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Abstract

Natural antioxidants derived from plants become increasingly popular as functional food and feed ingredients. This viewpoint article highlights the emerging antioxidant character of natural non-structural carbohydrates, with focus on those plant-derived compounds that have dual antioxidative and prebiotic properties. In parallel to more indirect action mechanisms, it is proposed here that such compounds are involved in direct ROS scavenging processes in plants, in food and in the gastrointestinal tract, counteracting oxidative stress and preventing pathogen outbreaks and the development of ROS related diseases.

Key words: antioxidant, colon, disease, inulin, oxidative stress, polyphenol, ROS.

Introduction

The use of antioxidants as food supplements has a very long tradition. However, there are great concerns on the safety of so called chemical or artificial antioxidants, similar to the ongoing debate on the use of artificial sweeteners (e.g. aspartame) versus natural sweeteners (Stoyanova *et al.*, 2011). Therefore, natural products derived from plants are becoming increasingly popular. The world-wide problems with obesity and other diseases have opened the eyes of a number of consumers, stimulating them to consume fibre and antioxidant enriched foods. As a result, functional foods are becoming increasingly accepted by the general public, and the growth potentials for functional food markets are still enormous. More than ever, consumers want safe, natural, good looking and tasteful foods that at the same time promote health. Functional foods include for instance probiotics (living bacteria), prebiotics (compounds selectively stimulating “beneficial” intestinal bacteria), and antioxidants (compounds counteracting oxidative stress caused by an imbalance of Reactive Oxygen Species: ROS).

Origin and identity of ROS in living beings

Since the emergence of photosystem II about 2.5×10^9 years ago, water has been used as electron donor for photoautotrophic life. Since then, oxygen levels have been raising in the earth’s atmosphere and aerobic metabolism evolved. As an inevitable consequence, oxygen derived hyperactive ROS (De Gara *et al.*, 2010) are generated in eukaryotic organelles such as mitochondria (respiration), chloroplasts (photosynthesis) and peroxisomes (fatty acid degradation) and in the electron transfer chains operating in prokaryotic membranes. ROS can also be produced extracellularly, by the action of

NADPH oxidases (e.g. in plants, phagocytes and colon epithelial cells) and other types of oxidases. ROS include the hydroxyl radical ($\cdot\text{OH}$), the superoxide radical ($\text{O}_2^{\cdot-}$), the singlet oxygen ($^1\text{O}_2$) and hydrogen peroxide (H_2O_2). Among these, the $\cdot\text{OH}$ is the most reactive (*in vivo* half-life 10^{-9} s) and dangerous species, immediately attacking virtually any molecule in its neighbourhood. By contrast, H_2O_2 is much more stable and it is able to cross membranes. ROS attacks various biomolecules (e.g. DNA, RNA, proteins, lipids, cofactors in enzymes), damaging them and disturbing normal cellular metabolism. Stress (heat, UV, drought and cold treatments on plants) generally increases ROS production. This can potentially be detrimental to the cell, leading to cell death. However, the generated ROS (e.g. H_2O_2) can also be used as signalling molecules for initiating ROS defence mechanisms (see below) to sustain ROS homeostasis. Moreover, ROS play crucial roles in developmental processes in all living organisms (Dowling & Simmons, 2009) and they are also important in plant pathogen responses and immune responses in mammals.

It is believed that organisms age because their cells accumulate ROS-based damage over time (free-radical aging theory; Chong-Han, 2010). Many diseases (such as arteriosclerosis, cancer, type 2 diabetes and neurodegenerative diseases) are believed to be initiated by ROS. Conversely, other diseases (such as HIV infection) result in a disturbance of ROS homeostasis, leading to a continuous oxidative stress in infected cells. Recent plant data strongly suggest that sugars and sugar metabolizing enzymes can regulate ROS formation in mitochondria, contributing to ROS homeostasis (Xiang *et al.*, 2011).

The Fenton reaction catalysed by transition metals such as ferrous ion ($\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{OH}^- + \cdot\text{OH} + \text{Fe}^{3+}$) is a famous chemical reaction resulting in the production of $\cdot\text{OH}$ radicals. In living cells, iron is generally sequestered in redox-inactive complexes to prevent as much as possible the oxidative damage via Fenton chemistry (Prousek, 2007).

Natural ANTioxidants (NANTs)

Identity of NANTs

ROS homeostasis in cells is reached at the balance between ROS production and ROS scavenging by antioxidants. The latter act as electron donors, reducing ROS to less harmful molecules; the oxidized products formed in the process are not very reactive or harmful. Higher eukaryotic organisms use an array of enzymatic and non-enzymatic mechanisms to detoxify ROS (Apel and Hirt, 2004). Non-enzymatic mechanisms involve ascorbate (Vit C), glutathione, tocopherol (Vit E), polyphenols, alkaloids and carotenoids. The enzymes involved include superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX) and peroxiredoxin (PrxR) (Foyer & Noctor, 2009).

In plants, it has become increasingly clear that a group of non digestible carbohydrates with “fibre-like properties” (NDCs: fructans, Raffinose Family Oligosaccharides or RFOs, arabinoxylans, β glucans) and their breakdown products (e.g. fructosyl oligosaccharides or FOS) as well as sugar-sterols and sugar-phenols might act as important ROS scavengers *in planta* (Nishizawa, Yabuta & Shigeoka, 2008), since they show strong antioxidant activities *in vitro* (Stoyanova *et al.*, 2011). The antioxidant capacity of inulin-type fructans can further be increased by grafting extra groups (e.g. phenols) on their structure (Ren *et al.*, 2011). Although the ROS scavenging capacity of natural sugar preparations might in some cases be overestimated by contamination with other strong antioxidants (e.g. polyphenols), synthetically produced, pure carbohydrates were also shown to exert considerable antioxidant activity *in vitro* and they also counteracted lipid peroxidation processes in mice *in vivo* (Li *et al.*, 2007). Strong antioxidant carbohydrates are also isolated from fungi, algae and other organisms (Zhang *et al.*, 2007).

Microorganisms, even strict anaerobic ones, also contain SOD, CAT, APX, GPX glutathione NADH oxidase, glutathione PrxRs and surface associated antioxidants such as carotenoids and others (Ginsburg, Kohen &

Koren, 2011), to protect themselves against ROS from endo- or exogenous origin.

