ESPOO 2003 VTT PUBLICATIONS 500

Sirpa Karppinen

Dietary fibre components of rye bran and their fermentation *in vitro*

VTT PUBLICATIONS 500

Dietary fibre components of rye bran and their fermentation *in vitro*

Sirpa Karppinen

VTT Biotechnology

To be presented with the permission of the Faculty of Science of the University of Helsinki, for public criticism in the Auditorium 1041 at the Department of Biosciences, Biocenter 2, Viikinkaari 5, Helsinki on August 15th, 2003, at 12 o'clock noon.

ISBN 951–38–6229–1 (soft back ed.)
ISSN 1235–0621 (soft back ed.)

ISBN 951–38–6230–5 (URL: http://www.vtt.fi/inf/pdf/)
ISSN 1455–0849 (URL: http://www.vtt.fi/inf/pdf/)

JULKAISIJA – UTGIVARE – PUBLISHER

VTT, Vuorimiehentie 5, PL 2000, 02044 VTT
puh. vaihde (09) 4561, faksi (09) 456 4374

VTT, Bergsmansvägen 5, PB 2000, 02044 VTT
tel. växel (09) 4561, fax (09) 456 4374

VTT Technical Research Centre of Finland, Vuorimiehentie 5, P.O.Box 2000, FIN–02044 VTT, Finland
phone internat. + 358 9 4561, fax + 358 9 456 4374

VTT Biotekniikka, Tietotie 2, PL 1500, 02044 VTT
puh. vaihde (09) 4561, faksi (09) 455 2103

VTT Bioteknik, Datavägen 2, PB 1500, 02044 VTT
tel. växel (09) 4561, fax (09) 455 2103

VTT Biotechnology, Tietotie 2, P.O.Box 1500, FIN–02044 VTT, Finland
phone internat. + 358 9 4561, fax + 358 9 455 2103

Technical editing Maini Manninen
Cover picture: Risto Karppinen

Otamedia Oy, Espoo 2003

Karppinen, Sirpa. Dietary fibre components of rye bran and their fermentation *in vitro*. Espoo 2003. VTT Publications 500. 96 p. + app. 52 p.

Keywords dietary fibres, rye bran, fermentation, *in vitro* fermentation, carbohydrates, metabolism, prebiotics, lignans, extraction, chemical analysis

Abstract

Dietary fibre (DF) is defined for nutritional purposes as the non-digestible part of plant food. Because DF has many physiological effects along the entire human gastrointestinal tract, it is important for human well-being. Many of the health effects are mediated by the microbial fermentation of DF carbohydrates in the large intestine.

Rye is the main source of DF in Finland. Rye bran, in particular, is rich in DF. According to the traditional method of estimating DF the content is about 38 g/100 g on a dry weight basis. Rye bran was also shown to contain a significant fructan concentration (7 g/100 g), which according to the suggested new dietary fibre concept is also a component of DF. Rye grain contained 4.6–6.6 g of fructan/100 g depending on the growth conditions. In traditional soft rye bread, the DF content increases from 11 to 14 g/100 g because of fructan. In the average Finnish rye intake (43 g/day), this means that the total DF intake increases from 7.7 g/day to about 10 g/day.

Different rye bran fractions and processed rye bran were prepared in order to study the effect of solubility and processing on the fermentability of rye bran. Before fermentation, substrates were enzymatically digested simulating conditions within the small intestine in order to remove starch and protein. Fermentability was studied *in vitro* using human faecal inoculum.

Soluble rye-bran fractions were fermented rapidly and completely. Rye bran and the rye-bran residue, after water- or alkali-extraction, were fermented at a slower rate, but the fermentation continued throughout the entire fermentation time (24 h). About half of the neutral sugars (arabinose, xylose and glucose) of the total fermentation of rye bran (including the neutral sugars of the inoculum) was consumed over 24 h. Xylanase treatment of rye bran increased the initial rate of

fermentation slightly, but after 24 h fermentation the consumption of carbohydrates was the same as in the fermentation of the original rye bran.

Short-chain fatty acids (SCFA) were produced, which were related to the consumption of carbohydrates: the higher the rate and quantity of carbohydrate consumption the higher the rate and quantity of SCFA production. However, the ratio of SCFA produced to carbohydrates consumed changed between experiments depending on the faecal inoculum. Butyric and propionic acids were produced in all rye fermentations, but xylanase treatment slightly decreased their production. The most rapid butyric-acid production was shown to be in the fermentation of soluble fractions, but the extent was similar in all rye fermentations.

The fermentation rate of rye bran was the same as that of wheat bran, but the extent of fermentation was higher in the case of rye bran. Rye bran was fermented at a slower rate than oat bran.

In *in vitro* experiments with single bacterial strains, all the *Bifidobacterium longum* strains and one of the *Bifidobacterium adolescentis* strains examined were able to grow using rye arabinoxylan as the sole carbon source. Arabinoxylan from rye may also have potential as a prebiotic substrate for the proliferation of *Bifidobacterium longum*, a numerically dominant *Bifidobacterium* species in the adult human colon. Many *Bifidobacterium* species were able also to efficiently ferment xylo-oligosaccharides.

Rye bran contained plant lignans secoisolariciresinol, matairesinol, syringaresinol, pinoresinol, lariciresinol and isolariciresinol, the sum of which amounted to 5 mg/100 g. The effect of processing of rye bran on the conversion of plant lignans to mammalian lignans (enterodiol, enterolactone) was studied *in vitro* using a human faecal inoculum. The highest enterodiol formation was found in the fermentation of soluble rye-bran extract. Also, xylanase treatment of rye bran slightly increased enterodiol formation. Enterolactone production was very slow and hardly detectable.

Academic dissertation

Faculty of Science, Department of Biosciences, Division of Biochemistry
University of Helsinki, Finland

Custos

Professor Carl G. Gahmberg
University of Helsinki, Faculty of Science,
Department of Biosciences, Division of Biochemistry

Supervisor

Professor Kaisa Poutanen
Technical Research Centre of Finland
VTT Biotechnology

Reviewers

Professor Seppo Salminen
University of Turku, Faculty of Mathematics and Natural Sciences,
Department of Biochemistry and Food Chemistry,
and Degree Programme on Health Biosciences

Professor Atte von Wright
University of Kuopio, Faculty of Natural and Environmental Sciences,
Institute of Applied Biotechnology

Opponent

Professor Hannu Salovaara
University of Helsinki, Faculty of Agriculture and Forestry,
Department of Food Technology

Preface

This study was carried out at VTT Biotechnology during the years 1998–2003. The research was part of the national projects entitled 'Cereal- and carbohydrate-based prebiotics' and 'Non-digestible carbohydrates and lignans in gut health' included in the Tekes 'Innovation in Foods' technology programme and in the VTT 'Future Foods' and 'Tailored Technologies for Future Foods' research programmes. Tekes, the Finnish companies and also research teams in Universities of Helsinki and Kuopio are gratefully acknowledged for pleasant collaboration during these projects.

I thank Professor Juha Ahvenainen, executive director of VTT Biotechnology, for providing excellent working facilities and also for the support in writing of the thesis. I thank Professor Carl G. Gahmberg, the head of the division of biochemistry at the University of Helsinki for his encouraging attitude during the process of this thesis. Very special thanks to the reviewers of this thesis, Professor Seppo Salminen and Professor Atte Von Wright, for constructive comments.

I am very grateful to Professor Kaisa Poutanen for supervising my work at VTT Biotechnology. Her advice and endless support and encouragement have been important during my work. Very special thanks are due to my colleagues and co-workers at VTT: Dr. Pirkko Forssell and Dr. Liisa Lähteenmäki for their supporting attitude as group leaders; Anna-Marja Aura and Dr. Kirsi-Helena Liukkonen for fruitful collaboration and advice concerning *in vitro* fermentations; Eeva Manninen for skilful technical assistance in carbohydrate analysis; Dr. Tapani Suortti for advice concerning HPLC; Olavi Myllymäki for technical advice on pilot extractions; Dr. Marjatta Salmenkallio-Marttila for microscopic studies of rye bran; Dr. Jaana Mättö and Dr. Ross Crittenden for their microbial work; Hannele Virtanen for analyses of short-chain fatty acids; and Teija Jokila, Leena Öhrnberg, Siv Matomaa and Katja Kiiliäinen for their technical assistance. I wish to express my appreciation for my co-authors Tiina Mattila-Sandholm, Maria Saarela, Suvi Ojanen, Maija Tenkanen, Richard Fagerström. I thank also Satu-Maarit Heinonen, Herman Adlercreutz and Tarja Nurmi at University of Helsinki for collaboration in the lignan part of this work.

I warmly thank the entire staff at VTT Biotechnology and, especially, the carbohydrate team, Tuomo Kiutamo, Heljä Heikkinen, Päivi Myllärinen, Jaana Lehtinen, Riitta Partanen, Piia Hakala and Ritva Hartikainen for their important contributions in creating a pleasant working atmosphere.

Finally, I am deeply grateful to my family and my mother for their support and encouragement.

Espoo, June 2003

Sirpa Karppinen

List of original publications

The present thesis is based on the following publications, which will be referred to in the text by their Roman numerals. Additional unpublished data are also presented.

I Karppinen, S., Liukkonen, K., Aura, A.-M., Forssell, P. and Poutanen, K. 2000. *In vitro* fermentation of polysaccharides of rye, wheat and oat brans and inulin by human faecal bacteria. J. Sci. Food Agric., Vol. 80, pp. 1469–1476.

II Karppinen, S., Kiiliäinen, K., Liukkonen, K., Forssell, P. and Poutanen, K. 2001. Extraction and *in vitro* fermentation of rye bran fractions. J. Cereal Sci., Vol. 34, pp. 269–278.

III Crittenden, R., Karppinen, S., Ojanen, S., Tenkanen, M., Fagerström, R., Mättö, J., Saarela, M., Mattila-Sandholm, T. and Poutanen, K. 2002. *In vitro* fermentation of cereal dietary fibre carbohydrates by probiotic and intestinal bacteria. J. Sci. Food Agric., Vol. 82, pp. 781–789.

IV Karppinen, S., Myllymäki, O., Forssell, P. and Poutanen, K. 2003. Fructan content of rye and rye products. Cereal Chem., Vol. 80, pp. 168–171.

V Karppinen, S., Aura, A.-M., Virtanen, H., Forssell, P., Heinonen, S.-M., Nurmi, T., Adlercreutz, H. and Poutanen, K. 2003. Processing of rye bran influences both the fermentation of dietary fibre and the bioconversion of lignans by human faecal flora *in vitro*. J. Sci. Food Agric., submitted.

The author of the present thesis had the main responsibility for fractionation of rye bran, for carbohydrate analyses and for writing of all of the above publications, except for Publication III where the responsibility of the author concerned carbohydrate analysis. *In vitro* fermentation of rye bran and interpretation of the *in vitro* results were performed in association with Anna-Marja Aura and Dr. Kirsi-Helena Liukkonen. Lignan analyses were carried out by Satu-Maarit Heinonen at the University of Helsinki.

Contents

Appendices of this publication are not included in the PDF version. Please order the printed version to get the complete publication (http://www.vtt.fi/inf/pdf/)

List of abbreviations

SCFA = short-chain fatty acids

DF = dietary fibre

HPLC = high-performance liquid chromatography

DP = degree of polymerization

AOAC = Association of Analytical Communities

APC^{Min} mice = adenomatous polyposis colimultiple intestinal neoplasia mice, a genetically manipulated animal model of colon cancer

1. Introduction

This dissertation concentrates on the dietary fibre (DF) derived from rye. DF, in general, has many positive health effects in the human gastrointestinal tract, and many of the health effects are due to the microbial fermentation of DF carbohydrates in the large intestine (van Loo et al. 1999). However, future success in the development of DF-containing functional foods for the gut will depend on more fundamental research being carried out on gastrointestinal functions (Salminen et al. 1998).

In Finland, whole-grain rye has been traditionally used in bread. Rye, and especially rye bran, is a good source of DF, but information concerning its fermentation in the human colon is scarce. *In vitro* fermentation experiments with the human faecal inoculum used in this study allow predictions of the fate of DF of rye in the human colon.

1.1 The concept of dietary fibre

DF is a nutritional definition for the non-digestible part of plant food. The concept of DF was defined in 1972 as the skeletal remains of plant cells that are resistant to hydrolysis by the enzymes found in man (Trowell 1972). Later DF was redefined as plant polysaccharides and lignin, which are resistant to hydrolysis by the digestive enzymes of man (Trowell et al. 1976). According to the suggested new definition, oligosaccharides are also included in DF (Anonymous 2001a): “Dietary fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibres promote beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation.”

Because DF is not one compound but a class of different compounds with different physiological and health effects, more attention has been focused on the type or 'quality' of DF. For example, in 1995, the new concept ‘prebiotic’ was launched (Gibson and Roberfroid 1995): ‘A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the

growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health'. In addition to prebiotics, the effects of DF, among other things, on lipid metabolism, calcium absorption and colon cancer are currently being researched. Some of the health effects of DF may also be due to the bioactive compounds that are attached to DF and are, therefore, eaten along with DF.

Many analytical methods have been developed for the determination of the DF content of foods, as reviewed by McCleary (2001). Traditional methods are based on the definition 'non-digestible in the human small intestine'. In general, protein and starch (and also fat, if necessary) are enzymatically digested and the remainder is quantitated gravimetrically or by gas or liquid chromatography. The enzymatic-gravimetric methods are most frequently used: the official AOAC method for estimating total dietary fibre (AOAC 985.29) and the method of Asp et al. (1983) for estimating soluble and insoluble dietary fibre. For small molecules, such as fructan, specific methods have been developed because such molecules cannot be quantitated by traditional analysis. The official AOAC method (AOAC 997.08) and the method of McCleary et al. (2000) can be used for fructan.

1.2 Rye and cereals as sources of dietary fibre

Cereals are good sources of dietary fibre (DF). In Europe, 50% of DF, on average, is derived from cereals, 31% from vegetables, 16% from fruit and 3% from other sources (Cummings 1993). In Finland, cereals comprise as much as 58% of the total DF intake (Cummings 1993). The consumption of cereals has remained at the same level from 1993 to 2000, but the consumption of vegetables and fruit has increased (Lahti-Koski and Kilkkinen 2001). Whole-grain rye is a traditional grain in Finland and it is mostly eaten as bread. The most typical rye breads in Finland are whole-grain soft sourdough rye bread, and rye crisp bread, which can be produced with or without sourdough fermentation. In addition to whole-grain rye flour, refined wheat flour is also often used in breads nowadays. According to the 'Food balance sheet for food commodities 1999 and 2000' the average rye intake in Finland was 43 g/day (Anonymous 2001b), which corresponds to an intake of 7.7 g DF/day. The amount of DF originating from rye comprises about 33% of the total DF intake, which has been

at a level of 22–23 g/day (Mälkki et al. 1993, Valsta 1999). The recommended intake of DF is, however, greater (25–35 g/day, Anonymous 1998) so the intake of DF in Finland should be increased.

Grains consist of endosperm, germ, and bran. The endosperm comprises the main part of the whole grain. Bran consists of the outer parts of the grain, and depending on the milling process, the bran fraction recovery accounts for approximately 10–20% of the entire rye grain. Rye and wheat grains behave differently during milling (Weipert 1997). The main components of rye grain are the same as in other cereals: starch 57–66%, DF 15–17% and protein 7–13% (Nilsson et al. 1997a). Bran is the most DF-rich part of the grain (Table 1): the DF content of the whole rye grain is 13–17 g/100 g and that of rye bran is 35–49 g/100 g (Graham et al. 1988, Nilsson et al. 1996, 1997a,b, 2000, Bach Knudsen 1997, Härkönen et al. 1997, Glitsø and Bach Knudsen 1999). Endosperm, on the other hand, is rich in starch. Rye grain, in general, contains less starch and protein than wheat, but more DF and free sugars, including fructans (Åman et al. 1995). The DF content of wheat is 12–14 g/100 g (Graham et al. 1988, Bach Knudsen 1997), whereas wheat bran contains c. 45 g of DF /100 g (Bach Knudsen 1997). The large variation in composition of cereals depends on both the cultivar and the growing conditions (Åman et al. 1995). The higher DF content in rye compared to wheat reflects a higher content of cell walls within the endosperm (Åman et al. 1995).

The main DF components of rye bran, as also in whole rye, are arabinoxylan, cellulose, β-glucan, lignin, and also, according to the suggested new definition (Anonymous 2001a), fructan. In rye bran, the DF components form the rigid mainly insoluble structure of plant cell walls. Some of the polysaccharides are, however, also found as soluble components, and there is no sharp distinction between the soluble and insoluble fractions (Åman and Westerlund 1996). Depending on the processing and extraction conditions, some of the 'insoluble' DF complex can be extracted. In rye, as also in wheat, the thickness of the cell walls in different parts of the grain shows little variation between different varieties (Autio 2001).

Table 1. Dietary fibre contents of rye and wheat (g/100 g).

Dietary fibre content	**Reference**
wheat: 12 g/100 g rye: 15 g/100 g	Graham et al. 1988
rye bran: 38 g/100 g rye flour: 8.4 g/100 g	Nilsson et al. 1996
whole-grain rye: 16 g/100 g rye bran: 41 g/100 g	Nilsson et al. 1997a
rye grain (7 varieties): 15–17 g/100 g	Nilsson et al. 1997b
rye: 17 g/100 g rye bran: 35 g/100 g	Härkönen et al. 1997
whole-grain rye 17 g/100 g rye bran: 49 g/100 g wheat: 14 g/100 g wheat bran: 45 g/100 g wheat flour: 3,5 g/100 g	Bach Knudsen 1997
whole rye: 15 g/100 g pericarp/testa: 73 g/100 g aleurone: 28 g/100 g endosperm: 6.5 g/100 g	Glitsø and Bach Knudsen 1999
rye grain (4 varieties): 13–16 g/100 g	Nilsson et al. 2000
rye whole meal: 19 g/100 g	Boskov Hansen et al. 2002

The main component of rye cell walls is arabinoxylan, which consists of a β(1→4)-linked backbone of xylose in which 0–2 arabinose units are attached to each xylose unit. The mono- and double-substituted xylose residues have been shown to be present in different polymers or in different regions of the same polymer (Bengtsson et al. 1992). Two different polysaccharide structures of water-soluble arabinoxylans have been shown to be present in rye (Bengtsson et al. 1992). In arabinoxylan I, c. 40% of the xylose units are substituted at the 3-position by arabinose residues (Åman and Westerlund 1996). In arabinoxylan II, c. 70% of the xylose units are substituted with arabinose residues at both the 2- and 3-positions; however, no xylose unit is monosubstituted. The distribution of arabinose units along the xylan backbone has been shown to be non-random. In

some regions, substituted xylose units are clustered together and the other regions contain relatively few arabinose units and are, therefore, susceptible to hydrolysis by xylanases (Åman and Westerlund 1996). The arabinose:xylose ratio has been shown to be highest in the endosperm of rye (1.1–1.2) and lowest in the bran (0.6–0.7) (Delcour et al. 1989, Härkönen et al. 1997).

Cellulose, a β(1→4)-linked glucose polymer, is a minor component of rye. Mixed linked β(1→3,4)-glucan (β-glucan) is a water-soluble polysaccharide composed of glucose units. Fructan is a β-D-linked polymer of fructose containing a terminal glucose unit. The fructans of rye have been shown to have a higher degree of polymerization (DP) than those of wheat and barley. Seventy-eight percent of fructans in rye have a DP greater than 5 (Henry and Saini 1989). Medcalf and Cheung (1971) have shown that in wheat the primary D-fructose linkage was β-2,6, and that the other linkage type was β-1,2.

1.3 Physiological and health effects of dietary fibre

DF has many physiological effects throughout the entire gastrointestinal tract. These effects depend on the physicochemical properties of the DF, such as hydration properties, viscosity and capacity to absorb organic compounds (Guillon and Champ 2000). Most DF is ingested as plant cell-wall material. In the mouth, food is first masticated into small pieces and lubricated with saliva. DF increases salivation and dilutes food mass. A diet which contains a substantial amount of cell walls requires, in general, a longer time to eat (McDougall et al. 1996). The particle size of cell walls and, in turn, the DF may vary during transit through the digestive tract as a result of chewing, grinding in the stomach and bacterial degradation in the large intestine (Guillon and Champ 2000).

In stomach, DF activates both excretion of gastric acid and digestive hormones. DF also increases satiety and may decrease overeating and obesity (Cummings and Englyst 1995, McDougall et al. 1996). Soluble DF, such as β-glucan, increases the viscosity of the food mass in the stomach, which in turn slows gastric emptying (Edward and Parrett 1996). The presence of viscous polysaccharides impairs mixing of the food mass and can markedly affect the degree of contact of food substrates with the enzymes that digest them in the

small intestine (McDougall et al. 1996). In this way, DF may slow the rate of digestion of carbohydrates. Viscosity may slow down nutrient absorption by shifting the sites of absorption further along the digestive tract, as in the case of products with a low glycemic index when glucose absorption is slow. Isolated insoluble fibres, in turn, have very little effect on the absorption of nutrients (Edwards 1995, Gurr and Asp 1996). In the small intestine, DF also has a buffering capacity. In addition, DF can bind bile acids and also glycerides, fatty acids and phospholipids. By binding bile acids, DF may have an effect on blood cholesterol (Cummings and Englyst 1995). There are several possible mechanisms by which soluble DF may affect serum cholesterol (Edward and Parrett 1996). Water-soluble DF binds more bile acids than insoluble DF.

