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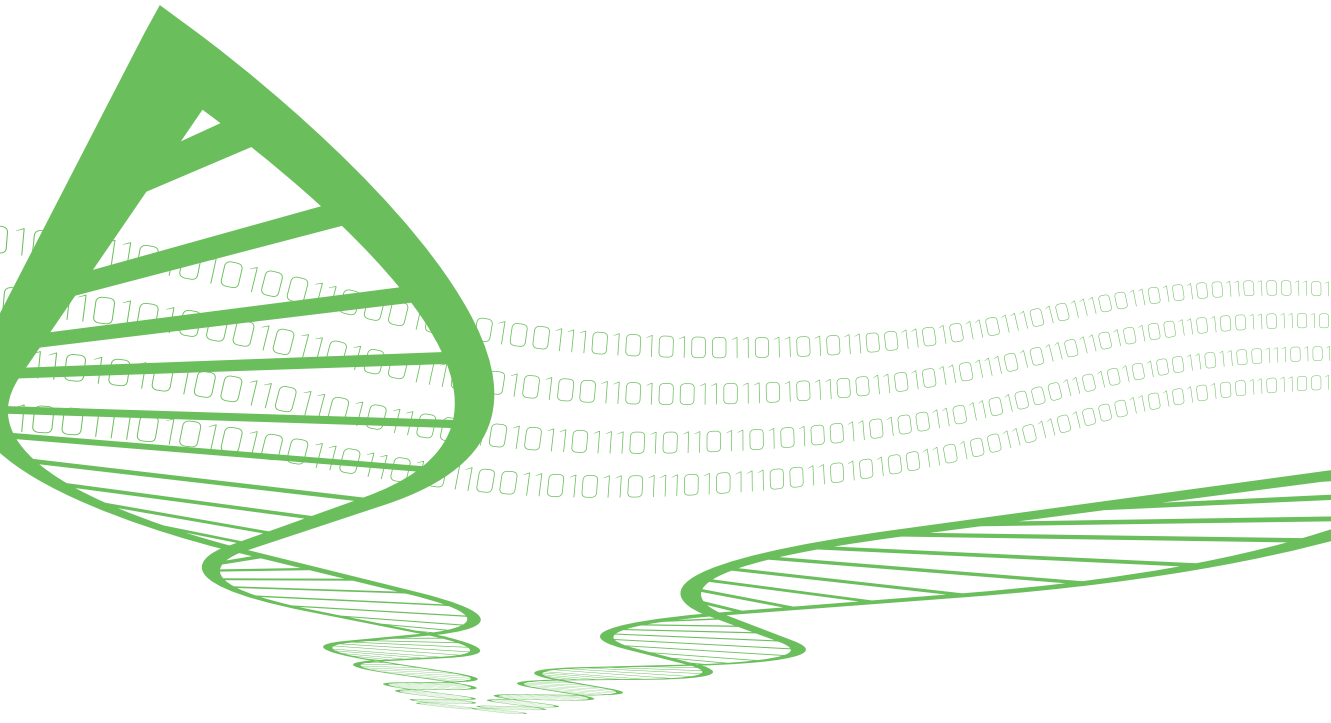
Impact of water content on enzymatic modification of wheat bran

Outi Santala



VTT SCIENCE 59

Impact of water content on enzymatic modification of wheat bran

Outi Santala

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Impact of water content on enzymatic modification of wheat bran

Vesipitoisuuden vaikutus vehnäleseen entsymaattisessa muokkauksessa. **Outi Santala.** Espoo 2014. VTT Science 59. 97 p. + app. 52 p.

Abstract

Enzymatic treatments of plant-based materials are generally conducted in excess water because reduction of water content usually decreases enzymatic conversion. Processing at high solids content would offer economical advantages, but in the area of enzymatic bioprocessing of plant materials for food applications, the role of water content has seldom been studied. Wheat bran is one of the most important by-products of the cereal processing industry and comprises the outer tissues of grain. Bran is a good source of dietary fibre (DF), protein and phytochemicals, but its use in food applications is limited because unprocessed bran is usually detrimental to product quality. The present work aimed to examine and develop techniques to utilize hydrolytic enzymes, especially xylanase, at reduced water content in order to increase the technological functionality of wheat bran in food applications.

The impact of water content on the action of xylanase was studied by treating wheat bran with a commercial xylanase enzyme preparation at water contents ranging from 20 to 92% using different processing methods including both continuous mixing and short pre-mixing combined with stationary incubation. The action of xylanase was measured by the solubilisation of bran arabinoxylan (AX), the main DF polysaccharide of wheat. The minimum required water content for the action of xylanase on wheat bran during continuous mixing was between 20 and 30%, corresponding to a water activity (a_w) of 0.83–0.89. Xylanase action was significantly enhanced at a water content of 40% (a_w 0.93), at which the granular material was transformed to a continuous paste. AX was solubilised at a similar level at 40 and 90% water contents when continuous mixing was used, but at water contents of 50–80% AX solubilisation was lower. Furthermore, it was shown that the use of an extruder for pre-mixing and forming a bran-water mixture increased the action of xylanase during stationary incubation at a water content of ≤54%, as compared to pre-mixing with a blade-mixer. The results indicated that the formation of a continuous paste is important for efficient enzyme action at low water content, and that it is possible to increase the enzyme action by changing the granular structure of the material to a continuous paste using an extruder, without increasing the water content. The extruder-aided pre-mixing process enabled efficient xylanase action at low water content without the requirement for continuous mixing.

Neither water content nor processing method affected the apparent average molecular weight (MW) of water extractable AX (WEAX) precipitated with 65% EtOH at water contents above 40%. When bran was treated with continuous mixing,

the A/X ratio of the bran water extract decreased similarly at both water contents of 40% and 90%, suggesting that AX was solubilised from the same bran tissues regardless of the processing conditions studied. The bran treated at a water content of 40% was characterized by higher solubilisation of DF polysaccharides, smaller average particle size, lower water holding capacity and more changes in bran proteins than the treatment at a water content of 90%. The more intensive changes in the properties of bran treated at low water content were related to the compact consistency and thus higher impacts of shear exerted on the bran-water mixture. Reduction of particle size, either prior to the treatment by grinding or during the treatment by intensive mixing and shear, was shown to enhance AX solubilisation and xylanase action, presumably due to improved substrate availability as a result of increased surface area. Small particle size also favoured the transformation of the bran-water mixture from granular mass to a continuous paste, which also enhanced enzyme action.

The technological functionality of modified bran was demonstrated in endosperm-flour based expanded extrudates supplemented with 20% of bran. Bran was treated with commercial xylanase and cellulase enzymes at a water content of 48% using the extrusion-aided low-water process, followed by oven or freeze drying. The modified bran ingredients increased the crispiness and reduced the hardness and bulk density of the bran-enriched expanded extrudates. The improvements in extrudate properties were attributed to the increased WEAX content and decreased water holding capacity of the modified brans.

The results of the work showed that enzymatic solubilisation of bran AX and improved technological functionality of bran can be achieved by enzymatic modification at a water content of 40–50%, which is well below the point of absence of free bulk water (70–80%). The consistency of the reaction mixture, mixing method and bran particle size were found to be important factors affecting the intensity of the modification process at low water content. The results can be utilized for improving the technological functionality of bran in food applications and for developing new processes for the enzymatic modification of plant raw materials at reduced water content.

Keywords wheat bran, arabinoxylan, xylanase, enzymatic modification, solubilisation, water content, high solids hydrolysis, bran particle size, high torque mixing, stationary incubation, extrusion, mechanical properties, extrudate structure

Vesipitoisuuden vaikutus vehnäleseen entsymaattisessa muokkauksessa

Impact of water content on enzymatic modification of wheat bran. **Outi Santala.**
Espoo 2014. VTT Science 59. 97 s. + liitt. 52 s.

Tiivistelmä

Kasviperäisten materiaalien entsymaattinen muokkaus tehdään yleensä suuressa vesimäärässä, koska vesipitoisuuden vähentäminen useimmiten heikentää entsyymien toimintaa. Teollisissa prosesseissa vesipitoisuuden vähentäminen toisi taloudellisia hyötyjä, mutta toistaiseksi vesipitoisuuden vaikutusta kasvimateriaalien entsymaattisessa muokkauksessa elintarvikesovelluksia varten on tutkittu hyvin vähän. Jyvän kuorikerroksista koostuva vehnälese on yksi viljateollisuuden tärkeimmistä sivutuotteista. Lese on hyvä ravintokuidun, proteiinin ja fytokemikaalien lähde, mutta sen käyttö elintarvikkeissa on hankalaa, koska käsittelemätön lese yleensä heikentää tuotteen laatua. Työn tarkoituksena oli tutkia ja kehittää menetelmiä hydrolyyttisten entsyymien, erityisesti ksylanaasien, käyttämiseen matalassa vesipitoisuudessa vehnäleseen teknologisen toimivuuden lisäämiseksi elintarvikesovelluksissa.

Vesipitoisuuden vaikutusta ksylanaasin toimintaan tutkittiin käsittelemällä vehnälesettä kaupallisella ksylanaasia sisältävällä entsyymiseoksella vesipitoisuuksissa 20–92 %. Työssä käytettiin erilaisia prosessointimenetelmiä, joissa lesettä sekoitettiin inkuboitaessa joko jatkuvatoimisesti tai vain lyhytaikaisesti käsittelyn alussa. Ksylanaasin toiminta mitattiin määrittämällä vehnän tärkeimmän ravintokuitukomponentin, arabinoksylaanin (AX) liukenemista. Vähimmäisvesipitoisuus, jossa ksylanaasi alkoi toimia jatkuvaa sekoitusta käytettäessä, oli 20:n ja 30 prosentin välillä, vastaten veden aktiivisuutta (a_w) 0.83–0.89. Ksylanaasin toiminta tehostui huomattavasti vesipitoisuudessa 40 % (a_w 0.93), jossa koostumukseltaan rakeinen materiaali muuttui yhtenäiseksi plastiseksi massaksi. AX:n liukeneminen jatkuvan sekoituksen prosessissa oli yhtä tehokasta 40:n ja 90 %:n vesipitoisuuksissa, mutta vesipitoisuuksissa 50–80 % AX:a liukeni vähemmän. Lisäksi osoitettiin, että verrattuna lapasekoittimeen lese-vesimassan esisekoitus ekstruuderilla ja sen aikaansaama plastisen massan muodostuminen tehosti ksylanaasin toimintaa ilman sekoitusta tapahtuvassa inkuboinnissa ≤54 %:n vesipitoisuudessa. Ekstruuderiavusteinen prosessi mahdollisti ksylanaasin tehokkaan toiminnan matalassa vesipitosuudessa ilman jatkuvaa sekoitusta. Tulokset osoittivat, että yhtenäisen, plastisen massan muodostuminen on tärkeää tehokkaalle entsyymin toiminnalle matalassa vesipitoisuudessa ja että entsyymin toimintaa on mahdollista tehostaa nostamatta vesipitoisuutta muuttamalla materiaali rakeisesta yhtenäiseksi massaksi ekstuuderin avulla.

Käsittelyn vesipitoisuus tai prosessointimenetelmä ei vaikuttanut 65 %:n etanolipitoisuudessa saostetun vesiliukoisen AX:n (WEAX) keskimääräiseen molekyyli-

painoon yli 40 %:n vesipitoisuudessa. Kun käytettiin jatkuvaa sekoitusta, leseen vesiuutteen arabinoosi-ksyloosisuhde laski samalla tavalla vesipitoisuuksissa 40 ja 90 %, viitaten siihen että liuennut AX oli lähtöisin samoista leseen soluseinän osista riippumatta käytetyistä prosessiolosuhteista. Verrattuna 90 %:ssa käsiteltyyn leseeseen 40 %:n käsittelyn jälkeen lese sisälsi enemmän liukoisia ravintokuitupolysakkarideja, leseen proteiineissa havaittiin enemmän muutoksia ja leseen partikkelikoko ja vedensidontakapasitetti oli pienempi. Suuremmat muutokset matalassa 40 %:n vesipitoisuudessa käsitellyssä leseessä johtuivat todennäköisesti seoksen kompaktista rakenteesta ja siitä johtuvasta suuremmasta leikkausvoimien vaikutuksesta leseeseen. Partikkelikoon pienentäminen joko ennen käsittelyä lesettä jauhamalla tai käsittelyn aikana tehokkaan sekoituksen ja leikkausvoimien vaikutuksesta ja siitä seurannut partikkelien pinta-alan kasvu lisäsi AX:n liukenemista ja ksylanaasin toimintaa, mikä todennäköisesti johtui substraatin saatavuuden parantumisesta. Pieni partikkelikoko myös edesauttoi seoksen rakenteen muuttumista rakeisesta yhtenäiseksi massaksi, mikä myös tehosti entsyymin toimintaa.

Muokatun leseen teknologinen toimivuus osoitettiin endospermijauhopohjaisissa puffatuissa ekstrudaateissa, joissa 20 % jauhoista oli korvattu leseellä. Lesettä käsiteltiin ensin kaupallisilla ksylanaasi- ja sellulaasientsyymeillä 48 %:n vesipitoisuudessa käyttämällä ekstruusioavusteista matalan vesipitoisuuden prosessia, jonka jälkeen lese kuivattiin joko uuni- tai kylmäkuivauksella. Muokatut lesetuotteet paransivat lesettä sisältävien ekstrudaattien rapeutta ja vähensivät niiden kovuutta ja tiheyttä. Ekstrudaattien ominaisuuksien parantumisen katsottiin johtuvan muokattujen leseiden kasvaneesta WEAX-pitoisuudesta ja pienentyneestä vedensidontakapasiteetista.

Tulokset osoittivat, että leseen AX:n entsymaattinen liuottaminen ja leseen teknologisten ominaisuuksien parantaminen on mahdollista tehdä entsymaattisella muokkauksella 40–50 %:n vesipitoisuudessa, joka on selvästi matalampi kuin rajapitoisuus (70–80 %) jossa kaikki seoksen vesi on sitoutuneena leseeseen. Reaktioseoksen fysikaalinen koostumus, sekoitusmenetelmä sekä leseen partikkelikoko todettiin tärkeiksi tekijöiksi, jotka vaikuttavat muokkausprosessin tehokkuuteen matalassa vesipitoisuudessa. Tutkimuksen tuloksia voidaan hyödyntää leseen teknologisen toimivuuden parantamiseen elintarvikesovelluksissa. Tulosten perusteella voidaan myös kehittää uusia entsymaattisia prosesseja kasvimateriaalien muokkaamiseksi matalassa vesipitoisuudessa.

Avainsanat wheat bran, arabinoxylan, xylanase, enzymatic modification, solubilisation, water content, high solids hydrolysis, bran particle size, high torque mixing, stationary incubation, extrusion, mechanical properties, extrudate structure

Preface

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My deepest gratitude goes to my thesis advisors, Academy Professor Kaisa Poutanen and Dr. Emilia Nordlund for giving invaluable guidance, support and motivation during this project. I thank Kaisa for introducing me into the fascinating world of food and cereal science; her passion and enthusiasm are truly inspiring. I am forever thankful to Kaisa for believing in me and for the time she has given me despite her extremely busy schedule. I warmly thank Emilia for always having the door open for my questions, for her excellent advice and for replying my emails within 24 h even during her two maternity leaves. One can truly admire her efficiency. I also wish to thank my former thesis advisor Dr. Pekka Lehtinen for guidance and support during the first two years of my studies.

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I extend my heartfelt thanks to my family, relatives and friends. I warmly thank my dear parents Helena and Kalevi and my dear brothers Tuomas and Samuli for their love and support throughout my life. A Huge thanks also goes to all my awesome friends for support and for bringing enlightenment and joy to my days!

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Espoo, May 2014

Outi Santala

Academic dissertation

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Supervising professor	Professor Heikki Ojamo Department of Biotechnology and Chemical Technology Aalto University Espoo, Finland

List of publications

This thesis is based on the following original publications which are referred to in the text as I–IV.The publications are reproduced with kind permission from the publishers.

I Santala, O., Lehtinen, P., Nordlund, E., Suortti, T., & Poutanen, K. (2011). Impact of water content on the solubilisation of arabinoxylan during xylanase treatment of wheat bran. Journal of Cereal Science, 54, 187–194.

II Santala, O., Nordlund, E., & Poutanen, K. (2013). Treatments with xylanase at high (90%) and low (40%) water content have different impacts on physicochemical properties of wheat bran. Food and Bioprocess Technology, 6, 3102–3112.

III Santala, O., Nordlund, E., & Poutanen, K. (2013). Use of an extruder for pre-mixing enhances xylanase action on wheat bran at low water content. Bioresource Technology, 149, 191–199.

IV Santala, O., Kiran, A., Sozer N., Poutanen, K., & Nordlund, E. (2014). Enzymatic modification and particle size reduction of wheat bran improves the mechanical properties and structure of bran-supplemented expanded extrudates. Journal of Cereal Science, in press.

Author's contributions

I The author planned the work together with Prof. Kaisa Poutanen, Dr. Pekka Lehtinen and Dr. Emilia Nordlund, and carried out the experimental work. The author interpreted the results together with the other authors. Dr. Tapani Suortti had the main responsibility for the HP-SEC analysis of arabinoxylan. The author was responsible of writing the publication in cooperation with Prof. Kaisa Poutanen, Dr. Emilia Nordlund and Dr. Pekka Lehtinen.

II The author planned the work together with the other authors and carried out the bran treatments with the help of a trainee (Ninon Piacere). The author carried out the analyses, except for the gas chromatography analyses, and interpreted the results together with the other authors and MSc. Ulla Holopainen (microscopy). The author wrote the publication in cooperation with the other authors.

III The author was responsible for planning the work together with the other authors. The author conducted the bran treatments with the help of a trainee (Anish Kiran) and carried out the analyses except for dietary fibre analyses. The author intreperted the results and wrote the publication in cooperation with the other authors.

IV The author had the main responsibility for planning the work in collaboration with the other authors. The author carried out the bran treatments and the production of the expanded extrudates together with an MSc student Anish Kiran, who performed the analyses with the help of technicians. The author supervised the MSc work of Anish Kiran together with Dr. Emilia Nordlund. The author interpreted the results together with Dr. Emilia Nordlund and Dr. Nesli Sozer and wrote the paper in cooperation with the other authors.

Contents

List of abbreviations

a_w	water activity
AX	arabinoxylan
A/X	arabinose to xylose ratio
AGP	arabinogalactan peptide
C_i	crispiness index
DF	dietary fibre
DM	dry matter
DTT	dithiothreitol
Dv50	median of the volumetric distribution of particle sizes
ER	expansion rate
F_{cr}	crushing force
F_{max}	maximum point of the force-deformation curve
GC	gas chromatography
GH	glycosyl hydrolase
HPLC	high performance liquid chromatography
HP-SEC	high performance size exclusion chromatography
MW	molecular weight
SDS	sodium dodecyl sulphate
WEAX	water extractable arabinoxylan
WBC	water binding capacity
WHC	water holding capacity
WUAX	water unextractable arabinoxylan

1. Introduction

Waste and side streams of agriculture, forestry and the food industry are currently considered as valuable resources for conversion to value added products such as food, feed, fuel and chemicals. These residues include renewable materials such as brans, straws, cobs, grasses and woody biomass, and are mainly composed of plant cell wall constituents, i.e. cellulose, hemicellulose and lignin (Singh nee' Nigam et al. 2009; Modenbach and Nokes 2013). Bran is one of the most important by-products of the cereal industry. Bran comprises the outer layers of grain separated in the milling process during the production of refined flours. High nutritional quality, especially the high content of dietary fibre, protein and phytochemicals, makes bran an interesting raw material for food products, but it is currently under-utilized as a food ingredient due to its technological and sensory challenges. In baked and extruded products bran is known to have an adverse effect on the volume, texture, flavour and appearance of the product (Coda et al. 2014; Lebesi and Tzia 2011; Robin et al. 2011a; Brennan et al. 2008).