Non Digestible Carbohydrates (NDCs) acting as prebiotics and antioxidants

In this paper, we will limit our discussions to plant-derived NDCs, with special focus on fructans as polyfructose compounds. Prebiotics are a special class of carbohydrates and soluble fibers that are not digested in the upper gastro-intestinal tract. In the colon, they are fermented and they selectively promote the growth of beneficial bacteria supporting overall good health. The criteria for being classified as prebiotics are strictly defined (Gibson *et al.*, 2004). Roberfroid (2007) defined “A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health”. Prebiotics are rapidly rising in popularity in functional food markets, thanks to applications in dairy products, health drinks, nutrition bars, breakfast cereals, beverages, bakery products, meat products, mineral supplements, weight loss products, green foods, infant food and pet food.

About 15% of plants accumulate fructans, including inulins, levans, graminans and neokestose-derived inulins and levans (Van den Ende, De Coninck & Van Laere, 2004). They occur in vegetables such as chicory, onion, asparagus and garlic but also in cereals (De Gara *et al.*, 2003). These compounds have a long tradition as prebiotics. They are not degraded by gastrointestinal enzymes since mammals do not have the appropriate β -fructosidase enzymes to do so. They selectively stimulate the growth of “beneficial” bacteria in the colon, which do contain specific enzymes for their degradation (belonging to glycoside hydrolase family 32, GH32). Inulin (higher DP β -2,1 type fructans) and fructo-oligosaccharides (FOS: lower DP β -2,1 type fructans, also termed oligofructose) are the most widely used prebiotics (Roberfroid *et al.*, 2010) and are commercially produced from chicory roots (*Cichorium intybus* L) or Jerusalem artichoke tubers (*Helianthus tuberosus*) (Van den Ende, De Coninck & Van Laere, 2004). Inulin and FOS consumption leads to increased short-chain fatty acids levels and decreased pHs in the colon (Tarini & Wolever, 2010), an improved mineral uptake (Scholz-Ahrens *et al.*, 2007), a reduction in serum triacylglycerol levels (Brighenti, 2007; Tarini & Wolever 2010), a stimulation of Bifidobacteria and Lactobacillae (De Preter *et*

al., 2007) and an overall stimulation of the immune system (Lomax & Calder, 2009). Inulin and FOS supplements have also been suggested to help in preventing colon cancer (Sauer, Richter & Pool-Zobel, 2007). Most of these points are further discussed below.

RFOs (also termed galacto-oligosaccharides or GOS) such as the trisaccharide raffinose and the tetrasaccharide stachyose are common reserves in higher plants and also known to have prebiotic properties. Similar benefits for health and well-being are reported for this class of carbohydrates (Roberfroid *et al.*, 2010). Raffinose is a moderate ROS scavenger *in vitro* (Stoyanova *et al.*, 2011).

Cereal arabinoxylans (AX) are plant cell wall derived molecules constituting part of the dietary fiber intake. Hydrolysis of AX leads to arabinoxylan-oligosaccharides (AXOS), consisting of arabinoxyloligosaccharides and xylooligosaccharides (XOS) (Broekaert *et al.*, 2011). AX hydrolysis occurs in cereal-derived food products such as bread and beer, but also in the colon. Substantial evidence is accumulating that also these oligosaccharides should be considered as true prebiotics. The antioxidant capacity of AX and AXOS appears to be correlated with their ferulic acid content (Broekaert *et al.*, 2007).

Overall, a systematic comparison of the prebiotic and antioxidative properties of inulin, levan, FOS, graminan, RFOs, AX, AXOS and XOS is now lacking. A plethora of different methods and approaches are now used for studying subsets of these compounds. Future work will have to deal with standardizing methods and comparing the effects of these compounds and their mixtures in well-designed experiments, both on animals and humans. Most probably, synergistic effects can be expected by using an optimal cocktail of prebiotics together with non-prebiotic soluble fibers and non-prebiotic antioxidants (e.g. polyphenols).

Polyphenols

A huge variety of secondary products can accumulate in plants. Polyphenols (polymeric phenols) form an important group with more than 10000 individual compounds currently characterized from plants (Quideau *et al.*, 2011). Anthocyanins are the most studied polyphenols (Shipp & Abdel-Aal, 2010). Many polyphenols are strong radical scavengers with health promoting effects. In foods, they are widely used as colorants, derived from grapes, red cabbage, radishes, elderberries, chokeberries, blackberries, black raspberries, and black currants. Depending on the specific polyphenol under consideration, partial breakdown may or may not occur by the gut microbial population. In human studies, it was found that only a very low percentage of polyphenols can enter the bloodstream (D'Archivio *et al.*, 2010). Feed and food supplementation studies of both NDCs and polyphenols showed increasing antioxidant capacities in the blood and in several organs. The extremely low levels of NDCs and low levels of polyphenols in the blood suggest that these compounds might not act directly as ROS scavengers but indirectly as signals initiating endogenous antioxidant systems.

NANTs as ROS scavengers

In plants

For decennia, the “classic” antioxidant mechanisms have mostly been studied in the cytosolic, mitochondrial and chloroplastic cellular environments (De Gara *et al.*, 2010). However, recent developments point at the existence of vacuolar antioxidant mechanisms in plants exposed to stress (Van den Ende & Valluru, 2009). Therefore, it was proposed that vacuolar compounds, such as fructans and polyphenols, might be effective candidates for capturing ROS in tissues exposed to a wide range of environmental stresses. Fructans are known to protrude deep between the head-groups of membranes. Under stress, excess cytoplasmic H_2O_2 can easily diffuse through the tonoplast where class III peroxidases might be involved in the production of more toxic ROS in the vicinity of the tonoplast. Recent models now include that both sugars and

polyphenols might act synergistically to detoxify these ROS, avoiding membrane leakage, and preserving vacuolar and cellular integrity under stress (Bolouri *et al.*, 2010).

