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DECIPHERING ANTIOXIDANT ADVANTAGE OF DIETARY FIBRE

Sheel Sharma, Shivanki Aggarwal¹ and Preeti Verma

Department of Food Science and Nutrition, Banasthali University, Rajasthan, India.

¹Corresponding author: shivankkiaggarwal88@gmail.com

ABSTRACT

Dietary Fibre (DF) has been well-documented to possess an inverse correlation with degenerative diseases of different etymology, especially colon cancer. Such appreciation has been hitherto explained due to its polysaccharide moiety. However recent reports suggest that polyphenols in general and non-extractable polyphenols (NEPP) in particular, present largely in plant based foods form an important constituent of dietary fibre. These substances perform an array of health enhancing acts ranging from free radical scavenging, metal chelation, enzyme or receptor modulation in cellular signaling pathways. Dietary fibre encompasses these phytochemicals in itself and makes them bioavailable too. Thus, it becomes prudent to include fruits and vegetables, whole grains, pulses and nuts in the diet in order to ensure the provision of synergistic antioxidative environment in the body for impeding diseases arising out of oxidative damage. Further, it is the type of DF that determines the extent of bioavailability of complex polyphenols and proanthocyanidins corroborated from the evidences saying that soluble fractions due to their higher fermentability render polyphenolic compounds more bioavailable as compared to highly crystalline insoluble counterparts.

Keywords: Antioxidants, Dietary fibre, Non extractable polyphenols, Non extractable proanthocyanidins **Abbreviations:** DF- Dietary fibre, IDF- Insoluble dietary fibre, IDF-PC- Insoluble dietary fibre phenolic compounds, NEPA- Non extractable proanthocyanidins, NEPP- Non extractable polyphenols, PC- Phenolic compounds, SDF- Soluble dietary fibre, SDF-PC- Soluble dietary fibre phenolic compounds.

INTRODUCTION

Accumulated literature advocates Dietary Fibre (DF) to provide shield against diseases of different etymology and colorectal cancer in particular (Seal, 2006; Anderson et al, 2009). Much of the aforesaid protective demeanour has been hitherto attributed to its polysaccharide moiety overshadowing the synergism of phenolic compounds associated with fibre fractions (Jiménez-Escrig et al, 2001; Martinez-Tome et al, 2004). Conventionally, the principal action of DF is to regulate intestinal functions via various mechanisms (Heredia et al., 2002). However, renaissance of DF as functional ingredient is due to the fact that it provides entrapping matrix to phenolic compounds and facilitate their bioavailability in the body. Earlier Non Extractable Polyphenols (NEPP) were not considered physiologically important and not analyzed in laboratory procedures but now-a-days they are gaining intense scientific interest (Arranz et al, 2010) linked with their substantial presence in fibre fractions and postulation that they, being structurally complex, are better free radical scavengers compared to simple phenolic compounds. A further, biological effect of

NEPP, like other phenolic compounds, depends upon their bioaccessibility and bioavailability which in turn relies profoundly upon presence, type, extent and site of degradation of DF. Thus to understand the action of DF one requires more holistic approach to understand its definition and principle actions within the human body.

DEFINITION

Hipsley (1953) coined the term Dietary Fibre (DF) as nondigestible constituents making up the plant cell wall. The definition witnessed several updations. Trowell (1976) defined it as "The remnants of plant foods remaining after hydrolysis by the enzymes of the digestive system" and hemicellulose, comprises of cellulose, oligosaccharides, pectins, gums and waxes. The principal function of DF is to provide substrates for fermentation in large intestine producing various end products and culminating in nourishment of intestinal ecosystem. Later it was observed that DF is not the only source of fermentable substrate for bacterial degradation in large intestine corroborated from the finding that the daily amount available



for fermentation in the large intestine in persons consuming Western diets was estimated close to 50g whereby DF intake represented about 39% of this amount and provided less than half of the energy supply to large bowel (Cummnings and Macfarlane, 1991). As revelations called for extension of DF concept by incorporating other indigestible food constituents (carbohydrates and non-carbohydrates), a new definition of DF emerged as ' A combination of chemically heterogeneous substance not absorbed in the small intestine' and includes non-starch polysaccharides, resistant starch. oligosaccharides, resistant protein, polyphenols, and others (Ferguson et al, 2001; AACC, 2001). The term 'heterogeneous' decodes that fibre is not an isolated, welldefined compound but a combination of chemical substances of distinct composition and structure. Based on simulated intestinal solubility, DF is classified as Soluble-DF (SDF) and insoluble-DF (IDF). IDF include lignin, cellulose, hemicellulose and have bulking action attributed to lower fermentability whereas SDF include pectins, β-glucans, galactomannan gums, inulin. SDF being highly fermentable in the colon has a function of decreasing cholesterol levels and adsorbing intestinal glucose (Scheneeman, 1987).