Enzymatic conversions have a fundamental role in several industrial food manufacturing processes and in the upgrading of agro-industrial residues with the aim of increasing the efficacy of the production processes and utilization rate of the raw material, and improving the sensory quality of the end product. Hydrolases are the most commonly used enzymes in industrial processes, and depending on the process the aim is either complete or partial degradation of the substrate. Enzymes are also commonly used in the cereal industry. Solubilisation and degradation of cell wall components of bran by bioprocessing with hydrolytic enzymes such as xylanases has shown potential as a means to improve the technological and nutritional properties of the bran in food applications (Coda et al. 2014; Lebesi and Tzia 2011; Mateo Anson et al. 2011; Nordlund et al. 2013).

In current industrial processes, enzymatic reactions are typically conducted in excess water due to technological considerations. In general, reduction of the water content leads to reduced enzymatic conversion. However, increasing interest in reduction of water content as a means of improving the overall economy of industrial processes has promoted research in the area of processing of lignocellulosic materials under high-solids conditions. In the area of enzymatic processing of plant materials for food applications, the role of water content has been studied to a much smaller extent.

1.1 Enzymatic processing of plant-based materials at low water content

1.1.1 Role of water in enzymatic processing

Water has a unique role in biological structure and function. Enzymes are generally stable and active catalysts in their natural, cellular microenvironments. In addition to the role of water in maintaining the structure of enzyme proteins, water molecules can mediate enzymatic catalysis either directly by taking part in the reaction or indirectly by providing a medium for the reactants and products (Simpson et al. 2012). In relation to its amount and availability, water affects several physicochemical properties of plant-based substrate materials, with effects on the rate of enzymatic reactions.

Biological function of water in retaining protein structure

Water plays a crucial role in maintaining the active conformation of enzymes through hydrophobic and other non-covalent interactions that provide thermodynamic stability to folded protein structures in aqueous solution. The degree of enzyme hydration required for function is not fully resolved, but despite the varying estimates, 0.2 g H_2O /g protein is generally accepted as a threshold value of water required for enzyme activity, which is actually less than the amount of water needed for the formation of a monomolecular layer of water molecules on the polar groups of protein (Lind et al. 2004; Beliz et al. 2009).

It is generally accepted that completely anhydrous solvents do not support enzymatic activity, but that enzymes may be active even in almost non-aqueous solvents containing only traces of water. The use of enzymes in non-aqueous solvents has been studied intensively since the 1980s as a means of producing chemicals by reactions that are not feasible in aqueous media. In non-aqueous solvents such as supercritical fluids, gases, ionic liquids and organic solvents, enzymes may exhibit altered selectivities, pH memory, increased activity and stability at elevated temperatures, and hydrolytic enzymes may carry out synthetic reactions, as reviewed by Hari Krishna (2002).

Water as a reactant

Hydrolases are the most commonly used enzymes in industry (Hari Krishna 2002), and in their reactions, water takes part as a reactant. In the hydrolysis reaction, chemical bonds are cleaved by the addition of water to the cleavage point. This means that a certain amount of bulk water is consumed during the reaction, thus increasing the mass of the solute. This phenomenon is referred to as the hydrolytic gain (Marchal and Tramper 1999). The role of the hydrolytic gain in the water usage of an industrial process was illustrated by the following example. Industrial processes producing glucose syrups from starch typically use an initial dry matter

content of about 35%. During hydrolysis the cleavage of each glycosidic linkage results in the net addition of one molecule of water. In a solution containing 35% starch, approximately 5% of the initial water concentration is used for the hydrolytic gain (Van der Veen et al. 2006).

Water as a solvent, reaction medium and plasticizer

Water is a good solvent due to its polarity, and it is a universal solvent in biological systems. Many biological catalyses involve soluble enzymes acting on insoluble substrates, and water is the medium for dissolution, dispersion, transition and reaction of the components.

Water has an essential role in the mass transfer of enzymatic processes, including the diffusion and convection of enzyme, substrate and product molecules in the reaction medium. Mass transfer impacts enzymatic hydrolysis rates mainly by affecting the rate at which (1) enzymes are distributed in the reaction medium and transferred to reaction sites and (2) solubilised hydrolysis products are transferred away from the reaction site (Roberts et al. 2011). Efficiency of mass transfer is affected by the viscosity and other rheological properties of the enzyme-substrate mixture, and it can be enhanced mechanically by mixing. Increase of water content generally decreases viscosity, which makes mixing easier and less energy demanding. However, viscosity also depends on the intrinsic characteristics of the solutes and solids, as well as on temperature (Guillon and Champ 2000; Modenbach and Nokes 2013).

The rate of biochemical reactions is also dependent on the physical state of the substrate, which can be affected by the amount of water in the system. Water can act as a plasticizer converting amorphous materials from a solid glassy state to a rubbery state (Ruan and Chen 1998). This conversion is known as glass transition, and it is accompanied by a considerable decrease in viscosity and increase in molecular mobility. Although many authors have suggested that reactant mobility and diffusion within a matrix is related to both solvent characteristics (i.e. water availability) and system mobility, described by glass transition temperature and viscosity, studies on the individual or synergistic effects of these parameters on enzyme activity are still scarce, as reviewed by Neri et al. (2010).

States of water in biomaterials and measurement of hydration

The properties of water are affected by its microenvironment. The amount and chemical nature of solutes and the sorption of water within the insoluble plant cell wall structures define the level of “free” or “available” water in these systems. The determination of the state and location of water in biomaterials is of importance when identifying their susceptibility to enzymatic action.

In food science, the state of water has classically been described by the concept of ‘‘water activity” (a_w), defined as the ratio of partial vapour pressure in equilibrium with the food to the saturation vapour pressure of pure water at the same temperature. Solutes decrease the vapour pressure of a solvent by imposing a

physical constraint on solvent molecules directly interacting with the solute molecules (Israelachvili 2006). The extent to which a solute reduces a_w is a function of the chemical nature of the solute. The relationship between water content and water activity in a specific material is indicated by the moisture sorption isotherm at equilibrium. Classically, water activity measurements have been used to determine the product quality and stability based on the "stability map" introduced by Labuza et al. (1970), which shows the general relationship between the occurrences of various reactions (chemical, physical, biochemical, microbial) as a function of a_w. According to the stability map, enzymatic activity is virtually non-existent below a_w 0.2 and starts to increase gradually at a_w 0.3, until a rapid increase occurs above a_w 0.8 (deMan 1999).

Three different conditions of water present in food are classically differentiated on the basis of the sigmoid shape of a typical sorption isotherm, i.e. 1) a monomolecular layer of water, 2) additional layers of water, 3) the water in capillaries and pores of the material (deMan 1999). However, this definition is not very precise, and as reviewed by Roberts et al. (2011), up to five different 'pools' i.e. states of water have been observed in cellulosic suspensions by using time-domain nuclear magnetic resonance, including: (1) primary bound water, also known as non-freezing bound water, which is constrained by interactions with the surface of cellulose; (2) secondary bound water, also known as freezing bound water, which is constrained by primary bound water; (3) water bound by capillary forces within cell wall lumens; (4) restricted bulk water or bulk water the movement of which is restricted by the presence of the other pools of water; and (5) free water. The division of water between the different states depends on a number of factors including the chemical structure of the components, the associations between molecules, the porosity and size of the particles, and temperature (Thebaudin et al. 1997).

The approximate areas of the different states of water in relation to the water content and physical state of the substrate-water mixture are presented in Table 1. According to Felby et al. (2008), below the saturation point of approximately 25–30% moisture, the major part of the water will be present as primary or secondary bound water within the cell wall. Similar values (28–30%) have also been reported for wheat flour, as reviewed by Lee (1970). Above the saturation point water fills the cell lumens until full saturation in the area of 60–70% moisture content (Felby et al. 2008). Even above a water content of 80% (the area of restricted bulk water in which the material typically is in the form of a slurry), the extent of the binding of water might be dependent on the water content of the system, as suggested by Roberts et al. (2011), who found that water was held more tightly in cellulose suspensions as the solids content increased from 5 to 20%. It has also been shown that it is possible to link the state and location of water within the cellulose fibre with structural changes occurring in enzymatic hydrolysis (Felby et al. 2008).

A practical approach to determine the water-substrate interactions is measurement of the hydration properties of the material. The water holding capacity of the substrate determines the point of disappearance of the free bulk liquid phase from the mixture, which has dramatic impacts on the rheological properties and physical form of the mixture (Table 1). When water content is reduced below this point, the

material changes from a pourable suspension into a thick, paste-like substance that can be moulded and formed into shapes (Coussot 2007; Stickel et al. 2009). When water content is further reduced, the material reaches a point at which the liquid is fully absorbed into the solids. At this point the mixture can no longer be called a slurry because it is unsaturated and acts more like a wet, granular substance (Stickel et al. 2009; Viamajala et al. 2009). The measurement of the kinetics of water absorption or the overall water holding capacity of the material can be made either without the application of external forces or by the application of external forces such as centrifugation or pressure (Guillon and Champ 2000; Robertson et al. 2000).

Table 1. Different states of water in relation to water content and physical form of the substrate-water mixture.

Water content	State of water	Physical form of the mixture
< 30%	Primary or secondary bound water, no liquid phase	Powder
30–60%	Water starts to fill the capillaries, no liquid phase	Powder / granular
60–80%	Full saturation of the capillaries, appearance of liquid phase	Granular
> 80%	Restricted bulk water / free bulk water	Paste / slurry

1.1.2 Effects of water content reduction on enzymatic processing

High-solids (i.e. low-water) enzymatic hydrolysis has been loosely defined to take place at solids contents at which significant amounts of free liquid are not initially present (Hodge et al. 2009). In the case of many lignosellulosic substrates, this corresponds to a solids content of 15–20%.The benefits of working at high solids concentrations include reduced downstream processing costs due to higher product concentrations, as well as reduced disposal and treatment costs due to the lower water usage. In addition, higher solids concentrations mean lower requirements for reactor volume and thus reduced capital costs, as well as reduced energy demands for heating and cooling. However, the reduction of water content is generally accompanied by reduced enzyme activity. The effect appears to be rather linear (Jørgensen et al. 2007) and to hold for a variety of substrate materials, as reviewed by Kristensen et al. (2009). Several studies have attempted to elucidate the mechanisms behind this phenomenon, as recently reviewed by Modenbach and Nokes (2013), but the exact cause has not been determined. Many of the encountered problems are related to mass transfer limitations in a direct or indirect way, although it appears to be difficult to quantify and assign the challenges of operating at high solids content to a specific factor because many of them are strongly interrelated. The benefits and challenges related to reduction of the water content of an enzymatic process are summarized in Table 2 and further discussed below.

Table 2. Benefits and challenges related to enzymatic processing at high-solids / reduced water content.

Benefits of low-water processing		**Challenges and related phenomena**	
Lower water usage	Enzyme performance	Properties of the substrate-water mixture	Enzyme performance
• lower requirements for reactor volume • reduced energy demands for heating and cooling • higher product concentrations • reduced downstream processing costs (e.g. drying, concentration) • reduced wastewater treatment and disposal costs • simpler process layout	• Increased enzyme stability	• increased viscosity and changes in the state of water → mass transfer limitations • mixing difficulties; increased shear and power requirements for mixing • increased concentrations of end-product inhibitors	• reduced rate of enzyme action → lower yield, long hydrolysis times • higher degree of polymerization of hydrolysis products • formation of side products • reduced enzyme adsorption on substrate • non-productive binding of enzymes • overcrowding of available substrate sites

Handling of the substrate mixture and mass transfer limitations

Inadequate mixing is considered to be one causative factor for reduced enzyme action at low water content. Mixing is important as a means to enhance the mass transfer of enzyme reactions. When water content is reduced, mixing becomes difficult due to changes in the rheological properties of the material. At high water content, molecules or particles are well separated from each other and are free to move independently. Reduction of water content increases the viscosity of slurry when the particles and/or solutes start to touch and entangle with each other causing increased friction, and consequently mixing and handling of the material become more difficult (Viamajala et al. 2009). Increased viscosity increases the required shear stress necessary to produce a given shear rate, necessitating higher power input for mixing (Hodge et al. 2009; Kristensen et al. 2009). However, increasing the shear rate is not always a viable option for enzyme stability; for example, cellulase enzymes have been shown to be sensitive to both shear and temperature (Reese and Ryu 1980; Gunjikar et al. 2001).

Roberts et al. (2011) found that water was more tightly bound to lignocellulose as solids loadings increased, and they hypothesized that the negative effects of reduced water content on the hydrolysis rate are caused by this ‘water constraint’. Their data suggested that the primary mechanism by which the increased constraint (caused either by increased solids content or increased monosaccharide content) results in decreased saccharification rates is probably the increase in

mass transfer resistances. Increased viscosity of the fluid in the suspensions resulted in decreased diffusivities of solutes such as protein and monosaccharides. They also pointed out that uniform distribution of enzymes in high solids saccharification reactions is difficult to achieve because mass transfer resistances in the reaction increase, and this might limit the synergistic action of enzymes.

Changes in enzyme performance

End-product inhibition by hydrolysis products can play an important role in enzymatic hydrolysis. For example, cellobiose and glucose have been demonstrated to significantly inhibit endoglucanases, cellobiohydrolases and β-glucosidase (Bezerra and Dias 2005; Xiao et al. 2004). At low water contents, the inhibition may become more apparent due to the increased concentration of the inhibiting sugars, especially since it is often coupled with reduced mass transfer rates, which hinder diffusion of the inhibitors away from the reaction site (Hodge et al. 2009). Another mechanism related to the increased concentration of hydrolysis products was suggested by Kristensen et al. (2009). They showed that increasing concentrations of glucose and cellobiose inhibited the adsorption of enzymes onto cellulose, and suggested this to be the main factor behind the “solids effect” limiting enzyme action (Kristensen et al. 2009). On the other hand, some authors have explained decreased enzyme action at higher solids contents by the non-productive adsorption of cellulases to insoluble solids, especially to lignin and cellulose (Rosgaard et al. 2007), and the same mechanism has also been suggested for xylanases binding to xylan substrates (Sørensen et al. 2006).

Long hydrolysis time is one of the challenges specific to high solids enzymatic hydrolysis (Modenbach and Nokes 2013). As pointed out by Kristensen et al. (2009), the inhibition effect of high solids content primarily affects the hydrolysis rate and not the maximum conversion or yield, given sufficient time. In principle the hydrolysis rates could be increased by an increase of enzyme dose, but as reviewed by Modenbach and Nokes (2013), recent studies have suggested that increased enzyme loading may not improve the hydrolysis rate as expected (Bommarius et al. 2008; Olsen et al. 2011; Xu and Ding 2007). It has been suggested that enzymes can completely saturate the substrate by binding to all the accessible sites, which would prevent achievement of the full hydrolytic potential of the given enzyme loading.

In addition to reduced enzymatic activity, the reduction of water content may cause changes in product composition. Van der Veen et al. (2005) investigated the hydrolysis of maltodextrins at dry matter contents of 30–70% w/w, and reported that increased dry matter content resulted in increased formation of condensation products so that both the quantity and the length of the product polymers increased. They concluded that the product composition was kinetically controlled, and that the yield of glucose showed an optimum in time so that the slow side reactions could virtually be excluded by short reaction times (van der Veen et al. 2005). Hardt et al. (2013) showed that wheat gluten can be hydrolysed at solids concentrations as high as 60%. However, they noted that high solids concentra-

tions resulted in a higher weight fraction of peptides with a high molecular mass of > 25 kDa than low solids concentrations. This was attributed to high viscosity and mass transfer limitations (Hardt et al. 2013).

The possible increase in enzyme temperature stability is one beneficial impact of increased substrate concentration. This phenomenon has been noted in non-aqueous media but also in systems with water as the reaction medium (Baks et al. 2008; de Cordt et al. 1994; Sola-Penna and Meyer-Fernandes 1998; Warmerdam et al. 2013). It has been suggested that the higher enzyme stability at higher substrate concentration might be caused by the mechanism of molecular crowding or by complexation with the substrate (Warmerdam et al. 2013). It has also been proposed that the protection of enzymes by soluble sugars is caused by the exclusion of water from the hydration layer of the enzymes, which would cause a decrease in enzyme flexibility, finally resulting in increased enzyme stability but reduced activity (Sola-Penna and Meyer-Fernandes 1998).

1.1.3 Process solutions for hydrolytic enzyme treatments at low water content

A number of processing strategies have been developed and studied as means of modifying different plant-based raw materials with hydrolytic enzymes at reduced water content for various purposes (Table 3). The features of the different processes are discussed below.

Table 3. Processes applying hydrolytic enzymes at low-water conditions for the modification of plant-based raw materials.

Process type	Aim	Raw materials	Mixing type / process solution	Solids content	Enzymes	References
High-solids hydrolysis of lignocellulosic biomass	Hydrolys to monosaccharides	Various lignocellulosic biomasses	Stirred tank reactor	up to 30%	cellulolytic enzymes	e.g. Zhang et al. 2010)
			Rotating drum	up to 40%		Jørgensen et al. (2007)
			Peg mixer	20%		Zhang et al. (2009)
			Roller bottles	15–30%		Roche et al. (2009)
			Fed batch feeding	up to 30%		e.g. Hodge et al. (2009): Yang et al. (2011)
Enzymatic hydrolysis of cereal materials using extruders	Production of glucose syrups	Native/pre-gelatinized starches	Extrusion or extrusion+batch incubation	30–80%	α-amylase, glucoamylase	e.g. Baks et al. (2008); Linko (1989)
	Modification of baking properties	Brewery spent grains		65%	xylanase and cellulase enzyme mixtures	Steinmacher et al. (2012)
	Partial depolymerisation of β-glucan	β-glucan-enriched oat bran fraction		50%	hydrolytic enzyme mixture	Sibakov et al. (2013)
Solid-state enzymatic bioconversions of agricultural food raw materials	Release of antioxidants	Wheat bran	Static incubation	57–70 %	β-glucanase, carboxylic esterase, polygalacturonase, aminopeptidase, cellulase	Moore et al. (2006)
	Hydrolysis of starch to monosaccharides	Chestnut		0.165–0.495 g/l	α-amylase, glucoamylase	López et al. (2005)
	Reduction of water absorbing capacity	Psyllium		*not specified*	xylanase enzyme mixtures	Yu and Perret (2003)
Baking and other low-water cereal food processes applying enzymes	Improved dough properties and/or end-product quality	Various cereal fractions from wheat, rye, oat etc.	Static incubation / intermittent mixing	20–50%	hydrolytic, oxidative and transferase enzymes	e.g. Martinez-Anaya and Jimenez (1997); Lebesi and Tzia (2012); Coda et al. (2014)
	Improved gluten functional properties	Wheat gluten	Continuous mixing	10–60%	protease mixture	Hardt et al. (2013)

High-solids hydrolysis of lignocellulosic biomass

Hydrolysis of lignocellulosic biomass is an area in which much research has been performed to target the challenges related to working at low water content. At high solids content, the method of mixing can have a substantial impact on the conversion of lignocellulosic substrates. Traditional mixing designs resembling a standard, vertical stirred tank reactor require excessive power for adequate mixing and generally perform poorly for high yield stress fluids (Ehrhardt et al. 2010; Lavenson et al. 2012; Modenbach and Nokes 2013; Roche et al. 2009; Saeed et al. 2008). Approximately 12–15% of insoluble solids is generally considered to be the upper limit at which biomass slurries can be mixed and hydrolysed effectively in conventional stirred-tank reactors (Hodge et al. 2009; Kristensen et al. 2009). However, the geometry of the impeller can play a significant role in the efficiency of the mixing, as shown by Zhang et al. (2010), who demonstrated the superior performance of a helical impeller as compared to the traditional Rushton impeller at high solids contents up to 30%. Several studies have shown that horizontal orientation of the reactor and free-fall mixing provide numerous advantages over typical stirred tank reactors, including minimized particle settling, easy scale-up and lower power requirements, as reviewed by Modenbach and Nokes (2013). Examples of this type of mixing systems are the peg mixer, roller bottles and the rotating drum, which have been used up to solids contents of 40% (Table 3).