In food

When natural products are harvested (e.g. fruit), extracted (e.g. juice production) or killed and processed (e.g. animals for meat production), wounding, loss of cellular integrity and increased oxygen levels and release of divalent cations can greatly contribute to ROS production (Fenton reactions, see above), irreversibly leading to quality losses (discoloration, lipid oxidation and vitamin losses). Oxidative processes can be particularly problematic in food products, unless they are produced and stored under low oxygen. NANTs such as polyphenols and inulin are increasingly used as food additives to increase food stability (Stoyanova *et al.*, 2011).

In the stomach

Kanner & Lapidot (2001) suggested that human gastric fluid may be an excellent medium for enhancing the oxidation of lipids and other dietary constituents. The acid environment of the stomach (pH 1.5-2) can lead to a partial hydrolysis of inulin and oligofructose. Thus, dietary antioxidants (among which inulin, steviol glycosides and polyphenols) could be involved in preventing lipid peroxidation in the stomach. Such reactions might be important to explain the strikingly low incidences of coronary heart diseases in France, despite intake of a high-fat diet (the “French paradox”: Renaud & Delorgeril, 1992; Corder *et al.*, 2006).

Prebiotic effects and immuno-modulatory roles

Inulin, FOS, GOS and AXOS are well-known prebiotics for healthy human subjects (considered as completely safe with minimal intestinal discomfort when supplemented at 10 g/day). The selective stimulation of beneficial bacteria in the colon has been repeatedly confirmed. These bacteria secrete extracellular β -fructosidases. The unique 3-D structure of the β -fructosidase of *Bifidobacterium longum*, the main inhabitants of the large intestine, has recently been unravelled (Bujacz *et al.*, 2011). Also *Lactobacillus* species are widely considered to exert a number of beneficial effects on human health. The probiotic role of several lactobacilli has been attributed to the synthesis of prebiotic fructans by fructosyltransferases (ftfs) of family GH68 (Cerning, 1990). Colonisation of the mouse gut by the wild-type strain of *Lactobacillus reuteri* increased the proportions of regulatory T cells in the spleen, whereas colonisation by the ftf mutant did not (Sims *et al.*, 2011). Overall, host immune reactivity responses might include the activation of macrophages, the inhibition of NF- κ B and the modulation of apoptosis (Rook & Brunet, 2005).

Next to probiotics (Veiga *et al.*, 2010), prebiotics (Hoentjen *et al.*, 2005) and polyphenols (Percival, 2009) are known to exert immunomodulating effects and to stimulate anti-inflammation responses. Early feeding of prebiotic oligosaccharides to babies can mimic some of the benefits inherent to breastfeeding (Arslanoglu, Moro & Boehm, 2007). The activation of “healthy” bacteria in the gut might actively contribute to a “healthy” immune system. Thus, this leads to the point of view that the mammalian immune system, specifically designed to control microorganisms, is in fact by itself controlled by (some beneficial) microorganisms (Round & Mazmanian, 2009). It was proposed that (at least part of the) immunomodulating effects are mediated by short chain fatty acids (SCFAs), the fermentation products of these prebiotics (Watzl, Girrback & Roller, 2005). SCFAs are also known to induce glutathione S-transferases (GSTs), important enzymes to counteract oxidative stress (Glei *et al.*, 2006). An increasing amount of data point to a combined antioxidant and immunomodulatory effect for prebiotics, suggesting that the underlying

mechanisms might be identical. The finding that both AXOS and inulin-type fructans can act as antioxidants themselves (Broekaert *et al.*, 2011; Stoyanova *et al.*, 2011), raises the question whether they could act directly as ROS scavengers (instead of indirectly through SCFAs and GSTs). For inulins it is well-known that they can be taken up by pinocytosis in liver cells (Scharschmidt *et al.*, 1986) and colon cells (Chambers & Serafini, 1985; Peng *et al.*, 2007). Similar to soluble fructans, also soluble glucans can be taken up efficiently by gut epithelial cells (Rice *et al.*, 2005). Since absorption of prebiotics in the gastro-intestinal tract is widely neglected, more specific studies on the uptake of inulin and other prebiotics in gut epithelial cells are needed. More in particular it would be interesting to compare (i) the uptake velocities of SCFAs, inulins and other prebiotics and (ii) compare how they affect GSTs or other factors involved in antioxidant defence. Intriguingly, both inulin and anthocyanins increases the cellular uptake of glucose (Yun *et al.*, 2009; Faria *et al.*, 2009). The process seems to be associated with the AMP-activated protein kinase (AMPK) and phosphatidylinositol 3-kinase (PI3-K) signalling pathways (Yun *et al.*, 2009). The same AMPK signalling pathway is involved in the development and maintenance of the intestinal barrier with butyrate as emerging regulator (Peng *et al.*, 2009). Conclusively, NANTs and their breakdown/fermentation products may not only stimulate beneficial bacteria in the colon, but they can also directly interfere with cellular metabolism and absorption processes in the colon, perhaps driven by (partial) uptake in epithelial cells. Some polyphenols were effective inhibitors of *Salmonella enterica* serotype Typhimurium (*S. Typhimurium*) adherence to Caco-2 enterocytes while they enhanced the adherence of the probiotic *Lactobacillus rhamnosus* (Parkar *et al.*, 2008). It was found that polyphenols can tightly bind to some bacteria (Koren *et al.*, 2009). So far, it is not known if there is a difference in binding properties among gut bacteria. But, when polyphenols

would preferentially bind to “beneficial” colon bacteria, such bacteria would be better protected against ROS. This new line of thinking requires further research, but such hypothesis would explain the observed synergistic effects of FOS and polyphenols on the oxidative stress related parameters in rats (Juskiewicz *et al.*, 2011). Clearly, a multidisciplinary approach is required to understand the molecular mechanisms underlying health beneficial effects arising from the interaction of microbes, diets and the human body.

