-oligosaccharides and fructans. High molecular weight povidextrose fractions are determined and quantified by HPAEC-ED chromatography.

In addition to these direct determinations of fibre, there are also other systems for determining fibre-related substances such as: starch in vegetables (ref. 948.02), cereals (refs. 979.10, 996.11), fruits (925.38), chocolate (920.84), peanut butter (ref. 954.08); lignin in plant food (refs. 932.01, 949.04) and feed (ref. 973.18); starch flour and soy flour in meat products (refs. 958.06, 935.49, 913.01); alginates in chocolate (ref. 959.06); gums in cheeses and ice-creams (refs. 937.06, 960.33); and agar in meat products (ref. 945.57).

Fibre content in foods and consumption

Intensive research has been carried out in relation to the effects of foods rich in dietary fibre and isolated fibre components (Mälkki, 2004). Dietary fibre is naturally present in cereals, vegetables, fruits, and nuts, and the amount and composition of fibres differ from food to food (Desmedt & Jacobs, 2001). Several non-starch foods provide up 20–35 g of fibre/100 g dry weight and other those containing starch about 10 g/100 g of dry weight; and the content of fibre of fruits and vegetables is 1.5–2.5 g/100 g of dry weight (Selvendran & Robertson, 1994). Among the different rich in fibre-foods, cereals are one of the main sources of DF, contributing to about 50% of the fibre intake in western countries (Lambo, Öste, & Nyman, 2005); 30–40% DF may come from vegetables, about 16% from fruits and the remained 3% from other minor sources (Gregory, Foster, Tyler, & Wiseman, 1990; Cummings, 1996).

Recent tendencies suggest a move towards diets that include higher amount of plant foods as they seem to be implicated in keeping and/or improving our health. According to the results from the European Cost-92 Programme there are significant differences in the consumption of fruits and vegetables between European countries (Cummings, 1996). So, the intake of DF is much

lower in Scandinavian countries than in South European countries such as France, Italy and Spain (Saura-Calixto & Goñi, 1993). The recommendations about which must be the intake of DF are not the same in all countries; while UK proposes 18 g/day of DF expressed as non-starch polysaccharides (NSP), this amount is increased to 30 g/d in Germany, and in USA is specified that the intake should be 38 g/d for men and 26 g/d for women (Miller, 2004). A Mediterranean diet, typical in Spain, Italy and Greece, provides a significant content of DF as it is rich in vegetables, cereals, fruits and legumes; the recommended intake in these countries being 20 g/d for men and 15.7 g/d for women (Capita & Alonso-Calleja, 2003)

The amount of DF coming from cereals differs to a great extent depending on the source and the processing of the product; so the content of DF in wheat flour varies from 2.5 g/100 g in refined flour to 12 g/100 g in unrefined flour obtained from wheat bran, in which most fibre consists in the insoluble fraction that is lost during the refining process. The content of DF in vegetables can represent to 28–30% of dry matter, although in some products, such as white and red beans, much higher values are reached. Similar to vegetables, fruits contain a significant percent of water together with small amounts of lignified vascular tissues, having thin cell walls, which is related to their lower contents of DF (1–3.5% dry matter) (Johnson & Southgate, 1994).

Functional foods

Currently ‘functional foods’ are defined as those that in addition to act as nutrients may positively affect specific biological functions, improving our general state of health and/or reducing the risk of suffering distinct diseases (Diplock, Aggett, Ashwell, Borneo, Fern, & Roberfroid, 1999). The International Life Science Institute—ILSI Europe (1999) established that “a food product can be considered as functional if it has satisfactorily been proved that it produces a beneficial effect on one or more physiological functions, besides its conventional nutritional effects, being this relevant for improving the human health and/or reducing the risk of suffering certain diseases.” It must be taken into account that functional foods, in the amounts that are usually ingested in our diet, must keep their nutritional role together with their capacity for producing beneficial effects on determined organic functions (López-Varela *et al.*, 2000; Roberfroid, 2000).

DF holds all the characteristics required to be considered as an important ingredient in the formulation of functional foods, due to its beneficial effects such as increasing the volume of faecal bulk, decreasing the time of intestinal transit, cholesterol and glycaemia levels, trapping substances that can be dangerous for the human organism (mutagenic and carcinogenic agents), stimulating the proliferation of the intestinal flora, etc. (Heredia *et al.*, 2002)

The importance of food fibres has led to the development of a large and potential market for fibre-rich products and

ingredients and, in recent years, there is a trend to find new sources of dietary fibre that can be used as ingredients in the food industry (Chau & Huang, 2003).