A special approach to overcome the challenges related to working at high solids content is the use of fed-batch feeding strategies, i.e. sequential loading of substrate or substrate plus enzymes during enzymatic hydrolysis (Hodge et al. 2009; Yang et al. 2011). One benefit of fed-batch feeding is lower initial viscosity as compared to simple batch processing. The viscosity of lignocellulosic substrates is known to decrease as a result of cellulolytic activity, and in fed-batch processing the initial viscosities can be low since this allows time for the slurry to liquefy before adding additional solids. However, when a fed-batch approach is selected, it must be considered how and when to add substrate, as well as enzymes, in order to maintain high rates of conversion (Modenbach and Nokes 2013). The possible problems include inability of the enzyme to desorb from partially hydrolysed substrate and find accessible cellulose sites in the fresh substrate (Chandra et al. 2011), but sequential addition of enzymes with each addition of fresh substrate has given positive results (Hodge et al. 2009; Yang et al. 2011). Although many studies support the use of fed-batch processing for high-solids hydrolysis, the results concerning its applicability are currently still inconclusive (Modenbach and Nokes 2013).

Specific viscosity-modifying additives, such as surfactants and water soluble polymers, have also been investigated as a means to decrease the viscosity effects in high-solid slurries, but the economics of their use in industrial scale are still to be validated (Knutsen and Liberatore 2010). Another studied approach is the reduction of particle size of the substrate (Dasari and Berson 2007). Viamajala et al. (2009) and Dasari and Berson (2007) reported that smaller particle sizes re-

sulted in lower apparent viscosities under equivalent conditions, as well as enhanced enzymatic hydrolysis. Size-reduction could have reduced the amount or size of macro-pores in the biomass particles, so that less liquid is entrained in these particles and thus more free water remains in the suspension, resulting in decreased interactions between particles and lower apparent slurry viscosities (Viamajala et al. 2009).

Hydrolysis of cereal materials using extruders as bioreactors

The enzymatic hydrolysis of starch to glucose syrups is an important industrial process that consists usually of two steps: 1) gelatinization and liquefaction, and 2) saccharification. The industrial gelatinisation and liquefaction with α-amylase are usually carried out in excess water (at 30–35% dry solids content) in order to facilitate gelatinisation and ensure sufficient mixing during the reactions (Van der Veen et al. 2006), but these processes can also be efficiently performed at dry matter contents from 50% up to 80% using extruders (Govindasamy et al. 1997a, 1997b; Linko 1989; Van Zuilichem et al. 1990). Extruders are able to continuously create high shear, which is required when the gelatinization process is performed at reduced water content (Barron et al. 2001; Van der Veen et al. 2006). An extruder comprises a horizontal barrel with one or two conveying screws and a die exit. The raw material becomes efficiently mixed and subjected to high pressure and shear as it is conveyed across the barrel to the die with a restricted opening. In a typical extrusion process temperatures are between 100 and 180°C and residence times range from seconds to a few minutes (Guy 2001). Extrusion cooking usually involves low moisture conditions between 10 and 40%, but since the 1980s, “wet extrusion” with a feed moisture content above 40% has been possible due to developments with twin screw extruders including sophisticated barrel designs, screws and dies (Akdogan 1999), which have enabled the use of extruders as bioreactors for enzymatic treatments.

As reviewed by Baks et al. (2008), extruders have been used for combined gelatinisation and enzymatic hydrolysis of native starches (Govindasamy et al.1997a, 1997b; Lee and Kim 1990; Vasanthan et al. 2001) and for hydrolysis of pregelatinised starch (Komolprasert and Ofoli 1991), as well as in combination with a batch reactor to increase the hydrolysis time (Chouvel et al. 1983; Linko 1989; Reinikainen et al. 1986; van Zuilichem et al. 1990). In many cases the enzymes have been added at the beginning of the extruder together with the starch-water mixture, but in order to avoid the shear-induced enzyme deactivation it is preferable to add the enzyme at the end of the gelatinisation (Baks et al. 2008; Grafelman and Meagher 1995; Van der Veen et al. 2006). The impact of water content has also been studied, and in most cases the conversion has been highest at the highest water content studied, typically at 55–70% water content, as reviewed by Linko (1989) and Akdogan (1999), although (Tomás et al. 1997) reported maximum starch hydrolysis at an intermediate water content of 60% when studying in the range of 55–65%.

Studies concerning the use of extruders for enzymatic modification of other cereal materials than starch or for other targets are rare. However, two recent papers reported the use of cell wall degrading enzymes for enzymatic hydrolysis of oat bran β-glucan at a water content of 50% (Sibakov et al. 2013) and for modification of brewer's spent grain at a water content of 65% (Steinmacher et al. 2012).

Solid-state enzymatic bioconversions

Solid-state (or substrate) fermentation (SSF) is generally defined as the growth of microorganisms on (moist) solid material in the absence or near-absence of free water (Pandey et al. 2008). In SSF processes, the growth and metabolic reactions of microorganisms are utilized for a variety of applications, including the production of industrial enzymes, organic acids and secondary metabolites such as antibiotics and ethanol, as well as for bioremediation and biotransformation of agro-industrial raw materials and residues (Krishna 2005). Enzymatic reactions initiated by the metabolic activity of microorganisms have a key role in many SSF processes, and the same approach, i.e. incubation of substrates in solid state, has been applied for enzymatic conversions of some agro-industrial materials for food applications (Table 2). These processes have been conducted without agitation, and the reaction times last up to several days. The benefits of this type of processing are that they do not require expensive equipment or post-reaction processing for product recovery (Moore et al. 2006). Solid-state enzymatic procedures have been used to improve the physiochemical and functional properties of psyllium (Yu and Perret 2003), to hydrolyse chestnut starch (López et al. 2005) and to release insoluble bound phenolic acids from wheat bran (Moore et al. 2006). These studies have reported that solid-state enzyme treatments have promoted beneficial changes in the raw materials, and also investigated the effect of water content (e.g. Moore et al. 2006). However, the long process times and the lack of comparison of the low-water process to optional process variations (high-water content with mixing) complicate evaluation of the overall efficiency and industrial applicability of the solid-state enzyme treatments in the field of food and feed processing.

Baking and other low-water cereal food processes

Enzymes are routinely used in the baking industry as processing aids and for improving product quality. Baking can be considered as a low-water process since the reactions take place in the dough, i.e. in the absence of free liquid. The water content of bread dough is typically around 50%. Wheat is by far the most important cereal grain in bread making, and the essential wheat flour constituents include starch, gluten proteins, arabinoxylans and lipids (Goesaert et al. 2005). In the baking process, enzymes are mixed together with the other ingredients into a visco-elastic dough, which is fermented and baked. The enzymes used and studied in baking include hydrolytic enzymes such as amylases, lipases, proteases, cellulases, β-glucanases and xylanases, as well as oxidative enzymes such as

lipoxygenase, glucose oxidase and laccase, and also the transferase enzyme transglutaminase (Caballero et al. 2007; Goesaert et al. 2005; Martínez-Anaya and Jiménez 1997). The use of hydrolytic enzymes has also been shown to improve the quality of high-fibre breads produced using whole-grain flours or brans (Katina et al. 2006; Laurikainen et al. 1998; Shah et al. 2006).

In addition to direct addition in the bread dough, enzymes have been used as a pre-treatment for modification of the baking raw materials. The wheat flour milling process includes a tempering step, during which the moisture content of wheat grains is increased to 15.5%. Haros et al. (2002) examined the impact of addition of cellulase, xylanase, and β-glucanase to the tempering treatment (20 °C, 16 h), and reported that the quality of fresh bread improved when using flours obtained from the carbohydrase-treated wheat. Several studies have shown that bioprocesssing of bran with cell wall degrading enzymes can modify the physicochemical properties and structure of bran and improve its technological functionality in baking processes (Coda et al. 2014; Lebesi and Tzia 2012; Nordlund et al. 2013). These bran fermentations have been performed at water contents of 65–80%. The recent paper of Hardt et al. (2013) is a rare example of a study in the field of food processing in which the impact of a range of water contents on an enzymatic treatment has been studied. They examined the enzymatic hydrolysis of wheat gluten at solids concentrations varying from 10% to 60%, and concluded that wheat gluten can be hydrolysed at solid concentrations as high as 60%, but that increased reaction times at very high solid concentrations result in optimum productivity at 40% solids content (Hardt et al. 2013).

1.2 Xylanolytic enzymes and degradation of plant cell walls

Xylanolytic enzymes are a widespread group of enzymes catalysing the hydrolysis of xylan, which is a major structural polysaccharide in plant cell walls. Xylanases together with other xylanolytic enzymes have an important role in the hydrolysis of lignocellulosics as well as in the processing of agro-industrial plant materials for food and feed applications.

Xylan is a constituent of the hemicellulose fraction of plant materials and accounts for one third of all renewable organic carbon available on earth (Prade 1996). Most xylans occur as heteropolysaccharides, containing different substituent side chains in the backbone chain composed of 1,4-linked β-D-xylopyranosyl units. The substituents found on the backbone are glucuronopyranosyl, 4-O-methyl-D-glucuronopyranosyl, α-L-arabinofuranosyl, acetyl, feruloyl and p-coumaroyl side-chain groups (Kulkarni et al. 1999; Liab et al. 2000). In cereals and other grasses, xylan occurs mainly as arabinoxylan (AX), and it is the predominant form of non-cellulosic polysaccharides of both primary and secondary cell walls (Izydorczyk 2009).

1.2.1 Xylanolytic enzymes

Xylanolytic enzymes are produced mainly by microorganisms but are also found in plants, marine algae, protozoans, crustaceans, insects and snails (Sunna and Antranikian 1997; Dekker and Richards 1976). They participate in the breakdown of plant cell walls, and also digest xylan during the germination of seeds (Polizeli et al. 2005; Dekker and Richards 1976). Since the 1980s, industrially produced xylanolytic enzymes have found use in numerous commercial applications such as bleaching of cellulose pulp, waste water treatment, hydrolysis of biomass for biofuels production, animal feeds, baking and other food, ingredient and drink manufacturing processes, as well as in the pharmaceutical, chemical and textile industries (Beg et al. 2001; Polizeli et al. 2005). In the cereal industry, xylanases are frequently used to adjust processing, yield, and/or end product quality (Dornez et al. 2009), as well as for the production of special ingredients such as prebiotic oligosaccharides (Van Craeyveld et al. 2010).

The most important xylanolytic enzymes are the endo-(1,4)-β-D-xylanases (EC 3.2.1.8), also called endoxylanases or simply xylanases. They catalyse the hydrolysis of 1,4-β-D-xylosidic linkages in xylan backbone. Based on amino acid sequence and structural similarities, endoxylanases have been classified in glycoside hydrolase (GH) families 5, 7, 8, 10, 11 and 43 (Collins et al. 2005). The majority of microbial xylanases belong to GH families 10 and 11. As reviewed by Courtin and Delcour (2002), the differences in size and complexity of the protein structure of GH family 10 and 11 xylanases apparently reflect their substrate specificity and selectivity. In general, GH10 xylanases have greater catalytic versatility and lower substrate specificity, and tend to produce oligosaccharides with a low degree of polymerisation, whereas GH11 xylanases are more specific for xylan and produce larger oligosaccharides (Biely et al. 1997). Xylanases may also have selectivity towards either water extractable or water unextractable xylan (Courtin and Delcour 2001; Maes et al. 2004). It is generally accepted that GH11 xylanases preferentially cleave in unsubstituted regions of the xylan backbone, whereas GH10 enzymes cleave in the decorated regions (Berrin and Juge 2008; Biely et al. 1997). Fungal endoxylanases are typically stable over a wide pH range (3.0–10.0), whereas their pH optimum is generally 3.5–5.5. Bacterial endoxylanases usually have somewhat higher pH optima (6.0–7.0), and narrower pH stability (5.0–7.3) (Courtin and Delcour 2002). The optimum temperature for xylanase action ranges between 35 and 60°C (Beg et al. 2001), but bacterial and fungal xylanases typically have temperature optima between 40 and 50 °C, above which their stability is limited (Dekker and Richards 1976; Reilly, 1981).

Due to the heterogeneity and complex chemical nature of plant xylan, its complete breakdown requires the action of an array of several hydrolytic enzymes with diverse specificities and modes of action (Beg et al. 2001). The presence of such a multifunctional xylanolytic enzyme system is widespread among fungi, actinomycetes and bacteria, as reviewed by Collins et al. (2005). The cleavage of xylose monomers from the non-reducing end of xylo-oligosaccharides and xylobiose is

catalysed by β-D-xylosidases (EC 3.2.1.37). Enzymes catalysing the removal of the side groups include α-L-arabinofuranosidases (EC 3.2.1.55), α-D-glucuronidases (EC 3.2.1.139), acetyl xylan esterases (EC 3.1.1.72), ferulic acid esterases (EC 3.1.1.73) and *p*-coumaric acid esterases (EC 3.1.1.). The sites of enzyme attack in xylan are presented in Figure 1 (Collins et al. 2005).

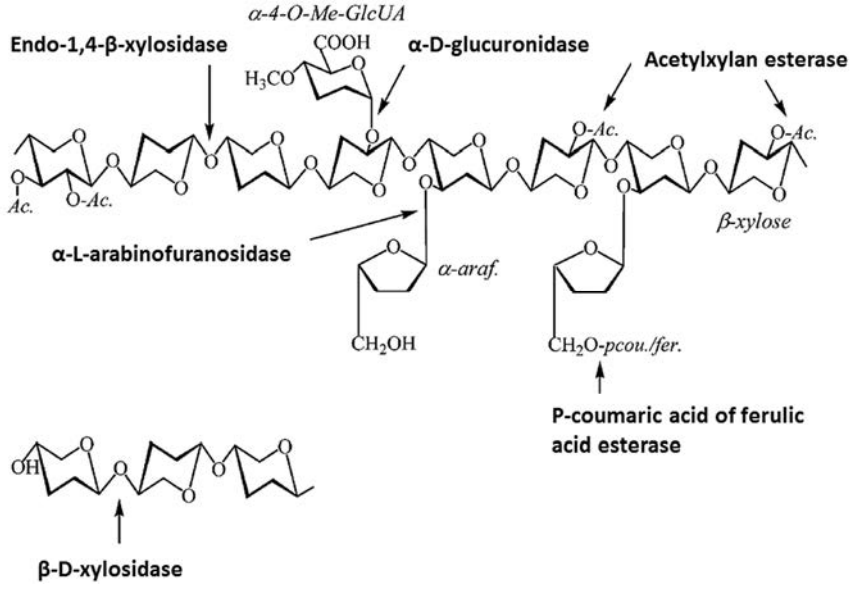

Figure 1. Structure of xylan and the sites of attack by xylanolytic enzymes. Adapted from Collins et al. (2005).

1.2.2 Enzymatic degradation of plant cell walls

Cell walls constitute the key structural components of plants and account for the bulk of lignocellulosic biomass. From a plant physiological perspective, the cell walls provide support and determine the cell shape and also take part in numerous activities in plant development, including cell growth and division, intercellular signalling and transport, protection against other organisms and environmental stresses and storage of food reserves (Waldron et al. 2003). The plant cell walls also have a major impact on the processing of plant materials for food and feed purposes (milling, malting, baking, juice processing etc.), (Cui and Wang 2009), and thus degradation of the cell walls with hydrolytic enzymes is often an essential step in the upgrading of plant-based materials for these applications.

Together with xylan, the major polymeric constituents of plant cell walls are cellulose (1,4-β-glucan) and lignin (a complex polyphenolic compound), and in cereal cell walls also β-glucan (1,3–1,4-β-glucan). The structure of a plant cell wall is generally described as a network of cellulosic microfibrils embedded in a matrix of

non-cellulosic polysaccharides, in which phenolic components, structural proteins and glycoproteins may also be present (Carpita 1996; Waldron et al. 2003). Within the cell wall matrix, different constituents are associated with each other through covalent and non-covalent linkages leading to the formation of a non-uniform, three-dimensional and compact structure (Andersson et al. 2003; Iiyama et al. 1994). The complex interaction between different components makes cell walls very resistant towards enzymatic action.

In the hydrolysis of biomass the use of a combination of different cell wall degrading enzymes is often feasible since enzymes may cooperate in a synergistic manner to degrade the substrate, meaning that the activity of enzymes working together is higher than the sum of their individual activities (Van Dyk and Pletschke 2012). Numerous examples of synergistic action of cellulases and xylanases and other enzymes in the degradation of lignocellulose were presented in the recent extensive review by Van Dyk and Pletschke (2012). Cellulose is a particularly difficult polymer to degrade, as it is insoluble and present as hydrogen-bonded crystalline fibres (Mansfield et al. 1999). It is generally accepted that three types of enzymes are required to hydrolyse cellulose into glucose monomers, namely exo-1,4-β-glucanases i.e. cellobiohydrolase (EC 3.2.1.91 and EC 3.2.1.176), endo-1,4-β-glucanases (EC 3.2.1.4) and β-glucosidases i.e. cellobiases (EC 3.2.1.21), as reviewed by Van Dyk and Pletschke (2012). Endoglucanases cleave cellulose chains in the middle and rapidly reduce the degree of polymerisation, whereas cellobiohydrolases attack the ends of cellulose chains.

In contrast to the hydrolysis of lignocellulosic biomass for e.g. biofuels production, in food processing the aim of plant cell wall degradation is not always complete hydrolysis of the cell wall constituents to monosaccharides. With targeted enzymatic modification of the plant cell walls, the quality of the end product, for example texture and sensory properties, as well as the nutritional properties of the product can be affected (Harris and Smith 2006; Lebesi and Tzia 2012; Nordlund et al. 2013). Cell walls also affect the release of potentially health-promoting components, such as phenolic compounds, which are bound to cell wall structures (Mateo Anson et al. 2009; Bunzel et al. 2001), and it has been shown that the bioavailability of ferulic acid in bran-supplemented bread can be increased by enzymatic bran pre-treatment (Mateo Anson et al. 2011). In baking the aim of the use of cell wall degrading enzymes is opening up of the cell wall structures and solubilisation of AX without too extensive depolymerisation (Courtin and Delcour 2002). Thus, specificity and selectivity are important criteria for the selection of enzymes for a specific application. Commercial enzyme products are typically mixtures of several hydrolytic activities that in theory enable the synergistic action of enzymes and thus effective plant cell wall degradation, but this might be a disadvantage when aiming for more specific targets.

1.3 Wheat bran: properties and processing strategies for food applications

Bran is the fraction of the outer layers of a grain which is removed in the milling process during the production of refined flour. The annual world production of wheat is currently about 650–690 million tons (Earth Policy Institute 2013), and the bran fraction accounts for 11–15% of the grain weight (Hemery et al. 2007). Most of the bran is used for animal feeding. Bran is also used in food products to increase the DF content of the end product or as a filler to reduce its energy density. Wheat bran also contains substantial amounts of protein and phytochemicals, which further motivates its use in food products. However, it is generally known that the use of unprocessed bran is usually detrimental for product quality, and it may also contain contaminants that need to be taken in consideration when used in foods. Numerous processing methods have been developed and are currently studied as potential means to facilitate the use of bran fractions in different types of food products.

1.3.1 Composition and nutritional properties of wheat bran and DF

Several different tissues can be distinguished in wheat (*Triticum aestivum L.*) grain from the centre to the periphery, i.e. the embryo, the endosperm (composed of the starchy endosperm and the single cell layered aleurone layer), the seed coats (composed of the nucellar epidermis and the testa), and the pericarp (composed of the tube cells, the cross cells, the hypodermis, and the epidermis). Wheat bran is composed of the pericarp, seed coats and aleurone layer, as well as variable amounts of remnants of starchy endosperm and germ depending on the separation efficiency of the milling process (Kamal-Eldin et al. 2009) (Figure 2). The main chemical constituents of wheat bran are cell wall carbohydrates (AX, β-glucan, cellulose), starch, fructan, lignin, protein, fat and minerals (Table 1). Wheat bran is also a source of vitamins, phenolic acids, alkylrecinols, plant sterols and lignans (Hemery et al. 2007). Variation in composition arises from differences in the genetic and agricultural backgrounds of wheat, as well as in the milling processes (Kamal-Eldin et al. 2009). In particular there can be wide variation in starch content, which directly affects the ash and fibre content of bran.

The structure and chemical composition of the cell walls vary in different bran layers. For example, the cell walls of the aleurone layer consist mainly of sparsely substituted AX and β-glucan, whereas the bran pericarp contains cellulose, lignin and highly substituted AX (Antoine et al. 2003). Most of the bran protein, lipids and phytochemicals are concentrated in the aleurone fraction (Hemery et al. 2007). The chemical, structural, and physical properties significantly affect the nutritional and technological functionality of the different bran layers, which can be separated using modern fractionation techniques (Hemery et al. 2007).