Though substantial information is available about the chemical, physiological and nutritional properties of the 'new' DF components i.e. resistant starch, resistant protein, oligosaccharides, and others, yet knowledge regarding dietary antioxidants associated with DF is scanty. In the context of food science, dietary antioxidant may be defined as 'A substance in foods that significantly decreases the adverse effects of reactive species on normal physiological function in humans (Institute of medicine, 2000) whereas biological antioxidant is defined as 'Any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate' (Halliwell and Gutteridge, 1995). Dietary antioxidants protect against oxidative damage to DNA, proteins, and lipids and regulate gene expression (Herrera et al, 2009; Dai et al, 2008). Vitamins (C,E,A), polyphenols (flavonoids, phenolic acids, stillbenes, tannins), and carotenoids (carotenes and xanthophylls) are the main groups of dietary antioxidants (Kaur and Kapoor, 2001). The general definition of a phenolic compound (PC) is any compound containing a benzene ring with one or more hydroxyl groups. Major categories involve phenolic acids, flavonoids, stilbenes, and lignin (Naczk and Shahidi, 2004). They are found in plant foods as free or bound cell wall constituents and are gaining incremental interest as 'life span essentials' due to their ubiquitous presence in plant foods, appreciable antioxidant properties and documented protection against degenerative diseases i.e. heart diseases, cancer etc (Rhodes and Price, 1997). Biological effects of polyphenols are not only confined to the modulation of oxidative stress via donating hydrogen atoms or chelating redox-active metal ions but also through their direct interactions with enzymes or receptors involved in cellular signaling pathways. Various in vitro and ex vivo biological effects of PC include inhibition of the proliferation of cancer cells, reduced vascularization, protection of neurons against oxidative stress, and stimulated vasodilation and improved insulin secretion (Ferguson et al, 2004; Silva et al, 2008). The biological properties of polyphenols depend not only on their concentration in plant foods but also on their bioaccessibility and bioavailability in the Bioaccessibility is defined as amount of ingested nutrient available for absorption in the gut after digestion while Bioavailability is defined as proportion of nutrient that is digested, absorbed, and utilized in normal metabolism. The bioaccessibility and bioavailability of each antioxidant differs greatly, and the most abundant antioxidants in ingested food are not necessarily those leading to highest concentrations of active metabolites in the target tissues (Manach et al, 2005). Ancient studies have cited DF and PC as separable terms. However recent scientific studies have marked both as closely related terms that can be approached jointly in nutrition studies. Explanation for such interrelation is the fact that PC are an important constituents of DF and that majority of the ingested PC and DF share a common metabolic fate in the human gut.

DIETARY FIBRE AS ENTRAPPING MATRIX

The characteristic feature of plant PC is that they are not evenly distributed throughout the plant food, with their higher concentrations in outer parts. Polyphenolic feature to accumulate in outer layers is due to the fact that PC has a tendency to chemically interact and form complexes with plant macromolecules. PC has both hydrophobic aromatic rings and hydrophilic hydroxyl groups with the ability to bind to polysaccharides, particularly carbohydrates at several sites on the cell wall surface. They can be linked by hydrogen bonding (between the hydroxyl group of PC and oxygen atoms of the glycosidic linkages of polysaccharides), hydrophobic interactions, and covalent bonds such as ester bonds between phenolic acids and polysaccharides (Hanlin et al, 2010; Bunzel et al, 2004). An array of examples connotes the aforesaid explanation. The parenchymal cell wall, most common cell type in plants, of certain families of food plants contain PC of hydroxycinnamic derivatives i.e. ferulic acid, p-coumaric acid and dehydrodiferulates which are covalently linked to specific polysaccharides (Smith and Harris, 2001; Hartley and Harris, 1981). About 80-95% of whole grains PC are located in bran portion. In chemical terms, they are covalently linked to the cell wall polysaccharides, mainly αarabinoxylans and are altogether indicated as dietary fibrephenolic compounds (DF-PC) (Vitaglione et al, 2008). Ferulic acid, cited as one of the most abundant PC in whole grains has almost all its part (98%) to be concentrated in aleurone and pericarp (Smith and Hartley, 1983). Such substantial PC presence in polysaccharide moiety affects the physical structure of DF by the formation of crosslinks



