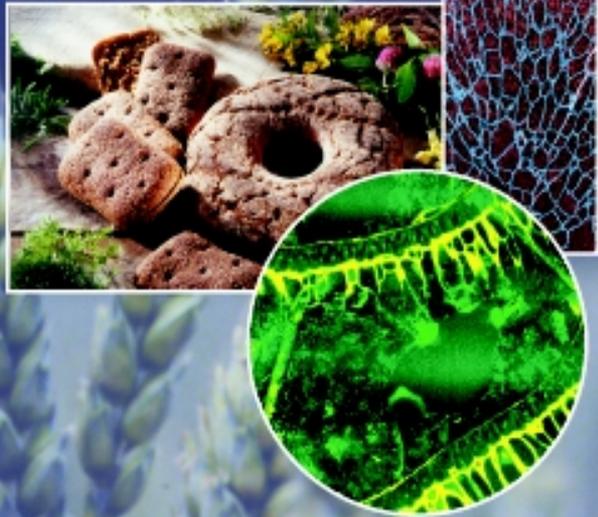


# Whole Grain and Human Health





VTT SYMPOSIUM 213

**Keywords:** meetings, food, whole grains, physiological effects, human health, diseases, dietary fibres, cereals, diabetes, cardiovascular diseases

# Whole Grain and Human Health International Symposium

Abstracts

Haikko Manor, Finland, June 13–15, 2001

Edited by

Kirsi Liukkonen, Annemari Kuokka & Kaisa Poutanen  
VTT Biotechnology, Finland

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Technical editing Maini Manninen

Otamedia Oy, Espoo 2001

## **Welcome to the world of whole grain!**

Intake of whole grains has in many studies been associated with health benefits, including improved regulation of blood glucose levels, decreased risk of coronary heart disease and different cancers. Researchers have found evidence that increased consumption of whole grains has great potential in improving public health. Important compounds and especially mechanisms behind the health benefits are yet only partly understood.

The intake of whole grain products, *ie.* foods containing all the parts of a grain, is inadequate. Wheat and rice are usually refined so that the outer layers rich in bioactive compounds are removed. Northern Europe is unique in having traditions of the use of whole grain rye breads and foods. There is a distinct use of whole grain oats and barley as well, but the use of these grains is minimal in comparison to their cultivation. Considering consumers' increasing interest in the health values of foods, it is a challenge and opportunity for the food industry to develop new, palatable whole grain foods.

This symposium features the latest results about the physiological effects of whole grains and their role in improving human health. It also reviews new types of food processing targeted for whole grain products, and discusses the role of health claims in promoting their use. About 70 contributions from 5 continents give a good picture of the prospects and potential of health promotion by whole grains all over the world. Thanks for the participants for their contribution to the scientific program.

We also present our sincere thanks to organizations and companies who have contributed to the success of this symposium: VTT Biotechnology, AACC, Finnish Food Research Foundation, Swedish Nutrition Foundation, Nordic Industrial Fund, Vaasan & Vaasan Oy, Fazer Bakeries Ltd, Avena Group, Wasabröd AB, Cerealia Group, Nestlé S.A., General Mills Inc, Raisio Group, Cargill Foods.

***Welcome to the world of whole grains to share with each other our knowledge and views. Let's reveal the secrets of what could and should be our everyday food!***

On behalf of the organizers

Kirsi Liukkonen, VTT Biotechnology  
Chairman,  
Organizing committee

Kaisa Poutanen, VTT Biotechnology  
Chairman,  
Scientific committee

# Whole Grain and Human Health International Symposium

13-15 June, 2001  
Hotel Haikko, Porvoo, Finland

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Dr. Knud Erik Bach Knudsen, Danish Institute of Agricultural Sciences, Denmark  
Dr. Len Marquardt, General Mills, USA  
Prof. Julie Jones, College of St Caitrine, USA

# PROGRAMME

WEDNESDAY, 13 JUNE

- 14.00 - 16.00 Visit to VTT Biotechnology
- 16.00 - 19.00 Registration
- 19.00 - 21.00 Get-together party

THURSDAY, 14 JUNE

- 8.00 - 8.30 Registration
- 8.30 - 8.45 Opening and welcome  
*Kirsi Liukkonen, VTT Biotechnology, Finland  
and Julie Jones, AACC*

## **The whole grain concept**

*Chairman: Kaisa Poutanen, VTT Biotechnology, Finland*

- 8.45 - 9.15 The health relevance of whole grains  
*David Jacobs, University of Minnesota, USA*
- 9.15 - 9.45 Whole grains: just for the health of it - who consumes  
adequate whole grain and who is missing the mark?  
*Julie Jones, College of St. Catherine, USA*
- 9.45 - 10.15 The dietary fibre complex  
*Per Åman, Swedish University of Agricultural Sciences,  
Sweden*
- 10.15 - 10.45 Coffee and posters

## **Bioactive compounds and mechanisms**

*Chairman: Knud Erik Bach Knudsen, Danish Institute of  
Agricultural Sciences, Denmark*

- 10.45 - 11.15 Cereal phytoestrogens and their association with human  
health  
*Herman Adlercreutz, Folkhälsan Research Centre and  
University of Helsinki, Finland*
- 11.15 - 11.45 Lignans and phytic acid  
*Lilian Thompson, University of Toronto, Canada*

- 11.45 - 12.15                    Colonic formation and absorption of mammalian lignans and butyrate  
*Knud Erik Bach Knudsen, Danish Institute of Agricultural Sciences, Denmark*
- 12.15 - 13.15                    Lunch
- 13.15 - 14.45                    Poster session; authors available by the posters for discussions

**Health claims: where are we?**

***Chairman: Nils Georg Asp, Lund University, Sweden***

- 14.45 - 15.15                    Effectiveness and acceptability of a dietary intervention to increase consumption of whole grain products in free living individuals  
*Ailbhe Smith, University of Newcastle, UK*
- 15.15 - 15.45                    Voluntary codes for health claims  
*Nils Georg Asp, Lund University, Sweden*
- 15.45 - 16.15                    Health claims in the USA  
*Len Marquardt, General Mills, USA*
- 16.15 - 16.45                    Coffee and posters
- 17.30 - 19.30                    Bus tour in old city of Porvoo
- 19.30                                Symposium dinner

FRIDAY, 15 June

**Diabetes and insulin metabolism**

***Chairman: Per Åman, Swedish University of Agricultural Sciences, Sweden***

- 8.30 - 9.00                        Wheat bran and whole grain cereals on blood glucose and cholesterol and diabetes and cardiovascular disease  
*David Jenkins, University of Toronto, Canada*
- 9.00 - 9.30                        Modulating the GI of cereal products; impact on glucose tolerance as measures in second-meal studies, and in a semi-long-term dietary intervention  
*Inger Björk, Lund University, Sweden*

9.30 - 10.00 Effects of rye on insulin metabolism  
*Hannu Mykkänen, University of Kuopio, Finland*

10.00 - 10.30 Coffee and posters

### **Cardiovascular disease**

***Chairman: Göran Hallmans, Umeå University, Sweden***

10.30 - 11.00 Whole grain, lipid metabolism and cardiovascular disease  
*Denis Lairon, INSERM, France*

11.00 - 11.30 Fibre and coronary risk: The role of enterolactone?  
*Meri Vanharanta, University of Kuopio, Finland*

11.30 - 12.30 Lunch

### **Cancers**

***Chairman: Len Marquardt, General Mills, USA***

12.30 - 13.00 Whole grain and risk of cancer  
*Joanne Slavin, University of Minnesota, USA*

13.00 - 13.30 Whole grain, lignans and prostate cancer  
*Göran Hallmans, Umeå University, Sweden*

13.30 - 14.00 Whole grains, cereal fibres and mechanisms of prevention of colorectal cancer  
*Graeme McIntosh, CSIRO Health Sciences and Nutrition, Australia*

14.00 - 14.30 Coffee

### **Discussion and conclusions**

***Chairman: Kaisa Poutanen, VTT Biotechnology, Finland***

14.30 - 16.00 ***Panel discussion:***  
The potential of whole grain in prevention of western diseases (Introduction D. Jacobs)  
How to promote the use of whole grains?  
(Introduction J. Jones)

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# **ABSTRACTS**



# THE HEALTH RELEVANCE OF WHOLE GRAINS

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Grains are seeds of grasses (monocotyledenous plants), including wheat, oats, rye, rice, barley, and corn. Grains are eaten in breads, rices, pastas, breakfast cereals, and cakes. Grains have a special botanical structure that, according to epidemiologic and feeding studies, is important to prevention of chronic diseases in humans. All grains have three major parts: bran, germ, and endosperm. The function of these grain components is to support the new grain plant until it roots. The bran and germ perform many botanical functions and are correspondingly fiber and nutrient- rich. The endosperm is primarily a food sack for the potential seedling. As a human food, it is energy-rich, consisting mostly of starch and cell walls. Although there is some fiber in the endosperm, it is nutrient-poor. The two major processes used in preparing grain for human consumption are refining (separating and discarding the bran and the germ, retaining only endosperm) and pulverizing to flour. Most nutrients and other bioactive constituents are contained in the bran and germ, and are lost in refining. Therefore refining of grain leads to a product that lacks many potentially beneficial nutritional properties. Pulverizing increases the glycemic index of the grain food, compared to the lower glycemic index when intact kernels are eaten. Flour made from whole grain therefore contains all the phytochemicals that intact kernels contain.

Whole grain flour appears to have health benefits in reducing risk of chronic diseases. Large prospective studies in the United States and Norway have found that risk of coronary artery disease and diabetes is reduced by 30–40% in those men and women who habitually eat whole grain foods. One two year clinical trial in male survivors of myocardial infarction yielded reduced risk when advice to increase fiber, decrease fat, and increase fish was followed, but not when advice was to increase fiber without the other two components. Cancer and whole grain intake has rarely been studied in prospective studies; breast cancer was unrelated to whole grain intake, while upper digestive tract and endometrial (the latter only in nonusers of hormone replacement therapy) cancers were inversely related to whole grain intake. Case-control studies find reductions in diverse cancers, such as soft tissue sarcoma, non-Hodgkin's lymphoma, bladder, myeloma, oral, pharyngeal, tongue, esophageal, larynx, gastric, liver, pancreatic, endometrial, ovarian, thyroid, Hodgkin's lymphoma, and brain. Case-control findings for whole grain intake and colon cancer are mixed. Clinical trials of high fiber diets did not show reduced risk for recurrent colon polyps. It is likely that whole grain intake reduces risk for nonmalignant digestive diseases, such as diverticulitis and

appendicitis, but there is very limited information on this topic. Whole grain intake is related to reduced coronary calcification, reduced microalbuminuria, higher lung function, and lower fasting insulin. Fasting insulin and plasminogen activator inhibitor were reduced and serum enterolactone increased in a whole grain feeding study.

There are many ways in which whole grain may positively affect health, and in which throwing away of the bran and germ seems counterproductive. This is especially so in Western cultures where energy expenditure is low and total energy intake is correspondingly low. Nevertheless, Scandinavian countries that eat considerable amounts of whole grain do not have lower rates of many chronic diseases than do other countries, e.g., in the Mediterranean area where the diet is rich in many other minimally processed plant foods, but bread is usually made from refined wheat, or in the Orient, where refined rice is a staple. We conclude that whole grain is an important part of a diet otherwise high in minimally processed plant foods and low in saturated and trans fats.

# WHOLE GRAINS: JUST FOR THE HEALTH OF IT - WHO CONSUMES ADEQUATE WHOLE GRAIN AND WHO IS MISSING THE MARK?

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Consumption of whole grain products has been shown in epidemiological studies to reduce the risk of many different chronic diseases. For example, in The Iowa Women's Health Study consumption of between 1–3 servings of whole grains reduced risk of obesity, Type II diabetes, and other diseases. Similar results have been shown in populations in other parts of the world. In these studies nutrients contained in the whole grains such as fiber and B vitamins could not fully account for the observations. However, there were many confounders. Those individuals who consumed lots of whole grain had a host of other healthy habits.

This paper will compare and contrast the whole grain intake of various groups around the world with a variety of recommendations regarding whole grain intake from government agencies and health advocacy bodies. For many populations in Western countries, consumption is far below the recommendations. It will compare differences in nutrient intake of consumers with high and low whole grain intake within and among populations. In instances where whole grain intake is difficult to ascertain, it will be extrapolated from marker nutrients such as dietary fiber and the analysis will compare effects of fiber, marker nutrients and phytochemicals to approximate whole grain consumption. The effects of individual components of whole grains will be compared at to see if the effects of ingesting them as a unified package shows the whole to be greater than the sum of its parts. Whole grain intake with respect to other healthy behaviors will also be analyzed.

Programs in place around the world to increase whole grain consumption such as Go Grains in Australia will be assessed. Recommendations for programs will be proposed that may increase grain consumption in many parts of the world.

# THE DIETARY FIBRE COMPLEX IN WHOLE GRAIN CEREALS

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In this paper I will present the structure, composition and content of the main dietary fibre components in whole grain cereals. Methods for definition and analyses of dietary fibre will be discussed. The term dietary fibre complex, including the dietary fibre and bioactive components present in fibre-rich structures, will be put forward. Finally bioactive components in the “Dietary Fibre Complex” will be exemplified.

Cereal grains are composed of pericarp, testa, aleurone, starchy endosperm and germ. All these structures contain cell walls with different composition and properties. In cereals the main cell wall components are  $\beta$ -glucan, xylans, cellulose and lignin. Associated substances may be waxes, cutin, suberin and substituents such as cinnamic acids and acetyl groups. The structure of the plant cell walls is not very well defined but several models have been put forward.

Trowell has defined dietary fibre as "... the remnants of edible plant cell polysaccharides, lignin and associated substances resistant to (hydrolysis) digestion by the alimentary enzymes of humans". Other definitions such as "Non starch polysaccharides, enzyme resistant starch and Klason lignin" or "Non-starch polysaccharides" are more chemical in nature. Today no generally accepted definition exists and in Europe efforts to adopt such a definition have failed. Therefore it is more or less the method used for the analysis of dietary fibre that will determine which components that will be included in the dietary fibre value. Examples of methods for the dietary fibre analysis, which have been collaboratively evaluated, are the enzymatic-gravimetric method for total dietary fibre (Prosky method) (AOAC 985.29, AACC 32-05), the enzymatic-gravimetric method for total and insoluble dietary fibre (MES Tris buffer method) (AOAC 991.43, AACC 32-07) and the gas chromatographic-colorimetric-gravimetric method for individual dietary fibre components (Uppsala method) (AOAC 994.13, AACC 32-25, NMKL 162). All these methods give similar values for dietary fibre. Fructans and fructooligosaccharides will generally not be included in these analyses, but can be determined in a separate analysis and then if desired can be added to the fibre value.

It is important to have a generally accepted definition for dietary fibre and an analytical method that corresponds to this definition for many reasons, such as for declaration of nutrient content in foods, for making dietary fibre values in

data bases which are used in eg. epidemiological studies useful and for making health claims related to the content of dietary fibre reliable. Recently AACC defined dietary fibre as "... 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". This definition will clearly also include more soluble substances such as fructo-oligosaccharides, galacto-oligosaccharides and polydextrose, which are not always included in the fibre values used today.

In the future more specific methods for individual dietary fibre components will probably be used more extensively. Examples of such components are  $\beta$ -glucan, arabinoxylan, cellulose and lignin. Also the properties of dietary fibre structures or individual components such as viscosity, gel forming properties, water-holding capacity, ion-exchange capacity and fermentability will be evaluated

With the dietary fibre complex we mean the dietary fibre and the biologically active substances such as vitamins, antioxidants and phytohormones present in fibre rich parts of the foods. These bioactive components will generally not influence the fibre value to any greater extent since they are present in low amounts but will have pronounced biological effects. The biologically active substances will be exemplified with tocopherols and tocotrienols in barley fractions, phenolic antioxidants such as cinnamic acids in maize bran and avenanthramides in oats as well as plant lignans and alkylresorcinols in rye.

Alkylresorcinols are a group of phenolic lipids which have been studied in the Nordic rye and health project. They are present in the outer parts of cereals, especially in rye, triticale and wheat. The content in rye range between 550 and 1120  $\mu\text{g/g}$  and in bran the content may be as high as 3000  $\mu\text{g/g}$ . Alkylresorcinols can be incorporated in lipid membranes and may influence lipid absorption and liposome structure. A study on the absorption of alkylresorcinols in humans was performed in collaboration with Professor Hallmans group at Umeå University. Ten ileostomy-operated subjects were fed diets containing rye bran bread which are rich in alkylresorcinols or a white wheat control bread with no alkylresorcinols. Diets and ileostomy-samples were analysed for alkylresorcinols and the apparent digestibility calculated. The average apparent digestibility of the different alkylresorcinols in humans was found to be around 60 % for all major homologues. Alkylresorcinols may, therefor, be a good marker for whole grain wheat and rye intake.

From declaration of nutrient, epidemiological and health claim points of view it is important to define the term dietary fibre and to develop methods in

accordance with a generally accepted definition. The term “Dietary Fibre Complex” includes dietary fibre and biologically active substances present in fibre rich structures. These biologically active substances are generally minor components and will therefore not influence the dietary fibre value, if included, to any greater extent. However their bioavailability and biological activity will greatly depend on the dietary fibre content, composition and structure. In order to understand the biological effects of fibre-rich foods, such as whole grain cereals, both the content and properties of dietary fibre and biologically active components need to be studied.

# CEREAL PHYTOESTROGENS AND THEIR ASSOCIATION WITH HUMAN HEALTH

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In 1982 studying women living in Boston [1] we found low excretion of enterolactone in healthy women with breast cancer and suggested that the lignans may be involved in protection against this disease. We also suggested later on that they may also be involved in the protection against prostate and colon cancer [2]. This hypothesis was supported by finding of higher urinary excretion of lignans in rural regions in north-east Finland with lower incidence of these cancers compared to more urban areas in south and southwest Finland [3]. The precursors of the biologically active compounds detected in man are found mainly in whole grain cereal food, seeds, berries, tea, and some vegetables, like carrots and Brussels sprouts. The lignans found in man and animals are called mammalian lignans, the two main compounds being enterolactone and enterodiol. These are formed by intestinal microbial metabolism mainly in the upper part of the colon [4, 5]. Previously only two plant precursors for enterolactone were known to occur, i.e. secoisolariciresinol and matairesinol. Of these two precursors secoisolariciresinol is by far the most abundant in human foods. This plant lignan is converted first to enterodiol, which then is oxidized in the colon to enterolactone. The relative amount of enterolactone increases distally in the large bowel. Recently we found a number of new lignans in rye bran of which pinoresinol, lariciresinol and syringaresinol are converted to enterolactone. A fourth lignan called isolariciresinol is not converted to enterolactone by human fecal flora [6]. Our most recent results give further support to the view that the intestinal microflora in the upper part of the colon plays a very significant role in mediating the beneficial effects of plant food to our body as suggested previously [7, 8]. Epidemiological as well as *in vitro* and *in vivo* experimental studies suggest that the lignans may protect against colon, breast and perhaps prostate cancer as well as coronary heart disease, but in most of the studies it has not been possible to separate the effect of the lignans from the effect of other components in the same food. Some of our studies in collaboration with other groups are briefly described below:

The recent development of rapid highly sensitive micromethods for plasma and urine enterolactone [9, 10] allowing large scale investigations has caused a rapid development of the field. In a study in 2383 Finnish subjects the main determinants of plasma enterolactone were smoking and obesity (negative

association), whole grain bread, vegetables, berries and fruit and obstipation (positive association) [11]. However, the great variation in plasma enterolactone observed could only to a little degree be explained by these determinants, which suggests that the main determinant is the composition of the intestinal microflora and its activity. The evidence obtained suggested that plasma enterolactone is a good biomarker of lifestyle and diet. The first large-scale studies in diseased subjects using the new methods were carried out to investigate the association of breast cancer with plasma enterolactone. In a case-control study in eastern Finland in 194 subjects with breast cancer and 208 community-based controls the odds ratio adjusted for all the known risk factors for breast cancer was 0.38 in the highest quintile of enterolactone values [12]. In a recent prospective study in northern Sweden low plasma levels of enterolactone below the 12.5th percentile (enterolactone below 5.7 nmol/l) were associated with increased breast cancer risk. The adjusted relative risk and 95 % CI at the lowest percentile were 2.0 (95% CI 1.1–3.5) [13]. Intake of antibiotics decreases enterolactone production for a very long time even up to 12–16 months [14] and recently it was found that frequent intake of such drugs increases risk of breast cancer in young women with urinary infections [15]. Furthermore, a high plasma enterolactone level protects men for acute coronary events [16] and plasma enterolactone shows an inverse association with F2-isoprostanes in plasma [17] (to be presented separately at this meeting). However, it should be emphasized that plasma enterolactone may not be the active substance reducing risk, but a good biomarker of lifestyle. It is likely that many other phytochemicals participate in reducing disease risk.

In collaboration with Dr K.E. Bach Knudsen and associates we have studied enterolactone formation in the colon of pigs *in vivo*. There is a highly significant association between butyrate and enterolactone production, which means that a high fermentation favors production of enterolactone in the colon. Butyrate is protective with regard to colon cancer [18] and perhaps also breast and prostate cancer [19, 20] and high microbial activity also increases bile acid esterification associated with lower risk of colon cancer [21–23].

It is concluded that the microbial activity of the colon leading to production of butyrate, enterolactone and esterified bile acids may be closely related to breast and colon cancer risk and may be risk of other Western diseases. Much further work is needed to verify our original hypothesis that the common denominator of these diseases is the dietary effects on the intestinal steroid, sterol and bile acid metabolism, and on the composition of the intestinal microflora and on its activities [2, 7].

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# LIGNANS AND PHYTIC ACID

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Many studies have indicated that whole grains are protective against chronic diseases such as cancer, cardiovascular disease and diabetes. Several components may be responsible for this effect and may include the lignans and phytic acid (PA; inositol hexaphosphate). This paper will describe some of our recent work supporting the potential role of these compounds in the prevention or treatment of cancer particularly of the colon or breast.

The major lignans found in whole grains are secoisolariciresinol diglycoside (SDG) and matairesinol which can be metabolized to the mammalian lignans enterodiol (ED) and enterolactone (EL) by the bacterial flora in the colon. In carcinogen-treated rats, purified SDG has been shown to reduce the early biomarkers of colon cancer. In *in vitro* studies, we have observed the ability of ED and EL to reduce the cell proliferation and increase the apoptosis of colon tumor cells particularly in the presence of calcium and short chain fatty acids (SCFA). Because the soluble fiber in whole grains can increase the amount of dietary minerals such as calcium that reach the colon and since calcium can be released upon fermentation of the soluble fiber to SCFA in the colon, the results suggest that the interactive effects of the lignans, calcium and SCFA may in part contribute to the protective effects of some whole grains. In other *in vitro* studies, we have observed that ED and EL can reduce the migration and adhesion of the human cancer cells MDA MB435, indicating that these lignans may also reduce the risk of breast cancer metastasis.

Many studies have shown that PA can reduce the early biomarkers of cancer risk or tumors in carcinogen-treated rats. However, in most cases, these studies were conducted using purified PA given either in the drinking water or diet. Thus it is unclear whether the naturally occurring or endogenous PA in whole grains will produce the same effect. To differentiate the effect of endogenous vs purified PA, we studied the effect of a low fiber diet with or without supplementation with either 25% wheat bran (WB), 1% pure PA (equivalent to the amount present in 25% WB), 25% dephytinized wheat bran (DWB), or DWB + 1% pure PA, on early biomarkers of colon cancer risk. All treatment diets significantly reduced the sialomucin producing aberrant crypt foci and cell proliferation, particularly in the top 40% of the crypt, versus the control diet. WB, with its endogenous PA, had a greater effect on cell proliferation and apoptosis than the purified PA. The

removal of endogenous PA caused an increase in cell proliferation suggesting that the colon cancer protective effects of WB may be due in part to the endogenous PA. The purified PA appears to be more protective when added to a low fiber diet than to the high fiber DWB diet. The different effects of endogenous and exogenous (purified) PA suggest that, although both are effective, they may be acting through different mechanisms. Many mechanisms have been suggested for the cancer protective effect of PA including its ability to act as an antioxidant by binding iron, a catalyst of lipid peroxidation. However no significant differences in antioxidant activities were observed between diet groups in this study.

