Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Institutionen för molekylära vetenskaper

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Therese Holmsten

Självständigt arbete • 15 hp
Agronomprogrammet – Livsmedel
Molekylära vetenskaper, 2018:24
Uppsala, 2018

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Handledare:	Roger Andersson, Sveriges Lantbruksuniversitet, Institutionen för molekylära vetenskaper
Examinator:	Jana Pickova, Sveriges Lantbruksuniversitet, Institutionen för molekylära vetenskaper

Omfattning:	15 hp
Nivå och fördjupning:	Grundnivå, G2E
Kurstitel:	Självständigt arbete i livsmedelsvetenskap
Kurskod:	EX0669
Program/utbildning:	Livsmedelsagronom
Utgivningsort:	Uppsala
Utgivningsår:	2018
Delnummer i serien:	2018:24
Elektronisk publicering:	https://stud.epsilon.slu.se
Nyckelord:	Cereals, dietary fibres, prebiotics, arabinoxylan, β-Glucan, short-chain fatty acids

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Swedish University of Agricultural Sciences

Fakulteten för naturresurser och jordbruksvetenskap
Institutionen för molekylära vetenskaper

Abstract

The human gut is colonized by an enormous amount of microorganisms, the so called microbiota. The microbiota degrades dietary components in the colon and utilize them as substrates in their metabolism. Dietary fibres are the most important energy source for the microbiota. Some dietary fibres are classified as prebiotics, as they have the ability to alter the composition and activity of the microbiota and promote health benefits.

Cereals are the major source of dietary fibres in Scandinavia. Among the cereals, rye (*Secale cereale)* and oat (*Avena sativa*) are most frequently consumed as whole grains. The aim of this literature study was to review the current knowledge about the prebiotic properties of arabinoxylan and β-Glucan, the major dietary fibres in rye and oats.

A prebiotic substrate is selective and confer health benefits. The result shows that arabinoxylan and β-Glucan fermentation display prebiotic properties. Both confer health benefits by indirectly stimulating beneficial metabolite production of the short-chain fatty acids acetate, propionate and butyrate. The literature shows that arabinoxylan particularly promotes butyrate production, while β-Glucan promotes propionate production and in some conditions butyrate as well.

Butyrate has been shown to improve intestinal integrity, prevent inflammation and exhibit an anti-carcinogenic effect. Propionate inhibits cholesterol synthesis. However, health effects of dietary fibres can depend on the individual responsiveness to a substrate, the so-called enterotype. In general, a diet with various dietary fibres has been connected to health benefits, partly because it prevents production of detrimental metabolites that is formed in the absence of fermentable carbohydrates.

Future studies will have to further confirm the prebiotic properties of arabinoxylan and β-Glucan *in vivo* with human subjects. If such studies would present enough scientific support for authority health claims, it may be a way to increase the consumption of dietary fibres in the population and contribute to improving public health.

Keywords: Cereals, dietary fibres, prebiotics, arabinoxylan, β-Glucan, short-chain fatty acids

Sammanfattning

Människans mag-tarmkanal är koloniserad av ett enormt antal mikroorganismer, den så kallade mikrobiotan. I tjocktarmen bryter mikrobiotan ner komponenter i maten och använder dem som substrat i sin metabolism. Kostfibrer är mikrobiotans viktigaste energikälla. Vissa kostfibrer klassas som prebiotika, eftersom de har förmågan att påverka kompositionen och aktiviteten hos mikrobiotan och ge positiva hälsoeffekter.

Cerealier är den största kostfiberkällan i Skandinavien, och av dem är det råg (*Secale cereale)* och havre (*Avena sativa*) som oftast konsumeras som fullkorn. Syftet med denna litteraturstudie var attsammanställa den nuvarande kunskapen om de prebiotiska egenskaperna hos kostfibrerna arabinoxylan och β-Glukan, som finns i högst mängd i råg och havre.

Prebiotiska substrat är selektiva och ger hälsofördelar. Resultatet visar att fermenteringen av arabinoxylan och β-Glukan har prebiotiska egenskaper. Båda ger hälsofördelar genom att indirekt stimulera gynnsam metabolitproduktion av de kortkedjiga fettsyrorna acetat, propionat och butyrat. Litteraturen visar att arabinoxylan särskilt gynnar butyratproduktion, medan β-Glukan gynnar propionatproduktion och under visa förhållanden även butyrat.

Butyrat har visats förbättra tarmbarriärens integritet, motverka inflammation och ha en anti-carcinogen effekt. Propionat inhiberar kolesterolsyntes. Men hälsoeffekter av kostfibrer kan även bero på individens mottaglighet för ett substrat, den så kallade enterotypen. Generellt har en varierad kost med många olika kostfibrer kopplats till bättre hälsa, vilket delvis beror på en minskning av skadliga metaboliter som kan produceras i tjocktarmen när det är brist på fermenterbara kolhydrater.

