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Cereal Dietary Fibre

Physicochemical Properties and Suitability for Addition
to Low-Fat Meat Products

Karin Petersson

2012



LUND
UNIVERSITY

Department of Food Technology, Engineering and Nutrition
Faculty of Engineering
Lund University, Sweden

Cereal Dietary Fibre - Physicochemical Properties and Suitability for Addition to
Low-Fat Meat Products

Doctoral thesis

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Abstract

It is well known that dietary fibre is good for the health. Cereals, and in particular the outer parts of the cereal kernels, are rich in dietary fibre. Rye bran, wheat bran, oat bran and barley fibre have been investigated regarding their suitability as additives in low-fat meat products. Two types of meat products, frankfurter-type sausages and meatballs have been evaluated in this thesis. In the sausages the meat protein network governs the texture and water-holding properties, whereas the meatballs have a more particulate structure, where a more crumble texture is perceived and the water-holding capacity is lower.

Cereal bran particles can be difficult to add to a food-system without also adding a disagreeable sensation of grittiness. Evaluations of how the size and concentration of small rye bran particles in a continuous phase of varying viscosity influence the sensory perception of grittiness were performed. The threshold for the sensation of grittiness resulting from rye bran particles in a starch gel was very low and independent of particle size. The particles were detected already at concentrations of 0.1-0.3%, despite the fact that they were so small (20-180 μm). The rheological properties of the suspension medium did not influence the detection threshold. One reason for this obvious perception of grittiness of rye bran particles could be their irregular shape and hardness.

One way of altering these adverse properties of the bran particles could be by treating them with hydrolytic enzymes. The xylanases and endoglucanases evaluated in this thesis did not increase the water-holding capacity of the rye or wheat bran or the viscosity of the aqueous phase. The water-holding capacity was probably reduced because of the lower amount of insoluble material after solubilization by the enzymes, while the lower viscosity showed that the solubilized dietary fibre were degraded into smaller molecules which could no longer contribute to the viscosity.

Due to the gelling ability of the inherent β -glucan of the oat bran upon heating, it was found to be the most suitable for addition to low-fat sausages. These sausages exhibited low process and frying losses, together with high values of both firmness and sensory acceptance. The barley fibre gave a poor texture of the sausages and was the least preferred. This barley β -glucan could not form a gel, probably because of a smaller molecular weight and a less favourable structure compared to the oat β -glucan. Rye bran is suitable for addition to meatballs, probably due to its particulate nature, which is more acceptable in this type of meat product, where the gelling properties are not as important as in sausages.

Populärvetenskaplig sammanfattning

Kostfiber kallas de kolhydrater från växtriket som inte bryts ner under matsmältningen och därför når tjocktarmen i stort sett opåverkad. Kostfiber finns till exempel i våra fyra sädeslag, havre, råg, korn och vete, vilka alla är cerealier. Framför allt är det de yttre delarna av cerealiekärnorna, klidelen, som innehåller mycket kostfiber. I cerealier är de vanligaste kostfiberna arabinoxylan, betaglukaner och cellulosa. Dessa kan ha olika egenskaper beroende på hur stora molekylerna är och om de är lösliga i vatten eller inte. Kostfiber har flera hälsosamma egenskaper och livsmedelsverket rekommenderar att vuxna ska få i sig 25-35 gram per dag. De flesta får dock inte i sig dessa mängder. Ett sätt att öka kostfiberintaget är att berika vanliga livsmedel med mer kostfiber. Det många av oss däremot får i oss för mycket av är fett. Genom att minska fetthalten och samtidigt öka fiberinnehållet i produkter som korv och köttbullar kan dessa produkter, som många äter mycket av, bli nyttigare.

För att livsmedel med mer kostfiber ska kunna accepteras av konsumenter bör varken smak eller konsistens av produkten förändras för mycket. Oavsett hur hälsosam en livsmedelsprodukt anses vara är det inte troligt att den kommer att bli särskilt väl mottagen om den inte smakar bra. Syftet med denna studie har därför varit att undersöka hur man kan påverka kostfibers egenskaper och hur dessa bör vara för att köttprodukter ska kunna berikas med mer kostfiber och ändå inte skilja sig allt för mycket från de produkter vi är vana vid.

Rågkli innehåller framför allt olösliga kostfiber. När dessa tillsätts i livsmedel är risken att de upplevs som en otrevlig sandighet i munnen. För att undersöka hur storleken av rågpartiklarna påverkar hur tydligt denna sandighet känns, fick en grupp människor smaka rågpartiklar av olika storlekar och koncentrationer. Resultatet visade att vi känner av partiklar väldigt tydligt även om dessa är så små som 20 μm och vid en så låg koncentration som 0.2%. Rågpartiklarna var blandade i saftkräm, gjord på olika koncentrationer av stärkelse och därmed med en varierande konsistens. Det visade sig att detta inte påverkade vid vilken koncentration av partiklarna sandigheten började kännas.

Enzymer är proteiner som kan påskynda en kemisk reaktion. Det finns enzymer som gör att kostfiber bryts ner till mindre molekyler. Detta kan leda till att arabinoxylaner eller betaglukaner som tidigare inte var lösliga i vatten, efter enzymbehandling nu blir det. Detta i sin tur kan göra att viktiga egenskaper förändras. En undersökning gjordes för att ta reda på om behandling med enzymer kunde vara en metod som gynnade tillsättningen av rågkli och vetekli till

livsmedel. Flera olika enzymer testades och förändringar i kostfiberinnehåll, vattenhållande förmåga, konsistens och partikelstorlek hos råg och vetekli undersöktes. Ingen av enzymerna som användes i studien hade någon positiv effekt vad gällde den vattenhållande förmågan. Effekterna av till exempel hur mycket kostfiber som lösliggjordes var dock olika för enzymerna och studien gav viktig kuskap om enzymernas egenskaper. Rågkli som behandlats med tre av dessa enzymer testades senare att användas som fibertillsats i korv och köttbullar.

Det slutliga målet var att tillverka korv och köttbullar med sänkt fetthalt och med tillsatt fiber. Korv och köttbullar skiljer sig åt på så sätt att ingredienserna i korv är mer finfördelade och en högre salthalt används, vilket leder till ett tätare nätverk mellan köttproteinerna. Köttbullar däremot har en mer partikular struktur där proteinnätverket inte dominerar lika mycket. Köttbullar och korv innehåller vanligtvis mycket fett och när fetthalten i en köttprodukt sänks kan flera problem uppstå. I korv av typen falukorv, förstärker fett det köttproteinnätverk som bildas under uppvärmning och som ger produkten dess konsistens och är avgörande för hur väl korven kan hålla kvar vatten och fett under tillagning. För att kunna göra en rättvis jämförelse mellan de olika cerealietillsatserna med avseende på kostfiberinnehållet hölls denna faktor konstant vid 1%. De flesta andra parametrar som påverkar konsistens och vattenhållande förmåga i köttprodukterna hölls också konstanta, som kvoten mellan vatten och köttproteiner och mängden stärkelse.

Rågkli som hade blivit behandlat på olika sätt tillsattes till korv och köttbullar och egenskaper som fasthet, vattenhållande förmåga och smak bedömdes. Rågkli som hade behandlats med de olika enzymerna visade sig ge en positiv effekt när de tillsattes i korv, jämfört med tillsats av rågkli utan någon behandling. Det visade sig dock att det alternativ som var allra bäst var när rågkli behandlats på liknande sätt, i 50 gradigt vatten i 4 timmar, fast utan några tillsatta enzymer. Troligtvis gynnade denna behandling de enzymer som naturligt finns i rågkli och de gjorde kostfibern mer vattenlöslig, samtidigt som den svällde och var på så sätt bättre på att hålla kvar vatten i köttprodukterna. Enzymerna som hade tillsats gjorde kostfibern också mer vattenlöslig, men var aningen för effektiva och bröt ner kostfiberna till för små molekyler som inte var lika bra på att förbättra konsistens eller vattenhållande förmåga hos köttprodukterna.

Havrekli, rågkli och kornfiber tillsattes i korv och köttbullar med sänkt fetthalt. Havrekli var det alternativ som passade bäst i en lågfettskorv. Dessa korvar tyckte smakpanelen smakade bäst och de hade en fastare konsistens än korvarna med rågkli eller kornfiber. Dessa korvar förlorade inte heller så mycket vatten när de stektes. Korvarna med havrekli var jämförbara med referenskorvar (med samma fett och stärkelsehalt men utan tillsatt havrekli) med avseende på stekförluster, smak och konsistens. När konsistensen hos en uppslammning av endast vatten,

potatisstärkelse och respektive cerealie undersöktes, visade det sig att havrekli kunde bilda en gel vid uppvärmning och gav en betydligt fastare konsistens än kornfiber, vilket gav förklaringen till de bättre korvarna. Kornfibern innehöll mycket lösligt betaglukan, men det visade sig att denna höga koncentration hade en negativ inverkan på korvarnas konsistens.

Råekli passade bättre som tillsats i köttbullar. Anledningen var troligtvis att partiklar av råekli passade bättre in i köttbullarnas köttstruktur som är av mer partikulär natur än korv, och förmågan att bilda gel och på så sätt påverka konsistensen är inte lika viktig för denna produkt.

List of papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.

I. Fibre content and composition – a comparison of a variety of dietary fibre sources and their characteristics

Petersson, K., Bengtsson, H., Nyman, M., Eliasson, A-C. and Tornberg, E.
Manuscript

II. Sensory perception of rye bran particles of varying size and concentration in a viscous phase

Petersson, K., Eliasson, A-C., Tornberg, E. and Bergenståhl, B.
Submitted for publication in Journal of Texture Studies August 2012

III. Impact of cell-wall-degrading enzymes on water-holding capacity and solubility of dietary fibre in rye and wheat bran

Petersson, K., Nordlund, E., Tornberg, E., Eliasson, A-C. and Buchert, J.
Journal of the Science of Food and Agriculture (2012), In press,
doi: 10.1002/jsfa.5816

IV. The effect of adding rye bran, non- and enzyme treated, in low-fat sausages and meatballs

Petersson, K., Godard, O., Eliasson, A-C. and Tornberg, E.
Submitted for publication in Meat Science June 2012

V. The effect of adding rye bran, oat bran and barley fibre to low-fat sausages and meatballs

Petersson, K., Godard, O., Eliasson, A-C. and Tornberg, E.
Submitted for publication in Meat Science June 2012

The author's contribution to the papers

- I.** K. Petersson designed the study together with the co-workers, performed the experimental work regarding the cereal materials, evaluated the results and wrote the paper in collaboration with H. Bengtsson.
- II.** K. Petersson designed the study together with the co-workers, performed the experimental work, evaluated the results and wrote the major part of the paper.
- III.** K. Petersson designed the study together with the co-workers, performed the experimental work, evaluated the results and wrote the major part of the paper.
- IV.** K. Petersson designed the study together with the co-workers, evaluated the results and wrote the major part of the paper.
- V.** K. Petersson designed the study together with the co-workers, evaluated the results and wrote the major part of the paper.

Abbreviations

AX	Arabinoxylan
Ara	Arabinose
DF	Dietary fibre
dm	Dry matter
Gal	Galactose
GC	Gas chromatography
Glu	Glucose
IM	Insoluble material
M _w	Molecular weight
Man	Mannose
PCA	Principal component analysis
PSD	Particle size distribution
Rha	Rhamnose
WHC	Water-holding capacity
W/P	Water/Protein
Xyl	Xylos

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1. Introduction

It is well known that dietary fibre (DF) is good for the health. The recommended daily intake of dietary fibre in Sweden is 25-35 g (Nordic Nutrition Recommendations, 2004). This is however rarely achieved in the populations of the Western world (Gray, 2006). Cereals, and in particular the outer parts of the cereal kernels, the bran, are rich in dietary fibre. The bran materials are by-products from the process of milling flour and new applications for these dietary fibre rich materials are requested.

Comminuted meat products, such as meatballs and sausages normally contain a rather high amount of fat. The task of developing new healthy products without sacrificing a good taste and texture is demanding. There are several challenges when reducing the fat content of a food product, since fat highly contributes to many positive attributes of foods concerning both flavour and texture. A reduced fat content of a meat product is often followed by an increased cooking loss (Cofrades, Hughes, & Troy, 2000; Jiménez-Colmenero, Barreto, Mota, & Carballo, 1995). To prevent this, additives that have the ability to keep the water in the product are needed. These additives should not change the attributes compared to the full-fat food item too much and preferably they should also be received as a positive additive by the consumers, something that add an extra value to the product.

If cereal dietary fibre rich materials could be successfully added to low-fat meat products the profits would be multiple. Not only would the meat product be healthier because of the lower fat content, but also due to increased dietary fibre content. Adding dietary fibre to products, which are frequently eaten, could be one way to get closer to the recommended dietary fibre intake. Furthermore, a by-product could be utilized and even give an added value to the food item.

1.1 Objectives

The main focus with this thesis was to make low-fat meatballs and sausages, with increased dietary fibre content, without sacrificing important quality attributes, such as cooking loss, texture and of course taste. The aim of the studies performed were to achieve a better understanding of the physicochemical properties of cereal dietary fibre and their suitability to be incorporated to comminuted meat products.

Four different dietary fibre rich cereal materials have been used in the studies, namely rye bran, wheat bran, oat bran and barley fibre. These were first characterized in terms of their solubility, dietary fibre content and composition and water-holding capacity. (**Paper I**).

Rye bran is an underutilized material and the one of the four cereal materials that was presumed to be the most challenging in terms of being successfully incorporated into food products. The bran is the part of the rye grain that has the most bitter taste and the strongest flavour and aftertaste, and darkens for example the colour of bread (Heiniö, Liukkonen, Katina, Myllymäki, & Poutanen, 2003). Adding bran particles may also produce an unpleasant feeling of grittiness to the product (Zhang & Moore, 1999). Therefore a lot of effort was made to improve the understanding of this material and to try to improve its potential to be used in foods.

Evaluations of how the size and concentration of small rye bran particles in a continuous phase of varying viscosity influence the sensory perception of grittiness were performed (**Paper II**). The results showed how sensitive we are to small particles in the mouth and that it is difficult to add rye bran particles, regardless of their size, to a system consisting of starch and water without creating a sensation of grittiness/sandiness. One reason for this enhanced perception of grittiness of rye bran particles could be their irregular shape and their hardness.

One way of altering these adverse properties of the rye bran particles could be by treating them with hydrolytic enzymes. Therefore several xylanases and endoglucanases were evaluated on how they could influence the physicochemical properties (water-holding capacity, viscosity, particle size and morphology) and the dietary fibre composition of rye and wheat bran (**Paper III**).