DF entering the large intestine (Figure 1) provides a substrate for the colonic microbiota (Hudson and Marsh 1995). Bacteriologically, the large intestine is an extremely complex ecosystem, which probably contains more than 400 different species of bacteria. During fermentation, the bacterial mass increases and consequently also the faecal bulk. The nonfermentable part of DF also adds to the faecal bulk (Figure 1). An increase in bulk in the large intestine results in a shorter transit time and prevents constipation. Large particles of plant cell walls have a greater effect on stool weight and higher faecal water output than fine particles (Brodribb and Groves 1978). The density of the microbial population in the large intestine amounts to 10^{11}–10^{12} cells per gram digesta. The human large bowel is about 150 cm long with a surface area of 1300 cm^2. Its contents, on average, amount to 220 g (58–908 g) of which bacteria constitute 40–55% in persons consuming Western-type diets. (Cummings and Macfarlane 1991)

Fermentation by microbiota results in the formation of a number of end products that influence large intestine physiology and metabolism. Short-chain fatty acids (SCFA) produced during fermentation reduce the pH and alter the balance of microbiota in the colon. SCFA are metabolized in different ways. Gases may cause flatulence and intestinal pain. In addition to the bulking effect, DF can bind and remove potentially harmful compounds in the colon. (Asp et al. 1993, Edwards 1995, Feldheim and Wisker 1995, McDougall et al. 1996)

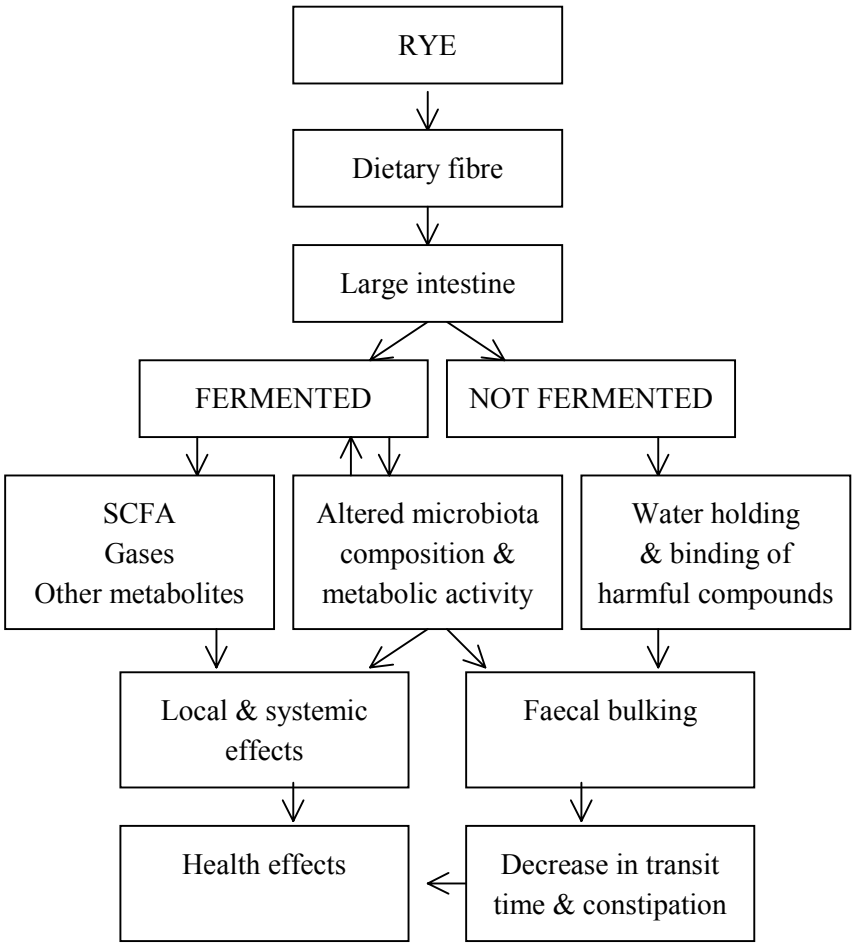


Figure 1. Possible effects of DF in the human large intestine.

Many review articles have been published containing data, which support the hypothesis that a diet rich in DF is associated with a decreased risk of cardiovascular disease and cancer (Reddy 1995, Hill 1998, Jenkins et al. 1998, Fuchs et al. 1999). In particular, the effects of whole-grain foods on cancer and heart disease risk have been reviewed (Jacobs et al. 1998, Anderson et al. 2000, Slavin 2000, Slavin et al. 2000). In Finland, independent of other risk factors, a greater intake of foods rich in fibre can substantially reduce the risk of coronary heart disease, and particularly coronary death, in middle-aged, smoking men (Pietinen et al. 1996). Rye bread has been shown to improve bowel function and decrease the concentrations of some compounds (bile acid, enzymes) that are putative colon cancer risk markers in middle-aged women and men (Gråsten et al. 2000). One mechanism by which DF may modulate carcinogenesis is related to the production of butyric acid produced during the colonic fermentation of carbohydrates (Russo et al. 1999).

1.4 Fermentation of dietary fibre

Many of the health effects of DF are related to the microbial fermentation of DF carbohydrates in the large intestine (van Loo et al. 1999). However, monitoring of fermentation in the human gut is very difficult. Different measurements can be made from blood and faeces, but they do not reflect the true situation in the colon. The residual DF after fermentation can be measured from faeces, but it does not give any information about the fermentation rate. SCFA produced in the fermentation are readily absorbed in the large intestine and, therefore, only part of the SCFA are found in faeces. Because of the limitations of clinical experiments, different *in vivo* animal models have been developed (Van Soest 1995). The rat (e.g. Nyman et al. 1986) and the pig (e.g. Glitsø et al. 1998) are the two main animals used in fermentation studies. However, clinical studies are time consuming and expensive, as are animal experiments, and, therefore, different *in vitro* models simulating conditions in the human gastrointestinal tract have been developed.

1.4.1 *In vitro* fermentation models

In vitro fermentation experiments performed under well-defined conditions offer appropriate means to measure fermentability of DF (Lebet et al. 1998a). In an *in vitro* study the consumption of polysaccharides and the production of SCFA and gases can be monitored as a function of time. Also, a large number of samples can be studied over a short time. Systemic effects, however, cannot be simulated by *in vitro* models. *In vitro* models can be divided into continuous, semi-continuous and batch models. However, because *in vitro* models are only predictive, the final experiments should always include studies with human subjects to document health effects.

Different *in vitro* models have been used to examine the consumption of DF and the production of SCFA. The production of gases can also be measured *in vitro*. Different experimental protocols have been reported. In general, fresh, pooled human faeces from healthy volunteers, who have been on a normal Western diet and have received no antibiotic treatment for 3 months, have been used in several studies (Englyst et al. 1987, Salvador et al. 1993, Casterline et al. 1997, Fardet et al. 1997, Lebet et al. 1998a). In another approach (Titgemeyer et al. 1991), the donors consumed >20 g DF/day for the 8 days prior to the onset of the experiment. The faeces were mixed anaerobically with the medium used in the experiment, filtered to remove particles and used immediately as the inoculum. The amount of inoculum varied from study to study. The amount of the substrate varies in general from 100 mg to 500 mg, and is hydrated overnight (Casterline et al. 1997, Lebet et al. 1998a, Wisker et al. 1998), but larger amounts have also been used (1.4 g) (Fardet et al. 1997). Also fermentation times have been variable: 3, 6, 12, 24 and 48 h (Titgemeyer et al. 1991); 6, 12 and 24 h (Salvador et al. 1993, Fardet et al. 1997); 0, 6, 24 and 48 h (Casterline et al. 1997); 0, 24 and 48 h (Wisker et al. 1998); and 0, 2, 4, 6, 8 and 24 h (Lebet et al. 1998a). Glitsø et al. (2000a) used faeces, which were collected from pigs that had adapted to a whole rye diet. In their experiment, 4–6 g substrate triplicates were incubated in a fermentor with 600 ml of faecal slurry for 0, 3, 7, 12, 24 and 48 h. The limitation of all these *in vitro* methods is that they are not standardized, which means that the results from different experiments cannot be compared.

Wisker et al. (1998) studied whether the extent of fermentation of non-starch polysaccharides in humans could be predicted by an *in vitro* batch system. The

results indicate that the *in vitro* batch system used could provide quantitative data on the *in vivo* fermentation of non-starch polysaccharides in mixed diets and some single fibre sources. An *in vitro* incubation time of 24 h was reported sufficient to mimic the non-starch polysaccharide degradation *in vivo*. Also, the good agreement between the *in vivo* and *in vitro* data in the study of Christensen et al. (1999) has demonstrated that the *in vitro* system is a useful method for predicting colonic fermentation *in vivo*.

The *in vitro* model used in this dissertation was the model tested by five European laboratories (Barry et al. 1995). In this model, the nutritive medium is a buffer solution containing different trace elements. The fairly high buffering capacity of the medium keeps the pH value within the physiological values found in the colon. Rather high concentration of faecal suspension (16.7 %, w/v) ensures sufficient degradation and conversion activities for samples. The amount of substrate is low (100 mg) because a greater amount would reduce the fermentation rate. In this model, the consumption of carbohydrates and the production of gases, SCFA and other metabolites are studied as a function of time. In the fermentation of carbohydrates, 24 h is generally adequate.

1.4.2 Fermentation of carbohydrates

DF carbohydrates are fermented in the colon. The amount of carbohydrates reaching the colon depends on the amount consumed. The recommended daily consumption of DF is 25–35 g (Anonymous 1998). Obviously, other carbohydrates that are not completely absorbed in the small intestine may also enter the colon (Guillon and Champ 2000, Conway 2001). In the colon, polymeric substrates are first degraded by the hydrolytic enzymes of the intestinal bacteria, and the released sugars are then fermented. The colonic microbiota and/or its activity may alter, depending on the nature of the substrate passing through. Many different bacterial species may be needed for hydrolysis and fermentation of a single complex polysaccharide (Macfarlane et al. 1992).

The fermentation of glucose can be described as follows (Levitt et al. 1995):

34.5 Glucose -> 48 Acetic acid + 11 Propionic acid + 5 Butyric acid + 58 Carbon dioxide + 95 Hydrogen + 10.5 Water

Acetic, propionic and butyric acids are the main SCFA that are formed during the colonic fermentation of carbohydrates. SCFA are formed in different molar ratios depending on the substrates available. SCFA are absorbed and metabolized in different ways that may have important implications for human health. Acetic acid is absorbed, transferred via circulation and metabolized in muscle, kidney, heart and brain tissues. Propionic acid is metabolized in the liver where it, among other things, may suppress cholesterol synthesis. Butyric acid is metabolized by the colonic epithelium; it regulates cell growth and differentiation, and thus it has been proposed to assist in maintaining a healthy mucosa (Cummings 1995). In addition to SCFA, hydrogen and carbon dioxide, as primary gas products, and methane or hydrogen sulphide, as secondary gas products, are produced. In the case of methanogenic bacteria, methane is also formed as an end product. Gases are excreted in breath or as flatus. Gases have been measured in only a few fermentation experiments (e.g. Marthinsen and Fleming 1982, and Lebet et al. 1998a).

The physical form, the three-dimensional arrangement of different components within the fibre and the physicochemical properties of DF have been suggested to be strongly involved in the degradability of different fibre forms by human faecal bacteria (Guillon et al. 1992). According to Edwards (1995), fermentation of carbohydrates is dependent on solubility, particle size, lignification, the induction of enzymes, and time.

Fermentation can be characterized by the rate, extent, and products of fermentation (Edwards 1995). The extent of fermentation and the profile of SCFA depend on the substrate (Salvador et al. 1993). The profiles of SCFA are different for carbohydrates and proteins. The rate of fermentation of a polysaccharide may be critical to its action in the colon. Slower fermentation produces SCFA in the distal colon and this may have a protective effect against colon carcinogenesis (Edwards 1995). Knowledge of both the rate and extent of fermentation and of the SCFA produced is, therefore, of great importance. Lebet et al. (1998b) showed that high amounts of starch mask the *in vitro* fermentation patterns of the fibre fraction. Therefore, when using an *in vitro* model, enzymatic digestion is needed to hydrolyse starch and protein before fermentation, or DF isolates, rather than whole foods, should be used to provide the best estimates of colonic SCFA production (McBurney et al. 1988).

1.4.3 Fermentation rate

The fermentation rate has been studied for both isolated carbohydrates and carbohydrates attached to the matrix, but there are not many studies that include cereal carbohydrates (Table 2). Englyst et al. (1987) suggested that in the large intestine a hierarchy of polysaccharide utilization may occur which may partly be a result of the physical and chemical characteristics of the individual polysaccharides. It has been shown that glucose is fermented faster than xylose (Barry et al. 1989), and glucose-containing soluble polysaccharides faster than xylose-containing polysaccharides (Englyst et al. 1987, Lebet et al. 1998a, Monsma et al. 2000). Non-substituted xylo-oligosaccharides and arabinoxylo-oligosaccharides were fermented more quickly than the more complex structures of xylo-oligosaccharides (Kabel et al. 2002).

Soluble fructans, inulin and fructo-oligosaccharides, which are composed of fructose units and are well documented to be prebiotic, were rapidly and completely fermented by human faecal bacteria (Roberfroid et al. 1998). The fermentation rate of fructo-oligosaccharides was dependent on the chain length; molecules with a degree of polymerization (DP) >10 were fermented, on average, only half as quickly as molecules with a DP <10 (Roberfroid et al. 1998).

Isolated starch and pectin are readily fermentable. They were fermented with human faecal inoculum faster than isolated arabinogalactan and xylan (Englyst et al. 1987). In a European interlaboratory study (Barry et al. 1995), five different isolated DFs were studied *in vitro* using a human faecal inoculum. Pectin and soybean fibre were fermented fastest and to the highest extent whereas cellulose and maize bran were fermented slowest and to the lowest extent. Fermentation of sugarbeet fibre was at an intermediate level. Pectin had the fastest fermentation rate and cellulose the slowest also in the study of McBurney and Thompson (1989), where DF digestibility differed significantly according to the fibre source. The resistant starch obtained from potato was fermented readily *in vitro* compared to the fermentation rate of the DF obtained from potato (Tschäppät et al. 1999). The fermentation rate of unmodified starch has been shown to depend on the source of starch. Wheat and rice starches were fermented fastest and completely after 12 h whereas other starches (kidney bean, rice, corn, amylopectin and amylose) were fermented completely after 24 h (McBurney et al. 1990).

Table 2. Different DF components and experimental variables (fermentation rates and/or production of SCFA) which have been studied.

Carbohydrate	**Study parameters**	**Reference**
Rye milling fractions	Rate+SCFA	Glitsø et al. 1998
Rye milling fractions	Rate	Glitsø et al. 1999
Rye milling fractions	SCFA	Glitsø et al. 2000a
Wholemeal rye bread, wheat-rye mixed bread	SCFA	Wisker et al. 2000
Oat DF	Rate	Bach Knudsen et al. 1993a
Oat bran, β-glucan-enriched oat fraction, insoluble oat residues	SCFA	Bach Knudsen et al. 1993b
Oat bran, wheat bran	Rate+SCFA	Monsma et al. 2000
Oat bran, wheat bran, corn fibre	SCFA	Bourquin et al. 1992
Wheat bran, sugar beet, maize, pea hulls, cocoa	SCFA	Salvador et al. 1993
Wheat bran, oat, carrot, cocoa, pea, inulin	SCFA	Roland et al. 1995
Oat bran, apple pomace, celery cell walls, pea hulls	Rate	Lebet et al. 1998b
Xylo-oligosaccharides, arabinoxylo-oligosaccharides	Rate+SCFA	Kabel et al. 2002
Oat fibre, corn bran, soy fibre, sugarbeet fibre, pea fibre, arabic gum, guar gum, apple pectin, citrus pectin	SCFA	Titgemeyer et al. 1991

Table 2. continues.

Carbohydrate	**Study parameters**	**Reference**
Oat, wheat, β-glucan, fig, soy, pea, apple, corn, pear, resistant starch, pectin	SCFA	Casterline et al. 1997
Maize bran, sugarbeet fibre, soybean fibre, cellulose, pectin	Rate+SCFA	Barry et al. 1995
Xylan, starch, arabinogalactan, pectin	Rate+SCFA	Englyst et al. 1987
Soy fibre, cellulose, pectin, tragacanth gum, psyllium gum, guar gum	Rate	McBurney & Thompson 1989
Potato fibre, potato resistant starch	Rate+SCFA	Tschäppät et al. 1999
Resistant potato starch	SCFA	Le Blay et al. 1999
Resistant potato starch, banana, wheat and maize	SCFA	Cummings et al. 1996
Starches	Rate	McBurney et al. 1990
Dextran, oligodextran, maltodextrin	Rate	Olano-Martin et al. 2000
Fructo-oligosaccharide, xylo-oligosaccharide, cellulose	SCFA	Campbell et al. 1997
Fructo-oligosaccharides	Rate	Roberfroid et al. 1998
Glucose, xylose	Rate	Barry et al. 1989
Mono- and disaccharides	SCFA	Mortensen et al. 1988

Fermentability of DF of plant foods may differ from that of isolated fibres. Soluble DF components, such as β-glucan and soluble arabinoxylan in cereals, are readily fermented (Bach Knudsen et al. 1997, Glitsø et al. 1999). Cell-wall polysaccharides that are not soluble in water are also known to be fermented in the large intestine, but at a slower rate than soluble polysaccharides because the complex cell-wall structure limits the accessibility of hydrolytic enzymes and bacteria (Lebet et al. 1998a, Glitsø et al. 1999). Insoluble DF, which is fermented only to a small extent, results in an increase in faecal bulk.

Bach Knudsen et al. (1993a) studied the digestibility of oat in experiments with ileum-cannulated pigs. Diets were prepared from oat groats, oat flour or oat bran. β-Glucan, which survived the small intestine, was degraded in the caecum and proximal colon while arabinoxylan was more slowly degraded. Four types of bread, based on rye milling fractions (whole rye, pericarp/testa, aleurone or endosperm) having different arabinoxylan structures, were fed to ileum-cannulated pigs (Glitsø et al. 1998, 1999). The endosperm arabinoxylan, which was water-extractable, was extensively and readily degraded in the caecum, whereas arabinoxylan of pericarp/testa, which was more complex, was not degraded in the intestinal tract of pigs. Arabinoxylan of aleurone was also degraded to a large extent but at a slower rate. So, in addition to solubility, the structural characteristics and links between polymers and lignin are also important for the fermentation rate (Glitsø et al. 1998, 2000a).

In *in vitro* fermentation studies using a rat faecal inoculum, regardless of the fibre source (wheat bran, oat bran), both the initial rate and maximum disappearance of glucose was significantly greater than those of arabinose and xylose (Monsma et al. 2000). Initial fermentation rates of arabinose and xylose were similar in the fermentation of both wheat and oat bran. The maximum disappearance of arabinose, but not of xylose, was significantly lower in the fermentation of wheat bran than in the fermentation of oat bran.

Treating the pericarp/testa of rye with alkali prior to incubation considerably increased fermentability and suggested that, at least in lignified tissue, alkali-labile cross-links were more important determinants of arabinoxylan degradability than the structure of arabinoxylan itself. Oxidative delignification increased fermentability too, but to a much lower degree, which indicates that

the type and extent of links between polymers and lignin rather than the amount of lignin are important in determining degradability (Glitsø et al. 2000a).

1.4.4 Fermentation metabolites

Short-chain fatty acids (SCFA) and gases are the main end products formed in the fermentation of carbohydrates. However, the formation of SCFA (Table 2) has been studied more than the production of gases. The molar ratio of acetic, propionic and butyric acids produced during fermentation, as also the gas composition, may vary from one fermentation to another depending, for example, on the substrate. Differences have been shown to depend on the monosaccharide composition of carbohydrate (Mortensen et al. 1988, Salvador et al. 1993). However, different pure monosaccharides (Mortensen et al. 1988) were shown to produce SCFA in different ratios than monosaccharides that were components of polysaccharides (Salvador et al. 1993). The difference in molar ratios may also be due to the interperson variation of the faecal inoculum. Interperson variation has been shown to occur both in the molar ratios of SCFA and in the amount of SCFA produced (Bourquin et al. 1992, Kabel et al. 2002).

Butyric acid and also propionic acid are the most interesting acids because of their possible health effects. However, very different estimates of SCFA production have been obtained. Butyric and propionic acids are produced in different quantities depending on the substrate, but also different amounts have been obtained with the same substrate (review of Cummings 1995). No significant differences in butyric acid proportions between pectin, soybean fibre, sugarbeet fibre, cellulose and maize have been shown, although total SCFA were produced in accordance with the consumption of carbohydrates (Barry et al. 1995). When comparing starch, arabinogalactan, xylan and pectin, it was shown that starch produced the most butyric acid and that arabinogalactan produced the most propionic acid (Englyst et al. 1987).

Arabic and guar gums have been shown to induce greater production levels of propionic and butyric acids than does apple pectin (Titgemeyer et al. 1991). Fermentation of DF fractions from fruits such as pear, apple and fig produced low amounts of butyric acid. In contrast, less acetic acid and more propionic and butyric acids were produced by fermentation of DF fractions from oat and soy.

Pure β-glucan produced propionic and butyric acids in higher amounts than did pectin, starch, and resistant starch (Casterline et al. 1997). The fermentation of the fibre fraction of potato led to higher propionic acid levels, but lower acetic-acid and butyric-acid production, compared to those from resistant starch of potato (Tschäppät et al. 1999). Resistant starch has also been studied *in vivo*. Twelve healthy volunteers ate resistant starch from potato, banana, wheat or maize. All forms of resistant starch increased faecal total SCFA excretion. Resistant starch from wheat and maize gave greater proportions of propionic acid in faeces than resistant starch from potato and banana, but in the case of butyric acid no differences were found (Cummings et al. 1996). Dextran and oligodextran have been shown to stimulate butyric-acid production (Olano-Martin et al. 2000).

Fermentative characteristics of different types of fibre have also been studied *in vivo* with rats (Roland et al. 1995, Campbell et al. 1997, Le Blay et al. 1999). Regarding the three major SCFA, rats fed on inulin had a large caecal concentration of butyric acid, while in the case of wheat bran and pea, the concentration of propionic acid was large (Roland et al. 1995). The fructo-oligosaccharide-containing diet has been shown to result in a higher caecal butyric acid concentration than the cellulose and xylo-oligosaccharide diets (Campbell et al. 1997). However, the total amounts of caecal SCFA were higher with fructo- or xylo-oligosaccharide-containing diets compared with control or cellulose diets (Campbell et al. 1997). Butyric-acid production was promoted also from the caecum of the rat towards the distal colon during a long-term consumption of potato starch, which suggests that a slow adaptive process occurs within the digestive tract (Le Blay et al. 1999).

In the fermentation of oat bran, the molar proportions of propionic/butyric acid were shown to be high (Titgemeyer et al. 1991, Bach Knudsen et al. 1993b, Casterline et al. 1997). In *in vivo* studies with ileum-cannulated pigs (Bach Knudsen et al. 1993b), the relative proportion of butyric acid in the luminal contents of the large intestine was greater in the case of oat bran and insoluble oat residues than in the case of wheat flour and the β-glucan-enriched fraction of oat. This means that the enhanced butyric-acid production was due to arabinoxylan, and not to β-glucan in the cell walls of oat bran. However, in the *in vitro* fermentation with rat caecal inocula, the source of fibre (oat bran/wheat bran) had no effect on the molar proportion of butyric acid (Monsma et al.

2000), although the amounts of butyric and propionic acids, produced during the fermentation were greater from the fermentation of oat bran than from that of wheat bran.

SCFA production in *in vitro* fermentation of rye has been studied by Glitsø et al. (2000a). Rye bread diets based on either whole rye or one of three rye milling fractions enriched in pericarp/testa, aleurone and endosperm were fed to cannulated pigs. Ileal effluent from the pigs was freeze-dried and fermented *in vitro* with faecal inoculum from pigs fed a whole-grain rye diet. The production of SCFA was highest from the diets high in fermentable carbohydrates (whole rye, aleurone and endosperm) and lowest in the fermentation of the pericarp/testa-containing diet. The *in vitro* molar distribution of acetic, propionic and butyric acids was largely similar between diets, in contrast to the *in vivo* results (Glitsø et al. 1998) where the pericarp/testa diet yielded more acetic acid between the caecal and rectal lumen than the other diets and, from the caecum onwards, butyric acid was significantly higher in the pigs fed on an aleurone-containing diet.

The type of bread, either as component of a human diet or as such, has an influence on the SCFA produced during *in vitro* fermentation (Wisker et al. 2000). Substrates in the study of Wisker et al. (2000) were DF residues obtained from specific diets and breads. The molar ratios of butyric acid were higher for the two high-fibre diets containing coarse or fine wholemeal bread than for the low-fibre diet containing wheat-rye mixed bread. The coarse wholemeal bread alone produced a higher molar ratio of butyric acid and a lower molar ratio of propionic acid than the fine wholemeal bread and the wheat-rye mixed bread. In an *in vivo* experiment, wholemeal rye bread increased faecal butyric acid content.