Framtida studier bör vidare bekräfta den prebiotiska effekten av arabinoxylan och β-Glukan *in vivo*. Om sådana studier skulle visa på tillräckligt vetenskapligt stöd för att utfärda hälsopåståenden, kan det vara ett sätt att öka kostfiberkonsumtionen I populationen och bidra till att förbättra folkhälsan.

Nyckelord: Cerealier, kostfibrer, prebiotika, arabinoxylan, β-Glukan, kortkedjiga fettsyror

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Abbreviations

AXOS	Arabinoxylo-oligosaccharides
GI	Gastrointestinal
SCFAs	Short-chain fatty acids
XOS	Xylo-oligosaccharides

1 Introduction

The human gut is colonized by an enormous amount of inhabitants - the so called microbiota. In the past few years the microbiota and its proclaimed impact on human health has become a hot topic in the Western society. The microbiota consists of over 10^{14} microbes, which is more than the number of cells in the human body. While the microbe community is called the 'microbiota' their common collection of genes goes under the name 'microbiome' (Clemente *et al.*, 2012).

The human existence is highly dependent on the symbiosis with the microbiota. The continuous host-microbiota interactions are part of a complex ecosystem, sometimes referred to as 'the forgotten organ', because of its extensive metabolic activity and essential role in the function of the human body (O'Hara & Shanahan, 2006). The microbiota composition is influenced by both internal and external factors, e.g. genetics, age, antibiotic usage and stress. One of the most influential factors is what the microbiota is fed with on a daily basis – the diet. The microbes use the dietary food components as substrates in their energy metabolism (Holscher, 2017).

The carbohydrate polymers that resist intestinal degradation, the dietary fibres, are important substrates for the microbiota (Holscher, 2017). The interaction between dietary fibres and the microbiota has gained more interest, as the concept of 'prebiotics' emerged about 20 years ago (Gibson *et al.*, 2017). Prebiotics are dietary fibres that have the ability to alter microbiota composition and activity (Holscher, 2017). Metabolites, mainly short-chain fatty acids (SCFAs), are the end-products of the microbial degradation and has been observed to have beneficial physiological effects (Knudsen & Erik, 2015)

Cereal dietary fibres are less mentioned in connection to the prebiotic concept. Although they have been shown to possess prebiotic properties they are not generally accepted as prebiotics (Kellow *et al.*, 2014). However, the functionality of cereal dietary fibres compared to well-established prebiotics, e.g. inulin and fructooligosaccharides, have been emphasized in the literature (Karppinen *et al.*, 2000; Rose, 2014). Especially since those prebiotics often origin from less commonly consumed foods.

Cereals are the major source of dietary fibres in Scandinavia (Andersson *et al.*, 2014) and the highest content of dietary fibres is found in whole-grain products (Egervärn *et al.* 2018). Rye (*Secale cereale)* and oat (*Avena sativa*) are the cereals most frequently consumed as whole grains (Welch, 2011; Andersson *et al.*, 2014). Rye in the form of whole-grain bread (Knudsen & Lærke, 2014) and oats mostly in the form of breakfast cereals (Welch, 2011). The major dietary fibres in rye and oat are arabinoxylan and β-Glucan, respectively. Both are known to influence gastrointestinal health (Carlson *et al.*, 2018).

The Western population consume less dietary fibres than recommended – the so called 'fiber gap' (Requena *et al.*, 2018). The recommended intake of fibre to maintain colonic health is 25 to 35g/day for adults, but the average intake in the Swedish population is 20g/day (Amcoff *et al.*, 2012).

Dietary fibre consumption within the population have important implications for public health. The purpose of this thesis is to review the knowledge about the prebiotic properties of cereal dietary fibres. It is a highly relevant topic considering its potential to point out more advantages of whole grain consumption and play a role in future diet recommendations.

1.1 Aim

The aim of this thesis is to review the current knowledge about the prebiotic properties of arabinoxylan and β-Glucan, as they are the major dietary fibres in rye and oat. The question to be answered is: How are arabinoxylan and β-Glucan metabolised by the human microbiota and can the fermentation end-products in extension affect human health?

In order to answer this question, the composition of dietary fibres, the diet-microbiota interactions and the definition of prebiotic properties will be described. The discussion on the impact on human health will be limited to production of short-chain fatty acids and their health effects. Other aspects of prebiotics, e.g. diet-induced shifts in the microbiota composition, is beyond the scope of this thesis and will not be considered.

1.2 Method

Information for this literature study was mainly scientific articles found by searching in the data bases *Google Scholar, Web of Science, Scopus* and *PubMed.* The following words were used in different combinations to make the literature search: *microbiota, gut, prebiotic*, "dietary fibre*", "dietary fiber*", *, cereal*, rye, oat*, arabinoxylan, β-Glucan*, enterotype*, "short-chain fatty acid*", SCFA*, butyrate, health*.