between DF and PC through the mechanism of ferulate dehydrodimerisation to yield diferulates (Bunzel et al, 2004), a complex structural scaffold consisting of cross-linked DF and PC in whole grains. Therefore most of the whole grains PC are reported to be present in bound form. Phenolic phytochemicals unique to specific grains i.e. 3deoxyanthocyanidins from sorghums (Awika et al, 2005) and alkylresorcinols from wheat, rye, barley are also reported to be concentrated in bran manifold. Furthermore refined wheat flour is reported to loss 83% of total phenolic acids, 79% of total flavonoids, 93% of ferulic acid, 78% of total zexanthin, 51% of total lutein, and 42% of total cryptoxanthin (Adom et al, 2005). Indeed, the antioxidant protective shield provided by fruit components such as mango peel, pineapple shell, guava pulp, grape pomace, acerola fruit has been heralded to the unique cocktail of DF and PC enabling the aforesaid fruit components to be known as 'Antioxidant Dietary Fibers'.

BIOAVAILABILITY OF PHENOLIC COMPOUNDS ASSOCIATED WITH DIETARY FIBRE

DF is explained as instrumental for rendering nonextractable polyphenols (NEPP) bioavailable (Saura- Calixto, 2011) culminating in systemic and local antioxidant activities in the body. Conceptually, NEPP is defined as a fraction of polyphenols not bioaccessible/degraded in small intestine either by direct solubilization in intestinal fluids in physiological conditions (37°C, Ph 1-7.5) or by the action of digestive enzymes (Rios et al, 2002) and consequently reach colon as nondegraded, unaltered bolus (Manach et al, 2005; Saura-Calixto et al, 2007). As DF provides fermentable substrates to intestinal microflora for their maintainence, PC that were bound with DF set free upon colonic degradation (Arranz et al, 2010) and become a good substrate for bacterial enzymes which ultimately leads to the slow and continuous release of PC. The bioavailability of PC associated with DF is determined by two factors-(a) concentration of PC in fibre matrix (b) type of DF (soluble or insoluble) to PC are linked. PC affluence in matrix suggests that the higher the concentration of PC in the matrix, the more probable the dimer formation, which implies a stronger physical structure of the fibre and the consequent higher difficulty to be degraded and utilized by intestinal microflora (Vitaglione et al, 2008). The high crosslinks suggest inefficient digestion by colonic microflora and therefore less release of PC units from indigestible matrix. Altogether it can be concluded that physical state of DF-PC determines the bioavailability of NEPP. Once free in gut lumen, PC can pass the colonic mucosa and absorbed into bloodstream after hepatic metabolism. Nonabsorbable microbial metabolites, being not able to pass through colon barrier, remain in the colonic lumen and exert local antioxidant activity by quenching free radicals and counteracting the effects of dietary pro-oxidants.

NEPP includes polymeric PP (hydrolysable and condensed tannins/ proanthocyanidins) and low molecular weight PP linked to DF and/or trapped in the cores of the fibre matrix (Saura-Calixto, 2011). Ferulic acid, caffeic acid, hesperidin, naringin, catechin, epicatechin, ellagic acid, gallic acid derivatives, protocatechin, and p-hydroxybenzoic acid are the major individual NEPP in plant foods of the diet (Arranz et al, 2010). The highest concentrations of NEPP are found in fruits, largely consisting of proanthocyanidins (NEPA). Legumes and nuts are also the sources of NEPA. Cereals and vegetables donot contain NEPA, but they are important sources of hydrolysable PP in the diet (Arranz et al, 2010; Arranz et al, 2009).