Finally, the suitability of the different cereal materials to be added to low-fat sausages and meatballs were evaluated. Rye bran, non-treated or treated with enzymes, which had been evaluated in **Paper III**, were added to the low-fat meat

products (**Paper IV**). The addition of rye bran, oat bran and barley fibre were also compared and evaluated based on cooking losses, texture and sensory analysis of the two meat products (**Paper V**).

1.2 Dietary fibre

Definition of dietary fibre

The definition of dietary fibre in EU since 2008 is (European Parliament, 2008): “fibre means carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories:

- edible carbohydrate polymers naturally occurring in the food as consumed;
- edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence;
- edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence.”

The definition adopted by the United Nations’ Food and Agriculture Organization and World Health Organization (FAO/WHO), Codex Alimentarius from 2008 is very similar, but does only include the carbohydrate polymers with 10 or more monomers (Codex Alimentarius Commission, 2008).

When discussing the dietary fibre content in this thesis, it is referring to the sum of the monosaccharides analysed by the Uppsala method (Theander, Åman, Westerlund, Andersson, & Pettersson, 1995), which does not include the polysaccharides which are smaller than 10 monomeres.

Traditionally, dietary fibre analysis does not include fructan and the dietary fibre content would be higher if these were included which is now accepted according to the EU definition. Rye whole meal for example contains about 5% fructan (R. Andersson, Fransson, Tietjen, & Åman, 2009).

Health benefits of dietary fibre

Dietary fibre can be classified as either water-soluble or water-insoluble. There are several beneficial physiological effects of dietary fibre which have been suggested.

The health promoting effects that have been associated with the soluble dietary fibres are the attenuation of postprandial plasma glucose and insulin levels and the control of cholesterol (Theuwissen & Mensink, 2008).

Some of the dietary fibres are fermented in the colon by microbiota. This will lead to production of short-chain fatty acids, which have been shown to lower the blood cholesterol level and act protective against colorectal cancer (Wong & Jenkins, 2007). The fermentation also favours the growth of health-promoting bacteria, which have been shown to protect against inflammation and colorectal cancer (Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004).

In May 2012 the European Commission established a new list of permitted health claims made on foods (The European Commission, 2012) and there are a few claims that include the intake of dietary fibre. Two health claims can be used for β -glucans: “Consumption of beta-glucans from oats or barley as part of a meal contributes to the reduction of the blood glucose rise after that meal” and “ β -glucans contribute to the maintenance of normal blood cholesterol levels”. In the first claim, 4 g of beta-glucans from oats or barley for each 30 g of available carbohydrates are needed, and in the second one at least 1 g β -glucan per portion.

One claim is valid for arabinoxylans produced from wheat endosperm: “Consumption of arabinoxylan as part of a meal contributes to a reduction of the blood glucose rise after that meal”. This claim may be used if the food product contains at least 8 g of arabinoxylan rich fibre produced from wheat endosperm (at least 60% AX by weight) per 100 g of available carbohydrates.

For wheat bran, barley grain fibre and oat grain fibre there is also a claim which concern the insoluble fibres: “Contribute to an increase in fecal bulk” and if rye bran is included in the food: “Rye fibre contributes to normal bowel function”. These two claims can be used if the amount of dietary fibre is at least 6 g per 100 g or 3 g per 100 kcal.

1.3 Cereal dietary fibre

The four important cereals grown in Sweden are: wheat, rye, oat and barley. These have all been included in this work. An introduction of them will follow here:

Wheat

Wheat (*Triticum aestivum*) production is widespread globally. It is the most common cereal cultivated in Sweden and covers about 415100 ha (Jordbruksverket, 2012), which correspond to about 16% of the total cultivated area in Sweden and 42% of the total amount of area where cereals are cultivated. Over 2 million ton of wheat was harvested in Sweden 2011.

Rye

Rye (*Secale cereale*) is grown on only about 23 900 ha in Sweden, which in 2011 resulted in a harvest of 127 000 ton (Jordbruksverket, 2012). The consumption of rye is most common in the north-eastern Europe and the Nordic countries, where it is frequently used in rye bread and crisp bread. Rye consumption varies greatly between countries with >35 kg per capita and year in Poland, Belarus, and Estonia, and 10-15 kg per capita and year in Finland, Denmark, Sweden and Germany compared with average world consumption of 1 kg per year (Kamal-Eldin, et al., 2009; Knudsen & Laerke, 2010). Rye is more tolerant to poor growing conditions than wheat and can withstand cold better than the other cereals (Sahlstrom & Knutsen, 2010).

Oat

Oat (*Avena sativa*) is commonly consumed as porridge and breakfast cereals. About 175 600 ha is used for oat in Sweden and 690 000 ton was harvested in 2011 (Jordbruksverket, 2012). There has been an increasing appreciation of oat as a part of our diet due to its positive health effects, mainly contributed by the oat β -glucans. Oat is also an important part of a gluten free diet for people with celiac disease, since it does not contain any gluten protein. Oat contains more lipids compared to the other cereals and is normally heat treated in order to reduce the enzymatic activity of lipase.

Barley

Barley (*Hordeum vulgare*) is a major animal feed crop. Barley is the best suited grain for malting, and is therefore used for beer and whiskey production. Barley is cultivated on 323 000 ha in Sweden and 1 400 000 ton was harvested in 2011 (Jordbruksverket, 2012). Other than in beers and whiskeys, there is no big consumption of barley products. Due to the big interest of the positive health effects by β -glucans, barley β -glucan enrichments of foods have increased.

An illustration of a rye kernel is shown in Figure 1. The bran fraction consists of the pericarp of the outer husk and the aleurone layer and is the most DF rich part of the grain kernel. The aleurone layer of wheat, rye and oat grain is one cell thick, and that of barley grain is two or three cells thick. The inner endosperm is rich in starch and is the fraction that is used for flour production. After the milling procedure to obtain the flour, the bran fraction is considered as a by-product. New applications of these fractions are therefore desired and because of the high content of DF, one area of interest is the DF fortification of foods.

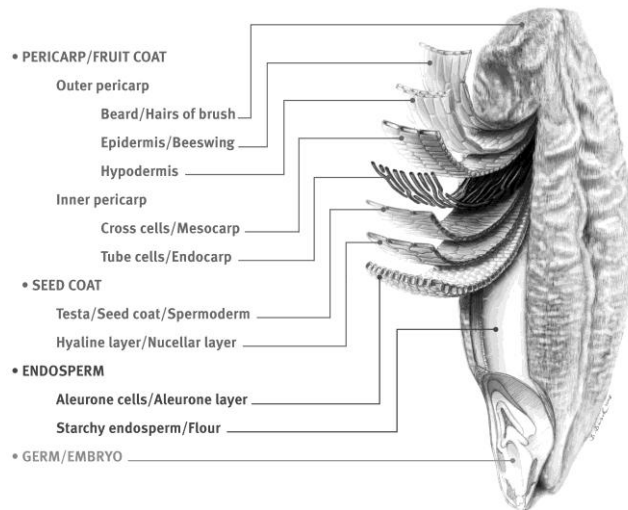


Figure 1. Illustration of the different layers of a rye kernel (Karmppfmeier Food Innovation GmbH, Hamburg, Germany).

Arabinoxylans

Arabinoxylans (AX) are branched heteropolysaccharides. AX consist mostly of arabinose and xylose residues, where the latter contains 5 carbon atoms and are therefore often referred to as pentosans. The pentosans also include arabinogalactan, which is present in small amounts in cereals. The AX backbone consists of β -D-xylopyranosyl residues connected via (1 \rightarrow 4) linkages. About 50% of the xylose residues are substituted with one α -L-arabinofuranose residue, while a smaller part carry two arabinose units (Vinkx & Delcour, 1996). AX also contain some ferulic acid. The structure of a part of an arabinoxylan molecule is shown in Figure 2. The degree of arabinose substitution is an important structural characteristic of AX, often expressed as the ratio of Arabinoses/Xyloses (Ara/Xyl). The higher this ratio is, the more branched are the molecules and the more soluble they are. Arabinoxylans are often divided on the basis of solubility. The AX in rye is more soluble than those in wheat, because of the more branched structure of rye AX (higher Ara/Xyl) (Roger Andersson, Eliasson, Selenare, Kamal-Eldin, & Åman, 2003; Maes & Delcour, 2002). The AX in the outer layers of the kernel is less branched than in the endosperm and therefore less soluble (Izydorczyk & Biliaderis, 1995).

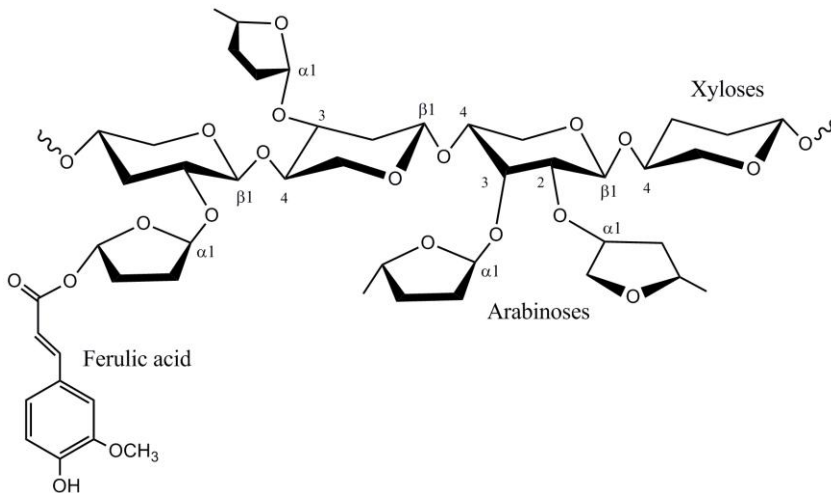


Figure 2. Structure of arabinoxylan, modified after Vinkx and Delcour (1996). The H and OH are not shown in the structure.

β -Glucans

β -Glucan is a linear unbranched homopolysaccharide of β -D-glucopyranosyl residues linked via (1 \rightarrow 3) and (1 \rightarrow 4) linkages. The structure of β -glucan can be seen in Figure 3. The polysaccharide mainly consist of trimer and tetramer blocks of (1 \rightarrow 4) linked glucose residues, separated by single (1 \rightarrow 3) linkages (Lazaridou & Biliaderis, 2007). β -Glucan is very similar to cellulose, with the difference that cellulose consist of only (1 \rightarrow 4) linkages, which make the molecules very regular and highly insoluble. Because of the (1 \rightarrow 3) linkages in β -glucan, the molecules get an irregularity and hence molecules that are more soluble in water. The solubility depends on the occurrence of the (1 \rightarrow 3) linkage and the ratio between the trimers and tetramers of glucose units. β -glucan is mainly present in oat and barley, where the amount is 5-6% (Beer, Wood, & Weisz, 1997).

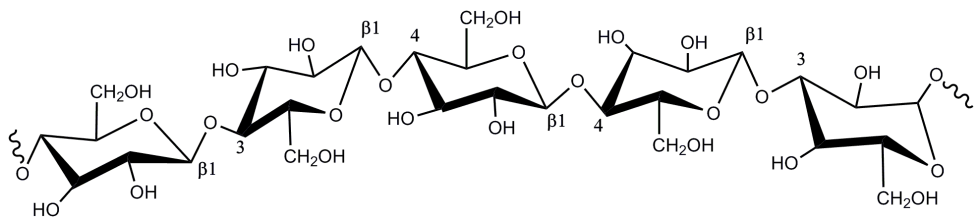


Figure 3. Structure of β -glucan. Modified from Cui and Wang (2009).

Dietary fibre content and composition of whole grains and cereal bran

Table 1 presents the dietary fibre content of the whole grain and of the bran fraction of the four cereals. The total amount of dietary fibre as well as the AX and β -glucan contents are presented and in some cases the amounts of soluble dietary fibres have been included (within brackets). As can be seen in the table, there is a variation within the same kind of cereal, both concerning the dietary fibre content of the whole grain, but even more so for the bran fractions. The composition depends on variety and environment (Hansen, Rasmussen, Bach Knudsen, & Hansen, 2003) and on the methodology of measuring the dietary fibre content. The chemical composition of cereal bran very much depends on the industrial milling process used and may therefore vary between the same kind of cereal fraction (Kamal-Eldin, et al., 2009).

As can be seen in the table, wheat and rye bran are very rich in dietary fibre, with a total amount of around 40%. Oat and barley bran have half that amount, or lower. Wheat and rye have a very high amount of AX, which is especially obvious when comparing the contents of the bran fractions. Oat and barley on the other hand have a higher content of β -glucan than wheat and rye.

Table 1. Dietary fibre (DF) content in cereal grains and bran (% of dry matter (dm)) numbers in brackets report the amount of soluble dietary fibres (% of dm).

	Total DF	Arabinoxylans	β-Glucans	Ref
Wheat	<u>Whole grain</u>			
	11.6 (1.4)	4.9 (1.0)	0.6	1
		4.0-9.0 (0.3-0.9)	0.5-2.3 (0.02)	2
	<u>Bran</u>			
	35 (3.1)	22.6 (1.8)	1.2 (0.3)	3
	39.9-53.1	22.4-29.8	2.2-2.6	4
	46.6 (2.0)	26.4 (0.7)	1.6	5
Rye	<u>Whole grain</u>			
	15.2 (4.1)	7.0 (1.8)	2.1	1
	20.5-25.2		1.7-2.0	6
	<u>Bran</u>			
	41.1-47.5	20.6-29.8	4.2-5.3	4
	42.0 (4.3)	24.1 (2.9)		7
37 (4.5)	22.7 (1.7)	3.5	5	
	12.1-14.8		6	
Oat	<u>Whole grain</u>			
		2.2-4.1 (0.2)		2
	10.6-23.4		4.5-5.6	8
	<u>Bran</u>			
	10.7-19.4	2.3-4.7	4.7-8.3 (1.7-2.9)	9
19.8 (6.7)	3.8 (0.2)	9.5	5	
	3.8-13.2 (0.2)	6.2-8.4	8	
Barley	<u>Whole grain</u>			
		3.4-8.0 (0.3-0.4)	2.0-20.0 (1.7-2.6)	2
	15.0-23.8		3.7-6.5	10
	<u>Bran</u>			
20.4 (6.9)		7.7	11	
	4.8-9.8		10	

1. Ragaee, Campell, Scoles, G, & Tyler (2001); 2. Collins, et al.(2010); 3. Maes & Delcour (2002); 4. Kamal-Eldin, et al. (2009); 5. Sirpa Karppinen, Liukkonen, Aura, Forssell, & Poutanen (2000); 6. Nyström, et al. (2008); 7. R. Andersson, Eliasson, Selenare, Kamal-Eldin, & Åman (2003); 8. Shewry, et al. (2008); 9. (Luhalo, Martensson, Andersson, & Aman (1998); 10. A. A. M. Andersson, et al. (2008); 11. Bhatta (1993)

Physicochemical properties of AX and β -glucans

Arabinoxylans have been widely studied because of their important contribution in the quality of breads (Courtin, Roelants, & Delcour, 1999). It has been shown that the soluble AX have the ability to improve the quality of rye bread by influencing the viscosity and gas-retention ability of the dough (Autio, 2006). Soluble AX have the ability to form highly viscous solutions, while the insoluble AX have a high water-holding capacity, and thus the capability to swell (Meuser & Suckow, 1986).