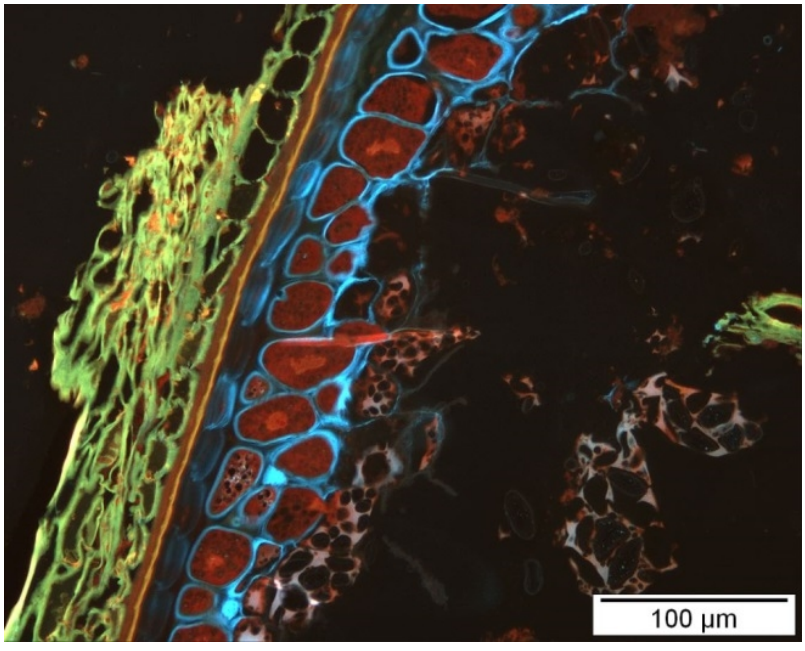

Figure 2. Microstructure of wheat bran stained with Acid Fuchsin and Calcofluor White. The β-glucan-rich aleurone cell walls appear in blue, the pigment strand (between the pericarp and the aleurone layer) in orange, the pericarp layer in light green and yellowish, and proteins in red (Courtesy of VTT Technical Research Centre of Finland, Ulla Holopainen).

Table 4. Main constituents of wheat bran.

Component	Content (% bran dry matter)
Arabinoxylan	19–30
Cellulose	9.3–12.
Klason lignin	3.0–4.9
Fructan	2.8–3.7
β-glucan	1.2–2.6
Starch	8.8–29
Protein	14–17
Fat	5.6–6
Ash	4–6.6

Values obtained from Kamal-Eldin et al. (2009); Maes and Delcour (2002); Swennen et al. (2006).

A major portion (40–50%) of wheat bran is dietary fibre (DF), i.e. those compounds of edible plants that are not digested in the human small intestine. The main DF polymers in wheat bran are AX, cellulose, lignin, fructan and β-glucan (Table 4). The importance of DF as a health protective component has long been recognized, but its analysis and definition based on chemical and physical properties has been a topic of intense debate and research, as reviewed by Raninen et al. (2011). DF is mainly formed from cell wall constituents and includes carbohydrate polymers with ≥ 3 or ≥ 10 monomeric units, depending on the definition, occurring naturally in food or those derived by modification and synthetic means (European Commission 2008; FAO/WHO 2009). Lignin and associated plant substances are also included in DF when existing naturally in the material. Dietary guidelines generally recommend a daily intake of 25–35 g of DF, or 3 g DF/1000 kJ (Raninen et al. 2011). Furthermore, EFSA has accepted several health claims related to the consumption of DF or wheat bran. A minimum of 3 g and 6 g of DF per 100 g of the product is required for claims of “source of fibre” and “high fibre”, respectively. Related to the consumption of wheat bran, the positive effect on human health can be due to the increase of faecal bulk and the reduction of intestinal transit time. To substantiate these claims, the daily wheat bran fibre consumption should be more than 10 g.

The substantial content of vitamins, phenolic acids, alkylrecinols, plant sterols, lignans and other potentially health-promoting components makes wheat bran a particularly interesting ingredient for food production. However, the accessibility of the compounds may be limited as they are trapped or encapsulated by complex cell wall structures. Wheat bran also contains phytate, mostly located in the aleurone layer. Phytate is able to bind minerals, and may thus reduce their availability in foods containing bran. Bran may also contain contaminants, such as mycotoxins, pesticide residues, heavy metals, etc., as well as yeasts and bacteria. However, it has been shown that these unwanted features can largely be overcome by using targeted processing and fractionation techniques (Hemery et al. 2007; Katina et al. 2007; Katina et al. 2012; Mateo Anson et al. 2011; Servi et al. 2008).

1.3.2 Technological properties of wheat bran and DF and methods for their modification

As in the case of many other DF-rich materials, addition of wheat bran usually causes significant and typically unwanted changes in the texture, flavour and colour of foods (Ortiz and Lafond 2012). Products to which wheat bran is generally added include bread and other baked products, extruded snacks and breakfast cereals, pasta and noodles. Depending on the product, increased hardness, brittleness and roughness is associated with bran addition. Wheat bran is known to have an adverse effect on volume, softness, flavour and appearance of bread and other baked products at 5–10% or higher levels (Pomeranz et al. 1977; Lebesi and Tzia 2011; Coda et al. 2014). Wheat bran addition to light coloured products such as crackers or rice cake leads to darker, browner or speckled appearance. Fur-

thermore, on the basis several studies it has been shown that cereal bran addition reduces volumetric expansion and increases hardness of extruded snacks (Robin et al. 2012).

The reasons behind the adverse effects of bran have not been fully elucidated, but both physical and chemical mechanisms have been suggested (Gan et al. 1992; Guy 2001; Lai et al. 1989; Moraru and Kokini 2003; Noort et al. 2010). One of these is the fact that bran addition decreases the concentration of the main structure-forming components, starch and gluten. The mechanisms behind the adverse effects of bran are generally related to its insoluble nature and coarseness, and its ability to bind large amounts of water and alter viscosity. These properties are affected by particle size, porosity, cell wall architecture, chemical composition, and molecular structure of the DF polymers. Processing methods aiming to improve the technological functionality of bran in baked, extruded and other products generally aim to modify these properties. The methods used for the modification of bran and other DF-rich plant materials include various enzymatic, chemical, mechanical, hydrothermal and thermo-mechanical methods (Guillon and Champ 2000). In addition to modification of the physicochemical properties, the processability and stability of bran have been targeted by inactivating endogenous microbes, enzymes or enzyme inhibitors of bran by e.g. heat treatments or fractionation techniques (de Kock et al. 1999; Hemery et al. 2007). The physicochemical properties of bran are strongly interrelated, and a change in one property typically also alters other physicochemical properties. However, some general relationships between the physicochemical properties and technological functionality of bran can be distinguished, as summarized in Table 5 and discussed further below.

Table 5. General relationships between physical, chemical, physicochemical and technological properties of bran and examples of methods for their modification.

Physical/ physicochemical property	Determinant physical / chemical properties	Technological impacts of modification of the property	Examples of methods used for modification of the property
Particle size	Surface area, number of particles	Reduction of particle size • positive/negative impact in bread (Noort et al. 2010; Lai et al. 1989; Coda et al. 2014) • positive impact in extruded products (Blake 2006; Alam et al. 2013)	• Grinding (Noort et al. 2010; Zhu et al. 2010) • Ball milling (Van Craeyveld et al. 2009)
Solubility of dietary fibre (DF)	Cell wall architecture, level of branching of DF polymers	Increase in DF solubility • positive impacts in baked products (Katina et al. 2012; Lebesi and Tzia 2012) • positive impact in extruded products (Pai et al. 2009)	• Enzyme treatments (Lebesi and Tzia 2012) • Fermentation (Katina et al. 2006; 2012) • Chemical alkaline treatment (Pai et al. 2009) • Grinding (Zhu et al. 2010) • Extrusion (Ralet et al. 1990; Wang et al. 1993)
Hydration properties	Particle size, porosity, cell wall architecture, level of branching of DF polymers	Decrease of bran water binding capacity • positive impacts in baked products (Lebesi and Tzia 2012)	• Enzyme treatments (Lebesi and Tzia 2012) • Grinding (Noort et al. 2010; Sanz Penella et al. 2008) • Extrusion, hydrothermal treatments (Caprez et al. 1986)
Viscosity of soluble phase	Concentration, molecular weight, degree of substitution and substitution pattern of soluble DF	High viscosity of dough/melt • positive impact in baking (Courtin and Delcour 2002; Lebesi and Tzia 2012) • positive or negative impact in extrusion (Pai et al. 2009)	• Enzyme treatments (Lebesi and Tzia 2012), • Chemical alkaline treatment (Pai et al. 2009), • Extrusion (Wang et al. 1993)

Particle size

The average particle size of native wheat bran is generally about 800–1000 µm (Auffret et al. 1994; Kamal-Eldin et al. 2009; Noort et al. 2010; Sanz Penella et al. 2008). Particle size is an important parameter for the use of bran, affecting both its physiological effects and technological functionality (Hemery et al. 2011; Robin et al. 2012; Zhang and Moore 1999). Particle size determines the surface area of the particles and may thus affect reactions that are dependent on the available surface area and surface characteristics. Reduction of particle size increases the total surface area and the number of the particles, and several studies have reported that decreasing the particle size of plant-based substrates may enhance their enzymatic hydrolysis (Dasari and Berson 2007; Mahasukhonthachat et al. 2010; Niemi et al. 2012; Silva et al. 2012). Reduction of particle size may also affect the release of components from the particles, and an increase in the level of soluble DF is often observed after intensive milling (Hemery et al. 2011; Zhu et al. 2010). Particle size is also a factor affecting hydration properties of bran and viscosity of doughs (Sanz Penella et al. 2008; Noort et al. 2010).

In food products, large bran particles may cause a gritty mouthfeel (Ortiz and Lafond 2012). Although reduction of particle size has been shown to alleviate this problem (Coda et al. 2014), it also alters other functional properties of the fibre particles. In baking, the impact of bran particle size is a controversial issue, as some studies indicate that reduction of bran particle size improves baking performance, such as dough mixing properties and loaf volume (Lai et al.1989; Moder et al. 1984), whereas others report the opposite (Zhang and Moore 1999; Noort et al. 2010). Coda et al. (2014) compared the baking performance of brans with mean particle sizes of 750, 400, 160 and 50 µm, and reported that the least detrimental particle size was 160 µm. The negative impact of particle size reduction has been ascribed to increased interaction surface and liberation of reactive components due to cell breakage (Noort et al. 2010). In extrusion, decreasing fibre ingredient particle size has been reported to increase the radial expansion of extrudates containing DF (Lue et al. 1991; Blake 2006; Alam et al. 2013). However, particle size reduction has not improved expansion when the size differences or bran addition levels have been low (Robin et al. 2011a; Blake 2006; Alam et al. 2013).

Particle size reduction equipment for cereal materials (wet and dry) in food and feed processing include hammer, roller and attrition mills, but blade, pin, ball and cryogenic mills are also used, especially for specialty products (Mahasukhonthachat et al. 2010). These mills differ in the effective operating force and the extent of frictional heat generation during grinding. Particle size of bran might also decrease as a consequence of other processes involving mechanical shear, e.g. extrusion (Wang et al. 1993; Robin et al. 2011a).

Solubility of DF

Solubility has a fundamental role in DF functionality. Fibres are generally classified as soluble or insoluble in water, although when considered soluble they might also

be present as a colloidal suspension as opposed to a true solution (Ortiz and Lafond 2012). Insoluble and soluble DF have typically very different impacts in cereal products. Insoluble fibre particles may physically interrupt the food macrostructure, causing potential weak points in the three-dimensional food matrix (Ortiz and Lafond 2012). Insoluble fibres can further negatively influence food texture by their water retention and swelling properties (Thebaudin et al. 1997). Soluble DF, on the contrary, is more easy to incorporate in foods and is characterized by its ability to increase viscosity in solution and to form gels and/or act as an emulsifier (Elleuch et al. 2011).

Solubility is related to the chemical structure of the polymers, and also to the extraction conditions used. In general, linear regions in polysaccharides are able to form strong interchain interactions allowing the formation of ordered crystalline structures that are insoluble in water (Ortiz and Lafond 2012). Branching limits the number of interchain interactions, and thus polysaccharides with some irregularities in their structure (in the backbone or as side chains) tend to be soluble (Guillon and Champ 2000). Wheat bran contains 1.5–4.0% soluble DF and 35–48% insoluble DF (Vitaglione et al. 2008). The main DF component in wheat bran is AX, and thus the chemical nature of AX has a major impact on bran functionality. Based on their solubility, wheat bran AXs are divided into two fractions, i.e. extractable (WEAX) and unextractable (WUAX) in water. The content of WEAX in wheat bran is approximately 0.3–0.9% of bran DM (Ward et al. 2008).

Processing of cereal bran with cell wall degrading enzymes has been shown to facilitate the addition of bran to baked products, and the beneficial effects of these processes have been related to the solubilisation of bran AX (Coda et al. 2014; Figueroa-Espinoza et al. 2004; Lebesi and Tzia 2012). Fermentation with microbes is another method in which enzymatic hydrolysis plays an important role, as the beneficial impact of fermentation on the quality of high fibre bread has been ascribed largely to the solubilisation of AX due to the action of endogenous hydrolytic enzymes naturally present in cereal materials (Katina et al. 2012). The solubilisation of AX and DF might also accompany mechanical or thermo-mechanical treatments, such as mixing, grinding or extrusion (Cleemput et al. 1997; Dornez et al. 2007; Ralet et al. 1990; Wang et al. 1993; Zhu et al. 2010).

In terms of extruded products, it has been reported that soluble DF, such as pectin, inulin or guar gum, generally performs better than fibres that are mostly insoluble such as wheat bran (Brennan et al. 2008; Yanniotis et al. 2007), and it has been suggested that increasing the solubility of DF prior to extrusion could be a means to improve the functionality of DF in extruded products (Robin et al. 2012). However, only a few studies have examined this possibility. Pai et al. (2009) showed that increasing the content of soluble DF in corn bran with concomitant reduction of insoluble DF by a chemical alkaline treatment resulted in higher expansion as compared to untreated bran.

Hydration properties

Insoluble fibres which also form the majority of wheat bran DF can hydrate and physically entrap water, but still be present in the food matrix as discrete particles (Ortiz and Lafond 2012). Hydration properties are considered important in terms of DF functionality, and the detrimental effect of addition of insoluble DF to both baked and extruded products has been partly attributed to the competition for water between the DF and other components (Gan et al. 1992; Robin et al. 2012; Moraru and Kokini 2003). A number of terms and methods are used interchangeably to describe and quantify the ability of a fibre to retain water, including *water uptake, hydration, adsorption, absorption, retention, binding, or holding* (Guillon and Champ 2000; Thebaudin et al. 1997). According to a common definition, the water binding capacity (WBC) refers to the quantity of water that is retained by the soaked sample following the application of an external force (pressure or centrifugation), whereas the water holding capacity (WHC) means the quantity of water that is absorbed into the fibres without the application of external forces after soaking (except for gravity and atmospheric pressure) (Thebaudin et al. 1997). Swelling capacity is also included in the hydration properties and is defined as the volume occupied by a known weight of sample after soaking under the conditions used (Guillon and Champ 2000; Robertson et al. 2000).

The ability of bran to retain water is mainly determined by its particle size, porosity and DF polymer chain length (Tungland and Meyer 2002). By modifying the physical properties of the fibre matrix, processes such as grinding, drying, heating or extrusion cooking all affect the hydration properties (Thibault et al. 1992). In general, particle size reduction of bran and other DF preparations decreases their water binding capacity (Noort et al. 2010; Auffret et al. 1994; Zhu et al. 2010). When particle size increases, so does the trapped volume due to imperfect packing and consequent apparent water binding (Thebaudin et al. 1997). However, grinding may also increase the hydration properties of DF preparations as a consequence of the increase in surface area (Elleuch et al. 2011). Furthermore, when added to bread dough, the water absorption of the dough has been found to increase along with reduction of bran particle size (Sanz Penella et al. 2008; Noort et al. 2010).

In baked products the reduction of water binding capacity of bran or the increase in the level of available water by enzymatic hydrolysis has been related to improved baking properties (Lebesi and Tzia 2012; Katina et al. 2006,). Insoluble DF such as WUAX are known to bind more water than their soluble counterparts (Courtin and Delcour 2002), and thus the solubilisation of DF by hydrolytic enzymes can reduce its water holding capacity (Yu and Perret 2003; Lebesi and Tzia 2012). On the other hand, treatment with hydrolytic enzymes has also been found to increase the bran swelling capacity and decrease batter a_w (Lebesi and Tzia, 2012). In terms of extruded products, several researchers have theorized that the binding of water to DF molecules might reduce the amount of water available for starch gelatinization, eventually reducing radial expansion (Yanniotis et al. 2007; Moraru and Kokini 2003). However, Robin et al. (2011b) reported that addition of

wheat bran resulted in increase in the level of available water. It can be concluded that interactions related to the hydration properties of DF, availability of water and the impacts of these factors on the quality of baked and extruded products are complex and partly unresolved.

Viscosity in solution

Soluble DF is able to thicken or form gels in fluids due to entanglement of the polysaccharide chains within the fluid. Viscosity depends on intrinsic characteristics of the polysaccharide (amount of space occupied by the polymer, generally characterised by intrinsic viscosity), its concentration, the solvent and temperature (Guillon and Champ 2000). Generally, as the molecular weight or chain length of DF increases, the viscosity in solution also increases (Tungland and Meyer 2002). The viscosity of AX in solution is also determined by its degree of substitution and substitution pattern (Courtin and Delcour 2002).

Addition of bran or other DF ingredients can have an essential impact on the viscosity and other rheological properties of the dough (Sanz Penella et al. 2008; Wang et al. 2002). These properties are affected by bran particle size and solubility of DF (Collar et al. 2006; Noort et al. 2010; Rosell et al. 2010; Sanz Penella et al. 2008). The beneficial impact of the use of xylanolytic enzymes in baking is largely related to the increase in dough viscosity due to the increase in the content of WEAX (Courtin and Delcour 2002). The increase in dough viscosity by an endoxylanase treatment of bran was also related to the improved baking properties of oat and rice bran (Lebesi and Tzia 2012). However, too extensive degradation of WEAX to low MW oligo- or monosaccharides can have a detrimental effect on bread due to the decrease in dough viscosity (Courtin and Delcour 2002), and thus the enzyme selection and dosing are important factors determining the impact of the use of xylanases in baking. In extrusion, melt viscosity is an important factor affecting expansion, and the effects of soluble and insoluble DF on expansion have also been related to their effects on melt viscosity (Moraru and Kokini 2003; Pai et al. 2009). The improved expansion due to solubilisation of corn bran DF by an alkaline treatment was related to favourable changes in melt viscosity (Pai et al. 2009).

2. Aims of the study

Wheat bran is a nutritionally appealing and vastly available raw material, but is currently under-utilized as a food ingredient due to its technological and sensory challenges. Treatments with hydrolytic enzymes such as xylanases have been studied as a means to modify the properties of bran, but very little focus has been given to the impact of water content on the enzyme function and efficiency of these processes. Processing at reduced water content could be economically beneficial, especially when targeting dry end products. However, reduction of water content of an enzymatic process presents several challenges that need to be studied in order to be able to develop new low-water processes.

The present work aimed to examine and develop techniques to utilize hydrolytic enzymes at reduced water content in order to increase the technological functionality of wheat bran. More specifically, the aims were:

1. To determine and control the interactions between water content and physical and physicochemical properties of bran-water mixtures.
2. To examine the effects of water content on the reaction kinetics, mode of action and interplay of exo- and endogenous cell wall degrading enzymes, especially xylanases.
3. To develop practical applications for enzymatic treatment at low water content in order to improve the technological functionality of wheat bran, and to study the effects of the process on the technological properties of bran in a food application.

3. Materials and methods

A general outline of the materials and methods used in the work is presented in this section. Detailed descriptions can be found in the original Publications I–IV.