Since the research field of prebiotics is relatively new, the literature that has been used is dominated by publications from the last 10 years. Books providing an overview of the nutritional aspects of rye and oats were also used in addition to the scientific articles.

2 Prebiotics

2.1 The human microbiota

Koropatkin *et al.* (2012) describes the importance of the co-evolved symbiosis between the human genome and the microbiome. Humans would only be able to degrade a few carbohydrate polymers, e.g. starch, lactose and sucrose, without microbial assistance in addition to the endogenous enzymes. Many plant polysaccharides are far more complex and contain a range of glycoside linkages, each requiring specific degrading enzymes that the inhabitants of the microbiota provide.

The microbiota contains both 'generalists' and 'specialists', which target different glycoside linkages (Koropatkin *et al.*, 2012). The phylum Bacteroidetes are generalists and Firmicutes are specialists, but they cooperate through cross-feeding. Bacteroidetes degrade the primary substrates and Firmicutes subsequently utilize their end-products (Requena *et al.*, 2018).

2.2 Microbial diversity

The broad taxonomic groups of the microbiota are quite stable through a lifetime. However, a proportion of the microbiota is constantly shifting in numbers depending on external factors, e.g. short-term changes of the composition have been observed within 24 hours after dietary interventions (Koropatkin *et al.*, 2012).

The dietary changes following industrialization in the Western population have resulted in a decreased diversity of the microbiota over time, which has been connected to a higher prevalence of e.g. metabolic diseases, inflammatory bowel disease and colon cancer (Requena *et al.*, 2018).

Egervärn *et al.* (2018) from the Swedish National Food Agency states that a diet rich in dietary fibres, together with a low intake of animal-derived fats and proteins, is related to increased microbial diversity and health benefits. That is evident in studies of populations eating plenty of dietary fibres, e.g. a small population in Amazonas was found to have the most species-rich microbiota so far investigated.

2.3 Definition of prebiotics

The concept of 'prebiotics' emerged in 1995 to describe fermentable carbohydrates, mainly fructooligosaccharides (FOS), inulin and galactooligosaccharides (GOS), that modulated microbiota composition. These compounds had been seen to promote growth of e.g. *Lactobacillus* and *Bifidobacterium* spp., which are considered beneficial for human health (Gibson & Roberfroid, 1995).

There are different opinions about the meaning and correct usage of the term 'prebiotics'. Delcour *et al.* (2016) argue that the most well-established prebiotics (inulin, FOS and GOS) should not be separated from other fermentable carbohydrates that also modulate microbiota composition and activity. Delcour *et al.* (2016) emphasize the importance of rather discussing the functionality of the microbiota and its fermentation patterns, than focusing on a narrow range of the microbial community. They argue that the meaning of the concept should evolve as knowledge about the microbiota increase.

Actually there is an ongoing development of the concept, as the definition was recently broadened and updated by an expert consensus panel from The International Scientific Association for Probiotics and Prebiotics. Gibson *et al.* (2017) state in the article that a prebiotic is defined as "a substrate that is selectively utilized by host microorganisms conferring a health benefit". This definition will be the starting point when discussing the prebiotic properties of arabinoxylan and β-Glucan in this literature study.

A prebiotic must fulfil two requirements according to this definition, i.e. to be selective and confer health benefits. Gibson *et al.* (2017) define selectively as a substrate that target a specific range of the microbiota. In contrast to the older definitions, the selectivity is no longer solely limited to promotion of beneficial microbial genera. In terms of metabolite production, a prebiotic would e.g. need to promote specific parts of the microbiota that are known producers of butyrate. A compound that stimulates growth of the microbial community in general is not qualified as a prebiotic.

Gibson *et al.* (2017) further describes the second requirement concerning health benefits, that can be fulfilled either if the substrate directly stimulates beneficial microbial genera or if it indirectly stimulates beneficial metabolite production. They also emphasize that the prebiotic effect should be confirmed in well-controlled *in vivo* studies. The studies need to show causality between microbial activity and health benefits, e.g. modulation of the metabolite production. The total microbial diversity of the community should be taken into account when evaluating the prebiotic effect, which is why *in vitro* systems is not considered sufficient (Gibson *et al.*, 2017).

2.4 Enterotypes

The individual microbiota composition is a significant aspect when discussing how diet influence the microbiota. According to Chen *et al.* (2017), the microbiota is not homogenous within the human population and can display different fermentation patterns even though the same dietary components are consumed. A classification of the microbiome in two 'enterotypes' have recently emerged (Arumugam *et al.*, 2011). As Requena *et al.* (2018) describes, the first enterotype is dominated by the genera *Prevotella* and is linked to a long-term consumption of complex carbohydates. *Bacteroides* dominates when the diet constitutes a higher proportion of animal fats and proteins, as is usually the case in industrialized populations.