A wide distribution of molecular weights has been reported for AX from rye and wheat. The estimated molecular weight is depending on the analytical technique and different reports may therefore vary a lot. One study by Andersson et al (2009) reported an average molecular weight of water extractable AX in whole-grain rye flour of about 200 000 g/mol.

The lowering of glucose response and cholesterol levels that have been observed after intake of β -glucans can be due to its viscous effects. The rheological properties of β -glucans are important not only for their physiological effects, but also to understand technological behavior when included in different foods. The rheological properties of β -glucans depend on concentration, molecular weight, the structure of the polysaccharide and their ability to form aggregates (Gómez, Navarro, Manzanares, Horta, & Carbonell, 1997).

β -glucan solutions have been shown to be highly shear thinning (pseudoplastic), and that this behavior is enhanced by an increased concentration. At low concentrations (<0.2%), the β -glucan solution behaves like Newtonian solution (an increasing shear rate does not influence the viscosity). (Autio, Myllymaki, & Malkki, 1987; Doublier & Wood, 1995).

Doublier and Wood (1995) showed that while the larger β -glucans forms pseudoplastic solutions, the lower molecular weight β -glucans can form soft gels at higher concentrations. Their explanation to this behaviour is that the smaller molecules are more mobile and diffuse more easily and therefore have a greater probability of forming aggregates.

In a study by Mikkelsen et al (2010) oat β -glucan was shown to be 100 times more viscous than barley β -glucan and oat β -glucan showed shear thinning behaviour, while the barley β -glucan showed Newtonian behaviour. Barley β -glucans have been shown to be able to form gels at concentrations above 4%, when allowed to set for 24 h at room temperature (Burkus & Temelli, 1999).

Oat β -glucans have a higher molecular weight than barley β -glucans (Beer, et al., 1997) and a lower ratio of tri- to tetramers; around 2, compared to 3 in barley (W. Cui, Wood, Blackwell, & Nikiforuk, 2000). This ratio is very important since it influence the gel forming ability of the β -glucans. It is the sequences of (1 \rightarrow 4)-linkages that can bind together and the higher quantity of the longer sequences, the stronger gel can be formed (Böhm & Kulicke, 1999).

A large variation of β -glucan molecular weights have been reported (20 000-3 000 000 g/mol) and this is due to the different extraction procedures and methodologies used (Autio, 2006). The molecular weights of β -glucan can be influenced during processing. Åman et al (2004) showed that there were large differences of the molecular weights of oat β -glucans depending on type of product and therefore the kind of processing that the β -glucan had been through. By heat treatment such as when making oat porridge, the M_w was not influenced, while during a prolonged treatments, such as baking when a fermentation step is included, the molecular weight (M_w) was clearly reduced by enzymatic degradation. Ulmius et al (2012) showed that by microwave heating to 100 °C, only the largest aggregates of β -glucans were reduced in size, while heating to 121°C decreased the M_w of the aggregates remarkably and the polymers were therefore also degraded after this treatment.

2. Cereal materials used in the studies

Three cereal brans were used in the studies: rye bran, wheat bran and oat bran (Lantmännen, Sweden). Also a barley fibre was used in these studies and provided by Lyckeby Stärkelse. It is a product, which is developed to contain a very high amount of β -glucans (32%).

2.1 The composition and solubility of the cereal materials

The total dietary fibre content as well as the amount of AX, β -glucan and lignin of the cereal materials are presented together with the starch and protein content in Table 2. The total dietary fibre content presented corresponds to the sum of monosaccharides measured by the Uppsala method (Theander, et al., 1995) which means that the lignin content is not included here and neither is the fructans. Oat bran was the material with the lowest DF content (18.4%), while the barley fibre had the highest (63.2%). The wheat and rye bran had similar dietary fibre content close to 40%, which is similar to what is found in literature (Table 1). Also the AX content was similar for these two materials and was the dietary fibre which was found in the highest quantities in wheat and rye bran. The β -glucan content was by far the highest in the barley fibre (32%), while the oat bran also contains a considerable amount (10%). Similar β -glucan content of oat bran has been reported by Karppinen et al (2000), while others have reported lower values of 4.7-8.4 (Luhalo, et al., 1998; Shewry, et al., 2008). The starch content of the different materials varied (12-44%) while the protein contents were rather similar with slightly higher protein content in oat bran and barley fibre compared to wheat and rye bran. The starch and protein content of the wheat, rye and oat bran are in the same range as previous reports (Kamal-Eldin, et al., 2009; Karppinen, et al., 2000; Luhalo, et al., 1998).

Table 2. Composition of the cereal materials (% of total amount of dm).

	DF	AX	β-glucan	Lignin	Starch	Protein
Wheat bran	40.6	28.4	1.5	3.7	11.6	14.3
Rye bran	36.0	23.2	4.2	5.7	32.7	14.4
Oat bran	18.4	5.1	10.2	1.5	44.0	16.3
Barley fibre	63.2	18.5	32.0	1.7	20.3	16.0

When these materials were added to the meat products, the amount of materials added were based on a constant dietary fibre content of 1% in the meat products. Since there is a great variation between the DF contents of the materials, the amounts of cereal material which needed to be added varied. The oat bran only had a third of the DF content of the barley fibre. Therefore, the protein content of the products with oat bran added was three times the amount in the barley products. On the other hand, the total amounts of β -glucan in the oat bran and barley fibre products were very similar. The starch content differed between the materials and the higher starch level in oat bran was even more pronounced since more oat bran needed to be added to the meat products compared to the others. This was however compensated by reducing the amount of potato starch to keep the total amount of starch at a constant level. These differences between the materials are important to bear in mind when their suitability as additives in low-fat meat products is discussed later on in this thesis.

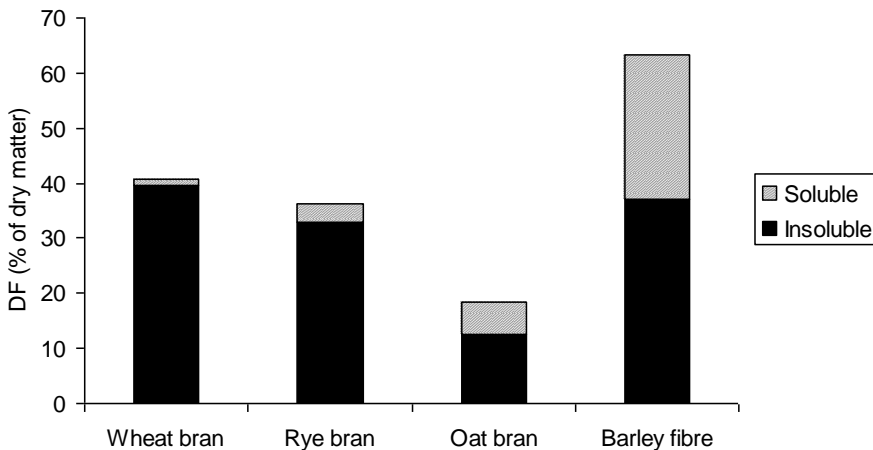


Figure 4. Amount of soluble and insoluble dietary fibre in the cereal materials.

The content, solubility and composition of the DF in both the soluble and insoluble part of the different cereal materials are shown in Figure 4, 5 and 6. The separation of the soluble and insoluble dietary fibres were made by mixing milled material (0.5 mm) in water over night at a cold temperature (7 °C), followed by separation with centrifugation at 3000g. The fractions were then freeze-dried prior dietary fibre analysis. The reason for this mild separation procedure was the wish to have a minimal influence on the solubility prior separation, with no influence of extra heat or processing involved. It is well known that the solubility of the DF increases with processing such as heat treatment (Graham, Grön Rydberg, & Åman, 1988).

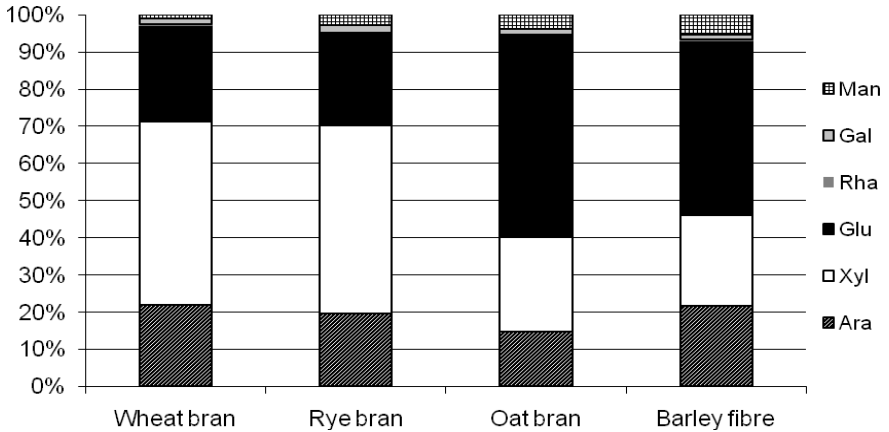


Figure 5. The monosaccharide composition of the insoluble dietary fibres of the four cereal materials.

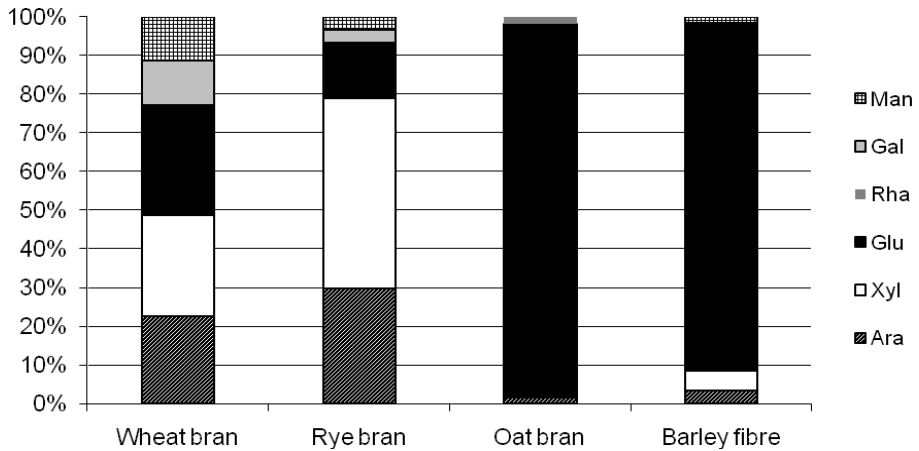


Figure 6. The monosaccharide composition of the soluble dietary fibres of the four cereal materials.

As can be seen in Figure 4, rye bran and wheat bran mainly consisted of insoluble DF. Oat bran and barley fibre had a higher soluble fibre content compared to the wheat and rye bran. Figure 5 and 6 describes the composition of insoluble and soluble fibre respectively. The majority of the DF in wheat and rye bran was insoluble AX and cellulose, analyzed as arabinose, xylose and glucose (Figure 5). Rye bran contains 3% of soluble DF, compared to the lower level of 1% in wheat

bran and AX is the main soluble DF in both materials. Oat bran has a soluble DF content of close to 6%, while the barley fibre has 26%. The soluble DF in both oat bran and barley fibre mainly consisted of β -glucans, 3.3% in oat bran and 22.3% in the barley fibre. The amounts of soluble fibre presented for wheat, rye and oat bran are lower than previous reports (Table 1). The reason for this is that the extraction was made in cold water and without any enzymes or heat added prior the separation by centrifugation (**Paper I**).

2.2 Physicochemical properties

The particle size distributions (PSD) of the milled materials (0.5 mm, Retch ZM1) are shown in Figure 7, where both the surface area weighted and the volume based diameters are displayed. The smaller particles have a larger influence on the area-weighted mean diameter (d_{32}), while the larger particles have a larger influence on the volume-weighted mean diameter (d_{43}). Oat bran had a d_{32} of only 15 μm , compared to the wheat bran which had 72 μm . In the graph of the volume-based particle size, there is a peak around 20 μm for oat bran, which is not seen for the other materials. One of the reasons for this could be the high starch content of oat bran, which moreover has smaller starch granules compared to rye, wheat and barley. The diameters of the oat starch granules are of the magnitude 2-14 μm , while rye, barley and wheat have a bimodal size distribution with the larger granules having diameters up to 32-36 μm (Lindeboom, Chang, & Tyler, 2004).

In the volume-weighted particle size distribution (Figure 7B), it can be seen that all graphs are truncated to different degrees and most for the wheat and oat bran, which suggest that these have particles that are larger than the 900 μm , which is the upper limit measured by the apparatus.

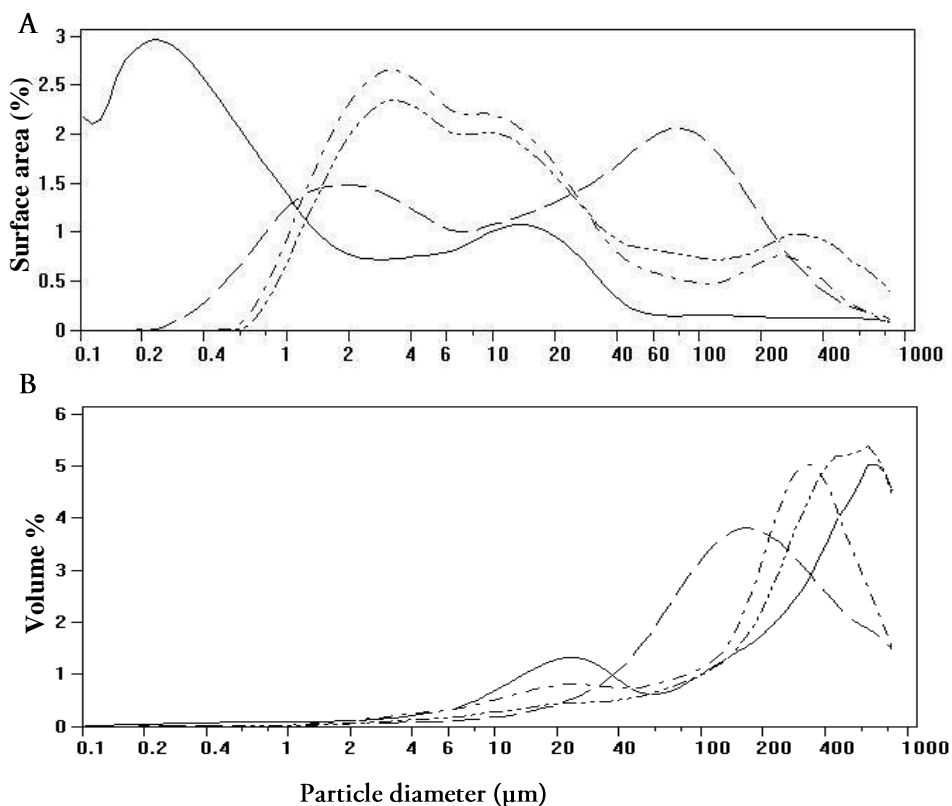


Figure 7. Surface-area-weighted particle size distribution (A) and volume-weighted particle size distribution (B) of the four cereal materials. — oat bran; --- barley fibre; - - - rye bran; - . - . - wheat bran.