3.1 Raw materials

3.1.1 Cereal raw materials

Two different brans ground to different levels of fineness were used in the study (Table 6). The bran in Publications I and II was obtained from mixed wheat varieties, and the grains were peeled by friction using the Bühler Peeling technology to remove 2–3% of the grain outer layers before bran removal in order to reduce the level of contaminating microbes and enzymes in the bran. The bran made from the peeled grains was ground in an impact sieve mill to finer particle size before use. In Publications III and IV, commercial native wheat bran obtained from Finnish spring wheat was produced using a conventional roller mill and ground by TurboRotor technology to three different levels of fineness. The Turborotor grinding process is based on high air throughput, that keeps the product airborne during the grinding. The intensity of size reduction is controlled by the number of grinding components and by the rotation speed and rate of air flow. Rye endosperm flour was used as a base material for the expanded extrudates in Publication IV. Average particle size and DF, AX and starch content of the wheat brans and rye flour, analysed as described in Publications I–IV, are presented in Table 6.

Table 6. Cereal raw materials used in the study. Dietary fibre (DF), arabinoxylan (AX) and starch content expressed as % of bran dry matter.

	Bran from peeled grains	Native bran				Rye endosperm flour
		Unground	Coarse	Fine	Ultrafine	
Publication	I and II	III and IV				IV
Grinding	Impact mill	-	Turborotor technology			-
Average particle size (µm)	113±9	1001±9	702±59	327±9	81±2	nd
Total DF	49.5	48.0	48.9	47.9	48.4	11.8
soluble DF	-[nd]	3.1	3.5	4.1	4.6	9.6
Total AX	21.7±0.6	20.6±0.4	20.5±0.3	20.3±0.6	20.6±0.2	-[nd]
Water extractable AX	0.6±0.1	0.5 ±0.1	0.5 ±0.1	0.6 ±0.1	0.8 ±0.1	-[nd]
Starch	11.6±0.1	16.3±0.1	16.7±0.1	16.8±0.2	16.5±0.1	84.7±0.3
Ash	6.7±0.1	6.4±0.1	6.4±0.1	6.3±0.1	6.3±0.1	0.6±0.1

-[nd] not determined

3.1.2 Enzymes

Commercial hydrolytic enzymes, Depol 761P (Biocatalysts Ltd, Cardiff, UK), a xylanase preparation derived from *Bacillus subtilis*, and Veron CP (AB Enzymes GmbH, Darmstadt, Germany), a cellulolytic enzyme preparation with hemicellulase side activities from *Trichoderma reesei*, were used either individually or in combination for the bran treatments. The activity profile of the enzymes is presented in Table 7. Depol 761P was selected based on a screening study (Petersson et al. 2013) and its activity profile: xylanase with only few side activities (Table 7). Veron CP enzyme preparation was selected because of its high cellulase (filter paper) and endoglucanase activities that were not found in Depol 716P (Table 7). All activity measurements were performed at pH 5, 50 °C.

Table 7. Activity profile of the enzyme preparations used in the study (nkat/g).

Activity	Depol 761P	Veron CP	Substrate	Reference
Endoxylanase	28660	14610	1% birch glucurone xylan	Bailey et al. (1992)
Polygalacturonase	1317	8469	0.4% polygalacturone acid	Bailey and Pessa (1990)
β-glucanase	1625	75760	1% barley β-glucan	Zurbriggen et al. (1990)
α-amylase	44	94	p-nitrophenyl maltoheptaoside	Megazyme Ceralpha method
β-xylosidase	2	257	5mMp-nitrophenyl-β-D-xylopyranoside	Poutanen and Puls (1988)
Cellulase / filter paper	-[a]	53[b]	filter paper	IUPAC (1987)
Endoglucanase	-[a]	18974	1% hydroxyethyl cellulose	IUPAC (1987)
Mannanase	-[a]	3022	0.5% locust bean gum	Stålbrand et al. (1993)
β-glucosidase	-[a]	528	1 mM p-nitrophenyl-β-D-glucopyranoside	Bailey and Linko (1990)
α-arabinosidase	-[a]	530	2 mM p-nitrophenyl-α-L-arabinofuranoside	Poutanen et al. (1987)
Ferulic acid esterase	-[a]	-[a]	4 mM ethyl ferulate	Forssell et al. (2009)
Endoprotease	-[a]	-[a]	azurine-crosslinked casein	Protazyme AK tablet method, Megazyme International Ireland

[a]no activity detected. [b]activity expressed as filter paper units/g.

3.2 Enzymatic treatments of bran

Different reactor and mixing systems used in the study are summarized in Table 8 and shown in Figure 3. The enzyme powders were mixed with bran before the addition of pre-heated water. All incubations were performed at 50 °C, which was the temperature recommended by the supplier of the Depol 761P enzyme preparation. The enzyme preparations were dosed according to their xylanase activity at 20 or 200 nkat/g bran dry matter (DM) (treatments with Depol 761P, Publications I–IV) or 100 nkat/g (treatments with Veron, CP, Publication IV). For the treatments with a combination of the two enzymes the dosages as xylanase activity were 200 nkat Depol 761P and 100 nkat Veron CP /g bran DM. Corresponding treatments without addition of enzymes were performed for each bran incubation process.

Table 8. Summary of the bran treatments.

	Reactor/ mixing type	Treatment water content (%)	Incubation time	Stirring during incubation	Publication
Low-water treatments	Horizontal mixing with z-blades	20–70	1–24 h	60–63 rpm	I and II
	Twin-screw extrusion[a]	37–60	0 or 4 h	no	III and IV
	Vertical mixing with K-blade[a]	37–60	0 or 4 h	no	III
High-water treatments	Vertical double-blade mixing	80 and 90	1–24 h	160 rpm	I and II
	Shaking	92	4 h	120 rpm	III

[a] Used for pre-mixing (3 min) and forming the material before stationary incubation.

In Publications I and II, the enzymatic treatments of bran at water contents of 20–70% were performed in a farinograph mixing bowl (Brabender Farinograph, mixer type S300 with z-blades) using continuous mixing (60–63 rpm). The bran was placed in the mixer, and pre-heated water was added by spraying during 1–3 min with rotating blades in order to obtain an even distribution of water. The treatments at water contents of 80 and 90%, due to the liquid form of the mixture, were performed with continuous double-blade mixing (speed 160 rpm) in a sealed vertical steel container placed in a water bath (50 °C). The reactions were stopped by cooling down and freezing the sample immediately. Subsequently, part of the frozen sample was freeze dried and ground in a laboratory mill (0.5 mm sieve) for later analyses.

In Publications III and IV, the enzymatic treatments at water contents of 37–60% were performed using two different pre-mixing and forming methods, blade-mixing and extrusion, followed by stationary incubation (i.e. without stirring), whereas the treatments at high water content (92%) were performed with continuous shaking. For the extrusion-aided treatments, a pre-conditioned (20% moisture) bran mixture was fed to the extruder (APV MPF 19/25, Baker Perkins Group Ltd, Peterborough, UK) at a rate of 26 g/min. The barrel temperature was 50 °C and screw speed was 65 rpm. Water was pumped to the barrel at an appropriate rate in order to obtain the intended moisture content in the bran mixture. The residence time inside the barrel was about 3 min. Bran mixture was collected from the die exit (diameter 3 mm) for 2 min and either immediately frozen in liquid nitrogen or transferred to incubation (4 h in sealed containers) or to drying, which was performed either in an oven (samples spread on metal trays and dried with air circulation at 50°C for 18–20 h) or by freezing the sample in liquid nitrogen for subsequent freeze drying. The incubated samples were also immediately dried by oven drying or by freeze drying. For the treatments without extruder treatment (hereafter referred to as 'blade-mixed treatments') at water contents of 37–60%, the pre-conditioned bran sample was pre-heated in a heating chamber at 55 °C for 12 min, after which it was brought to the intended moisture content by spraying pre-heated water while mixing with a Kenwood mixer (K-blades, speed setting 2) for 3 min at 55 °C. The bran mixture was divided into samples which were either frozen immediately in liquid nitrogen or incubated further in sealed containers at 50 °C for 4 h and then frozen in liquid nitrogen. The treatments at high water content (92%) were performed in centrifuge tubes with continuous shaking (120 rpm), after which the reaction was stopped by freezing the sample in liquid nitrogen.

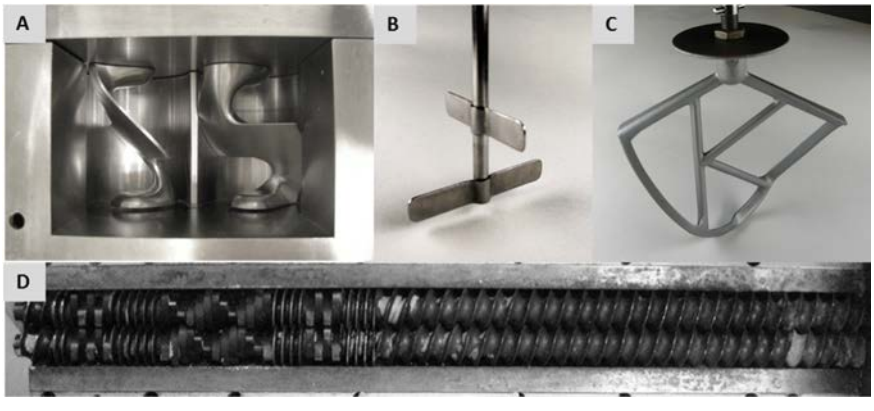

Figure 3. Mixing equipment used in the study. Horizontal z-blades (Farinograph) (a), vertical double-blade (b), vertical K-blade (c), twin-screw extruder (d).

3.3 Analysis of bran samples

3.3.1 Consistency, water activity and pH of bran-water mixtures

Changes in textural properties of the bran sample during the first 60 min of the treatments in Publication I were followed as Farinograph torque values reflecting the resistance of the bran-water mixture to the mixing blades. The water activity at the end of 24 h bran treatment was determined from a fresh sample using AquaLab CX2 (Decagon Devices Inc., USA). The pH of treated bran was measured by solubilising 0.2 g of the freeze dried sample in 10 ml of distilled water and stirring for 15 min prior to measurement.

3.3.2 Particle size, microstructure and hydration properties of bran

Average bran particle size was measured by laser light diffraction using either a Coulter LS320 particle size analyser (Coulter Corporation, Miami, FL, USA) or a Mastersizer 3000 (Malvern, Worcestershire, UK). Samples were measured either from dry bran dispersion (Publication I and untreated brans in Publication III) or after dispersing the samples in water (Publication II and treated samples in Publication III) or in ethanol (Publication IV). Mean or median particle sizes were calculated from the volumetric distribution of the particles using the Fraunhofer optical model.

For the microscopy analyses in Publication II, the bran samples were pretreated as described by Dornez et al. (2011) and stained and imaged using exciting light as described by Andersson et al. (2011). For imaging the sample, sections (2 µm) were stained either with Light Green and Lugol's iodine solution, which stain protein green and starch purple, respectively, or with Acid Fuchsin and Calcofluor White, which stain protein red and β-glucan-rich cell walls light blue, respectively.

Water binding capacity (WBC, termed as approximate WHC in Publication I) of the bran was analysed by dispersing the bran sample in water, followed by 60 min incubation (Publication I) or 30 min shaking (Publication II) at room temperature, and centrifugation. After removing the supernatant, the weight addition (plus the water contained initially in the sample) per gram of bran sample dry matter was the WBC of the sample. Water holding capacity (WHC) and its kinetics were determined using the Baumann apparatus (Baumann 1966) as described in Publication II using a sample size of 75 mg (Publication II) or 50 mg (Publications III and IV). The water uptake was recorded for 25 or 30 min at room temperature.

3.3.3 Analysis of arabinoxylan and monosaccharides

For the analysis of WEAX in Publications I and IV, dry bran sample was extracted with distilled water for 15 min in cold water (4 °C) in order to avoid enzymatic activity

during the extraction. After centrifugation, the supernatant was boiled and centrifuged again. In Publication III, the wet (frozen) bran samples were dispersed and extracted with glass beads as described in Publication III. The contents of AX and pentose monosaccharides in bran water extracts (i.e. WEAX), were determined by a colorimetric phloroglucinol method (Douglas, 1981) using xylose as a standard (Publications I, III and IV). Free pentose sugars were corrected by a factor of 0.88 to anhydro sugars. The degree of solubilisation (DS) was calculated by dividing the WEAX content of the sample by the total AX content of the bran. For the quantification of total AX (Publications I and III), bran sample was mixed with 0.5 M H_2SO_4 and boiled for 20 or 30 min and centrifuged, followed by the colorimetric determination (Douglas 1981).

For the analysis of water-extractable monosaccharide composition (Publication II), the dry bran sample was extracted as described above and the supernatant was hydrolysed with 3.75 M H_2SO_4 at 100° C for 2 h. The hydrolysate and monosaccharide standards were analysed as their alditol acetates as described by Blakeney et al. (1983) by gas chromatography (GC) using an Agilent 6890 GC equipped with a flame ionization detector (Agilent, Palo Alto, CA, USA). The column was a DB-225 [30 m × 0.32 mm; film thickness 0.15 µm (Agilent)]. The monosaccharides were identified according to their retention times and quantitated with corresponding standard curves. Free hexose sugars were corrected by a factor of 0.9 to anhydro sugars, and pentose sugars by factor of 0.88. For the analysis of total monosaccharide composition, dry bran sample was hydrolysed with H_2SO_4 at 100 °C as described in Publication II and the supernatant was acetylated and analysed by GC.

3.3.4 Molecular weight analysis of WEAX by HP-SEC

The apparent WEAX molecular weight (MW) distribution of bran in Publications I and III was analysed by high performance size exclusion chromatography (HP-SEC). In Publication I, the samples were extracted in boiling water, and starch and β-glucans present in the water extract were hydrolysed by a saccharifying enzyme solution (Optidex L-400, Genencor International). In Publication III, the analysis of the apparent MW distribution of WEAX was performed by also removing other poly- and oligomeric compounds from water extracts by an enzymatic procedure, followed by EtOH precipitation, using a method modified from that of Andersson et al. (2009) as described in Publication III. The liquid chromatograph with Alliance 2690 separation module and M-2414 refractive index detector consisted of three columns (7.8 × 300 mm) µHydrogel 500, µHydrogel 250 and µHydrogel 120 (Waters Inc., Milford, MA, USA). The suitability of the conditions of the HP-SEC analysis was previously checked using a dual angle laser light scattering detector (Precision Detectors, USA) that is sufficiently sensitive to detect aggregates (not observed) and by using flow rates of 0.2 ml/min and 0.5 ml/min. The results were similar at both flow rates, indicating that the sample does not hydrolyse during the analysis. Pullulan standards and maltopentaose were used for calibration. In Publication III, the average MW of the sample was calculated between 32.7 min (the

elution point of the largest standard) and 53.0 min (corresponding to a MW of approx. 2 kDa).

3.3.5 Analysis of residual endoxylanase activity in bran

For the calculation of the percentage of residual endoxylanase activity in the enzyme-treated bran samples in Publication I, the bran samples were extracted with phosphate buffer (25 mM, pH 6.0) during 60 min at room temperature, and the endoxylanase activity in the supernatant was analysed by Xylazyme AX Tablet assay (Megazyme, Ireland) using a reaction time of 60 min. The initial activity was calculated as the sum of the activity of the added xylanase plus the endogenous activity analysed for the bran.

3.3.6 Sequential extraction and analysis of proteins

Proteins of the bran samples were examined in Publication II using a sequential buffered extraction procedure. Salt-soluble proteins were extracted at 4 °C with 0.5 M Tris-HCl buffer (pH 8) containing 1 M NaCl and the residual protein fraction was extracted from the unsoluble sediment at 50 °C with a mixture containing 2% sodium dodecyl sulphate (SDS), 10% glycerol, 1.5% dithiothreitol (DTT), and 0.05 M Tris-HCl pH 8 buffer (repeated once). The different protein fractions were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli 1970) and visualized with a Criterion stain-free imaging system (Bio-Rad, Hercules, CA, USA) as described in Publication II. The protein content of all extracts was analysed by a commercial kit (RC DC Protein Assay, Bio-Rad).

3.4 Production and analysis of expanded extrudates

In Publication IV, bran-supplemented expanded extrudates were produced from mixtures of bran and rye endosperm flour (20:80 ratio) using a twin screw extruder (APV MPF 19/25, Baker Perkins Group Ltd, Peterborough, UK) with a 3 mm diameter die. Rye endosperm flour was selected as base material because of its good expansion properties and nutritional profile (DF content 11.8%). Extrusion parameters were selected based on pre-trials performed with the flour base material to obtain maximum expansion (feed rate 60 g/min, screw speed 450 rpm, temperatures at the barrel zones 1 to 4 set at 80, 90, 120 and 130 °C). The water feed rate was adjusted to attain a moisture content of 16%. The extrudates were collected in trays and dried at 50°C for 30 min in an oven drier with air circulation. The extrusions were performed in duplicate.

For the analysis of macrostructural parameters, the extrudates were cut into 20 pieces each of 5 cm length using a band saw (Scheppach, Germany). Expansion rate (ER), specific length and piece density of each sample were calculated as described by Alam et al. (2013).

Mechanical properties of the extrudates were measured by applying uniaxial compression using a texture analyser (TA.XT plus, Stable Micro Systems Ltd., United Kingdom) containing a 30 kg load cell and a 25 mm aluminium probe under 70% strain with a test speed of 1 mm/s. All extrudates were cut into 10 mm pieces (radial section) and equilibrated at 43% relative humidity (RH) at 21 °C prior to analysis. Measurements were made for 20 replicates. Exponent software version 6.0.7.0 (Stable Micro Systems Ltd., United Kingdom) was used to obtain values for calculation of the hardness indicators, F_{max} (the maximum point of the force-deformation curve) and crushing force (F_{cr}), i.e. average puncturing force (van Hecke et al. 1998), and crispiness index (C_i) (Heidenreich et al. 2004).

For imaging of the radial cross-sections of the extrudates, the samples were cut into 10 mm pieces and examined with a SteREO Discovery.V8 stereomicroscope (Carl Zeiss MicroImaging GmbH, Germany) and imaged using a DP-25 single chip colour CCD camera (Olympus Life Science Europa GmbH, Germany) and the Cell^P imaging software (Olympus).

For the analysis of total and soluble DF contents, the extrudates were ground in a laboratory mill with a 0.5 mm sieve and analysed by AOAC method no. 2009.01 (McCleary et al. 2013).

3.5 Statistical analysis

All bran treatments were made in duplicate, and each sample was analysed at least in duplicate. Thus all the results were calculated as means of at least four analysis results. In Publication III, the parameters of macrostructure and mechanical properties of the extrudates were calculated as means of 35–40 results. Data were subjected to analysis of variance using IBM SPSS Statistics 19 (IBM Corporation, Somers, NY, USA), and significant differences ($P < 0.05$) between individual means were identified by the Tukey's test (Publications II, III and IV). All replicates (analytical and treatment replicates) were considered as equivalent replicates in the variance analysis. In Publication IV, correlations between different variables were determined by subjecting the mean result values to the 2-tailed Pearson's bivariate correlation analysis.

4. Results and discussion

4.1 Impact of water content on xylanase action and properties of bran during continuous mixing (Publications I and II)

4.1.1 Consistency, microstructure and pH of bran-water mixtures

The impact of water content, ranging from 20 to 70%, on the action of xylanase and on the properties of bran-water mixtures during continuous mixing was studied using a horizontal mixer (Farinograph) with z-blades. The WHC of the bran used was 3.6 g water/g bran DM, from which it can be calculated that bran could hold all the added water up to a water content of 78%. Thus, at the water contents between 20 and 70% there was no free bulk water, which was reflected in the appearance and consistency of the mixtures (Table 9). When bran was mixed in the Farinograph at different water contents between 20 and 70%, the mixture transformed from granular powder to a paste at a water content of 40%. At this 'transition' moisture content, the bran-water mixture formed a very compact, plastic-like mass during the 24 h treatment (Figure 4). According to the Farinograph resistance values recorded during the first 60 minutes of the treatments (Table 9), the viscosity of the paste increased with increasing water content up to a water content of 50%, and then at higher water contents the viscosity again decreased. Addition of xylanase increased the resistance values, especially at a water content of 40%, but did not affect the water activity or the visual appearance of the mixtures (Table 9). For comparison, the bran was also treated with a traditional high-water treatment using vertical blade-mixing, since the free water present in the bran mixtures prevented the use of the Farinograph as a mixing device at water contents of 80 and 90% because of leakage of water. A solids content of 15% (i.e. water content of 85%) is generally considered to be the upper limit of efficient mixing of lignocellulosic slurries in conventional stirred-tank reactors (Hodge et al. 2009; Kristensen et al. 2009). In the current study the use of a blade mixer for wheat bran slurries of water content below 80% was not possible because of too high viscosity of the mixtures.