# COLONIC FORMATION AND ABSORPTION OF MAMMALIAN LIGNANS AND BUTYRATE

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There is increasing evidence that the microbial flora of the large intestine (caecum and colon) produce compounds that can have positive health effects in humans. For instance, butyrate and mammalian lignans are products of microbial fermentation in the large intestine from non-digestible carbohydrates (NDC i.e. non-digestible oligosaccharides, resistant starch and non-starch polysaccharides) and plant lignans. Mammalian lignans are believed to play a preventive role in the development of breast and prostate cancer and perhaps also colon cancer. Butyrate is the main oxidative substrate of the colonic epithelium and there is increasing evidence that some pathogenic conditions such as ulcerative colitis and colon cancer are related to impaired butyrate formation and metabolism. Moreover, it is now clear that the production of both mammalian lignans and butyrate in the large intestine is influenced by the dietary composition.

Because of the inaccessibility of the human large intestine, much of our knowledge concerning formation and absorption of mammalian lignans and butyrate derives either from *in vitro* fermentation studies or from *in vivo* studies with model animals such as rats or pigs.

## MAMMALIAN LIGNANS

Plant lignans occur as glycosides in the fibre rich matrix in a number of plant-based foodstuffs including whole grain cereals. The plant lignans are converted into mammalian lignans, enterodiol (End) and enterolactone (Enl), by microbial action in the gut with End being oxidised further to Enl. Until recently, it was believed that secoisolariciresinol (Seco) and matairesinol (Mata) were the only two plant precursors that were converted to mammalian lignans, but new studies have revealed that the same may be true for other plant lignans - lariciresinol and pinoresinoldiglucoside. *In vitro* fermentation studies show that the total formation of mammalian lignans is primarily determined by the level of plant lignans explicitly pointed out by the production being several folds higher when fermenting a whole grain rye diet compared to a refined endosperm diet. The *in vitro* fermentation studies also show that the conversion of plant to mammalian lignans is a relative slow process compared to the fermentation of NDC. For

instance, relative proportion of short-chain fatty acids (SCFA) formed during the first 3 hours of a 48 hours' batch incubation was 55 % compared to only 7 % of Enl during the same time span. *In vitro* fermentation studies further suggest that while the rate of SCFA formation was influenced by the cellular structure of the fibre fraction the same was not the case with Enl, which was formed at a rate independent of the fibre composition. Enl is most likely taken up by passive diffusion driven by the concentration difference between the lumen side and the portal vein. Results from experiments with catheterised pigs show that the portal and arterial levels of Enl were relatively stable with no difference between the absorptive phase (high influx of nutrients) and the post-absorptive phase (low influx of nutrients). The foremost important factor influencing the portal level of mammalian lignans is the concentration of plant lignans and in particular the dietary level of Seco, which was stronger, correlated to the portal level of Enl than the total level of plant lignans.

## BUTYRATE

The main substrate for butyrate formation is NDC. The rate and total production of butyrate is primarily influenced by type and levels of NDC entering the large intestine with some types of NDC having specific stimulatory effects on butyrate formation. For instance, arabinoxylans present in the cell walls of cereals stimulate butyrate formation as do resistant starch. It is generally believed that most butyrate is efficiently metabolised by the colonic epithelium. However, results from an *in vivo* experiment with catheterised pigs show that when the colonic formation of butyrate reaches a certain level, the relative proportion of butyrate in the portal vein increases relatively more than acetate and propionate. The nutritional significance of this finding is at present unknown, but it may indicate that at high production rates of butyrate, the molecule is distributed to cell not in direct proximity to the gut. It was also found that there was a relatively strong correlation between the net absorption of butyrate and mammalian lignans. The reason for that is at present not known but it could possibly be that the same type of plant tissues stimulates the formation of both butyrate and mammalian lignans.

## CONCLUSION

Mammalian lignans and butyrate are produced by microbial action in the large intestine. The colonic formation and concentration in the circulation are related to the dietary composition in particular the concentration of fibre. The implication of such findings is that the consumer, by choice of foodstuffs, has a great control over the supply to the body of health promoting compounds. Recent results suggest that the mammalian lignans serve as biomarkers of a healthy lifestyle that by its integrated approach may protect against chronic diseases like coronary heart disease and certain cancer forms.

# EFFECTIVENESS AND ACCEPTABILITY OF A DIETARY INTERVENTION TO INCREASE CONSUMPTION OF WHOLEGRAIN PRODUCTS IN FREE LIVING INDIVIDUALS

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## BACKGROUND

Consumption of foods rich in wholegrains has been advocated in health promotion strategies based on evidence linking increased wholegrain consumption with reduced risk of several non-communicable diseases such as heart disease, Type 2 diabetes and some cancers (Richardson, 2000). Despite attempts to raise awareness and increase wholegrain consumption, current intakes in the US population remain below recommended amounts (Krauss *et al.*, 1996). There are no comparable data for the UK population. Strategies aimed at increasing wholegrains consumption present an alternative 'positive' approach to eating a healthy diet compared with the 'negative' messages associated with reducing dietary fat consumption. Such an approach in which consumers are encouraged to increase consumption of wholegrains by concentrating on the benefits of these foods may be more acceptable and effective than stressing the harmful effects of diets high in fat.

## OBJECTIVES

To evaluate the effectiveness and acceptability of a dietary intervention aimed at increasing consumption of foods rich in wholegrains and to investigate barriers to increasing consumption of wholegrains in free living individuals. The results presented here represent part of a larger study comparing the effectiveness of negative and positive health promotion strategies.

Ethical approval for the study was provided by the Joint Universities and Newcastle & North Tyneside Health Authority Ethics Committee. All volunteers gave written, informed consent before taking part in the study.

## METHODS

Seven healthy female volunteers mean age 33.8 years (range 25 to 40) were recruited by advertisement from the Newcastle University staff population. Volunteers were contacted by telephone and those following therapeutic diets and those claiming they were following diet restriction were excluded from the study. Each volunteer completed a 7 day food diary with estimated food portion sizes which were confirmed at interview using a photographic atlas of food portion sizes (Nelson *et al.*, 1997). A 24 h collection of urine was also completed during the 7 d period. Following completion of the baseline food diary volunteers took part in a session during which the intervention was delivered by a nutritionist. The intervention involved delivery of the positive health messages arising from consumption of wholegrain products, advice on how to increase wholegrains consumption with examples of wholegrain products. Each volunteer was then provided with a range of wholegrain products sufficient to incorporate one item of wholegrain at each meal into their diet for 14 days (Table 1), with a target of 5 portions per day. A second ‘intervention’ diary was during the last 7 days of the intervention period. On six of the evenings during the intervention period volunteers completed a short ratings questionnaire to assess satiety, snacking habits and acceptability of their diet.

**Table 1.** Wholegrain foods provided for volunteers

Breakfast cereals	Shredded Wheat (Nestlé) Fruitful (Nestlé) Cheerios (Nestlé)
Pasta	Wholegrain pasta shapes Wholegrain Spaghetti
Rice	Brown rice Brown Basmati rice
Snacks	Rye crispbread (Ryvita) Nutri-Grain Bars (Kellogg’s)

One month following the intervention volunteers took part in a semi-structured focus group discussion exploring their attitudes towards the intervention, their ability to change their diet and perceived barriers to change. Nutrient intakes were calculated from food diaries using Microdiet Plus for Windows (Salford, UK). Comparison between baseline and intervention data was by Paired *t* test using SPSS version 10.0. Qualitative data was assessed using QSR NUD\*IST 4 (Sage Publications, CA, USA).

## RESULTS AND DISCUSSION

Nutrient intakes for volunteers at baseline and during the last seven days of the intervention are shown in Table 2. Energy intakes were within normal ranges

[ratio of energy intake: calculated BMR; 1.39 SD 0.38], and the pattern of nutrient intake at baseline was in line with current UK population data. The proportion of energy from total fat fell during the intervention period, although this was not statistically significant ( $P = 0.093$ ). This was balanced by a small rise in the percentages of energy from carbohydrate and protein. Fibre (as measured with Englyst methodology) intake, as a surrogate marker of wholegrain intake, rose from 12.8 to 16.5 g per day ( $P = 0.096$ ).

	<i>n</i> = 7	Baseline	Intervention
Energy Intake (mJ/day)		7.71 ± 1.87	8.12 ± 2.67
<i>% of daily energy from:</i>			
Carbohydrate		47.0 ± 2.8	50.2 ± 7.2
Total fat		35.1 ± 2.7	31.0 ± 5.6
Protein		16.1 ± 1.9	16.6 ± 2.3
Alcohol		3.3 ± 2.9	3.5 ± 5.1
Fibre (Englyst) intake (g/day)		12.8 ± 5.8	16.5 ± 5.9

<sup>1</sup>Mean values with SD

In the discussion session the volunteers expressed an overall positive attitude towards the study. They found the foods acceptable in taste and a convenient and welcome improvement to their previous dietary patterns. Their rating of the new dietary pattern averaged 6.2 (range 4.7 to 8.4 where 0 = ‘I really hate it’; 10 = ‘I really love it’). It was felt that five portions of whole grain foods per day were generally easily achieved during the intervention, the exceptions being situations in which the volunteers were not in control of meal preparation (e.g. eating out), or where other peoples’ preferences needed to be considered. All participants were happy to maintain at least some of the changes, though to a lesser extent than recommended in the intervention. The main barriers to the long-term inclusion of the foods included personal preferences for other products, the limited variety and availability of the foods, intestinal discomfort [bloating] experienced during the two week intervention period, and concerns that maintaining intervention consumption levels could compromise intakes of other important dietary constituents, such as fruit.

The positive messages used to increase wholegrains consumption were effective in promoting dietary change. Increased consumption of wholegrain products at a level consistent with acceptability and convenience could contribute to the lowering of the percentage of dietary energy derived from fat. Use of this type of intervention may be more successful in changing dietary habits at the population level than negative health promotion strategies aimed at reducing fat intake.

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# VOLUNTARY CODES FOR HEALTH CLAIMS

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Health claims in the labelling and marketing of food products is one way to win the attention of health-conscious consumers. Provided that such claims are used in a truthful and responsible way, and put into the context of the complete diet, they may contribute to consumer education on important diet-health relationships. Furthermore, the possibility to use well-substantiated health claims is an important incentive for food manufacturers to develop foods with health benefits in addition to providing nutrients. This possibility, however, is limited by the general prohibition of health claims on foods with reference to the medicinal products legislation.

## CODEX ALIMENTARIUS

According to the Codex general Guidelines on Claims (CAC/GL 1-1979, Rev. 1-1991) the following claims are prohibited: those which cannot be substantiated and those which claim a “suitability of a food for use in the prevention, alleviation, treatment or cure of a disease, disorder, or particular physiological condition”, unless they are permitted e.g. for foods for special dietary uses or “under the laws of the country in which the food is distributed”. Thus Codex leaves room for differences in legal regulations between countries.

In the present work within the Codex Committee on Food Labelling (ALINORM 01/22, APPENDIX VIII) “health claim means any representation that states, suggests, or implies that a relationship exists between a food or a constituent of that food and health”. The following three types of health claims are defined: Nutrient Function Claims – describe the physiological role of the nutrient in growth, development and normal functions of the body, Enhanced Function Claims – specific beneficial effects on physiological functions or biological activities, and Reduction of disease risk claims - relating the consumption of a food to reduced risk of developing disease. These must consist of two parts, information on an accepted diet-health relationship, followed by information on the composition of the product relevant to the relationship.

## VOLUNTARY CODES IN EUROPE

In the absence of a European Community legislation regarding health claims on foods, some EU countries have developed voluntary codes of conduct in order to complete the legislation and allow provisionally for some health claims.

### Sweden

The Swedish Food Industry's Rules (Self-Regulating Programme) *Health Claims in the Labelling and Marketing of Food Products* was established in 1990. In the revised programme applicable from January 1, 1997 (1), the claim must consist of two parts: information on one of eight listed, generally recognised diet-health relationships, followed by information on the composition of the product.

The eight connections between diet and health, on which health claims in two steps can be based are:

1. Obesity – energy content
2. Cholesterol level in the blood – low saturated fat, some types of soluble, gel-forming dietary fibre
3. Blood pressure - salt (sodium chloride)
4. Atherosclerosis – factors decreasing blood cholesterol and/or blood pressure; naturally occurring omega-3-fatty acids, as found in fat fish and fish products
5. Constipation – dietary fibre
6. Osteoporosis – calcium
7. Caries – absence of sugars and other easily fermented carbohydrates
8. Iron deficiency – iron content with consideration of components that stimulate and inhibit iron absorption

General requirements include that "Health claims must be formulated to take into account the need for a balanced diet providing all the different nutrients. Such claims should only be made in the marketing of products, the normal consumption of which has substantial effect on the diet as a whole."

The two-step principle means that only generic claims are allowed in the present Swedish code. Products eligible for health claims must fulfil certain compositional criteria, but no effect documentation of the product is required. Probiotic products are not to be handled within the programme, and diet-cancer claims are not allowed at present.

Two-step health claims according to the Swedish code are used at present mainly for yellow fats with low total and saturated fat content, and for cereal products with oat fibre regarding blood cholesterol level and risk for atherosclerosis (connections 2 and 4), and for cereal products regarding dietary fibre and bowel function (connection 5) (2).

In 1998, an extension of the code to “Product-specific Physiological Claims” was suggested (3-5), and in March 2001 the SNF Swedish Nutrition Foundation got the assignment to implement such an extension in collaboration with the food industry and retail organisations, and consumers, and in contact with the National Food Administration. Product-specific claims have to be based on scientifically acceptable human nutrition studies. A peer-reviewing procedure will be established for pre-marketing evaluation of the scientific documentation, as well as “The Assessment Board for Diet-Health Information” to evaluate the use of health claims in the marketing of food products. A temporary implementation of an extended self-regulating programme is in agreement with a recent report from the National Food Administration to the Swedish Government (6) on product-specific health claims.

#### The Netherlands

The Dutch “Code of practice assessing the scientific evidence for Health benefits stated in Health claims on food and drink products” (7) was established in 1998 with the objectives “to provide market parties with an efficient means of assessing the scientific evidence for health benefits stated in health claims”, and “to boost consumers’ confidence in information that is provided by health claims on food and drink products”.

After an application, the Netherlands Nutrition Centre appoints a panel of experts, in concert with the applicant, with at least three members. The task of the panel is to assess the scientific evidence for a health benefit as a basis for a health claim. The literal text of the health claims, and the safety aspects, are not to be assessed by the panel, however.

The panel’s judgement is based on relevant and reproducible data on human subjects relevant to the target population. It applies to food products (or group of products) and a general requirement is that the health benefit “must not clash with dietary guidelines”. Until October 2000, seven procedures had started, and two were completed. The assessment report is publicly available only when the applicant has set the product on the market with a health claim according to the assessment. The Dutch code is currently under evaluation.

#### United Kingdom

The UK Joint Health Claims Initiative (8), JHCI, was established in 1997 and officially launched in December 2000. It is a joint venture between consumer organisations, enforcement authorities and industry trade associations. The initiative arose from “recognition of the role diet has in maintaining good health and anticipation of growth in the ‘functional foods’ market, and that existing laws are both incomplete and inflexible, limiting the communication of the role of a healthy diet in reducing the risk of disease”.

A Code Administration Body of the JHCI has been established, comprised of the Council with equal representation of the three parties behind the Code, the Executive Secretary, and the Expert Committee. This panel of independent experts will advise on the establishment of a list of generic claims, and consider the scientific evidence submitted by companies wishing to make an innovative, i.e. product specific claim.

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# HEALTH CLAIMS IN THE U.S.A

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Health claims on food products can attract the attention of health-conscious consumers. They can positively influence consumer attitudes toward a product as evidenced by the recent claims placed on whole-grain foods. Since 1993, the Food and Drug Administration (FDA) has presided over what is allowed on food labels, in terms of how nutrients or certain substances in a food affect disease or health-related conditions. The growing relationship between food and health has led to a growing number of claim proposals being forwarded to FDA for review. This session details the different types of claims FDA has permitted including nutrient content, structure function, authoritative statement and health claims – and it presents the processes involved in preparing claims for submission. Approaching FDA for approval of a health-related claim is an intricate process requiring the efforts of many including manufacturers, scientists and health professionals. The time commitment, data and scientific evidence required for each type of claim differs, yielding pros and cons to each. These issues are discussed at this presentation and case studies on two recently approved claims -- the oat health claim and the whole grain authoritative statement are used to illustrate such points. Health claims are an important tool for communicating health information. A rigorous process for maintaining the integrity of food label claims helps to ensure a valuable resource for consumers.

# WHEAT BRAN AND WHOLE GRAIN CEREALS ON BLOOD GLUCOSE AND CHOLESTEROL AND DIABETES AND CARDIOVASCULAR DISEASE

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Cereal fiber has been shown in a significant number of cohort studies to reduce the risk of developing diabetes and cardiovascular disease (CHD). The mechanism(s) by which this might occur remain obscure. However wheat bran added to test meals has little effect on blood glucose and in the longer term, although some studies have reported improved glucose tolerance after wheat bran, no studies have reported improved HbA1C levels. Similarly the effects of wheat bran on serum lipids is generally unremarkable. This lack of effect of wheat bran contrasts with the effect of minimally processed whole grain cereals.

There have also been reports of reductions in blood pressure on wheat bran, however these data are limited since the majority of studies either have not measured blood pressure or appear to detect no difference. Similarly although high fruit and vegetables fiber intakes have been associated with improvements in clotting factors, studies focussing on wheat bran and clotting factors have not been undertaken. A similar picture consists for the lack effect of wheat bran on homocysteine and inflammatory biomarkers, such as c-reactive protein both of which have been linked to CHD. For these reasons the exact mechanism by which wheat bran exerts a protective effect in reducing the risk of diabetes and CHD remains unclear.

More recently emphasis has been focused on whole grain cereals. This term refers to the use of all the component parts of the cereal grain e.g. the wheat germ, the aleurone layer at the endosperm. More correctly the term whole grain should be reserved for the intact seed. Nevertheless emphasis is now being placed on whole grain product consumption in cohort studies, where a protective effect in reducing diabetes incidence has been reported. Again few controlled trials exist documenting the effect of whole grain (whole meal) products on carbohydrate and lipid metabolism, although beneficial metabolic effects have been reported for the whole (intact) grain. However true whole (intact) grain breads have been shown to have remarkably lower glycemic indices than milled flour breads of the same macronutrient composition. Furthermore altering the glycemic index by altering particle size has been shown to improve diabetes control, blood lipids and PAI-1. These data support findings from cohort studies

indicating that low glycemic index and glycemic load diets offer protection from development of diabetes and CHD.

Further studies are therefore required to define the metabolic effects of wheat bran, whole meal flours and their individual components (e.g. wheat germ) and whole (intact) grain products. These data would then form the basis for the design of large randomized controlled trials to assess effectiveness in reducing the risk for chronic diseases including diabetes and CHD.

# MODULATING THE GI OF CEREAL PRODUCTS; IMPACT ON GLUCOSE TOLERANCE AS MEASURED IN SECOND-MEAL STUDIES, AND IN A SEMI-LONG-TERM DIETARY INTERVENTION

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Several semi-long term dietary interventions are at hand demonstrating not only a therapeutic potential of a low Glycaemic Index (GI) diet in the treatment of diabetes and dyslipidemia, but also a preventive potential against NIDDM and cardio-vascular disease. The implementation of the GI concept in dietary advice will, however, require an extended list of low GI foods. In particular, there is a need to develop bread products and breakfast cereals with low GI features.

## GI-OPTIMISATION OF CEREAL PRODUCTS

A number of food factors have been identified that modulates the Glycaemic Index (GI) of carbohydrate foods. In cereal foods, structure related factors are of particular importance, and the maintenance of an intact botanical tissue, the formation of macromolecular interactions, and the maintenance and/or creation of a high starch crystallinity promote a lower GI. In addition, enrichment with certain cereal fibre or addition of organic acids of the type formed upon fermentation may have a similar blunting effect on postprandial glycemia to cereal products. Over all, the choice of traditional types of processing, bulgur, pumpernickel-baking and sour-dough fermentation reduce GI properties. In addition, the choice of new cereal genotypes with elevated contents of amylose or  $\beta$ -glucans also offer a potential in this respect.

## DIFFERENCES IN SECOND-MEAL IMPACT OF LOW GI CEREAL PRODUCTS

As judged from so called “second-meal” studies in healthy subjects, also low GI cereal foods appears to differ in metabolic impact at a subsequent meal. Consequently, e.g. a pasta breakfast meal (GI = 52), characterised by a prolonged digestive phase with a sustained late net increment in blood glucose appears to substantially reduce blood glucose and insulin responses by 35 and 30%, respectively at a standardised lunch meal ingested 4 h later. A pasta meal ingested in the evening did, however, not influence glucose tolerance at a subsequent standardised breakfast, 10h later. In contrast, an evening meal with boiled barley kernels with a similar GI (GI = 53), but rich in indigestible

carbohydrates significantly reduced metabolic responses at a subsequent breakfast by approximately 25%. If indicative of mechanisms for metabolic benefits noted in long-term studies, low GI cereal products should preferably promote an extended digestive phase, and/or contain a high content of indigestible carbohydrates.

#### SEMI-LONG TERM DIETARY INTERVENTION WITH HIGH VS LOW GI BREAD PRODUCTS IN WOMEN WITH A HISTORY OF GESTATIONAL DIABETES

To assess the metabolic potential of low GI cereal foods, bread products of high or low GI character were included in the diet of 8 women with a history of gestational diabetes. Two types of experimental bread products were distributed during the low GI period (GI = 48 and 57, respectively). The different test periods were evaluated with a cross-over design of 3 weeks each and with a 3w wash-out period. Insulin sensitivity was estimated by use of intravenous glucose tolerance test. The test period with high GI bread products did not significantly affect the insulin area. In contrast, the insulin area to an intravenous glucose challenge was significantly reduced over the test period with low GI bread. Consequently, the exchange of high GI bread of the type commonly consumed, for the low GI experimental bread products during a 3w period significantly improved insulin economy in women predisposed for type II diabetes.

It is concluded that 1; it is possible to reduce GI of cereal products by a conscious choice of raw material and processing conditions 2; that low GI products may differ in second-meal effects and 3; that the GI character of the bread products may influence insulin economy in women predisposed for development of NIDDM, and the development of low GI bread products offers an interesting potential in the combat of the insulin resistance syndrome.

# EFFECTS OF RYE ON INSULIN METABOLISM

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Whole grain cereals are among the foods considered beneficial for health due to their effects on glucose and insulin metabolism. There is both experimental evidence on whole grain cereals producing lower postprandial blood glucose and insulin responses, and epidemiological studies showing protective effects by whole grain cereals against many chronic diseases. We have studied the effects of various types of rye breads on glucose and insulin metabolism in healthy subjects. Using commercial breads we showed that a single meal of whole kernel rye bread produces lower postprandial insulin response in healthy subjects than white wheat bread, but there is no difference in plasma glucose response, indicating that less insulin is needed postprandially for the regulation of plasma glucose after consumption of wholekernel rye bread than after white wheat bread (Leinonen et al., 1999). This was later confirmed with two experimental rye breads with different characteristics, one containing 60% rye kernels (high content of whole kernels) and the other  $\beta$ -glucan concentrate (high content of viscous fiber) (Leinonen et al, in press). Since duodenal hormones (GIP, GLP-1) play a role in insulin secretion, we also determined the postprandial responses of these hormones to wheat and rye products. Similarly to the response of plasma insulin to rye breads, the levels of GIP and GLP-1 were lowered after a meal containing rye bread. Unlike in the postprandial responses, no differences in fasting plasma glucose and insulin levels were observed in healthy subjects consuming rye or wheat breads in 2 x 4-week crossover intervention. These findings indicate that whole grain rye beneficially influences postprandial insulin metabolism, but the significance of these effects in long-term need to be determined.