ROS and pathogens: the case of *Salmonella*

The perhaps most common and widely distributed food-borne diseases are caused by *Salmonella* infections. Babies, young children, older people and adults with a weakened immune system (e.g. HIV) are vulnerable to these infections causing severe diarrhoea. Next to their capacity for extracellular survival, Salmonellae are facultative intracellular bacteria and their ability to survive within cells is essential for their virulence. Surprisingly, a recent finding highlights the importance of gastro-intestinal ROS, generated during inflammatory responses (see higher), to promote the growth of pathogenic bacteria such as *Salmonella* (Winter *et al.*, 2010). According to this study, *Salmonella* uses ROS to produce the unique electron acceptor tetrathionate from thiosulphate, allowing the pathogen to generate ATP more efficiently than its competitors. It is widely accepted that *Salmonella* infections occur mainly in the small intestine, although some colon infections are also possible. What could be the effect of dietary prebiotics on the risk of a *Salmonella* outbreak? As well-known stimulators of “beneficial” bacteria, prebiotics are widely believed to decrease the risk on outbreaks of pathogens. GOS type of prebiotics may counteract infections by pathogens due to their competition for pathogen binding sites that coat the surface of gastrointestinal epithelial cells (Shoaf *et al.*, 2006; Searle *et al.*, 2010). Inulin has been found to reduce the mortality of mice challenged with *S. Typhimurium* (Buddington, Donahoo & Buddington, 2002). Also FOS and AXOS were reported to counteract *Salmonella* infections in poultry (Donalson *et al.*, 2008; Eeckhaut *et al.*, 2008). As explained above, these prebiotic compounds might be taken up in gut epithelial cells where they might act as powerful ROS scavengers (Stoyanova *et al.*, 2011), preventing the

formation of tetrathionate from thiosulphate, arresting the growth of *Salmonella*. Similarly, prebiotics might also scavenge extracellular ROS produced by oxidases in the gut epithelial membranes. Consistently, FOS supplementation led to a more than 100-fold reduction in *S. Enteritidis* counts in rat faeces (Bovee-Oudenhoven *et al.*, 2003).

Prevention of (ROS-related) diseases

Colon diseases: IBD and colon cancer

A human being can in fact be considered as a ‘symbiosis’ between 1 eukaryotic organism and billions of prokaryotes. These are in a delicate balance that can easily be disturbed by suboptimal (Western) diets. In inflammatory bowel diseases (IBD: Crohn’s disease and ulcerative colitis), the interactions between the gut microbiota and the host’s immune system are dysregulated (Leenen & Dieleman, 2007). Metagenomic and other analyses confirmed that faecal microbiota of IBD patients are indeed different, suggesting that microbial communities can be restructured, for instance with the help of functional food or pharmacological agents, in order to prevent and treat disease. IBD patients are at increased risk to develop colon cancer. Policymakers worldwide support a shift to a preventive strategy, termed chemoprevention, in order to decrease the risks of cancer development.

An inverse relationship has been reported between colon cancer and prebiotic dietary fiber intake (Pool-Zobel, 2005). Cancer is a ROS based disease, the damaged biomolecules (e.g. mutated DNA) leading to a malignant transformation. As already described above, prebiotics and their derived SCFAs (mediated by GSTs) counteract oxidative stress, protecting the cells from the genotoxic activity of ROS. Intriguingly, colon cancer cells take up inulin at a

much higher rate than normal cancer cells (Chambers & Serafini, 1985), similar to the typically increased glucose uptake rates observed in all tumor cells (El Mjiyad *et al.*, 2010). Since inulin cannot be metabolized by these cells, we speculate here that inulin accumulation might slow down tumor proliferation and even induce apoptosis. Apoptosis and reduced fermentation have been observed *in vitro*, when human colon tumor cells at different stages of carcinogenesis were challenged with a mixture of inulin fermentation products (Munjal *et al.*, 2009). In line with previous paragraphs, fungal carbohydrates and phenolic antioxidants are also emerging as promising food additives in chemoprevention (Zhang *et al.*, 2007; McDougall *et al.*, 2008).

Metabolic syndrome and diabetes

Metabolic syndrome is a collection of common pathologies, including central obesity, glucose intolerance, impaired fasting glycemia, insulin resistance, hyperinsulinemia, dyslipidemia and hypertension. Sedentary life-style and abundant intake of fat and refined sugars together with a susceptible genetic background are believed to play a role in the development of metabolic syndrome. Subjects with metabolic syndrome have an increased risk on type 2 diabetes (Gobinath *et al.*, 2010) and cardiovascular diseases (see below). This is associated with altered gut microbiota, endothelial dysfunction and oxidative stress (Puchau *et al.*, 2010). Therefore, intake of prebiotic oligosaccharides could prevent metabolic syndrome. Moreover, diabetic Wistar rats showed a reduction of diabetic complications when adding FOS and AXOS to their diets (Gobinath *et al.*, 2010). In rats, a fructose-rich diet promotes the metabolic syndrome. In severe contrast, supplementation with inulin-type fructans is efficient against fructose-induced hypertension (Rault-Nania *et al.*, 2008). Dietary supplementation of FOS and inulin were reported to counteract appetite in human subjects (Cani *et al.*, 2009). Moreover, oligofructose supplementation promoted weight loss and improved glucose responses in overweight adults (Parnell & Reimer, 2009).

Cardiovascular diseases

Increased levels of circulating free fatty acids (FFAs) and cholesterol can lead to atherosclerosis. Evidence is accumulating that intake of oligofructose and inulins have beneficial effects on blood lipid changes both in animals and in humans (Brigenti, 2007). Studies on apolipoprotein E-deficient mice showed that inulin and inulin+oligofructose supplementation resulted in significantly lowered hepatic cholesterol concentrations and an inhibition of atherosclerotic plaque formation, probably linked to an altered lipid metabolism (Rault-Nania *et al.*, 2008). Also in humans, the effect of inulin on postprandial glucose, insulin, SCFA, FFA, and gut hormone responses was recently investigated (Tarini & Wolever, 2010). Glucose and insulin responses did not differ from the control diet but serum acetate, propionate, and butyrate were significantly higher and FFAs were significantly lower. Moreover, inulin significantly increased plasma glucagon-like peptide-1 concentrations and reduced ghrelin concentrations. Results are consistent with the hypothesis that dietary fiber increases the production of colonic SCFAs, which may reduce type 2 diabetes risk by reducing postprandial FFAs and favorably affecting gut hormones that regulate food intake.