Table 9. Water activity and appearance of bran after 24 h treatment with continuous mixing without added xylanase, and resistance values of bran mixtures after 60 minutes with different xylanase dosages of Depol 761P (compiled from Publication I).

Water content	Water activity	Appearance	Resistance value at 60 min (FU)		
			No added enzyme	Depol 761P xylanase 20 nkat/g	Depol 761P xylanase 200 nkat/g
20	0.83	powder	60	60	– [b]
30	0.89	powder	110	150	200
40	0.93	paste	240–270	330–400	400–550
50	0.96	paste	280–310	310–340	310–340
60	0.97	paste	140	130	– [b]
70	0.98	paste	40	40	– [b]
80	≥0.98[a]	slurry	– [b]	– [b]	– [b]
90	> 0.98[a]	liquid	– [b]	– [b]	– [b]

[a] With the measurement system used it was not possible to determine the accurate water activity of samples with high amounts of free water. [b] Not determined.

Figure 4. Bran mixture at a water content of 40% in a Farinograph mixer after 24 h treatment with xylanase enzyme preparation Depol 761P (20 nkat/g) (Publication I).

Examination of the microstructure of the bran by staining protein and cell wall structures with specific dyes showed that the layered cell wall structure of the bran treated at 40% water content with or without added xylanase preparation was much more degraded as compared to the bran treated at 90% water content (Figure 5). In the bran treated at 40% with no added enzymes, the structure of aleurone cells had broken, and seed coats with pigment strand and nucellar epidermis had separated from the aleurone layer. The compact consistency of the sample at a water content of 40% presumably enhanced the impact of mixing shear and caused the physical degradation of bran cell walls. The use of xylanase enzyme preparation Depol 761P (200 nkat/g) caused further degradation of the bran cell wall structure and the separation of pigment strand and nucellar epidermis from

each other, whereas in the samples treated at 90% water content the layer of pericarp, pigment strand and nucellar epidermis still remained attached to the aleurone cell layer and intact aleurone cells were still detectable, especially in the sample treated with no added xylanase. Processing of bran also caused a significant release of proteins from the aleurone cells of the bran particles, especially after the treatments at 40% water content, but the change was also detectable (although to a lesser extent) at 90%. The bran micrographs stained with Light Green and Lugol's iodine solution further showed that starch granules present in the bran sample were not significantly affected by any of the treatments (data not shown).

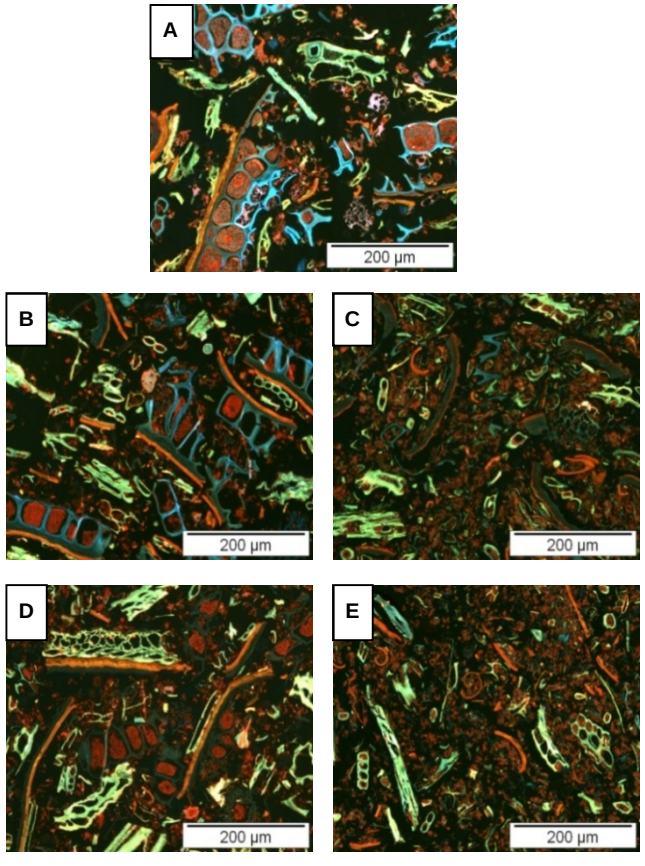

Figure 5. Microstructure of untreated bran (a) and bran samples treated for 24 h without added enzymes at 90% water content (b) and without added enzymes at 40% (c), and bran samples treated for 24 h with xylanase at 90% (d) and with xylanase at 40% water content (e). Micrographs, prepared from fresh (frozen) bran samples, were stained with Acid Fuchsin and Calcofluor White. β-Glucan-rich endosperm and aleurone cell walls appear in blue, pigment strand (between the pericarp and aleurone layers) in orange, pericarp layer in light green and yellowish, and proteins in red and reddish brown (Publication II).

The initial pH of the bran-water mixture was 6.8, and during the first 16 h of incubation the pH remained between 6.2 and 6.8 regardless of water content (Publication I). After 24 h, however, pH decreased to 4.7 at the highest water content (90%), indicating the growth and metabolic activity of acid-producing bacteria, such as lactic acid bacteria. Bran material naturally contains rather high amounts of yeasts and bacteria, but peeling of grain before bran removal is known to decrease the microbial load of bran (Katina et al. 2007). The use of bran from peeled grains and the relatively high temperature of the treatment (50 °C) were assumed to restrict the interference of microbes, but apparently some microbial growth occurred after long incubation times at the high water content. By contrast, in the treatments at decreased water contents the low a_w probably restricted the activity of these microbes, as the pH did not significantly change at the lower water contents (Publication I).

4.1.2 Action of xylanase during continuous mixing

The effect of water content on the action of xylanase on bran during continuous horizontal mixing with z-blades (Farinograph) was examined by analysing the content of WEAX after 24 h treatment (Publication I). WEAX content did not increase during the treatment at the lowest water content studied (20%), but remarkable increase in WEAX content occurred already at a water content of 30% (Figure 6). This indicates that the minimum required water content for the action of xylanase in the bran-water mixture was between 20 and 30%. The availability of water is better indicated by water activity, and most enzymes generally require a_w ≥0.85 for catalytic activity (Simpson et al. 2012). This was also the case in the current study, as a_w was 0.83 at a water content of 20% and increased to 0.89 at 30% (Table 9). Lee (1970) also reported that the limiting water content for hydrolytic enzyme reactions in wheat flour was about 30% of water, although the a_w at this point was higher (0.958) than in the current study. The range of approximately 25–30% of moisture has been defined as the saturation point below which the major part of the water in lignocellulose is present as primary or secondary bound water (Felby et al. 2008), and similar values (28–30%) have also been reported for wheat flour, as reviewed by Lee (1970).

Solubilisation of AX was highest at the water contents of 40 and 90% (6.9% WEAX of bran DM at both water contents, corresponding to DS of 32%), whereas at intermediate water contents (50–80%) the solubilisation was lower (Figure 6). Effects of treatment water content and the use of xylanase on AX solubilisation were also studied as a function of incubation time and xylanase dosage (20 and 200 nkat/g) at water contents of 30, 40, 50 and 90% (Publication I). No unexpected differences were observed in the rate of AX solubilisation with different xylanase dosages at different water contents. Generally the higher dosage caused significantly higher WEAX content and most of the WEAX was hydrolysed within 4 h incubation time, after which the solubilisation rate clearly reduced. An exception was the faster initial AX solubilisation at 90% water content during the first hour,

and the relatively higher increase in AX solubilisation with the higher enzyme dosage at the water content of 30% as compared to the other water contents studied (Publication I).

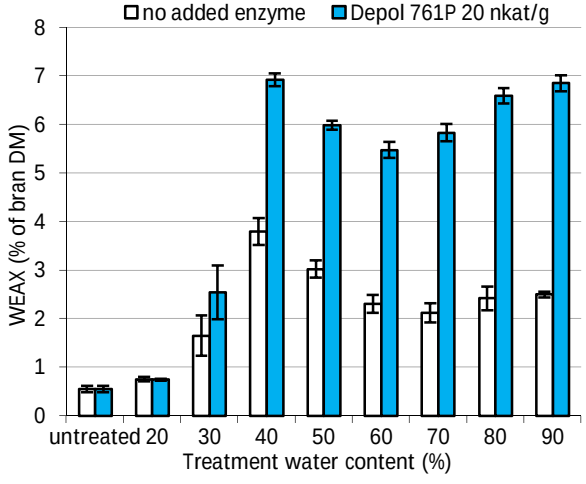

Figure 6. Effect of treatment water content and Depol 761P xylanase (20 nkat/g) on the WEAX content of wheat bran after 24 h incubation (Publication I).

The enhanced AX solubilisation at 40% water content was interesting, because decreasing the water content below 85% is known to decrease enzyme action on lignocellulosic biomass (Kristensen et al. 2009; Modenbach and Nokes 2013). There are only a few previous publications in which the modification of plant-based substrates with hydrolytic enzymes has been studied at solids contents above 40%, i.e. below the water content of 60% (Moore et al. 2006; Steinmacher et al. 2012), except for the use of enzymes in baking and hydrolysis of starch in screw reactors (Baks et al. 2008; Linko 1989). The reasons for the decreased enzyme action at increased solids content are generally related to the lack of available water, difficulties with mixing, insufficient mass and heat transfer, and increased concentration of inhibitors (Modenbach and Nokes 2013). It is probable that increased amount of available water and decreased viscosity at the water contents between 60 and 90% have improved the mass transfer and diffusion of components, and thus improved AX solubilisation. In this respect, the enhanced AX solubilisation at the water content of 40% was unexpected. However, the increase in AX solubilisation at the water content of 40% was evidently caused not only by the action of added xylanase but also by other factors, as even without added xylanase the solubilisation was also notably higher at the water content of 40% (3.8% WEAX in bran DM) than e.g. at 90% (2.5% WEAX) (Figure 6). It could be concluded that the compact consistency of the material at 40% water content probably enhanced AX solubilisation by physical breakdown of bran cell walls due

to shear forces, which was also indicated by the microscopic analysis. It has been shown that mechanical size reduction may cause solubilisation of DF and AX in wheat bran (Hemery et al. 2011; Zhu et al. 2010; Van Craeyveld et al. 2009).

The content of WEAX in selected bran samples was analysed by GC (Table 10), and the results were well in accordance with those determined by the colorimetric method (Publication I). The fact that some of the arabinose residues might have originated from arabinogalactan peptides (AGP) was not taken into account in the calculations of AX levels. No data is available concerning the content or presence of AGP in bran, but in wheat flour the contents of WE-AGP have been reported to be around 0.15–0.38% of flour DM (Andersson et al. 1994; Loosveld et al. 1997), which is very low compared to the level of AX in bran (generally in the range of 13–30%). Thus, it can be concluded that the increase in the content of water extractable pentose sugars (Publication I), or of arabinose and xylose (Publication II), was due to solubilisation of AX.

The analysis of A/X ratio (Publication II) provided new information about the role of enzymes in the solubilisation of AX during the treatment at the water content of 40%. The A/X ratio of the water extract of bran treated for 24 h at 40% was 0.32 (Table 10), which was in the range of values reported in the literature for enzymatically solubilised wheat bran AX (0.27–0.32) (Beaugrand et al. 2004; Swennen et al. 2006), whereas AX oligosaccharides produced by mechanical treatment have been reported to have much higher A/X ratios (0.65–0.72) (Van Craeyveld et al. 2009). The result suggested that although physical shear enhanced AX solubilisation at 40%, the origin of WEAX was presumably still due to enzymatic action on AX fragments with low arabinose substitution, occurring mostly in the aleurone (A/X 0.3–0.5) and in the nucellar epidermis (A/X 0.1), rather than to direct mechanical solubilisation of AX from the more substituted outer tissues (A/X≥1.0) (Antoine et al. 2003; Barron et al. 2007; Van Craeyveld et al. 2010). During the treatments at both water contents with and without added enzymes, the A/X ratio of the bran water extract decreased almost identically with increasing WEAX content, suggesting that AX was solubilised from the same bran tissues regardless of the processing conditions used (Publication II). The shear forces and the physical breakdown of bran particles could have enhanced the action of endo- and exogenous enzymes by improving the availability of the substrate, e.g. by increasing its surface area. It has been shown that decreasing the particle size of plant materials may enhance their enzymatic hydrolysis (Silva et al. 2012; Niemi et al. 2012; Mahasukhonthachat et al. 2010; Dasari and Berson 2007). The impact of bran particle size was further studied in Publication III and is discussed in section 4.2.2.

Table 10. Content and A/X ratio of WEAX in bran after 4 and 24 h treatment with Depol 761P xylanase at water contents of 40 and 90% as analysed by GC (Publication II) and the level of residual endoxylanase activity in bran water extract (Publication I).

Treatment water content (%)	Treatment time	WEAX[a] (% of bran DM)	A/X[a]	Endoxylanase activity (% of initial[b])
90	4 h	9.1±0.6	0.40±0.02	70±2
	24 h	11.1±0.5	0.36±0.01	64±4
40	4 h	8.5±1.1	0.41±0.02	45±3
	24 h	12.1±0.5	0.32±0.01	10±1

[a] xylanase dosage 200 nkat/g, [b] xylanase dosage 20 nkat/g.

The mode of action of xylanase was further studied by analysing the residual endoxylanase activity and the apparent MW distribution of WEAX in purified bran water extracts. The recovery of xylanase activity in water extracts of enzyme-treated (200 nkat/g) bran samples was only 10% of the initial activity after 24 h treatment at the water content of 40% (Table 10). The recovery was higher both at lower and higher water contents than 40%, and the highest recovery after 24 h treatment, 64%, was detected in bran treated at 90% water content. This was unexpected in view of the degree of AX solubilisation, but it is possible that the compact structure of the material and efficient binding of enzyme to the substrate at the water content of 40% prevented the extraction of the enzyme from the freeze dried sample in the enzyme activity assay used, thus giving lower activity values.

The analysis of the apparent MW distribution of purified bran water extract indicated that the amount of large WEAX polymers was higher after treatments with continuous mixing at 40% than at 90%, both with and without added xylanase (Publication I). This suggests that although the degree of AX solubilisation was the same (when added xylanase was used) or even higher (without added xylanase) at the low water content as compared to the high water content treatments, the depolymerisation efficiency of xylanase was higher at the water content of 90% than at 40% when continuous mixing was used. According to Sibakov et al. (2013), the use of a water content of 90% for enzymatic hydrolysis of β-glucan resulted in rapid breakdown into short oligosaccharides, whereas low water content (50%) enabled a more easily controlled depolymerisation of high MW β-glucan. Van der Veen et al. (2005) also reported that increased dry matter content in the hydrolysis of maltodextrins resulted in increased formation of condensation products so that both the quantity and the length of the product polymers increased. Similarly, Hardt et al. (2013) reported that high solids concentrations in wheat gluten hydrolysis resulted in a higher weight fraction of peptides with a high molecular mass than low solid concentrations. This was ascribed to high viscosity

and mass transfer limitations (Hardt et al. 2013), and the same attributes probably caused the decreased depolymerisation efficiency of xylanase in the current study.

4.1.3 Physicochemical properties of bran treated with continuous mixing

Solubility of carbohydrates

The impact of enzyme treatment of bran with continuous mixing at high (90%) and low (40%) water content on the solubilisation of carbohydrates was followed by analysing the monosaccharide composition of the water extracts of freeze dried bran samples after acid hydrolysis (Publication II). After 24 h treatment at a water content of 40% the total water-extractable monosaccharide content (19.0% of bran DM) was higher than after the treatment at a water content of 90% (10.0% of bran DM). This was mainly due to the glucose content, which increased at 40% but did not significantly change at 90% after 4 h treatment. An increase in the soluble glucose content at 40% indicates the degradation of starch, cellulose, or β-glucan, probably due to the physical degradation of the bran. The total monosaccharide composition of bran (glucose 24.0±0.8%, galactose 1.16±0.04%, mannose 0.63±0.04%, AX 22.8±0.6%) was not affected by any of the treatments, except for the glucose content, which decreased to 21.3±1.3% after 24 h treatment at 90% both with and without added xylanase. The decrease in glucose content during the treatment at the water content of 90% was probably caused by metabolic activity of contaminating microbes, as was also indicated by the decrease of pH after 16 h treatment at the water content of 90% (section 4.1.1).

Solubility and molecular weight of bran proteins

The impact of bran treatment with continuous Farinograph mixing on the solubility and molecular weight of bran proteins was analysed by sequential extraction and electrophoretic analysis of salt-extractable and SDS+DTT-extractable (residual) proteins (Publication II) and by the spectrophotometric quantification of protein in the extracted fractions (Figure 7). The physicochemical characteristics of bran proteins are not yet well established, although the protein content of bran is relatively high, generally around 15–17% (Kamal-Eldin et al. 2009; Maes and Delcour 2002). The spectrophotometric quantification indicated that the concentration of salt-extractable proteins decreased during the bran treatments, especially during the treatments at 40% water content, whereas the concentration of SDS+DTT-extractable residual protein increased during the bran treatments (Publication II). The results of the SDS-PAGE analysis (Figure 7) were in agreement with the quantification results. In the salt-soluble fraction the proteins above 25 kDa had disappeared or appeared as lighter bands in the treated bran samples. In the residual protein fraction of the treated bran samples, protein “dust” occurred in the area above 100 kDa, whereas in the untreated bran no protein was observed in this area. Furthermore, especially in the case of the samples treated at 40%, a

large amount of protein had remained in the wells of the electrophoresis gel, indicating the formation of large protein-containing aggregates. The SDS+DTT-extractable fractions also contained small proteins of molecular weight 10–20 kDa not detectable in the initial bran (Publication II).

Protein aggregation and depolymerisation were probably caused by the high shear exerted on the bran especially during the treatment at a water content of 40%. Because electrophoresis was performed in denaturing conditions, which leads to the reduction of disulphide bonds during preparation of samples for SDS-PAGE, the high molecular weight protein aggregates were presumably caused by the formation of covalent bonds during the bran treatments. It is known that heating and shearing may cause degradation or aggregation of proteins by disulphide or covalent bond formation, for example in extrusion processing, and several authors have reported a decrease in protein extractability after extrusion, as reviewed by Anderson and Ng (2000). Bran is known to contain endogenous proteases, but they generally activate below pH 5 (Loponen et al. 2004) and thus the depolymerisation observed in the residual fraction was probably not caused by endogenous proteases, since the pH decreased below 5 only after 24 h treatment at 90% (Publication I). The observed decrease in the solubility of bran proteins supports the hypothesis that the decreased level of residual endoxylanase activity in the water extract of bran treated at 40% was caused by limited extractability of enzyme proteins from the bran sample. The recent study of Nordlund et al. (2013) showed that bioprocessing by sequential treatments with cell-wall hydrolysing enzymes and yeast fermentation caused release of protein from aleurone cells, assessed as a higher content of soluble protein in bran and a higher hydrolysis rate *in vitro*. The solubilisation and digestion of bran-associated proteins were explained both by the yeast fermentation and the treatment with the hydrolytic enzyme mixture, which also contained low endoprotease activity. In the current study, the enzyme preparation used was free of endoprotease activity, and the use of the enzyme had no visible effect on the electrophoretic patterns of proteins at either water content. Similarly, the use of xylanase had no significant impact on the content of protein in the salt-extractable and residual fractions analysed by the spectrophotometric quantification.