# WHOLE GRAIN, LIPID METABOLISM AND CARDIOVASCULAR DISEASE

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Converging recent epidemiological data show that whole grain intake dietary fiber intake is negatively correlated to the risk for coronary heart disease (CHD). Moreover, other epidemiological evidences have been obtained on the negative relationship between dietary fiber intake (especially from cereals) and CHD risk in both sexes. Finally, experimental and clinical evidence has been obtained in the last decades on existing mechanisms of action of whole grain sources of dietary fibers supporting the concept of a protective role. The most studied risk factor for CHD was plasma and LDL cholesterol.

For most soluble fibers as provided by some whole grain sources, the potential mechanisms can be listed as follows: dietary fibers can alter energy intake and gastric emptying; dietary fibers can alter emulsification of dietary fat in the conditions prevailing in the stomach and small intestine, alter the secretion rate of pancreatic enzymes and reduce the activity of gut lipases ; dietary fibers can slow and/or reduce the intestinal absorption of dietary fatty acids and cholesterol as well as glucose absorption and insulin response ; dietary fibers can bind bile salts in the small intestine ; as a result, dietary fibers can increase ileal and fecal excretion of fat, cholesterol and bile salts ; dietary fibers can alter chylomicron secretion and thus, postprandial lipid and lipoprotein metabolism ; dietary fibers can alter hepatic levels of LDL receptor ; most soluble viscous dietary fiber sources lower fasting plasma and LDL cholesterol after chronic intake ; dietary fibers can favorably alter risk factors such as hyperinsulinemia or thrombotic indexes. Depending on the particular type of fiber, some mechanisms can have more or less importance.

Regarding wholewheat fractions providing fibers, such as bran and germ, interferences with lipid assimilation and metabolism have been observed *in vitro* as well as in animal models and humans.

Overall, epidemiological, clinical and mechanistic evidences have been obtained supporting the concept that whole grains can play a significant role in the reduction of risk for coronary heart disease.

# FIBRE AND CORONARY RISK: THE ROLE OF ENTEROLACTONE?

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Growing evidence from prospective studies has provided confirmation for the prevailing view that diets rich in fruits, vegetables and whole grains may reduce the risk of coronary heart disease and ischemic heart disease death. Whole grain foods as well as fruits and vegetables are major sources of dietary fiber and provide vitamins, minerals, and complex carbohydrates thus several substances together might be responsible for their favorable health effects. However, these previously established beneficial nutrients in the whole grain foods do not appear to account fully for the protective effect, leaving room for novel explanations.

Of the potential new antioxidative substances found in diet, prospective population based data is available only for flavonoids and for a lignan, enterolactone. Dietary intake of a variety of flavonoids estimated by dietary history interview has been associated with coronary mortality in previous studies. However, the advantage of determining enterolactone from human samples has increased specificity of the dietary exposure. Accurate measurement tools enabling focusing on a single compound has allowed investigation of a specific fiber related substance. In our studies low serum enterolactone concentration is in association with higher risk of acute coronary events, coronary death and with increased lipid peroxidation. Fiber is a *cluster* of many potential health related compounds that most likely needs to be breakdown in order to find out the substances explaining the beneficial health effects seen with high fiber diets.

# WHOLE GRAIN AND RISK OF CANCER

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Dietary guidance recommends consumption of whole grains for the prevention of cancer. Epidemiologic studies find that whole grains are protective against cancer, especially gastrointestinal cancers such as gastric and colonic, and hormonally dependent cancers including breast and prostate. Protection against cancer in epidemiological studies is stronger for whole grains than the sum of its parts, including dietary fiber, antioxidants, and other phytochemicals, suggesting that it is the combination of components in whole grains that are most protective. Different biological mechanisms have been proposed for the protectiveness of whole grains against cancer. First, whole grains are concentrated sources of dietary fiber, resistant starch, and oligosaccharides, fermentable carbohydrates thought to protect against cancer. Fermentation of carbohydrates in colon results in production of short chain fatty acids (SCFA) that lower colonic pH. SCFAs serve as an energy source for the colonocytes. Secondly, whole grains are rich in antioxidants including trace minerals and phenolic compounds and antioxidants have been proposed to be important in cancer prevention. Thirdly, whole grains are significant sources of phytoestrogens that have hormonal effects related to cancer protection. Phytoestrogens are thought to be particularly important in prevention of hormonally dependent cancers such as breast and prostate. Finally, whole grains mediate glucose response, which has been proposed to protect against colon and breast cancer. Whole grain foods are known to slow digestion and absorption of carbohydrates, thereby slowing glycemic response.

Thus, whole grains are rich in many components, including dietary fiber, starch, fat, antioxidant nutrients, minerals, vitamins, lignans, and phenolic compounds, all of which have been linked to reduced risk of different types of cancer. Most of these protective components are found in the germ and bran, which are reduced in the grain refining process. The most potent protective components of whole grains need identification so efforts can be directed to minimize the losses of physiologically important constituents of grains during processing. Additional research on the mechanisms by which whole grains protect against cancer is needed.

# WHOLE GRAIN, LIGNANS AND PROSTATE CANCER

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The observation that a western diet high in fat and meat is associated with a number of chronic diseases like cancer, cardiovascular disease and diabetes is strongly supported by evidence from a variety of studies. It has been difficult to identify individual food items or food groups as causative or protective for various diseases. There is however strong evidence supporting a protective effect on myocardial infarctions of plant foods based on whole grain cereals and vegetables while the overall picture what regards cancer is much more complex.

Concerning the two major cancer forms in the two sexes, breast and prostate cancer, they both vary very much between countries and migration studies clearly show that the western lifestyle has adverse effects on their incidence.

## STUDIES IN ANIMALS

In our experiments with rats and nude mice we have now repeatedly seen that rye bran and soy protein intake delays the early growth of hormone-sensitive prostate cancer. We have also observed that rye and soy treatment markedly inhibited the prostate specific antigen (PSA) secretion of human LNCaP tumours transplanted to nude mice and that these diets increased tumour cell apoptosis. The active component(s) of the dietary complex of rye bran may be lignans, but other substances and/or mechanisms may be involved like effects on insulin and IGF-1. Our experiments also showed that addition of fat to a rye diet abolishes the beneficial effects on tumour take, tumour cell apoptosis and tumour growth. Fat also decreased the urinary excretion of enterolactone, which shows that fat interacts with the formation of enterolactone in the gut, possibly by a reduced rate of fermentation.

The observation that the cancer-protecting effects of components in the traditional diets can be reduced just by adding fat, may be of considerable importance for the understanding of the geographical and temporal changes in

the incidence of prostate cancer. The experimental studies also show that the effect of diet is transient on prostate tumour growth and that no effect is seen on aggressively growing tumours. The results are however supported by effects of purified extracts of lignans on experimental breast- and colorectal cancer found by Thompsons group in Canada as well as by results from our own laboratory using purified lignans in experimental prostate cancer.

## STUDIES IN HUMANS

At present the aggregated results from studies in humans are insufficient to permit any conclusions indicating that rye or phytoestrogens are cancer-protective. There are however some studies pointing in that direction. A delay in clinical cancer may be very important in a public health perspective. Dietary intervention studies in humans are therefore needed using various forms of endpoints like apoptosis in tumours and changes in PSA concentration. An intervention study has therefore been performed within the framework of a Nordic rye and health project. The results of this project will be given at this symposium concerning the effect of a diet with a high concentration of rye bran on cell proliferation and apoptosis.

Some recent epidemiological studies using biomarkers have shown that a high blood concentration of insulin-like growth factor (IGF-I) is associated with an increased risk for breast, colon and prostate cancer. Enterolactone is a biomarker for both the function of the intestinal microflora as well as intake of lignan rich food. In case control studies a protective association has been observed between high enterolactone concentrations in blood or urine and breast cancer. A number of prospective studies using enterolactone as a biomarker is ongoing and some preliminary results on breast-, colon- and prostate cancer will be given at the symposium.

# WHOLE GRAINS, CEREAL FIBRES AND MECHANISMS OF PREVENTION OF COLORECTAL CANCER

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Whole cereal grain foods are emerging alongside fruits and vegetables as dietary constituents which can deliver significant health benefits. Burkitt as early as 1971 hypothesized that dietary fibre (DF) provides significant protection against colon cancer. This hypothesis has been tested over years, with the more recent addition that the dietary fibre more effective in large bowel health and colon cancer prevention is insoluble fibre (Jacobs and Lupton 1986, McIntyre et al. 1993). Cereal grains provide one of the richest sources of insoluble dietary fibre in the diet. The hypothesis involving fibre rich cereal foods has now been supported by reviews of a number of case-control studies (Howe et al. 1992, Kritchevsky 1995, Jacobs et al. 1998, Hill 1998). Data from animal colon cancer modelling has been consistent in showing a protective effect, particularly when milled components such as wheat or barley bran were added to high fat diets (20%w/w) at levels equivalent to 5 to 10% as dietary fibre. Wheat, barley, rye and triticale look to be desirable food sources.

The outer layers of the cereal grains from which fibre rich brans (wheat bran has approximately 50% DF) and foods are produced include associated components which also offer potential protection against cancers: phytate, lignans (diphenolics), flavonoids, phytosterols, vitamins (E and B group), and certain trace elements (eg selenium). Influences of fibre on bulking, fermentation and binding of mutagenic/toxic agents have been frequently reported as playing a significant role in protection. The antioxidant potential of some of the associated components (eg phenolics, phytate) is one of several possible mechanisms which have been studied (Thompson and Zang 1991, Tanaka et al. 1993, Vucenik et al. 1997). The production of short chain fatty acids and in particular butyrate from microbiological breakdown of cereal fibres, and its potential to inhibit colon cancer development in rat cancer models is another (Cummings 1997). Butyrate modulates DNA synthesis and repair, and has been shown to diminish aberrant crypt formation (early preneoplastic markers) in the colon, as well as reducing or preventing adenocarcinoma development in the azoxymethane (AOM) colon cancer model in rats (Wargovitch et al. 1996, Medina et al. 1998, McIntosh et al. 2000). We recently reported that butyrate when provided in the diet as tributyrin, or as butyrate in butterfat (4%w/w) significantly diminished mammary cancer induced by NMU in rats (Belobrajdic and McIntosh, 2000). The issue as to

whether butyrate generated in the colon is absorbed in sufficient quantity to have a significant effect at mammary level, needs more investigation.

An important influence underlying this mechanism and other potentially protective effects is the presence of a desirable gastrointestinal flora. Microbial fermentation and modification of dietary lignans leads to mammalian lignans (enterodiol and enterolactone) with a variety of potential influences, antiproliferative and antioxidant influences and anti-estrogenic activity which provides another area of active interest in anticancer terms. However the latter activities relevance to colon cancer needs more investigation. We examined wheat aleurone flour\* included in diet at 5% dietary fibre in the rat AOM model, and showed a significant increase in  $\beta$ -glucuronidase which correlated with reduced adenomas in the rat colon (McIntosh et al. 2000). This agrees with results of Jenab and Thompson (1996) which showed that increased  $\beta$ -glucuronidase in the colon was associated with increased release of lignans measured as urinary lignan in rats, providing a possible mechanism of protection against colon carcinogenesis. However, studies of probiotic bacteria in cancer prevention have indicated an opposite view, that reduced  $\beta$ -glucuronidase activity seen with introduction of probiotics or prebiotics is associated with reduced risk of cancer (Goldin et al 1980, Rowland 1996, Kulkarni and Reddy 1994). Clearly this needs to be resolved with further studies.

We studied the influence of three sources of dietary fibre and two sources of fat on aberrant crypt foci (ACF) induced in rats with AOM (Coleman et al. 2000). The fibre sources used in an AIN purified diet were: soy bean fibre (fibrim™),  $\alpha$  cellulose and resistant starch (RS) derived from high amylose maize and were added at 10% dietary fibre. The two fat sources were fish oil and sunflower seed oil (SSO) added at 20% w/w. Three measures of risk were assessed: caecal water cytotoxicity was assessed using an in vitro assay with HT 29 colon cancer cells, and caecal butyrate concentration and  $\beta$  glucuronidase activity. Fish oil and  $\alpha$ -cellulose provided the most protective diet with lowest numbers of ACFs, while RS and SSO provided the least protection, with most ACFs. Caecal water cytotoxicity correlated with ACF numbers. Caecal butyrate concentration and  $\beta$ -glucuronidase activity were highest in the rats fed the most protective diet and were lowest in rats fed the least protective diet. Highest butyrate was associated the lowest pH in caecal contents, which would significantly reduce solubility of secondary bile acids potentially toxic to the colon wall. The data supports an hypothesis that insoluble fibres such as  $\alpha$ -cellulose ferment slowly but effectively to butyrate which is delivered to the distal colon where most ACF and potential cancer risk occurs. They offer significantly more protection from large bowel cancer than the soluble sources of fibre: soy and resistant starch.

In two other AOM induced rodent colon cancer studies we showed that chickpeas offered little protection relative to whole wheat (McIntosh et al. 1998)

and that soybean meal offered less protection than  $\alpha$  cellulose (McIntosh et al. 1995). The pectic polysaccharides of legume dietary fibre appear to be significantly less protective than the fibres of some cereals in the colon cancer model. Higher fecal concentrations of the mutagenic secondary bile acid deoxycholic acid were associated with the chickpea diet. When comparing only cereal sources of fibres in the colon cancer model, McIntyre et al. (1993) came to the conclusion that oat bran was significantly less protective than wheat bran, an effect that was attributed to the soluble nature of oat bran fibre present mainly as soluble  $\beta$  glucan. Wheat bran however has insoluble fibre ( $\alpha$ -cellulose and arabinoxylan) and has an effect in the colon of increasing bulk, reducing transit time, and increasing binding of toxic/mutagenic metabolites. Other aspects however are also potentially relevant eg. lignans, phytate, phytosterols, but were not evaluated in the above mentioned studies.

The development of relevant biomarkers of colon cancer risk provides a means for more effectively testing dietary hypotheses in human intervention studies, as well as in epidemiological studies. Recognition in humans of aberrant crypt foci (Takayama et al. 1999), first identified in the rodent colon cancer model provides a useful marker for future human investigation. Examination of a number of components of cereals with regard to cancer risk has supported the view that a number of components of the cereal grain are desirable in the diet, and provide protection against cancer. Certainly inclusion of the outer aleurone layers of the grain look to be more important than endosperm in this respect. Components such as cereal proteins should also be examined for their possible contribution. They like non-starch polysaccharides undoubtedly reach the colon in significant amounts, and may also be influencing microbial activity significantly.

The convergence of epidemiological information regarding potential for protection by cereal grains in several areas of health (colon cancer, maturity onset diabetes and heart disease) provides incentive for more research investigation to better understand mechanisms, and for recommending more whole grain food usage by the wider community.

\* Processed wheat aleurone supplied as Natures Gold™ by Goodman Fielder Aust.

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# WHOLE GRAIN ANTIOXIDANTS AND HEALTH

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## INTRODUCTION

Scientific studies show that regular consumption of whole grain cereal products reduces the incidence of chronic diseases such as heart disease and some cancers. Whole grain fiber is generally considered a primary factor contributing to the observed health benefit. Grains are a source of many phytonutrients with potential for health benefit. These include lignans, antioxidants, sterols, tocotrienols, phytates, sphingolipids and vitamins and minerals. The potential contribution of grain antioxidants to the healthfulness has had sparse attention.

## OBJECTIVES

To quantify content and define chemical characteristics of antioxidants in grains and grain products.

## METHODOLOGY

Diphenylpicrylhydrazyl (DPPH), an intensely colored, stable free radical was used as the indicator reagent for antioxidants. Finely ground samples were dispersed in a 50% aqueous methanol solution of DPPH and stirred for 4 hours at 35° C. Samples were filtered and absorption of the filtrate measured at 515nm with a spectrophotometer. Loss of absorption relative to starting value was due to antioxidant activity. This activity was compared to standard Trolox solutions and calculated as Trolox equivalents/100 g of sample. This technique was used to compare antioxidant activity of grain products, fruits, vegetables and other foods and worked well for either dry or wet materials.

## RESULTS

Antioxidant activity was compared for grains, grain products, fruits, and vegetables. Whole grain products contained much higher levels of antioxidant activity than most all fruits and vegetables when compared as consumed. On an average serving basis, whole grain products had about the same antioxidant content as fruits and more than vegetables. These findings suggest whole grain products are equal to fruits or vegetables as a source of antioxidants that may have health significance. Antioxidants found in grains, as reported in the literature, make an extensive list of compounds. These include derivatives of

cinnamic acid, benzoic acid and flavonoids. Fat soluble antioxidants such as tocopherols, tocotrienols, alkylresorcinols and alkyl esters of ferulic and caffeic acid are common to grains. Avenanthramides appear to be unique to oats. Ferulic acid is an abundant phenolic antioxidant in grains and is about 90% esterified to cell wall hemicellulose. Bound ferulic acid can be released by the action of colon microflora and thus become bioavailable. The DPPH method of analysis does not measure insoluble antioxidants, although overall efficiency is not known. Samples first extracted with aqueous methanol retained about two-thirds of total activity in the insoluble residue. These results are consistent with known estimates of fiber bound ferulates. Grains have a range of antioxidant content. In general rye is highest, followed by barley, red wheat, white wheat, oats and brown rice. For white wheat, refined flour has about half the antioxidant activity as the whole grain. Red wheat refined flour has about a third the activity of whole grain. Purified red wheat bran has about ten times the activity of refined flour.

Grains are not eaten raw, as is the case for most fruits and many vegetables. Analysis of breakfast cereals and bread products showed that antioxidant activity does not decrease during processing. For a whole wheat breakfast cereal, antioxidant activity increased by about 30%. In the case of white bread, the increase was nearly 50%. It is assumed that this is due to Maillard reaction products. Whole grain products contain a variety of antioxidant compounds, all of which have potential for health benefit.

## CONCLUSION

Whole grains are rich in antioxidants and this may be a significant part of their positive health attributes along with fiber and other phytonutrients. Grain antioxidants can be water soluble, fat soluble or insoluble. Insoluble materials can be made bioavailable by activity of colon bacteria. Whole grain products, such as breads or breakfast cereals contain a substantial amount of antioxidant activity generated during processing. Antioxidants are among the phytonutrients in whole grain that need further study to better understand their contribution to good health.

# RYE GRAIN PRODUCTS EXHIBIT ANTIOXIDANT ACTIVITY AGAINST FREE RADICAL DPPH<sup>•</sup> *IN VITRO*

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## INTRODUCTION

Wholemeal rye products have an important role in daily Finnish diet as a source of dietary fiber. In addition to dietary fiber, rye products contain a wide range of nutrient and non-nutrient phytochemicals. These non-nutrient substances include phenolic compounds known to be capable of effective antioxidant action.

In this study, different rye varieties and selected rye products were screened for their ability to act as antioxidant by scavenging the nitrogen-centered free radical DPPH<sup>•</sup> (1,1-diphenyl-2-picrylhydrazyl) widely used in radical-based antioxidant assays.

## METHODS

### Extraction

Methanolic extracts of freeze-dried and ground rye samples were obtained by an ultrasonication-assisted extraction procedure.

### DPPH<sup>•</sup> scavenging assay

The assessment of the radical scavenging activity of the samples was based on spectrophotometric detection of the absorbance at 517 nm caused by DPPH<sup>•</sup> radicals, which was decreased or inhibited by antioxidants capable of radical scavenging in the system. This assay was performed using two slightly different methods both in a cuvette-based (1) and a microtiter-plate (2,3) procedure.

### Determination of the total phenolic content

The content of total phenolics (gallic acid equivalents) in methanolic extracts was determined using Folin-Ciocalteu procedure (4).

## RESULTS AND CONCLUSIONS

Rye samples tested in these experiments exhibited varying degrees of radical scavenging activity. Bran obtained from the outer, phenolic-rich layer of the grain showed highest radical scavenging activity in the assay, whereas flour (low total phenolic content) obtained from the inner parts of grain exhibited activity weakest in the whole set of samples. Between different rye varieties no clear difference in activity was observed. Malting seemed to increase the antioxidant effectiveness probably by increasing the amount of total phenolics.

As a general rule, the radical scavenging activity was connected to the total phenolic content found in the sample. Compounds contributing the radical scavenging activity seemed to be relatively stable during the baking process, as activity was also detected in different rye breads.

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# THE MINERAL AND TRACE ELEMENT COMPOSITION OF ORGANIC AND CONVENTIONAL CULTIVATED CEREALS

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## INTRODUCTION

The fertilization practice between the organic and conventional cultivation is different. It is possible that some mineral or trace elements accumulate to cereal grains from industrial fertilizers. A very well known example is Se. The supplementation of all commercial fertilizers with Se has increased the Se content of Finnish cereal grains remarkably. The Se content is highly depended on the Se amount of the fertilizers almost completely. In organic cultivation the crop yields are lower and the size of grains smaller compared to the conventional cultivation. That may affect the mineral and trace element composition of these cereals. There are very little research results from the mineral and trace element composition of organic foods.

The aim of this study was compare the mineral and trace element composition of cereals originated from organic and conventional cultivation.

## MATERIALS AND METHODS

The samples of wheat and rye flour, oat flakes and barley pearl were collected from seven organic farmers. The same food items originated from conventional cultivation were purchased from eight retail food stores in the Helsinki area and pooled to one analysable sample.

The dried samples will be digested in concentrated  $\text{HNO}_3$  and the mineral and trace elements composition will be determined with inductively coupled plasma – mass spectrometer (ICP-MS) using TotalQuant technique. Then it is possible to determinate all mineral and trace elements of the samples.

## RESULTS

The mineral and trace element composition of organic and conventional cultivated cereal samples will be presented and it is possible to compare the results.

# SELENIUM IN OAT AND RYE - RESULTS OF THE OFFICIAL VARIETY AND ORGANIC CULTIVATION TRIALS IN FINLAND

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This study is part of the two larger surveys focusing on chemical composition of oat and rye in Finland. The objectives of these surveys were to study basic quality of oat and rye varieties and consequently find those varieties and cultivation techniques that produce best nutritional and technological quality. The present work describes the selenium (Se) content of Finnish oats and rye in official variety and organic cultivation trials conducted by MTT during 1997–2000.

Se was determined by graphite furnace atomic absorption spectrometry after wet digestion and extraction into MIBK (Kumpulainen et al. 1983).

Finland is one of the low Se regions in the world. Since 1984 the inorganic compound fertilisers have been supplemented with Se to raise the Se content of domestic agricultural products and thus increase the Se intake of Finnish population. The Se supplementation level has been revised twice and is now 10 mg/kg fertiliser.

Se content of oat groats in official variety trials during 1997–1999 varied considerably between the cultivars, years and the locations. The range of the Se content was 0,016–0,460 mg/kg DM and the mean values in 1987–99 were 0.110, 0.035 and 0.160 mg/kg DM respectively. The effect of the 1998 increased Se supplementation level is clearly seen as higher average Se contents of oat in 1999. Rainy and cold growing season 1998 resulted lower Se contents than warm and dry seasons 1997 and 1999. The cultivars Leila and Veli had significantly ( $p < 0,001$ ) higher Se content than other cultivars studied. Lowest Se contents were in the cultivar Salo.