1.5 Prebiotic and bifidogenic carbohydrates

Prebiotic is a new concept launched in 1995: a **prebiotic** is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health (Gibson and Roberfroid 1995). Closely related to prebiotics are probiotics and synbiotics. A **probiotic** is a live microbial feed supplement

that beneficially affects the host animal by improving its intestinal microbial balance (Fuller 1989). A **synbiotic** is a mixture of probiotics and prebiotics (Gibson and Roberfroid 1995).

Any dietary ingredient that reaches the colon, e.g. non-digestible carbohydrates, some peptides and proteins, as well as certain lipids, is a candidate prebiotic. In order for a food ingredient to be classified as a prebiotic, it must according to Gibson and Roberfroid (1995):

- be neither hydrolysed nor absorbed in the upper part of the gastrointestinal tract,
- be a selective substrate for one or a limited number of beneficial bacteria commensal to the colon, which are stimulated to grow and/or are metabolically activated, especially lactobacilli and bifidobacteria (Gibson 1998, Roberfroid 1998),
- consequently, be able to alter the colonic microbiota towards a healthier composition,
- induce luminal or systemic effects that are beneficial to the host's health.

The Consensus Report from the year 1999 (van Loo et al. 1999) contains results from a European-Commission-funded project on non-digestible oligosaccharides. The prebiotic effect of non-digestible oligosaccharides has been studied by means of both *in vitro* and human *in vivo* experiments. The only prebiotic effect that has been fully demonstrated is the selective stimulation of growth of bifidobacteria. *In vitro* results are important, but they cannot be considered adequate for studying the complex ecosystem of the colon. In particular, results with pure cultures cannot be used to demonstrate a prebiotic effect. There is a need for more clinical studies to show the prebiotic and health effects of non-digestible carbohydrates. (van Loo et al. 1999)

Of the candidate prebiotics, the inulin-type fructans have been the most thoroughly investigated. According to the Consensus Report (van Loo et al. 1999), there is strong evidence, based on many different *in vivo* studies, that $\beta(2\rightarrow1)$-type fructans fulfil the prebiotic criteria. A limited number of human feeding studies indicate that galacto-oligosaccharides may have a prebiotic effect and a few animal studies indicate that soybean oligosaccharides may be prebiotics. Very little scientific information on the prebiotic effect of xylo-oligosaccharides and pyrodextrins is available. (van Loo et al. 1999)

In 1999, it was proposed to modify the definition of a prebiotic effect to the following: a prebiotic effect is a food-induced increase in numbers and/or activity predominantly of bifidobacteria and lactic acid bacteria in the human intestine (van Loo et al. 1999). The definition incorporates the bifidobacteria and the lactic acid bacteria because they are considered good biomarkers of a well-balanced intestinal flora. The health aspect is omitted from the definition, because, to date, no information is available which could support such a statement (van Loo et al. 1999). The current status of prebiotics was reviewed by Conway (2001). According to Conway, prebiotics need to be broadly classified based on microbiological and physiological functions. However, confirmation of effects in humans is needed from clinical studies. With the availability of a variety of prebiotics and probiotics, the potential exists for providing combinations targeted for special health-related benefits (Conway 2001).

The definition of prebiotics more or less overlaps with the definition of DF, with the exception of its selectivity for certain bacterial species (Schrezenmeier and de Vrese 2001). In the large intestine, prebiotics, in addition to their selective effects on bifidobacteria and lactobacilli, influence many aspects of bowel function through fermentation. SCFA are a major product of prebiotic breakdown, but as yet, no characteristic pattern of fermentation acids has been identified. Through stimulation of bacterial growth and fermentation, prebiotics affect bowel habit and are mildly laxative. (Cummings et al. 2001)

Many commercial carbohydrates and oligosaccharides have been reported to be bifidogenic (Kleessen et al. 1997, Bouhnik et al. 1997, 1999, Brighenti et al. 1999, Kruse et al. 1999), which may be one criterion for prebiotics. Most of the studies have been made with inulin, fructo-oligosaccharides or transgalacto-oligosaccharides. Okazaki et al. (1990) reported that xylo-oligosaccharides can selectively promote the growth of bifidobacteria. Esteves et al. (1999) produced oligomers from brewery's spent grain, a by-product of the brewing industry that has a high xylan content. *B. longum* fermented these oligosaccharides, even though the overall growth was poor compared with that obtained with oligofructose. Lactulose may be considered as a bifidogenic or lactogenic factor capable of promoting the growth of probiotic microbiota in the colon (Ballongue et al. 1997, Salminen and Salminen 1997).

After birth, the digestive tract of a child is rapidly colonized by bacteria. Bifidobacteria appear a few days after birth and become dominant (10^{10}-10^{11}/g stool) barely 1 week after birth. The number of bifidobacteria falls significantly in adult stools, particularly in those of the elderly. The proportions of the various species of the genus *Bifidobacterium* also vary with age and each age group has its characteristic species. (Ballongue 1998)

Bifidobacteria are known to have positive health effects. The potentially positive effects of bifidobacteria on human health include the following (Gibson and Roberfroid 1995):

- Bifidobacteria produce strong acids (acetic acid, lactic acid) as end products of fermentation. They lower the pH in the colon and may thus exert an antibacterial effect.
- An added effect of acid production is the protonation of potentially toxic ammonia and amines to produce NH_4^+, which is non-diffusible and thus lowers blood ammonia levels.
- Bifidobacteria produce some vitamins, largely of the B group, as well as digestive enzymes.
- Certain cellular components of bifidobacteria act as 'immunomodulators', i.e. they promote immunological response against malignant cells. This activation of the immune system will also contribute towards improved host resistance to pathogens.
- Bifidobacteria have been used to restore the normal intestinal microbiota during antibiotic therapy.

In addition to the prebiotic effect, fermentation of carbohydrates always produces also systemic effects. The effects of non-digestible carbohydrates among other things on lipid metabolism, calcium absorption and colon cancer have been studied. The results are mainly preliminary and partly incompatible. However, many of the results support each other by showing improved calcium absorption by using fructo-oligosaccharides (e.g. Ohta et al. 1998, Coudray et al. 1997). The review article of Scheppach et al. (2001) describes the beneficial health effects of low-digestible carbohydrate consumption.

1.6 Lignans

Plant lignans have been shown to contribute to the health-protective properties of whole-grain foods (Adlercreutz 2002). Plant lignans are one group of the phyto-oestrogens, which have been suggested to have, among other things, a protective effect against various cancers (Cassidy et al. 2000). Plant lignans are converted to mammalian lignans by bacterial fermentation in the human large intestine, producing enterodiol and enterolactone, which undergo enterohepatic circulation (Setchell et al. 1981). Enterolactone excretion and elevated enterolactone in plasma have been associated with a reduced risk of breast cancer and acute coronary events (Vanharanta et al. 1999, Pietinen et al. 2001, Hultén et al. 2002). Also, lignan-rich diets have been shown to inhibit colon cancer growth in test animals (Davies et al. 1999).

Plant lignans are abundant in flax. However, the low consumption of flaxseed in most human populations cannot explain the general occurrence of mammalian lignans in human tissues (Nicolle et al. 2002). In Finland and other Northern European countries, rye is one good source of lignans. Earlier studies showed that rye, and especially rye bran, contain matairesinol and secoisolariciresinol (Nilsson et al. 1997b, Mazur and Adlercreutz 1998). Four new plant lignans have been recently identified in rye (Heinonen et al. 2001): syringaresinol, pinoresinol, lariciresinol and isolariciresinol. A diet rich in rye bread has been shown to produce significantly higher serum levels of enterolactone than a diet containing white wheat bread (Juntunen et al. 2000).

It has been reported that during bacterial fermentation secoisolariciresinol is converted to enterodiol through several intermediates (Wang et al. 2000). Enterodiol in turn is oxidated to enterolactone by facultative aerobes (Borriello et al. 1985). Matairesinol is directly converted to enterolactone. It has been suggested that the metabolism of pinoresinol involves the formation of lariciresinol followed by secoisolariciresinol (Heinonen et al. 2001). Syringaresinol as a pure substrate was only partly converted to enterodiol/enterolactone and isolariciresinol was not converted at all (Heinonen et al. 2001). The metabolic pathway, however, has not been fully established.

The efficiency of enterolactone formation shows a large interindividual variation. This may largely be caused by differences in the activities of the

microbiota (Rowland et al. 1999, Adlercreutz 2002). Carbohydrates may also have a role in lignan conversion. Evidence from an *in vitro* fermentation model suggests that the conversion of plant lignans to their mammalian metabolites is enhanced by the presence of a high-carbohydrate substrate (Cassidy 1991). DF intake has also been shown to be positively correlated to the excretion of enterodiol in humans (Rowland et al. 1999).

1.7 Aims of the present study

The general objective of this study was to characterize the DF properties of rye using an *in vitro* fermentation method. To achieve this the chosen steps of the study were:

- To produce, based on solubility, rye-bran fractions with different DF properties.
- To characterize the fermentation rate of carbohydrates of different DF-rich rye-bran fractions.
- To assess the ability of bifidobacteria to use rye DF carbohydrates.
- To study the interactions of rye DF carbohydrates and lignan bioconversion by intestinal microbiota.

2. Materials and methods

A detailed description of the experimental procedures is presented in the original Publications I–V.

2.1 Raw materials

Commercial rye, wheat and oat brans were obtained from Melia Ltd, Raisio, Finland and were dry milled to a particle size < 0.5 mm (I, II). Commercial inulin, Raftiline HP (Orafti), was used as a reference material in the fermentation experiment (I). Wheat pentosan was obtained from Pfeifer & Langen (Germany) (II).

For fructan analysis, samples of Finnish rye cultivars (Akusti, Anna and Bor 7068) were kindly donated by Dr Simo Hovinen, Boreal Plant Breeding Ltd, Jokioinen, Finland. The cultivars were grown at the same locations in Jokioinen over the 3 years, and were harvested in 1998–2000. Commercially available rye products included whole-grain rye flour, light refined rye flour, rye flakes, rye crisp bread and soft rye bread (IV).

Commercially available pure carbohydrates were used as substrates for pure bacterial strains *in vitro*: D-glucose, D-xylose, L-arabinose, arabinoxylan (from rye; Megazyme), xylan (relatively unsubstituted, from oat spelt; Sigma), β-glucan (from barley; Megazyme), fructo-oligosaccharide (Raftilose P95; Orafti) and xylo-oligosaccharide (Xylo-oligo 70; Suntory).

2.1.1 Preparation of water-extractable, alkali-extractable and unextractable fractions of rye bran (II)

The water-extractable fraction was prepared on a pilot scale by extracting 10 kg milled rye bran with 100 l of water (Figure 2) modifying the method of Annison et al. (1992). Starch and protein were removed by α-amylase and protease treatment. After centrifugation, the water-extractable material was precipitated with 60% ethanol and the precipitate was finally air-dried. The water-unextractable fraction was extracted with an alkali (Annison et al. 1992, Bergmans et al. 1996), the solution was precipitated with 50% ethanol and the precipitate was finally air-dried. The unextractable residue was freeze-dried.

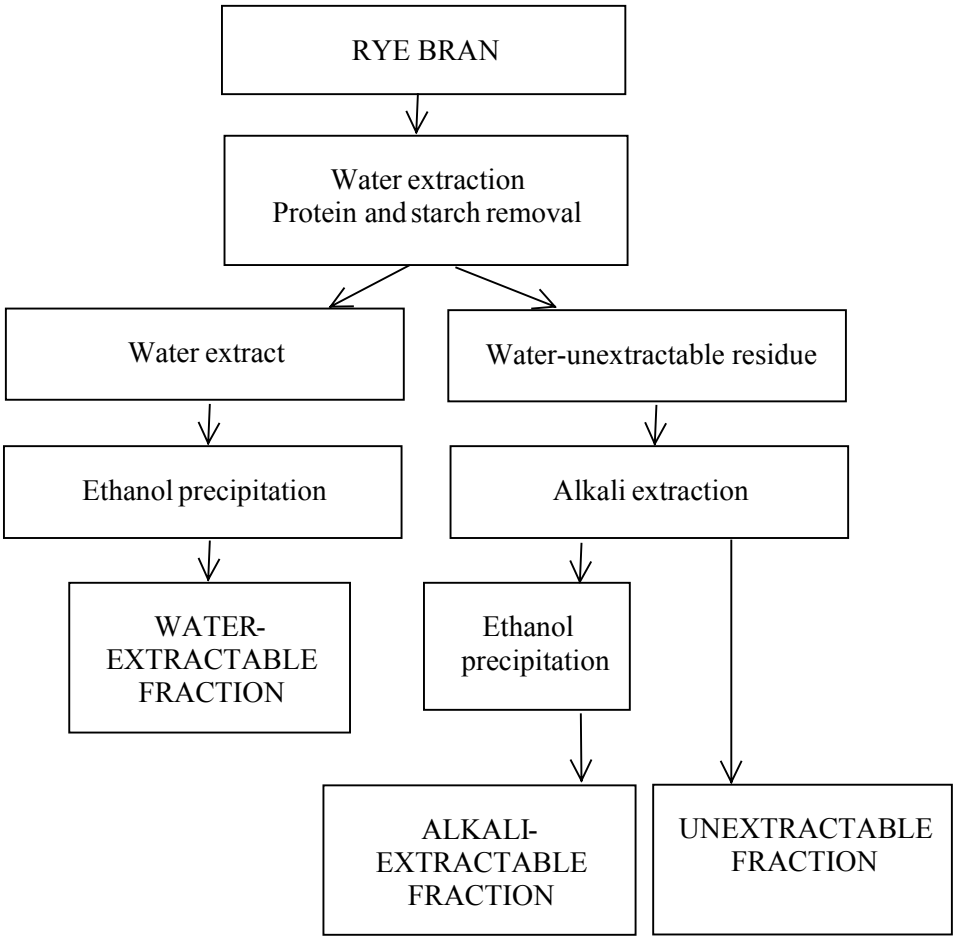


Figure 2. Schematic presentation of the extraction of water- and alkali-extractable rye-bran fractions.

2.1.2 Preparation of water-extractable rye bran concentrate (IV)

The water-extractable rye bran concentrate was prepared on a pilot scale (Figure 3) in order to get enough material for both *in vitro* and *in vivo* fermentation studies. Extruded rye bran (160 kg) was mixed with 1500 l water. Xylanase was added to increase the amount of soluble components. The solid material was allowed to cool and settle overnight and after that the supernatant was centrifuged, evaporated and lyophilized.

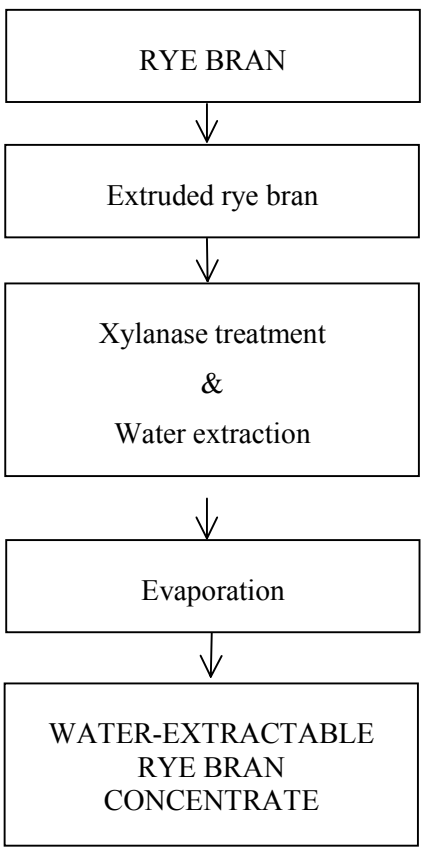


Figure 3. Schematic presentation of the preparation of water-extractable rye-bran concentrate.

2.1.3 Preparation of enzyme-treated rye bran and fractions (V)

Enzyme-treated rye-bran samples were prepared on a laboratory scale (Figure 4): 200 g of extruded rye bran and 2 l of water were mixed and xylanase was added. After centrifugation, fractions from five batches were mixed and lyophilized and the rye-bran extract (supernatant) and rye-bran residue (precipitate) were obtained. One batch from the enzyme treatment was frozen and lyophilized without centrifugation to obtain the xylanase-treated rye bran. All the samples were passed through a 0.5-mm sieve.

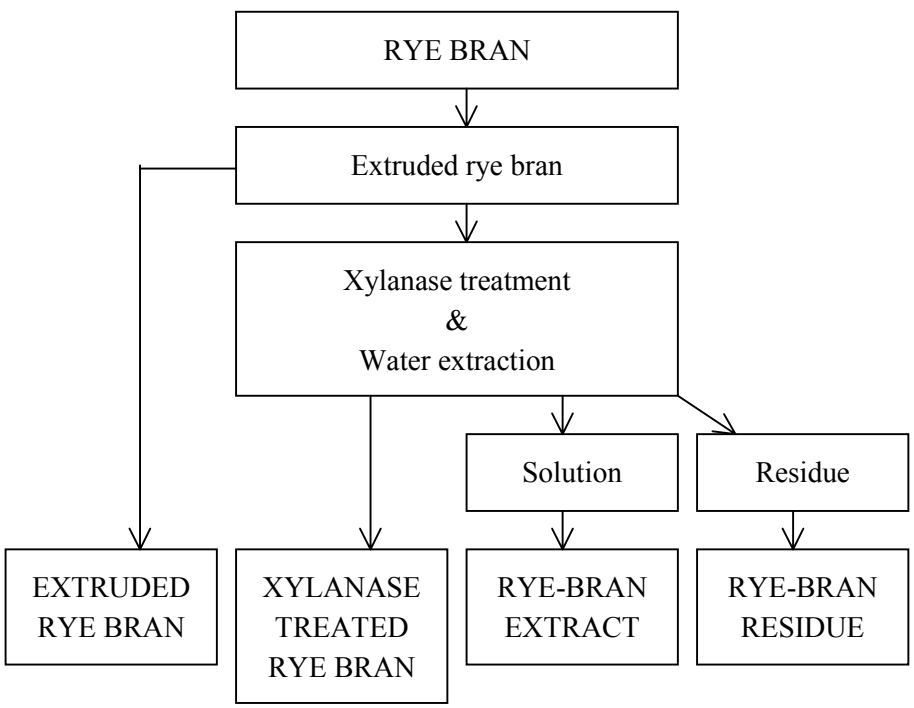


Figure 4. Schematic presentation of the preparation of xylanase-treated rye-bran fractions.

2.1.4 Digestion of substrates (I, II, V)

All the samples were digested before fermentation. Starch and protein in rye samples were partly removed by enzymatic digestion according to the method of Aura et al. (1999) (Figure 5). Samples were incubated first with salivary α-amylase at pH 6.5 (I, II) or pH 6.9 (V) at 37°C for 5 min simulating conditions in the mouth, secondly with pepsin at a pH below 2.5 at 37°C for 2 h simulating conditions in the stomach and finally with bile, pancreatin and mucin at pH values ranging from 6.5 to 7.5 for 3 h at 37°C simulating conditions in the small intestine (duodenum and jejunum). After that, samples were dialysed simulating the distal part of the small intestine (ileum) and finally freeze-dried.

2.2 *In vitro* fermentation (I, II, V)

2.2.1 Inoculum

Fresh faeces were collected from three (I, II) or four (V) healthy human volunteers, who usually consumed a normal diet, had no digestive disease and had not received antibiotics for at least 3 months. Freshly passed faeces were immediately taken in an anaerobic chamber and homogenized with the an equal weight of a buffer containing minerals. The slurry was diluted with the buffer to working concentration, filtered through a 1-mm sieve and used immediately as an inoculum.

2.2.2 Fermentation experiments

Fermentations were performed *in vitro* as batch fermentation under anaerobic conditions (Figure 6). One-hundred milligrams of each sample (I, V) were weighed into the fermentation bottles and wetted with 2 ml of the culture medium 1 day before incubation to reduce the lag in fermentation rate due to the wetting of the samples. In Publication II, the amount of digested rye bran was 130 mg and the amounts of the other substrates were adjusted so that the amount of arabinoxylan was the same in all the fermentations (water-extractable fraction 110.9 mg, alkali-extractable fraction 91.7 mg, unextractable fraction 90.9 mg,

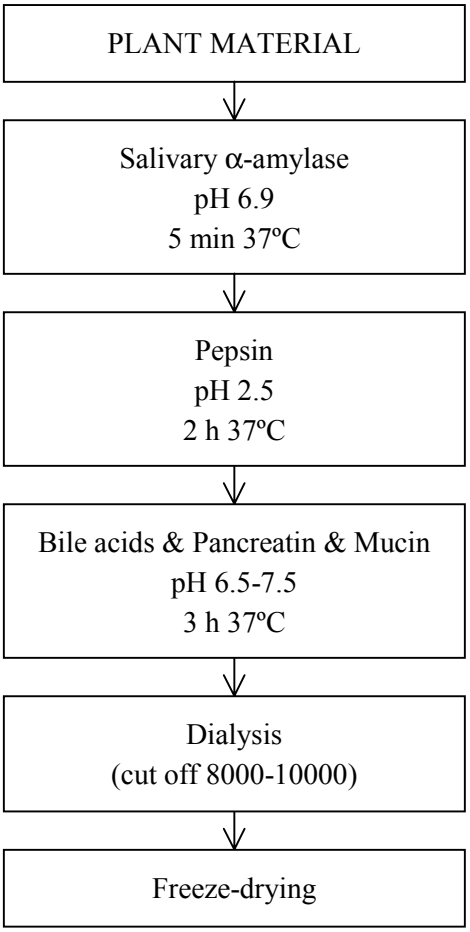


Figure 5. Schematic presentation of the in vitro *enzymatic digestion (Aura et al. 1999).*

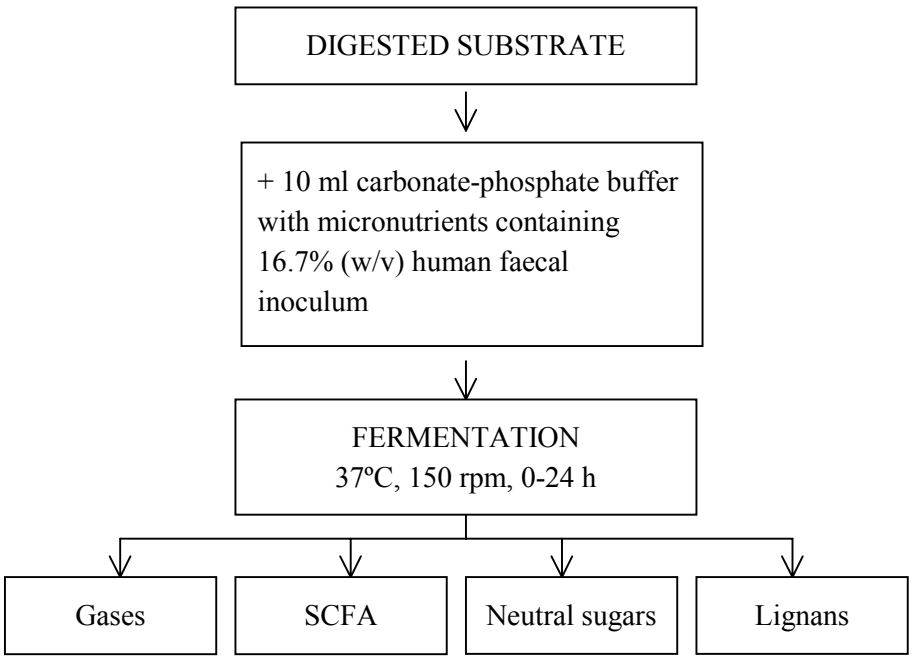


Figure 6. Schematic presentation of the in vitro *fermentation model.*

wheat pentosan 64.1 mg) while the glucose contents were very similar to each other except in the case of the alkali-extractable fraction. Another amount (127.8 mg) of alkali-extractable rye-bran fraction was fermented in an experiment where the sum of arabinose, xylose and glucose was the same as in rye bran.