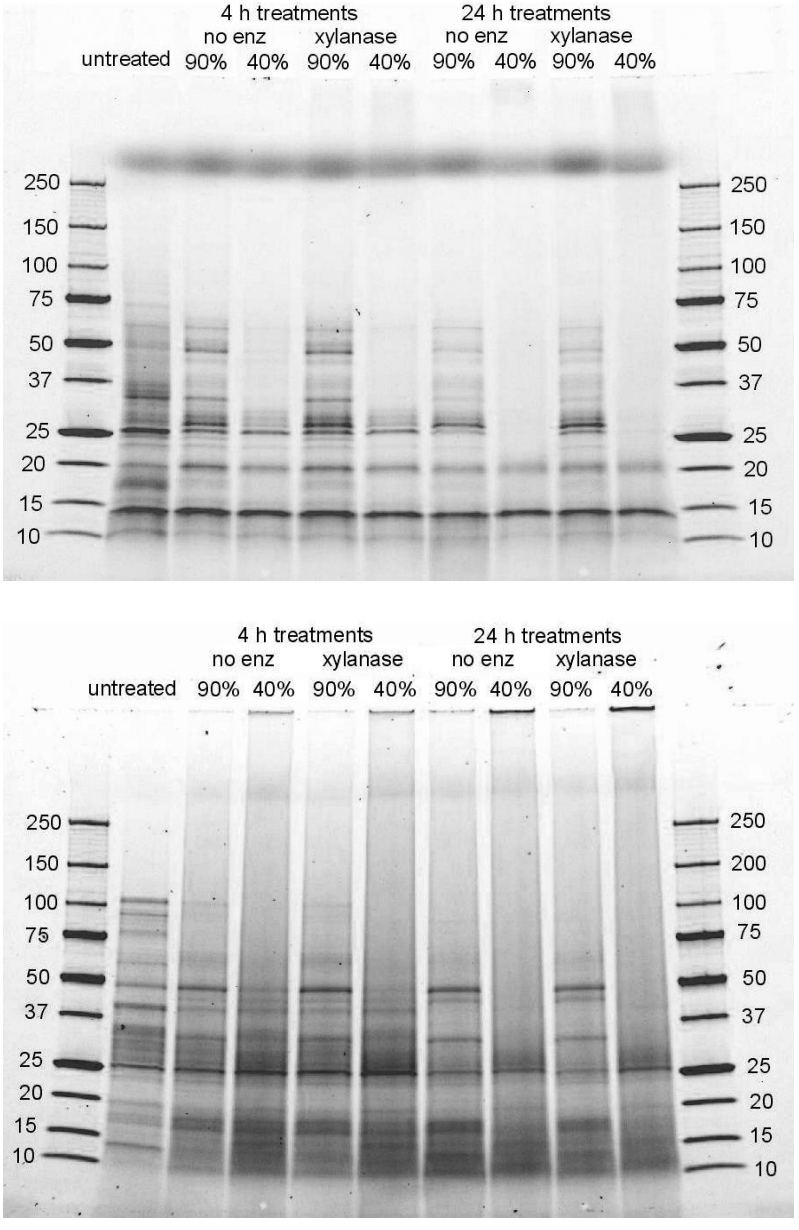

Figure 7. SDS-PAGE patterns of salt extractable (a) and SDS+DTT extractable (b) (residual) proteins of untreated bran and bran samples treated at water contents of 40% and 90% with and without Depol 761P xylanase (200 nkat/g) (Publication II).

Hydration properties and particle size of xylanase-treated bran

Analysis of the hydration properties of brans treated at 40% and 90% (Publication II) showed that the treatments at 40% water content resulted in lower WHC and WBC of the freeze dried sample than the corresponding treatments at 90% water content (Table 11). This may be due to the significant decrease of the bran particle size (especially upon grinding the freeze dried processed sample) during the treatment at 40% (Table 11). Reduction of particle size of bran and other DF preparations generally decreases WBC due to the decrease in the trapped volume as a consequence of structure collapse (Noort et al. 2010; Zhu et al. 2010; Thebaudin et al. 1997). During the treatment at 40% water content, transformation of the bran-water mixture into a compact, plastic-like mass probably enhanced the impact of the mechanical shear during the treatment in the Farinograph mixer, and caused more severe particle size reduction than the blade-mixing treatment at the water content of 90%.

The WHC of the bran increased at 90% after 4 h treatment without enzymes. This might be due to swelling of the bran in water. The use of freeze drying preserved the sample with minimal structural damage. The use of xylanase decreased WHC at both water contents as compared to the corresponding treatments with no added enzymes (Table 11), which was expected as WUAX is known to bind more water than WEAX (Courtin and Delcour 2002). The presumed degradation and solubilisation of other DF components, such as β-glucan, probably also affected the WHC in the same way as the solubilisation of AX by binding less water.

Hydration properties were measured using two different methods; WHC with the Baumann method and WBC with a centrifugation method. In the Baumann method no external force is used and the measurement is based on the principle of the diffusion of a liquid by capillary action, and thus it also describes the kinetics of water movement. However, the samples did not significantly differ in the kinetics of water holding, as observed from the estimated slopes of the WHC curves (Publication II). WBC of the bran changed during the treatments in a similar manner as the WHC (with minor exceptions), but WBC was always 0.6–0.9 units lower than the WHC of the same sample (Table 11), probably because WHC also includes the proportion of water loosely associated with the fibre matrix, whereas WBC includes only strongly absorbed water. WBC might have greater practical significance than WHC, because food manufacturing processes typically utilise some form of physical stress such as mixing, stirring, kneading or homogenization (Tungland and Meyer 2002). On the other hand, in the centrifugation method, the water-soluble components of the sample are lost in the supernatant, and thus the result describes only the hydration properties of the insoluble solids and may also depend on the g-force used (Chaplin 2003).

Table 11. Mean particle size (µm), water binding capacity (g water / g bran DM) and water holding capacity (g water / g bran DM) of untreated bran and bran samples treated at 40% and 90% water content (determined from freeze dried and ground samples). Values marked with different letters within the same column are significantly different (P < 0.05) (Compiled from Publication II).

	Treatment water content (%)	Treatment time	Mean particle size (µm)	Water binding capacity (g water /g bran DM)	Water holding capacity (g water /g bran DM)
		untreated	113 a	2.8 a	3.6 b
With no added enzyme	90	4 h	110 a	3.0 a	3.9 a
		24 h	94 b	3.0 a	3.6 ab
	40	4 h	56 c	2.5 b	3.2 c
		24 h	37 d	2.3 bc	3.0 d
With Depol 761P xylanase (200 nkat/g)	90	4 h	65 c	2.1 c	2.9 d
		24 h	60 c	2.3 bc	2.9 d
	40	4 h	37 d	1.7 d	2.4 e
		24 h	26 e	1.3 e	2.0 f

4.2 Impact of properties of bran-water mixture on xylanase action during stationary incubation (Publications III and VI)

In the first part of the work (Publications I and II), the efficient xylanase action at a water content of 40% was attributed to the compact consistency of the bran-water mixture that enhanced physical degradation of bran during continuous mixing. The role of the physical form of the substrate mixture (continuous mass vs. powdery/granular material), and the impact of bran particle size on xylanase action were further studied by comparing two different pre-mixing and forming methods, blade-mixing and extrusion, on xylanase action during stationary incubation of wheat bran (Publication III).

4.2.1 Physical form and particle size of bran after pre-mixing by extrusion and blade-mixing

The consistency of bran-water mixtures by vertical blade-mixing and extrusion was examined at water contents of 37–60% (Table 12). In the extrusion treatments, the bran mixture was forced through a small die after intensive mixing in the extruder barrel, causing the formation of uniform ‘sticks’ of moist bran mass (Figure 8). When using vertical blade-mixing with the coarsely ground bran, the material remained in the form of moist granular material at all water contents studied (37–60%)

(Table 12, Figure 8). By contrast, in the case of the fine bran, the blade-mixed bran-water mixture remained in the form of a moist granular powder up to a water content of 48%, but at 54% it formed a continuous, plastic mass during the 3 min blade-mixing. The higher tendency of the ultrafine bran to form a continuous mass might be explained by its lower water holding capacity, which was 3.3 g water /g bran DM as compared to 3.6 g/g bran DM for the coarse bran (Publication III). When a material binds less water, more water remains to act as a plasticizer. Furthermore, when the bran and water were mixed using an extruder, the reduction of particle size caused a reduction in the torque values of the extruder, especially at water contents of 54–60% (data not shown), indicating a reduction of viscosity with decreasing bran particle size. Dasari and Berson (2007) and Viamajala et al. (2009) also reported a reduction in viscosity as a result of decreasing particle size. Reduction in viscosity due to smaller particle size may allow for higher solids loading (i.e. lower water content) in enzymatic processing (Dasari and Berson 2007).

The particle size of the coarse bran had decreased during the extrusion, especially at the low water contents (Table 12). Reduction in particle size was presumably due to the higher shear forces, indicated by higher torque values (data not shown) exerted on the bran mixture at low water content. Particle size was reduced similarly or even slightly more in the samples with no added enzyme (data not shown). By contrast, when bran was processed with continuous mixing in the Farinograph mixer (Publication I), the particle size decreased more with the use of xylanase, and the samples with xylanase also increased the torque values (Table 9). WEAX is known to increase viscosity in solution, depending on its molecular weight. In the current study, however, the samples were heterogeneous mixtures with high contents of insoluble material, which also affects the rheological properties e.g. by its water absorption properties and friction caused by particles. The different impact of xylanase addition in the two processing systems, extruder and Farinograph, may be due to the essentially different time scales of the measurement (one hour in the Farinograph and about 3 minutes in the extruder). Contrary to the extruder-treated samples, the particle sizes of the blade-mixed samples (892–938 µm for coarse and 84–85 µm for ultrafine bran) were slightly higher than those of the untreated brans measured with the dry method (702 and 81 µm), which is probably due to swelling of the blade-mixed brans by the water of the wet method used for the particle size analysis.

Table 12. Appearance and particle size of coarse and ultrafine bran after extrusion and blade-mixing. Values marked with different letters within the same bran type (coarse/ultrafine) are significantly different (P < 0.05).

		Coarse (702 µm)		Ultrafine (84 µm)	
		Appearance	Particle size	Appearance	Particle size
37	Extruded	solid	348 ± 13 a	solid	55 ± 2 a
	Blade-mixed	granular	902 ± 35 d	granular	84 ± 5 c
42	Extruded	solid	-[a]	solid	-[a]
	Blade-mixed	granular	-[a]	granular	-[a]
48	Extruded	solid	603 ± 21 b	solid	76 ± 2 b
	Blade-mixed	granular	938 ± 31 d	granular	84 ± 5 c
54	Extruded	solid	-[a]	solid	-[a]
	Blade-mixed	granular	-[a]	solid	-[a]
60	Extruded	solid	770 ± 32 c	solid	85 ± 3 c
	Blade-mixed	granular	892 ± 41 d	solid	85 ± 5 c

[a] not determined

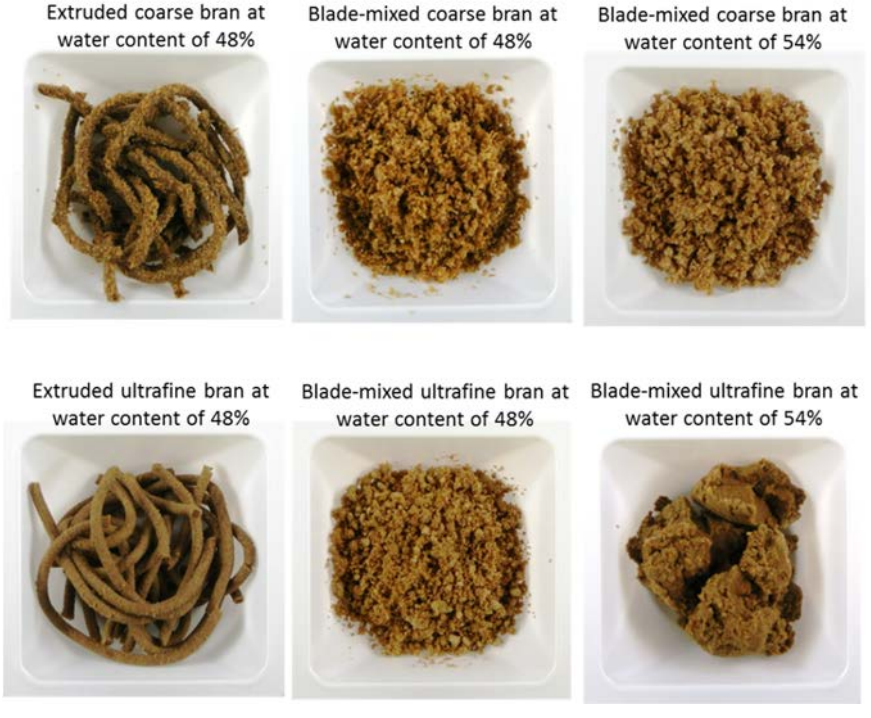

Figure 8. Coarse and ultrafine wheat bran-water-xylanase mixtures after blade-mixing or extrusion at water contents of 48 and 54% (before incubation).

4.2.2 Action of xylanase after pre-mixing by extrusion and blade-mixing

Impact of pre-mixing method and particle size on AX solubilisation

The impact of treatment water content, pre-mixing method and bran particle size on xylanase action was evaluated by analysing the amount of WEAX in the processed bran samples (Figure 9). The level of WEAX after the treatments varied between 2.6% (blade-mixed coarse bran treated at a water content of 37%) and 6.3% (extruded ultrafine bran treated at a water content of 60%) of bran DM, corresponding to DS of 13–30%. In both fine and coarse brans the solubilisation of AX was higher after the extrusion-aided treatments than after the corresponding blade-mixed treatments, especially below the water content of 54% (Figure 9). With the coarsely ground bran, the solubilisation of AX increased rather linearly with the increase of water content in the blade-mixed treatments. However, in the case of the fine bran, there was a notable increase in the AX solubilisation between 48% (WEAX 4.7% of bran dm) and 54% (WEAX 5.9%) water content, which was the water content at which the bran-water mixture of the ultrafine bran formed a continuous, plastic mass during the blade mixing (Table 12, Figure 8). A similar observation was also made earlier when studying the solubilisation of bran AX during continuous mixing (Publication I). The enhanced enzyme activity in the extrusion-aided treatment below the water content of 54%, as compared to the blade-mixed treatments at the same water content, might similarly be due to the formation of plastic mass by the pressure and mechanical shaping in the extruder. It has previously been pointed out that the absence of continuous free water phase may cause the bulk to behave as a wet granular material when portions of the ‘‘void’’ volume contain air rather than liquid, which is detrimental to efficient mixing, and consequently to efficient enzyme action (Viamajala et al. 2009). Hence, the continuous consistency of the material formed during the extrusion probably enhanced enzyme action, for example by improving diffusion, which is considered a major factor affecting enzymatic reaction rates especially at high solids concentration (Lavenson et al. 2012). However, the particle size reduction observed in the extruder (Table 12) could also have enhanced AX solubilisation, but the reduced particle size of the coarse bran was still much higher than that of the blade-mixed ultrafine bran (84–85 µm), which showed lower AX solubilisation after blade-mixed treatment than the coarse bran in the extruder. This confirms that the enhanced AX solubilisation in the extruder-aided treatment was not merely caused by the particle size reduction in the extruder.

The impact of bran particle size on the solubilisation of AX was further studied using two additional bran particle sizes, i.e. unground bran (Dv50 = 1001 µm) and fine bran (Dv50 = 327 µm), in addition to the coarse (Dv50 = 702 µm) and ultrafine (Dv50 = 81 µm) brans. Reduction of bran particle size enhanced solubilisation of bran AX, but in most points studied the differences between different brans were statistically significant only when comparing the ultrafine bran (Dv50 = 81 µm) with the coarse (Dv50 = 702 µm) and unground bran (1000 µm) (Publication III). The

results showed that the impact of bran particle size on AX solubilisation with added xylanase was similar in all the processing methods and water contents studied, including a treatment at a water content of 92% with continuous shaking, and it was also similar without added enzymes (Publication III). The only notable exception was in case of the blade-mixed treatments at a water content of 54%, when the difference between the ultrafine and coarsely ground bran was higher than in the other water contents (Figure 9). The enhanced AX solubilisation of the ultrafine bran was most probably caused by plasticization of the ultrafine bran mixture in these conditions, as already discussed.

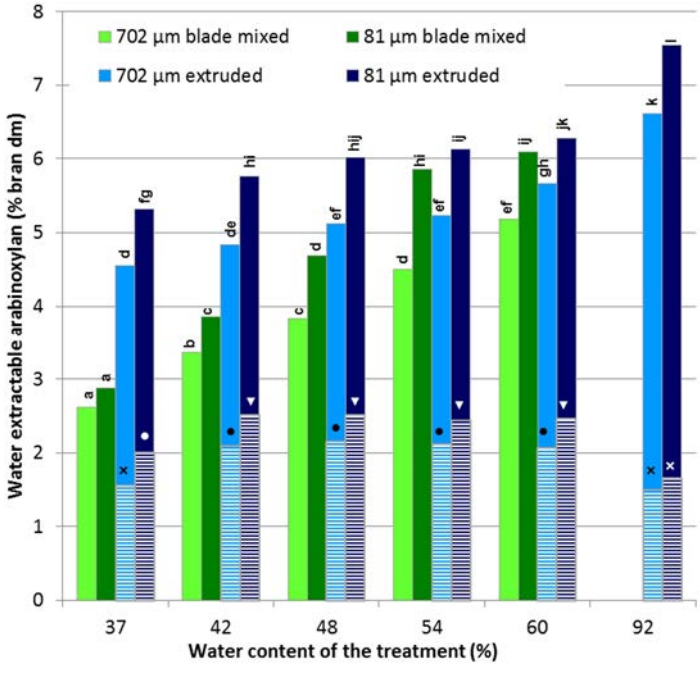

Figure 9. The amount of water extractable arabinoxylan after blade-mixing and extrusion-aided treatments (including 4 h stationary incubation) at water contents of 37–60% and after shaking treatments at a water content of 92%. The solid columns represent samples treated with Depol 761P xylanase (200 nkat/g) and the patterned columns represent samples without added enzymes. Values marked with different letters within the samples with xylanase and with different symbols within the samples without added enzymes are significantly different ($P < 0.05$) (Compiled from Publication III).

When solubilisation of AX at the low-water content (37–60%) was compared to a high-water (92%) system with continuous shaking, the solubilisation of AX with xylanase was higher in the high water content treatment (WEAX 6.4% of DM for coarsely ground bran) than in the corresponding low water treatments in the ex-

truder (4.4–5.7% at water contents of 37–60%) (Figure 9). By contrast, when xylanase action was studied at water contents of 40 and 90% using continuous mixing (Publications I and II), the solubilisation of AX was similar at both water contents (Table 10). This was probably due to the continuous mixing, whereas in the extrusion-aided treatments there was no mixing during the incubation. As recently reviewed by Lavenson et al. (2012), efficient mixing and mass transfer are generally considered to be essential for the efficient performance of enzymatic reactions, and in that respect even higher differences would be expected because of the lack of mixing during the low water content incubations. Probably the efficient initial mixing was sufficient to facilitate the enzyme action during the extrusion-aided treatment. It has been reported that effective initial mixing to promote good enzyme distribution and continued, but not necessarily continuous mixing is necessary in order to facilitate high biomass conversion rates at high solids concentration (Roche et al. 2009).

Contrary to the treatments with added xylanase, in the blank treatments with no added enzymes the solubilisation of AX was notably higher in the extrusion-aided processes at the water contents of 42–60% than in the high water content (92%) treatments (Figure 9). The solubilisation of AX was most probably caused by the action of endogenous hydrolytic enzymes of the bran material (Dornez et al. 2009), and the results suggest that low moisture content could be favourable for the solubilisation of AX by endogenous bran enzymes. This would be logical, as the natural environment and activation of the endogenous enzymes is not necessarily highly aqueous.