The Se contents of rye were generally lower than in oats. Rye is a winter cereal which has different fertilisation and cultivation practices compared to oat. The mean Se contents of rye grain in official variety trials in 1998–2000 were 0,040, 0,050 and 0,060 mg/kg DM respectively. The range of the Se content was 0,006–0,130 mg/kg DM. Differences between the cultivars are not yet tested, variation between the years was large and the cultivar differences were not very clear in

rye. Akusti, Anna, Riihi and Elvi were higher and Esprit lower in Se than other cultivars.

Se supplementation does not reach the organically grown products as the use of inorganic fertilisers is forbidden. Consequently, in organic cultivation the Se content of oat and rye were very low, generally <0.01 mg/kg DM. This corresponds to the Se levels in cereals before the use of Se fertilisation (Eurola et al. 1990).

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# WHEAT VARIETY HAS A MAJOR INFLUENCE ON MINERAL BIOAVAILABILITY IN RATS

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## OBJECTIVES

The aim of the study was to compare mineral bioavailability of four soft wheat varieties (Soissons, Hardi, X and Y) in rats.

## METHODOLOGY

Four varieties used in the study were cultivated in the same place (Chartainvilliers, France) in the same year (1999) with the same agronomic conditions. The different whole wheats were incorporated into rat diet at the rate of 70%. The animals were adapted for 21 days and during this period, feces and urine were collected for mineral balance determination.

## RESULTS

If caecal fermentation was stimulated by four diets compared to control diets, the incorporation of 70% of whole wheat flour in the rat diet did not modify Ca bioavailability. Nevertheless, Mg absorption and accumulation in bone were stimulated by the ingestion of X and Y varieties. Feeding X or Y varieties significantly increased Fe absorption and status compared to three other groups. Zn absorption was significantly depressed by Soissons flour (-28%) whereas three other whole wheat flour diets led to a better Zn assimilation compared to control group. The consumption of Hardi, X or Y wheat varieties had repercussions on liver Zn (+29, +36% and +33% compared to control group). Cu absorption significantly increased by X or Y varieties (+93% and +88% respectively) but Cu status was not modified by nutritional differences. Moreover phytate breakdown was higher when the diets contained X or Y varieties.

## CONCLUSION

Mineral bioavailability from X or Y varieties are higher than that observed with Soissons or Hardi varieties. Genetic origin thus plays a major role in determining mineral bioavailability in whole wheat.

# STUDIES ON A PHYTASE INHIBITING COMPOUND IN OATS

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## BACKGROUND

Oat has a very low level of phytase activity compared with other cereal grains. Because of its relatively high phytate content this might lead to an impaired mineral status among subjects consuming high amounts of oat products. Traditionally, most of the world oat production has been used as animal feed, but in recent years the interest has shifted towards food usage. Research has focused on the potential health benefits of oats, due to the growing body of evidence suggesting a protective effect of dietary fibre against atherosclerosis and other diseases common to Western society. While nutritional interest in oats has concentrated on oat products as a source of dietary fibre, the reasons for the low phytase activity have not been elucidated. Therefore, it is important to study the mechanism behind phytate hydrolysis in oats and its biochemical background.

## OBJECTIVES

There are a few reports on compounds present in oats which negatively interfere with phytate degradation, but neither the compound nor the mechanism of influencing phytate hydrolysis is known. Thus, the aim of this investigation was to identify the repressing compound from oats.

## MATERIAL AND METHODS

Malted oats were obtained from Tärnö Säteri (Nyköping, Sweden). The experiments also included dehulled, unmalted oats. During the course of previous experiments to purify oat phytase, an increase in total phytase activity by about 50 % was observed after acetone precipitation. This may be due to a separation from a phytase inhibitor occurring in oats. To study the repressing compound in more detail, a inhibitor assay was developed. Different experiments were performed to investigate the stability and extractability of the inhibitor at different pH-values, its thermal stability and dialysability.

## RESULTS

The assay system was linear with increasing amount of inhibitor added. In addition, the inhibitor solution did not have any effect on the colour development as studied with pure phosphate solutions. The repressing compound could be extracted from unmalted as well as from malted oats, but a higher inhibitor activity was found in the extract from malted oats (unmalted oats: 100 %, malted oats: 141 %). The repressing compound was found to be stable under extreme acidic and alkaline conditions and also to be heat stable. The inhibitor retained its activity even when held at 100°C for 30 min. Furthermore, the compound is soluble in a buffer solution and in an acetone:buffer mixture. Thus, having hydrophobic as well as hydrophilic properties or its binding form changes under hydrophilic in comparison to hydrophobic conditions. In addition, dialysis of the solution did not remove its repressing capacity, indicating it has to be a high molecular mass compound or very strongly bound to other compounds in the solution.

## CONCLUSIONS

The nature and identity of the inhibiting compound still needs to be further investigated. However, the low enzyme activity of oats might only partly be attributed to the presence of a phytase inhibitor and it is possible that the phytase and the repressing compound are located in different parts of the oat kernel and therefore meet only after disruption of the cell walls.

# OXIDATION OF POLYPHENOLS IN HIGH-TANNIN CEREALS AND THE EFFECT ON IRON BIOAVAILABILITY

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## BACKGROUND

Sorghum and millets are commonly grown for human consumption in many arid and semi arid areas in the world. They are the principal sources of energy, protein, vitamins and minerals for a large proportion of people in these areas. The bioavailability of essential minerals like iron is, however, very low because of the high content of compounds that inhibit iron absorption. Colored varieties of sorghum and millets contain high amounts of polyphenols or tannins. They form insoluble complexes with iron in the gastrointestinal tract, rendering it unavailable for absorption. A vegetable diet with a low bioavailability of iron is considered as one of the main causes of iron deficiency anemia in developing countries. The bioavailability of iron can be improved by processing methods that affect the ability of polyphenols to bind iron. Polyphenols can be oxidized by polyphenol oxidases, enzymes that can be found more or less in all fruits and vegetables. Treatment of cereals rich in polyphenols (high-tannin cereals) with polyphenol oxidases may affect the bioavailability of iron, and thereby the nutritional value of these grains.

## AIMS

The aim of the study was to investigate how addition of polyphenol oxidase to high-tannin cereals affects the amount of phenolic groups and the *in vitro* availability of iron.

## METHODS

The cereal flour was mixed with water and incubated with phytase to degrade the phytate before addition of polyphenol oxidase. The bioavailability of iron was estimated by an *in vitro* method that simulates the human digestion system. The total iron content and the *in vitro* available iron were analyzed with atomic absorption spectrometry. The phenolic content was estimated by spectrophotometric means.

## RESULTS

Treatment of high-tannin cereals with polyphenol oxidase had a reducing effect on the phenolic content. A significant increase in *in vitro* available iron could be seen when the cereals were incubated with both phytase and polyphenol oxidase. Incubation of a red variety of sorghum with wheat phytase (2 u/g cereal) and mushroom polyphenol oxidase (1500 u/g cereal) decreased the phenol content with as much as 60%, and the *in vitro* available iron increased from 3.5% to 6.4%.

## CONCLUSION

Processing of high-tannin cereals with enzymes that reduce the content of phytate and polyphenols can increase the bioavailability of iron in these foods, and may therefore be used as a method to improve their nutritional value.

# PLANT STEROLS IN MILLING FRACTIONS OF WHEAT AND RYE

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Since plant sterols have positive health effects, a lot of research is focused on them. At our department, we have conducted plant sterol analyses of a representative selection of food items for food compositional purposes, and studied the effects of several food processing and storage conditions on the contents of plant sterols. The aim of this study was to follow how plant sterols are distributed in the different milling fractions of the two major cereals, i.e. wheat and rye. This study was a part of a collaboration project of University of Helsinki and VTT financed by National Technology Agency of Finland and Finnish food industry.

Samples from milling processes of wheat and rye were taken from the industry and sampling was repeated twice. Whole grain samples as well as four milling fractions of both cereals were studied. Plant sterols were analyzed by gas chromatography. The sample preparation method used included both acid and alkaline hydrolysis to liberate sterols from their derivatives and from food matrix, purification of the unsaponifiable matter by solid-phase extraction, and derivatization to trimethylsilyl ethers.

Whole grain wheat and rye are good sources of plant sterols. The mean total plant sterol content of whole grain wheat samples was 78 mg/100 g and whole grain rye samples 111 mg/100 g. Sitosterol was the major sterol in the both cereals contributing to 57% and 52% of total sterols in wheat and rye, respectively. Unlike most other natural sources of plant sterols, they were also rich in stanols, i.e. campestanol and sitostanol, containing 20% of them.

Total plant sterol content of various milling fractions of wheat ranged from 40 to 185 mg/100g and those of rye from 47 to 188 mg/100 g. There was a positive relationship between the ash content and the sterol content in the milling fractions. Thus, the lowest levels of sterols occurred in the most refined milling fractions with small ash contents and the highest levels in the bran fractions. There was also a difference in the plant sterol profile of various parts of grains, since bran fractions contained stanols almost twice the proportion than the most refined fractions did. Whole meal wheat and rye flours and, especially, bran fractions are good sources of natural plant sterols in our diet.

# COMPARATIVE STUDY ON PROTEIN AND STORAGE QUALITY OF SUPPLEMENTED *UPPUMA* OF DICOCCUM AND DURUM WHEAT

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## OBJECTIVES

To evaluate the protein quality of dicoccum wheat semolina, *uppuma* and developed supplemented *uppuma* in comparison with durum wheat and shelf-life study of ready-to-use supplemented dicoccum *uppuma* mix.

## METHODOLOGY

The proximate composition of semolina and *uppuma* of dicoccum and commercial durum wheat and *in vitro* protein digestibility of *uppuma* was analyzed as per the standard AOAC procedures(1985). The quality of dicoccum semolina was assessed biologically for protein efficiency ratio(PER), net protein ratio(NPR), biological value(BV), and digestibility coefficient (DC) and net protein utilization (NPU) by animal studies. Further, the protein quality of dicoccum semolina was improved by supplementation with black gram dhal, bengal gram dhal and ground nut in the proportion of 70:10:10:10 to meet the recommended protein-energy per cent of pre-school children(Gopalan et al. 1996), and *uppuma* prepared from this was evaluated for its nutrient composition and *in vitro* protein digestibility as per the standard procedures(Mouliswar et al. 1993) and its acceptability and impact on nutritional status was tested on pre-school children in comparison with *uppuma* of dicoccum semolina and commercial durum semolina. Ready-to-use(RTU) *uppuma* mix was prepared from this supplemented semolina by the addition of dehydrated vegetables and was studied for its storage quality at weekly intervals.

## RESULTS

The proximate composition of dicoccum and durum semolina differed significantly. The protein, ash and crude fiber contents of dicoccum semolina were higher than of commercial durum semolina. Dicoccum semolina protein had higher PER (1.9%), NPR (3.4%), BV (88.2%) and NPU (72.3%) compared to durum semolina (1.4, 2.3, 84.7% and 70.7%, respectively). Whereas, DC was

significantly higher in commercial durum semolina (83.5%) than dicoccum semolina (81.3%).

*Uppuma* of dicoccum semolina provided moisture 62.6%, protein 5.2%, fat 3.1%, ash 1.4%, crude fiber 0.4% and total carbohydrates 27.1%, which were higher than both *uppuma* of dicoccum and durum semolina. Dicoccum *uppuma* provided higher protein, fat, crude fiber and total carbohydrates but lower moisture (3.3%, 1.6%, 0.24%, 22.3% and 71.2%, respectively) compared to durum *uppuma* (2.04%, 0.91%, 0.11%, 20.4% and 75.4% respectively). However, *in vitro* protein digestibility of *uppuma* of durum semolina 92.1% was maximum followed by dicoccum *uppuma* (89.1%) and dicoccum supplemented *uppuma* (85.1%).

Pre-school children highly accepted the *uppuma* of commercial durum semolina followed by supplemented and non supplemented dicoccum *uppuma* during 45 days of short- term feeding trial while protein contribution per day was the highest by supplemented dicoccum *uppuma* (6.4 g) followed by dicoccum *uppuma* (4.0 g) and durum *uppuma* (3.8 g). Nutritional status assessed by anthropometric measurements and hemoglobin level, improved in all pre-school children at the end of feeding trial irrespective of the test foods consumed. Supplemented dicoccum *uppuma* group showed maximum improvement in weight, height, mid upper arm circumference and triceps skin fold followed by dicoccum *uppuma* and durum *uppuma*. Hemoglobin level improved in all three tests food groups with significant increase being in durum *uppuma* and supplemented dicoccum *uppuma* fed children.

The moisture and FFA content of RTU *uppuma* mix increased with increase in storage period. The moisture content increased by 27.3% and 98.6% for RTU dicoccum and durum *uppuma* mix respectively while FFA content increased by 38.9% in the former and 94.6% in the latter. During storage of RTU *uppuma* mixes under ambient condition there was degradation of colour, development of bland taste and rancid flavour. Organoleptically RTU supplemented dicoccum *uppuma* mix was acceptable up to 10 weeks while durum *uppuma* mix was accepted up to 8 weeks only.

## CONCLUSION

Nutritional quality of semolina of dicoccum wheat is better than commercially available durum semolina. Supplementation with pulses and oil seed further improved the protein quality of dicoccum semolina. This supplemented semolina can be made in to RTU *uppuma* mix. This can be commercially popularised and distributed as nutrient rich food product having better shelf-life, in feeding programmes as geriatric food and also for preparation of various dense products.

# EFFECTIVE RELEASE OF BIOACTIVE NUTRACEUTICALS FROM THE INSOLUBLE CELL WALL MATRIX OF CEREALS

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It has been well documented that ferulic acid and arabinoxylan present in the cell wall of cereals possess a variety of physiological activities such as antioxidant, immunostimulating, anticancer, etc. However, ferulic acid and arabinoxylan are covalently crosslinked to the insoluble matrix in the cereal cell wall. Accordingly, their bioavailability may be very low, since human does not provide the digestive enzymes to hydrolyze the insoluble linkages.

## OBJECTIVES

This research aimed to develop a protocol process to effectively release nutraceuticals from the insoluble cell wall of cereals, using rice bran as a raw material. The combined effects of mechanical and enzymatic treatment on solubilization of the rigid cell wall were investigated to find optimum conditions to release ferulic acid and arabinoxylan from rice bran.

## METHODOLOGY

Defatted rice bran was extruded at 130°C of temperature, 250 rpm of screw speed and 25% of moisture content, using co-rotating intermeshing type twin-screw extruder. The extrudate was dried, ground and stored at -4°C prior to use.

The extrudate was destarched by using Termamyl 120L (Novozyme A/S, Denmark), followed by treating with a hemicellulase complex, Fiberase R (BioCare Co., Korea) to hydrolyze the insoluble cell wall matrix of extruded and destarched rice bran.

Ferulic acid released was measured by capillary electrophoresis (Beckman P/ACE, USA) equipped with a 57 cm × 75 µm I.D. fused-silica capillary column. The yield of ferulic acid of extruded and enzyme treated samples was compared to that of alkaline hydrolysis (1.0N NaOH).

Water soluble polysaccharide (WSP) was isolated from extruded rice bran using hot water, which was then treated with a ultrafiltrator (TCF 10, Amicon Inc.,

USA) with a membrane of MWCO 50,000 and freeze-dried. Neutral sugar composition of WSP was measured by Bio-LC (DX-500, Dionex, USA) equipped with Carbopac™ PA1 column (25cm × 4mm I.D.) and with ECD detector.

## RESULTS

Ferulic acid present in rice bran was released up to 85.2% of the alkali-extractable ferulic acid when extrusion and enzyme (Fiberase R) treatment were used in combination. In contrast, extrusion without enzyme treatment did not nearly release ferulic acid, while enzyme treatment alone released approximately 30.1%. The results indicate that physiological activity of rice bran can be enhanced after extrusion and enzyme treatment due to activated ferulic acid. The DPPH test revealed that free radical scavenging activity of rice bran after extrusion and enzyme treatments increased up to 40 times compared with that of raw rice bran.

Fiberase R treatment of extruded rice bran resulted in producing 23.5% of water soluble polysaccharide (WSP). Based on the arabinoxylan content, the purity of WSP was 87.2%, which is higher than 75.0% of commercial product. In contrast, the yield of WSP and purity for other commercial enzymes ranged 4.2–7.8% and 46.3–64.8%, respectively. The results indicate that the yield and purity of arabinoxylan isolated from rice bran are significantly dependent on the types of enzymes employed.

## CONCLUSIONS

It was clearly observed that extrusion and subsequent plant cell wall hydrolase treatments enabled to effectively release ferulic acid and arabinoxylan from rice bran. The rigid cell wall structure of rice bran was significantly loosed due to the shearing force generated by the extrusion process, which allowed enzymes to penetrate the cell wall matrix and hydrolyze the insoluble linkages of ferulic acid and arabinoxylan. It is anticipated that this process could be successfully applied to other cereal based products to enhance bioavailability of nutraceuticals present in cereals.

# LARGE PARTICLES OF MAINLY FOOD ORIGIN ARE RESPONSIBLE FOR VISCOELASTIC PROPERTIES OF PIG CECAL CONTENTS

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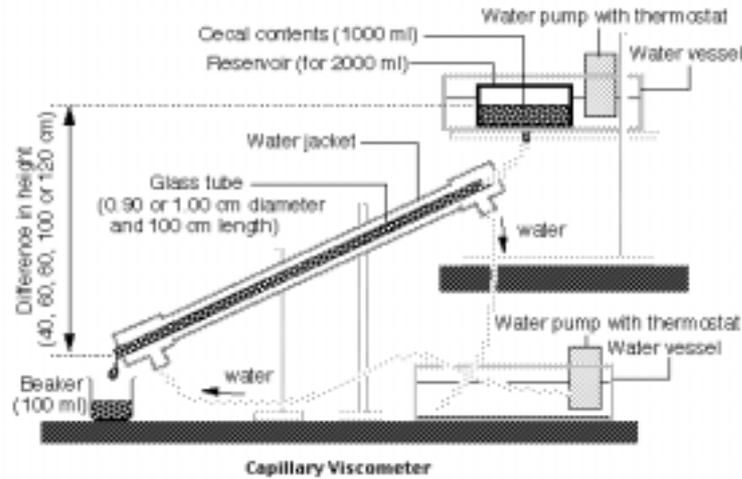
## INTRODUCTION

One of the key roles of whole grain diet should be to provide indigestible solid particles to the gut lumen. Apart from their roles to expand or abrade gut wall such solid particles may alter the viscoelastic properties of gut contents in a way that blood cells define the viscosity and non-Newtonian nature of the whole blood.

The viscosity of gut contents should define the mixing, diffusion and flow of nutrients in the contents. In this regard, earlier works measured the viscosity of gut contents only after the removal of solid particles. However, the removal of solid particles from pig cecal contents vigorously lowered the viscosity of the contents and entirely altered viscoelastic character of the contents in our recent study. Accordingly, we examined the contribution of large solid particles of mainly food origin to viscoelastic parameters of gut contents semi-quantitatively.

## METHODS

We filtered pig cecal contents freshly sampled at a local slaughterhouse through two layers of surgical gauze. Then, we reconstructed the cecal contents by returning all, one half or none of the original amount of large particles to the filtrate. We measured the viscosity of the reconstructed cecal contents using a self made tube-flow viscometer. We computed the coefficient of viscosity from the volume flow rate and pressure drop of cecal contents through glass tubes of known inner diameter.




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## RESULTS

The coefficient of viscosity of pig cecal contents increased as the large particles increased. Cecal contents behaved as a non-Newtonian fluid even after the total removal of large particles. The large particles elevated the coefficient of viscosity of cecal contents considerably in a wide range of shear rates. Cecal contents showed Bingham plastic nature in all samples. The yield stress of cecal contents increased with an increase in the large particle contents. This suggests that required stress to flow cecal contents drastically increase as the large-particle content increases. Therefore, the contracting force of the intestine required to squeeze cecal contents should increase considerably with modest increase in the content of large particles.

## CONCLUSIONS

Large particles elevated the viscosity and yield stress of gut contents, however, without changing basic viscoelastic characteristics of pig cecal contents as non-Newtonian fluid and as Bingham plastic.

## IMPLICATIONS

Modest change in large particle content in gut contents can considerably affect the digestion and absorption by modifying the rate of diffusion across the contents and the ease to mix or squeeze gut contents. In this regard, it is very likely that whole grain diet can modify the rate of digestion and absorption in the but by changing the viscoelastic parameters of the digesta.

# THE SYNTHESIS OF <sup>13</sup>C LABELLED LIGNANS

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Wholegrains contain relatively high concentrations of plant lignans, which after conversion to the hormone like compounds enterolactone and enterodiol have the ability to bind to oestrogen receptors with low affinity and have weak oestrogenic activity. Lignans have been shown to have oestrogenic, antioestrogenic, antioxidative, antiviral and antibacterial properties and are antiproliferative in relation to many types of tumour cell cultures. Also high levels of enterolactone excretion are found in populations in areas with a low incidence of hormone related cancers, suggesting that consumption of lignans may offer some protection against these diseases.

Investigations on the biological effects of lignan phytoestrogens on human health have been hindered by the lack of availability of pure lignans. Also it is necessary to have stable, isotopically labelled lignans as internal standards for accurate GC-MS and LC-MS analysis. As interest in lignans has increased this problem has become more acute.

We have been interested in the synthesis of lignans and the development of routes for labelling lignans with carbon-13. For use as internal standards it is necessary to have at least three carbon-13 atoms incorporated into the lignan skeleton. These carbon-13 labelled standards have major advantages over deuterium labelled standards. The incorporation of deuterium involves exchange methods under acidic conditions to put the deuterium atoms into the reactive positions on the aromatic rings. However, these are not chemically stable and will exchange back out, albeit slowly, in aqueous conditions during the analysis procedure. However when the carbon-13 atoms are incorporated into the framework of the molecule these are not able to be lost and the standards are chemically stable.

Synthetic routes have now been established for the plant lignans, secoisolariciresinol and matairesinol and the mammalian lignans, enterolactone and enterodiol in an unlabelled form. Subsequently we have synthesised all four lignans with three carbon-13 atoms in the structure, which have been specifically and quantitatively incorporated. Work is now continuing on the synthesis of enterofuran and shonanin and other lignan metabolites.

# LC-MS/MS ANALYSIS OF PLANT LIGNAN METABOLITES IN PLASMA AND URINE

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Plant Lignans undergo a complex series of metabolic conversions during their passage through the mammalian digestive tract and a second series of conversions in the liver after absorption. The plant lignans such as secoisolariciresinol diglucoside are converted to their corresponding aglycones which are further metabolized to the mammalian lignans enterodiol and enterolactone. After absorption, these metabolites are eventually converted to glucuronides, sulfates and other metabolites to facilitate excretion. The traditional approach to the analysis of these metabolites in human and animal tissues and fluids is to hydrolyze the conjugates to their corresponding aglycones and then analyze volatile derivatives by GC-MS. Characterization of the metabolites can only be achieved by a complex process of chromatographic fractionation. LC-MS/MS analysis of these metabolites can be achieved directly without hydrolysis and derivitization. This allows us to measure directly the glucuronides, sulfates and other metabolites and to detect the presence of metabolites not previously observed.

# ANALYSIS OF PLANT LIGNANS IN CEREAL SAMPLES

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Well-known plant lignans in cereals are secoisolariciresinol (SECO) and matairesinol (MAT)(Mazur, 1998). These lignans are precursors of mammalian lignans enterodiol (END) and enterolactone (ENL)(Axelson and Setchell, 1981) (Borriello et al., 1985). Enterolactone has been associated with decreased risk of breast cancer (Hulten et al., 1998) and acute coronary event (Vanharanta et al., 1999). Recently we identified new lignans in rye, which shown to be also precursors of mammalian lignans (Heinonen et al., 2000). These lignans were pinoresinol (PIN), lariciresinol (LAR) and syringaresinol (SYR). Furthermore, we found also an abundant lignan isolariciresinol (ISO), which was not a precursor of mammalian lignans. These new precursors are one possible explanation to the discrepancy obtained in rye feeding experiment between the plant lignan input and mammalian lignan output. We found the present methodology inapplicable to determine the new precursor in cereal samples.