The implication for the metabolite production, as decribed by Chen *et al.* (2017), is that the same substrate may give rise to different SCFA profiles depending on the enterotype. Thus, possible health effects of dietary fibres are not only dependent on the substrate supply, but also the individual responsiveness to the substrate. It has been suggested that customized dietary strategies to promote health will emerge in the future, as the knowledge about the enterotypes increase (Chen *et al.*, 2017; Kovatcheva-Datchary *et al.*, 2015).

3 Dietary fibres

3.1 Definition

The definition of dietary fibre was traditionally based solely on chemical properties, but have more recently evolved to include more physiologic aspects (Carlson *et al.*, 2018). The European Union regulation (EU) No 1169/2011 defines dietary fibre as ”carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine”. The carbohydrate polymers are either consumed in raw form in food, modified from natural carbohydrate sources or synthetically produced. The two latter are also required to “have a beneficial physiological effect demonstrated by generally accepted scientific evidence” to be classified as dietary fibres.

3.2 Sources and properties

Dietary fibres are a diverse group of carbohydrates originating from the plant kingdom, consumed in the human diet in the form of cereals and grains, legumes, nuts, fruits and vegetables. Solubility, fermentability and viscosity affect the physiological effects of dietary fibres (Mudgil, 2017). Chemical properties, such as molecular weight, also influence the physiological effect (Rakha *et al.*, 2010).

Dietary fibres are most commonly classified by their solubility, according to Mudgil (2017). The soluble dietary fibres are either viscous or non-viscous. The main soluble dietary fibre in cereals are β-Glucan. The majority of the cereal grain cell-walls consists of insoluble fibres (Mudgil, 2017), that are mostly located in the outer layers of the kernel (Daou Cheickna & Zhang Hui, 2012) and are further divided into celluloses, which are totally insoluble, and hemicelluloses, which are partially soluble (Coultate, 2015). A major insoluble fibre in whole grain cereals is the hemicellulose arabinoxylan (Mudgil, 2017). However, both soluble and insoluble arabinoxylans are found in rye (Knudsen & Erik, 2015).

Insoluble fibres have a substantial bulk effect due their hydrophilic molecular structure that can bind water and increase faecal mass. The bulking also has the favourable effect of diluting potential toxic metabolites in the colon content and limit their contact with the epithelial cells (Mudgil, 2017).

3.3 Gastrointestinal degradation

Microbial fermentation of dietary components occurs in the colon, the final part of the gastrointestinal tract (GI) (Koropatkin *et al.*, 2012). Several studies have shown that there are regional differences in the microbiota composition and fermentation pattern along the GI tract. The microbial population gradually increase from a limited number of species in the small intestine, to a very dense and diverse community in the distal colon (Koropatkin *et al.*, 2012). Fermentation of soluble fibres, such as β-Glucan, occurs in the proximal colon where microbial activity is high (Knudsen & Erik, 2015). Fermentation of insoluble fibres occurs in the distal parts of the colon, where the fermentation rate and transit time is slower (Koropatkin *et al.*, 2012).

The microbial degradation process is affected by the molecular structure of the dietary fibre as well as the complexity of the plant material, e.g. cell wall polysaccharides may be folded in a matrix structure (Koropatkin *et al.*, 2012) that delays substrate availability (Mudgil, 2017). Food processing, e.g. cooking and milling, also affect substrate availability and affect the fermentation process (Koropatkin *et al.*, 2012).

Fermentation of dietary fibres results in SCFA production and a decrease of the colonic pH. The SCFA concentration varies along the GI tract (Hamer *et al.*, 2008). It is highest in the proximal colon and decreases subsequently. The pH follows the opposite pattern with the lowest pH being found in the proximal colon (Glitsø *et al.*, 1998). The mean pH of the proximal and distal colon is approximately 5.6 and 6.3, respectively (Hamer *et al.*, 2008).

3.4 Dietary fibres in cereals

3.4.1 Composition

Nonstarch polysaccharides, mainly arabinoxylans, β-Glucan and cellulose, together with resistant starch, fructans and lignin constitutes the dietary fibres in cereals. Most dietary fibres are found in the outer tissues, which means that the consumed amount will depend on how the grain has been processed (Knudsen & Erik, 2015).

The composition (Table 1) varies among rye, wheat, oat and barley. It should be noted that the content differs greatly between measurements and the values listed here are only one example. Rye have a higher content of arabinoxylan than any of the other cereals. It also contains most dietary fibres in total, between 13-17% according to Rakha *et al.* (2010). Oats have a significantly smaller content of dietary fibre, between 6-9%, but a high content of soluble fibres in the form of β-Glucan (Daou Cheickna & Zhang Hui, 2012).