Among foods enriched in fibre, the most known and consumed are breakfast cereal and bakery products such as integral breads and cookies (Cho & Prosky, 1999; Nelson, 2001), as well as milk and meat derived products. Enrichment of bakery products has traditionally consisted of the addition of unrefined cereals; however it is starting to use other DF sources, mainly fruits, which present better nutritional quality, higher amounts of total and soluble fibre, less caloric content, stronger antioxidant capacity and greater grade of fermentability and water retention (Grigelmo-Miguel & Martín-Belloso, 1999b; Larrauri *et al.*, 1996; Saura-Calixto, 1998). The addition of DF to bakery products also improves their nutritional quality since it makes possible to decrease the fat content, by using DF as substitutive of fat without loss of quality (Byrne, 1997; Martin, 1999). Isolated fibre components such as resistant starch and β -glucans are also used for increasing fibre content in pastries, breakfast cereal, etc. (Knuckles, Hudson, Chiu, & Sayre, 1997).

In the case of beverages and drinks, the addition of DF increases their viscosity and stability, SF being the most used because it is more dispersible in water than IF. Some examples of these SFs are those from fractions of grains and multi-fruits (Bollinger, 2001), pectins (Bjerrum, 1996), β -glucans, cellulose beet-root fibre (Nelson, 2001), polidextrose (Mitchell, 2001), etc.

Some types of soluble fibres, such as pectins, inuline, guar gum and carboxymethyl-cellulose, are utilized as functional ingredients in milk products (Nelson, 2001). Guar gum, pectins and inuline are added during cheese processing to decrease its %fat without losing its organoleptic characteristics, such as texture and flavour; on the other hand, the addition of DF into yogurts and ice-creams improves the stability of these emulsions.

DFs based on pectins, cellulose, soy, wheat, maize or rice isolates and beet fibre can be used for improving the texture of meat products, such as sausages, pates and salami; and, at the same time, are adequate to prepare low-fat products, such as 'dietetic hamburgers'. Also, since they have the ability of increasing the water retention capacity, their inclusion in the meat matrix contributes to maintain its juiciness, which implies that the volatile compounds responsible for the flavour of the product are more slowly released (Chevance, Farmer, Desmond, Novelli, Troy, & Chizzolini, 2000; Desmond, Troy, & Buckley, 1998; Mansour & Khalil, 1999).

For the elaboration of jams and marmalades, the most common added-fibres are those consisting of pectins with different degree of esterification, which mainly comes from fruits and are a factor in keeping the stability of the final product. (Grigelmo-Miguel & Martín-Belloso, 1999b; 2000). In the case of low-calorie chocolates and derivatives, fibre compounds such as inuline and oligofructose are used as sugar substitutes (Gonze & Van der Schueren, 1997).

Although great achievements have been made by using

fibres as functional foods and ingredients, further investigations about DF structure and functionality within the food matrix are needed. Only after getting a better knowledge about the subject, will it be possible to 'design' new food systems that consider the precise functionality of DF from both technological and physiological points of view (Guillon & Champ, 2000).

Isolation of dietary fibre from plant-food residues

Plant biomass constitutes a primary and unlimited source of human resources, thanks to its bioavailability and renewable character.

Market for DF is highly competitive and new fibres with healthy properties that satisfy the growing consumer requests that are demanded every day; there are a great variety of agronomic subproducts that are available (Chi-Fai, Ya-Ling, & Mao-Hsiang, 2003). The residual substances that remain after isolating the main component of the total subproduct are abundant, and represent an inexpensive material that has been undervalued until now, being only used as combustible or fertilizer (Grigelmo-Miguel & Martín-Belloso, 1999a). There is an increasing interest in recovering that material, which may be used, among other uses, as source of DF destined to supplement low-in-fibre food products (Fernández-Bolaños, Rodríguez, R., Saldaña, C., Heredia, a., Guillén, R., & Jiménez, A., 1990). While a few years ago the subproducts generated during the processing of plant food constituted an economic and environmental problem, today they are considered a promising source of functional compounds (Carle *et al.*, 2001).

They are many fruits, for example orange, apple, peach and olive, which are used for the extraction of their juices. They all contain a by-product from which can be recovered different high-added value compounds; among those, it is remarkable the fibre fraction that has a great potential in the preparation of functional foods. There are also several vegetables, such as pepper, artichoke, onion and asparagus that originate a waste during their processing that in the case of asparagus spears can represent upto 40–50% of their fresh weight (Rodríguez, Jiménez, Guillén, Heredia, & Fernández-Bolaños, 1999a) and it contains both soluble and insoluble fibre compounds that can be used for designing new 'functional foods'.