SECO and MAT had been determined using GC-MS method developed by Mazur et al (Mazur et al., 1996). Samples were hydrolysed first with enzymes then with hydrochloric acid and finally purified with ion exchange chromatographies. Chemical nature of the new precursor lignans is different from SECO and MAT. Compounds tend to decompose easily in the conditions, which were used in previously developed GC-MS method. We developed a new pre-treatment for cereal samples to obtain more precise data about the lignan content. Enzyme hydrolysis, acid hydrolysis and purification chromatographies were optimised. Optimisation criteria and new pre-treatment method for lignans are presented.

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# LIGNANS IN OATS - COMPARISON OF CULTIVATION TECHNIQUES AND CULTIVARS

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In a three year study conducted by MTT the quality parameters of Finnish oat and suitability of different oat cultivars as food and feed was studied keeping in mind the demands for the raw material set by the processing industry. As a marker for quality the following chemical analyses were done from several oat cultivars: beta-glucan, oil, minerals, selenium, cadmium, thiamine, mycotoxins and lignans. The content of lignans in Veli cultivar grown in 1997 and 1998 in different locations will be presented as an example. In these field trials organic and traditional cultivation techniques were also compared. Finally comparison of 7 cultivars grown in two locations in 1997 will be made.

Lignans, namely secoisolariciresinol and matairesinol, were analysed by gas chromatograph-mass spectrometry after sample preparation which consisted of three hydrolysis steps followed by ion-exchange chromatography and silylation.

The content of secoisolariciresinol and matairesinol in different oat cultivar grown in different locations and using organic or traditional cultivation techniques was < 0.5–3.2 mg / kg f.w. and < 0.5–0.9 mg/kg f.w. respectively (limit of quantitation 0.5 mg/kg ). The results showed that the content of lignans is not connected to cultivation technique nor to the cultivars. Also the effect of latitude on lignan content could not be seen.

# THE OCCURRENCE OF NEW MAMMALIAN LIGNAN PRECURSORS IN WHOLE GRAINS

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Lignans are phytoestrogens that have gained recently considerable attention as it has been suggested that the diet rich in lignans is associated with lower incidence of hormone-dependent diseases. Mammalian lignans enterolactone (ENL) and enterodiol (END) are formed from plant lignans by the action of colonic bacteria. It has been long assumed that matairesinol (MAT) and secoisolariciresinol (SECO) are the only plant precursors of the mammalian lignans. We have very recently shown that the intestinal microflora is capable to transform also other plant lignans, such as pinoresinol (PIN), lariciresinol (LAR) and syringaresinol (SYR), to ENL and END. The aim of this investigation was to determine the occurrence of new mammalian lignan precursors and other plant lignans, such as isolariciresinol (ISO), in whole grains (rye, oat, wheat, barley, millet and buckwheat).

The lignan composition of the whole grain samples was determined by GC-MS. The pre-treatment method was specially developed for new plant lignans, since the compounds were found to be very sensitive to acidic and enzymatic hydrolyses. The structures of the isolated lignans were characterized with authentic synthetic reference compounds when available.

PIN, LAR, ISO, SYR, MAT and SECO were the most abundant lignans present in whole grains. The lignan composition and some quantitative data of new mammalian lignan precursors in different grains are presented. The high amounts of newly identified precursors of ENL and END in rye will explain the discrepancy accounted in rye feeding experiments in which the excretion of mammalian lignans was notably higher, than the intake of MAT and SECO. It is concluded that the new precursors of mammalian lignans seem to be quantitatively more important than MAT and SECO. In the case of rye, PIN, LAR and SYR contribute more to the formation of mammalian lignans than MAT and SECO.

# COMPLETE PATHWAY OF MAMMALIAN LIGNAN FORMATION FROM SECOISOLARICIRE SINOL DIGLUCOSIDE

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## INTRODUCTION

The mammalian lignans enterodiol and enterolactone are produced in the large bowel by intestinal bacteria [1–3]. A major dietary precursor as evinced by several research groups is secoisolariciresinol diglucoside (SDG) which is a major component of the phenolic fraction of flaxseed. The mammalian lignans are also produced from a variety of other dietary components including wheat bran in far lower yield, however [4]. Enterodiol and enterolactone show antitumorigenic effects presumably mediated by their anti-oestrogenic and antioxidant activity and are therefore regarded to represent a link between healthy diets and their chemopreventive effects against breast and colorectal cancer in particular [5–8].

## OBJECTIVES

Although a partial pathway of mammalian lignan formation from flaxseed by the intestinal flora has been forwarded a number of the possible intermediates have not been isolated and identified. Therefore the aim of this study was to elucidate more fully the route of mammalian lignan formation specifically from SDG.

## METHODOLOGY

Secoisolariciresinol diglucoside was isolated and purified to homogeneity from flaxseed according to the protocol described by Thompson *et al.* [7]. The metabolism of SDG by intestinal bacteria was studied in both aerobic and anaerobic conditions using Brain Heart Infusion (BHI) as the basal medium. Aerobic experiments were conducted in Erhlenmeyer flasks (250 ml) incubated at 37°C for 72 h on a shaking water bath. Anaerobic experiments (100 ml) were conducted in 100 ml bottles containing BHI fortified with cysteine-HCl and sodium formaldehyde sulphonylate in an anaerobic churn at 37°C for 72 h. The

substrate SDG was added at a concentration of 0.05 % and the media was inoculated with freshly voided stool (1 g). After incubation the cultures were extracted on extrelute columns using methanol (5 %) in ethyl formate. The extracts were concentrated to dryness on a rotary evaporator and suspended in methanol prior to analysis by analytical high performance chromatography (HPLC). Thereafter the methanolic extracts were immobilised on silica gel, freeze-dried and subjected to column chromatography using increasing concentrations of methanol in dichloromethane. Fractions enriched in intermediates of SDG transformation were subjected to semi-preparative HPLC, and, isolated and purified compounds were studied by gas-chromatography mass spectrometry (GC-MS), liquid chromatography electrospray ionisation (LC-ESI), nanoelectrospray ionisation (ESI), and nuclear magnetic resonance spectroscopy (NMR) for confirmation of their structures.

## RESULTS

The metabolism of SDG by the faecal bacteria proceeded smoothly in both aerobic and anaerobic conditions. In aerobic conditions metabolism was restricted however to deglycosylation yielding secoisolariciresinol (SILR) as the only product (100%) at 72 h. Time course experiments revealed the transient presence of the intermediate SILR-monoglucoside (SMG) with a yield of 20 and 15% at 24 and 48 h respectively. In anaerobic conditions the fermentation pathway was more complex and rarely reached completion at 72 h. The major end-products at this time were identified as SMG, SILR, monomethoxy-SILR (MMSILR), enterodiol and enterolactone. Three further intermediates were also detected and isolated in lower yield and one of these was confirmed by GC-MS and NMR to be enteroaldehyde (ENNA). The remaining two have been tentatively assigned by GC-MS data as demethyl-SILR (SD1) and demethyl-MMSILR (SD2). Based on the data accrued, a complete pathway of SDG transformation to the mammalian lignans is proposed (Figure).

## DISCUSSION

The production of mammalian lignans from dietary precursors in the large bowel by intestinal bacteria is regarded to afford chemopreventive effects against colorectal and breast cancer especially. To date the previously identified enterodiol and enterolactone have been shown to elicit a range of metabolic consequences such as antioestrogenic, antioxidant and antitumourigenic activity. Studies are in progress to assess the anticarcinogenic potential by a battery of screening tests of all of the intermediates of SDG metabolism described in this study.

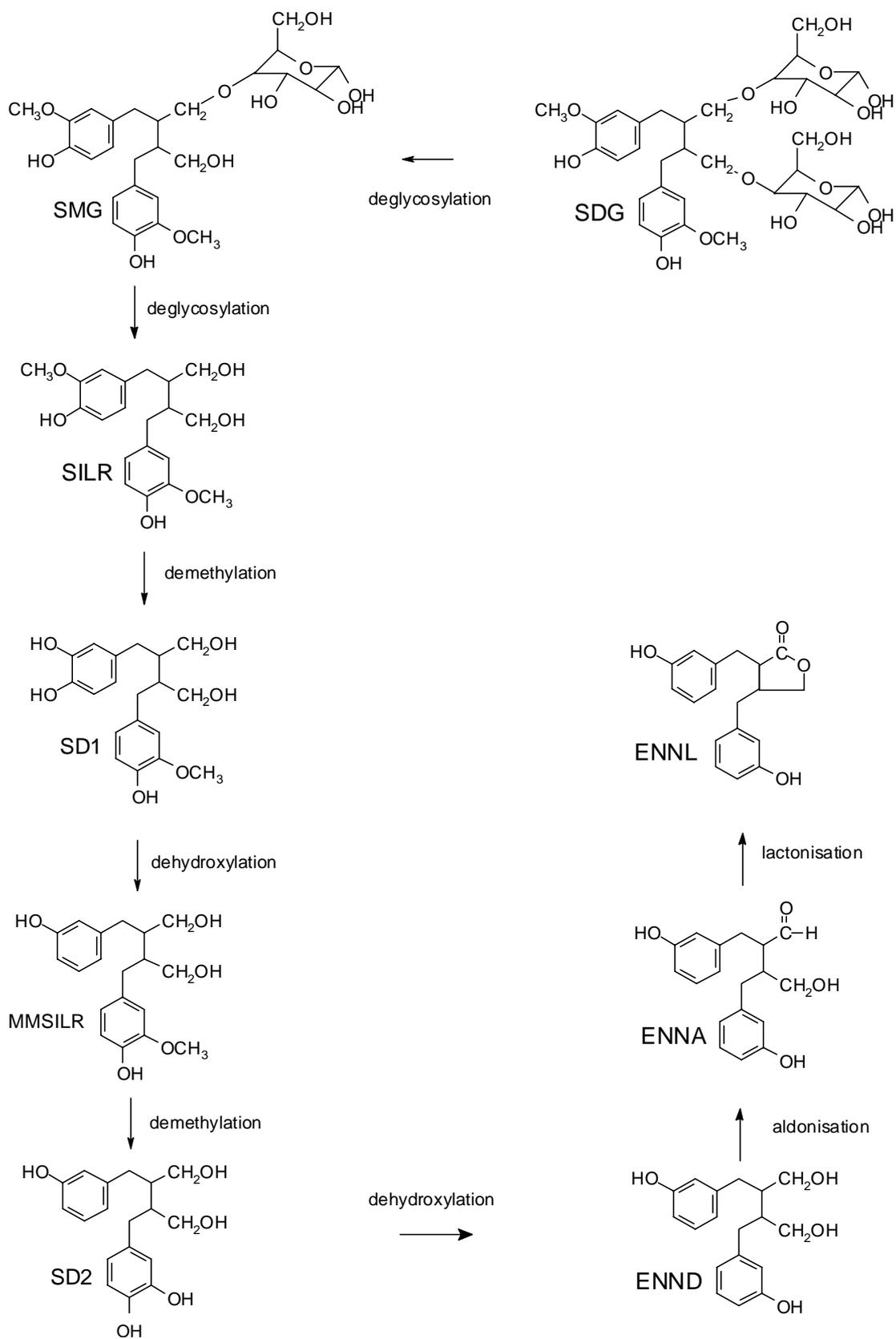


Figure. Pathway of SDG transformation by human faecal bacteria.

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# FORMATION OF THE MAMMALIAN LIGNANS ENTERODIOL AND ENTEROLACTONE FROM (+)- PINORESINOL, A MAJOR LIGNAN PRESENT IN OLIVE OIL

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## INTRODUCTION

Formation of the mammalian lignans enterodiol and enterolactone in the large bowel by intestinal bacteria is regarded to be a consequence in the main of transformation of secoisolariciresinol diglucoside a major component of the phenolic fraction of flaxseed. However discrepancies exist between the nutritional data relating intake of mammalian lignan precursor to the yield of enterodiol and enterolactone detected in the faecal matrix. Recently the presence of the lignans (+)-1-acetoxypinoresinol and (+)-pinoresinol have been described as major components of the phenolic fraction of olive oil. Extra virgin olive oils show concentrations up to 100 mg/kg total lignans [1-4]. However the presence of these lignans in the faecal matrix has eluded detection.

## OBJECTIVES

No data exists on the potential of the lignans present in the phenolic fraction of olive oil to serve as substrates for the intestinal microflora. Therefore the objective of this study was to assess the potential of (+)-pinoresinol, a major lignan identified in olive oil as a precursor for mammalian lignan formation.

## METHODOLOGY

(+)-Pinoresinol was isolated and purified to homogeneity from extravirgin olive oil according to the protocol described by Owen *et al.* [2]. The metabolism of (+)-pinoresinol by intestinal bacteria was studied in anaerobic conditions (100 ml) using Brain Heart Infusion (BHI) fortified with cysteine-HCl and sodium formaldehyde sulfoxylate as the basal medium. Experiments (100 ml) were conducted in 100 ml bottles in an anaerobic churn at 37°C for 72 h. The substrate (+)-pinoresinol was added at a concentration of 0.05 % and the media was

inoculated with freshly voided stool (1 g). After incubation the cultures were extracted on extrelute columns using methanol (5 %) in ethyl formate. The extracts were concentrated to dryness on a rotary evaporator and suspended in methanol prior to analysis by analytical high performance chromatography (HPLC). Thereafter the methanolic extracts were immobilised on silica gel, freeze-dried and subjected to column chromatography using increasing concentrations of methanol in dichloromethane. Fractions enriched in intermediates of (+)-pinoresinol transformation were subjected to semi-preparative HPLC and isolated and purified compounds were studied by gas-chromatography mass spectrometry (GC-MS), liquid chromatography electrospray ionisation (LC-ESI), nano-electrospray ionisation (ESI), and nuclear magnetic resonance spectroscopy (NMR) for confirmation of their structures.

## RESULTS

The metabolism of (+)-pinoresinol by faecal bacteria was essentially complete after 72 h incubation at 37°C. The major metabolite was enterolactone (> 90%) but a range of other intermediates in the pathway were also identified by GC-MS and NMR. These included monomethoxy-secoisolariciresinol (MMSILR), enterodiol and enteraldehyde and an isomer of enterolactone. Five other metabolites were detected by LC/ESI and GC/MS and a preliminary structural assignment is given in the Figure. Of these, like MMSILR, P1 is an early intermediate in the pathway in which both methoxy groups have been demethylated and ring opening of one half of the dioxabicyclo ring has occurred to form a methylene hydroxy moiety. P2 has a similar structure in which both methoxy groups have been demethylated and ring-opening of both halves of the dioxabicyclo ring has occurred with fusion of C6 to phenolic ring A at C6'. P3 is a similar metabolite with one of the methoxy groups intact. The two remaining metabolites are closely related to enterolactone with P4 containing an extra methoxy group and P5 containing an extra hydroxyl group.

## DISCUSSION

(+)-Pinoresinol which is a major lignan of the phenolic fraction of olive oil is extensively transformed by the human intestinal microflora into the mammalian lignan enterolactone. A number of minor intermediates in the pathway can also be detected and identified. Therefore it is likely that the phenolic fraction of olive oil, which escapes absorption in the upper gastrointestinal tract, becomes a substrate for the intestinal microflora and production of mammalian lignans ensues. This may be a further reason for the health protective effects of the Mediterranean diet. Studies are in progress to elucidate fully the pathway of (+)-pinoresinol transformation to the mammalian lignans with complete confirmation of intermediate structure. Studies on the transformation of additional dietary phenolic precursors is also continuing.

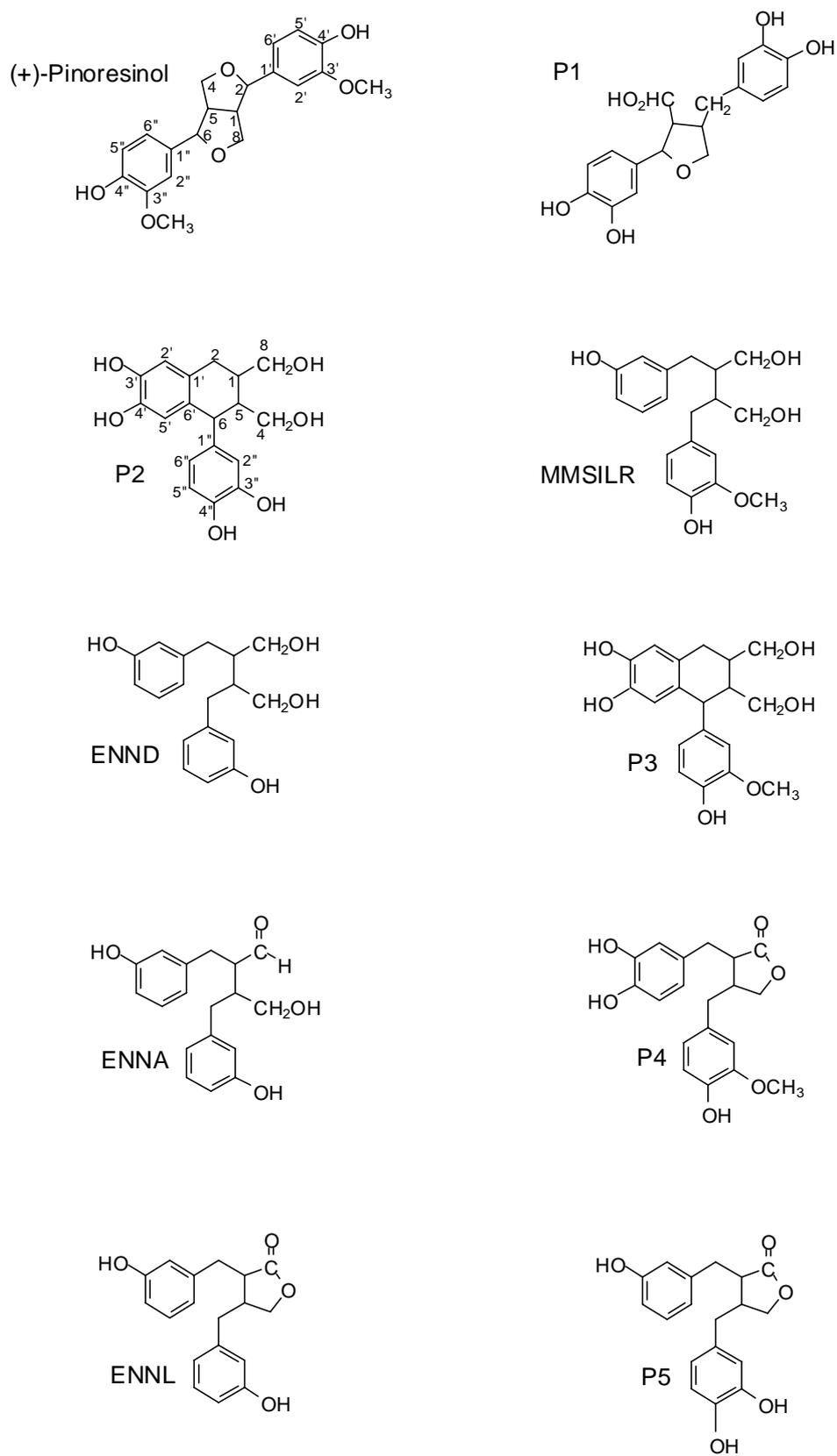


Figure. Metabolites produced from (+)-pinoresinol by human faecal bacteria.

In conclusion the transformation of the phenolic fraction of olive oil in the human large intestine by the resident flora may explain the previously observed discrepancy between dietary substrate intake and the extent of mammalian lignan excretion.

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# IN VITRO FERMENTATION OF DIETARY FIBRE AND LIGNANS OF RYE BRAN BY HUMAN FAECAL BACTERIA

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## INTRODUCTION

Rye is an important source of dietary fibre in the Nordic countries. Lignans are found in the same parts of the kernel as dietary fibre, i.e. in the outer layers of kernel. In the colon, dietary fibre is partly fermented to short chain fatty acids, and plant lignans are converted to mammalian lignans enterolactone (ENL) and enterodiol (END) by colonic bacteria. The fermentation products are absorbed and metabolised by the host and have important implications for human health.

## AIM

The aim was to study the consumption of carbohydrates and the production of short chain fatty acids and mammalian lignans during the *in vitro* fermentation of rye bran.

## METHODS

Rye bran was digested enzymatically to remove starch and protein, and subsequently fermented (100 mg) with human faecal inoculum *in vitro* (1). The consumption of carbohydrates was followed by measuring the content of neutral monosaccharides (arabinose, xylose, glucose) by HPLC after hydrolysis with sulphuric acid. The formation of short chain fatty acids (acetic acid, butyric acid, propionic acid) was measured by gas chromatography. Quantitative analysis of mammalian lignan precursors and mammalian lignans, ENL and END, was performed by HPLC with coulometric electrode array detection. In addition, pH and production of total gas was measured.

## RESULTS

During the 24-h fermentation, 50, 83 and 74% of rye bran arabinose, xylose, and glucose, respectively, were consumed, and short chain fatty acids, gases and ENL and END were formed. Acetic, propionic and butyric acids were produced

in the molar ratio of 57:19:24, and the pH decreased from 7.2 to 6.0. Conversion of plant lignans to ENL occurred concurrently with the formation of short chain fatty acids. The formation of ENL and END after 24 h fermentation exceeded almost 10 times the amount of matairesinol and secoisolariciresinol detected in rye bran. New lignans, also acting as ENL precursors, have recently been identified in rye (2).

## CONCLUSIONS

Rye dietary fibre is fermented by the intestinal bacteria with concurrent conversion of the rye lignans to enterolactone. The *in vitro* fermentation model is a good tool in studying the rates of fermentation and metabolite production. The interaction between fermentation of carbohydrates and bioconversion of lignans and other rye phytochemicals, as well as the role of the different gut bacteria, need to be studied further.

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# NO SEASONAL VARIATION IN PLASMA AND URINE ENTEROLACTONE

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## BACKGROUND

The lignan enterolactone may be protective against some cancer-types and coronary heart disease. In assessing the role of enterolactone in the epidemiology of these diseases, the majority of the studies have measured enterolactone concentrations in single blood or urine samples.

## AIM

The aims of the study were 1) to determine the seasonal variation of enterolactone concentration in plasma and urine in Finnish subjects, and 2) to see whether any correlation between enterolactone plasma concentration and urinary excretion exists.

## SUBJECTS

Of the 40 healthy, Finnish study subjects, 20 were omnivores and 20 vegetarians. Each subject gave a 72-hour urine sample and three venous samples, taken in three successive days and pooled, four times during one year.

## METHODS

Enterolactone concentrations in plasma and urine were measured by time-resolved fluoroimmunoassay. The samples were first hydrolysed with  $\beta$ -glucuronidase and sulfatase, after which the plasma samples were extracted with diethyl ether. An enterolactone derivative coupled to Europium was used as a label. Victor 1420 Multicounter (WALLAC OY, Turku, Finland) counted the fluorescence.

## RESULTS

No differences existed in the median enterolactone concentrations collected in different seasons. The median concentrations of enterolactone in spring, summer, autumn, and winter seasons were 24.9, 21.8, 20.7, and 22.5 nmol/l in plasma ( $p = 0.96$ ), and 14.7, 13.6, 13.9, and 17.0  $\mu\text{mol}/72\text{h}$  in urine ( $p = 0.54$ ). A linear

correlation existed between enterolactone urine excretion and plasma concentration ( $R = 0.88$ ).

## CONCLUSION

No seasonal variation exists in plasma or urine enterolactone concentrations in Finnish subjects. One epidemiological study can, therefore, in order to analyse enterolactone concentration, use samples collected in different seasons.