Micrographs of the four milled cereal materials are shown in Figure 8. As can be observed, the wheat and rye bran (A and B) have higher frequency of large particles probably containing most of the insoluble AX and cellulose, which is in accordance with the peak in the volume-based PSD around 200-800 µm for these two brans. For the oat bran (C) the frequently occurring small starch granules of 20µm can mostly be observed. The insoluble part of the barley fibre has a more smooth appearance.

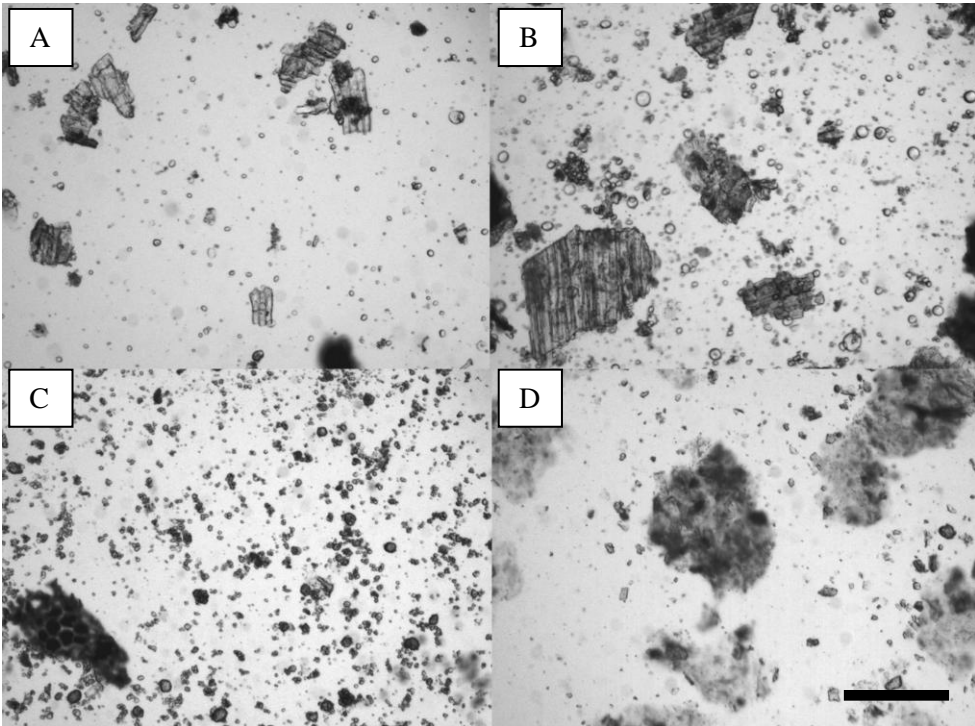


Figure 8. Micrographs of the four cereal materials. A) wheat bran, B) rye bran, C) oat bran and D) barley fibre. The bars in micrographs indicate 200 μm .

The WHC of the cereal materials used in the study increase in the order rye bran < wheat bran < oat bran < barley fibre (3.0, 4.7, 5.1 and 8.2 g water /g dry material) (**Paper I**). The WHC were determined by soaking the materials in cold water over night, followed by centrifugation (10 000g). The rather low WHC of cereal brans are in agreement of previous studies where wheat and oat bran have been reported to have WHC around 4 g water/g bran or lower (Auffret, Ralet, Guillon, Barry, & Thibault, 1994; Grigelmo-Miguel & Martin-Belloso, 1999). The barley fibre which had higher soluble DF content compared to the other three materials had the best WHC. It has previous been suggested that the WHC is related to the amount of soluble DF (Grigelmo-Miguel & Martin-Belloso, 1999). This was however not consistent for the wheat and rye bran where the wheat bran had the higher WHC even though it had a lower amount of soluble dietary fibre. A relationship between the amount of insoluble material and the WHC was seen when this was studied in more detail for wheat and rye bran (**Paper III**), which will be described later on in the thesis.

3. Sensory evaluation methodology

It is important that the addition of a new ingredient to a food product does not change the textural properties in a negative way. The texture of food is a very important sensory property (Szczesniak, 2002). The texture of foods can to some extent be instrumentally measured with a rheometer or a texture analyzer. However, to assess the actual texture perceived in the mouth, sensory evaluations should be performed.

There are a number of different methods to use when evaluating sensory properties. Discrimination tests should be used if the question is whether there is a difference in how samples are perceived (Lawless & Heymann, 1998). Some examples of discriminating tests are the triangular test (which sample is different compared to the other two?), the duo-trio test (which of two samples matches the reference?) and the paired comparison test (which of the two samples has the strongest perceivment of a specific attribute?). Triangular tests were used to find out if there were perceived grittiness of the added rye bran particles, of a certain size and concentration, to a starch gel of varying consistency (**Paper II**).

In a ranking test, assessors evaluate a number of samples in random order and are asked to place them in rank order based upon a specified criterion. This method is useful if several samples are going to be evaluated and compared for a certain attribute. Time can be saved and more samples can be analysed without making the assessors fatigue. Ranking tests were used to evaluate whether an increased concentration of rye bran particles in a starch gel could be detected by a sensory panel (**Paper II**).

A 9-point hedonic scale is very often used for evaluating foods. This scale is commonly used for assessing the degree of liking of food products (Lawless & Heymann, 1998). A hedonic scale was used to evaluate the overall acceptability of low-fat sausages and meatballs and also specific attributes, such as crumbliness, compactness, juiciness, meat taste, colour and off-flavour (**Paper IV and V**).

4. Sensory perception of rye bran particles

Perceived grittiness of a product is usually considered as something negative. When adding hard particles to a food product, there is a risk of simultaneously adding an unpleasant feeling of grittiness. It has been shown that addition of particles to a starch-based custard increased the roughness and reduced more potentially favourable attributes such as smoothness and creaminess (Engelen, de Wijk, et al., 2005).

Previous studies on the perception of particles and the threshold of grittiness are in agreement that round particles are perceived as smaller than hard irregular ones in the size range 2-230 μm (Engelen, Van Der Bilt, Schipper, & Bosman, 2005; Imai, Hatae, & Shimada, 1995; Tyle, 1993). The influence of the viscosity of the continuous phase in which the particles are suspended on the oral perception of grittiness is however not clear. Imai et al. (1995) reported a difference in the threshold for grittiness depending on the viscosity of the continuous phase, while Engelen et al. (2005) found no such relation. Imai et al. (1995) compared two suspensions of different concentrations of xanthan gums, with apparent viscosities of 0.02 and 0.1 Pa·s, in which particles of microcrystalline cellulose of different sizes and concentrations were suspended. They reported that the sample with the higher viscosity resulted in a lower perceived grittiness. The same study showed that the number of people who perceived the solutions as gritty increased with increasing particle size and concentration. Engelen et al. (2005) investigated a custard desert with varying concentrations of carboxymethyl cellulose with viscosities of 3 and 6 Pa·s, to which SiO_2 or polystyrene particles of different sizes had been added. They reported that the hard, irregularly shaped SiO_2 particles were perceived as being larger than the soft, round polystyrene particles, and the viscosity of the media did not influence the result.

In the present study, the perceived grittiness of small rye bran particles was evaluated (**Paper II**). Rye bran was milled and air-classified in order to get fractions of different particle sizes. Three fractions were obtained, which had clearly different size distributions, determined by laser diffraction using a Coulter LS130 instrument (Beckman Coulter, High Wycombe, UK), with median particle sizes of 20, 60 and 180 μm . The insoluble parts of the rye bran fractions were boiled to minimize the floury taste. The samples were then prepared by adding the particles to flavoured starch gels, made of different concentrations of potato starch (4, 5 and 6%). Micrographs of the mixtures of starch gel and the different rye bran

fractions are shown in Figure 9. The large difference in size of the fractions is clear when studying the micrographs in Figure 9A, B and C, where the same magnification has been used. In Figure 9D, the sample of 20 μm has been enlarged to get a better picture of these small particles. All fractions appear to have irregular shaped particles with sharp edges. The large and smooth swelled potato starch granules can also be seen (the paler structures).

Two kinds of sensory test were used: triangular test and ranking test. In the triangular test, the thresholds of grittiness were sought. Three samples were given simultaneously to the assessors: one starch gel with added rye bran particles and two without. An untrained sensory panel was asked to point out the sample which was grittier than the other two. Four groups, consisting of eight panel members, were evaluating six concentrations between 0.10 and 1.5% for two combinations of rye bran fraction and starch concentration. Six of the eight panel members

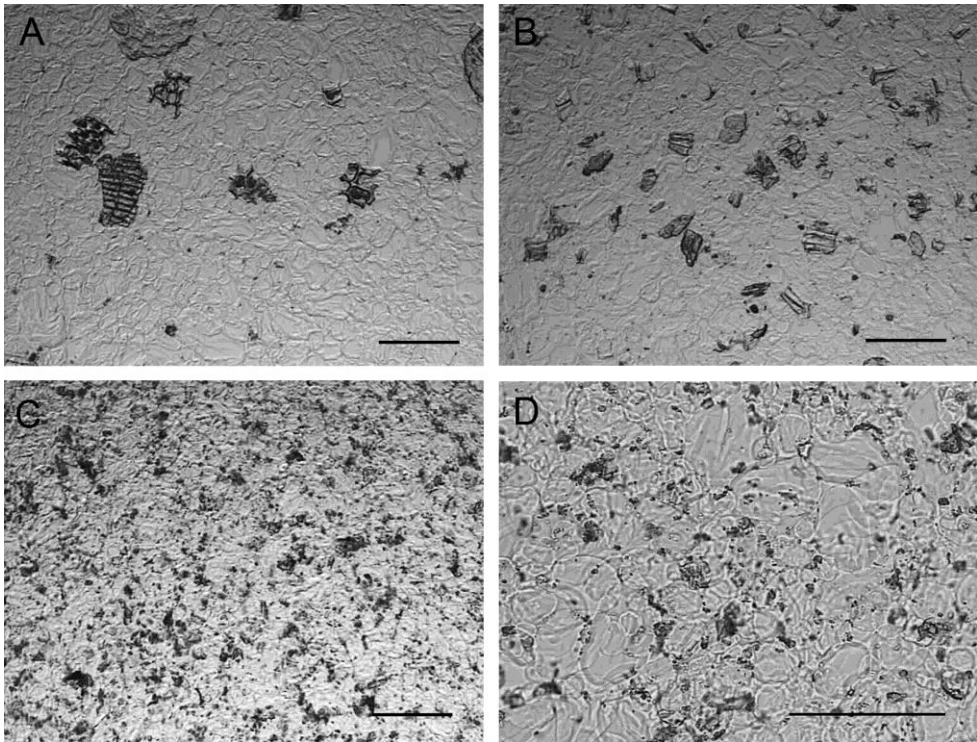


Figure 9. Micrographs of the rye bran fractions used in the triangular tests (i.e. only the insoluble fractions). A) fraction 180 μm , B) fraction 60 μm and C) and D) fraction 20 μm . All fractions were added at a concentration of 0.67% to a gel containing 4% starch. The scale bar corresponds to 100 μm .

needed to point out the increased grittiness correctly in order to significantly approve the difference (Roessler, Pangborn, Sidel, & Stone, 1978). The threshold of the grittiness was determined as the lowest concentration where the difference was significant.

The threshold for the sensation of grittiness resulting from rye bran particles in a starch gel was very low, as can be seen in Figure 10. The particles were detected already at concentrations of 0.1-0.3%, despite the fact that they were so small. When designing the experimental plan, it was assumed that there would be a difference in the threshold of grittiness depending on the viscosity of the medium in which the particles were included. This was however not the case which can be seen in Figure 10. The reason for this behaviour is discussed below.

The rheological properties of the starch gels used, including the elastic modulus (G'), the viscous modulus (G''), the yield stress and apparent viscosity (η), were measured by an oscillatory stress sweep at 20°C (StressTech, Reologica AB, Lund, Sweden). In a stress sweep an increased shear stress is applied on the sample at a constant frequency, in this case 1 Hz. All three starch gels were indeed gels ($G' > G''$) in the linear region. The yield stresses were recorded at the end of the linear region of G' , where the second derivate of the G' is the greatest (Figure 11A) and they increased with the starch content (4, 12 and 20 Pa).

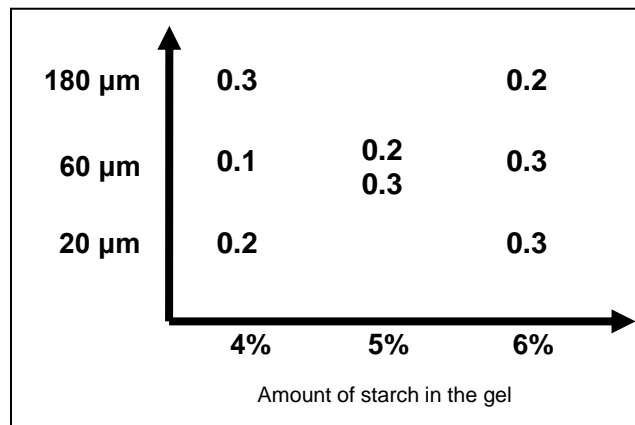


Figure 10. The grittiness threshold values (% w/w) for the different particle sizes of rye bran evaluated by the triangular test (**Paper II**).

It might be so that the stress applied in the mouth is so high that it has surpassed the yield stress and then the difference in viscosity is not so obvious. The shear stress applied in the mouth is dependent on the properties of the food sample. Shama and Sherman (1973) suggested that low viscosity liquids ($<0.1 \text{ Pa}\cdot\text{s}$) are evaluated at a constant shear stress of about 10 Pa while products with a viscosity above 10 Pa·s are evaluated at a constant shear rate of about 10 s^{-1} .