In the first fermentation experiment with different cereal brans, 8 ml of a 20.8% faecal suspension were added to the pre-wetted samples (2 ml) to get a 16.7% faecal suspension (I). All the experiments were performed in triplicate. The bottles were closed tightly and incubated in a water bath at 37°C with magnetic stirring for 0, 2, 4, 6, 8 and 24 h. In the second fermentation study (II), 10 ml of a 16.7% faecal suspension were added directly onto non-wetted samples and the incubation was as in the first experiment. In this experiment, all the samples were fermented in duplicate in order to increase the number of substrates to be studied with the same inoculum. In the third fermentation experiment (V), 8 ml of a 12.5% faecal suspension were added to the pre-wetted samples to give a 10% faecal suspension which was thought to be a better concentration for the bioconversion of lignans. All samples were fermented triplicate. Incubation was carried out as in the first two fermentations except that samples were taken also

after 1 h. Faecal blanks, without the added substrate, were performed in all the fermentation experiments.

The total gas production (I, II) was measured immediately after removal of the bottle from the water bath. After the gas measurement, the fermentation was stopped by plunging the vials into iced water. The vial contents were mixed, the pH measured and the slurry was centrifuged. The supernatants were used for determination of SCFA and neutral sugars. The residues were dried after ethanol treatment, weighed and used for neutral sugar analysis (I, II). In Publication V, the samples were removed after the desired incubation time, placed on ice and poured into a tared centrifugation tube and rapidly frozen with liquid nitrogen. On the following day, the samples were thawn and the pH was measured. After centrifugation, the volume of the supernatant was measured and the precipitate was freeze-dried and weighed. The supernatant was used for the determination of SCFA, neutral sugars and mammalian lignans and the residue for the determination of neutral sugars and mammalian lignans (V).

Mean values were calculated from replicated determinations and they were expressed as a function of fermentation time.

2.3 Bacterial substrate specificity (III)

The ability of probiotic and other intestinal bacteria to ferment indigestible cereal DF components as well as their component monosaccharides was investigated. Different bacteria (III) were grown separately in duplicate fermentations in the appropriate base medium containing 5 g/l of carbohydrate. The level of bacterial growth was determined by measuring the optical density of samples taken immediately following inoculation and after 24 and 48 h of fermentation. Free monosaccharides, disaccharides and oligosaccharides in the fermentation sample were quantified by high-performance anion-exchange chromatography (HPLC). The concentration and monosaccharide composition of the xylan and arabinoxylan in fermentation samples were determined by analysis of neutral sugars after sulphuric acid hydrolysis.

2.4 Chemical analyses

Total starch, β-glucan and fructan contents were determined by specific enzymatic kits (Megazyme, Ireland). The pentosan content was measured by the colorimetric phloroglucinol method of Douglas (1981). The protein content was determined by the Kjeldahl method (6.25 x nitrogen). Dietary fibre was measured according to Asp et al. (1983) (I, II, V).

Fructo-oligosaccharides were quantitated by a HPLC method using 1-kestose, 1,1-kestotetraose and 1,1,1-kestopentaose (Megazyme) as standards (IV). Xylo-oligosaccharides were quantitated as fructo-oligosaccharides using xylobiose, xylotriose, xylotetraose and xylopentaose (Megazyme) as standards.

For optimal hydrolysis conditions with sulphuric acid, different times and temperatures were tested with commercial arabinoxylan (Megazyme) and rye bran. The best recovery was found by determining the neutral sugars (glucose, arabinose, xylose) in the following conditions: 25 mg substrate or dry insoluble residue from the fermentation experiment were weighed accurately into 25-ml hydrolysis tubes. One millilitre of 12 M H_2SO_4 was added and prehydrolysis was carried out for 2 h at room temperature. The main hydrolysis was carried out for 2 h in a boiling water bath after dilution to 1 M H_2SO_4. Hydrolysis of soluble polysaccharides was accomplished directly with 1 M H_2SO_4. For the fructose analysis, a sample was hydrolysed with 0.5 M HCl at 80°C for 20 min. These conditions were tested with inulin (Orafti). The hydrolysates were diluted to the level of 5-200 mg kg^{-1}, filtered through 0.45-μm filters and analyzed by HPLC with pulsed amperometric detection (Lebet et al. 1997). (I, II, V)

Gases (H_2, CO_2, H_2S, CH_4) were analysed isothermically at 30°C by gas chromatography using the static headspace technique (I, II). SCFA (acetic, propionic and butyric acids) were extracted with diethyl ether and analysed using gas chromatography (I, II, V). Plant lignans in rye samples were determined by a gas chromatographic-mass spectrometric method and mammalian lignans by a HPLC method.

2.5 Cell-wall staining

For the microscopic investigation of cell walls of rye bran, samples were prepared and stained according to Fulcher and Wong (1980) and Parkkonen et al. (1994). Calcofluor stained β-glucan in cell walls blue and acid fuchsin stained protein red.

3. Results and discussion

3.1 Dietary fibre content of rye bran (I, II)

Rye bran had a high dietary fibre (DF) content which varied between 37 and 39 g/100 g depending on the batch studied (Table 1 in Publications I and II). The content was in accordance with the earlier results of 35–41 g/100 g (Nilsson et al. 1996, 1997a, Härkönen et al. 1997). According to the earlier results (Härkönen et al. 1997, Nilsson et al. 1997a), bran was the richest DF part of the rye grain. Because of the high DF content, bran was selected to be the substrate for the *in vitro* fermentation studies instead of whole rye grain. In rye bran, the proportion of soluble DF of the total DF was 11–12%.

Rye bran was different from wheat and oat brans. In wheat bran, the DF content was higher (47 g/100 g) than in rye bran, and, in oat bran, the DF content was lower (20 g/100 g) (Table 1 in Publication I). Bach Knudsen (1997) has also shown the DF content of wheat bran to be high (45 g/100 g). In spite of the high DF content, the soluble proportion of the total DF content in wheat bran was only 4%. In the case of oat bran, as much as 34% of the total DF content was soluble.

The main fermentable DF polysaccharide in rye bran was arabinoxylan (pentosan) (19–23 g/100 g) of which 7% was soluble (I, II). In wheat bran, the pentosan content was higher than in rye bran, but the soluble portion of pentosan was only 3%. β-Glucan was a minor component in rye bran (3.5 g/100 g). In oat bran, β-glucan was the main DF carbohydrate analysed (9.5 g/100 g). Rye bran also contained much fermentable fructan (6.6–7.7 g/100 g) (I, II, IV) unlike wheat and oat brans (I). These differences in chemical compositions affect the solubility of DF and its carbohydrate components.

Before fermentation brans were digested enzymatically in conditions simulating those of the small intestine (Figure 1 in Publication I). Forty-two percent of the mass of rye bran was digested and discarded during dialysis. Most of the starch (91%) and a smaller part of the protein (51%) were hydrolysed. The recovery of pentosan after digestion and dialysis was 99% and that of β-glucan was 98%. In the case of fructan, the recovery was only 48% because of the small molecular weight of fructans. Most of the pentosan and β-glucan was recovered in the

digestion of wheat and oat brans (I). Fructan recovery was lower in the digestion of wheat bran (34%) than in the digestion of rye bran (48%). The difference in fructan recovery rates may be due to the molecular weight of fructans, which is lower in wheat than in rye (Henry and Saini 1989).

3.2 Fructan content of rye and rye products (IV)

The fructan content of three different cultivars of Finnish rye (Akusti, Anna and Bor 7068), harvested in 1998-2000, varied between 4.6 and 6.6 g/100 g (Table 1 in Publication IV). There were small differences between the cultivars, but the greatest differences were found between the harvest years, which apparently is due to the different growth conditions. The results in the present study showed higher fructan contents in rye grain than were recorded in some earlier studies (1.7–3.9 g/100 g) (MacLeod and Preece 1954, Henry and Saini 1989, Glitsø and Bach Knudsen 1999). Wheat in turn has been shown to contain 0.9 g fructan/100 g, barley 0.8 g/100 g and oat 0.1 g/100 g (MacLeod and Preece 1954, Henry and Saini 1989). However, Fretzdorff et al. (Fretzdorff, Kuhlmann and Betsche 2000, unpublished results) analyzed enzymatically the fructan content of 25 rye samples and found them to vary between 4.8 and 7.4g/100 g, which is in close agreement with the results presented here.

The fructan content of commercial whole-grain rye flour was 4.5 g/100 g (Table 2 in Publication IV), which was at the same level as in rye grains from the years 1999 and 2000. The fructan content of rye bran (6.6–7.7 g/100 g) (I, II, IV) was slightly greater than that of whole grain. This indicates that fructan accumulated in the outer parts of rye kernel; this was also reported by Glitsø and Bach Knudsen (1999). The fructan content of rye flakes was slightly lower, 3.7–4.2 g/100 g, and the lowest fructan content (3.1 g/100 g) was found in light refined rye flour, from which the outer parts of the grain were removed.

The fructan content of soft rye breads, analysed in this study, was 2.1–2.8 g/100 g (on a dry weight basis) and that of rye crisp bread was 2.2–2.6 g/100 g (Table 2 in Publication IV). Rye breads in Finland are usually made from whole-grain rye flour, but also wheat flour can be used which decreases the fructan content compared to wholemeal rye bread. Rye breads are usually made by the sourdough method, with or without added yeast. Boskov Hansen et al. (2002)

have shown that during the bread-making process the content of fructan decreased from 6.2 g/100 g in the rye wholemeal to 3.4 g/100 g in rye bread crumb. The differences between the breads analysed in this study may relate to the differences in the amounts of rye flour used, in the fructan content of the flours and in differences in the baking conditions.

According to the suggested new definition of DF, fructan is included in the total DF content of rye products. This means that the calculated total DF intake is about 10 g/day instead of 7.7 g/day as previously calculated.for the average rye intake per Finn (43 g/day, Anonymous 2001b). This means that the daily DF intake, c. 25 g/day, would be within the recommended level. In traditional soft rye bread, where 78 g rye flour are used per 100 g bread, the DF content is 14 g/100 g instead of 11 g/100 g, if fructan is included. In soft rye-wheat bread, where 51% rye has been used, the apparent increase is from 8.1 to 10 g/100 g. In rye crisp bread, the corresponding increase is from 16 to c. 18 g/100 g. In conclusion, because rye products are an important part of Finnish food intake, the fructan content of rye has a great impact on the total DF intake. The high fructan content also increases the known amount of readily fermentable carbohydrates in rye products.

3.3 Rye bran fractionation (II, IV, V)

3.3.1 Water-extractable, alkali-extractable and unextractable fractions (II)

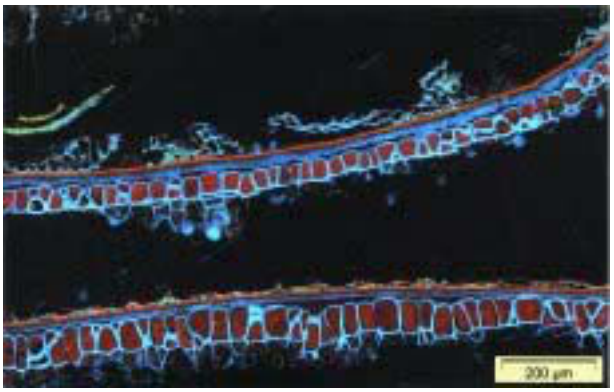
Three qualitatively different rye-bran fractions (water-extractable, alkali-extractable and unextractable) were prepared (Figure 2). Pilot-scale extraction was needed, because the content of extractable carbohydrates in rye bran was small. Annison et al. (1992) used a pilot-scale extraction method for water-extractable wheat pentosans and this method with minor modifications was used in this study. Because the purpose was to prepare qualitatively different fractions, but not necessarily pure products, this method was the most suitable and the easiest to perform. In the case of rye, only laboratory-scale extractions were reported until Delcour et al. (1999) extracted water-extractable arabinoxylans from rye on a pilot scale. The alkali-extraction in the current study

was performed by modifying the methods of Annison et al. (1992) and Bergmans et al. (1996) so that all alkali were food grade.

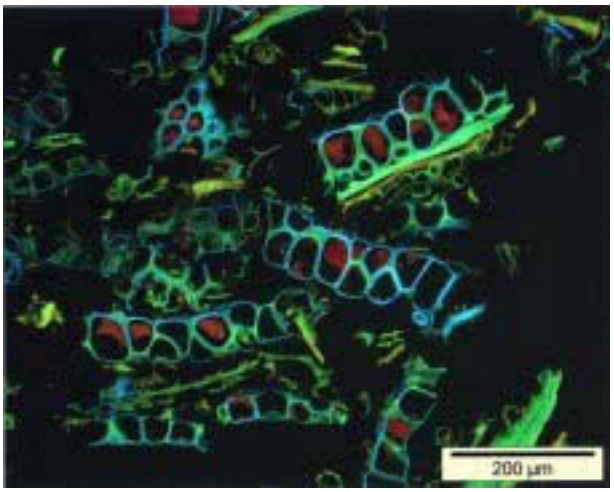
Rye bran was extracted with water after heat treatment and hydrolysis of the starch and protein. One kilogram of dry water-extractable fraction was obtained from 10 kg of rye bran. Under the microscope, it was observed that some small particles remained suspended in the water-extractable fraction because of the incomplete separation by the separator used. Alkali extraction was performed after water extraction, upon which some of the water-unextractable arabinoxylans were solubilized, and 0.3 kg of alkali-extractable fraction was obtained. The amount of the unextractable fraction was 6 kg.

Cell-wall structures of rye bran remained after extraction with water and alkali (Figure 7). In the micrograph of rye bran, large particles of cell walls were seen, and in the case of the unextractable fraction the large particles were split into differently sized pieces. In the unextractable fraction, some of the protein remained in the cells.

In all fractions, the total DF content was higher (50–75 g/100 g) than in rye bran (39 g/100 g) (Table 1 in Publication II). During the ethanol precipitation of water- and alkali-extractable fractions, all the small molecules (e.g. some of the fructans) were removed and this increased the DF concentration. In the case of the unextractable fraction, soluble components were washed away during the fractionation process. The same effect was seen also in the pentosan content: in all fractions the pentosan content was higher (25–37 g/100 g) than in rye bran (19 g/100 g). Thirteen percent of the pentosans in rye bran were obtained in the water-extractable fraction and 5% in the alkali-extractable fraction. The amounts were more than could be presumed according to the analysis of soluble pentosan. Therefore, the solubility/extractability is dependent on the extraction method. The β-glucan content of the fractions was 3–9 g/100 g and the recovery from rye bran was 19% in the water-extractable fraction and 3% in the alkali-extractable fraction. Most of both pentosan and β-glucan remained in the unextractable fraction. The fructan content was 0.9–8.1 g/100 g, the highest content being in the water-extractable fraction. However, because of the small molecular weight, most of the fructan was removed during ethanol precipitation (II).



a.



b.

Figure 7. Micrographs of a) rye bran and b) unextractable fraction of rye bran. Cell walls are seen as blue and protein as red.

After the enzymatic digestion of the substrates (Table 2 in Publication II), most of the pentosan (86–100%), β-glucan (84–100%) and fructan (60–100%) were recovered. The ratio of soluble to total pentosan increased during the enzymatic digestion. During the digestion, the most of the bran starch was hydrolysed (91%), whereas the protein was only partly hydrolysed (51%). Also, in the study of Hoebler et al. (1998), the removal of protein by *in vitro* enzymatic treatment was not very efficient, although enzymatic treatment correctly simulated the passage of DF through the digestive tract.

3.3.2 Water-extractable concentrate of rye bran (IV)

Extrusion and xylanase treatment were used as an aid to solubilize cell-wall structures and, in this way, to increase the recovery of soluble components. In the preparation of the water-extractable rye bran fraction (II), there were difficulties with the separation of the suspension because of the high viscosity of the solution. Xylanase treatment decreased the viscosity of the water-extract of rye bran, thus making the separation easier. In the preliminary laboratory experiments, extrusion and xylanase pretreatment produced an approximate 4-fold increase in the content of soluble pentosan (arabinoxylan and arabinoxylo-oligosaccharides) in the extract. (Figure 8.) The fructan content also increased because of the xylanase treatment, but the effect was not as great as in the case of arabinoxylan. A 9.4 kg quantity of water-extractable concentrate was obtained from 160 kg of rye bran (Figure 3). However, only about half of the rye-water suspension was separated; the extraction was, therefore, not quantitative. Food-grade xylanase was used in this extraction to construct a diet for mice. This material has been used in animal experiments to study the influence of diet on colon carcinogenesis (Oikarinen et al. 2003). Later, a similar extraction was performed on a laboratory scale (V) when also the rye-bran residue was freeze-dried. The fructan content of the water-extractable concentrate was as high as 23 g/100 g (Table 3 in Publication IV). The pentosan content was 28 g/100 g and the β-glucan content was 4.7 g/100 g. However, the DF content, according to the method of Asp et al. (1983), was only 11 g/100 g because of the large amount of small molecules which are not included in the classical DF determination. The total DF content of water-extractable concentrate of rye bran, calculated as a sum of pentosan, β-glucan and fructan, was 55g/100 g.

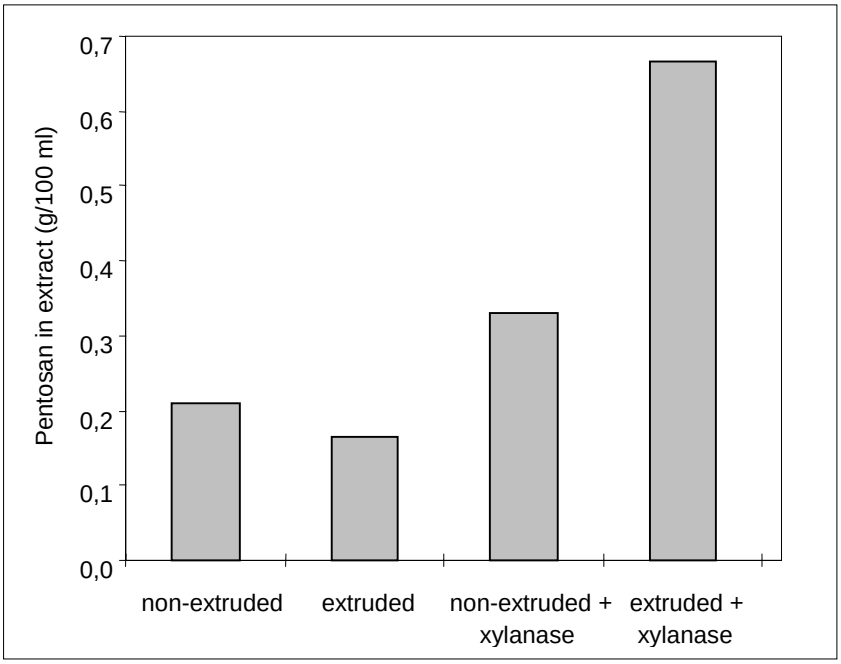


Figure 8. The effect of extrusion and xylanase treatment on the pentosan content in the water extract.

Three small fructo-oligosaccharides were quantitated from the water-extractable rye bran concentrate (Table 3). Commercial standards were available only for oligosaccharides with a degree of polymerization (DP) of 3–5. They comprised 33% of the total fructan content in the water-extractable concentrate of rye bran. In the case of whole grain, these three fructo-oligosaccharides formed 24% of the total fructan content. This is in close agreement with Henry and Saini (1989). The remainder of the fructan was apparently composed of longer oligosaccharides. The larger proportion (33%) of the three fructo-oligosaccharides of the total fructan content in the water-extractable concentrate than in rye grain (24%) may be due to the processing of rye bran – fructan may be hydrolysed during processing or only the smallest fructans may be solubilized.

Table 3. The content of analyzed oligosaccharides of water-extractable concentrate of rye bran (g/100 g dry weight). (IV)

	Fructo-oligosaccharides
1-kestose	6.0
1,1-kestotetraose	0.4
1,1,1-kestopentaose	1.0
	Xylo-oligosaccharides
xylobiose	5.2
xylotriose	2.4
xylotetraose	1.0
xylopentaose	0.7

The amount of the three fructo-oligosaccharides was 1.2 g/100 g in rye grain and 7.4 g/100 g in the water-extractable concentrate of rye bran. Campbell et al. (1997) and Hogarth et al. (2000) have quantitated the fructo-oligosaccharides of rye grain and they found these small fructo-oligosaccharides at levels of 0.4–1.2 g/100 g, which is in the same range as the results of the current study. The structures of fructo-oligosaccharides were not determined in this study. Structurally two kinds of fructans have been detected in wheat (White and Secor 1953, Medcalf and Cheung 1971).

Xylo-oligosaccharides were also quantified according to the available commercial standards (Table 3). The amount of xylobiose, xylotriose, xylotetraose and xylopentaose was about 9 g/100 g, which formed 33% of the total pentosan content. In addition to xylo-oligosaccharides, there were also arabino-xylo-oligosaccharides but no commercial standards were available for them. According to these results, the pentosan in the water-extractable rye bran concentrate was hydrolysed to a large extent.

3.3.3 Xylanase-treated rye bran and rye-bran fractions (V)

The same xylanase treatment as in the previous extraction (IV) was used in the laboratory-scale rye-bran fractionation (Figure 4) (V). Using this method, 207 g of rye-bran extract and 645 g rye-bran residue were obtained from 1 kg of rye

bran. Xylanase-treated rye bran was dried without any separation; it, therefore, contained both fractions in their original proportions. Subsequently, the samples were digested enzymatically *in vitro* to reduce the levels of starch and protein in the fermentation experiments.

The total content of non-digestible carbohydrates (DF + fructan) of the processed rye-bran samples was as follows: 51 g/100 g for extruded rye bran, 42 g/100 g for xylanase-treated rye bran, and 47 g/100 g for the insoluble rye-bran residue (Table 4). In the case of the rye-bran extract, the total content of non-digestible carbohydrates (fructan + pentosan + β-glucan) was 44 g/100 g. The pentosan content in all samples was 16–20 g/100 g. The pentosan content of the rye-bran extract on a laboratory scale (V) was slightly less than that obtained in the pilot-scale extraction (IV). The reason apparently is that in the pilot scale the xylanase was not denatured, so it had a longer time to split arabinoxylan. The recovery of pentosan in rye-bran extract was 17% of the total pentosan content in rye bran. This recovery was higher than was obtained in the preparation of the water-extractable fraction from rye bran (13%, II) because of the solubilizing effect of the xylanase treatment. The β-glucan content varied between 4.2 and 5.7 g/100 g and the recovery in the rye-bran extract was 28% of the total β-glucan content in rye bran, which was also higher than the recovery from rye bran without xylanase (19%) (II). Fructan was clearly concentrated in the rye-bran extract; its content was 22 g/100 g and its recovery was as high as 64%. The starch content in the rye-bran extract was only 6.5 g/100 g, while it was 20-24 g/100 g in the other rye-bran samples (Table 4).