Taking the bioavailability of hydroxycinnamates, studies show that they cannot be absorbed as such because of their structural complexity but human gastrointestinal esterase from intestinal mucosa (Andreasen et al, 2001a; Andreasen et al, 2001c) and intestinal microbiota (Kroon et al, 1997) are able to cleave the ester bonds, preferentially from SDF compared to IDF, and release into lumen a proportion of the free hydroxycinnamic and diferulic acids. Bioavailability studies on the proanthocyanidins (NEPA), oligomers or polymers of flavonol units, pinpoint DF as to enhance the absorption of NEPA. The effect of DF is apparent from the study which on initial use of whole grape extract reported the absorption and metabolism of proanthocyanidins in rats while later experiments using purified proanthocyanidin fractions failed to demonstrate the absorption of these food components on the same subjects (Santos-Buelga and Scalbert, 2000). Similarly another study has reported the higher yield of phenolic metabolites in proanthocyanidin-rich DF samples than in purified proanthocyanidin samples (Saura- Calixto et al, 2010) which indicates that DF enhances colonic fermentation of proanthocyanidins.

Since the colonic absorption is slower due to smaller exchange area and low density of transport systems, consistent intake of fibre-associated polyphenols could be most promising mechanism to provide steady, constant, and cumulative antioxidative ambience (Poquet et al, 2008). The biological significance of DF is reiterated from the fact that polyphenols constitute the only dietary antioxidants present in the colon (Scalbert and Williamson, 2000) and provide antioxidant protection through its absorbable and nonabsorbable fractions.

INFLUENCE OF DIETARY FIBRE TYPE ON PHENOLIC BIOAVAILABILITY

Since the substantial presence of PC in plant cell walls enroutes them to chemically interact and form crosslinks with polysaccharide moiety (Bunzel et al, 2001), IDF being more crystalline than SDF is readily available for cross linking with phenolic constituents culminating in the formation of highly complex scaffold (Vitaglione et al,



2008). SDF is reported to contain amount of diferulates 100fold lower than the amount present in corresponding IDF (Bunzel et al, 2001). In biological terms, it is hypothesized that highly cross linked IDF-PC is inversely correlated to the fermentability by intestinal microflora (Wang et al, 2004) attributed to the inefficiency of bacterial β-glucosidases and esterases to attack on highly lignified IDF-PC complex which leads to the slow release of PC from IDF matrix. A typical example of the influence of DF type on phenolic bioavailability is discrepancy in FA bioavailability from endosperm and bran portion of cereal grain (Adam et al, 2002). Recent study suggests that low to moderate levels of diferulates do not interfere in degrading nonlignified cell walls by human gut microbiota (Funk et al, 2007) and it is generally accepted that the higher the SDF/IDF ratio of the cereal product is, the higher the DF-PC bioaccessibility (Funk et al, 2007). Another example is the study which reported higher fermentability of apple polyphenols when fermentation was performed in the presence of pectins the effect of which was pronounced improvement in plasma lipid profile (Aprikian et al, 2003). Another study showed that the extent of degradation of proanthocyanidins into aromatic amino acids decreased as the degree of polymerization increased (Serrano et al, 2009) and it was found to be 21 times lower for polymers than for the catechin monomer (Gonthier et al, 2003).

Although IDF-PC and polymerized phenolics i.e. proanthocyanidins are not as bioavailable as SDF-PC and simpler phenolics, recent findings have deciphered indigestible food components as to possess marked antioxidant activity by solid-liquid interactions and impede carcinogenesis by absorbing intestinal carcinogens, especially hydrophobic carcinogens (Serpen et al, 2007). Indeed Butyrate produced from partial fermentation of IDF in colon is cited as anti-proliferative and anti-apoptotic agent by modulating gene expression and metabolic changes (McIntyre et al, 1993; Augenlicht et al, 1999). Such mechanisms open up a new perspective about the physiological relevance of food insoluble matter.

HEALTH-RELATED PROPERTIES OF DF-PC

The ability of DF to elicit biological effects and keep degenerative maladies at bay has been attributed to unique presence of wide phenolic profile impregnated in it (Chen et al, 2004; Ardiansyah et al, 2006). Kroon and coworkers (1997), on reexamination of literature on DF and cancer protection, suggested that the release of cinnamic phenolics (ferulic acid, p-coumaric acid and ferulic acid dehydrodimers) in colon from fibre matrix is an important pathway to impede cancer initiation and progression. The mechanisms by which they produce a chemopreventive effect for colorectal cancer are generation of an antioxidative environment in the colon, inhibition of DNA oxidative damage and inflammation, induction of apoptosis, and

prevention of polyp formation (Pérez- Jiménez et al, 2009). Further, antioxidant capacity emanated from DF-PC is explained to be more constant, prolonged and associated with higher potential benefit than the abrupt increase and obsolescence of plasma antioxidant concentration after the ingestion of a free phenolic compound-rich food (Vitaglione et al, 2008). A study on rats upon the administration of single dose of wheat bran or an equivalent amount of free ferulic acid demonstrated that plasma ferulic acid from wheat bran remained constant upto 24hr after meal but completely disappeared 4hr after free refulic acid ingestion (Rondini et al, 2004).