Orange and lemon sub-products, which are abundant and cheap, also constitute an important source of fibre since they are very rich in pectins (Askar, 1998). Other fruits such as grapes, apples, bananas, mango, guava, etc. which are mainly commercialized in processed form originate great amounts of sub-products consisting of peels, bones and seeds. This material could be a restrictive factor in the commercialization of these products if it is not usefully recovered, because it represents significant losses with respect to the raw material, which considerably increases the price of the processed products (Schieber *et al.*, 2002). Among many other bioactive compounds, significant

amounts of pectins and polyphenols can be recovered from apple by-products (Carle *et al.*, 2001); and different types of fibres are isolated from grapes, after the extraction of their juice, as well as from guava skin and pulp (Schieber *et al.*, 2002). Since these fibres are associated with antioxidants compounds acids derivatives, they constitute a multiple and complete dietary supplement. Other fibres of interest are those rich in highly branched pectins that can be isolated from the mango skin (Sudahakar & Maini, 2000).

Other waste products are those coming from the kiwi that contain about 25% fibre referred to dry matter (Martin-Cabrejas, Esteban, López-Andreu, Waldron, & Selvendran, 1995) and from the pineapple shell that has a high percentage of insoluble fibre (70% TF), which is mainly composed of neutral sugars, such as xylose and glucose, and presents a great antioxidant capacity (Salvi & Rajput, 1995; Larrauri, Rupérez, & Saura-Calixto, 1997). Olives that are largely destined for the production of olive oil also leave a by-product that is rich in different bioactive components, including phenolics and fibre (Heredia *et al.*, 1993).

Influence of food processing in fibre properties

The processed foods of major interest for the human consumption are those whose elaboration implies fermentative and heating processes, which may alter the chemical composition and the physico-chemical, nutritional and functional properties of their fibre fraction, and so affect its physiological effects on the human body (Nyman, Schweizer, Palsson, & Asp, 1991).

Fermentation is a type of plant food processing that causes modifications of the composition and structure of DF. These changes are motivated by the enzymatic activity developed during the fermentative process and principally consist of solubilization of different cell wall polysaccharides. The main enzymes that have been detected in the fermentative brines are amylase, proteinase, poligalacturonase, cellulase and β -galactosidase that selectively degrade distinct cell wall polysaccharides and as a consequence provoke a decrease of the fibre content that mainly consists of losses of SF, although a portion of the IF can also be released. The polysaccharides that are released into the brines include both soluble compounds such as neutral polysaccharides and pectins; and constituents of the IF such as hemicelluloses and cellulose (Jiménez, Sánchez-Romero, Guillén, Fernández-Bolaños, & Heredia, 1998). Table olives and sauerkraut are some of the most known and appreciated fermented plant foods. Olive fruits that are processed for their consumption as table olives suffered a significant loss of DF during that elaboration process (Durán-Quintana, García, & Garrido, 1999; Sánchez, García, Rejano, Brenes, & Garrido, 1995; Sánchez, Rejano, Montaña, & Castro, 2001), (Fernández-Bolaños *et al.*, 2002; Guillén, Heredia, Felizón, Jiménez, & Fernández-Bolaños, 1991, 1992; Heredia, Ruiz-Gutierrez, Felizón, Guillén, Jiménez, & Fernández-Bolaños, 1993; Jiménez, Rodríguez, Fernández-Caro, Guillén, & Fernández-Bolaños, 2000);

whereas the content of DF from the sauerkraut is approximately the same as that from the raw cabbage (Halasz, Barath, & Holzapfel, 1999; Petaja, Millyniemi, Petaja, Ollilainen, & Piironen, 2000; Roch, Yung, & I, 2001). This is explained by the fact that this food is consumed with a great part of its processing liquid, where the portion of fibre lost from the vegetable has been solubilized (Soo & Hong, 1997).

The most used processes of elaboration of plant foods that consist of applying thermal treatments are boiling, cooking and canning. It is well documented that heating can considerably change the texture of plant tissues and that this modification depends on the composition and structure of the fibre components. So, it is crucial to study the effect of thermal treatments on the physico-chemical and physiological properties of the fibre in order to establish the precise amount and bio-availability of the DF that remains in the final product that gets to the consumer.

Boiling is usually a not very prolonged treatment that implies the heating of the samples with water at 100 °C, steam water, or in microwave. This process results in the inactivation of practically all the enzymes that could negatively affect the organoleptic properties of the final products. Some of the undesirable effects that could happen are excessive softening of the plant tissues, loss of colour and flavour and/or development of odd colours and flavours. Cooking is usually longer than the above process and can be realized at boiling temperature or at lower values, with or without applying high pressures, and by using conventional oven or microwave apparatus (Heredia *et al.*, 2002). Canning is usually carried out by the application of high temperature and pressure during short periods of time.