Perspectives: the challenges of a personalized food (feed) and medicine

The effect of NDCs are species-specific and may vary greatly among individuals

It should be noted that some of the above-mentioned health-promoting, prebiotic effects in humans and animals such as rats, mice, chickens, cats, dogs and pigs cannot be extrapolated to other species. For instance, grazers such as cattle and horses react in a completely different way on higher doses of

prebiotic fructans. For feeding cattle, breeding programs focus on developing grasses accumulating increasing amounts of water-soluble carbohydrates such as fructans, in order to increase milk and meat production (Miller *et al.*, 2001). By contrast, short chain fructans (oligofructose, FOS) are detrimental for horses and ponies (van Epps & Pollitt, 2006), which are sensitive to very low doses. The microbial population and digestive system is completely different between horse and cattle, and apparently the equine microbiota changes in a way that is not beneficial for these animals. Oligofructose is now widely accepted as one of most dangerous triggers for pasture-induced laminitis, a common painful and exhausting condition resulting from cellular damage and inflammation within the hoof (van Epps & Pollitt, 2006).

Since each individual has a unique genetic background, and a unique population of gut microbes (Turnbaugh *et al.*, 2009), prebiotic effects are well-known to vary significantly from one individual to another (Roberfroid & Delzenne, 1998). Therefore, each individual will have its own “optimal dose” with maximal health benefits and minimal intestinal discomfort (flatulence, diarrhoea). As the age of personal genomes sequencing approaches, some aspects of diet and medical therapies will be customized based on genotype. Diet can also be personalized to optimize microbiota function and interaction with the host based on the metagenomic analysis of an individual’s microbiota (Sonnenburg *et al.*, 2010).

Manipulation of the microbial population by modifying the DP and branching of NDC: the case of fructans

Intriguingly, it was recently demonstrated that a dietary shift from inulin (β -2,1 linkages) to levan-type fructans (β -2,6 linkages) resulted in a shift in the *Bacteroides* subpopulation (Sonnenburg *et al.*, 2010). Structural and genetic analyses within *Bacteroides* species disclosed a fructose binding, hybrid two-component signalling sensor that controls the fructan utilization locus. At this locus, *Bacteroides thetaiotaomicron* contains a unique GH32 levanase while all the other *Bacteroides* species contain a GH32 inulinase or a mixed type GH32 β -fructosidase. Unravelling the functionality of GH32 genes by heterologous expression is a time-consuming task. It would be extremely helpful to accurately predict the GH32 enzyme functionality for new GH32 genes derived

from metagenomic sequencing data (Qi *et al.*, 2010; see also below). The GH32 family harbours an array of distinct enzyme functionalities varying in their preference for linkage type and DP and in their ability to handle branching points (Van den Ende *et al.*, 2009). It can be expected that higher DP and branched type fructans (e.g. graminans derived from cereals) are processed more slowly throughout the colon by bacterial β -fructosidases (Van den Ende *et al.*, 2003). Therefore, mixtures of linear and branched type fructans with varying DP might not only “protect” the proximal but also the distal part of the colon. This urges further research on the prebiotic properties of branched-type fructosyl oligo- and polysaccharides as compared to the linear, inulin-type fructosyl oligo- and polysaccharides. The first *in vitro* results with cereal fructans are very promising (Jenkins *et al.*, 2011).

Rational enzyme design: production of tailor-made fructans

Within GH32 and the related family GH68 enzymes, information on 3D structure has become increasingly available in the last few years. This boosted structure-function research in the area. The amino acids specifically involved in donor substrate specificity (sucrose versus fructan as donor) and acceptor substrate specificity (sucrose or fructan as acceptor versus water as acceptor) have been unravelled (Le Roy *et al.*, 2007; Schroeve *et al.*, 2008). Intriguingly, in all cases only a few amino acids in the vicinity of the active site appear to control substrate specificity. Using simple rules, we are now close to predicting the functionality of new plant GH32 members (Van den Ende *et al.*, 2009). Pending some more data on structure-function relationships in microbial GH32 members, these models can hopefully be extended to the whole GH32 family in the near future. However, major challenges remain, such as understanding which residues are critical to explain the formation and

degradation of β -2,1 versus β -2,6 linkages in fructans and understanding better why some sugars act as inhibitors rather as substrates. Finally, such additional insights could lead to a true rational enzyme design for creating novel fructosyltransferases synthesizing a set of tailor-made fructans with superior prebiotic properties. In the future, such an array of tailor-made fructans may be applied to individuals with a specific (disease susceptible) genotype, in order to program their microbiome to optimize health benefits and prevent development of diseases. Alternatively, it would also allow rationalized re-programming of the microbiome after disease or treatment with antibiotics, in order to restore the most optimal host-microbial superorganism.

Concluding remarks

NANTs including (prebiotic) NDCs from plants are emerging as powerful, but rather cheap; health promoting food and feed supplements. They might be taken up (at least partly) in gut epithelial cells and might counteract oxidative stress via direct (ROS scavenging) and more indirect (e.g. SCFA, GST) mechanisms, preventing ROS-related diseases such as colon cancer and other diseases. They stimulate the immune system and might decrease the risks on pathogen outbreaks (e.g. *Salmonella*) by scavenging ROS. However, more fundamental research is needed to fully understand their precise antioxidative action mechanisms in plants and in the gastrointestinal tract of animals and humans. Production of different kinds of NANTs could be optimized in superior transgenic plants or by creating superior enzymes in bioreactors. By varying NANT ratios in individual diets, one can specifically manipulate the microbiome of an individual with a specific genotype. This will pave the way to a personalized and preventive medicine.

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References

Apel, K., & Hirt, H. (2004). Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology*, 55, 373-399.

Arslanoglu, S., Moro, G. E., & Boehm, G. (2007). Early supplementation of prebiotic oligosaccharides protects formula-fed infants against infections during the first 6 months of life. *Journal of Nutrition*, 137, 2420-2424.

Bolouri-Moghaddam, M. R., Le Roy, K., Xiang, L., Rolland, F., & Van den Ende, W. (2010). Sugar signalling and antioxidant network connections in plant cells. *Febs Journal*, 277, 2022-2037.

Bovee-Oudenhoven, I. M. J., ten Bruggencate, S. J. M., Lettink-Wissink, M. L. G., & van der Meer, R. (2003). Dietary fructo-oligosaccharides and lactulose inhibit intestinal colonisation but stimulate translocation of salmonella in rats. *Gut*, 52, 1572-1578.