After digestion the total content of non-digestible carbohydrates (DF + fructan) was 59 g/100 g for extruded rye bran, 51 g/100 g for xylanase-treated rye bran, and 49 g/100 g for rye-bran residue (Table 1 in Publication V). In the case of the rye-bran extract, the total content of non-digestible carbohydrates (fructan + pentosan + β-glucan) was 39 g/100 g.

Table 4. Components of the processed rye-bran samples (g/100 g dry weight).

	Rye bran			
	Extruded	**Extruded, xylanase-treated**	**Extract**	**Insoluble residue**
Dietary fibre*	44	35	12	44
*Soluble dietary fibre**	*4.5*	*2.9*	*12*	*1.5*
*Insoluble dietary fibre**	*39*	*32*	*0*	*43*
Pentosan	*20*	*20*	*16*	*20*
β-Glucan	*4.2*	*4.5*	*5.7*	*4.3*
Fructan	7.1	6.7	22	3.0
Starch	20	20	6.5	24
Protein	16	16	14	17
Fat	4.4	5.3	2.6	6.4
Ash	6.5	6.4	13	5.2

*According to Asp et al. (1983).

The highest content of plant lignans was in rye-bran extract (9 mg/100 g) and in the case of other substrates the content was 4–6 mg/100 g (Table 5). In all substrates, the main part of these plant lignans comprised syringaresinol (63–68% of the total lignans), of which, however, only a small part was convertible to enterodiol and enterolactone (Heinonen et al. 2001). The isolariciresinol content of rye-bran substrates was also high (12–19% of the total plant lignans), but has not been shown to be converted at all to enterodiol or enterolactone (Heinonen et al. 2001). The remainder of the plant lignans analysed in this study consists of secoisolariciresinol, matairesinol, lariciresinol and pinoresinol, which are precursors of enterodiol/enterolactone. During the digestion, the pinoresinol content was decreased in all substrates. After digestion, the rye-bran extract contained plant lignans at 10 mg/100 g and the other samples at 3–5 mg/100 g (Table 2 in Publication V).

Table 5. Plant lignan contents of the processed rye-bran samples, mg/100g dry weight.

	Rye bran			
	Extruded	**Extruded, xylanase-treated**	**Extract**	**Insoluble residue**
Secoisolariciresinol	0.13	0.15	0.32	0.13
Matairesinol	0.15	0.14	0.21	0.13
Lariciresinol	0.14	0.16	0.30	0.25
Pinoresinol	0.32	0.51	0.92	0.43
Isolariciresinol	0.84	1.16	1.12	0.68
Syringaresinol	3.02	3.87	6.17	2.75
Plant lignans, total	**4.6**	**6.0**	**9.0**	**4.4**

3.4 Fermentation of rye-bran substrates (I, II, V)

Samples to be compared were fermented with the pooled inoculum in the same fermentation experiment. Depending on the interperson differences in human colonic microbiota and, thus, in the metabolic activity of the bacteria, the fermentability of the same carbohydrate may vary with different inocula (Kabel et al. 2002). Digested rye bran was the common substrate in all fermentations performed in this study in order to make comparisons between different fermentation experiments.

The fermentation of the faecal inoculum is included in all the results of this study, because synergistic effects of the substrate and inoculum are difficult to predict in fermentation and they may be different for each substrate. The faecal inoculum contained undigested carbohydrates, which comprised virtually half of the total content of neutral sugars in the fermentations. Xylose and arabinose of the inoculum were not fermented, but glucose and glucose-containing polysaccharides were fermented to different degrees: 18% (II), 57% (I), and 75% (V) of the original glucose in the inoculum was fermented during 24 h

fermentation. This may be due to different microbiota, but also the type of glucose-containing polysaccharide may impact on the fermentability. For example, cellulose is an indigestible polysaccharide that has not been shown to be fermentable. In addition to cellulose, the inoculum also contained other, fermentable polysaccharides.

The fermentation progress was followed by measuring carbohydrate consumption (I, II, V), gas production (I, II) and SCFA production (I, II, V) as a function of time. Also, the bioconversion of plant lignans of rye bran was followed (V).

3.4.1 Fermentability of carbohydrates (I, II, V)

The rate and extent of fermentation were estimated as the consumption of carbohydrates by determining the neutral sugar content after sulphuric acid hydrolysis of fermentation residues as a function of fermentation time. The monosaccharide after the hydrolysis of β-glucan, resistant starch and cellulose was glucose, while arabinose and xylose were derived from arabinoxylan. In addition, galactose and mannose were liberated during sulphuric acid hydrolysis, but their amounts were very low and they had no significant effect on the total consumption of neutral sugars. Fructose was partly destroyed in sulphuric acid, so HCl hydrolysis was used to estimate the fructan content.

The figures of carbohydrate consumption and of SCFA production were reproduced in the following pages to allow direct comparisons of the different experiments (Figures 9–17) (I, II, V). All fermentations contained digested rye bran as the substrate in order to make a comparison between different fermentation batches. Rye bran was fermented at a slow rate, but the fermentation continued up to 24 h (Figures 9–11). From 45% (II) to 56% (I) of the neutral sugars (arabinose, xylose, glucose) of the whole fermentation were consumed. If the fermentability was calculated without the neutral sugars of the inoculum, the fermentability of the digested rye bran would be 62% (II) to 75% (I). Glucose was fermented at a faster rate than xylose (Figure 12) (I). This faster fermentation rate of glucose or glucose-containing polymers compared to xylose or xylose-containing polymers has also been reported earlier (Englyst et al.

1987, Barry et al. 1989, Lebet et al. 1997). Arabinose was fermented only to a very small extent (I).

Because the main part of the fermentable carbohydrates in the intact rye bran are attached to the cell-wall structures, they must first be released during fermentation and/or degraded enzymatically by colonic microflora and then consumed to produce SCFA and other metabolites (Hudson and Marsh 1995). The released sugars were consumed readily because they were not found in the fermentation solution (I).

The soluble fractions, made from rye bran by different methods, were fermented very fast when compared to rye bran (II, V). Most of the neutral sugars of the water- and alkali-extractable fractions of rye bran disappeared within the first 2 h of fermentation, as was case also for commercially available soluble wheat pentosan (Figures 10, 13) (II). The same was also shown in the fermentation of the rye-bran extract (Figures 11, 14) (V): all of the arabinose, xylose and, also, fructose were consumed to the level of the faecal blank within 2 h. The consumption of glucose was slower than that of other sugars, but it was also consumed to the level of the faecal blank within 24 h (Figure 14) (V). This was contradictory to the results of the fermentation of rye bran (I), where the consumption of glucose was faster than that of xylose. This may be due to the faecal inoculum. Sixty percent of the glucose in the fermentation of the rye-bran extract originated from the inoculum, and apparently the inoculum contained glucose-containing polymers, which were not readily fermented and, also, polymers which were not fermented at all, for example, cellulose.

The unextractable fractions of rye bran (II, V) were fermented at a slower rate than the extracted, soluble fractions (Figures 10, 11). This was also seen in the formation of gases and SCFA. The fermentation rate of the unextractable fraction was similar to that of rye bran, because most of the carbohydrates in the rye bran and the unextractable fraction were the same. Micrographs were prepared from the fermentation suspension of the unextractable fraction at the beginning of fermentation and after 24 h fermentation (Figure 18). At the beginning of fermentation, cell walls were observed but during the fermentation the cell-wall structures were destroyed.

There are only a few published articles concerning the fermentation rate of rye (Table 2). Knowledge about the fermentation of rye in the human colon is nationally important, because rye is widely eaten in Finland. In general, the effect of cereal carbohydrates in colonic fermentation in humans has not been sufficiently examined. Glitsø et al. (1998, 1999) studied *in vivo* fermentation of different milling fractions of whole rye grain in the large intestine of pigs. The water-extractable arabinoxylan of the endosperm of rye was extensively and readily degraded in the caeca of pigs, whereas the outer parts of the grain (bran) was fermented at a slower rate and the outermost part not at all. In this *in vitro* study, however, human faecal inocula has been used in order to predict the fate of carbohydrates in human colon.

Xylanase treatment slightly increased the fermentation rate of rye bran making cell-wall structures more accessible for colonic microflora (Figure 11) (V). However, enzymatic treatment did not change the extent of fermentation. Xylanase treatment only partly hydrolysed cell walls, because the fermentation pattern of extruded, xylanase-treated rye bran was very similar to that of extruded rye bran. The increase in the fermentation rate was, at least, partly due to solubilized xylo-oligosaccharides and arabinoxylo-oligosaccharides formed during the enzyme treatment, which in turn were more readily fermented than polysaccharides. After 24 h fermentation, 67–71% of the neutral sugars in the fermentations (including the inoculum) of rye bran, extruded rye bran, extruded and xylanase-treated rye bran and rye-bran residue were consumed (V), which was slightly more than in the fermentation of unprocessed rye bran in the first two fermentation experiments (45–56%) (I, II). The difference was due to the different inocula, not to the processing, because the fermentability of rye bran varied in these experiments. In the case of the rye-bran extract, 81% of the neutral sugars in the whole fermentation were consumed, after which the residual content of the neutral sugars was at the level of the inoculum (V).

In summary, and with regard to the rye-bran fermentations, it can be said that at least three properties affect the fermentability of rye bran in addition to the effect of the bacterial composition and the activity of the inoculum: 1) its chemical composition, 2) the presence of quickly fermented soluble carbohydrates, and 3) the structure of the plant cell walls. In addition, the matrix of rye bran cell walls also impact on the extent of fermentation. It is dependent on the type and extent of linkages between polymers in cell walls, e.g. between arabinoxylans and

lignin (Glitsø et al. 2000a). By changing of rye cultivars or for example by genetic modification of rye different results may be obtained.

The main fermentable component of rye bran was arabinoxylan. The arabinose: xylose ratio at the beginning of the fermentation was 0.51. During the fermentation, more xylose was consumed than arabinose, and after 24 h the arabinose:xylose ratio increased to 1.2 (I). In the second fermentation of rye bran (II), the increase in the arabinose:xylose ratio was similar, from 0.57 to 1.1. This indicates that the unfermented arabinoxylan, the main part of which came from the inoculum, had a branched structure that was highly resistant to hydrolysis and fermentation and so remained unused by colonic microflora. In *in vivo* experiments (Glitsø et al. 1998, 1999), pericarp/testa arabinoxylan, which had a high degree of xylose substitution, remained undegraded in the large intestine of pigs. Aleurone arabinoxylan, characterized by a very low degree of substitution, was extensively degraded but at a slower rate.

The arabinose:xylose ratios were higher in the rye-bran extract (V) and the water-extractable rye-bran fraction (II) than in rye bran. In the endosperm of rye, the arabinose:xylose ratio has been shown to be 0.71–0.83 and in the aleurone 0.35–0.57 (Glitsø and Bach Knudsen 1999). In commercial rye bran, there may be endosperm residues which are easily solubilized during extraction. In the fermentations of xylanase-treated samples (V), the arabinose:xylose ratio was increased more extensively than in the fermentation of rye bran (I, II). This may mean that the xylanase treatment has modified the structure of arabinoxylan making it more easily fermented.

Fermentation of rye bran was different from that of wheat or oat bran (Figure 9) (I). The fermentation rate of rye bran was rather similar to that of wheat bran. The arabinose: xylose ratio at the beginning of the fermentation was 0.51 for rye-bran fermentation and 0.71 for wheat-bran fermentation, which means that, in wheat bran, the xylan backbone was more substituted than in rye bran. The extent of fermentability of rye bran was different from that of wheat. Fifty-six percent of the original neutral sugars (arabinose + xylose + glucose) in the fermentation of rye bran were consumed, but in the fermentation of wheat the consumption was only 41% (I). The differences in the composition and chemical structure of rye and wheat brans, and also the differences in the solubilities of carbohydrates may be reasons for different extents of fermentation.

The fermentation rate of oat bran was faster than that of rye and wheat bran (Figure 9) (I). The fermentation rate was faster despite the fact that there was a reduced amount of fermentable carbohydrates at the beginning of the fermentation. The faster fermentation rate of oat bran was best shown in the production of gases and SCFA. The faster fermentation rate of glucose may be a reason for the faster fermentation of oat bran, which is rich in glucose-containing polysaccharides (β-glucan), compared to the fermentations of rye and wheat bran, which are rich in arabinoxylan. Sixty-four percent of the original neutral sugars (arabinose + xylose + glucose) of oat bran fermentation were fermented during 24 h (I).

The fermentation rates of rye, wheat and oat brans were very different from that of commercial inulin. Inulin was consumed at a faster rate than cereal brans, and 99% of the original inulin was consumed during the first 4 h (I). The same cereal brans and inulin studied in this dissertation were also used as substrates for the APC^{Min} mice and it was shown that rye bran prevents the formation of intestinal polyps in APC^{Min} mice, but inulin did not have any significant effect (Mutanen et al. 2000). Later it was shown that fractionating of rye influences adenoma formation in mice (Oikarinen et al. 2003). The main result regarding adenoma formation was the observation that the soluble extract promoted the number and growth of adenomas in the distal small intestine when compared with the non-fibre group, but the effect was not mediated by plasma enterolactone levels or the production of bifidogenic bacteria in Min mice.

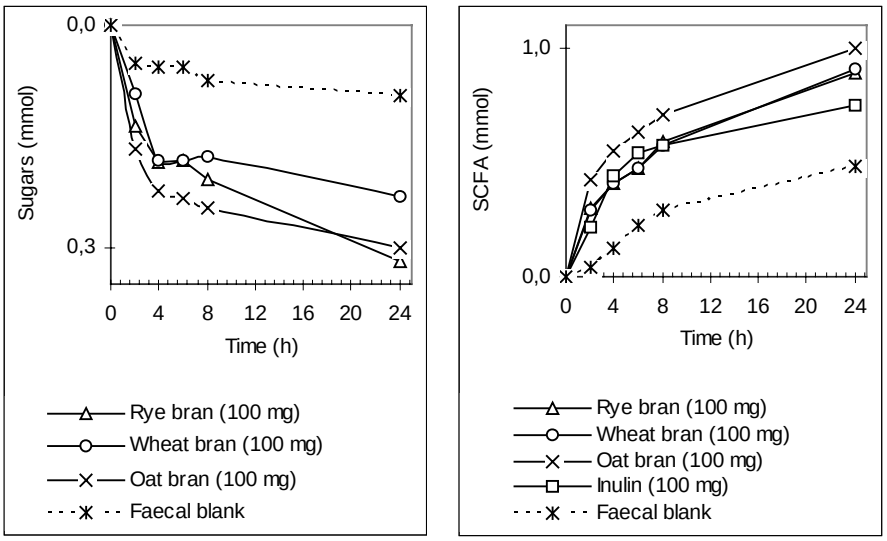


Figure 9. ***Consumption*** *of the neutral sugars (arabinose+xylose+glucose) and* ***production*** *of SCFA (acetic+propionic+butyric acids) in the fermentations of cereal brans (I). The amount of substrate is in the brackets.*

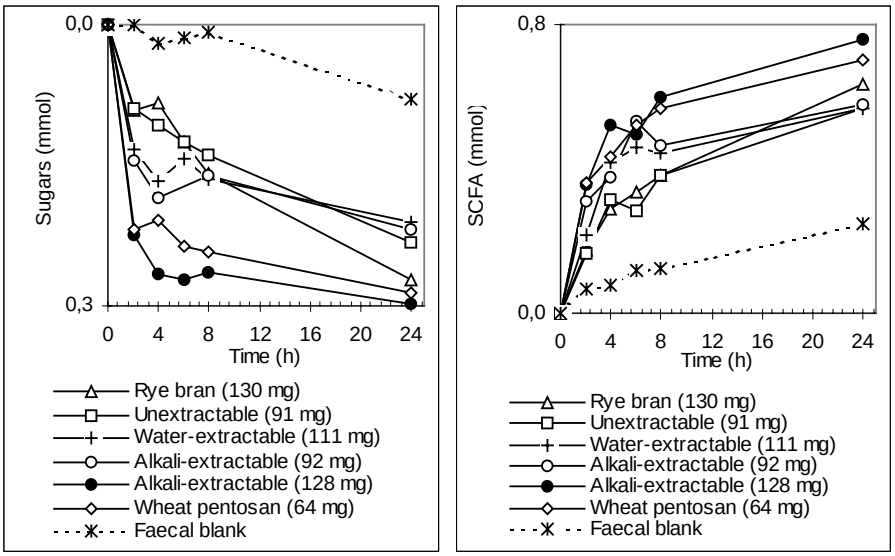


Figure 10. ***Consumption*** *of neutral sugars (arabinose+xylose+glucose) and* ***production*** *of SCFA (acetic+propionic+butyric acids) in the fermentations of rye bran, water- and alkali-extractable fractions, unextractable fraction and wheat pentosan (II). The amount of substrate is in the brackets.*

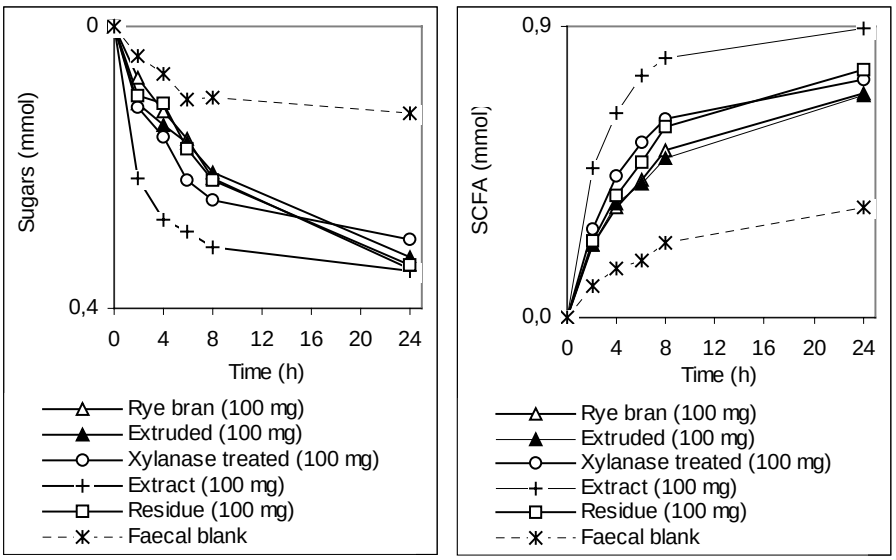


Figure 11. ***Consumption*** *of neutral sugars (arabinose+xylose+glucose) and* ***production*** *of SCFA (acetic+propionic+butyric acid) in the fermentations of rye bran, extruded rye bran, xylanase-treated rye bran, rye-bran extract and rye-bran residue (V). The amount of substrate is in the brackets.*

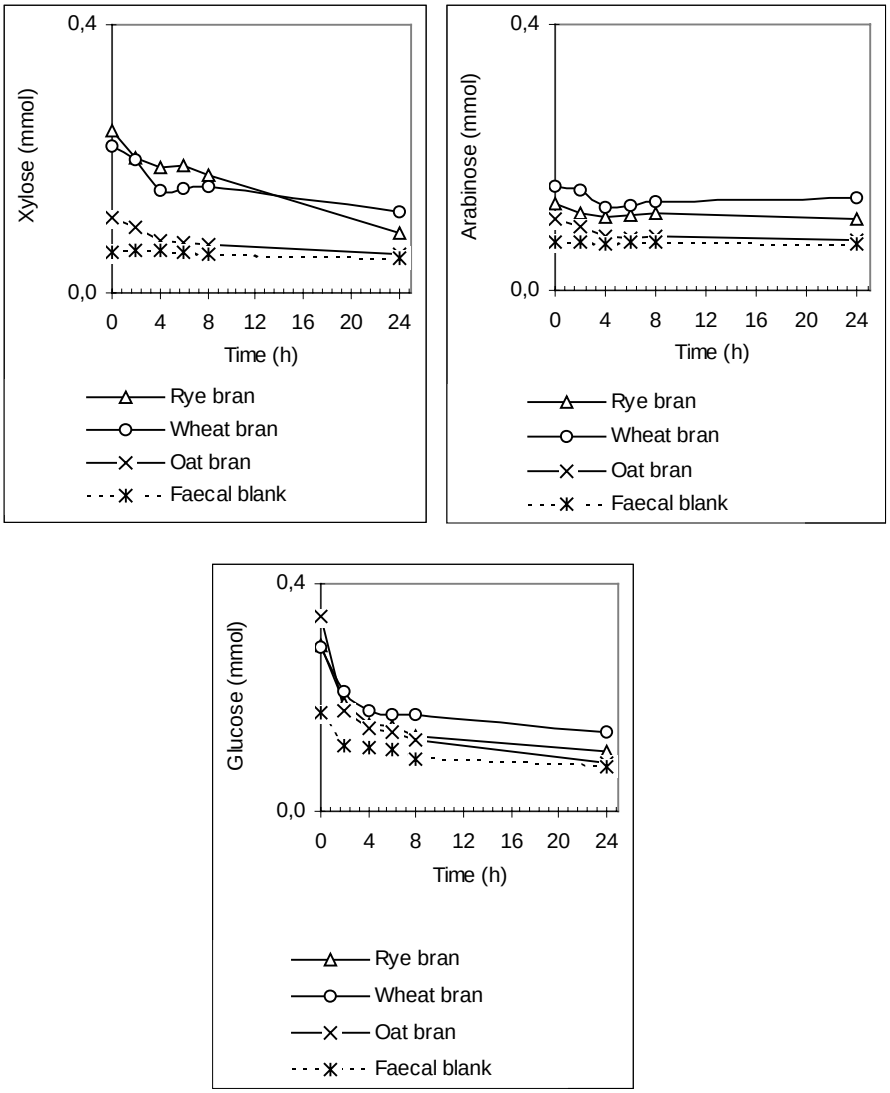


Figure 12. ***Residual*** *xylose, arabinose and glucose in the fermentations of cereal brans (I).*

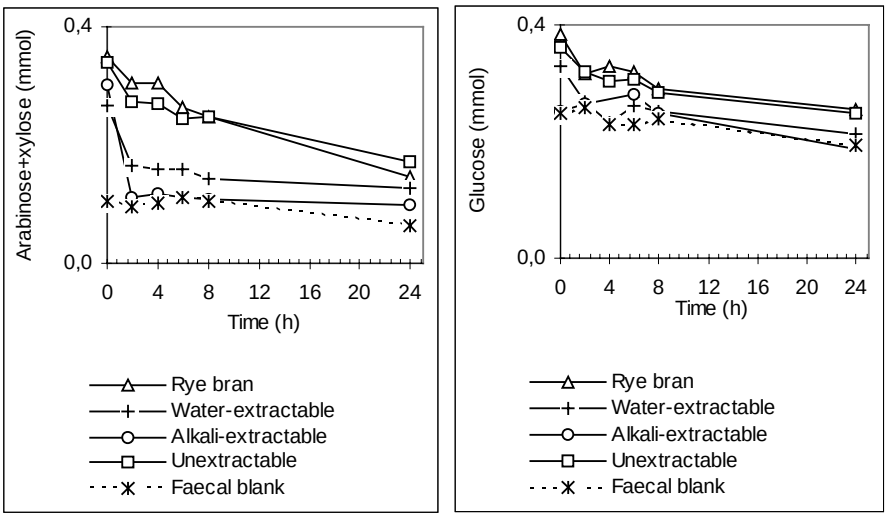


Figure 13. ***Residual*** *arabinose+xylose and glucose in the fermentation of enzymatically digested rye bran, water- and alkali-extractable and unextractable rye-bran fractions (II).*