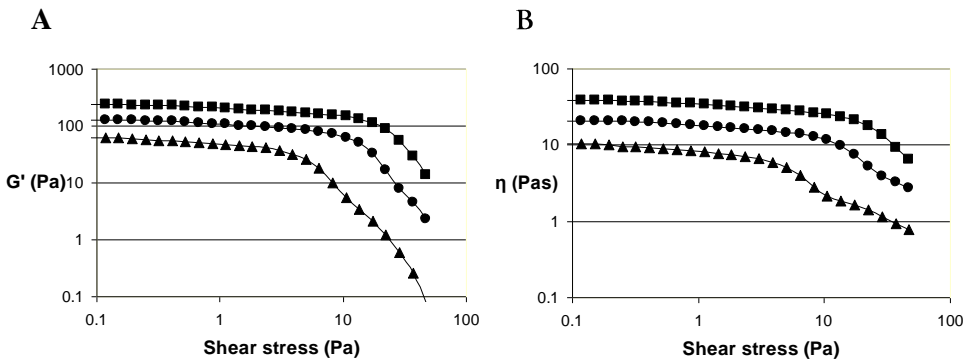


Figure 11. A) The elastic modulus (G') and B) the apparent viscosity (η) of an oscillatory measurement of gels made of 4, 5 and 6% (w/w) starch (\blacktriangle , \bullet and \blacksquare , respectively).

A more advanced calculation of the shear stress on the tongue was proposed by Kokini et al (1977), who also stated that the orally perceived thickness was proportional to this calculated shear stress applied in the mouth. Their results fitted quite well with those of Shama and Sherman. The implication by Terpstra et al (2005) was that the linear relationship between the thickness and the shear stress according to Kokini et al (1977) was valid but only within a limited range of shear stresses ($<30 \text{ Pa}$ for custard, and $>150 \text{ Pa}$ for mayonnaise).

To make an approximation of the shear stress in the mouth for the starch gels used in this study, the model of Shama and Sherman was used. From the oscillatory measurements on the starch gels, described in **Paper II**, the shear rate was not given. The measurements were based on an increasing shear stress and the apparent viscosity was calculated (Figure 11B). From this information the shear stress could be estimated by searching the point where the shear stress divided by the viscosity gave a shear rate of 10 s^{-1} . The shear stress applied in the mouth for the different gels would then be about 17, 37 and 61 Pa (increasing with the starch content). From Figure 11A it then becomes obvious that at the appropriate shear stress for each starch gel, G' has markedly decreased and the shear stress in the

mouth has exceeded the yield stress. The experienced viscosity in the mouth would then be around 1.5, 3 and 5 Pa·s for the three gels of 4, 5 and 6% starch.

There are also several other mechanisms influencing the structure of the food items in the mouth and the texture perceived during sensory evaluation. Firstly, the sample will be diluted with saliva. This may be followed by enzymatic breakdown, which may result in decreased viscosity and, possibly, mechanical breakdown of structures due to mastication. It has been shown that even small amounts of saliva can have a drastic effect on the viscosity of starch-based foods, and that this effect occurs within the time which is reasonable for food to be processed in the mouth before being swallowed (Prinz, Janssen, & de Wijk, 2007). The amount of saliva added depends on both the taste and texture of the food (Mackie & Pangborn, 1990). Dry products, such as crispbread, give rise to a high saliva production (Pangborn & Lundgren, 1977). Dilution and breakdown of the starch gel may be other reasons for the observation that the viscosity, measured prior to ingestion, did not influence the grittiness threshold.

In the ranking test, described in **Paper II**, it was evaluated whether the panel (23 members) could distinguish between the increasing concentrations of rye bran particles added to a starch gel (5% starch). Five samples with varying concentration (0.1-1.2%) of the same size fraction were evaluated together and the panel was asked to rank the sample from 1-5, from the least gritty to the most gritty.

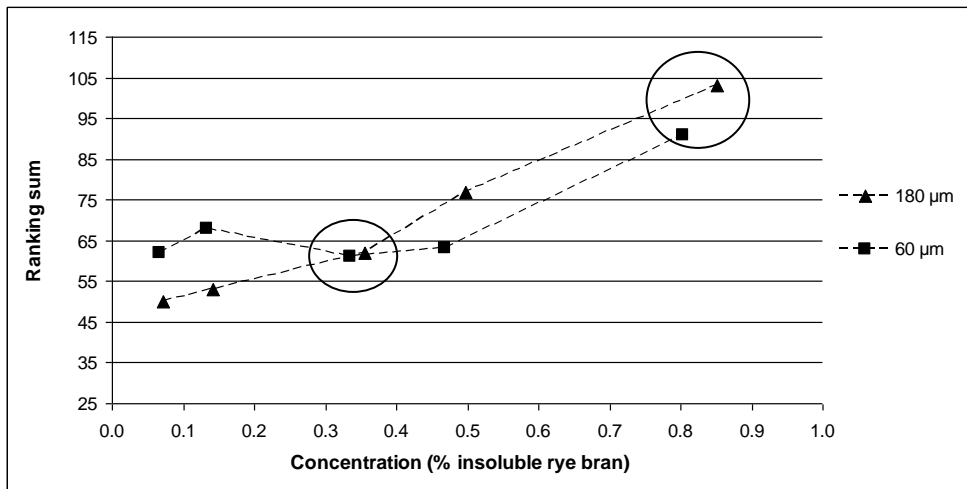


Figure 12. Ranking sums of the two coarse rye bran fractions (60 and 180 μm) at different concentrations of insoluble rye bran added to a starch gel of 5% (**Paper II**). The encircled points imply significant differences of the ranking sums between these samples.

The results of the ranking test were evaluated by comparing the sums of the scores of all the members in the panel. Significant difference between the concentrations were detected if the differences between the sums were greater than the critical value of 29.3 (Basker, 1988). The sums for each of the concentrations used in the ranking tests are illustrated in Figure 12. In the ranking test, the whole rye bran fraction was added to the starch gels and not only the insoluble part which was the case in the triangular test.

The concentrations used in the graph in Figure 12 are based on the amount of insoluble material added to the starch gels to be able to make a comparison of the two sensory assessments. The smallest fraction of rye bran was not included in this test. For both size fractions, there were significant difference between the highest concentration (1.2%) and the concentration of 0.5% which are indicated by circles in Figure 12. The sensory panel was able to detect an increase in particle concentration in the ranking test, but the ability of the panel to detect the increase in concentration was not influenced by particle size, as can be seen in the two graphs, which are quite similar (Figure 12).

There was no significant difference observed between the concentrations between 0.1-0.7 for any of the two size fractions used in gels made of 5% starch. The thresholds of grittiness found for the same starch gel when the fraction of 60 μ m was used in the triangular test were an insoluble rye bran concentration of 0.2 and 0.3%. These results suggest that we are able to feel the grittiness at very low concentrations, but we do not distinguish an increased perceived grittiness until the concentration is at least 1.2%.

5. Enzymatic treatment of rye and wheat bran

One way of changing the physicochemical properties of cereal particles and thereby possibly also the perceived grittiness, could be by the treatment with hydrolytic enzymes. It is possible to increase the solubility of DF by treating cereal bran with hydrolytic enzymes. The use of endoxylanase to hydrolyse water-insoluble AX may allow more water to be absorbed by the cell wall material due to the cleavage of AX, thereby opening up the structure, and increasing the degree of swelling (Gruppen, Kormelink, & Voragen, 1993). However, when attempting to increase the viscosity of the soluble fraction, the degree of hydrolysis of the solubilized molecules must be considered, as the viscosity depends on the size of the molecules released (Härkönen, et al., 1995; Ragae, et al., 2001). Endoxylanases have different substrate selectivity and vary in their preference for the hydrolysis of water-insoluble and water-soluble AX (Courtin & Delcour, 2001; Moers, Celus, Brijs, Courtin, & Delcour, 2005), thus, understanding the specificity and selectivity of the enzymes is crucial to achieve the desired characteristics of AX. The variation in the functionality of xylanases also depends on interactions with inhibitors present in cereal materials (Goesaert, et al., 2004).

Rye bran and wheat bran were treated with a number of hydrolyzing xylanases and endoglucanases to evaluate how they could influence the physicochemical properties of the bran materials (**Paper III**). The goal was to see if any of the enzymes had the ability to improve important physicochemical properties of the bran, and thereby improve their suitability to be added in the meat products. Solubility, water-holding capacity, particle size, viscosity and dietary fibre content were investigated. The enzymes used varied in their origins and enzymatic activities.

An overview of the results of this study can be seen in Figure 13, where plots of Principal Component Analysis (PCA) of the results are presented. When data from both rye and wheat bran were included in the PCA (Figure 13A and B), the two materials were separated in two groups in the score plot (Figure 13B). The wheat bran had a higher content of insoluble material, larger particle sizes and a higher water-holding capacity than rye bran. A higher water-holding capacity of wheat bran compared to rye bran was also presented in **Paper I**. Rye bran on the other hand gave a higher viscosity to the aqueous phase.

Separate PCA plots were done for the two materials and the ones for rye bran are shown in Figure 13C and D. The enzymes originating from *Bacillus subtilis* (EDS91 and Depol 761) were more effective than the others in solubilizing DF from wheat and rye bran. These enzymes were however also efficient in degrading

the soluble dietary fibre into small fragments not detectable in the fibre analysis and thereby also decreasing the total amount of DF. The water-holding capacity was strongly correlated to the amount of insoluble material ($p < 0.001$, $r = 0.990$). The enzymes originating from *Trichoderma reesei* (EGI and EGII) had a very small effect on both wheat and rye bran, probably due to the presence of inhibitors in the cereals (Goesaert, et al., 2004).

None of the enzymes could increase the water-holding capacity or the viscosity of the aqueous phase of the rye or wheat bran. The water-holding capacity were probably reduced because of the lower amount of insoluble material after solubilization by the enzymes, while the lower viscosity showed that the solubilized dietary fibre were degraded into smaller molecules which could no longer contribute to the viscosity.

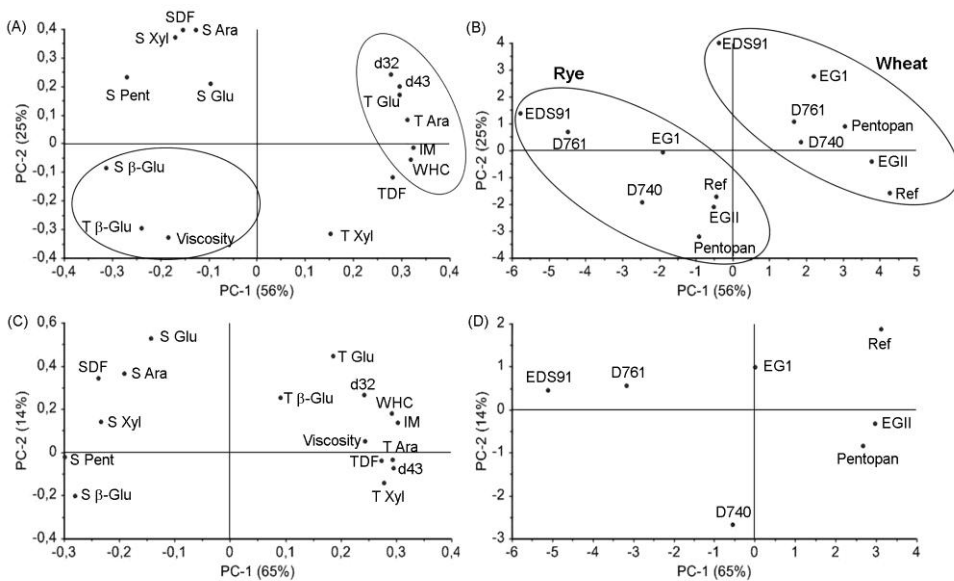


Figure 13. PCA of the results of the hydrolysis of 20% cereal bran treated for 4 hours. The PCA plots A (loading plot) and B (score plot) show the results for rye and wheat bran analysed together. The variables correlated to the materials are encircled in the loading plot (A). Plots C and D show the results of the analysis of rye bran only. SDF = soluble dietary fibre, TDF = total dietary fibre, S Ara = soluble arabinose, T Ara = total arabinose, S Xyl = soluble xylose, T Xyl = total xylose, S Glu = soluble glucose, T Glu = total glucose, S Pent = soluble pentosans, S β-Glu = soluble β-glucans, T β-Glu = total β-glucans, IM = insoluble material, WHC = water-holding capacity, d43 = volume-weighted mean particle size, d32 = area-weighted mean particle size.

The effects of the treatments with the different enzymes were also studied by microscopy. The effects of the different enzymes were seen when the bran samples were studied using fluorescence microscopy with Calcofluor and Acid Fuchsin staining (Figure 14). The intact cell walls stained with Calcofluor appear blue and the proteins stained with Acid Fuchsin appear red. In the micrograph of the reference, which had been treated in water without any enzymes added, structures from the pericarp and the aleuron layer were clearly seen (Figure 14A). The enzyme EG I did not influence the rye bran structure to any big extent (Figure 13B). The enzymes EDS91 (Figure 14C) and Depol 761P (Figure 14D), both originating from *Bacillus subtilis*, had larger impacts on the cell wall structures. EDS91 degraded the aleuron cell wall the most (seen as the decreased intensity of the blue colour), which was in agreement with the high amount of soluble dietary fibre in this sample. The pericarp and aleuron layer were mainly still intact after treatment with EDS91, while the micrograph suggest a fragmentation of the bran material after treatment with Depol 761P.