Brighenti, F. (2007). Dietary Fructans and serum triacylglycerols: A meta-analysis of randomized controlled trials. *Journal of Nutrition*, 137, 2552S-2556S.

Broekaert, W. F., Courtin, C. M., Verbeke, K., Van de Wiele, T., Verstraete, W., & Delcour, J. A. (2011). Prebiotic and other health-related effects of cereal-derived arabinoxylans, arabinoxylan-oligosaccharides, and xylooligosaccharides. *Critical Reviews in Food Science and Nutrition*, 51, 178-194.

Buddington, K. K., Donahoo, J. B., & Buddington, R. K. (2002). Dietary oligofructose and inulin protect mice from enteric and systemic pathogens and tumor inducers. *Journal of Nutrition*, 132, 472-477.

Bujacz, A., Jedrzejczak-Krzepkowska, M., Bielecki, S., Redzynia, I., & Bujacz, G. (2011). Crystal structures of the apo form of β -fructofuranosidase from

Bifidobacterium longum and its complex with fructose. *Febs Journal*, 278, 1728-1744.

Cani, P. D., Lecourt, E., Dewulf, E. M., Sohet, F. M., Pachikian, B. D., Naslain, D., et al. (2009). Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. *American Journal of Clinical Nutrition*, 90, 1236-1243.

Cerning, J. (1990). Exocellular polysaccharides produced by lactic-acid bacteria. *Fems Microbiology Reviews*, 87, 113-130.

Chambers, T. J., & Serafini, E. P. (1985). The permeability of normal, adenomatous, ulcerative colitic and malignant large bowel epithelial-cell membranes to inulin. *British Journal of Experimental Pathology*, 66, 309-315.

Chong-Han, K. (2010). Dietary lipophilic antioxidants: implications and significance in the aging process. *Critical Reviews in Food Science and Nutrition*, 50, 931-937.

Corder, R., Mullen, W., Khan, N. Q., Marks, S. C., Wood, E. G., Carrier, M. J., et al. (2006). Red wine procyanidins and vascular health. *Nature*, 444, 566-566.

D'Archivio, M., Filesi, C., Vari, R., Scazzocchio, B., & Masella, R. (2010). Bioavailability of the polyphenols: status and controversies. *International Journal of Molecular Sciences*, 11, 1321-1342.

De Gara, L., de Pinto, M. C., Moliterni, V. M. C., & D'Egidio, M. G. (2003). Redox regulation and storage processes during maturation in kernels of *Triticum durum*. *Journal of Experimental Botany*, 54, 249-258.

De Gara, L., Locato, V., Dipierro, S., & de Pinto, M. C. (2010). Redox homeostasis in plants. The challenge of living with endogenous oxygen production. *Respiratory Physiology & Neurobiology*, 173, S13-S19.

De Preter, V., Vanhoutte, T., Huys, G., Swings, J., De Vuyst, L., Rutgeerts, P., et al. (2007). Effects of *Lactobacillus casei* Shirota, *Bifidobacterium breve*, and oligofructose-enriched inulin on colonic nitrogen-protein metabolism in healthy humans. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 292, G358-G368.

Donalson, L. M., McReynolds, J. L., Kim, W. K., Chalova, V. I., Woodward, C. L., Kubena, L. F., et al. (2008). The influence of a fructooligosaccharide prebiotic combined with alfalfa molt diets on the gastrointestinal tract

fermentation, Salmonella Enteritidis infection, and intestinal shedding in laying hens. *Poultry Science*, 87, 1253-1262.

Dowling, D. K., & Simmons, L. W. (2009). Reactive oxygen species as universal constraints in life-history evolution. *Proceedings of the Royal Society B-Biological Sciences*, 276, 1737-1745.

Eeckhaut, V., Van Immerseel, F., Dewulf, J., Pasmans, F., Haesebrouck, F., Ducatelle, R., et al. (2008). Arabinoxyloligosaccharides from wheat bran inhibit Salmonella colonization in broiler chickens. *Poultry Science*, 87, 2329-2334.

El Mjiyad, N., Caro-Maldonado, A., Ramirez-Peinado, S., & Munoz-Pinedo, C. (2011). Sugar-free approaches to cancer cell killing. *Oncogene*, 30, 253-264.

Faria, A., Pestana, D., Azevedo, J., Martel, F., de Freitas, V., Azevedo, I., et al. (2009). Absorption of anthocyanins through intestinal epithelial cells - Putative involvement of GLUT2. *Molecular Nutrition & Food Research*, 53, 1430-1437.

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A., & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews*, 17, 259-275.

Ginsburg, I., Kohen, R., & Koren, E. (2011). Microbial and host cells acquire enhanced oxidant-scavenging abilities by binding polyphenols. *Archives of Biochemistry and Biophysics*, 506, 12-23.

Glei, M., Hofmann, T., Kuster, K., Hollmann, J., Lindhauer, M. G., & Pool-Zobel, B. L. (2006). Both wheat (*Triticum aestivum*) bran arabinoxylans and gut flora-mediated fermentation products protect human colon cells from genotoxic activities of 4-hydroxynonenal and hydrogen peroxide. *Journal of Agricultural and Food Chemistry*, 54, 2088-2095.

Gobinath, D., Madhu, A. N., Prashant, G., Srinivasan, K., & Prapulla, S. G. (2010). Beneficial effect of xylo-oligosaccharides and fructo-oligosaccharides in streptozotocin-induced diabetic rats. *British Journal of Nutrition*, 104, 40-47.