Table 1. *Dietary fibre content in cereals on g/100g dry-matter basis.*

Dietary fibre[a]	**Rye**	**Wheat**	**Oat**	**Barley**
Total, excl. fructans	13.0	10.6	7.7	11.7
Fructan	2.4-3.1	1.5-2.0	0.6-1.0	0.6-1.0
Soluble DF	4.5	2.3	4.5	3.9
Insoluble DF	8.5	8.3	3.2	7.8
Cellulose	1.3	1.7	0.6	1.4
Other insoluble	7.2	6.5	2.6	6.4
β-Glucan	2.1	0.83	4.4	4.2
Arabinoxylan[b]	6.5-12.0	5.8-6.5	2.0-4.5	4.0-7.0

[a] Numbers are for whole-grain rye, wheat, oat-meal and pearled barley and derived from Welch (2011) for all dietary fibres except arabinoxylan. [b] Numbers from Rose (2014).

3.4.2 Arabinoxylan

Arabinoxylan is a type of xylan, which is the major group of hemicellulose found in cereal grains (Coultate, 2015). The highest content of arabinoxylan is found in rye, where it contributes to more than half of the dietary fibres. The molecule, showed in Figure 1, is based on a linear xylose backbone with $\beta 1 \rightarrow 4$ linkages and arabinose side chains (Kellow & Walker, 2018). The arabinose substituents are linked at one of the O-2 and O-3 positions or at both of them (Andersson *et al.*, 2014).

Phenolic compounds can also be linked to the xylose backbone (Kellow & Walker, 2018). Ferulic acid is the most common phenolic substituent in the arabinoxylan structure found in rye. Ferulic acid has the ability to form cross-linkages, which highly influence the viscosity and solubility properties of the molecule (Andersson *et al.*, 2014).

Arabinoxylans are found in a range of molecular weights and substitution patterns. The A/X ratio describes the proportion of arabinose substituents in relation to the xylose backbone. Arabinoxylans in different parts of e.g. the rye kernel have different A/X ratios, which influence the physiochemical properties of the tissues (Andersson *et al.*, 2014). The A/X ratio is closely related to solubility and explains why arabinoxylans exist in both soluble and insoluble forms. A high A/X ratio, i.e.

many arabinose side chains in relation to the xylose units, increase the solubility (Kellow & Walker, 2018).

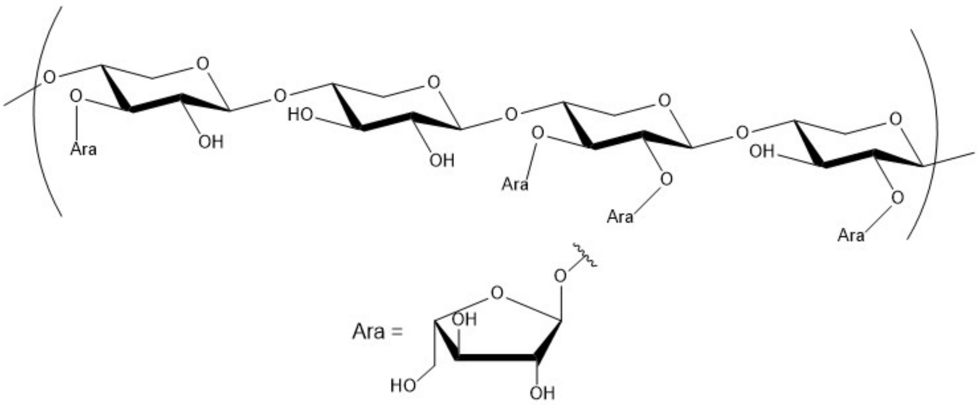

Figure 1. Molecular structure of arabinoxylan.

3.4.3 β-Glucan

The major soluble dietary fibre of cereals is the linear (1→3)(1→4)-β-D-glucan, the short name used is β-Glucan. The structure of the molecule is shown in Figure 2. The molecule is highly soluble because of the (1→3) link, that makes the molecule less tightly packed than e.g. cellulose (Andersson *et al.*, 2014). β-Glucan occurs in the highest concentrations in oats and barley (Welch, 2011).

Most of the β-Glucan in oats are located in the walls of the endosperm, the sub-aleurone layer. The viscosity of β-Glucan has been widely examined in relation to the health-promoting properties of oats. β-Glucan has a well-established effect of lowering serum cholesterol (Daou Cheickna & Zhang Hui, 2012), which has been recognized in health claims stated by both U.S. Food and Drug Administration and EFSA (Cloetens *et al.*, 2012).

The viscosity of β-Glucan increases exponentially with molecular weight (Mälkki & Virtanen, 2001). The viscosity has been found to be lower in barley than in oats, which may be related to the lower molecular weight found in barley (Cloetens *et al.*, 2012). The chemical properties determine the physiological activity and should be considered when discussing health effects (El Khoury *et al.*, 2012).