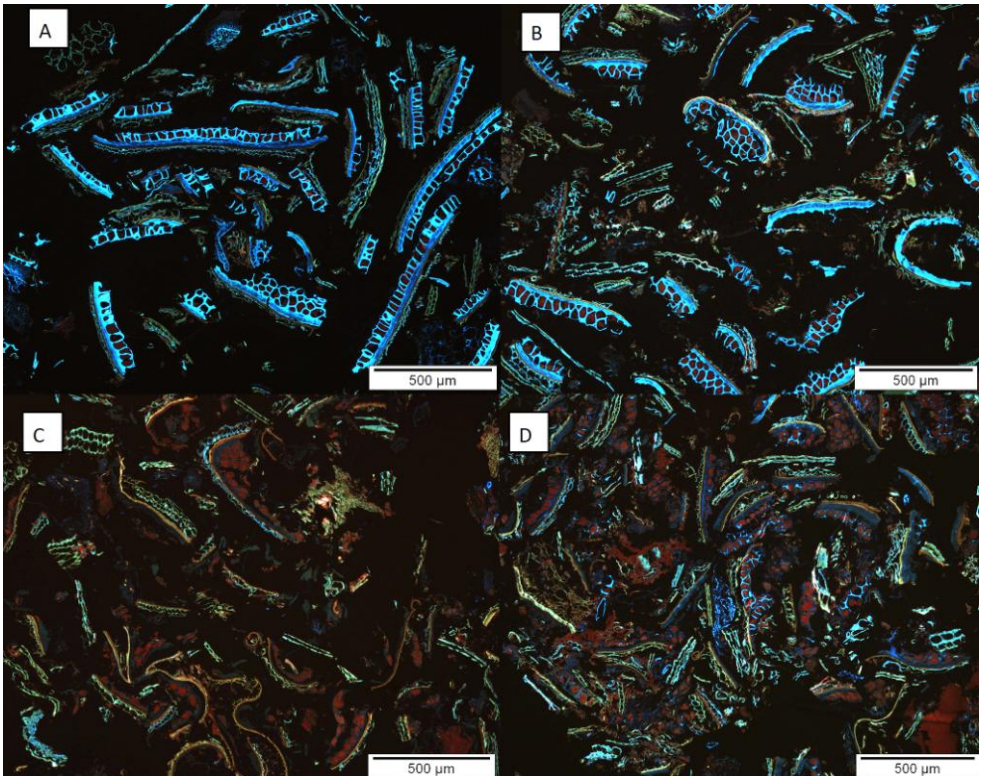


Figure 14. Micrographs of rye bran stained with Acid Fuchsin and Calcofluor. A) reference, treatment with B) EG I, C) EDS91 and D) Depol 761P. Intact cell walls stained with Calcofluor appear blue and proteins stained with Acid Fuchsin appear red.

The most obvious difference in Figure 14 is between the top row (A and B) and bottom row (C and D). The reference and the EGII had intact cell wall materials, while the EDS91 and Depol 761P hydrolysed the material more effectively. This differences are also seen in the PCA (Figure 13C and D), where the reference and EGII are to the right in the score plot (Figure 13D) while the other two are to the left. In the loading plot (Figure 13C) it can be seen that the left side correspond to a higher amount of soluble dietary fibre.

6. Low-fat sausages and meatballs

6.1 Effects of fat and starch content in meat products

Sausages and meatballs are two common meat products, which are similar in their recipes, but still differ a lot in their appearance and microstructure. Sausages are more comminuted, and have a higher salt content than meatballs, creating a strong meat protein network. This meat protein network determines the texture and sensory properties of sausages (K. Andersson, Andersson, & Tornberg, 1997). Meatballs, on the other hand, are a mixture of minced meat and other ingredients, leading to a more particulate structure, and the protein network does not determine the water-holding and fat-holding capacity of the product to the same degree as in sausages (A. Andersson, Andersson, & Tornberg, 2000; Tornberg, 2005).

Sausages and meatballs normally contain relatively high amounts of fat and therefore low-fat alternatives are frequently developed. There are however several challenges when reducing the fat content of meat products, since the fat makes a considerable contribution to the texture and taste of food products.

The results of several studies where the amount of fat has been reduced in sausages or meatballs/hamburgers are given in Table 3. Since it is mainly the shape of the meatballs and hamburgers that differ, they are discussed as being the same type of product here. The references listed in the table have compared samples with different fat levels, but without any special additive to replace the fat. Still, there are different ways of reducing the fat and the results may therefore differ between the studies. The reduction of fat in meat products can be done by using leaner meat; this however normally includes a higher price. Adding more water is another option, which is normally followed by a simultaneous addition of a suitable material that can prevent the water to be lost upon heating the product. In the case where more water is added, the water/protein (W/P) ratio will increase and the protein network will be weaker and a higher water and fat loss will be followed (Claus, Hunt, & Kastner, 1989).

There is a tradition in Sweden of making sausages and meatballs with lower meat content and with a rather high content of potato flour. In Table 3 it can be seen that the W/P ratios used in our studies are higher than the ones used in the other studies, which is a result of the lower meat content. The “Falukorv” is a Swedish, very popular sausage, which is protected by the European Commission as “traditional specialities guaranteed”. The sausages should have a meat content not

lower than 40% to be able to be called a “Falukorv”. The sausage recipe used in the present studies resembles the one for “Falukorv” with a meat content of about 50%. The meatballs were also made with about 50% meat, which is a common level of the industrially made meatballs.

The meat protein content has been shown to be more important than the fat content for the texture of sausages (Claus, et al., 1989; Jiménez-Colmenero, et al., 1995), and it is therefore important to maintain a constant water/meat (W/P) protein ratio in the products when studying the influence of changing the recipes. Not keeping the W/P ratio constant may cause erroneous results.

The ratio of the W/P used in the sausages in the present study was kept constant at 7.9 while the W/P for the meatballs was set to 7.4. The salt content is another important variable that may influence the result and was set constant to 2% in the sausages and 1.3% in the meatballs.

Effects of reducing the fat content of sausages

A reduced fat content in sausages resulted in an increased cooking loss for all studies included in Table 3. Fat stabilizes the protein network of the sausages, which therefore is weakened when the amount of fat is reduced (A. Andersson, et al., 2000). Moreover, many of the reported studies have an increased W/P ratio for the low-fat products, which means that the water will be more easily lost, due to a less dense protein network.

The effects on the firmness when reducing the fat content is reported to decrease when the fat is removed (Table 3). The reason for this is the same as described above for the increased cooking loss. A less dense protein network is developed and a less firm texture is therefore measured (Jiménez-Colmenero, et al., 1995). Our study differed from the others and the firmness was unchanged when reducing the fat content. Two reasons for this might be the following: The fat content of the high fat sample in our sausages was not very high and the change in fat content was therefore not as drastic as for the recipes used in the other studies. Moreover, the W/P was kept constant in our investigation which was not always the case for the other ones. When the compactness was sensory evaluated for the samples of different fat content, only one study reported a decreased value for the low-fat sample (Claus, 1991), while the other studies reported an unchanged compactness.

The juiciness is either increased or unchanged for all samples. The juiciness is closely connected to the water-holding capacity of the meat system and thereby the cooking loss. If water is easily lost during cooking, the remaining water is most likely not very tightly bound to the system either and will be experienced as a high juiciness in the mouth.

The results of the colour of the sausages are diverse. Two of the reported effects are results of instrumentally measured darkness, while the other two derive from visual observations. The crumbliness of the sausages was only evaluated in two of the studies. In the present study, there were no significant change in crumbliness caused by a reduced fat content, while the crumbliness was reported to increase according to Cofrades et al (2000). The larger differences of the fat content of the recipes could explain these varying results.

None of the reduced fat samples were more liked than the sample with a higher fat content. These results suggest that low-fat sausages are not successfully produced by simply reducing the fat content of the product. Some kind of additive that may aid in keeping the water in the product are therefore needed.

Effects of reducing the fat content of meatballs/hamburgers

The effects when decreasing the fat level is not the same for the meatballs/hamburger as for the sausages. The cooking loss was increased in the study presented in **Paper IV**. The other references included in Table 3, reported reduced cooking losses, although not statistically significant in all cases. The difference between our results compared to the others could be due to the lower fat level used as high-fat control. Andersson et al (2000) reported losses of less than 10% of the fat in hamburgers with a fat content below 10%, during frying, whereas the fat losses increased exponentially with increasing fat content. They concluded that the fat content of the hamburger mixture influenced the fat-retention ability during frying, and that the dominating factor in such meat products was the probability of encounter between fat droplets. The decreased cooking loss reported in Table 3 could therefore mostly be due to a reduced fat loss. The 9% fat level which was used for the meatballs evaluated in **Paper IV** was lower than the 10%, which was reported to be an important limit by Andersson et al (2000). The fat lost from this “high fat” sample upon cooking is therefore lower than for the other samples with a higher fat content.

The texture of the meatballs/hamburgers became more firm as the fat contents were reduced. In **Paper IV** an unchanged value of the firmness when the fat level is lowered was found.

As for the sausages, the juiciness is related to the cooking loss. The samples with a reduced perceived juiciness have cooking loss that is either decreased or unchanged. **Paper IV** is the only study in which an increased juiciness is reported for the meatballs with a decreased fat content. In this study, two different cooking methods have been performed, pan-frying and deep-fat frying. The sensory evaluated samples were the deep-fat fried ones and in this case there were no

significant increase of the cooking loss, which was the case for the pan-fried samples. This is explained by a fat uptake during deep-fat frying.

Starch content in sausages and meatballs

Potato starch is often used in Sweden in comminuted meat products due to its high water-holding capacities. Potato starch granules have the ability to swell about 100 times of its size, when treated in water at 80°C (Tornberg, Andersson, & Asplund, 1998). Cereal starch granules such as rye, oat and barley have been reported to have a swelling factor of 7-14 at the same temperature (Buksa, et al., 2010; Tester & Karkalas, 1996; Tester & Morrison, 1992). When reducing the starch content from 8 to 4% in low-fat meatballs, the firmness was reduced markedly both when measured with texture analyser and in the sensory analysis (**Paper IV**). The frying losses were increased, however only significantly when meatballs were fried in a pan and not when deep-fat fried (Paper IV). When the potato starch level was reduced from 6.5 to 3.2% in low-fat sausages, the frying loss increased significantly, while no change in firmness was measured by the texture analyser (**Paper IV**). These results suggest that swollen starch granules contribute to the texture of a meat product when it is more particulate in structure, such as in meatballs, whereas this is not the case in sausages, where the meat protein network dominates the structure. In both cases, the potato starch granules contribute to a lower frying loss of low-fat comminuted meat products.

Table 3. Change of properties in low-fat meat products compared to high fat meat products.

First author and year	High fat		Low fat		Sensory properties									
	Fat (%)	W/P	Fat (%)	W/P	Cooking loss	Texture/firmness	Crumbliness	Tenderness	Compactness	Juiciness	Meat taste	Colour	Off-flavour	Total impression
Sausages														
Peterson (Paper IV)	5	7.9	2	7.9	↑	≈	≈	-	≈	-	≈	→	≈	≈
Cofrades (2000)	30	3.6	5, 12	4.3-4.5	-	↓	↑	-	≈	↑	-	-	-	→
Hughes (1997)	30	3.6	5, 12	4.3-4.5	↑	-	-	-	-	-	-	↑ ^a	≈	→
Jimenez-Colmonero (1995)	22	4.5	10-20	4.0-6.7	-	↓	-	-	≈	≈	-	-	-	≈
Claus (1991)	30	5.1	10	6.7	↑	↓	-	-	↓	↑	-	↓ ^a	≈	-
Claus (1989)	30	4.9	5-20	4.7-7.3	↑	↓	-	-	≈	↑	-	↑	-	-
Meatballs/hamburgers														
Peterson (Paper IV)	9	7.4	1.5	7.4	↑ ^b	≈	≈	-	≈	↑	≈	↓	≈	≈
Troy (1999)	23	3.4	9	3.7	↓	↑	-	↓	-	↓	≈	-	≈	≈
Desmond (1998)	23	3.3	9	3.4	≈	↑	-	≈	-	→	≈	-	≈	≈
Brewer (1992)	20	-	8	-	≈	-	-	-	-	↓	≈	-	≈	-
Troutt (1992)	30	3.3	5-25	3.2-3.4	↓	↑	-	-	↑	→	≈	≈	≈	-

↑: increased value when the fat content is reduced, ↓: decreased value when the fat content is reduced

≈ : no significant difference

^a : Instrumentally measured, ^b : statistically different only for pan-frying, not for deep-frying

Table 4. Addition of cereal dietary fibre in sausages and meatballs

First author and year	Fat level control (%)	Fat level (%)	Additive (Information about the amounts added in meat products are within brackets)	Cooking loss	Texture/firmness	Sensory properties							
						Crumbliness	Tenderness	Compactness	Juiciness	Meat taste	Colour	Off-flavour	Total impression
Sausages													
Özvural (2009)	18	15	Brewer's spent grain (4% DF) ^c	-	↓	-	-	↓	-	-	↓	-	→
Aleson-Carbonell (2005)	14	13	Oat fibre (0.7% β-glucan)	↓	↓	-	-	-	↑	-	→	-	≈
Morin (2002, 2004)	14	12	Barley β-glucan (0.3, 0.8%)	↓ ^b	↓ ^b	-	-	↓	≈	↓	≈ ^a	↑	↓ ^b
Cofrades (2000)	5-30	5-30	Oat fibre (1.8% DF)	-	↑	-	-	↑	↓	-	-	-	≈
Hughes (1997)	5-30	5-30	Oat fibre (1.8% DF)	↓	-	-	-	-	-	-	≈ ^a	≈	≈
Meatballs/hamburgers													
Pinero (2008)	20	<10	Oat fibre (0.4% β-glucan)	↓	↓	-	≈	-	↓	-	≈	-	↑
Kumar (2004)	19	9	Barley flour (4-10% flour)	↓	↓	-	-	↓	≈	-	↓	-	→
Yilmaz (2004)	11	9-10	Rye bran (5-20% bran)	≈	↑	-	-	≈	↓	-	↑ ^a	-	→
Yilmaz (2003)	25	5-20	Oat bran (5-20% bran)	-	↑	-	-	↑	↓	-	↑ ^a	-	≈
Mansour (1999)	17	4-13	Wheat bran (5-15% bran)	↓	↑	-	≈	-	≈	≈	-	-	≈
Desmond (1998)	9	11	Oat fibre (0.9% DF)	↓	↓	-	↑	-	↑	≈	-	↑	≈

↑: increased value when the fat content is reduced, ↓: decreased value when the fat content is reduced, ≈: no significant difference

^a Instrumentally measured, ^b Decreased values for 0.8% β-glucan, while unchanged values for 0.3% β-glucan, ^c Brewer's spent grain is the main by-product from the brewing process and it contains a high amount of insoluble DF from barley (composition not reported).

6.2 Dietary fibre in sausages

Due to the interest in making new healthier products, there have been a number of studies where the effects of adding dietary fibre to meat products have been evaluated. In Table 4 some of the references where cereal materials have been added to sausages or meatballs/hamburgers are summarized.

Adding β -glucans rich materials from oat or barley in sausages seem to be especially popular according to the number of papers published. Morin et al (2002) found a higher limit of how much soluble barley β -glucan they could add to sausages without destroying its texture. Addition of 0.8% soluble barley β -glucan decreased the firmness of the sausage, while adding 0.3% had a similar texture as the reference.