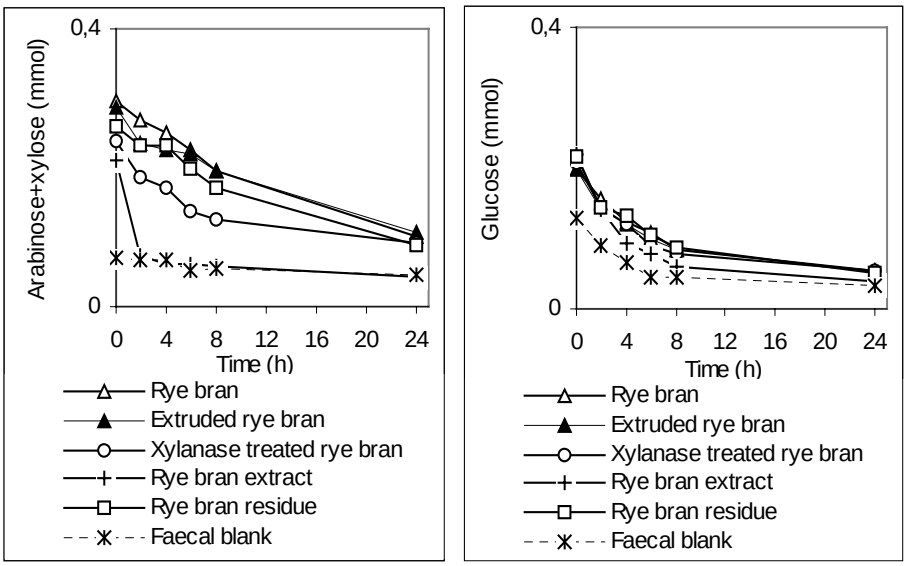


Figure 14. ***Residual*** *arabinose+xylose and glucose in the fermentations of rye bran, extruded rye bran, xylanase-treated rye bran, rye-bran extract and insoluble rye-bran residue (V).*

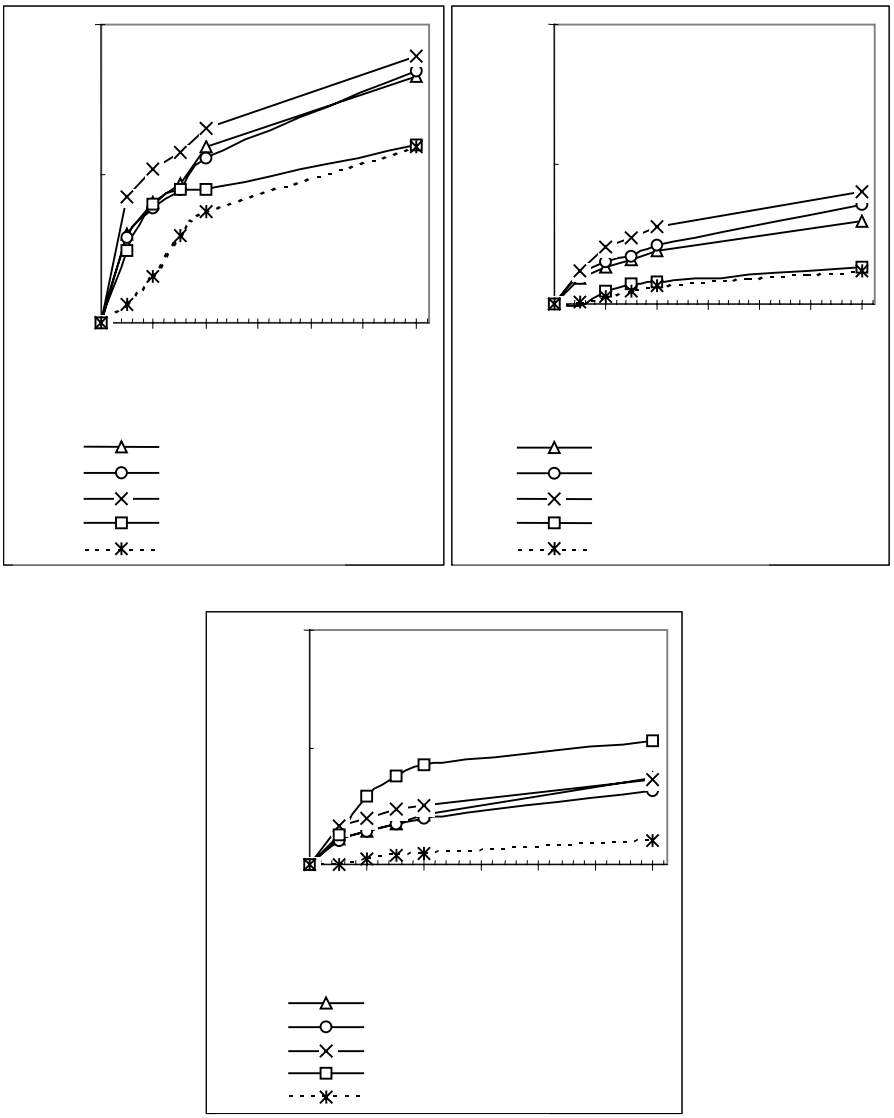


Figure 15. ***Production*** *of acetic, propionic and butyric acids in the fermentations of cereal brans and inulin as a function of time (I). Inulin was used without digestion.*

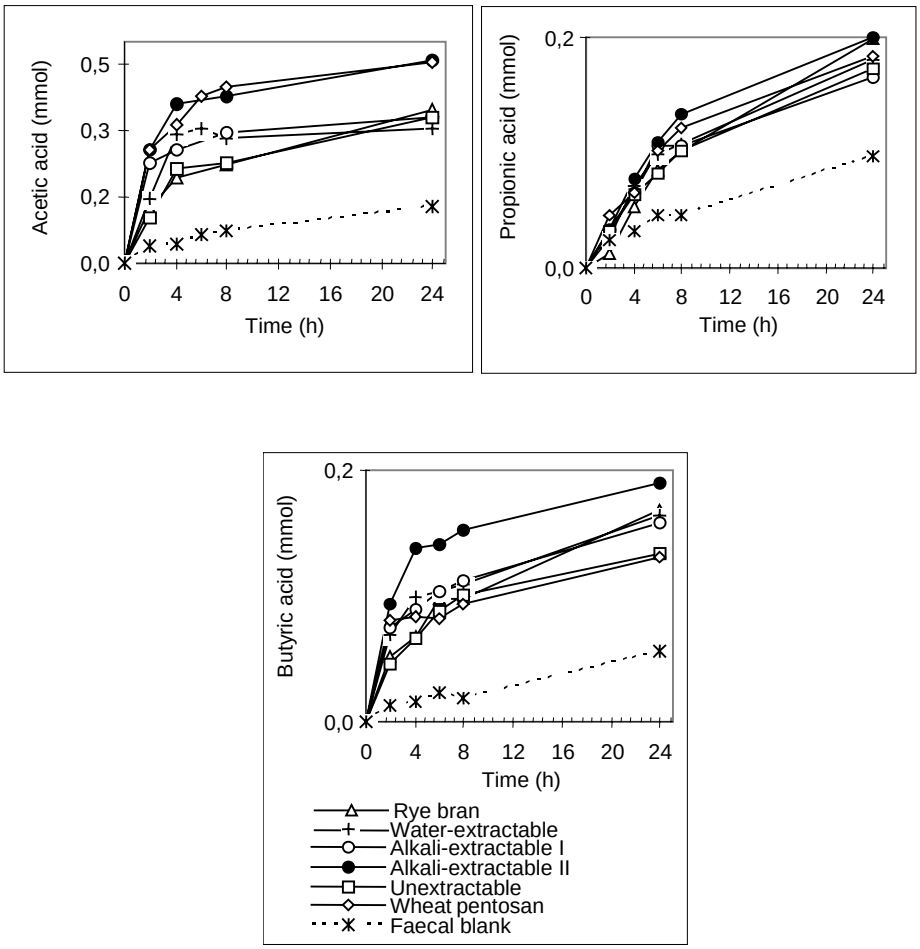


Figure 16. ***Production*** *of acetic, propionic and butyric acids in the fermentation of rye bran (130 mg), water- (111 mg) and alkali-extractable (I: 92 mg; II: 128 mg) and unextractable (91 mg) rye-bran fractions and commercial wheat pentosan (64 mg) (II).*

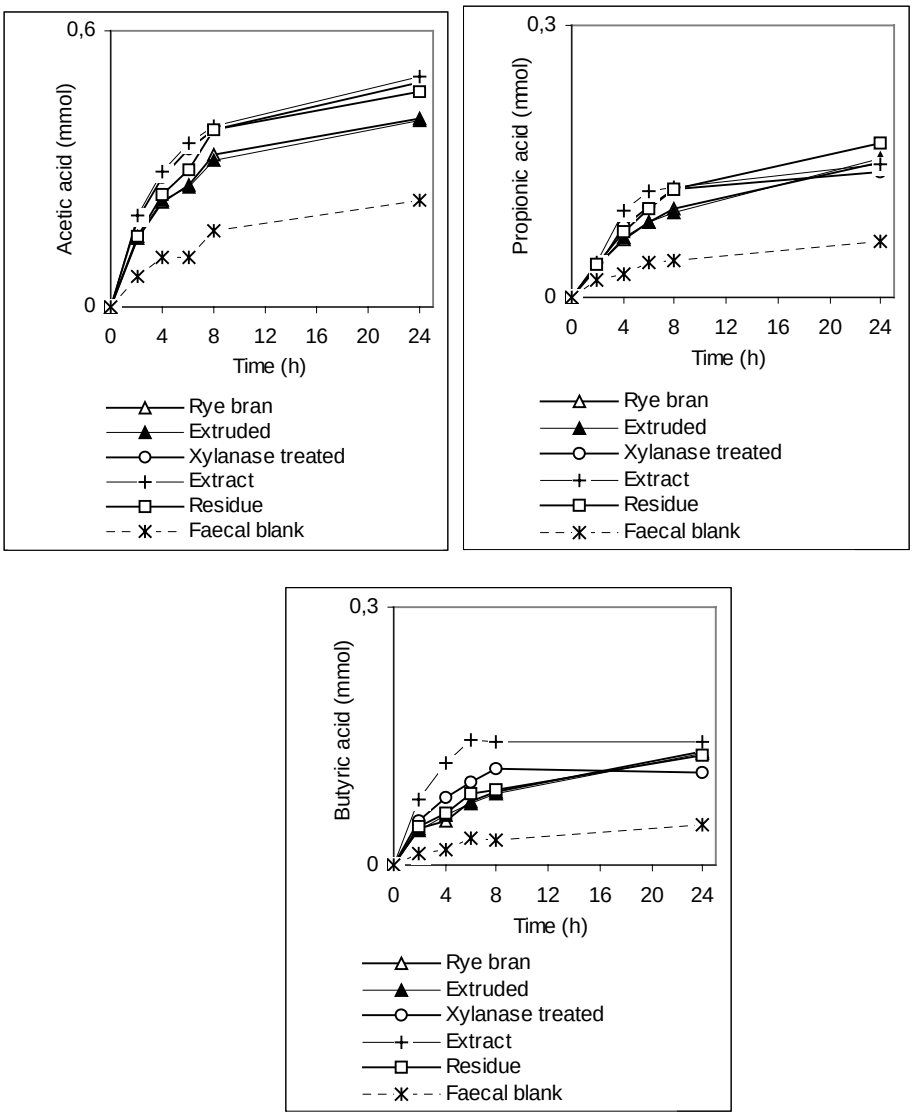
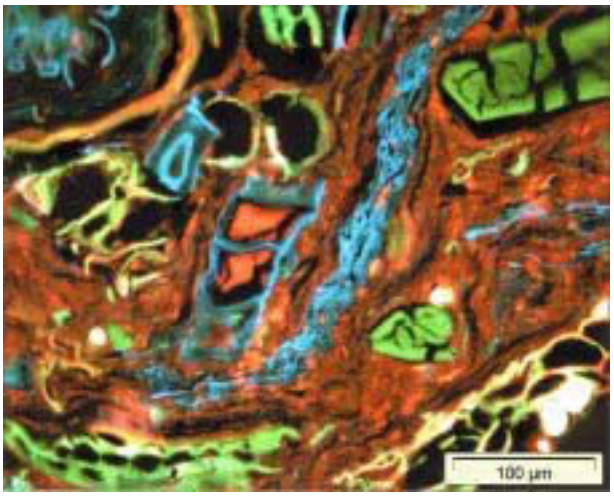
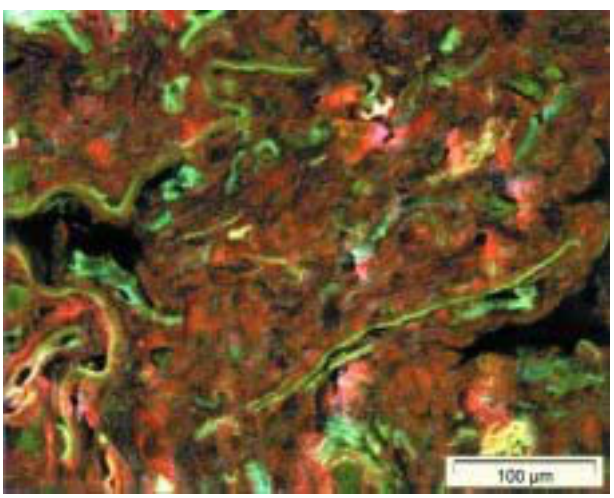


Figure 17. ***Production*** *of acetic, propionic and butyric acids in the fermentation of rye bran, extruded rye bran, xylanase-treated rye bran, rye-bran extract and rye-bran residue (V).*



a.



b.

Figure 18. Micrographs of a) the unextractable fraction of rye bran at the beginning of fermentation with the inoculum and b) the fermentation residue of unextractable fraction after 24 h fermentation. Cell walls are seen as blue and protein as red.

3.4.2 Gas production (I, II)

The production of gas was studied only in the first two fermentations. Hydrogen and carbon dioxide are the primary gas products, and methane or hydrogen sulphide the secondary gas products. Methane was not found in these experiments because of the non-methanogenic donors.

After 24 h fermentation, the amount of total gas produced was the same for rye and oat brans, although the oat bran had a faster initial rate (I). In the fermentation of wheat bran, the total gas production, as also the production of hydrogen, was slightly lower than in the case of rye bran. Total gas production was in accordance with the consumption of carbohydrates. The production of hydrogen sulphide was similar both in fermentations of rye and wheat brans, while oat bran produced more hydrogen sulphide. When comparing cereal brans to inulin, all brans produced gases at slower rates and at lower levels. Inulin had a very fast total gas production rate and the amount of gas produced was also high, which was in accordance with the rapid and total consumption of carbohydrates. Hydrogen production was also high as was shown by Roland et al. (1995). However, inulin produced a smaller amount of hydrogen sulphide than cereal brans (I).

The initial rate of total gas production was faster in the fermentations of water- and alkali-extractable fractions than in the fermentations of rye bran and the unextractable fraction due to the rapid carbohydrate fermentation (II). Although the initial rate of gas production was slower in the fermentation of rye bran, it continued throughout the fermentation, so that after 24 h the total gas volume was largest for rye bran. Very similar results were also seen in the formation of carbon dioxide and hydrogen sulphide (II). This is, however, a property of the unprocessed rye bran, because the unextractable fraction of rye bran produced all gases in smaller amounts than rye bran. Therefore, the reason for the high gas production was due to an unknown factor which was solubilized during extraction and obtained in the extract. Such a high level of gas in the human colon is, at least, inconvenient and it may also cause flatulence.

3.4.3 Short-chain fatty acid production (I, II, V)

The production of SCFA in the fermentations of rye bran followed the consumption of carbohydrates: the higher the rate and quantity of carbohydrate consumption the higher the rate and quantity of SCFA production (Figures 9-11). The initial rate of SCFA production in the fermentation of rye bran was surprisingly similar to the fermentations of wheat and oat brans and also to that of inulin (Figure 9) (I). Of the rye-bran substrates, the fastest production rate of SCFA was in the fermentations of extracted, soluble fractions (Figures 10, 11) which also had the fastest consumption of carbohydrates (II, V). In the fermentations of rye bran and the unextractable residue (II), SCFA production occurred at a slower rate than in the fermentations of the alkali-extractable and water-extractable fractions (II), but after 24 h fermentation the amount of total SCFA found was the same with all these rye substrates. The ratio of SCFA produced to monosaccharides consumed was very similar between samples: 2.2–2.5 mol/mol (II).

The production of SCFA in the fermentation of the rye-bran extract, which was prepared using a xylanase treatment (V), was faster than in the case of rye bran or xylanase-treated rye bran. Also, the amount of SCFA produced was highest following the fermentation of the rye-bran extract. However, the ratio of SCFA produced to monosaccharides consumed was 2.1–2.4 mol/mol in all of the fermentations (V) which is the same as in the fermentation of rye bran and water- and alkali-extracted fractions (II). The ratio is, however, dependent on the inoculum, because for rye bran the ratio of 2.8 mol/mol was obtained (I). Bourquin et al. (1992) have shown that the extent of the fermentation of oat and wheat brans varied among inoculum donors, implying that colonic microbial activities differ among individuals.

During the 24 h fermentation of rye bran, as well as of wheat and oat brans, butyric and propionic acids were produced (Figure 15) (I). The greatest butyric-acid production in this fermentation experiment, however, was due to inulin, which was fermented as a control. The molar ratio of butyric acid in the fermentation of rye bran after 24 h was 19%, which was lower than that of inulin (35%). The molar ratio of propionic acid was 24%, which in turn was more than obtained in the fermentation of inulin (14%). According to Mortensen et al. (1988), the relatively high proportion of propionic acid is typical for xylose

fermentation. Also *in vivo* in rats, inulin produced more butyric acid than wheat bran, and in the case of wheat bran more propionic acid was produced (Roland et al. 1995). However, Glitsø et al. (1998) have shown *in vivo* that a diet containing aleurone in rye can increase the production of butyric acid and Bach Knudsen et al. (1993b) have shown that arabinoxylan, but not β-glucan, in the cell walls of oat bran enhances butyric-acid production in the large intestine of pigs. The cereal brans studied in the present experiment were studied also *in vivo* in rats (Gråsten et al. 2002). They showed that rye bran increased the molar proportion of butyric acid and inulin that of propionic acid. Different results between *in vivo* and *in vitro* experiments has been shown also by Glitsø et al. (1998, 2000a). It is difficult to compare fermentation experiments with each other, because results are influenced by different parameters like the origin and preparation of the inoculum, diets of the donors and fermentation conditions. Thus all substrates under investigation should be included in the same experiment.

In the fermentations of water-extractable, alkali-extractable and unextractable fractions (II), the molar ratio of propionic acid after 24 h was 21–23%, and the molar ratio of butyric acid was 18–21%, which were rather similar to the molar ratios of rye bran (I). The fastest butyric-acid production was in the fermentation of soluble fractions and the highest amount was obtained in the case of the alkali-extractable fraction (Figure 16). Wheat pentosan, which was used as a control in this fermentation, produced less butyric acid than the alkali-extracted rye-bran fraction, although it was fully fermented. In the fermentation of rye bran with a smaller inoculum (V), the molar ratio of SCFA was similar to the other rye-bran fermentations: 21% propionic acid and 19% butyric acid. The fastest butyric acid production was obtained in the fermentation of rye bran extract, but the extent was similar to that of rye bran (Figure 17). Extrusion did not have an effect on the molar ratio. Of the xylanase-treated rye-bran substrates (V), more acetic acid was formed than in the fermentation of the other substrates (Figure 17) (I, II). By the end (24 h) of the fermentation of xylanase-treated substrates, the molar ratio of propionic acid was 16–21% and the molar ratio of butyric acid was 16–17%. Apparently, the xylanase treatment of rye bran modified the structure of arabinoxylan. In the fermentation of xylo- or arabinoxylo-oligosaccharides, both propionic and butyric acid were shown to be produced depending on the inoculum used (Kabel et al. 2002).

The health effects of DF are believed to be related to the butyric acid produced during fermentation (Cummings 1995). Butyric acid may have a protective effect on colon cancer (Russo et al. 1999). The molar ratios of SCFA, however, is not the only important consequence of fermentation, as the amount of SCFA produced also plays an important role. It may be that a small amount of SCFA with a high molar ratio of butyric acid may have the same health effect as a high amount of SCFA containing a little butyric acid.

3.5 Bacterial substrate specificity (III)

A range of probiotic and other intestinal bacteria was examined for their ability to ferment the DF carbohydrates. Knowledge of the fermentative capacity of individual species within the intestinal microbiota assists in the understanding of the mechanisms of polysaccharide fermentation in the human colon.

All the *Bifidobacterium longum* strains and one of the *Bifidobacterium adolescentis* strains examined were able to grow using rye arabinoxylan as the sole carbon source. The determination of the neutral sugar composition after fermentation by the *Bifidobacterium longum* strains showed that these bacteria almost completely hydrolysed and fermented the arabinosyl residues from arabinoxylan (III). However, they did not efficiently utilize the xylose backbone of the arabinoxylan, and unsubstituted xylan was not fermented at all by these bacteria.

Arabinoxylan was not fermented by the other species of bifidobacteria examined or by any of the lactobacilli. Importantly, arabinoxylan was not fermented either by the potentially harmful intestinal bacteria *Escherichia coli, Clostridium perfringens* or *Clostridium difficile*. Arabinoxylan from rye may, therefore, have potential as a prebiotic substrate for the proliferation of *Bifidobacterium longum*, a numerically dominant *Bifidobacterium* species in the adult human colon. It would be interesting to study if arabinoxylan attached to rye cell walls has a similar effect. Arabinoxylan may have the potential to be an applicable carbohydrate to complement probiotic *Bifidobacterium longum* strains in synbiotic combinations. However, pure culture in *in vitro* studies cannot predict which bacterial species will play the major role in the fermentation of complex carbohydrates in the intestinal tract. Polysaccharide degradation and

fermentation in the colon and also in *in vitro* fermentation studies with human faecal inocula is certainly a co-operative process involving consortia of different bacterial species. In *in vivo* mice experiments rye-supplemented diets have been shown to support the growth of *Bifidobacterium* (Oikarinen et al. 2003).