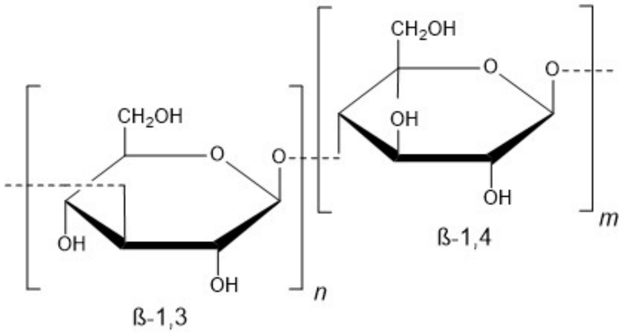

Figure 2. Molecular structure of β-Glucan.

4 Fermentation and metabolite production

4.1 Fermentation products

Acetate, propionate and butyrate are the main end-products of microbial fermentation in the gut (Koh *et al.*, 2016). Besides the dietary substrates, endogenous material of the colon also acts as substrates for fermentation, e.g. glycoproteins and exfoliated epithelial cells (Knudsen *et al.*, 2003).

The SCFAs are formed by different microbial species and reaction pathways. Acetate is produced with pyruvate as the building block, by most species of the microbiota and constitutes the largest portion of the SCFAs (Koh *et al.*, 2016).

A smaller range of the microbiota produce propionate and butyrate (Knudsen & Erik, 2015). Propionate is produced mainly by *Bacteroides* spp. (Koh *et al.*, 2016), with the assistance of several Firmicutes that supply vitamin B_{12} as a cofactor (Requena *et al*., 2018). Butyrate is produced with either acetate or lactate as substrate (Koh *et al.*, 2016). The most prevalent butyrate-producing species of the human microbiota belong to the Firmicutes phylum (Requena *et al*., 2018).

Different substrates give rise to different concentrations of total SCFAs as well as ratios of acetate, propionate and butyrate. The concentration of SCFAs is measured in the faeces, which is a representational model of the actual fermentation occurring in the colon (Valeur *et al.*, 2016).

4.1.1 Absorption of SCFAs

The SCFAs, mainly butyrate, act as the primary energy source for the epithelial cells of the colon that metabolise them through beta-oxidation (Knudsen *et al.*, 2003). Over 95% of the butyrate is immediately metabolized by the epithelial cells (Hamer *et al.*, 2008) and the rest is metabolized in the liver along with propionate.

Acetate is the SCFA found in highest concentration in the peripheral circulation since it is not as readily metabolized (Koh *et al.*, 2016), although small concentrations of propionate and butyrate is also found (Morrison & Preston, 2016).

The SCFAs influence pH, bioavailability of minerals and inhibition of pathogenic bacteria (Gullón *et al.*, 2014) as well as acting as signalling molecules between the microbiota and the host. They can bind to peripheral receptors and influence functions in other parts of the body, besides acting as an energy source for the epithelial cells (Morrison & Preston, 2016).

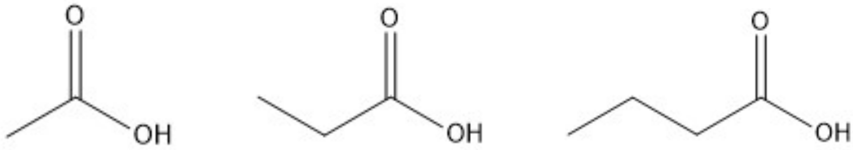

Figure 3. Acetate, propionate and butyrate.

4.2 Arabinoxylan fermentation

4.2.1 Degradation process

The microbial degradation of arabinoxylan involves several enzymatic steps. It occurs along a wider range of the colon length compared to e.g. the oligosaccharides, that are more readily fermented in the proximal colon (Salden *et al.*, 2018). Once arabinoxylan enters the colon it is first degraded by *Roseburia* and *Bacteroides* spp. that produce endoxylanase enzymes (Broekaert *et al.*, 2011). The enzymes hydrolyse the polymer into the shorter arabinoxylo-oligosaccharides (AXOS) and xylo-oligosaccharides (XOS) chains. These are further degraded to their constituting arabinose and xylose units by the enzymes arabinofuranosidase and xylanase, that are formed by *Bifidobacterium* spp. among others. About 15% of the consumed arabinoxylan is never degraded but will instead contribute to faecal bulk and reduced transit time (Kellow & Walker, 2018).

4.2.2 Fermentation profile

In the recent review by Knudsen & Erik (2015) they conclude that the fermentation of arabinoxylan results in an increased total SCFA production. It has been observed as a result of arabinoxylan, AXOS and XOS fermentation in animal studies (Broekaert *et al.*, 2011). It was also observed to increase in human intervention studies (Hald *et al.*, 2016) together with a faecal pH reduction (Salden *et al.*, 2018). The pH reduction favours the abundance of beneficial saccharolytic species, e.g. *Bacteroides, Roseburia* and *Lactobacillus*, which has been observed both *in vitro* and *in vivo* (Kellow & Walker, 2018).