# ALKYLRESORCINOLS ARE ABSORBED BY HUMANS

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Alkylresorcinols are amphiphilic compounds found in high levels in the aleurone layers of rye and wheat. They are membrane forming compounds claimed to have anticancer and antioxidant activities. There is no published information about the absorption of these compounds *in vivo*.

An ileostomy study was performed on 10 humans with different diets – one high in whole grains (and high in alkylresorcinols) and one with only white wheat flour (no alkylresorcinols). Analysis of the ingredients and resulting ileostomy samples was done by gas chromatography. The rate of absorption of alkylresorcinols was shown to be about 50 % with some interpersonal variation. As alkylresorcinols are absorbed, it may be possible that they may be bioactive *in vivo*, and be used as biomarkers of whole grain cereal intake.

## BAKING WITH OATS

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The nutritional and health promoting properties of oats will only be fully manifested if a variety of oat-based foods are available. These foods need to be attractive and physiologically functional, e.g. the cholesterol lowering property of oat beta-glucan must be sustained in the process. The major food-use of cereals is as bread and other baked goods. However, the use of oats in baking seems to be restricted to muffins or scones, and only low amounts of oats are used in industrial breadmaking. We have experimented with various baking processes, including non-wheat baking technologies, and used high amounts of oats or oat bran. The results show that whereas it is possible to achieve relatively high concentrations of beta-glucan in bread, the sensory properties require addition of other ingredients. In terms of the physiological effects, the critical issue is that the viscosity of the beta-glucan may be lost because of hydrolysis induced by enzymatic activity originating from other ingredients. A viscosity index in addition to beta-glucan content would be informative when considering the justification of beta-glucan related health benefits in baked oat foods.

# AN OPPORTUNITY TO ENGINEER NUTRITIONAL PROPERTIES OF FOODS BY STRUCTURE ENGINEERING

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With most starchy foods the rate of enzymatic digestion of starch is a key determinant of the glycaemic response. Many food factors that reduce the rate of amylolysis, also lower total starch digestibility in the small intestine. In a whole cereal grain the intact cell walls protect starch from digestion. The characteristics of starch *per se* and heating conditions are also of crucial importance. Starch granules that are only slightly gelatinized are less hydrolyzed by  $\alpha$ -amylase than starch granules that are more gelatinized. Further heating can decrease accessibility of starch granules to hydrolysis, because amylose will leach out of starch granules. We have studied the effect of processing on the microstructure of cell walls and starch. Malting caused cell wall degradation, but starch was not gelatinized. Autoclaving did not break cell walls, but starch was gelatinized. Milling liberates starch from cells and organic acids and heat treatment affects greatly the degree of starch swelling. The degree of starch hydrolysis by  $\alpha$ -amylase can be engineered by processing.

# GERMINATION INCREASED BIOACTIVITY AND STABILITY OF WHOLE GRAIN OATS

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Germination was used as a tool to produce new, healthy and tasty food ingredients from oats. With the developed short germination programme combined with heat treatment the stability of oats during storage was improved and the amount of bioactive compounds was increased. However, the content and molecular weight of oat  $\beta$ -glucans was only slightly influenced. The improvement in storage stability was seen as a slower formation of lipid originated aldehydes and retarded development of rancid flavors in germinated oats during storage. Antioxidativity, sterol content and the content of oat specific phenolic compounds, avenantramides, were increased significantly during germination. Some increase was also seen in vitamin content. The increase in bioactive compounds was especially seen in the studied naked cultivar Lisbeth.

# MALTING OF BARLEY; OPTIMISATION OF PHYTATE DEGRADATION AND PHYTASE ACTIVITY

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## BACKGROUND

Phytate (*myo*-inositolhexaphosphate) is naturally present in plants and occurs in large quantities in cereals. Due to its strong chelating properties, phytate is often considered as an anti-nutrient. It forms insoluble complexes with essential minerals, such as iron and zinc, making them unavailable for absorption in the human intestine. The consumption of barley as food is nowadays low but an extended usage is of great interest due to the nutritional value and the high content of soluble  $\beta$ -glucans. Since a high intake of cereals can cause impaired absorption of minerals it is important to find methods that increase the bioavailability of naturally occurring minerals and preserve the health beneficial properties of the cereal. Malting is a well established process, which involves soaking (steeping), germination and drying of whole kernels. The process promotes many chemical and biochemical changes in the seed, which may increase the nutritional value in the final product. The enzymatic activity in the seed is increased during germination. This includes phytase that hydrolyses phytate to lower inositol phosphates, which may render important minerals more accessible for absorption. Thus, optimisation of the malting conditions may be a possible way to achieve effective phytate degradation and obtain a malt or final food product with improved mineral bioavailability and preserved content of  $\beta$ -glucans.

## AIMS

The aims are to study the effect of different malting conditions on the phytase activity and phytate degradation in barley and to optimise a malting process for increased mineral availability and with preserved content of  $\beta$ -glucans and antioxidants.

## MATERIAL AND METHODS

Conditions during steeping and germination were varied using multivariate experimental design for optimisation. Major parameters studied were steeping at 15 and 48°C and addition of lactic acid to steeping water. Phytate content was

analysed using HPC and the decrease of phytate during incubation at pH 5.0 and 48°C for 15 min was used as a measurement of phytase activity.

## RESULTS

Two varieties of barley, one naked and one hulled, were selected for high phytase activity and low  $\beta$ -glucanase activity. Variations in steeping conditions were found to influence the degradation of phytate and preservation of  $\beta$ -glucans to a greater extent than other parameters studied. Steeping in lactic acid gave a significant increase in phytate degradation. The greatest degradation, 63% compared to raw material, was obtained in the hulled variety after steeping in lactic acid at 48°C.

## CONCLUSION

The present result show promising evidence that further optimisation of the malting conditions can be a feasible way to produce malted products with high mineral availability. The possibility to correlate this with a preserved  $\beta$ -glucan content is shown in parallel studies within the project.

# POPPING OF PEARL MILLET (*Pennisetum americanum*) AND DEVELOPMENT OF A SUPPLEMENTARY FOOD BASED ON POPPED MILLET

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## OBJECTIVES

To study the popping condition and functional and nutritional quality of popped millet. Development of popped millet based ready to eat supplementary food and its quality evaluation.

## METHODOLOGY

### Optimization of popping conditions

A popular pearl millet variety CO 6 identified to possess good popping quality was used for the studies. The influence of moisture content of grains, conditioning time and popping temperature on the quality of popped millet were determined by the methods described by Malleshi (1983).

### Nutritional and functional qualities

Proximate composition, solubility, swelling power and pasting characteristics of popped samples were determined according to the method of AOAC (1985), Leach et al. (1959) and Halick and Kelly (1959) respectively. Nitrogen solubility by standard AACC procedure (1983). *In vitro* protein digestibility was analyzed as per the procedure of Mouliswar et al. (1993). Amino acid analysis was done by automatic amino acid analyzer (Spackman et al. 1958).

### Development and quality evaluation of supplementary food

Popped pearl millet flour (65%), popped chickpea flour (25%) and skimmed milk powder (10%) were blended to prepare popped food (PSF). The amino acid profile of the food protein was comparable to that suggested by foods FAO/WHO/UNO (1985) for supplementary foods. Toasted millet flour (65%), toasted chickpea dhal flour (25%) and skimmed milk powder (10%) were blended for preparation of roller dried supplementary food.

Chemical composition, viscosity, *in vitro* protein and carbohydrate digestibilities were analyzed as per the standard procedures mentioned earlier. Protein quality of supplementary foods was evaluated by animal feeding experiments as per the ISI (1974) guide lines. Developed products were packed in polypropylene (PP) and metallised polyester (MP) and assessed for storage quality at ambient (27°C and 65% RH) and accelerated (38°C and 92% RH) storage conditions, by analyzing the moisture, free fatty acids and peroxide values as per AACC (1983). Stored products were also evaluated for sensory qualities by a panel of 30 judges, by hedonic scale (Watts et al. 1989). A short term acceptability and tolerance study of the food on 37 pre-school children of age 12-72 months was conducted. Food was served to children *ad libitum* for each sitting. The anthropometric measurements and haemoglobin level of the subjects at the beginning and at the end of the study were taken by the accepted standard techniques (Jelliffe 1966).

## RESULTS

The optimum conditions for preparation of well popped millet were found to be, equilibrating the grains to 16% moisture and popping in sand heated to about 2500°C. Popping the millet did not affect the over all nutrient composition of the grain, however, it increased the total and soluble dietary fiber contents and decreased the polyphenol content. Popped millet exhibited higher swelling power and solubility and improved protein digestibility. Popped meal was hygroscopic and absorbed moisture even at 22% RH. Scanning electron micrograph of endosperm portion of millet revealed that the starch granules were blown up forming honey comb structure.

Popped millet (65%) was blended with popped chick pea dhal (25%) and skim milk powder (10%), to prepare a supplementary food. The physico-chemical, nutritional and storage qualities of the popped supplementary food (PSF) were compared with roller dried supplementary food (RDSF) of similar composition. The protein and calorie contents of 100 g PSF were 18.4 g and 355 Kcal respectively. The composition of PSF in general was in accordance with Indian and International standards for cereal based weaning and supplementary foods. The cooked paste viscosity of popped food was considerably high. Addition of about 5% barley malt to a 30% slurry reduced its viscosity (dietary bulk) from semi solid consistency to free flowing consistency (1200 cpu) and provided about 1Cal/ml of food. The essential amino acid content of the supplementary food met the FAO/WHO/UNO (1985) recommended levels for pre-school children except for lysine, which was about 80% of the recommended level. The PER (3.2) of PSF was comparable to reference protein diet and its TD and BV were 82.0 and 91.6% respectively. The popped supplementary food was comparable to RDSF with respect to nutritional quality.

The critical moisture and humidity for safe storage of popped supplementary foods were 10.97 and 56%, respectively. The shelf-life of PSF packed in polypropylene and metallised polyester pouches were 7 months at ambient and 5 months at accelerated storage conditions.

A short term acceptability and tolerance study on PSF was conducted on pre-school children. The food provided about 5.0 g protein and about 185 Calories per child of 12–36 months age group and about 7.0 g protein and 257 Calories for children of 36–72 months of age group. The food was readily accepted and tolerated by the children. Supplementary feeding improved the haemoglobin level and overall nutritional status of the children.

## CONCLUSION

Popping the millet at 16% moisture at about 250°C yielded fully popped ready to eat product. The functional properties of popped millet indicated a suitable base for nutritious ready to eat supplementary food formulation and suitable in nutrition feeding programmes.

# QUALITY OF LESS EXPENSIVE AND MINIMALLY PROCESSED POPPED SORGHUM CULTIVARS FOR IDENTIFICATION AND CONSERVATION OF NATIVE DIVERSITY

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## OBJECTIVES

To assess the popping quality of pop sorghum cultivars grown in Kharif and Rabi seasons for commercial exploitation by conserving the native diversity .

## METHODOLOGY

### Sample Selection

Pop sorghum cultivars of different regions of North Karnataka, grown in Kharif and Rabi seasons were collected from Sorghum scheme, Main Research station, UAS, Dharwad.

### Popping Quality

The pop sorghum grains were moistened by boiling in water for one minute and dried for 15 minutes after draining. Tempered grains were popped by conventional method of dry heat in thick frying pan at 250°C. The per cent yield, expansion ratio and were determined.

### Proximate Composition

Fifteen cultivars each of both the seasons and their popped grains were analysed for proximate composition as per the standard AOAC (1985) procedures.

### Dietary fibre content

Three cultivars of kharif, and their popped grains were analyzed for total, soluble and insoluble dietary fibre contents as per the procedure of Asp et al. (1983).

## RESULTS

There was a wide range in the colour of the pop sorghum grains grown in Kharif and Rabi seasons. Kharif grown cultivars were brownish and purple red and Rabi cultivars were creamish white in colour. Hundred kernel weights of Kharif cultivars ranged from 1.3–2.4 g and Rabi from 1.6–3.6 g. Popping yield of Kharif cultivars ranged from 43.3–90.0% with a mean of 70.3% whereas, Rabi cultivars ranged from 64.8–99.7% with a mean of 90.4%. Similarly expansion ratio and flake size of Kharif and Rabi grown cultivars ranged from 4.1–11.96 0.16–0.53 ml/popped grain and 4.3–20.7, 0.42–0.72 ml/popped grain, respectively. Popping did not affect the proximate composition, however Rabi cultivars were nutritionally superior over Kharif cultivars. The total and soluble dietary fiber contents of raw and popped grains of pop sorghum were higher than grain sorghum (M-35-1) and popping enhanced the total and soluble dietary fiber contents.

## CONCLUSION

Thus the result reveals that though popping is a simple, less expensive, high temperature short time (HTST) processing method has not been fully exploited for pop sorghum similar to maize for development of value added health products, supplementary foods, functional foods and ready to eat breakfast cereals. Cereal fibers are very effective in controlling blood sugar and serum lipids, and popped sorghum provides more dietary fiber in soluble form. This quality has been emphasized in developing therapeutic foods for metabolic disorders. Since popping is done by different heating methods, there is a need for in depth study in the cost and quality of popped cereals produced by different methods and their suitability for commercial utilization. As popped foods are highly acceptable and easily digestible, there is a need to focus on this area for the development of supplementary foods to children and geriatric segment for their better health.

# THE ORIGINAL METHOD FOR INDUSTRIAL PRODUCTION OF WHOLE GRAIN PRODUCTS

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## OBJECTIVES

The main aim of this paper is to present the original and conceptually new method for processing of whole grains (wheat, rye, etc) that allows:

- (a) preserving and using all the components of the whole grain (including the germ, all grain outer covers and inner layers);
- (b) producing various bakery, confectionary and macaroni products from whole grain in the early stage of grain's sprouting (germination) rather than from milled dry whole grain.

The aim is two-fold. On one hand we will demonstrate the new method (technology) as such, and on the other hand it is important to emphasize all the unique characteristics of the products produced with the herein described technology (using the example of the bread, named The New Whole Grain Bread with trade mark "GraiNew").

## METHODOLOGY

This paper is based on the experience from the industrial production facilities of the company's licensed manufacturer of the New Whole Grain Bread.

Dr. Vladimir Antonov invented the main idea of the original method and special machines. Dr. Grigory Kalnish and his company (Bulgarian Trade House – Sofia, Ltd.) developed production technology and production line of the special equipment suitable for industrial scale of production. Dr. Kalnish and Dr. Antonov have jointly patented the technology and special equipment under the Patent Cooperation Treaty.

Medical research of the New Whole Grain Bread properties was undertaken by independent research institutions and confirmed positive effect of this type of bread on the human health.

## RESULTS

In the process of development of the bread baking technology bakers were using different types of flour - from wholly milled grains in ancient history to first class white flour produced with modern technology. However, with all the development more and more parts of the grain structure were wasted in the flour milling process. In line with this more and more vitamins, mineral matter and proteins were wasted. Recent researches have confirmed that usage of all parts of the whole grain would be highly beneficial for the functioning of a human body (including digestive system, cardiovascular system, etc). That is why many technologies strive to utilize the idea of using all the components of whole grains including seed and fruit covers, the alleurone coating and the germ.

The herein presented original method offers unique opportunity to use all the natural power captured in the whole grains. The main merit of the method is that it uses sprouted grain, i.e. whole grain at the germ germination stage. At this stage all the natural processes associated with germ growing and whole grain itself are activated.

This method is fully compatible with traditional processes of bread baking, while fully substituting flour with so-called grain paste. The finely dispersed grain paste is produced from sprouted (germinated) grain using special patented equipment. The method involves following processes:

1. Grain peeling stage. At this stage the grain outer husk /the hull/ (up to 1% of the total grain weight) is being peeled, while preserving all other parts of the grain structure. The process is undertaken in specially designed and patented equipment. The equipment allows peeling outer husk of the grain while preserving the structure of the whole grain seed. That includes all grain covers and layers, the alleurone coating and hyaline membrane.
2. Grain washing stage. At this stage peeled grain seeds are being rinsed on special equipment (hydrodynamic separator). In the process all foreign substances and mineral dust are being removed.
3. Grain sprouting (germination) stage. At this stage the grain seeds are soaked in water until the sprouting of the germ starts (the stage at which the activation of the germ /germination/ starts). The original technology prescribes special parameters, such as temperature, air supply, grain moisture, time duration and acidity level under which the sprouting (germination) process should be taking place. The technology for manufacturing of the New Whole Grain Bread also prescribes characteristics of the time when the grain is fully germinated.
4. Grain dispersing process – *preparation of the finely dispersed grain paste*. At this stage the whole germinated grain is being pulverized on a special patented piece of equipment (*Dispergator*) that allows deriving at a finely dispersed grain paste.

The grain paste then undergoes traditional bread baking processes using traditional equipment: dough mixing, dividing, fermentation and baking. The original technology prescribes specific technological parameters (including temperature, humidity level and time) for each of the traditional stages.

As can be seen from the description of the technological processes involved there are three main differences embedded in the original technological process:

- Usage of *all parts of the Whole Grain* – 99% of the grain structure (apart from certain part of the Epidermis);
- Usage of the *grain at the stage of germination*, when it is activated and vital chemical and biological processes start to function thus releasing whole range of vitamins, microelements, etc that are otherwise tied up in the dry grain.
- *No addition of any other components*, including flour, gluten, etc.

Therefore, the composition, unique characteristics and properties of the New Whole Grain Bread can be attributed to the original technology that allows preserving and biological activation of all parts of the whole grain and thus ensuring that its natural power is accumulated in the end product.

The unique composition of the New Whole Grain Bread can be summarized as following:

**Fibre.** The New Whole Grain Bread has an increased content of fibres. It is known that fibres are an important constituent of the human diet and have a positive effect on the health and functioning of numerous organs. The recommended daily allowance of fibers is no less than 30 grams – just as much as a 150–200 gram loaf of the New Whole Grain Bread contains.

**Vitamins.** The New Whole Grain Bread has an increased content of vitamins, and in particular group B, E and PP, as well as mineral matter. All of those are derived from the germ, seed and fruit covers. Whereas in the dry grain these elements are tied up, in the process of germination they are being released. That is why the content of these substances is much higher in the New Whole Grain Bread than in the traditional breads from dry milled grain or flour.

**Proteins.** The New Whole Grain Bread has increased content of proteins (due to the use of whole grain) with a balanced content of amino acids (due to the use of whole grain at the stage of germination).

Various medical researches have been undertaken in Bulgaria and Russia on the *Grain TONUS*<sup>®</sup> – one of the breads manufactured under this original technology. The main institutions that have undertaken the research so far were: Diabetes Center (Sofia, Bulgaria), Research Institute of Nourishment attached to Russian Academy of Science (Moscow), The Ministry of Health of Russian Federation, Biophysics institute of the Siberian Department attached to Russian Academy of Science (Novosibirsk, Russia), General Junior Hospital (Krasnojarsk, Russia), Specialised Cancer Centre (Krasnojarsk, Russia), General Hospital (Tver, Russia). All researches had proven positive effect the product has on the human body.

## CONCLUSIONS

The evidence outlined in this paper undoubtedly shows that the herein described original method for manufacturing products from whole grains offer a whole new possibility for increasing consumption of whole grain among wider population groups by means of incorporating whole grains in such staple product as bread and bakery products.

# A MULTIFUNCTIONAL HEALTH PROMOTING BREAD

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## OBJECTIVES

The main objective of this paper is to present the original multifunctional health promoting bread that aims to resolve the so-called diseases of civilisation.

The main goal of our invention was to combine in one universal nutritional product all health promoting potentials of the cereal grains (such as wheat, rye, oat) with the potentials of lentil, soybeans and sunflower kernels. The main goal was to achieve the maximum potential for reducing the risk of dangerous chronic diseases. The hypercholesterolaemic and carcinogenic effects of the animal proteins have allowed achieving high biological value of the vegetable protein in our product. Such a product will permit to reduce to a larger degree and even totally eliminate the animal protein in the diet without the risk of developing protein malnutrition.

## BACKGROUND

During the last century, and especially during the past 40 years, we have witnessed remarkable permanent increase in the number of heavy chronic diseases, such as cardiovascular diseases, cancer, type 2 diabetes, metabolic syndrome, obesity, all of which are clearly linked to the lifestyle. The comprehensive review of the scientific data from the past 20–25 years leads to the unequivocal conclusion that the main reason for the unceasing augmentation of these diseases is not the affluent western lifestyle and richness of the western diet, as argued by some scientists, but rather the substantial changes that have occurred in human nutrition during the last 100–150 years.

An increasing amount of studies during the last two decades have shown that the most harmful and injurious consequences of these changes in nutrition are the often, sharp, and high postprandial fluctuation in the level of blood glucose, insulin, and lipids. These fluctuations lead after time, especially if there is genetic predisposition, to insulin resistance, which is considered to play the key role in developing of the most prevalent diseases of our time. The main conclusion of these studies is that effective combat of the epidemic of the chronic diseases is only possible on the basis of a new nutritional practice, which will eliminate the dangerous disturbances in carbohydrate and lipid metabolism.

In recent years the need of such changes in our nutrition has become to be a widely recognized view. Now there are many national programmes for achieving permanent healthful dietary changes. These programmes have also aim at encouraging the food industry to increase production of wider range of healthy foods that help to meet dietary guidelines for chronic disease prevention.

For the greater part of the population almost in all countries it is extremely difficult to achieve substantial change in the diet in accordance with the national recommendation for a healthy nutrition, and attain sufficient disease reducing results. Based on that assumption, we have set ourselves the goal to create a universal nutritional product with potentials to improve and correct the most dangerous disturbances in the metabolism provoked by the western manner of nutrition.

In order to achieve an effective reduction of the most dangerous chronic diseases we analyzed the health and biological potentials of the nutritional products, which may permit us to receive the following effects:

- a) flatten the postprandial glucose and insulin blood levels, the high and frequent elevation of which is the main reason for developing insulin-resistance, glucose intolerance and elevated levels of blood glucose;
- b) prevent development of hypercholesterolaemia and hypertriglyceridaemia, which are the major risk factor for CHD;
- c) prevent uncontrolled generation of reactive oxidative species and developing of oxidative stress, which is a major risk factor for arteriosclerosis and cancer;
- d) improve the gastrointestinal physiology and bowel function;
- e) diminish the risk of colon, breast and prostate cancer;
- f) exert a prebiotic effect.

The recent analysis of the scientific data of the physiological effects of the nutritional products lead to the conclusion that it is possible to achieve such multifunctional effects only with the help of the potentials of the whole cereal grains. Whole grain products have as a rule low glycemic index and have the potentials to lower the postprandial blood glucose and insulin response. They are the most important source of dietary fibre, resistant starch, oligosaccharides and vegetable protein. They are also a rich source of numerous vitamins, minerals and wide range of photochemical and bioactive compounds with high antioxidant and anticarcinogenic effects.

Despite the fact that the composition of all cereal grains is similar, they differ in the quantity of the ingredients and some bioactive component. The prevalence of one or another ingredient in the seed determine the specific properties, the

glycemic index, and the health and protective potentials of the different cereal grains. The legumes, sunflower and some other seed kernels represent another group of vegetable nutritional products with powerful and in some aspects unique health properties, including the reducing of the chronic diseases risk.

We have concluded that bread would be the best solution for such a nutritional product for a number of reasons. The bread is a basic component of the human diet, satisfying from 15 to more than 50 per cent of the energy and protein needs of the organism. Moreover most people consume it two to three times a day as an accomplishment to their main meals. And what is of much greater importance is that it can be prepared from a combination of products that have proven potential to improve and correct the harmful changes in carbohydrate and lipid metabolism.