When adding β -glucan rich cereal materials to sausages, it has been shown that the cooking loss can be reduced (Table 2). On the other hand, the hardness is often reduced as an effect of the addition of DF. Cofrades et al (2000) reported an increased firmness when oat fibre was added to sausages. 2% of the oat fibre was added, which included about 1.8% dietary fibre. The composition of this fibre was not reported, but since it is an oat fibre, it can be presumed that the dietary fibre to a great extent consist of β -glucans. It could be that these sausages got an increased firmness, which was not the case for the other ones, because of the greater content of β -glucan compared to the others and also that the source of β -glucan was oat and not barley. The differences between these β -glucans have been mentioned earlier and will be further discussed later on. Comparison of the results from different studies is difficult since there are many important factors that may vary and influence the results, the meat quantity, quality and fat content, the methodology of making the sausages, the degree of comminution and salt content. To be able to compare the effects of different additives in a sausage, these should be compared under the same conditions for the meat proteins, as the latter are so crucial in determining the physicochemical properties of the sausages.

Rye bran, oat bran and barley fibre in low-fat sausages

All additives used in the present studies, were added to give the product a constant dietary fibre content of 1%. The recipes used when making the sausages are shown in Table 5, where also the amount of soluble DF, soluble β -glucan, total β -glucan and arabinoxylan are presented.

Table 5. Recipes for sausages (g ingredient per 100 g batter)

Ingredient	HFHS ¹	LFHS ¹	LFLS ¹	Rye bran	Oat bran	Barley fibre
Water/ice	41.1	43.9	46.2	45.2	44.7	45.5
Meat ²	48.4	45.6	47.3	46.4	45.8	46.7
Spices and additives ³	2.57	2.57	2.57	2.57	2.57	2.57
Potato flour	8.0	8.0	4.0	2.9	1.0	3.6
Cereal additive	-	-	-	2.9	6.0	1.7
Soluble DF ⁴	-	-	-	0.08	0.31	0.39
Soluble β -glucan	-	-	-	0.03	0.18	0.33
Total β -glucan	-	-	-	0.11	0.55	0.48
Total AX	-	-	-	0.68	0.28	0.31

¹ HFHS-High Fat, High Starch, LFHS-Low Fat, High Starch, LFLS-Low Fat, Low Starch

² 60% pork and 40% low- or high-fat beef

³ Black pepper (0.1 g), nitrite salt (0.72 g), vacuum salt (1.28 g), ascorbic acid (0.02 g), polyphosphate (0.15 g) and liquid smoke (0.3 g)

⁴ Amount of soluble DF added by the cereal additive

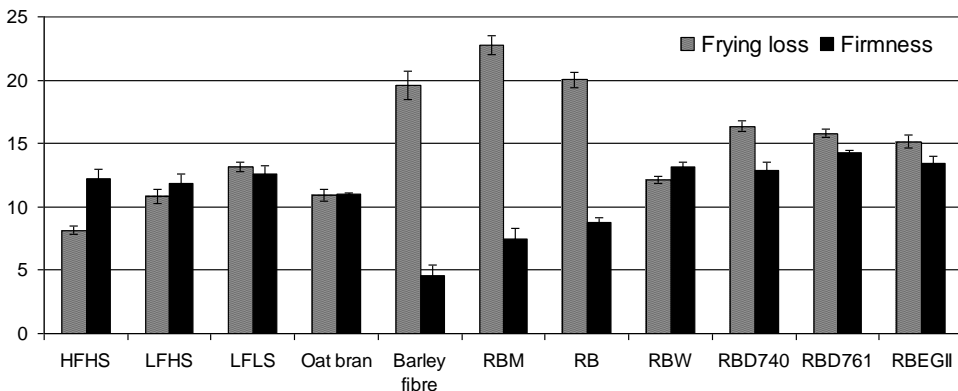


Figure 15. Frying loss (%) and the firmness (N) of the sausages.

HFHS-High Fat, High Starch; LFHS-Low Fat, High Starch; LFLS-Low Fat, Low Starch; RBM-Rye bran milled; RB-Rye bran; RBW-Rye bran treated in water; RBD740-Rye bran treated with the enzyme Depol D740; RBD761-Rye bran treated with the enzyme Depol D761; RBEGII-Rye bran treated with the enzyme EGII

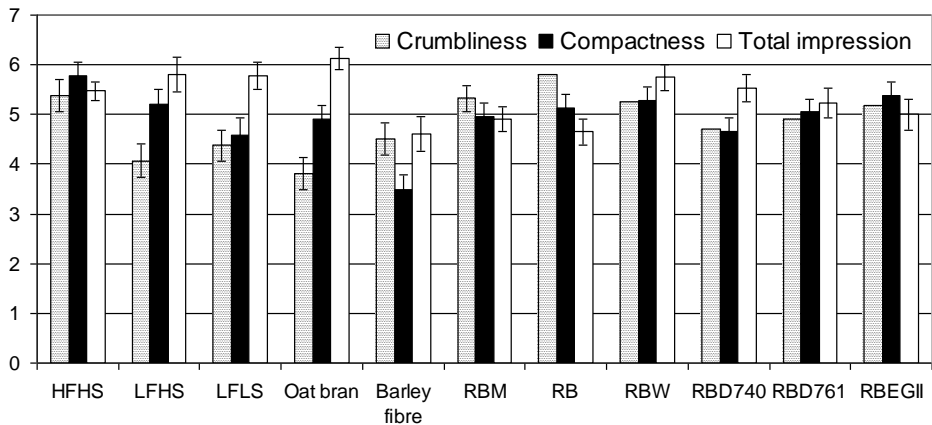


Figure 16. Mean values for some attributes from the sensory analysis of the sausages. HFHS-High Fat, High Starch; LFHS-Low Fat, High Starch; LFLS-Low Fat, Low Starch; RBM-Rye bran milled; RB-Rye bran; RBW-Rye bran treated in water; RBD740-Rye bran treated with the enzyme Depol D740; RBD761-Rye bran treated with the enzyme Depol D761; RBEGII-Rye bran treated with the enzyme EGII

All together, eight sausages with different additives were evaluated. The results of the frying loss and firmness are shown in Figure 15, while the results of the sensory evaluation are shown in Figure 16. In Table 6, an overview of the effects of the additives on the sausages and meatballs can be seen.

Six different rye bran alternatives were evaluated as additives to low-fat sausages: milled and not milled dry rye bran (RBM and RB), and rye bran pre-treated in water (50 °C, 4 hours) with or without enzymes (**Paper IV**). The rye bran treated in water without any enzymes are denoted RBW in Figure 15, while the enzymatic treated brans are denoted RBD740, RBD761 and RBEGII. The enzymes used have been discussed earlier and are included in the study presented in **Paper III**. It was found that the pre-treatment in water had a big impact on the performance of the rye bran, concerning the texture and frying losses, when added to low-fat sausages. The addition of enzymes to the water did not improve the results any further, but rather the contrary (Figure 15). The samples with enzymes added are however better than the RB and RBM. By soaking the rye bran in water at 50°C, the endogenous enzymes may have been active and solubilised some of the dietary fibres. When adding the hydrolysing enzymes, there was degradation and solubilisation of insoluble dietary fibre, but a simultaneous degradation of the soluble dietary fibres into smaller fragments. If the fragments were too small they could no longer have any impact on the gel forming ability and thereby the texture of the sausages. For the sausage with rye bran treated in only water (RBW), the highest value of total impression was perceived by the sensory panel, when

compared to the other rye bran samples. The differences between the values of the sensory attributes were however not significant for the different rye bran samples.

When barley fibre was added to low-fat sausages (**Paper V**), it resulted in high cooking losses and a soft texture of the sausages (Figure 15 and 16). Oat bran on the other hand was found to be the most suitable for addition to low-fat sausages. These sausages exhibited low process and frying losses and had high values of both firmness and sensory acceptance as can be seen in Figure 15 and 16. None of the attributes of the oat bran sausages were improved compared to the low fat, low starch (LFLS) sample, but they were however not reduced compared to the values of the low fat, high starch (LFHS) samples. The milled rye bran without any other treatment (RBM) is the rye bran sample which should be compared to the oat bran and barley fibre, since they were prepared in the same way (just milled). The firmness of the sausages with rye bran included was in between the barley fibre and oat bran sausages. The rye bran had the highest cooking loss of the three materials, which could explain the higher firmness compared to the barley fibre.

Rye bran, Oat bran and barley fibre were mixed together with water and potato starch in the same amounts as in the sausages, but without meat and salt, to investigate the influence on the texture by the different cereal materials. These mixtures were heated in the same manner as the sausages (in plastic cups, put in a water bath until a temperature of 75°C of the mixture was reached) and then stored at 7 °C over night. Rheological measurements were performed at 20°C (Figure 17) and the morphologies were studied (Figure 18).

In Figure 17, the stress sweeps of the heated mixtures of cereal materials, water and with or without potato starch are shown. The mixtures containing rye bran did not create a gel, with or without the addition of potato starch (results not included in the figure). In both cases the rye bran particles sedimented rapidly. Barley fibre in water did not form a gel either, and very low values of the elastic modulus (G') and the viscous modulus (G'') were recorded (not shown). When a mixture of oat bran, water and potato starch was heated, a strong gel was created, with a high value of G' , as can be seen in Figure 17. The value of G' was much higher than G'' in the linear viscoelastic region, where G' is independent of shear stress, indicating a gel network. The mixture of oat bran and water also created a gel with a G' almost as high as that of barley fibre together with water and potato starch. The ability to form a gel upon heating is probably the reason to why oat bran was the most suitable cereal material to be used in low-fat sausages.

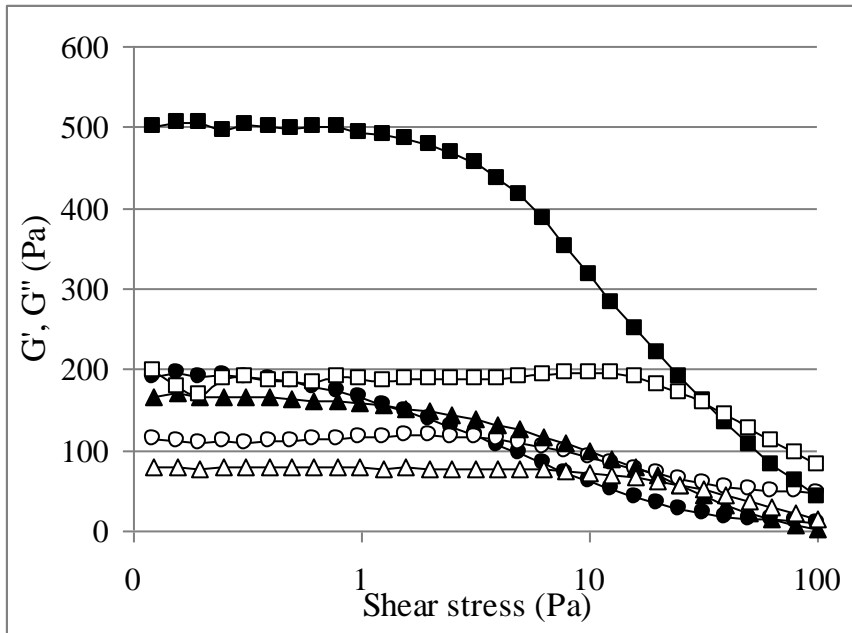


Figure 17. Rheological properties of the heated cereal fibre additives mixed with water and with or without added potato starch. Filled symbols show G' and open ones G'' . \square - oat bran + potato starch, \circ - barley fibre + potato starch, Δ - oat bran without potato starch. The suspensions of cereal fibres and potato starch were mixed in the same proportions as in the sausage recipes and heated as in the preparation of the sausages.

The total amount of starch was set to be constant in the sausages, meaning that the amount of potato starch depended on the level of cereal starch added (Table 5). The amount of potato starch was lowest in the oat bran sausages, but still these had the lowest cooking loss of the sausages with cereal additives and those sausages also had a firmness comparable to the high starch references (**Paper V**). Oat bran had the highest amount of starch among the cereal additives but the swelling ability of the starch is much less than for the potato starch, so there must be an additional factor of the oat bran that makes it so suitable in sausages.

Mixtures with only potato starch and water were also prepared and heated, using the same amount of potato starch as in the mixtures when also cereal materials were included. After heating, followed by cold storage over night, the potato starch in all cases, except for the low amount used together with oat bran, had formed strong gels which were capable of holding the structure together, so that the cylinder of gel could stand up alone. Together with barley fibre and rye bran, the potato starch in the same amounts does not form such strong gels. The particles lie in between the starch granules (Figure 18) and interfere with the gel

forming ability. In the case of rye bran, enzymatic activity and hydrolyzation of the starch cannot be excluded since the rye bran material has not been heat treated.

Another factor which differs between the samples with added cereal materials is the amount of cereal proteins. Since the oat bran had a low DF content, a high amount of bran was added to receive 1% DF in the sausage and therefore also a relatively high amount of proteins (three times more than in the barley fibre sausage). Oat proteins has been shown to have a high solubility only if the pH is low (optimum at pH 2.2) or high (optimum at pH 9.2) (Wu, Sexson, Cluskey, & Inglett, 1977), and should not have any big impact on the gel forming ability of the oat bran in sausages.

The concentrations of dietary fibre in the mixtures used for the rheological measurements were about twice the one used in the sausages, since the same amounts of water and cereal materials were used and the meat (which was about 50% the sausage ingredients) was excluded. The amount of the available water for the dietary fibre should thus be similar in this mixture as in the sausages. The total dietary fibre concentration of the mixtures with cereal materials, potato starch and water was 2.0, 1.9 and 2.0% for rye bran, oat bran and barley fibre respectively. The small differences are due to the varying amount of material added to reach the 1% dietary fibre level of the sausage. Since the soluble amount of β -glucan given in Table 5 is measured after extraction in cold water, the true amount of soluble β -glucan in the sausages will most likely be higher due to the heat treatment. The total amounts of β -glucan in the oat bran and barley fibre mixtures are 1.1% and 0.9%.

Oat β -glucan is known to have higher molecular weights compared to barley β -glucan. That together with the lower ratio of tri- to tetramers in oat β -glucan, makes the gelling ability greater for the oat β -glucan (Ryu, Lee, You, Shim, & Yoo, 2012). Moreover, the barley fibre is a more processed material compared to the oat bran, and the molecular weight might have been reduced. The difference in gel forming ability is clearly seen in the rheological measurements (Figure 17) and explains why the oat bran is the most suitable additive in the sausages.

The same mixtures of cereal material, potato starch and water were also studied in microscope, prior and after heating to 75°C. Clear differences are seen between the three mixtures in Figure 18. Upon heating, the oat bran material itself seems to create a gel network in water and the swollen potato starch lie within the network and thereby improving the strength of the gel even further (Figure 18D). The mixture with barley fibre has the highest content of potato starch added, which is clearly seen in Figure 18A and E. In these two figures, the swelling capacity of the potato starch granules is also obvious. Even so, this mixture was not capable to

create a gel as strong as the one with oat bran, which could be seen in Figure 17. In the micrographs of the rye bran mixture (Figure 18C and F), large particles are seen, which seem to be rather unaffected by the heating and no gel network formation is observed.