Although there is less evidence for the prebiotic properties of arabinoxylan compared to e.g. inulin and FOS, it has been argued that all the requirements for a full prebiotic status is fulfilled (Broekaert *et al.*, 2011). One example of such an argument is an *in vitro* study that showed a higher SCFA production from wheat-derived AXOS than fermentation of FOS, which is considered the 'golden standard' of prebiotics (Gullón *et al.*, 2014).

4.2.3 Butyrate production

The *in vitro* studies of the SCFA ratio have shown some contradicting results, but most human *in vivo* studies indicate that arabinoxylan particularly favours production of butyrate (Knudsen & Erik, 2015).

Both butyrate and acetate production increased when arabinoxylan consumption was compared to a low-fibre Western diet (Hald *et al.*, 2016). Though, as Knudsen & Erik (2015) mentions, some animal studies did not result in a higher butyrate ratio. They suggest that the butyrate production depends on the food matrix. Arabinoxylan in whole-grain foods are more slowly fermented and decrease pH in a wider length of the colon, which may favour the proportion of butyrate in the SCFA ratio.

4.2.4 Physiological effects

Several studies have been performed recently of wheat-derived arabinoxylan and AXOS and their potential link to beneficial health effects in humans. Although, further human *in vivo* studies will be necessary to establish causal effects.

AXOS was shown to have a preventive effect against obesity and metabolic disorders in obese mice fed with a western diet (Neyrinck *et al.*, 2018). High molecular weight arabinoxylan has been shown to influence the gut barrier and immune system *in vitro* and in animal studies. On the contrary, when the effect of a 6-week arabinoxylan treatment to improve gut permeability was tested in a human intervention, it failed to show any significant results, apart from a generally increased SCFA production (Salden *et al.*, 2018).

4.3 β-Glucan fermentation

4.3.1 Fermentation profile

β-Glucan is more rapidly fermented than arabinoxylan (Karppinen *et al.*, 2000) and can be 100% fermented, since its chemical structure makes it readily degraded by the microbiota. The major products of the fermentation are acetate, propionate and butyrate (Drzikova *et al.*, 2005). An increased total SCFA concentration has been observed for β-Glucan in oat bran compared to rye bra, wheat bran and inulin (Karppinen *et al.*, 2000).

Knudsen & Erik (2015) mentions that the molecular weight of β-Glucan influences the fermentation profile. Low molecular weight β-Glucan appears to produce a higher total SCFA concentration and lower pH than high molecular weight β-Glucan (Dong *et al.,* 2017*)*. In animal studies, a higher ratio of propionate and butyrate to acetate has been observed with increasing molecular weight (Immerstrand *et al.*, 2010).

4.3.2 Propionate production

Several studies have shown that β-Glucan has a propionate-rich fermentation profile compared to FOS and inulin (Nordlund *et al.*, 2012) as well as pectin and resistant starch (James L. Casterline *et al.*, 1997). *In vitro* faecal fermentations have also acknowledged that β-Glucan produces a high proportion of propionate (Hughes *et al.*, 2008; Carlson *et al.*, 2017).

4.3.3 Butyrate production

The literature is not consistent regarding the butyrate production of β-Glucan fermentation. The fermentation profile for thick and thin oat flakes tested in an *in vitro* system, was considered to be abundant in propionate but also showed an increased proportion of butyrate in the thicker flakes (Connolly *et al.*, 2010). Mice fed with a diet based on oat flour and oat bran showed a higher butyrate production in the caecum compared to the control group (Drzikova *et al.*, 2005). Knudsen & Erik (2015) also points to several *in vivo* studies that show enhanced faecal butyrate formation as a result of β-Glucan fermentations.

Whole-grain oats seem to provide a more butyrate-rich profile than fermentation of β-Glucan alone. The butyrate enhancement has been suggested to origin from the high content of resistant starch found in some oat-based products (Rose, 2014). This corresponds to the previously mentioned suggestion that the SCFA profile is highly dependent on the food matrix (Knudsen & Erik, 2015)

Only a few *in vivo* studies on humans relating β-Glucan consumption to metabolite production were found in the literature. In a 3-month oat bran intervention in ulcerative colitis patients, the faecal butyrate concentration increased after 4 weeks (Nie *et al.*, 2017). Another 3-month intervention with β-Glucan bread showed an increased butyrate ratio after 1 month, compared to the placebo group, which thereafter declined (Turunen *et al.*, 2011). However, no significant increase in total SCFA concentration was observed in a 1-week study, when oat-meal porridge was consumed by 10 human subjects (Valeur *et al.*, 2016). These studies are relevant if there is an aim to establish β-Glucan as a prebiotic, more human *in vivo* studies are needed.