## RESULTS

Our bread is a totally new type of bread. It represents the combination of the health benefits and disease reducing properties of two already patented and industrially produced in Bulgaria functional breads: the New Whole Grain Bread “GraiNew” from sprouted whole grains, and the bread “Health” based on the combination of functional components including cereal grains and legumes. The new type of bread is derived at by carefully balancing the specific properties of all the ingredients for attaining the overall augmented protective effect with the ability to have all the herein mentioned effects.

## CONCLUSIONS

The new type of multifunctional bread produced without flour from sprouted grain with added functional constituents has the following benefits:

- a) it contains all components of the whole grain cell and botanical structure;
- b) it contains the activated germ of the grain, as well as dietary fibre (both water soluble and non water soluble);
- c) it contain high level of vegetable protein with high biological value.
- d) it has very low glycemic index;
- e) it is rich in photochemical and bio-active compounds with a proven track of decreasing the level of blood sugar, cholesterol and lipid concentration as well as diminish the risk of colon, breast and prostate cancer;
- f) it has a prebiotic effect;
- g) it also has a antioxidant effect.

# DEVELOPMENT OF DICOCCUM WHEAT BASED THERAPEUTIC BUN

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## OBJECTIVES

Development of nutri enriched dicocum wheat based bun and to evaluate nutritional, therapeutic and storage qualities of developed bun.

## METHODOLOGY

Development of dicocum wheat based bun was standardized for optimum incorporation of whole wheat flour, yeast, sugar and oil and were evaluated for physical and organoleptic characteristics by standard procedures and semi-trained panelists respectively. Commonly available local pulses(in the proportion of 4:1 to flour mix) and spices were selected and suitable spice addition was tested by organoleptic evaluation. Proportion of carrot was added without affecting the textural quality of the bun. Nutritional quality of enriched bun was assessed for its proximate composition (AOAC,1985), total dietary fiber (Asp et al. 1983).  $\beta$ -carotene and  $\alpha$ -tocopherol content of bun were calculated. Therapeutic quality of the nutri enriched bun was assessed by analyzing the post-prandial blood glucose response of the developed bun in 18 diabetic subjects in comparison with commercially available refined flour bun as control. Blood samples were analyzed for glucose by using enzymatic diagnostic kit (Span diagnostic Ltd., India). The shelf-life of the product was assessed by analyzing moisture, free fatty acids (FFA) and organoleptic quality till the product developed off-flavor.

## RESULTS

The spice based nutri-enriched bun, prepared by the addition of 40:40:20 dicocum whole wheat flour, refined flour and black gram dhal flour respectively, 20 g grated carrot, 4 g yeast, 10 g sugar, 8 g vegetable oil and 4 g ginger, was highly acceptable. Enriched bun was nutritionally superior as compared to dicocum whole wheat flour bun, as it not only increased the protein and dietary fiber contents by 7.5 and 20.0 per cent, respectively, but also improved the quality of the bun by increasing the soluble dietary fiber by 32.0 per cent. Per serving the bun provided 13.7 per cent of daily requirement of protein, 41.0 per cent of dietary fiber, 6.3 per cent  $\beta$ -carotene and 11.7 per cent of

Vit E requirement for a reference man. The post prandial blood glucose of enriched bun was lowered by 20.0 per cent as compared refined flour bun. The developed bun possessed the shelf-life of 3 days.

## CONCLUSION

Nutritious dicoccum wheat with special therapeutic quality can be incorporated in refined wheat flour for development of ready to use, convenient, therapeutic bakery products similar to commercial bakery products. Foods rich in other nutrients can be easily added to develop organoleptically acceptable enriched dicoccum wheat based products in the management of diabetes more efficiently avoiding further secondary complications. Thus, commercial value added varieties of dicoccum wheat products can be exploited and used regularly for effective cost management in diabetes.

# EFFECT OF GENOTYPE AND HARVEST YEAR ON DIETARY FIBRE CONTENT AND BAKING PROPERTIES OF RYE (*SECALE CEREALE L.*) WHOLE MEAL FLOUR

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## INTRODUCTION

The positive health effects of dietary fibre are well documented and an increased intake of whole grain products is recommended especially in the western industrialised countries<sup>1</sup>. Rye bread provides a substantial amount of the total dietary fibre intake in many Central, Northern and Eastern European countries<sup>2</sup>. However, very limited information exist on the variation in dietary fibre content among rye varieties and harvest years. Regarding the baking quality of rye flour the amount and physical properties (especially water-holding capacity) of the cell wall components arabinoxylan and mixed-linkage  $\beta$ -glucan are very important parameters. Some differences among varieties of rye have been reported<sup>3</sup>, whereas replicated studies used to quantify the effects of genotype and year are limited.

Thus the aim of the present study was to determine the effect of genotype and harvest year on dietary fibre content and composition as well as the baking quality of rye whole meal flour.

## MATERIALS AND METHODS

Grain samples from 19 different varieties were grown in the same location (Rønhave experimental station in Denmark) in 3 seasons (1996/97, 1997/98 and 1998/99). Each variety was grown in at least 3 plots of 18 m<sup>2</sup> size in each of the 3 years. Grain samples from different plots were harvested, milled and analysed separately. Original seed material was used each year.

The amounts of total and water-unextractable (WAUX) dietary fibre were determined as non-starch polysaccharides (NSP) and lignin by a modified Uppsala-method<sup>4, 5</sup>. The content of water-extractable (WAX) fibre components was calculated as the difference between the total and water-unextractable parts. All varieties from 1997 and 1998 and 9 varieties from 1999 harvest were

investigated. The content of mixed-linkage  $\beta$ -glucan was measured enzymatically by the ICC-method no. 166 (Megazyme kit). The content of fructan was measured enzymatically by the Megazyme fructan assay procedure, which measures fructan separate from sucrose and reducing sugars (AOAC; Recommended for First Action Adoption).

The baking quality measurements included Falling Number (FN), amylograph measurements, water absorption and swelling curves. A baking test was developed and used for measuring baking quality of 9 varieties x 3 years. Bread volume, crumb structure, crumb firmness and elasticity, crumb water content and sensory parameters were measured.

Data were treated by a 2-way analysis of variance and covariance (SAS, GLM procedure). Components of variance were calculated by means of the procedure VARCOMP in SAS.

## RESULTS

The dietary fibre content of the rye whole meal flour ranged from 14.7–20.9 g/100g d.m. from which 3.4–6.6 g/100g d.m. were water-extractable and 10.8–15.9 g/100g d.m. water-unextractable. Significant effects of harvest year, rye genotype and less significant covariation year\*genotype were found for almost all dietary fibre components. The variation due to yearly differences contributed with about 40–60% of total variation, genotype effects were 10–20% and year\*genotype variation was lower. The main dietary fibre component was arabinoxylan with 8.0–12.1 g/100g d.m. of rye flour and the proportion of water-extractable to total arabinoxylan was 30–35%. The content of mixed linkage  $\beta$ -glucan was 1.4–1.9 g/100g d.m. and a relatively high content of fructan (5–6 g/100g d.m.) was found.

The baking quality, measured as Falling Number (FN), Water absorption, amylograph viscosity and gelatinization temperature and swelling properties of the flour were significantly influenced by both genotype, harvest year and covariation genotype\*year. Regarding the starch properties (FN and amylograph viscosity) the variation due to yearly differences was generally higher (50–60%) than genotype effects (10–20%). Water absorption and swelling properties were stronger influenced by genotype effects, which amounted to about 40–70% of the total variation, compared to 15–46% contribution from yearly differences. The results of the baking test showed significant yearly differences in crumb firmness and elasticity and a significant effect of the genotype on crumb firmness with about 50% explained variation.

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# NEW CHOLESTEROL-LOWERING DIETARY FIBER FROM CERATONIA SILIQUA

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## BACKGROUND

Carob bean or St. John's bread (*Ceratonia siliqua*), the brown pod of the carob tree, is a fruit rich in carbohydrates which, for centuries, has been used by humans as a source of nutrition, especially in the Mediterranean countries.

Approximately 10 % of carob's weight is composed of seeds which are rich in water-soluble non starch polysaccharides that have shown cholesterol-lowering potential in different studies (locust bean gum). The husk of the pod, which makes up about 90 % of the fruit, stands out due to its high content of carbohydrates, minerals, protein, insoluble dietary fibres and tannic acid. An insoluble dietary fibre (Carob fibre is marketed under the trademark Caromax™ by Nutrinova) can be obtained from the husk by a mild process that results in a product that with a dietary fibre content of more than 80 % (determined according to AOAC method). This Mediterranean fibre is characterised by especially high contents of lignin and polyphenols and also exhibits very high antioxidative activity. Different animal studies showed a cholesterol-lowering effect of dietary fibre from carob pulp. The objective of this clinical study was to prove the effects of Caromax™ on blood lipids in human.

## METHODS

This human clinical trial was conducted as an open label study with 49 healthy volunteers with mild to moderately raised cholesterol levels. In addition to the normal diet, Caromax™ was administered three times daily in the form of foods such as breakfast cereals, fruit bars and drinking powders. The daily dose was 15 g with 5 g of Caromax™ per serving. The products were recommended to be consumed just before meal.

## RESULTS

After 6 weeks of treatment highly significant reductions of total cholesterol and LDL-cholesterol 7,8 % and 12,2 % respectively were found. The products were very well accepted by the participants and the compliance was very high.

## CONCLUSIONS

Caromax™ incorporated into the normal diet exhibited a highly significant reduction of total cholesterol levels, especially of LDL cholesterol in humans.

Since activity of this nature is predominantly known for soluble dietary fibres, this insoluble Mediterranean product provides an unique value to human nutrition and all kind of food products such as bakery products, breakfast cereals etc. Caromax™ special composition could be the key for this extraordinary effect of an insoluble fibre.

# FUNCTIONALITY AND APPLICATION OF RICE, CONTRIBUTION OF WHOLE RICE GRAIN TO HEALTH, BEAUTY AND RECOVERY

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Rice plant has long been applied wholly and functionally since old days in Asia especially in Japan for non-food use (*e.g.* feed, biomass *etc.*), semi-food-use (*e.g.* food-aid, beauty aid *etc.*) and food-use (*e.g.* *gohan*<sup>1</sup>, *sake*<sup>2</sup> *etc.*).

## OBJECTIVES

Many studies have been aimed to elevate the healthful values in food-use of whole rice grain (brown rice). In case of food-use, the grains are employed as brown (whole) rice or white rice. It is generally said that the former is nutritive but not so delicious with fibrousness, thus less popular, whereas the latter is delicious, thus more popular, but not so nutritive. Procedures which could control the undeliciousness of brown rice have been studied and developed for years. Principals are high pressure method (general), powdering (Cigario Co.), germinating (Funcl Co.), gene technological modification (under investigation) and so on. Here, the germinated-brown-rice are outlined to the points.

## METHODOLOGY

The germinated-brown-rice has simply been prepared by a small germination of the original brown-rice (whole grain), followed by gentle drying and a vacuum package. The germinated-brown-rice was boiled with water to obtain *gohan*. Food values were estimated mostly by chemical and instrumental analyses of nutritional and or bioactive compounds in the *gohan*.

## RESULTS

Boiling of the germinated-brown-rice gave peculiar *gohan*, which was found to be of moderate texture, softness and flavor. It was also detected in the composition of the *gohan* that some functional factors as  $\gamma$ -aminobutyric acid (hypertension-effective), prolylendopeptidase inhibitor (*Alzheimer*-effective) were newly generated and that the various nutritional components in the original grain became quite easily digestible and absorbable.

## CONCLUSIONS

The germinated-brown-rice could be said to have the moderate deliciousness, excellent nutritionalities and the characteristic functionalities (regulatory bioactivities). The brown rice *gohan*, mostly recommendable to health, beauty, recovery *etc.*, is being positively utilized by public schools, cultured cities and educated people in Japan.

Further, several attempts are being possibly done to prepare the other variously-processed foods from the germinated-brown-rice to enlarge the utilization (*e.g.* rice powder to apply to bread, *sake* to brew old-style of Japanese alcoholic drink and so on).

Key word: brown rice, germinated-brown-rice, health, beauty, recovery, hypertension-effective, *Alzheimer*-effective.

- 1) *gohan*: respectful term in Japanese for boiled-rice.
- 2) *sake*: typical Japanese alcoholic drink brewed from rice.

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# COMBINATIONS OF WHOLE GRAIN IN THE FINNISH DIET

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Whole grains have traditionally been consumed mostly as rye bread in Finland. The aim of this study was to find out if separate food groups contribute to whole grain consumption and fiber intake in different population groups.

In the study we aggregated consumption of food items and dishes containing whole grain. Whole grain was determined as cereal product that contains the bran of the kernel wholly or partly. Study subjects represented five areas of Finland in a cross-sectional population survey carried out in local health centers in 1997. Background data like education was obtained from a questionnaire. Dietary data was collected with a 24-h recall for 1501 women and 1361 men, age varying from 25 to 64 years. For a subsample (n = 242) three days' food dairies were collected. The fiber intake was determined from four aggregated food groups. The correlation of intake of fiber and consumption of food groups were used to study differences in dietary combinations.

The consumption of rye bread was higher among men (11.6 g/MJ) vs women (9.2 g/MJ), higher with age, higher in the Northern parts vs southern parts of Finland, but lower with education. The consumption of wheat breads containing whole grain was higher in southern parts of Finland (5.7 g/MJ) vs northern parts (3.8 g/MJ) and with higher education. The consumption of dishes containing whole grain followed mostly the differences in rye consumption. The average fiber intake varied between 16.7 g/day and 22.4 g/day by sex and age group. The Finnish men got 40 % of fiber intake from rye products, women 30 % and on the northern areas the proportion of fiber intake from rye was even 47 %. On average more than 70 % from fiber was obtained from cereal products. Whole grains contain other potential health promoting factors. In order to study dietary combinations, correlation of fiber intake with consumption of food groups was studied. In addition to rye bread fiber intake correlated positively with other sources of fiber like fruits, berries (Northern Finland), and in population groups with consumption of vegetables. In some population groups high correlation was also found between fiber intake and high-fat and fat-reduced milk, fish, cooked potato, coffee bread and porridge. The highest negative correlation was found between fiber intake and beer consumption. In Finland, ryebread seems to be the dominating source of dietary fiber in all population groups. However, the dietary combinations seem to vary from vegetable-based prudent diets to traditional milk-based diets in different populations.

# DIETARY FIBER INTAKE OF 13-MONTH-OLD TO 8-YEAR-OLD CHILDREN IN THE STRIP PROJECT

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Dietary fiber has important health benefits in childhood. It promotes optimum gastrointestinal function in childhood, and also may help in reducing the risk of future cardiovascular disease, obesity, some cancers and adult-onset diabetes. Health benefits are indisputable, but how much should children consume dietary fiber? According to the American Health Foundation (AHF) recommendation children older than 3 years of age should consume a minimum (age in years + 5) and a maximum (age in years +10) grams dietary fiber. The Finnish and Nordic Nutrition Recommendations contain no exact dietary fiber recommendations, and data about children's dietary fiber intake are scanty. Consequently the recommended dietary fiber intakes in childhood are based on different loose estimations.

The aim of this study was to evaluate dietary fiber intake in childhood prospectively when the age of the children increased from 13 months to 8 years. The children in this study were participants in the STRIP project (Special Turku coronary Risk factor Intervention Project for children), which is a prospective randomized long-term atherosclerosis prevention trial that began 11 years ago at children's age of 7 months and will continue until the age of 20 years.

Families were recruited for the trial by nurses at the well-baby clinics in the city of Turku. 1062 children were randomized to the intervention group (n = 540) or to the control group (n = 522). The intervention families received individualized counseling aimed at decreasing the child's intake of saturated fat and cholesterol and at increasing the intake of monounsaturated and polyunsaturated fat correspondingly. When the children have reached the age of seven years direct counseling of children was also started. The aims in this counseling of the children were the same as before decrease intake of saturated fat and increase intake of monounsaturated and polyunsaturated fat. Counseling concerning salt intake also began at the age of eight years. The control families received only general dietary information as currently delivered at Finnish well-baby clinics

and in the school health -care. In the STRIP project the control families did not received any detailed nutritional counseling.

All families kept a food record four days in every 6 months yearly until the age of seven years, after which the intervention children kept records half yearly but control children once yearly. Nutrient intakes were analyzed with Micro Nutrica® program (Research and Development Centre of Social Insurance Institution, Turku, Finland). T-test was used to analyze differences between the two groups. Differences were considered significant at  $P < 0.05$ .

Dietary fiber intake (as grams per day) differed between the boys and the girls at all ages studied. The dietary fiber intake of the boys increased from 7.5 g/d at 13 months to 13.3 g/d at 8 years, while that of the girls increased from 6.9 g/d to 12.6 g/d. The intervention children received in the mean more dietary fiber (g/d) than the control children at the ages of two (9.3 vs. 8.4 g/d), three (10.2 vs. 9.5 g/d), five (12.0 vs. 11.4 g/d), six (13.2 vs. 12.1g/d), seven (13.5 vs. 12.9 g/d) and eight (13.3 vs. 12.6 g/d) years than the control children ( $p$  for all differences  $< 0.05$ ).

The boys and girls showed closely similar dietary fiber intakes (as g/MJ) before the age of seven years. At the age of seven years the mean fiber intakes of the boys and girls were 1.9 g/MJ (SD 0.4) and 2.0 g/MJ (SD 0.5) ( $p = 0.03$ ), respectively. At the eight years of age, the boys received 1.8 g/MJ (SD 0.5) and girls 1.9 g/MJ (SD 0.5) of dietary fiber ( $p = 0.01$ ), respectively. The energy-intake corrected fiber intake of the intervention children was constantly higher than that of the control children. The dietary fiber intake of the intervention children increased from 1.8 g/MJ at 13 months to 2.0 g/MJ at 8 years, while that of the control children changed from 1.7 g/MJ at 13 months to 1.8 g/MJ at 8 years.

The Nordic Nutrition recommendations propose that children should eat daily about 2 g/MJ of dietary fiber. The mean dietary fiber intake of all study children was 2 g/MJ between the ages of four and eight years, but individual intakes varied greatly from 0.5 to 4.8 g/MJ.

Children received most of their dietary fiber from cereal products. Intake from cereal products decreased from 33 % of all fiber at 13 months to 27 % of all fiber at 7 years. Bread was also one of the main dietary fiber sources. Fiber intake from bread increased from 13 % at 13 months to 30 % at 7 years. Additional important sources of fiber were vegetables, fruits and berries. Fiber intake from vegetables decreased from 30 % at 13 months to 23 % at 7 years, while intake from fruits and berries decreased from 23 % at 13 months to 17 % at 7 years. Other fiber sources worth mentioning were beverages, sweets and sausages.

We conclude that the average dietary fiber intake of the children in this study was well within the current recommendations, but a rather large group of children is getting surprisingly small amounts of dietary fiber daily. Our study also shows that use of a low-fat diet increases the intake of dietary fiber automatically without any specific counseling.

# INTAKE AND SOURCES OF LIGNANS IN THE FINNISH DIET

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## OBJECTIVE

To compile a lignan database for Finnish foods, to estimate the lignan intake of Finnish adults, and to evaluate the main sources of lignans in the diet.

## METHODS

The Finnish lignan food database was compiled to contain values for plant lignans, matairesinol and secoisolariciresinol. The values (expressed as aglycones) were based on food analyses (GC-MS) of about 140 foods, imputed values based on the analyses as well as imputed values derived from the recipe database with over 1000 foods of the National Food Composition database Fineli<sup>®</sup>. Food consumption data were obtained from the National FINDIET 1997 Study carried out in a random sample of the population of 25 to 64 years of age in five areas in Finland. The dietary data was collected by 24-h recall (n = 2862) and sociodemographic data were collected by a structured questionnaire.

## RESULTS

The mean total lignan intake was 28 µg/MJ (245 µg/day) in men and 55 µg/MJ (376 µg/day) in women (p < 0.05). The median intakes of lignans were lower than the mean intakes. Secoisolariciresinol comprised most part of the lignans. The main sources of lignans were legumes and seeds (49 % of total intake) and cereals (22 %). Also berries (12%), fruit (6%) and vegetables (5%) contributed to the total intake. Rye products were the main source of matairesinol in the diet (79 % of total matairesinol).

## CONCLUSIONS

The range in lignan intake in Finland is large. Women seem to have a higher lignan density in their diet than men. Lignan intake can be considered as a marker of a recommended healthy diet.

# CORRELATION BETWEEN PERCEIVED FLAVOUR AND VOLATILE COMPOUNDS OF PROCESSED OAT

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Although oat is perceived as a healthy and tasty cereal, its bitter off-flavour and tendency to rancidity limits its use. In addition of using oat in cereals and flaked products, entirely new food applications could be developed through tailoring of its sensory properties. Germination, used to date mostly for barley, is a well-known process for intensifying both the colour and flavour of grain products. In this investigation it was specified how and why the sensory profile of oat is altered by the germination process followed by heat-treatment.

The sensory profile of oat changed significantly during the germination, when two oat cultivars, Veli and Lisbeth, were studied. The native grain was cereal-like, tough and hard, whereas the germinated, undried grain had a moist, musty and earthy flavour and a soft, moist texture. The most salient sensory attributes for the processed oat were a roasted odour and flavour, a sweet taste, an intense odour and aftertaste, and a hard, crisp and brittle texture ( $p < 0.05$ ). High temperatures exceeding 85°C, were necessary for the formation of the sensory attributes described above, and quick drying after germination resulted in higher attribute intensities of the favourable sensory descriptors.

The identified volatile compounds responsible for odour of oat were dimethyl sulphide, hexanal, pentanal and isobutanal. The relative amount of dimethyl sulphide increased as a function of temperature in drying, whereas hexanal, pentanal and isobutanal disappeared during heating. Several of the identified volatile compounds correlated significantly with the sensory attributes evaluated.

The germinated oat dried at high temperatures was perceived as being roasted, sweet and nutty, and it was clearly related to dimethyl sulphides and isobutanol when sensory and instrumental profiles of selected volatile compounds were analysed using statistical multivariate techniques (PLS). A moist and earthy odour correlated with cymene, limonene and isobutanal. In addition, phenolic compounds significantly influenced the oat flavour.

The composition of volatile compounds obtained is dependent on the heat-treatment used. For example in Maillard reaction, which requires a high

temperature and a dry atmosphere to occur, heterocyclic products, such as pyrazines, pyrroles and furans, mainly affect the roasted flavour. As a consequence of relatively low temperatures and high moisture levels in this study, detectable amount of these compounds were not formed although a roasted odour and flavour developed.

# POSITIVE HEALTH AND TECHNOLOGY ATTITUDES AS PROMOTERS OF ACCEPTANCE OF NEW FOOD PRODUCTS

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The aim of this study was to examine how consumers' background attitudes towards healthy eating and towards the use of new technology in food processing influence their acceptance of new foods in which these new technologies have been used.

Two types of products were examined: one everyday product (wholemeal roll) with a healthy image, and the other a delicacy product eaten occasionally (strawberry jam). One conventional and two new versions of both product types were included.

The data were collected by two questionnaires, which consumers filled in before and after a short home use period. In the first session, the 200 participants tasted the products. After this blind tasting they were informed about the production technologies used (use of enzymes, high-pressure technology) and their implications for the nutritional quality (high fibre, vitamins) of the products. A subsequent questionnaire inquired purchase intents, attitude measures, such as consumer's health orientation and attitudes towards food technology. These measurements were replicated after two weeks period of home use, with the exception that the tasting was not blind. The final sample consisted of 183 subjects.

Consumers' positive attitudes towards healthy eating and food technology promoted especially the acceptance of products, which were relevant for these motivational bases. High health interest was related to higher intentions to purchase high-fibre rolls, and positive technology attitudes to higher intentions to purchase "high-pressure" jams. Whereas, in case of conventional products, the background attitudes did not relate to the purchase intentions. Home exposure did not significantly change the intentions to purchase the products, except slightly in case of one of the 6 product alternatives.