In contrast to growth on xylan, many *Bifidobacterium* isolates were able to efficiently ferment xylo-oligosaccharides (III). *Bifidobacterium* spp. have also previously been observed to utilize xylo-oligosaccharides and also wheat arabinoxylo-oligosaccharides which have been proposed as potential prebiotics (Van Laere et al. 2000). Using a human faecal inoculum, Kabel et al. (2002) studied fermentation of xylo-oligosaccharides and arabinoxylo-oligosaccharides. They showed that in the first stage of fermentation mainly acetate and lactate were formed and, according to them, *Bifidobacterium* spp. may play an important role in this part of fermentation, because bifidobacteria do not produce butyric acid. In the second stage of fermentation, propionic and butyric acids were produced (Kabel et al. 2002). The amount of bifidobacteria was shown to increase with xylo-oligosaccharides in *in vivo* human experiments also (Okazaki et al. 1990). During the xylanase treatment of rye bran (V), xylo- and arabinoxylo-oligosaccharides are formed which, according to the results presented here (III), could also be substrates for bifidobacteria. Xylo-oligosaccharides (III) were also efficiently fermented by some *Bacteroides* isolates but not by *Escherichia coli*, enterococci, *Clostridium difficile, Clostridium perfringens* or by the majority of intestinal *Lactobacillus* species examined.

None of the lactobacilli and bifidobacteria examined utilized β-glucan as a sole carbon source. However, they may be able to utilize oligomers resulting from the hydrolysis of β-glucans by other intestinal bacteria.

3.6 Interactions of dietary fibre and lignan bioconversion (V)

In the fermentations of extruded and xylanase-treated rye bran, 11–12 nmol of plant lignans originated from the substrate (Table 3 in Publication V). In the fermentation of rye-bran extract there were more lignans, 23 nmol, although the total DF content of this substrate was lower than in other substrates. The lowest plant lignan content was in the fermentation of the rye-bran residue.

Both the fastest and most extensive enterodiol formation was detected in the fermentation of the rye-bran extract, the most active formation period being between 4 and 8 h (Figure 4 in Publication V). This substrate was also the only fully soluble substrate in this experiment and it was fermented rapidly and completely. After 8 h fermentation, 51% of the total plant lignans were converted to enterodiol (Table 3 in Publication V). With rye bran, the xylanase treatment slightly increased enterodiol formation. The maximal bioconversion was at 4 h in the fermentation of extruded rye bran and at 8 h in the fermentation of other substrates. The maximal bioconversion in the case of extruded rye bran was 35% and in the case of xylanase-treated rye bran it was increased to 50% which was the same as in the fermentation of rye-bran extract. In the case of the rye-bran residue, the bioconversion was 43%. Enterodiol was not formed in the faecal slurry without the addition of rye samples.

Heinonen et al. (2001) have studied *in vitro* the conversion of pure plant lignans to mammalian lignans and they have shown that most of the matairesinol, secoisolariciresinol and lariciresinol and a small amount of syringaresinol were converted to enterodiol and/or enterolactone, while isolariciresinol was not converted at all. According to these results, more enterodiol was produced in the fermentations of rye-bran substrates (V) than could be expected. This means that perhaps more syringaresinol was converted than with the pure substrate (Heinonen et al. 2001) because of the matrix effect, or because of different inocula. It may be also that in rye bran there are some, as yet unanalyzed, plant lignans which are converted to enterodiol.

Enterolactone production could be detected only from the fermentations of extruded rye bran and rye-bran extract. Enterolactone formation was highest after 24 h fermentation of rye-bran extract when 20% of the plant lignans were converted to enterolactone (Table 3 in Publication V). In the case of extruded rye bran, 4% of the plant lignans were converted to enterolactone.

Glitsø et al. (2000b) studied the influence of the rye-bread diets on the metabolism of lignans *in vivo* and they found that the fermentation pattern of DF had no effect on the intestinal metabolism of lignans. In the study of Nicolle et al. (2002), diet has been shown to influence the formation of lignans from wheat bran in rats. In the present study, the solubility and xylanase treatment impacted on the bioconversion, and the enterodiol formation correlated well with the

consumption of carbohydrates. This is in accordance with the results of Nicolle et al. (2002), and also with those of Cassidy (1991).

Bach Knudsen et al. (2001) showed that the conversion of plant to mammalian lignans was a relatively slow process compared to the fermentation of carbohydrates. In their *in vitro* fermentation study, enterolactone was formed at a rate independent of the fibre composition. The slow enterolactone production in the fermentation in this study is in accordance with the results of Bach Knudsen et al. (2001).

4. Conclusions

The rate and extent of *in vitro* fermentation of rye bran and its fractions were clearly dependent on the solubility and structure of the polysaccharides and the cell-wall matrix. Dietary fibre polysaccharides of rye bran can be divided into three groups: 1) fermentable, soluble polysaccharides that are rapidly fermented, 2) fermentable cell-wall associated polysaccharides that are gradually released from the cell-wall matrix and then fermented, and 3) polysaccharides and cell-wall structures that are not fermented at all.

Rye bran, as also whole-grain rye, might serve as a more balanced source of dietary fibre than readily fermentable isolated, soluble poly- and oligo-saccharides. The minor soluble part of the rye bran is fermented rapidly *in vitro*. Most dietary fibre carbohydrates are attached to cell-wall structures. These carbohydrates are fermented slowly *in vitro*. However, most of these cell-wall carbohydrates of rye bran were eventually fermented. According to these results, the soluble carbohydrates of rye bran could provide substrates for colonic microbiota in the caecum and the poorly extractable 'insoluble' part of rye bran could provide substrates for colonic microbiota lower down in the colon. The unfermentable part of rye bran could have a bulking effect in the colon.

Propionic and butyric acids were produced in the fermentation of rye bran. Because of the slow fermentation of rye bran, propionic and butyric acids would also be produced in more distant areas of the colon. This in turn ensures that butyric acid could be metabolized by colonic epithelium, thus maintaining and improving mucosal health further in the colon.

The fermentation of rye bran to SCFA, particularly to butyric acid, is accomplished by a mixture of many different faecal bacteria. In addition to butyric-acid production, growth promotion of bifidobacteria (which do not produce butyric acid) is another way by which non-digestible food components may promote gut health. Rye arabinoxylan was found to be fermented *in vitro* by *Bifidobacterium longum*, a numerically dominant *Bifidobacterium* species in the adult human colon. Also, xylo-oligosaccharides were found to be fermented by many *Bifidobacterium* strains. It is, therefore, possible that xylo-oligo-saccharides as well as arabinoxylo-oligosaccharides formed during the xylanase

treatment, because of their rapid fermentation rates, could select for bifidobacteria able to ferment arabinoxylan.

Rye, and especially rye bran, are rich in fermentable dietary fibre, and rye is an important natural source of dietary fibre in Finland and in Northern Europe. Rye was shown to also contain much fructan, which according to the suggested new dietary-fibre concept increases the calculated daily dietary-fibre intake among people who eat rye bread. Rye is rich in plant lignans and, in the present study, solubility and carbohydrate fermentation had an impact on the conversion of plant lignans to mammalian lignans.

The present study has demonstrated that rye fermentation may have important effects on gut health. Rye bran has both rapidly and slowly fermentable carbohydrates and also non-fermentable carbohydrates, all of which have their specific effects on the human colon with potential health benefits. An interesting observation is the effect of xylanase treatment which deserves more careful examination. Rye as such may be a candidate prebiotic but the effects should be verified in human studies. Fermentation of rye lignans should also be assessed in detail to characterise the potential health implications.

References

Adlercreutz, H. 2002. Phyto-oestrogens and cancer. Lancet Oncology, Vol. 3, pp. 364–373.

Anderson, J.W., Hanna, T.J., Peng, X. and Kryscio, R.J. 2000. Whole grain foods and heart disease risk. J. Am. Coll. Nutr. Vol. 19, pp. 291S–299S.

Annison, G., Choct, M. and Cheetham, N.W. 1992. Analysis of wheat arabinoxylans from a large-scale isolation. Carbohydr. Polym., Vol. 19, pp. 151–159.

Anonymous. 1998. Suomalaiset ravitsemussuositukset (in Finnish). Maa- ja metsätalousministeriö, Komiteamietintö 1998:7. Helsinki: Oy Edita Ab.

Anonymous. 2001a. The definition of dietary fiber. Report of the Dietary Fiber Definition Committee to the Board of Directors of the American Association of Cereal Chemists. Cereal Foods World, Vol. 46, pp. 112–129.

Anonymous. 2001b. Food balance sheet for food commodities 1999 and 2000. Information Centre of the Ministry of Agriculture and Forestry. Helsinki 2001.

Asp, N.-G., Johansson, C.-G., Hallmer, H. and Siljeström, M. 1983. Rapid enzymatic assay of insoluble and soluble dietary fiber. J. Agric. Food Chem., Vol. 31, pp. 476–482.

Asp, N.-G., Björck, I. and Nyman, M. 1993. Physiological effects of cereal dietary fibre. Carbohydrate Polymers, Vol. 21, pp. 183–187.

Aura, A.-M., Härkönen, H., Fabritius, M. and Poutanen, K. 1999. Development of an *in vitro* enzymatic digestion method for removal of starch and protein and assessment of its performance using rye and wheat breads. J. Cereal Sci., Vol. 29, pp. 139–152.

Autio, K. 2001. Light microscopic investigations on dietary fibre. In: McCleary, B.V. and Prosky, L. (eds.) Advanced Dietary Fibre Technology. Oxford: Blackwell Science. Pp. 25–29.

Bach Knudsen, K.E., 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Animal Feed Sci. Technol. Vol. 67, pp. 319–338.

Bach Knudsen, K.E., Jensen, B.B. and Hansen, I. 1993a. Digestion of polysaccharides and other major components in the small and large intestine of pigs fed on diets consisting of oat fractions rich in β-D-glucan. Br. J. Nutr., Vol. 70, pp. 537–556.

Bach Knudsen, K.E., Jensen, B.B. and Hansen, I. 1993b. Oat bran but not a β-glucan-enriched oat fraction enhances butyrate production in the large intestine of pigs. J. Nutr., Vol. 123, pp. 1235–1247.

Bach Knudsen, K.E., Johansen, H.N. and Glitsø, V. 1997. Rye dietary fiber and fermentation in the colon. Cereal Foods World, Vol. 42, pp. 690–694.

Bach Knudsen, K.E., Serena, A., Glitsø, V. and Adlercreutz, H. 2001. Colonic formation and absorption of mammalian lignans and butyrate. In: Liukkonen, K., Kuokka, A. and Poutanen, K. (eds.). VTT Symposium 213, Whole grain and human health, International symposium, June 13–15, 2001, Finland. Espoo: VTT. Pp. 30–31.

Ballongue, J., Schumann, C. and Quignon, P. 1997. Effects of lactulose and lactitol on colonic microflora and enzymatic activity. Scand. J. Gastroenterol., Suppl., Vol. 222, pp. 41–44.

Ballongue, J. 1998. Bifidobacteria and probiotic action. In: Salminen, S. and von Wright, A. (eds.). Lactic Acid Bacteria, Microbiology and Functional Aspects. New York: Marcel Dekker, Inc. Pp. 519–587.

Barry, J.L., Chourot, J.M., Bonnet, C., Kozlowski, F. and David, A. 1989. *In vitro* fermentation of neutral monosaccharides by ruminal and human fecal microflora. Acta Vet. Scand., Vol. 86, pp. 93–95.

Barry, J.L., Hoebler, C., Macfarlane, G.T., Macfarlane, S., Mathers, J.C., Reed, K.A., Mortensen, P.B., Nordgaard, I., Rowland, I.R. and Rumney, C.J. 1995. Estimation of the fermentability of dietary fibre *in vitro*: a European interlaboratory study. Br. J. Nutr., Vol. 74, pp. 303–322.

Bengtsson, S., Åman, P. and Andersson, R.E. 1992. Structural studies on water-soluble arabinoxylans in rye grain using enzymatic hydrolysis. Carbohydrate Polym., Vol. 17, pp. 277–284.

Bergmans, M.E.F., Beldman, G., Gruppen, H. and Voragen, A.G.J. 1996. Optimization of the selective extraction of (glucurono)arabinoxylans from wheat bran: use of barium and calcium hydroxide solution at elevated temperatures. J. Cereal Sci., Vol. 23, pp. 235–245.

Borriello, S.P., Setchell, K.D.R., Axelson, M. and Lawson, A.M. 1985. Production and metabolism of lignans by the human faecal flora. J. Appl. Bacteriol. Vol. 58, pp. 37–43.

Boskov Hansen, H., Andreasen, M.F., Nielsen, M.M., Larsen, L.M., Bach Knudsen, K.E., Meyer, A.S., Christensen, L.P. and Hansen, Å. 2002. Changes in dietary fibre, phenolic acids and activity of endogenous enzymes during rye bread-making. Eur. Food Res. Technol. Vol. 214, pp. 33–42.

Bouhnik, Y., Flourie, B., D'Agay-Abensour, L., Pochart, P., Gramet, G., Durand, M. and Rambaud, J.C. 1997. Administration of transgalacto-oligosaccharides increases fecal bifidobacteria and modifies colonic fermentation metabolism in healthy humans. J. Nutr. Vol. 127, pp. 444–448.

Bouhnik, Y., Vahedi, K., Achour, L., Attar, A., Salfati, J., Pochart, P., Marteau, P., Flourie, B., Bornet, F. and Rambaud, J.C. 1999. Short-chain fructo-oligosaccharide administration dose-dependently increases fecal bifidobacteria in healthy humans. J. Nutr. Vol. 129, pp. 113–116.

Bourquin, L.D., Titgemeyer, E.C., Garleb, K.A. and Fahey, C., Jr. 1992. Short-chain fatty acid production and fiber degradation by human colonic bacteria: effects of substrate and cell wall fractionation procedures. J. Nutr. Vol. 122, pp. 1508–1520.

Brighenti, F., Casiraghi, M.C., Canzi, E. and Ferrari, A. 1999. Effect of consumption of a ready-to-eat breakfast cereal containing inulin on the intestinal milieu and blood lipids in healthy male volunteers. Eur. J. Clin. Nutr. Vol. 53, pp. 726–733.

Brodribb, A.J.M. and Groves, C. 1978. Effect of bran particle size on stool weight. Gut Vol. 19, pp. 60–63.

Campbell, J.M., Fahey, G.C., Jr. and Wolf, B.W. 1997. Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. J. Nutr., Vol. 127, pp. 130–136.

Cassidy, A. 1991. Plant oestrogens and their relation to hormonal status in women. PhD Thesis, Cambridge University.

Cassidy, A., Hanley, B. and Lamuela-Raventos, R. 2000. Isoflavones, lignans and stilbenes – origins, metabolism and potential importance to human health. J. Sci. Food Agric., Vol. 80, pp. 1044–1062.

Casterline, J.L., Jr., Oles, C.J. and Ku, Y. 1997. *In vitro* fermentation of various food fiber fractions. J. Agric. Food Chem., Vol. 45, pp. 2463–2467.

Christensen, D.N., Bach Knudsen, K.E., Wolstrup, J. and Jensen, B.B. 1999. Integration of ileum cannulated pigs and in vitro fermentation to quantify the effect of diet composition on the amount of short-chain fatty acids available from fermentation in the large intestine. J. Sci. Food Agric. Vol. 79, pp. 755–762.

Conway, P.L. 2001. Prebiotics and human health: the state-of-the-art and future prespectives. Scand. J. Nutr. Vol. 45, 13–21.

Coudray, C., Bellanger, J., Castiglia-Delavaud, C., Remesy, C., Vermorel, M. ja Rayssignuier, Y. 1997. Effect of soluble or partly soluble dietary fibres supplementation on absorption and balance of calcium, magnesium, iron and zinc in healthy young men. Eur. J. Clin. Nutr. Vol. 51, pp. 375–380.

Cummings, J.H. 1993. Dietary fibre intakes in Europe: an overview. In: Cummings, J.H. and Frølich, W. (eds.). Dietary fibre intakes in Europe. A survey conducted by members of the management committee of Cost 92. Luxembourg: Commission of the European Communities. Pp. 11–19.

Cummings, J.H. 1995. Short chain catty acids. In: Gibson, G.R. and Macfarlane, G.T. (eds.). Human Colonic Bacteria. Role in Nutrition, Physiology, and Pathology. Boca Raton: CRC Press. Pp. 101–130.

Cummings, J.H. and Macfarlane, G.T. 1991. The control and consequences of bacterial fermentation in the human colon. J. Appl. Bacteriol. Vol. 70, pp. 443–459.

Cummings, J.H. and Englyst, H.N. 1995. Gastrointestinal effects of food carbohydrate. Am. J. Clin. Nutr. Vol. 61, pp. 938S–945S.

Cummings, J.H., Beatty, E.R., Kingman, S.M., Bingham, S.A. and Englyst, H.N. 1996. Digestion and physiological properties of resistant starch in the human large bowel. Br. J. Nutr. Vol. 75, pp. 733–747.

Cummings, J.H., Macfarlane, G.T. and Englyst, H.N. 2001. Prebiotic digestion and fermentation. Am. J. Clin. Nutr. Vol. 73, pp. 415S–420S.

Davies, M.J., Bowey, E.A., Adlercreutz, H., Rowland, I.R. and Rumsby, P.C. 1999. Effects of soy or rye supplementation of high-fat diets on colon tumour development in azoxymethane-treated rats. Carcinogenesis Vol. 20, pp. 927–931.

Delcour, J., Vanhamel, S. and de Geest, C. 1989. Physicochemical and functional properties of rye nonstarch polysaccharides. I. Colorimetric analysis of pentosans and their relative monosaccharide compositions in fractionated (milled) rye products. Cereal Chem. Vol. 66, pp. 107–111.

Delcour, J.A., Rouseu, N. and Vanhaesendonck, I.P. 1999. Pilot-scale isolation of water-extractable arabinoxylans from rye. Cereal Chem. Vol. 76, pp. 1–2.

Douglas, S.A. 1981. A rapid method for the determination of pentosans in wheat flour. Food Chem. Vol. 7, 139–145.

Edwards, C.A. 1995. The physiological effects of dietary fiber. In: Kritchevsky, D. and Bonfield, C. (eds.). Dietary Fiber in Health and Disease. St. Paul, Minnesota: Eagan Press. Pp. 58–71.

Edwards, C.A. and Parrett, A.M. 1996. Plant cell wall polysaccharides, gums, and hydrocolloids: nutritional aspects. In: Eliasson, A.-C. (ed.). Carbohydrates in Food. New York: Marcel Dekker, Inc. Pp. 319–346.

Englyst, H.N., Hay, S. and Macfarlane, G.T. 1987. Polysaccharide breakdown by mixed populations of human faecal bacteria. FEMS Microbiol. Ecol. Vol. 95, pp. 163–171.

Esteves, M.P., Calvarheiro, F., Moura, P., Amaral-Collaço, M.T. and Girio, F.M. 1999. Use of arabino-xylo-oligosaccharides from brewery's spent grains as growth substrate for probiotic strains. Proceedings of Euro Food Chem X, Functional Foods – A new challenge for the food chemists, 22-24 September, 1999, Budapest, Hungary.

Fardet, A., Guillon, F., Hoebler, C. and Barry, J.-L. 1997. *In vitro* fermentation of beet fibre and barley bran, of their insoluble residues after digestion and of ileal effluents. J. Sci. Food Agric. Vol. 75, pp. 315–325.

Feldheim, W. and Wisker, E. 1995. Nutritional aspects of rye. In: Poutanen, K. and Autio, K. (eds.). VTT Symposium 161, International rye symposium: Technology and products, Helsinki, Finland, 7–8 December, 1995. Espoo: VTT. Pp. 93–102.

Fuchs, C.S., Giovannucci, E.L., Colditz, G.A., Hunter, D.J., Stampfer, M.J., Rosner, B., Speizer, F.E. and Willett, W.C. 1999. Dietary fiber and the risk of colorectal cancer and adenoma in women. N. Engl. J. Med. Vol. 340, pp. 169–176.

Fulcher, R.G. and Wong, S.J. 1980. Inside cereals – A fluorescence microchemical view. In: Inglett, G.E. and Munck, L. (eds.). Cereals for Food and Beverages. New York: Academic Press. Pp. 1–26.

Fuller, R. 1989. A review: probiotics in man and animals. J. Appl. Bacteriol. Vol. 66, pp. 365–378.

Gibson, G.R. 1998. Dietary modulation of the human gut microflora using prebiotics. Br. J. Nutr. Vol. 80, pp. S209–S212.

Gibson, G.R. and Roberfroid, M.B. 1995. Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics. J. Nutr. Vol. 125, pp. 1401–1412.

Glitsø, L.V. and Bach Knudsen, K.E. 1999. Milling of whole grain rye to obtain fractions with different dietary fibre characteristics. J. Cereal Sci. Vol. 29, pp. 89–97.

Glitsø, L.V., Brunsgaard, G., Højsgaard, S., Sandström, B. and Bach Knudsen, K.E. 1998. Intestinal degradation in pigs of rye dietary fibre with different structural characteristics. Br. J. Nutr. Vol. 80, pp. 457–468.

Glitsø, L.V., Gruppen, H., Schols, H.A., Højsgaard, S., Sandström, B. and Bach Knudsen, K.E. 1999. Degradation of rye arabinoxylans in the large intestine of pigs. J. Sci. Food Agric. Vol. 79, pp. 961–969.

Glitsø, L.V., Jensen, B.B. and Bach Knudsen, K.E. 2000a. *In vitro* fermentation of rye carbohydrates including arabinoxylans of different structure. J. Sci. Food Agric. Vol. 80, pp. 1211–1218.

Glitsø, L.V., Mazur, W., Adlercreutz, H. Wähälä, K., Mäkelä, T., Sandström, B. and Bach Knudsen, K.E. 2000b. Intestinal metabolism of rye lignans in pigs. Br. J. Nutr. Vol. 84, pp. 429–437.

Graham, H., Grön Rydberg, M.-B. and Åman, P. 1988. Extraction of soluble dietary fiber. J. Agric. Food Chem. Vol. 36, pp. 494–497.

Gråsten, S.M., Juntunen, K.S., Poutanen, K.S., Gylling, H.K., Miettinen, T.A. and Mykkänen, H.M. 2000. Rye bread improves bowel function and decreases the concentrations of some compounds that are putative colon cancer risk markers in middle-aged women and men. J. Nutr. Vol. 130, pp. 2215–2221.

Gråsten, S.M., Pajari, A.-M., Liukkonen, K.-H., Karppinen, S. and Mykkänen, H.M. 2002. Fibers with different solubility characteristics alter similarly the metabolic activity of intestinal microbiota in rats fed cereal brans and inulin. Nutr. Res. Vol. 22, pp. 1435–1444.

Guillon, F. and Champ, M. 2000. Structural and physical properties of dietary fibres, and consequences of processing on human physiology. Food Res. Int. Vol. 33, pp. 233–245.

Guillon, F., Barry, J.L. and Thibault, J.F. 1992. Effects of autoclaving sugar-beet fibre on its physico-chemical properties and its in vitro degradation by human faecal bacteria. J. Sci. Food Agric. Vol. 60, pp. 69–79.

Gurr, M.I. and Asp, N.-G. 1996. Dietary fibre. Washington: ILSI Press. ISBN 0-944398-24-3. 23 p.

Heinonen, S., Nurmi, T., Liukkonen, K., Poutanen, K., Wähälä, K., Deyama, T., Nishibe, S. and Adlercreutz, H. 2001. *In vitro* metabolism of plant lignans: new precursors of mammalian lignans enterolactone and enterodiol. J. Agric. Food Chem. Vol. 49, pp. 3178–3186.