- Hoentjen, F., Welling, G. W., Harmsen, H. J. M., Zhang, X. Y., Snart, J., Tannock, G. W., et al. (2005). Reduction of colitis by prebiotics in HLA-1327 transgenic rats is associated with microflora changes and immunomodulation. *Inflammatory Bowel Diseases*, 11, 977-985.
- Jenkins, C. L. D., Lewis, D., Bushell, R., Belobrajdic, D. P., & Bird, A. R. (2011). Chain length of cereal fructans isolated from wheat stem and barley grain modulates *in vitro* fermentation. *Journal of Cereal Science*, 53, 188-191.
- Juskiewicz, J., Zary-Sikorska, E., Zdunczyk, Z., Krol, B., & Jurgonski, A. (2011). Physiological effects of chicory root preparations with various levels of fructan and polyphenolic fractions in diets for rats. *Archives of Animal Nutrition*, 65, 74-87.
- Kanner, J., & Lapidot, T. (2001). The stomach as a bioreactor: Dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants. *Free Radical Biology and Medicine*, 31, 1388-1395.
- Koren, E., Kohen, R., Ovadia, H., & Ginsburg, I. (2009). Bacteria coated by polyphenols acquire potent oxidant-scavenging capacities. *Experimental Biology and Medicine*, 234, 940-951.
- Le Roy, K., Lammens, W., Verhaest, M., De Coninck, B., Rabijns, A., Van Laere, A., et al. (2007). Unraveling the difference between invertases and fructan exohydrolases: A single amino acid (Asp-239) substitution transforms Arabidopsis cell wall invertase1 into a fructan 1-exohydrolase. *Plant Physiology*, 145, 616-625.
- Leenen, C. H. M., & Dieleman, L. A. (2007). Inulin and oligofructose in chronic inflammatory bowel disease. *Journal of Nutrition*, 137, 2572S-2575S.
- Li, X. M., Shi, Y. H., Wang, F., Wang, H. S., & Le, G. W. (2007). *In vitro* free radical scavenging activities and effect of synthetic oligosaccharides on antioxidant enzymes and lipid peroxidation in aged mice. *Journal of Pharmaceutical and Biomedical Analysis*, 43, 364-370.
- Lomax, A. R., & Calder, P. C. (2009). Prebiotics, immune function, infection and inflammation: a review of the evidence. *British Journal of Nutrition*, 101, 633-658.
- McDougall, G. J., Ross, H. A., Ikeji, M., & Stewart, D. (2008). Berry extracts exert different antiproliferative effects against cervical and colon cancer cells grown *in vitro*. *Journal of Agricultural and Food Chemistry*, 56, 3016-3023.

Miller, L. A., Moorby, J. M., Davies, D. R., Humphreys, M. O., Scollan, N. D., MacRae, J. C., et al. (2001). Increased concentration of water-soluble carbohydrate in perennial ryegrass (*Lolium perenne* L.): milk production from late-lactation dairy cows. *Grass and Forage Science*, 56, 383-394.

Foyer, C. H., & Noctor, G. (2009). Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. *Antioxidants & Redox Signaling*, 11, 861-905.

Munjal, U., Glei, M., Pool-Zobel, B. L., & Scharlau, D. (2009). Fermentation products of inulin-type fructans reduce proliferation and induce apoptosis in human colon tumour cells of different stages of carcinogenesis. *British Journal of Nutrition*, 102, 663-671.

Nishizawa, A., Yabuta, Y., & Shigeoka, S. (2008). Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. *Plant Physiology*, 147, 1251-1263.

Parkar, S. G., Stevenson, D. E., & Skinner, M. A. (2008). The potential influence of fruit polyphenols on colonic microflora and human gut health. *International Journal of Food Microbiology*, 124, 295-298.

Parnell, J. A., & Reimer, R. A. (2009). Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. *American Journal of Clinical Nutrition*, 89, 1751-1759.

Peng, L. Y., He, Z. J., Chen, W., Holzman, I. R., & Lin, J. (2007). Effects of butyrate on intestinal barrier function in a Caco-2 cell monolayer model of intestinal barrier. *Pediatric Research*, 61, 37-41.

Percival, S. S. (2009). Grape consumption supports immunity in animals and humans. *Journal of Nutrition*, 139, 1801S-1805S.

Pool-Zobel, B. L. (2005). Inulin-type fructans and reduction in colon cancer risk: review of experimental and human data. *British Journal of Nutrition*, 93,

S73-S90.

Prousek, J. (2007). Fenton chemistry in biology and medicine. *Pure and Applied Chemistry*, 79, 2325-2338.

Puchau, B., Zulet, M. A., de Echavarri, A. G., Hermisdorff, H. H. M., & Martinez, J. A. (2010). Dietary total antioxidant capacity is negatively associated with some metabolic syndrome features in healthy young adults. *Nutrition*, 26, 534-541.

Qin, J. J., Li, R. Q., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., et al. (2010). A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, 464, 59-U70.

Quideau, S., Deffieux, D., Douat-Casassus, C., & Pouysegou, L. (2011). Plant polyphenols: chemical properties, biological activities, and synthesis. *Angewandte Chemie-International Edition*, 50, 586-621.

Rault-Nania, M. H., Demougeot, C., Gueux, E., Berthelot, A., Dzimira, S., Rayssiguier, Y., et al. (2008). Inulin supplementation prevents high fructose diet-induced hypertension in rats. *Clinical Nutrition*, 27, 276-282.

Ren, J. M., Liu, J. L., Dong, F., & Guo, Z. Y. (2011). Synthesis and hydroxyl radicals scavenging activity of N-(aminoethyl)inulin. *Carbohydrate Polymers*, 85, 268-271.

Renaud, S., & Delorgeril, M. (1992). Wine, alcohol, platelets and the French paradox for coronary heart disease. *Lancet*, 339, 1523-1526.

Rice, P. J., Adams, E. L., Ozment-Skelton, T., Gonzalez, A. J., Goldman, M. P., Lockhart, B. E., et al. (2005). Oral delivery and gastrointestinal absorption of soluble glucans stimulate increased resistance to infectious challenge. *Journal of Pharmacology and Experimental Therapeutics*, 314, 1079-1086.

Roberfroid, M. (2007). Prebiotics: The concept revisited. *Journal of Nutrition*, 137, 830S-837S.

Roberfroid, M. B., & Delzenne, N. M. (1998). Dietary fructans. *Annual Review of Nutrition*, 18, 117-143.

Roberfroid, M., Gibson, G. R., Hoyles, L., McCartney, A. L., Rastall, R., Rowland, I., et al. (2010). Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition*, 104, S1-S63.

Rook, G. A. W., & Brunet, L. R. (2005). Microbes, immunoregulation, and the gut. *Gut*, 54, 317-320.