In spite of the fact that large amounts of plant food are consumed after being thermally treated (Block & Lance, 1987; Reistad & Frölich, 1984), there are not many references to the modifications that DF suffers during thermal processing. Also, it has not yet been clearly established which fibre constituents experience the greatest changes as a consequence of heating, although it has been reported that hemicelluloses and pectic substances seem to be the most affected components (Ben-Shalom, Olat, Levi, & Pinto, 1992; Massiot, Guiller, Baron, Drilleau, 1992). Tatjana *et al.*, have investigated the modifications that happen during the thermal processing of kidney-beans, and these authors have reported that the solubilization of those polysaccharides results in a decrease of the content of total fibre, mainly caused by the loss of SF (Tatjana, Terezija, Milica, & Plestenjak, 2002).

The apparent increases of fibre observed in some cases, are normally a consequence of the formation of complexes between polysaccharides and other components of the food, such as proteins and phenolic compounds, which are measured as fibre (Takeyama, Yokokawa, & Tanimura, 1996).

Processing required to make some vegetables and legumes (chick-pea, bean, lentil, etc) suitable for eating causes a decrease of several components of the fibre. For example, during the cooking of lentils previously dipped, the quantity of fibre diminishes, due fundamentally to a great decrease of hemicelluloses (Vidal-Valverde & Frías, 1991; Vidal-Valverde, Frías, & Esteban, 1992). In wheat bran it has been found that thermal treatments (boiling, cooking or roasting) originate an increase of total fibre that is not due to new synthesis, but rather to the formation of fibre - protein complexes that are resistant to heating and as quantified as DF (Caprez, Arrigoni, Amado, & Neucom, 1986).

Since the cooking of the vegetable products implies the employment of different acid and basic solutions, the pH is a key factor on the changes of texture that the plant tissues experience during this process. For example, it has been reported that potatoes, cauliflower, kidney-beans and peas keep higher values of firmness when they are cooked at pH 4 than if the process occurs at pH 10. That is explained by the fact that the solubilization of fibre components that results in a decreasing of texture is more pronounced in basic conditions. This effect is especially significant in products such a cauliflower, which presents a high content of fibre together with a poor resistance to the penetration of alkaline solutions through their tissues. In general, the final effect of pH conditions will depend on the fibre composition, time of treatment, size of the plant food and grade of penetration of the processing liquids (Brand, Jeltema, Zabik, & Jeltema, 1984).

The post-harvest storage of fresh fruits and vegetables tries to keep the organoleptic (color, texture and flavour) and nutritional properties of the final product in optimal conditions. Changes in fibre quantity and quality depend to a great extent on storage conditions; for example in apples stored in controlled atmosphere to assure their stability, no changes in the content of total food were observed; nevertheless in onions, which are usually stored in less restrictive conditions, there was a general increase of the fibre components that is more pronounced for the uronic acids that constitute the pectic polysaccharides (Marlett, 2000). During the storage of several vegetables such as cauliflower, broccoli and asparagus, a process of toughening takes place, associated with an increase of fibrousness, which devalues the quality of the final product. This process is more pronounced in asparagus, which suffers a quick hardening, mainly located in the basal portions of the spears, and related to modifications of fibre components that consist on the deposition of lignin, cellulose and hemicelluloses (Bernalte-García, Hernández-Méndez, & Carballo García, 1995; Rodríguez, Jiménez, Guillén, Heredia, & Fernández-Bolaños, 1999a, b).

Freezing is other method that can be used for preserving the quality attributes of fresh vegetables. Although there are not many studies about the influence of this process on the fibre properties and, furthermore, the results are contradictory, investigations carried out in tropical fruits have

shown that the content of fibre is lower in the frozen than in the fresh fruits (Salgado, Guerra, & Melo-Filo, 1999). Contrary to those results, freezing did not provoke significant changes to the fibre contents of specific vegetables, such as carrots, peas, Brussels sprouts and green beans (Nyman, Palsson, & Asp, 1987).

Final remarks

After reviewing all the topics about fibre that are included in the present paper, it can be concluded that there are still many aspects within this research field that need further investigation. In order to get a better knowledge of DF composition and structure together with an understanding of its physiological effects on the human body, the design of collaborative studies is required with the participation of researchers from different scientific areas: chemistry, biochemistry, biotechnology, biology, physiology, nutrition and medicine. At present, what is clearly established and well-documented is that DF possesses a great potential as a functional ingredient that may produce distinct beneficial effects on human health.

As a final conclusion it can be proposed that from the present review of DF, and despite the abundant scientific literature that currently exists, it is still necessary to build a larger and multi-discipline database that will facilitate a universal agreement on the definition of DF as well as about its methods of analysis.

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