Proanthocyanidins (NEPA), despite being scarcely bioavailable, have been considered as vibrant polyphenolics (Santos-Buelga and Scalbert, 2000). This is due to the fact that NEPA are major units of DF, are substantially consumed in the diet (Saura- Calixto and Goni, 2006), and are explained better antioxidants attributed to their structural complexity. Despite its limited absorption, it exerts antioxidant function as free radical scavenger, cardioprotective agent, metal chelator and impediment to carcinogenesis (Beecher, 2004) through its indigestible (that exist in stomach and upper intestine) (Serpen et al, 2007) and non absorbable fractions (that exist in colon) (Saura- Calixto, 2011) respectively. A study designed to observe any effect of grape antioxidant dietary fibre (GADF) on the risk of colon cancer found that the administration of lyophilized red grape pomace containing proanthocyanidin-rich DF on female (strain C57BL/6J) mice showed alterations in the expression of tumour suppressor genes and proto-oncogenes, modulation of genes from various pathways, over expression of xenobiotic detoxifying enzymes and endogenous antioxidant cell defenses. NEPA are generally regarded as anti-nutrients as they reduce feed efficiency, cause weight loss and lower nutrient utilization (Butler and Rogler, 1992). However, anti nutritional effects in these studies may not be totally due to them, but may be due to non-tannin materials that occur exclusively with tannin (Butler, 1989) and thus keeping mere anti-nutrient connotation could be too restrictive for the consideration of wide health effects of NEPA.

Altogether, the main properties derived from the presence of appreciable amounts of DF-associated NEPP reported in animal experiments are (a) enhancement of the excretion of lipids, protein, water, and total fecal output (b) positive effects on lipid metabolism, reduced lipid peroxidation, total cholesterol, LDL-cholesterol, and triglycerides in hypercholesteromic rats (c) increase of antioxidant activity in the large intestine and cecum (e) inhibition of proliferation in the colonic epithelium by reducing the total number of crypts in rats (Saura- Calixto, 2011). These results suggest positive effects on cardiovascular disease prevention and also in gastrointestinal health, including prevention of colon cancer risk.



RECOMMENDED DIETARY INTAKE (RDI) OF DF

Daily intake of fibre should be 20-35g/day for healthy individuals and age plus 5g/day for children (NIN, 2010). In relation to caloric intake, DF intake should be 14g per 1,000 calories (USDA, 2005; Derek and Joanne, 2008). DF intake should be met on holistic approach which calls for inclusion of whole grains, nuts, legumes along with fruits and vegetables (FV) rather advocating increasing the portion sizes of only FV.

Early research indicates that high fibre diet might have inhibitory effect on mineral bioavailability (Reinhold et al, 1976, Spencer et al, 1991). However scientific evidences are not convincing in demonstrating obnoxious effect of high-fibre diet (over 50g/day) on mineral absorption (Gordon et al, 1995). Indeed both soluble and insoluble fibres provide short chain fatty acids which contribute directly to the enhancement of mineral absorption via a cation exchange mechanism (apical presence of Mg²⁺/H²⁺ antiporters) (Trinidad et al, 1993) and indirectly by causing acidification of luminal contents (Heijnen et al, 1993) which augments mineral absorption in large intestine. Furthermore, health risks of high-fibre diet are significantly less deleterious than worrisome low-fibre diet in an era of peaked degenerative maladies.

CONCLUSION

DF is not an isolated compound, rather a blend of chemically heterogeneous compounds. In context of phenolic compounds, DF has been reported to impregnate an appreciable amount of indigested phenolics and therefore help in their release in large intestine by providing fuel/fermentable substrates to colonic microflora culminating in systemic and local antioxidant effects. Both SDF and IDF is documented to bestow antioxidant protection with the SDF being more able to maximize the bioavailabilty of associated polyphenols. Rather being robust prescriptionist for total DF, more emphasis should be placed on specific sources of fiber i.e. whole grains, fruits and vegetables, nuts because of their close structural association with plant phenolics.

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