Henry, R.J. and Saini, H.S. 1989. Characterization of cereal sugars and oligosaccharides. Cereal Chem. Vol. 66, pp. 362–365.

Hill, M.J. 1998. Cereals, dietary fibre and cancer. Nutr. Res. Vol. 18, pp. 653–659.

Hoebler, C., Guillon, F., Fardet, A., Cherbut, C. and Barry, J.-L. 1998. Gastrointestinal or simulated *in vitro* digestion changes dietary fibre properties and their fermentation. J. Sci. Food Agric. Vol. 77, pp. 327–333.

Hogarth, A.J., Hunter, D.E., Jacobs, W.A., Garleb, K.A. and Wolf, B.W. 2000. Ion chromatographic determination of three fructooligosaccharide oligomers in prepared and preserved foods. J. Agric. Food Chem. Vol. 48, pp. 5326–5330.

Hudson, M.J. and Marsh, P.D. 1995. Carbohydrate metabolism in the colon. In: Gibson, G.R. and Macfarlane, G.T. (eds.). Human Colonic Bacteria. Role in Nutrition, Physiology, and Pathology. Boca Raton: CRC Press. Pp. 61–74.

Hultén, K., Winkvist, A., Lenner, P., Johansson, R., Adlercreutz, H. and Hallmans, G. 2002. An incident case-referent study on plasma enterolactone and breast cancer risk. Eur. J. Clin. Nutr. Vol. 41, pp. 168–176.

Härkönen, H., Pessa, E., Suortti, T. and Poutanen, K. 1997. Distribution and some properties of cell wall polysaccharides in rye milling fractions. J. Cereal Sci. Vol. 26, pp. 95–104.

Jacobs, D.R., Marquart, L., Slavin, J. and Kushi, L.H. 1998. Whole-grain intake and cancer: an expanded review and meta-analysis. Nutr. Cancer Vol. 30, pp. 85–96.

Jenkins, D., Kendall, C. and Ransom, T. 1998. Dietary fibre, the evolution of the human diet and coronary heart disease. Nutr. Res. Vol. 18, pp. 633–652.

Juntunen, K., Mazur, W., Liukkonen, K., Uehara, M., Poutanen, K., Adlercreutz, H. and Mykkänen, H. 2000. Consumption of wholemeal rye bread increases serum concentrations and urinary excretion of enterolactone compared with consumption of white wheat bread in healthy Finnish men and women. Br. J. Nutr. Vol. 84, pp. 839–846.

Kabel, M.A., Kortenoeven, L., Schols, H.A. and Voragen, A.G.J. 2002. *In vitro* fermentability of differently substituted xylo-oligosaccharides. J. Agric. Food Chem. Vol. 50, pp. 6205–6210.

Kleessen, B., Sykura, B., Zunft, H.J. and Blaut, M. 1997. Effects of inulin and lactose on fecal microflora, microbial activity, and bowel habit in elderly constipated persons. Am. J. Clin. Nutr. Vol. 65, pp. 1397–1402.

Kruse, H.P., Kleessen, B. and Blaut, M. 1999. Effects of inulin on faecal bifidobacteria in human subjects. Br. J. Nutr. Vol. 82, pp. 375–382.

Lahti-Koski, M. and Kilkkinen, A. 2001. Ravitsemuskertomus 2000 (in Finnish). Kansanterveyslaitoksen julkaisuja B1/2001. Helsinki: Hakapaino Oy.

Lebet, V., Arrigoni, E. and Amado, R. 1997. Neutral-sugar determination by HPAEC-PAD to characterise polysaccharide degradation by colonic bacteria. Z. Lebensm. Unters. Forsch. Vol. 205, pp. 257–261.

Lebet, V., Arrigoni, E. and Amado, R. 1998a. Measurement of fermentation products and substrate disappearance during incubation of dietary fibre sources with human faecal flora. Lebensm. Wiss. u. Technol. Vol. 31, pp. 473–479.

Lebet, V., Arrigoni, E. and Amado, R. 1998b. Digestion procedure using mammalian enzymes to obtain substrates for *in vitro* fermentation studies. Lebensm. Wiss. u. Technol. Vol. 31, pp. 509–515.

Le Blay, G., Michel, C., Blottiere, H.M. and Cherbut, C. 1999. Enhancement of butyrate production in the rat caecocolonic tract by long-term ingestion of resistant potato starch. Br. J. Nutr. Vol. 82, pp. 419–426.

Levitt, M.D., Gibson, G.R. and Christl, S.U. 1995. Gas metabolism in the large intestine. In: Gibson, G.R. and Macfarlane, G.T. (eds.). Human Colonic Bacteria. Role in Nutrition, Physiology, and Pathology. Boca Raton: CRC Press. Pp. 131–154.

Macfarlane, G.T., Gibson, G.R. and Cummings, J.H. 1992. Comparison of fermentation reactions in different regions of the human colon. J. Appl. Bacteriol. Vol. 72, pp. 57–64.

MacLeod, A.M. and Preece, I.A. 1954. Studies of the free sugars of the barley grain. V. Comparison of sugars and fructosans with those of other cereals. J. Inst. Brew. Vol. 60, pp. 46–55.

Marthinsen, D. and Fleming, S.E. 1982. Excretion of breath and flatus gases by humans consuming high-fiber diets. J. Nutr. Vol. 112, pp. 1133–1143.

Mazur, W. and Adlercreutz, H. 1998. Naturally occurring oestrogens in food. Pure & Appl. Chem. Vol. 70, pp. 1759–1776.

McBurney, M.I. and Thompson, L.U. 1989. *In vitro* fermentabilities of purified fiber supplements. J. Food Sci. Vol. 54, pp. 347–350.

McBurney, M.I., Thompson, L.U., Cuff, D.J. and Jenkins, D.J.A. 1988. Comparison of ileal effluents, dietary fibers, and whole foods in predicting the

physiological importance of colonic fermentation. Am. J. Gastroenterol. Vol. 83, pp. 536–540.

McBurney, M.I., Cuff, D.J. and Thompson, L.U. 1990. Rates of fermentation and short chain fatty acid and gas production of six starches by human faecal microbiota. J. Sci. Food Agric. Vol. 50, pp. 79–88.

McCleary, B.V. 2001. Measurement of dietary fibre components: the importance of enzyme purity, activity and specificity. In: McCleary, B.V. and Prosky, L. (eds.) Advanced Dietary Fibre Technology. Oxford: Blackwell Science. Pp. 89–105.

McCleary, B.V., Murphy, A. and Mugford, D.C. 2000. Measurement of total fructan in foods by enzymatic/spectrophotometric method: collaborative study. J. AOAC Int. Vol. 83, pp. 356–364.

McDougall, G.J., Morrison, I.M., Stewart, D. and Hillman, J.R. 1996. Plant cell walls as dietary fibre: range, structure, processing and function. J. Sci. Food Agric. Vol. 70, pp. 133–150.

Medcalf, D.G. and Cheung, P.W. 1971. Composition and structure of glucofructans from durum wheat flour. Cereal Chem. Vol. 48, pp. 1–8.

Monsma, D.J., Thorsen, P.T., Vollendorf, N.W., Crenshaw, T.D. and Marlett, J.A. 2000. *In vitro* fermentation of swine ileal digesta containing oat bran dietary fiber by rat cecal inocula adapted to the test fiber increases propionate production but fermentation of wheat bran ileal digesta does not produce more butyrate. J. Nutr. Vol. 130, pp. 585–593.

Mortensen, P.B., Holtug, K. and Rasmussen, H.S. 1988. Short-chain fatty acid production from mono and disaccharides in a fecal incubation system: implications for colonic fermentation of dietary fiber in humans. J. Nutr. Vol. 118, pp. 321–325.

Mutanen, M., Pajari, A.-M. and Oikarinen, S. 2000. Beef induces and rye prevents the formation of intestinal polyps in APC^{Min} mice: relation to β-catenin and PKC isoenzymes. Carcinogenesis Vol. 21, pp. 1167–1173.

Mälkki, Y., Korpela, R. and Karaila, P. 1993. Dietary fibre intake in Finland. In: Cummings, J.H. and Frølich, W. (eds.). Dietary fibre intakes in Europe. A survey conducted by members of the management committee of Cost 92. Luxembourg: Commission of the European Communities. Pp. 41–47.

Nicolle, C., Manach, C., Morand, C., Mazur, W., Adlercreutz, H., Rémésy, C. and Scalbert, A. 2002. Mammalian lignan formation in rats fed a wheat bran diet. J. Agric. Food Chem. Vol. 50, pp. 6222–6226.

Nilsson, M., Saulnier, L., Andersson, R. and Åman, P. 1996. Water unextractable polysaccharides from three milling fractions of rye grain. Carbohydrate polymers Vol. 30, pp. 229–237.

Nilsson, M., Åman, P., Härkönen, H., Hallmans, G., Bach Knudsen, K.E., Mazur, W. and Adlercreutz, H. 1997a. Nutrient and lignan content, dough properties and baking performance of rye samples used in Scandinavia. Acta Agric. Scand., Sect. B, Soil and Plant Sci. Vol. 47, pp. 26–34.

Nilsson, M., Åman, P., Härkönen, H., Hallmans, G., Bach Knudsen, K.E., Mazur, W. and Adlercreutz, H. 1997b. Content of nutrients and lignans in roller milled fractions of rye. J. Sci. Food Agric. Vol. 73, pp. 143–148.

Nilsson, M., Andersson, R., Andersson, R.E., Autio, K. and Åman, P. 2000. Heterogeneity in a water-extractable rye arabinoxylan with a low degree of disubstitution. Carbohydrate Polym. Vol. 41, pp. 397–405.

Nyman, M., Asp, N.-G., Cummings, J. and Wiggins, H. 1986. Fermentation of dietary fibre in the intestinal tract: comparison between man and rat. Br. J. Nutr. Vol. 55, pp. 487–496.

Ohta, A., Ohtsuki, M., Hosono, A., Adachi, T., Hara, H. and Sakata, T. 1998. Dietary fructooligosaccharides prevent osteopenia after gastrectomy in rats. J. Nutr. Vol. 128, pp. 106–110.

Oikarinen, S., Heinonen, S.-M., Karppinen, S., Mättö, J., Adlercreutz, H., Poutanen, K. and Mutanen, M. 2003. Plasma enterolactone or intestinal

Bifidobacterium levels do not explain adenoma formation in Min mice fed with two different types of rye bran fractions. Br. J. Nutr. In press.

Okazaki, M., Fujikawa, S. and Matsumoto, N. 1990. Effect of xylo-oligosaccharide on the growth of bifidobacteria. Bifidobacteria Microflora Vol. 9, pp. 77–86.

Olano-Martin, E., Mountzouris, K.C., Gibson, G.R. and Rastall, R.A. 2000. *In vitro* fermentability of dextran, oligodextran and maltodextrin by human gut bacteria. Br. J. Nutr. Vol. 83, pp. 247–255.

Parkkonen, T., Härkönen, H. and Autio, K. 1994. The effect of baking on microstructure of rye cell walls and proteins. Cereal Chem. Vol. 71, pp. 58–63.

Pietinen, P., Rimm, E.B., Korhonen, P., Hartman, A.M., Willett, W.C., Albanes, D. and Virtamo, J. 1996. Intake of dietary fibre and risk of coronary heart disease in a cohort of Finnish men. Circulation Vol. 94, pp. 2720–2727.

Pietinen, P., Stumpf, K., Männistö, S., Kataja, V., Uusitupa, M. and Adlercreutz, H. 2001. Serum enterolactone and risk of breast cancer: a case-control study in eastern Finland. Cancer Epidemiology, Biomarkers & Prevention, Vol. 10, pp. 339–344.

Reddy, B.S. 1995. Nutritional factors and colon cancer. Crit. Rev. Food Sci. Nutr. Vol. 35, pp. 175–190.

Roberfroid, M.B. 1998. Prebiotics and synbiotics: concepts and nutritional properties. Br. J. Nutr. Vol. 80, pp. S197–S202.

Roberfroid, M.B., van Loo, J.A.E. and Gibson, G.R. 1998. The bifidogenic nature of chicory inulin and its hydrolysis products. J. Nutr. Vol. 128, pp. 11–19.

Roland, N., Nugon-Baudon, L., Andrieux, C. and Szylit, O. 1995. Comparative study of the fermentative characteristics of inulin and different types of fibre in rats inoculated with a human whole faecal flora. Br. J. Nutr. Vol. 74, pp. 239–249.

Rowland, I., Wiseman, H., Sanders, T., Adlercreutz, H. and Bowey, E. 1999. Metabolism of oestrogens and phytoestrogens: role of the gut microflora. Biochem. Soc. Transact. Vol. 27, pp. 304–308.

Russo, G.L., Pietra, V.D., Mercurio, C., Palumbo, R., Iacomino, G., Russo, M., Tosto, M. and Zappia, V. 1999. Protective effects of butyric acid in colon cancer. In: Zappia et al. (eds.) Advances in Nutrition and Cancer 2. New York: Kluwer Academic: Plenum Publishers. Pp. 131–147.

Salminen, S. and Salminen, E. 1997. Lactulose, lactic acid bacteria, intestinal microecology and mucosal protection. Scand. J. Gastroenterol., Suppl., Vol. 222, pp. 45–48.

Salminen, S., Bouley, C., Boutron-Ruault, M.-C., Cummings, J.H., Franck, A., Gibson, G.R., Isolauri, E., Moreau, M.-C., Roberfroid, M. and Rowland, I. 1998. Functional food science and gastrointestinal physiology and function. Br. J. Nutr. Vol. 80, pp. S147–S171.

Salvador, V., Cherbut, C., Barry, J.L., Bertrand, D., Bonnet, C., Delort-Laval, J. 1993. Sugar composition of dietary fibre and short-chain fatty acid production during *in vitro* fermentarion by human bacteria. Br. J. Nutr. Vol. 70, pp. 189–197.

Scheppach, W., Luehrs, H. and Menzel, T. 2001. Beneficial health effects of low-digestible carbohydrate consumption. Br. J. Nutr. Vol. 85, pp. S23–S30.

Schrezenmeier, J. and de Vrese, M. 2001. Probiotics, prebiotics, and synbiotics – approaching a definition. Am. J. Clin. Nutr. Vol. 73, pp. 361S–364S.

Setchell, K.D.R., Lawson, A.M., Borriello, S.P., Harkness, R., Gordon, H., Morgan, D.M.L., Kirk, D.N., Adlercreutz, H., Andersson, L.C. and Axelson, M. 1981. Lignan formation in man – microbial involvement and possible roles in relation to cancer. Lancet Vol. 2, pp. 4–7.

Slavin, J.L. 2000. Mechanisms for the impact of whole grain foods on cancer risk. J. Am. Coll. Nutr. Vol. 19. pp. 300S–307S.

Slavin, J., Marquart, L. and Jacobs, D., Jr. 2000. Consumption of whole-grain foods and decreased risk of cancer: Proposed mechanisms. Cereal Foods World Vol. 45, pp. 54–58.

Titgemeyer, E.C., Bourquin, L.D., Fahey, G.C. Jr. and Garleb, K.A. 1991. Fermentability of various fiber sources by hunan fecal bacteria *in vitro*. Am. J. Clin. Nutr. Vol. 53, pp. 1418–1424.

Trowell, H. 1972. Crude Fibre, dietary fibre and atherosclerosis. Atherosclerosis Vol. 16, pp. 138–140.

Trowell, H., Southgate, D.A.T., Wolever, T.M.S., Leeds, A.R., Gassull, M.A. and Jenkins, D.J.A. 1976. Dietary fibre redefined. The Lancet Vol. 1, p. 967.

Tschäppät, S., Arrigoni, E. and Amado, R. 1999. *In vitro* fermentability of indigestible potato carbohydrates. Sciences des Aliments Vol. 19, pp. 301–309.

Valsta, L.M. 1999. Food-based dietary guidelines for Finland – a staged approach. Br. J. Nutr. Vol. 81, pp. S49–S55.

Vanharanta, M., Voutilainen, S., Lakka, T.A., van der Lee, M., Adlercreutz, H. and Salonen, J.T. 1999. Risk of acute coronary events according to serum concentrations of enterolactone: a prospective population-based case-control study. Lancet Vol. 354, pp. 2112–2115.

Van Laere, K.M., Hartemink, R., Bosveld, M., Schols, H.A. and Voragen, A.G. 2000. Fermentation of plant cell wall derived polysaccharides and their corresponding oligosaccharides by intestinal bacteria. J. Agric. Food Chem. Vol. 48, pp. 1644–1652.

Van Loo, J., Cummings, J., Delzenne, N., Englyst, H., Franck, A., Hopkins, M., Kok, N., Macfarlane, G.H., Newton, D., Quigley, M., Roberfroid, M., van Vliet, T. and van den Heuvel, E. 1999. Functional food properties of non-digestible oligosaccharides: a consensus report from the ENDO project (DGXII AIRII-CT94-1095). Br. J. Nutr. Vol. 81, pp. 121–132.

Van Soest, P.J. 1995. Comparative Aspects of Animal Models. In: Kritchevsky, D. and Bonfield, C. (eds.). Dietary Fiber in Health and Disease. USA: Eagan Press. Pp. 321–339.

Wang, L.-Q., Meselhy, M.R., Li, Y., Qin, G.-W. and Hattori, M. 2000. Human intestinal bacteria capble of transforming secoisolariciresinol diglucoside to mammalian lignans, enterodiol and enterolactone. Chem. Pharm. Bull. Vol. 48, pp. 1606–1610.

Weipert, D. 1997. Processing performance of rye as compared to wheat. Cereal Foods World Vol. 42, pp. 706–712.

White, L.M. and Secor, G.E. 1953. Occurrence of two similar homologous series of oligosaccharides in wheat flour and wheat. Arch. Biochem. Biophys. Vol. 43, pp. 60–66.

Wisker, E., Daniel, M. Rave, G. and Feldheim, W. 1998. Fermentation of non-starch polysaccharides in mixed diets and single fibre sources: comparative studies in human subjects and *in vitro*. Br. J. Nutr. Vol. 80, 253–261.

Wisker, E., Daniel, M. Rave, G. and Feldheim, W. 2000. Short-chain fatty acids produced *in vitro* from fibre residues obtained from mixed diets containing different breads and in human faeces during the ingestion of the diets. Br. J. Nutr. Vol. 84, pp. 31–37.

Åman, P. and Westerlund, E. 1996. Cell wall polysaccharides: structural, chemical, and analytical aspects. In: Eliasson, A.-C. (ed.). Carbohydrates in Food. New York: Marcel Dekker, Inc. Pp. 191–226.

Åman, P., Nilsson, M. And Andersson, R. 1995. Chemical composition of rye grain. In: Poutanen, K. and Autio, K. (eds.). VTT Symposium 161, International rye symposium: Technology and products, Helsinki, Finland, 7-8 December, 1995. Espoo: VTT. Pp. 33–38.

Appendices of this publication are not included in the PDF version. Please order the printed version to get the complete publication (http://www.vtt.fi/inf/pdf/)

Published by

Series title, number and
report code of publication

VTT Publications 500
VTT–PUBS–500

Author(s)
Karppinen, Sirpa

Title

Dietary fibre components of rye bran and their fermentation *in vitro*

Abstract

Dietary fibre (DF) is important for human well-being. It has many physiological effects along the entire human gastrointestinal tract. Many of the health effects are mediated by the microbial fermentation of dietary fibre in the large intestine. Rye is the main source of DF in Finland. Rye bran, in particular, is rich in DF. Rye was also shown to contain a significant fructan concentration, which according to the suggested new dietary fibre concept is also a component of DF.

In this study different rye bran fractions were prepared in order to study the effect of solubility and processing on the fermentability of rye bran in vitro. Before fermentation, substrates were enzymatically digested simulating conditions within the small intestine in order to remove starch and protein. Fermentability was studied in vitro using human faecal inoculum. Dietary fibre polysaccharides of rye bran could be divided into three groups: 1) fermentable, soluble polysaccharides that are rapidly fermented, 2) fermentable cell-wall associated polysaccharides that are gradually released from the cell-wall matrix and then fermented, and 3) polysaccharides and cell-wall structures that are not fermented at all. Short-chain fatty acids (SCFA) were produced, which were related to the consumption of carbohydrates: the higher the rate and quantity of carbohydrate consumption the higher the rate and quantity of SCFA production. Butyric and propionic acids were produced in all rye fermentations.

Arabinoxylan of rye was found to be fermented in vitro by Bifidobacterium longum, a numerically dominant Bifidobacterium species in the adult human colon. Thus, rye as such may be a candidate prebiotic. Rye is rich in plant lignans, and solubility and fermentation of carbohydrates had an impact on the conversion of plant lignans to mammalian lignans.

Keywords
dietary fibres, rye bran, fermentation, *in vitro* fermentation, carbohydrates, metabolism, prebiotics, lignans, extraction, chemical analysis

Activity unit
VTT Biotechnology, Tietotie 2, P.O.Box 1500, FIN–02044 VTT, Finland

ISBN	Project number
951–38–6229–1 (soft back ed.) 951–38–6230–5 (URL:http://www.vtt.fi/inf/pdf/)	

Date	Language	Pages	Price
July 2003	English	96 p. + app. 52 p.	C

Name of project	Commissioned by

Series title and ISSN	Sold by
VTT Publications 1235–0621 (soft back ed.) 1455–0849 (URL: http://www.vtt.fi/inf/pdf/)	VTT Information Service P.O.Box 2000, FIN–02044 VTT, Finland Phone internat. +358 9 456 4404 Fax +358 9 456 4374

Dietary fibre is important for human well-being. It has many physiological effects along the entire human gastrointestinal tract. Many of the health effects are mediated by the microbial fermentation of dietary fibre in the large intestine. Rye is the main source of dietary fibre in Finland. In this study different rye bran fractions were prepared in order to study the effect of solubility and processing on the fermentability of rye bran *in vitro*. Dietary fibre polysaccharides of rye bran could be divided into three groups: 1) fermentable, soluble polysaccharides that are rapidly fermented, 2) fermentable cell-wall associated polysaccharides that are gradually released from the cell-wall matrix and then fermented, and 3) polysaccharides and cell-wall structures that are not fermented at all. Arabinoxylan of rye was found to be fermented *in vitro* by *Bifidobacterium longum*, a numerically dominant *Bifidobacterium* species in the adult human colon. Thus, rye as such may be a candidate prebiotic. Rye is rich in plant lignans, and solubility and fermentation of carbohydrates had an impact on the conversion of plant lignans to mammalian lignans.

Tätä julkaisua myy
VTT TIETOPALVELU
PL 2000
02044 VTT
Puh. (09) 456 4404
Faksi (09) 456 4374

Denna publikation säljs av
VTT INFORMATIONSTJÄNST
PB 2000
02044 VTT
Tel. (09) 456 4404
Fax (09) 456 4374

This publication is available from
VTT INFORMATION SERVICE
P.O.Box 2000
FIN–02044 VTT, Finland
Phone internat. +358 9 456 4404
Fax +358 9 456 4374

ISBN 951–38–6229–1 (soft back ed.)
ISSN 1235–0621 (soft back ed.)

ISBN 951–38–6230–5 (URL: http://www.vtt.fi/inf/pdf/)
ISSN 1455–0849 (URL: http://www.vtt.fi/inf/pdf/)

Sirpa Karppinen