# IMPACT OF ORGANIC ACIDS IN WHOLE GRAIN BREAD PRODUCTS ON THE GLYCAEMIC AND INSULINAEMIC RESPONSES IN HEALTHY SUBJECTS

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During the last 10 years an important number of studies have identified a low GI diet as beneficial in relation to the insulin-resistance syndrome. Several semi-long term dietary interventions are at hand in healthy subjects and in subjects with metabolic disease. With few exceptions, these studies have shown that a low GI diet, not only improves certain metabolic ramifications of insulin resistance, but also reduce insulin resistance *per se*.

Several food factors have been identified and could thus be used as tools to optimise the GI of food products. Some food factors are related to the raw material, and others to the choice of food process and processing conditions. In case of dietary fibre, it has been shown that a high content is not a prerequisite for low GI properties, and the naturally occurring levels of viscous fibre in common cereals have but marginal impact on glycaemia. Whole meal cereal products thus produce equally high GI's as white bread. However, when exchanging flour for 80 % intact wheat, rye or barley kernels, it was possible to lower the GI of bread products (49–73). Consequently, an intact botanical structure plays an important role in the digestive tract, acting as an enzymatic barrier protecting starch from degradation.

Another food factor of importance is organic acids. It has been established that the presence of certain organic acids like e.g. those produced upon sourdough fermentation, may reduce glycaemia. The physiological mechanisms for the acute effects appear to vary, and whereas lactic acid lowers the rate of starch digestion in bread, acetic and propionic acids instead prolong gastric emptying rate.

In a recent study the effect of organic acids on glycaemia and insulinaemia in healthy subjects was further investigated. Lactic acid, produced during sourdough fermentation was added, in two concentrations to kernel and flour based rye breads, respectively (final pH 4.2 and 4.8). The two lactic acid fermented kernel based breads lowered GI and II with 40 % ( $p < 0.05$ ), respectively. Consequently, the higher concentration of lactic acid did not further

improve the metabolic responses. Although not statistically significant, the lactic acid fermented flour based breads tended to reduce the glycaemia (- 18 %). However, an important lowering of the II (-30 %,  $p < 0.05$ ) was seen, showing a beneficial effect of lactic acid in flour based bread.

In order to investigate a possible additive effect of a mixture of lactic and acetic acid, two bread meals dressed with vinegar were served. One of the bread products was sourdough fermented and thus contained lactic acid, while the other bread was baked without sourdough. Both types of bread were soaked in a sauce containing vinegar before ingestion. It could be concluded that the addition of acetic acid to a lactic acid containing bread meal, did not further lower the glycaemic response.

In addition to the acute effects of organic acids on the metabolic responses, a possible second meal effect of a lactic acid containing barley bread was evaluated. The bread containing lactic acid and a reference bread, respectively, were served as a breakfast to healthy subjects. Four hours after the breakfast, a standardised high GI lunch was served to the subjects. The glucose and insulin levels after the lunch meal were significantly lowered when preceded by a lactic acid containing bread, compared with the reference bread.

It is concluded that both the acute and second meal glycaemia and insulinaemia to whole meal bread products, can be reduced by addition of organic acids. In order to further extend the knowledge about metabolic effects of organic acids, long term studies will be required.

# NUTRITIONAL QUALITY OF ANCIENT WHEAT: CHOICE OF BAKING PROCESSES IS AN IMPORTANT DETERMINANT FOR POSTPRANDIAL METABOLIC RESPONSES IN HEALTHY MEN

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## INTRODUCTION

Consumers claim that the nutritional quality and appetite sensations of bread products made from ancient wheat Einkorn (*Triticum monococcum*) are different from bread produced from modern wheat varieties. However, no scientific evidence for these claims has been reported.

## OBJECTIVE

To evaluate the nutritional quality of bread baked from Einkorn (*Triticum monococcum*) compared to bread made from modern wheat. The evaluation is based on measurements of the postprandial insulinaemic and glycaemic response as well as the gastrointestinal hormonal response of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) in healthy subjects.

## MATERIAL AND METHODS

Eleven healthy men (age  $25 \pm 2$ , BMI  $23 \pm 4$ ) participated in the study. Three processing methods applicable to production of einkorn breads were used: A) Honey-salt fermentation for 17 hours and baking for 5 hours at  $150^{\circ}\text{C}$ , B) Soaking of the grains for 12 hours followed by grinding and baking for 2.5 hours at  $150^{\circ}\text{C}$ , and C) Yeast fermentation using modern conventional procedure of raising for 1 hour and baking for  $\frac{1}{2}$  hour at  $220^{\circ}\text{C}$ . A reference bread was made from modern wheat using the same processing method as C. The four different breads were served in random order in portions of 50 g of available carbohydrates together with 250 ml of water to the participants with a wash out period of one week. Before and up to 180 minutes after the meal blood samples were collected for analyses of insulin, glucose, GIP and GLP-1.

## RESULTS

When compared to the yeast fermented breads made from either Einkorn or modern wheat, the postprandial GIP response calculated as incremental area under curve (iAUC) was significantly ( $p < 0.001$ ) reduced for the Einkorn bread processed by Honey-salt fermentation and by using Whole grain bread. No significant differences between breads were found in the postprandial responses of GLP-1, insulin or glucose.

## CONCLUSIONS

The postprandial response of the gastrointestinal hormone GIP differed significantly between breads processed in different ways. This finding should be explored further with respect to the prevention of the metabolic syndrome.

# GLYCAEMIC AND LIPIDEMIC RESPONSE TO DICOCCUM WHEAT (*Triticum dicoccum*) IN THE DIET OF DIABETIC PATIENTS

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## OBJECTIVES

To evaluate the glycaemic and lipidemic response to incorporation of dietary fibre rich dicoccum whole wheat flour in the diet of diabetic patients in comparison with common bread wheat flour.

## METHODOLOGY

### Selection of subjects

Sixteen non insulin dependent diabetic mellitus (NIDDM) subjects, taking oral hypoglycemic drugs and six insulin dependent diabetic mellitus (IDDM) subjects inclusive of both genders belonging to 40–70 years were selected purposively for the study from Diabetes Specialities Center, Hubli. Approval of the study was given by the Ethics Committee of the University.

### Dietary modification

Subjects were informed to collect the whole wheat flour in the beginning and once in 2 weeks and asked to include around 200–300g of flour daily in any two meals (breakfast/lunch/supper) in the form of unleavened bread for six weeks. Common bread wheat flour was supplied to nine subjects as control group and dicoccum wheat flour to thirteen subjects as test group.

### Analysis of biochemical parameters

The selected subjects were requested to report the clinic four times with two weeks interval, in the morning after 12hrs of fasting. On the first day and once in two weeks the fasting and post prandial (after 2hrs) blood was drawn and analyzed for glucose by using enzymatic diagnostic kit (Span Diagnostic Ltd., India). Fasting blood drawn on the first and last day of the experiment was also analyzed for total cholesterol, triglycerides and HDL-C by using diagnostic kit (Span Diagnostic Ltd., India). LDL-C was estimated using the formula. For statistical analysis, Student's t-test for paired data was carried out.

## RESULTS

The incorporation of dietary fiber rich dicoccum whole wheat flour (200–300g /day) in the regular diet of 13 diabetic subjects for 45 days reduced significantly the fasting blood glucose level ( $P \leq .01$ ). Similarly there was significant decrease in the lipid profile of total cholesterol ( $P \leq .01$ ), triglycerides ( $P \leq .01$ ) and LDL-C ( $P \leq .05$ ). However the incorporation of common bread wheat flour in the diet of 9 diabetic subjects did not show any significant variation in glycemic and lipid profile.

## CONCLUSION

Dietary fiber rich dicoccum wheat can reduce effectively the cardiovascular risk factors contributing to morbidity and mortality in diabetes.

# CONSUMPTION OF HIGH-FIBER RYE BREAD IMPROVES INSULIN SECRETION BUT NOT INSULIN SENSITIVITY OR GLUCOSE EFFECTIVENESS IN POSTMENOPAUSAL WOMEN

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It has been shown that the modification of carbohydrate intake affects the postprandial glucose and/or insulin responses, but little is known about the longer-term effects of different cereal products on insulin secretion and sensitivity and glucose effectiveness in peripheral tissues. In the present study the intake of carbohydrates was modified by replacing almost all bread cereals in the diet with high-fiber rye and white wheat breads in a randomized crossover trial. The subjects were 20 postmenopausal women,  $59 \pm 6.0$  years old, BMI  $27.5 \pm 2.9$  kg/m<sup>2</sup> and baseline fS-cholesterol  $6.5 \pm 0.8$  mmol/L. Three of the subjects had impaired glucose tolerance as determined by a 2-hour oral glucose tolerance test. After a 2–3-week run-in period the women were divided into two groups and advised to consume either rye bread or wheat bread for 8 weeks. After an 8-week wash-out period, the groups were reversed. Fasting blood samples were collected for the determination of plasma glucose and insulin at the beginning and at the end of both bread periods. The frequently sampled intravenous glucose tolerance test was performed at the run-in and at the end of both bread periods. Acute insulin response (AIR) was calculated from the area under the insulin curve above the baseline level from 0 to 10 minutes. Insulin sensitivity ( $S_I$ ) and glucose effectiveness ( $S_G$ ) were calculated with the Minmod program (Pacini et al., 1986).

There were no differences in body weight, intake of energy or reported frequency of exercise among the run-in and bread periods. The rye bread made up  $23.4 \pm 4.3$  % and wheat bread  $26.7 \pm 8.2$  % from the total intake of energy. The intake of fiber from the breads was  $35.5 \pm 7.3$  g and  $4.7 \pm 1.0$  g during the rye and wheat bread periods, respectively. The analysis of proportional changes in AIR during the rye bread period compared to run-in ( $9.9 \pm 24.2\%$ ) and during the wheat bread period compared to run-in ( $2.8 \pm 36.3\%$ ) tended to be significantly different ( $P = 0.062$ ). In pairwise comparisons only the consumption of rye bread increased AIR ( $P = 0.033$ ). The consumption of wheat bread did not affect any of the variables studied. The present study indicates that modification of the

carbohydrate intake by high-fiber rye bread may improve acute insulin secretion, but does not alter peripheral insulin sensitivity, glucose effectiveness or fasting concentrations of plasma glucose and insulin in postmenopausal hypercholesterolemic women.

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## THE INTAKE OF RYE IS DIRECTLY RELATED TO INSULIN SENSITIVITY AMONG NON-DIABETIC RELATIVES OF SUBJECTS WITH TYPE 2 DIABETES - THE BOTNIA DIETARY STUDY

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The Botnia Dietary Study investigates relationships between diet and disorders of glucose metabolism in a high risk population for type 2 diabetes. Here we present cross-sectional relations of cereal intake to insulin sensitivity. Of the invited 746 first- or second-degree relatives of subjects with type 2 diabetes, aged 20 to 75 yrs, and earlier diagnosed as having normal or impaired glucose tolerance (WHO 1985), 555 (74 %) completed two 3-day estimated food records, 5 to 11 months apart, and had data on clinical variables. The mean (SD) age and body mass index of men (N = 250) was 50.0 (10.2) yrs and 26.5 (3.0) kg/m<sup>2</sup> and those of women (N = 305) 51.4 (10.4) yrs and 26.3 (4.0) kg/m<sup>2</sup>, respectively. Cereal intake, calculated as the mean of six recording days, was divided into three groups: rye, wheat, and other cereals; the mean (SD) daily intakes of which were 42 (26), 105 (44), and 22 (19) g, respectively. Insulin sensitivity was described by the insulin sensitivity index based on glucose and insulin concentrations during an oral glucose tolerance test (Matsuda and DeFronzo 1999).

In linear regression models, adjusting for age, body mass index, waist-to-hip ratio, physical activity, length of education, and fasting and 2-h plasma glucose concentration, both in men and women, the energy-adjusted intake of rye was directly related to insulin sensitivity: regression coefficient (SE) in men 0.14 (0.05), P = 0.01 and in women 0.14 (0.04), P < 0.01. The intakes of wheat and other cereals were not associated with insulin sensitivity.

The results support the notion that rye intake is associated with insulin sensitivity. Thus, the possible role of rye in the development and prevention of type 2 diabetes deserves further research.

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# SUITABILITY OF DICOCCUM WHEAT (*Triticum dicoccum*) BASED PASTA AS CARBOHYDRATE LOADING FOR LONG DISTANCE RUNNERS

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## OBJECTIVE

To evaluate the carbohydrate profile of dicoccum wheat pasta product and its suitability as carbohydrate loading for long distance runners in comparison with durum wheat.

## METHODOLOGY

### Procurement of Sample

Commercially available pasta making durum wheat semolina was collected in a single lot from the local market. The commercial dicoccum wheat DDK-1001 was procured from wheat laboratory, University of Agricultural Sciences, Dharwad and semolina was prepared at commercial mill.

### Preparation of pasta product

Durum and dicoccum vermicelli were prepared by extruding the dough in a commercial vermicelli machine (Star company, Belgaum, with a capacity of 1-10kg), sun dried and stored in air tight aluminium box.

### Nutritional profile of pasta product

Wheat vermicelli was pulverized in a laboratory model wiley mill and the whole meal was analyzed for proximate composition by standard AOAC procedures (AOAC,1985). Total sugars were estimated by Nelson Somogyis method (Hawk et al. 1965). Starch was analyzed according to the method of Bhasarkar and Srinivasan (1997). Total and soluble amylose was determined according to the method of Sowbhagya and Bhattacharya (1979). The total soluble and insoluble dietary fiber contents were estimated by enzymatic method ( Asp et al., 1983).

## Evaluation of pasta product as carbohydrate loading for long distance runners

The trained athletes of 16–20 yrs including male and female long distance runners (1,500–10,000mts) were selected for the study. The subjects daily intake of energy, protein and fat from three day food record were computed and calculated for the percentage of carbohydrate, protein and fat. The total carbohydrate was up to 70 per cent with addition of test foods (dicocum and durum vermicelli) by modifying the normal diet. Carbohydrate loading was followed as per the procedure of Karlson and Saltin (1971). The subjects consumed a normal diet for the first three days of the week. For the next three days, the test foods were given as breakfast and snack along with their habitual diet. During these three days, the subjects were asked to taper down their regular workouts to a mild jog and on the seventh day the performance was tested. Two hundred gram raw vermicelli of durum and dicocum wheat given in the form of *uppuma* was used as test food for carbohydrate loading. Endurance capacity of five subjects each was tested by running on the track and by cycling (Hero Alegro cycle) in the laboratory. In both the experiments, the subjects performed three trials and each trial was separated by one week apart. The test foods were given along with their routine diet, which was control trial in a random order. After the subjects completed each trial, their time to exhaustion and distance covered was recorded. Data from these trials were compared using one way analysis of variance for repeated measures.

## RESULTS

Proximate composition of dicocum and durum wheat vermicelli varied significantly. Commercially available durum wheat vermicelli had protein content of 10.0% which was lower as compared to dicocum wheat (12.80%). The dicocum vermicelli was low in fat (1.88%), ash (1.49%) and moisture content (10.67%) as compared to durum (2.24%, 1.51% and 11.84% respectively). The carbohydrate profile of vermicelli revealed that the total carbohydrate content in durum wheat (74.31g/100g) was higher than in dicocum (73.21g/100g). Higher content of sugars, starch and amylopectin was also observed in durum vermicelli than dicocum. Dicocum vermicelli had significantly ( $P<.01$ ) higher (8.33g/100g) total dietary fiber content compared to durum (3.87g/100g).

The mean exercise time to fatigue in durum (112.2 min) and dicocum (119.0 min) trials was significantly higher than control trial (81.4min) in running. The mean running time to exhaustion in dicocum vermicelli fed trial was 7 per cent more than durum vermicelli trial. The mean exercise time to fatigue during cycling in durum (59.0 min) and dicocum (68.0 min) vermicelli trial was significantly higher compared to control trial (49.0 min). The mean riding time to

exhaustion in dicoccum vermicelli fed trial was 18 per cent more compared to durum wheat trial. The mean distance covered during running in durum (22.20 km) and dicoccum (25.40 km) vermicelli fed trial was significantly higher compared to control trial (16.96 km) and the mean distance covered during cycling was 22.32 km and 24.20 km in durum and dicoccum vermicelli fed trials respectively, which was significantly higher compared to control trial (18.18 km). The mean distance covered in dicoccum wheat vermicelli trial was 18.9 per cent more in running and 10.4 per cent more in cycling compared to durum vermicelli trial.

## CONCLUSION

The dicoccum pasta product rich in protein and complex carbohydrate can be recommended as a health food for athletes and there is a scope for developing value added products from dicoccum wheat.

# IMPACT OF WHEAT SOLUBLE FIBER ON THE LIPID METABOLISM

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Many works have shown the importance of soluble fibers, like pectine, guar gum, psyllium, to increase the steroids excretion and induce an hypocholesterolemic effect (Moundras et al., 1997). Other cereal fibers found in oat, barley and rye are recognized as effective agents on digestive fermentation and lipid metabolism (Kahlon et al., 1990), whereas the effectiveness of whole wheat flour is still discussed. The aim of our study was to show that a wheat variety, suitable for bread-making, with a high specific viscosity could have these properties.

To address this question, cecal digestion, short chain fatty acids (SCFA) metabolism and cholesterol metabolism were thus investigated in four groups of rats adapted to semi-purified diets differing in their carbohydrate sources: a control diet (purified wheat starch) and three whole cereal flour diets (Valoris wheat, Soissons wheat and Carnac triticale). Valoris is particularly viscous and Carnac is more rich in hemicellulose than wheat.

Rats fed with the whole cereal diets showed an hypertrophy of the caecal wall and a moderate acidification of the bulk pH (about 6.4) proving a strong fermentation activity due to the presence of fermentable and soluble fibers (Favier et al., 1995; Younes et al., 1995). These products of fermentation in volatile fatty acids form have caused an increase of the intestinal pool of acetate, propionate and butyrate, with a molar ratio reflecting propionic/butyric acid-rich fermentations. We observed as well that the more the flour presented strong viscous properties, the more the plasma cholesterol and triglyceride levels decreased. It was the same for the liver cholesterol and triglycerides concentrations. An analysis of the lipoproteins has showed that only the VLDL cholesterol cut down although the HDL cholesterol was not altered. The fecal excretion of steroids increased also with the viscosity of the flour, limiting in this way the intestinal cholesterol re-absorption. These results indicate that whole wheat flours can cause striking changes in caecal short chain fatty acids, especially butyrate, and are effective cholesterol-lowering agents.

In prospect, it would be interesting to find the same properties with a bread-making product, made with the same flour. It would be also noticeable to observe a positive impact on the glycemic response.

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# WATER SOLUBLE AND INSOLUBLE FRACTIONS OF RYE BRAN HAVE DISTINCT EFFECTS ON ADENOMA GROWTH IN MIN MICE

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Rye is a promising candidate for anticarcinogenic components in human diet. Two recent studies using different animal models both suggest that rye bran may contain substances which prevent intestinal tumour formation in animals (Davies et al. *Carcinogenesis* 1999;20:927–931; Mutanen et al. *Carcinogenesis* 2000;21:1167–1173). In this study, we wanted to see if there exist differences between whole bran and water soluble (WS) and water insoluble (WIS) fractions of rye bran on intestinal adenoma formation in adenomatous polyposis coli (APC) knockout mice. These mice spontaneously develop multiple intestinal neoplasia (Min). Male heterozygous Min mice (n = 11-12/group) were fed one of the following high-fat AIN93G-based diets for five to six weeks: a casein based diet with 10% (w/w) rye bran, or 7.9% (w/w) WS or WIS fractions of rye bran. This way all three diets had the same amount of undigestible components (4.14%, w/w). In rye bran and in the WIS fraction dietary fiber and fructan contents were 34.3/7.1% and 51.1/1.2%, respectively. The WS fraction was prepared using xylanase treatment and it contained mainly fructan, pentosane-oligosaccharides, and beta-glucan (21.2/26.7/4.4%). The fat (40% of energy) used in the diets was a mixture of butter, rapeseed oil, and sunflower seed oil. The intake of fatty acids corresponded to that in the Western type diet.

The rye bran, WIS and WS diets produced similar number of adenomas in the small intestine (37±28; 38±34; 38±30, mean±SD) and in the colon+caecum (0.8±1.0; 1.0±1.1; 0.7±0.6). Opposite effect between WIS and WS diets were, however, seen on adenoma growth when comparing duodenum and the distal small intestine. In the duodenum the mean size of adenomas were 2.22±0.35 mm and 1.42±0.70 mm after the WIS and WS diets (p < 0.05, Mann-Whitney) while the corresponding values in the distal part of the small intestine were 0.99±0.21 mm and 1.20±0.17 mm (p < 0.05). The number of large (>1.5 mm) adenomas either in the duodenum or in the distal small intestine supported these results; in the duodenum WIS diet significantly increased the number of large adenomas (% of total) when compared with the whole rye bran (35±13% vs. 17±17%,

$p < 0.05$ ), while it decreased the number of large adenomas in the distal small intestine ( $1.0 \pm 0.4\%$  vs.  $17 \pm 14\%$ ,  $p = 0.052$ ).

The results suggest that both WIS and WS fractions of rye bran probably contain substances which when separated from whole bran may, depending on the site of the intestine, have either favourable or unfavourable effects on the growth of adenomas in Min mice.

# AN INCIDENT CASE REFERENT STUDY ON PLASMA ENTEROLACTONE AND BREAST CANCER RISK

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## OBJECTIVE

Using a nested case-referent design, we evaluated the relationship between plasma levels of the lignan enterolactone and the risk of developing breast cancer.

## METHODS

167 cases and 328 referents were selected from the population-based cohorts in northern Sweden. Blood samples were donated at enrolment. All blood samples were stored at  $-80^{\circ}\text{C}$ . Cases and referents were matched for age, date of blood sample and sampling centre. Breast cancer cases were identified through the regional and national cancer registries.

## RESULTS

Plasma enterolactone was lower among smokers and subjects with BMI  $< 23$  and BMI  $> 28$ . Low plasma concentrations of enterolactone, below the 12.5<sup>th</sup> percentile, were significantly associated with an increased risk of breast cancer. Also, high values of plasma enterolactone, above the 87.5<sup>th</sup> percentile were non-significantly associated with an increased breast cancer risk. Pre-menopausal women mainly represented the increased breast cancer risk associated with high enterolactone levels.

## CONCLUSION

Low plasma concentrations of enterolactone were associated with a significant increased breast cancer risk. A tendency of an increased risk was seen also with very high plasma enterolactone values particularly among pre-menopausal women.



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## Whole Grain and Human Health

Abstract

Intake of whole grains has in many studies been associated with health benefits, including improved regulation of blood glucose levels, decreased risk of coronary heart disease and different cancers. Researchers have found evidence that increased consumption of whole grains has great potential in improving public health. Important compounds and especially mechanisms behind the health benefits are yet only partly understood.

The intake of whole grain products, *ie.* foods containing all the parts of a grain, is inadequate. Wheat and rice are usually refined so that the outer layers rich in bioactive compounds are removed. Northern Europe is unique in having traditions of the use of whole grain rye breads and foods. There is a distinct use of whole grain oats and barley as well, but the use of these grains is minimal in comparison to their cultivation. Considering consumers' increasing interest in the health values of foods, it is a challenge and opportunity for the food industry to develop new, palatable whole grain foods.

This symposium features the latest results about the physiological effects of whole grains and their role in improving human health. It also reviews new types of food processing targeted for whole grain products, and discusses the role of health claims in promoting their use. About 70 contributions from 5 continents give a good picture of the prospects and potential of health promotion by whole grains all over the world. Thanks for the participants for their contribution to the scientific program.

Keywords

meetings, food, whole grains, physiological effects, human health, diseases, dietary fibres, cereals, diabetes, cardiovascular diseases

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