Round, J. L., & Mazmanian, S. K. (2009). The gut microbiota shapes intestinal

immune responses during health and disease. *Nature Reviews Immunology*, 9, 313-323.

Sauer, J., Richter, K. K., & Pool-Zobel, B. L. (2007). Physiological concentrations of butyrate favorably modulate genes of oxidative and metabolic stress in primary human colon cells. *Journal of Nutritional Biochemistry*, 18, 736-745.

Scharschmidt, B. F., Lake, J. R., Renner, E. L., Licko, V., & Vandyke, R. W. (1986). Fluid phase endocytosis by cultured rat hepatocytes and perfused-rat-liver-implications plasma-membrane turnover and vesicular trafficking of fluid phase markers. *Proceedings of the National Academy of Sciences of the United States of America*, 83, 9488-9492.

Scholz-Ahrens, K. E., Ade, P., Marten, B., Weber, P., Timm, W., Asil, Y., et al. (2007). Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. *Journal of Nutrition*, 137, 838S-846S.

Schroeven, L., Lammens, W., Van Laere, A., & Van den Ende, W. (2008). Transforming wheat vacuolar invertase into a high affinity sucrose:sucrose 1-fructosyltransferase. *New Phytologist*, 180, 822-831.

Searle, L. E. J., Cooley, W. A., Jones, G., Nunez, A., Crudgington, B., Weyer, U., et al. (2010). Purified galactooligosaccharide, derived from a mixture produced by the enzymic activity of *Bifidobacterium bifidum*, reduces *Salmonella enterica* serovar Typhimurium adhesion and invasion *in vitro* and *in vivo*. *Journal of Medical Microbiology*, 59, 1428-1439.

Shipp, J. & Abdel-Aal, E-S. M. (2010). Food applications and physiological effects of anthocyanins as functional food ingredients. *The Open Food Science Journal*, 4, 7-22.

Shoaf, K., Mulvey, G. L., Armstrong, G. D., & Hutkins, R. W. (2006). Prebiotic galactooligosaccharides reduce adherence of enteropathogenic *Escherichia coli* to tissue culture cells. *Infection and Immunity*, 74, 6920-6928.

Sims, I.M., Frese, S.A., Walter, J., Loach, D., Wilson, M., Appleyard, K. et al. (2011). Structure and functions of exopolysaccharide produced by gut commensal *Lactobacillus reuteri* 100-23. *ISME J.* doi:10.1038/ismej.2010.201.

Sonnenburg, E. D., Zheng, H. J., Joglekar, P., Higginbottom, S. K., Firbank, S. J., Bolam, D. N., et al. (2010). Specificity of polysaccharide use in intestinal *Bacteroides* species determines diet-induced microbiota alterations. *Cell*, *141*, 1241-U1256.

Stoyanova, S., Geuns, J., Hideg, E., & Van den Ende, W. (2011). The food additives inulin and stevioside counteract oxidative stress. *International Journal of Food Sciences and Nutrition*, *62*, 207-214.

Tarini, J., & Wolever, T. M. S. (2010). The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. *Applied Physiology Nutrition and Metabolism-Physiologie Appliquee Nutrition Et Metabolisme*, *35*, 9-16.

Turnbaugh, P. J., Ridaura, V. K., Faith, J. J., Rey, F. E., Knight, R., & Gordon, J. I. (2009). The effect of diet on the human gut microbiome: A metagenomic analysis in humanized gnotobiotic mice. *Science Translational Medicine*, *1*(6).

Van den Ende, W., Clerens, S., Vergauwen, R., Van Riet, L., Van Laere, A., Yoshida, M., et al. (2003). Fructan 1-exohydrolases. beta-(2,1)-trimmers during graminan biosynthesis in stems of wheat? Purification, characterization, mass mapping, and cloning of two fructan 1-exohydrolase isoforms. *Plant Physiology*, *131*, 621-631.

Van den Ende, W., De Coninck, B., & Van Laere, A. (2004). Plant fructan exohydrolases: a role in signaling and defense? *Trends in Plant Science*, *9*, 523-528.

Van den Ende, W., Lammens, W., Van Laere, A., Schroeve, L., & Le Roy, K. (2009). Donor and acceptor substrate selectivity among plant glycoside hydrolase family 32 enzymes. *Febs Journal*, *276*, 5788-5798.

Van den Ende, W., & Valluru, R. (2009). Sucrose, sucrosyl oligosaccharides, and oxidative stress: scavenging and salvaging? *Journal of Experimental Botany*, *60*, 9-18.

van Eps, A. W., & Pollitt, C. C. (2006). Equine laminitis induced with oligofructose. *Equine Veterinary Journal*, *38*, 203-208.

Veiga, P., Gallini, C. A., Beal, C., Michaud, M., Delaney, M. L., DuBois, A., et al. (2010). *Bifidobacterium animalis* subsp lactis fermented milk product

reduces inflammation by altering a niche for colitogenic microbes. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 18132-18137.

Watzl, B., Girrbach, S., & Roller, M. (2005). Inulin, oligofructose and immunomodulation. *British Journal of Nutrition*, 93, S49-S55.

Winter, S. E., Thiennimitr, P., Winter, M. G., Butler, B. P., Huseby, D. L., Crawford, R. W., et al. (2010). Gut inflammation provides a respiratory electron acceptor for *Salmonella*. *Nature*, 467, 426-429.

Xiang, L., Le Roy, K., Bolouri-Moghaddam, M-R., Vanhaecke, M., Lammens, W., Rolland F., et al. (2011). Exploring the neutral invertase-oxidative stress defence connection in *Arabidopsis thaliana*. *Journal of Experimental Botany*. In press. doi:10.1093/jxb/err069.

Yun, H., Lee, J. H., Park, C. E., Kim, M. J., Min, B. I., Bae, H., et al. (2009). Inulin increases glucose transport in C2C12 myotubes and HepG2 cells via activation of AMP-activated protein kinase and phosphatidylinositol 3-kinase pathways. *Journal of Medicinal Food*, 12, 1023-1028.

Zhang, M., Cui, S. W., Cheung, P. C. K., & Wang, Q. (2006). Polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity. *Trends in Food Science & Technology*, 18, 4-19.