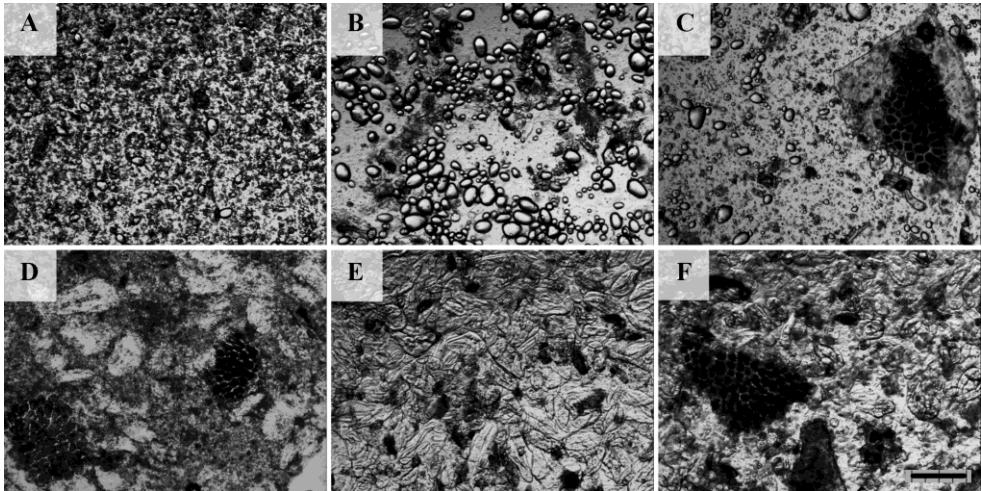


Figure 18. Micrographs of cereal materials mixed with water and potato starch. The top row shows the non-heated mixtures; the bottom row the mixtures heated to 75°C. Left: oat bran, centre: barley fibre, right: rye bran. The scale bar corresponds to 200 μm .

Table 6. Changes of attributes when adding cereal materials corresponding to 1% dietary fibre in sausages and meatballs (**Paper IV** and **V**).

Fat level control (%)	Fat level (%)	Additive	Frying loss	Texture/firmness	Crumbliness	Compactness	Juiciness	Meat taste	Colour	Off-flavour	Total impression	Sensory properties					
Sausages																	
2	2	Rye bran	↑	→	≈	≈	-	≈	←	≈	≈	→					
2	2	Rye bran soaked in water	≈	≈	↑	≈	-	≈	↑	≈	≈	≈					
2	2	Oat bran	≈	≈	≈	≈	-	≈	≈	≈	≈	≈					
2	2	Barley fibre	↑	→	≈	(↓)	-	≈	←	≈	≈	→					
Meatballs (deep-fat fried)																	
1.5	1.5	Rye bran	≈ (↑)	≈ (↓)	≈	≈ (↓)	↓ (≈)	≈	←	≈	≈	≈					
1.5	1.5	Rye bran soaked in water	≈	≈ (↓)	≈	≈	≈	≈	←	≈	≈	≈					
1.5	1.5	Oat bran	≈	≈ (↓)	↓	≈ (↓)	↓ (≈)	≈	≈	≈	≈	≈					
1.5	1.5	Barley fibre	≈	≈ (↓)	≈	≈ (↓)	≈ (↑)	≈	←	≈	≈	≈					

↑: increased value compared to the low-fat reference (LFLS)

↓: decreased value compared to the low-fat reference (LFLS)

≈ : no significant difference

Signs within brackets: the changes compared to the low-fat, high starch references (LFHS) if different from the change compared to LFLS

6.3 Dietary fibre in meatballs

Rye bran, oat bran and barley fibre in low-fat meatballs

The recipes used when making the meatballs are shown in Table 7. The additives used were the same as for the sausages: oat bran, barley fibre and the 6 samples of non-treated or enzyme treated rye bran. All additives were added to give a constant dietary fibre content of 1%.

As discussed previously, the starch content of the meatballs is the dominating variable concerning the frying losses and the texture. None of the additives improve the firmness, measured with the texture analyser, or the frying loss compared to the low-fat, low-starch reference (Figure 19). The oat bran and barley fibre meatballs have lower frying losses compared to the rye bran meatballs and the barley fibre was the only additive which improved the loss compared to the low-fat, low-starch reference (LFLS). The values of the firmness of all sausages with additives were low and similar to the LFLS.

Table 7. Recipes for meatballs (g ingredient per 100 g batter)

Ingredient	HFHS ¹	LFHS ¹	LFLS ¹	Rye bran	Oat bran	Barley fibre
Water	17.9	24.7	27.6	26.9	26.5	27.1
Meat ²	51.3	44.5	46.7	46.0	45.3	46.3
Onion	11.6	11.6	11.6	11.6	11.6	11.6
Potato	9.2	9.2	9.2	9.2	9.2	9.2
Potato flour	8.7	8.7	3.6	2.8	1.5	3.4
Salt	1.3	1.3	1.3	1.3	1.3	1.3
White pepper	0.1	0.1	0.1	0.1	0.1	0.1
Cereal additive	-	-	-	2.1	4.3	1.3
Soluble DF ³	-	-	-	0.06	0.22	0.31
Soluble β -glucan	-	-	-	0.02	0.13	0.27
Total β -glucan	-	-	-	0.08	0.40	0.38
Total AX	-	-	-	0.49	0.20	0.25

¹ HFHS-High Fat, High Starch, LFHS-Low Fat, High Starch, LFLS-Low Fat, Low Starch

² Low- or high-fat beef

³ Amount of soluble DF added by the cereal additive

In Figure 20 the sensory attributes of crumbliness, compactness, juiciness and total impression are presented for all meatball samples. Meat taste, colour and off-flavour were also evaluated. There were no significant difference between the samples concerning the meat taste and off-flavour. All samples received values of above 5 for the meat taste and no sample had a value above 3.7 for the off-flavour. This should be considered positive since apparently none of the samples resulted in high off-flavours and therefore no strong cereal flavour.

There were some variations considered the colour of the meatballs. The high fat, high starch sample received a higher value together with the meatballs, where rye bran and barley fibre had been added (5.4-6.2). The oat bran and the two references with a lower fat content got lower values of the colour (4.3-4.5).

Addition of oat bran to low-fat meatballs resulted in a smoother texture, i.e. lower crumbliness, than for the other meatballs. This did not appear to be a preferable attribute for meatballs, since the oat bran meatballs also received lower value of the total impression by the sensory panel.

The differences seen in the sausages, concerning the effects of the rye bran that had been soaked in water (RBW) and the one that had not (RBM), were not seen in the meatballs. But in this case the rye bran gave in general the best total impression among the cereal additives studied. The meatballs with RBD761 added received the highest value of the total impression (6.4). This was not significantly different from the LFLS, but increased however compared to the milled, not treated rye bran (RBM). Rye bran was suitable for addition to meatballs, probably due to its particulate nature, which is more acceptable in this type of meat product, where the gelling properties are not as important as in sausages.

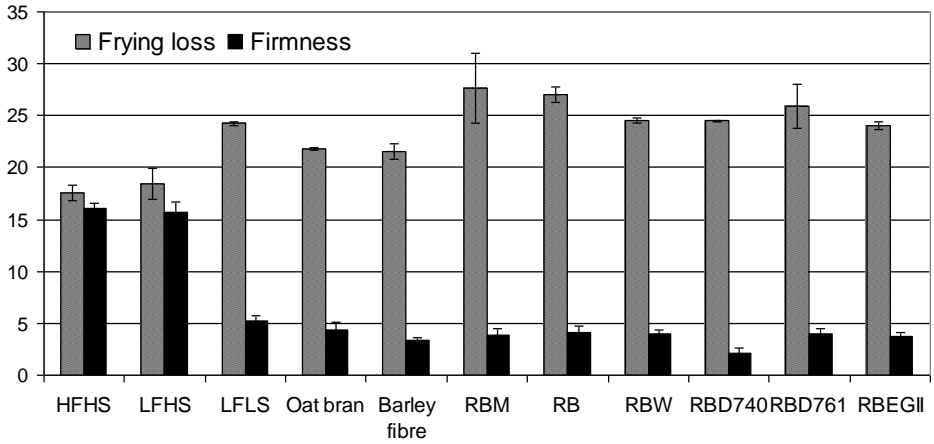


Figure 19. Frying loss (%) and firmness (N) of deep-fat fried meatballs. HFHS-High Fat, High Starch; LFHS-Low Fat, High Starch; LFLS-Low Fat, Low Starch; RBM-Rye bran milled; RB-Rye bran; RBW-Rye bran treated in water; RBD740-Rye bran treated with the enzyme Depol D740; RBD761-Rye bran treated with the enzyme Depol D761; RBEGII-Rye bran treated with the enzyme EGII

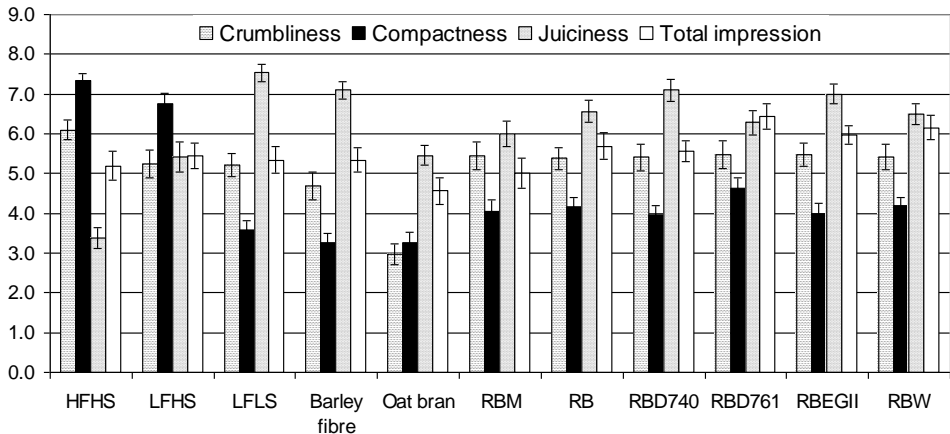


Figure 20. Mean values for some attributes from the sensory analysis of the meatballs. HFHS-High Fat, High Starch; LFHS-Low Fat, High Starch; LFLS-Low Fat, Low Starch; RBM-Rye bran milled; RB-Rye bran; RBW-Rye bran treated in water; RBD740-Rye bran treated with the enzyme Depol D740; RBD761-Rye bran treated with the enzyme Depol D761; RBEGII-Rye bran treated with the enzyme EGII

7. Conclusions

Four cereal materials have been studied for their physicochemical properties and suitability for addition to low-fat meat products. The materials had a large variation in dietary fibre content and composition. **Wheat and rye bran** had a high amount of DF (around 40%), which were mainly insoluble arabinoxylan and cellulose. **Oat bran** had a lower amount of DF (18%), while it had a higher content of soluble β -glucan (10%). The **barley fibre** was very rich in soluble β -glucan (22%).

Rye bran mainly consists of insoluble dietary fibre and was expected to be the most challenging material to include in new food products. The threshold for the sensation of grittiness resulting from rye bran particles in a starch gel was very low. The particles were detected already at concentrations of 0.1-0.3%, despite the fact that they were very small (20-180 μm). A sensory panel was able to detect an increase in particle concentration in a ranking test, but their ability to detect the increase in concentration was not influenced by particle size. The rheological properties of the suspension medium did not influence the detection threshold. One reason for this obvious perception of grittiness of rye bran particles could be their irregular shape and their hardness.

One way of altering these adverse properties of the bran particles could be by treating them with hydrolytic enzymes. None of the several xylanases and endoglucanases evaluated in this thesis did however increase the water-holding capacity of the bran or the viscosity of the aqueous phase, when evaluating the effects on **rye and wheat bran**. The water-holding capacity were probably reduced because of the lower amount of insoluble material after solubilization by the enzymes, while the lower viscosity showed that the solubilized dietary fibre were degraded into smaller molecules which could no longer contribute to the viscosity.

Two types of meat products, frankfurter-type sausages and meatballs have been evaluated in the thesis. In the sausages the meat protein network governs the texture and water-holding properties, whereas the meatballs have a more particulate structure, with higher frying losses and harder texture.

Due to its gelling ability upon heating, **oat bran** was found to be most suitable for addition to low-fat sausages, and these exhibited low process and frying losses, together with high values of both firmness and sensory acceptance. When adding **oat bran** to the meatballs, the result was a smoother texture, i.e. lower

crumbliness, than for the other meatballs; however, this did not appear to be a preferable attribute for meatballs.

The addition of untreated **rye bran** to sausages was detrimental, causing a substantial increase in frying loss. However, when rye bran that had been treated in water was added, the frying loss and firmness of the sausages were considerably improved. Enzymatic treatment of the rye brans did not improve the WHC or the texture of the sausages compared to the rye bran that had only been soaked in water. The enzymes probably degraded the solubilized fraction of the DF leaving only small fragments that cannot contribute to the texture and WHC of the sausages. **Rye bran** is suitable for addition to meatballs, probably due to its particulate nature, which is more acceptable in this type of meat product, where the gelling properties are not as important as in sausages.

The **barley fibre** was not a good additive in sausages despite its high content of soluble β -glucan. The addition of **barley fibre** led to high process and frying losses and a very low firmness of the sausages. This barley β -glucan could not form a gel, probably because of a smaller molecular weight and a less favourable structure, compared to the oat β -glucan. However, the addition of barley fibre may be suitable in meatballs, where it reduced the frying loss.

8. Future outlook

Oat bran could successfully be added to low-fat sausages, while rye bran and barley fibre were more suitable for the low-fat meatballs. In this investigation, only one concentration of dietary fibre has been used (1%). More information about how the different dietary fibre sources affect the meat systems could be given by also using other concentrations of the fibre sources.

In the study, only rye bran was treated in different ways before it was added to the meat products. It would be interesting to study how similar treatments would affect the oat bran and barley fibre and if this would influence their suitability as additives.

In the beginning of this study, the barley fibre was anticipated to be a good alternative to add to low-fat sausage, due to the high β -glucan content. This was however not the case, probably due to a low molecular weight of the β -glucan. This material was provided as a high content β -glucan material and we do not know much about the process it has been through. It would be very interesting to investigate if this process could be optimized to give an improved barley β -glucan, with better rheological properties.

To be able to add a health claim concerning the β -glucan content on the package, 1% β -glucan per portion is needed. The oat bran sausage contained 0.55 g β -glucan per 100 g sausage. A portion of 180 g sausage would then be needed to include 1% β -glucan. A more realistic portion size would be smaller and the concentration of added β -glucan would have to be increased. This is another reason to investigate varying concentrations of the addition of cereal dietary fibre to meat products.

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