5 Health effects

5.1 Fermentation patterns

Hamer *et al.* (2008) describes that saccharolytic fermentation is preferred by the microbiota, since carbohydrates are most often their preferred energy source. Fermentation of proteins, proteolytic fermentation, dominates when fermentable carbohydrates are not available. The produced proteolytic metabolites, e.g. branch-chained fatty acids, ammonia and amines, are considered unfavourable for host health. These metabolites have been linked to several GI diseases that are usually more prevalent in the distal colon. According to Hamer *et al.* (2008), it correlates to the fact that proteolytic fermentation is more likely to occur in the distal colon if the supply of fermentable dietary fibres is low. Dietary fibre consumption has been observed to decrease the proteolytic metabolite concentration, e.g. in a dietary intervention with arabinoxylan and resistant starch (Hald *et al.*, 2016).

A versatile plant-based diet offers the microbiota a diverse collection of dietary fibres with various physiochemical properties. Such a diet provides substrates along a broader region of the colon and promotes a more diverse fermentation profile by the microbiota (Holscher, 2017). The conventional diet in industrialized populations contains a small variety of dietary fibres and does not promote a beneficial fermentation pattern (Requena *et al.*, 2018).

5.2 Metabolic effects

The SCFAs act on peripheral tissues and may impact insulin sensitivity and glucose homeostasis (Knudsen & Erik, 2015). Morrison & Preston (2016) describes that the circulating SCFAs, dominated by acetate but also smaller amounts of propionate and butyrate, induce differentiation of fat cells making them smaller in size. Acetate also inhibits the breakdown of lipids into free fatty acids in the adipose tissue, which have implications for glucose homeostasis. Morrison & Preston

(2016) as well as Koh *et al.* (2016) mention that acetate may influence appetite regulation, by stimulating the hormone leptin or by acting on hypothalamus. Propionate also exhibit metabolic effects, e.g. by modifying lipid metabolism in the liver, as animal studies have shown an inhibitory effect on cholesterol synthesis (Kellow *et al.*, 2014).

5.3 Immune function

Butyrate regulates tight junction proteins and is involved in improving the integrity of the intestinal barrier. Increased permeability of the barrier is connected to intestinal inflammation, which in turn has been linked to obesity and insulin resistance (Morrison & Preston, 2016). A few clinical trials have been performed to the test the anti-inflammatory effect of butyrate in treatment of GI inflammatory diseases. Some mechanisms behind this effect have been suggested, but further studies are required to confirm this relationship (Hamer *et al.*, 2008).

5.4 Colon cancer

Epidemiological studies have shown a correlation between dietary fibre consumption and decreased incidence of colon cancer. It has been suggested that butyrate concentration in the colon might play a role in this correlation (Hamer *et al.*, 2008).

Butyrate act inhibitory on colon cancer in animal studies and *in vitro*, but human intervention studies are still lacking. Addition of butyrate to cancerous cells *in vitro* induce an anti-carcinogenic effect. It is thought that butyrate inhibits the enzyme histone deacetylase (HDAC), subsequently altering the expression of genes involved in cell proliferation and apoptosis (Hamer *et al.*, 2008). Propionate has also been seen to have a smaller but similar effect (Koh *et al.*, 2016).

Cancerous cells and noncancerous cells respond differently to butyrate, a phenomenon called 'the butyrate paradox' (Hamer *et al.*, 2008). Proliferation of noncancerous cells is not inhibited by butyrate and in some studies, on the contrary, it has been observed to be stimulated by butyrate (Donohoe *et al.*, 2012). The mechanisms of butyrate as a signalling molecule in this interaction is still uncertain (Koh *et al.*, 2016).

6 Discussion

Dietary fibres have many positive health benefits, one of them is the production of favourable metabolites in the colon. The result of this literature study shows that both arabinoxylan and β-Glucan fermentation display prebiotic properties. They are selective by influencing microbial activity of limited parts of the microbiota. Arabinoxylan seems to particularly promote parts of the microbiota that produce butyrate and β-Glucan especially promotes propionate production. The effect of β-Glucan on butyrate production is inconsistent and seems to depend on the food matrix as well as molecular weight. Both arabinoxylan and β-Glucan increase the production of SCFAs, which indirectly confers health benefits, since the SCFAs are involved in interactions that promote health.

The understanding of the diet-microbiota-health interactions is still in its infancy. The Swedish National Food Agency recently published a report about the current knowledge in the area, concluding that there are connections indeed, but the mechanisms are still unknown (Egervärn *et al.* 2018). This report further enhances the notion that prebiotics are of particular interest and there are strong reasons to focus on this field of research.

In conclusion, there is much potential in broadening the prebiotic concept with a higher emphasis on the functionality of foods that commonly occur in the Western diet. Future studies will have to confirm the prebiotic properties of arabinoxylan and β-Glucan in controlled *in vivo* studies of human subjects. Preferably future studies will also focus on the enterotypes and their responsiveness to arabinoxylan and β-Glucan interventions. If such studies would present enough scientific support for authority health claims, it may be a way to overbridge the 'fiber gap' and improve public health.

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