Impact of water content and mixing method on molecular weight distribution of WEAX

The HP-SEC analysis of WEAX indicated that the apparent average MWs of WEAX in the untreated brans were 158 kDa for coarse and 143 kDa for ultrafine bran (Table 13), which were very close to the values obtained by Zhang et al. (2011) for wheat bran WEAX using a different HP-SEC method (152 kDa). When bran was treated without added enzymes at a water content of 37%, the MW of AX of the processed brans did not markedly change from that of the untreated brans, but at a water content of 48% the MW was already significantly lower (79 and 76 kDa for coarse and ultrafine bran, respectively), and at 60% water content the MW was further decreased to the same level as after the shaking treatment at high water content (66–68 kDa). These results indicated that without added enzymes the depolymerisation of WEAX increased with increasing water content, presumably due to the action of endogenous bran enzymes, although the solubilisation of AX without added enzymes was favoured by the low water content process (extrusion-aided treatment) (Figure 9). Similarly, when bran was processed with continuous mixing, solubilisation of AX without added enzymes was higher and less depolymerisation occurred at low (40%) water content than at high (90%) water content (Publication I). These results may indicate that endogenous enzymes causing AX solubilisation preferably act at lower water activity, whereas the

enzymes that further depolymerize solubilised AX require more water. However, due to the higher shear exerted on the bran at low water content, this needs to be further confirmed.

In contrast to the treatments without enzymes, with added xylanase the MW of WEAX was not dependent on the used water content when comparing the extrusion-aided and shaking treatments (WEAX range 48–56 kDa with no statistically significant difference) (Table 13). However, with continuous mixing the depolymerisation of bran WEAX with xylanase was lower at the water content of 40% than at 90% (Publication I). The different results are probably caused by the different processing methods (extrusion and shaking vs. Farinograph-mixing and blade-mixing). However, the difference may also be due to the different purification procedures used for preparation of the samples for HP-SEC analysis. In Publication III, the removal of other components than AX in the sample was ensured by a more complete enzymatic purification method. The presence of high MW components in the bran treated at 40% and analysed in Publication I may thus be caused by incomplete removal of interfering components, such as proteins, which also showed aggregation as indicated by the SDS-PAGE analyses (Figure 7).

In order to learn how well the determined MW represented the total WEAX of the bran sample, the content of WEAX in the HP-SEC sample was also analysed (Publication III), since the method used for the MW analysis applies only to the WEAX fraction precipitating at 65% EtOH. When compared to the total level of WEAX in the original sample, the precipitated WEAX amounts in the HP-SEC samples corresponded to 24–35% of the total WEAX when bran had been processed with added xylanase, whereas without added enzymes and in the untreated bran, the analysed MW of WEAX represented as much as 57–86% of the total WEAX (Table 13). The rest of the WEAX were smaller oligosaccharides which were not present in the MW chromatograms. The contents of WEAX in HP-SEC samples are important because the level of WEAX in the original sample and the analysed MW are not necessarily comparable as such, especially in the case of enzymatic treatments. It is known that both the level of AX solubilisation (WEAX content as compared to water insoluble AX) and the MW of the WEAX are important factors affecting the technological properties of cereal AX (Courtin and Delcour 2002). In many food applications, the preferred xylanolytic reaction is the solubilisation of insoluble bran AX without intensive depolymerisation of WEAX (Courtin and Delcour 2002). In this study the xylanase enzyme dose used (200 nkat/g) was relatively high, since most of the solubilised AX was hydrolysed to oligosaccharides.

Table 13. Average apparent molecular weight (MW) of water extractable AX (WEAX) precipitated at 65% EtOH and its content (as % of total WEAX) in the brans after different treatments. Values marked with different letters are significantly different (P < 0.05) (Compiled from Publication III).

			Coarse bran		Ultrafine bran	
		Treatment water content (%)	Average MW (kDa)	% of total WEAX	Average MW (kDa)	% of total WEAX
Untreated bran			158 g	75	143 f	83
Without added enzymes	Extrusion-aided treatment	37	131 e	85	133 e	86
		48	79 d	60	76 cd	68
		60	68 b	57	68 b	58
	Shaking treatment	92	66 b	57	68 bc	67
With Depol 761P xylanase 200 nkat/g	Extrusion-aided treatment	37	51 a	25	52 a	32
		48	52 a	27	51 a	31
		60	56 a	24	52 a	31
	Shaking treatment	92	51 a	29	48 a	35

4.2.3 Impact of incubation time and drying on modification of bran by the extrusion-aided pre-mixing process

In order to study the impact of incubation time (0 or 4 h) and drying on bran after the extrusion-aided pre-mixing process, coarse (Dv50 = 700 µm) and ultrafine (Dv50 = 80 µm) bran were treated at a water content of 48% either without enzymes followed by direct freeze or oven drying, or with Depol 761P xylanase preparation followed by 4 h stationary incubation and drying by both techniques (Publication IV). In order to gain better understanding of the effects of the use of incubation and different types of enzymes, the fine bran was additionally treated by different combinations of the process parameters (4 h incubation without enzymes and direct drying with Depol 761P) and by two different enzyme preparations, Depol 761P and Veron CP, and their combination.

The effects of different process variations on the level of WEAX, bran particle size and WHC are shown in Table 14. The particle size reduction caused by the processing was higher than that observed in Publication III (Table 12), obviously due to the regrinding after drying, since in Publication III the bran samples produced by the extrusion-aided pre-mixing process were analysed without any drying step. Extrusion treatment without enzymes with direct drying increased the WEAX content of both coarse and ultrafine bran, indicating that some AX was

solubilised by the extrusion process itself, probably due to the shear exerted on the bran mixture. However, when the fine bran sample was further incubated for 4 h the WEAX content increased to 2.7%, indicating the action of bran endogenous enzymes since there was no shear during the stationary incubation. With Depol enzyme preparation, significant increase in the WEAX content (to 4.2%) occurred already without incubation, indicating that the added enzymes started to act immediately during mixing in the extruder, despite the relatively low water content used (48%). When incubated with Depol for 4 h, the WEAX content further increased to levels (4.8–4.9 and 5.6–5.7% in the coarse and ultrafine brans, respectively) that were slightly lower than those analysed in Publication III from the fresh samples (5.1 and 6.0% of bran DM, respectively) after similar treatment (Figure 9).

The fine bran treated with the combination of Veron and Depol enzyme preparations had a slightly higher WEAX content (6.2%) than the brans treated with Depol (5.7%) or Veron (4.3%) alone. This was obviously due to the different doses of endoxylanase and other enzyme activities in the treatments. Depol treatment contained mainly endoxylanase (200 nkat/g), whereas Veron treatment contained 100 nkat/g endoxylanase and additionally 130 nkat/g endoglucanase and 465 nkat/g β-glucanase, as well as higher levels of other side activities (Publication IV). The enzyme dose was highest in the combination treatment since it contained both enzyme preparations dosed at the same level as in the individual enzyme treatments. The use of multiple hydrolytic enzyme activities is generally considered beneficial in degradation and solubilisation of DF due to the synergistic action of different enzymes specific for certain cell wall components (Faulds and Williamson 1995; Petersson et al. 2013). In the current study, however, instead of studying the synergistic action of the enzymes, the dosages were selected aiming to obtain brans with different levels of AX degradation, in order to elucidate the impact of AX solubilisation on the functionality of bran in extrusion.

Brans were dried either by freeze drying or by oven drying. Freeze drying is known to cause minimal structural damage to the products, whereas oven drying is considerably cheaper. When bran was treated without enzymes, the higher content of WEAX in the oven dried brans was probably caused by the action of the bran endogenous enzymes in the beginning of the oven drying, whereas the use of liquid nitrogen for the samples that were freeze dried obviously stopped the enzyme reactions immediately. However, when the brans were treated with Depol 761P, the drying method did not have a significant impact on the content of WEAX, probably due to the high enzyme activity already during the extruder mixing and incubation, which probably obscured the possible impact of the short continuation of the enzyme action in the oven.

Similarly to the results of the bran treatment with continuous mixing (Table 11), WHC of the bran was generally reduced during the extruder-aided bran treatments with stationary incubation, especially with added enzymes, and it was always lower in the ultrafine brans than in the coarse brans (Table 14). However, the WHC of the coarse bran was significantly lower after oven drying than after freeze drying, both with and without the use of enzymes. It has also been reported previously that freeze dried DF ingredients are capable of holding more water than

those dried in an oven (de Escalada Pla et al. 2012; Massiot and Renard 1997). This can be explained by the fact that forced heated air dehydration (oven drying) can collapse the structure of DF, whereas freeze drying preserves their structural features, resulting in higher porosity that allows more water entrapment. However, the drying method did not have an impact on the WHC of the fine bran, probably because it already underwent significant structural collapse due to severe grinding conditions both before and after the treatments.

Table 14. Properties of the untreated and modified (0 h = treated without incubation, 4 h = treated with 4 h incubation, OD = oven dried, FD = freeze dried) coarse and ultrafine bran ingredients. Values marked with different letters within the results are significantly different ($P < 0.05$). For median particle size, the statistical analysis was performed separately for fine and coarse bran, and thus the letters indicate significant differences ($P < 0.05$) within each bran type (Publication IV).

			WEAX (% bran dm)		Median particle size (µm)		WHC (g water /g bran dm)	
			Coarse	Ultrafine	Coarse	Ultrafine	Coarse	Ultrafine
Untreated bran			0.5 a	0.8 b	702 a	84 a	3.7 a	3.3 b
Treated with no added enzymes	0 h	OD	1.4 de	1.6 e	279 b	68 ab	3.0 c	3.1 bc
	0 h	FD	1.1 c	1.3 cd	318 b	61 ab	3.7 a	3.1 bc
	4 h	OD	-	2.7 f	-	69 ab	-	3.0 c
Treated with Depol 761P	0 h	OD	-	4.2 g	-	70 ab	-	2.6 d
	4 h	OD	4.8 h	5.6 i	205 c	52 ab	2.5 d	2.4 d
	4 h	FD	4.9 h	5.7 i	285 b	45 b	3.1 bc	2.4 d
Treated with Veron CP	4 h	OD	-	4.3 g	-	62 ab	-	2.4 d
Treated with Veron CP +Depol 761P	4 h	OD	-	6.2 j	-	57 ab	-	2.4 d

4.3 Impact of enzymatically modified bran on the quality of bran-enriched expanded extrudates (Publication IV)

The technological functionality of enzymatically modified dry bran ingredients was examined in bran-supplemented expanded extrudates (Publication IV). Bran ingredients were produced at the water content of 48% by different variations of the extrusion-aided modification process followed by oven or freeze drying, and the effects of the distinct properties of the bran ingredients (Table 14) on the quality of rye endosperm-flour based expanded extrudates were examined at a bran supplementation level of 20%.

4.3.1 Structure of bran-supplemented extrudates

The impact of bran modifications and particle size on the macrostructure of endosperm-flour based extrudates was analysed by measuring the expansion rate (ER), specific length and piece density of the extrudates, which represented the radial, longitudinal and volumetric expansion, respectively. Addition of untreated or modified brans caused a significant reduction in the ER and piece density and an increase in the specific length of the extrudates as compared to that of the control extrudate without bran (Table 15), which is in accordance with information from the literature on the effects of wheat bran or insoluble DF addition in cereal extrudates (Robin et al. 2012; Yanniotis et al. 2007; Brennan et al. 2008; Lue et al. 1991; Karkle et al. 2012). Addition levels up to 50% of wheat bran have previously been studied and it has been reported that the negative effects of insoluble DF on extrudate quality, such as expansion volume and density, increase with increasing DF addition level, as reviewed by Robin et al. (2012).

ER remained generally rather similar when modified brans were used as compared to the use of untreated brans, but the ER was generally higher when bran of fine particle size was used as compared to the use of coarse bran. Indeed, there was a significant ($P < 0.01$) negative correlation between ER and bran particle size (Table 16). Smaller fibre particle size has also previously been reported to favour radial expansion and reduce the density of rye bran extrudates (Alam et al. 2013) and corn meal extrudates containing sugar beet fibre (Lue et al. 1991) or corn bran (Blake 2006). It has been suggested that coarse particles may cause early rupture of gas cells before their optimal expansion, or that reduction of particle size may improve expansion by providing more nucleation sites, and thus more air cells, than coarse fibre particles (Lue et al. 1991). Bran particle size also affected the cell structure as observed from the radial cross-section images obtained by stereomicroscopy (Figure 10). In the samples with coarse bran, the cells were small and the large bran particles were clearly visible, whereas in the samples with fine bran, the cell size distribution was less homogeneous due to the presence of some large cells, and the bran particles were less visible. It was also observed that particle size and WHC of the bran were significantly correlated (Table 16), and it is thus possible that the effect of fibre particle size on expansion and structure was not only related to their physical dimensions, but also to their different hydration properties and their impact on melt rheology, as previously pointed out by Sozer and Poutanen (2013).

Table 15. Macrostructural and mechanical properties of the extrudates with and without modified (0 h = treated without incubation, 4 h = treated with 4 h incubation, OD = oven dried, FD = freeze dried) coarse and ultrafine bran ingredients. Values marked with different letters within the same parameter are significantly different ($P < 0.05$) (Compiled from Publication IV).

			Expansion rate (%)		Specific length (m/kg)		Piece density (kg/m^3)		Crushing force (N)		Crispiness index (×10^3)	
Control (no bran)			452 a		54 a		130 c		18.3 c		5.8 bc	
			Coarse	**Fine**	**Coarse**	**Fine**	**Coarse**	**Fine**	**Coarse**	**Fine**	**Coarse**	**Fine**
Untreated bran			354 gh	390 bcd	68 c	62 b	169 a	155 b	22.5 a	21.8 ab	3.4 a	3.9 a
Treated with no added enzymes	0 h	OD	371 ef	401 b	78 efg	79 efgh	132 c	113 e	16.6 cd	15.3 def	6.3 cd	7.8 de
	0 h	FD	351 h	404 b	74 de	69 cd	155 b	126 cd	20.6 b	18.3 c	4.2 ab	5.8 bc
	4 h	OD	-	371ef	-	82 ghi	-	128 cd	-	15.1 def	-	10.8 gh
Treated with Depol 761P	0 h	OD	-	399 bc	-	83 ghi	-	108 e	-	16.3 de	-	9.7 fg
	4 h	OD	355 gh	404 b	84 hij	83 ghi	136 c	106 e	15.4 def	13.2 g	8.4 ef	12.1 hi
	4 h	FD	367 fg	384 cde	80 fgh	74 ef	133 c	129 c	15.9 de	13.8 fg	9.6 fg	12.9 i
Treated with Veron CP	4 h	OD	-	372 ef	-	89 j	-	116 de	-	14.6 efg	-	10.7 gh
Treated with Veron CP +Depol 761P	4 h	OD	-	379 def	-	86 ij	-	116 de	-	14.8 defg	-	11.7 hi

It has previously been reported that soluble DF generally produces higher radial expansion than insoluble DF (Pai et al. 2009; Yanniotis et al. 2007; Brennan et al. 2008), but in the current study no correlation was found between radial expansion and WEAX content of the bran (Table 16). In accordance, clear differences were not observed when comparing the radial cross-sectional images of the samples with treated brans to those of the corresponding untreated brans. This could be due to the relatively low differences in the chemical composition of the flour-bran mixtures in the current study as compared to those of the previous studies. The content of insoluble DF in the extrudates varied between 8.3 and 9.7% and the content of soluble DF (including oligosaccharides) between 6.5 and 7.9% (Publication IV). For example, in the study of Pai et al. (2009), who studied the impact of alkali-solubilised corn bran on extrusion, the differences in soluble DF content ranged from 1.6 to 64%, whereas other studies have mainly compared the impacts of addition of bran to the impacts of oligosaccharides or gums with no insoluble DF and essentially different chemical composition to that of the bran (Brennan et al. 2008; Yanniotis et al. 2007). The total DF content in all extrudates was between 15.8 and 16.4% (data not shown), indicating that the bran treatments had only minor or no impact on the total DF content of the bran.

In contrast to the less significant effects on the ER, it was noted that the bran treatments had a clear impact on the volumetric and longitudinal expansion. Compared to the untreated bran, the piece density decreased and the specific length increased when the modified brans were used. Melt viscosity and the level of available water are considered to be important factors affecting expansion, and the different effects of insoluble and soluble DF on expansion have also been related to these properties (Robin et al. 2011a; Moraru and Kokini 2003; Pai et al. 2009). In the current study, specific length was significantly correlated with both WEAX content and WHC (Table 16). It appears probable that the increase in longitudinal expansion was caused by altered melt viscosity due to increase in WEAX content and/or by increasing level of available water in the system due to reduced WHC. WEAX is known to affect viscosity in solution, depending on its molecular weight (Courtin and Delcour 2002). Robin et al. (2011a, 2011b) reported that addition of wheat bran resulted in an increase in water activity and decrease in the glass transition temperature of the melt, which would decrease the starch viscosity at constant temperature. Thus, it is also possible that in the current study, the increase in the level of available water (decrease in bran WHC) might also have promoted longitudinal expansion due to decreased melt viscosity by reducing melt glass transition temperature. However, the above mentioned possible mechanisms behind the observed effects of bran WHC and WEAX content on expansion remain to be tested experimentally.

Table 16. Pearson's correlation matrix for physicochemical properties of the bran ingredients and the macrostructural and mechanical properties of the bran-supplemented extrudates (Compiled from Publication IV).

	WHC	Particle size	Expansion ratio	Specific length	Piece density	Crushing force	Crispiness index
WEAX	-0.864**	-0.454	0.127	0.709**	-0.617*	-0.799**	0.898**
WHC	1	0.666**	-0.355	-0.710**	0.775**	0.858**	-0.866**
Particle size		1	-0.682**	-0.355	0.755**	0.636*	-0.608*
Expansion ratio			1	-0.045	-0.655*	-0.319	0.285
Specific length				1	-0.716**	-0.808**	0.747**
Piece density					1	0.852**	-0.764**
Crushing force						1	-0.927**
Crispiness index							1

** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.

4.3.2 Mechanical properties of bran-supplemented expanded extrudates

Bran addition increased the hardness of the extrudates as shown by the increased crushing force (F_{cr}) from that of the control extrudate (Table 15). By contrast, when the treated brans were used, the hardness generally decreased below that of the control (extrudate without bran), and it was decreased most when the brans were incubated with Depol 761P. Furthermore, the extrudates with modified fine bran were generally less hard than the corresponding coarse bran extrudates. Crispiness, analysed by measuring the crispiness index (C_i), decreased from that of the control when untreated coarse or fine bran was added, whereas bran treatment with enzymes produced more crispy extrudates than control, untreated bran or bran treated without enzymes with direct drying. C_i was also increased even when Depol 761P enzyme preparation was used without 4h incubation (Table 15).

Hardness and crispiness of cereal extrudates are mainly determined by their cellular structure, formed during the expansion of the extrudate, and by the phase properties and composition of the solid matrix (Moraru and Kokini 2003). It has been reported that addition of DF increases the hardness and decreases the crisp-

iness of expanded extrudates due to higher cell density and shorter cell diameter (Robin et al. 2011a; Karkle et al. 2012; Yanniotis et al. 2007; Jin et al. 1995). The samples with coarse bran had more homogenous and smaller cell size than the samples with fine bran, as visually observed from the stereomicroscopy images (Figure 10), but the improvements in the mechanical properties of the extrudates with treated brans were not clearly reflected in the cellular structure. However, the improved mechanical properties correlated with decreased piece density and increased specific length, as well as with increased WEAX content and decreased WHC of the bran. Decreased hardness and increased crispiness also correlated with decreased bran particle size, as also reported previously for rye bran (Alam et al. 2013). However, the properties of the extrudates prepared with untreated fine bran with mean particle size of 84 µm were significantly inferior to those of the extrudates made with modified coarse brans with particle size of 205–318 µm. Thus it can be concluded that the reduction of bran particle size after the bran treatments was not the primary reason for improved mechanical properties of the extrudates with modified bran. Rather, the extrudates with modified brans had improved mechanical properties probably due to the effects of the increased WEAX content and decreased WHC of the brans on the extrudate expansion. The differences in the extrudates due to the drying method can also be attributed to the slightly different impacts that the drying methods had (or did not have, as in case of the Depol 761P treatments) on the WEAX content and WHC of the brans. However, due to the complexity of the phenomena governing the expansion and texture formation, the precise mechanism behind the effect of the bran treatments on expansion and mechanical properties of the extrudates remains to be further elucidated. In addition to the analysed bran features, the changes in other bran properties, such as MW of WEAX and other DF components, as well as the solubility and molecular properties of bran proteins, may provide valuable information in this respect.

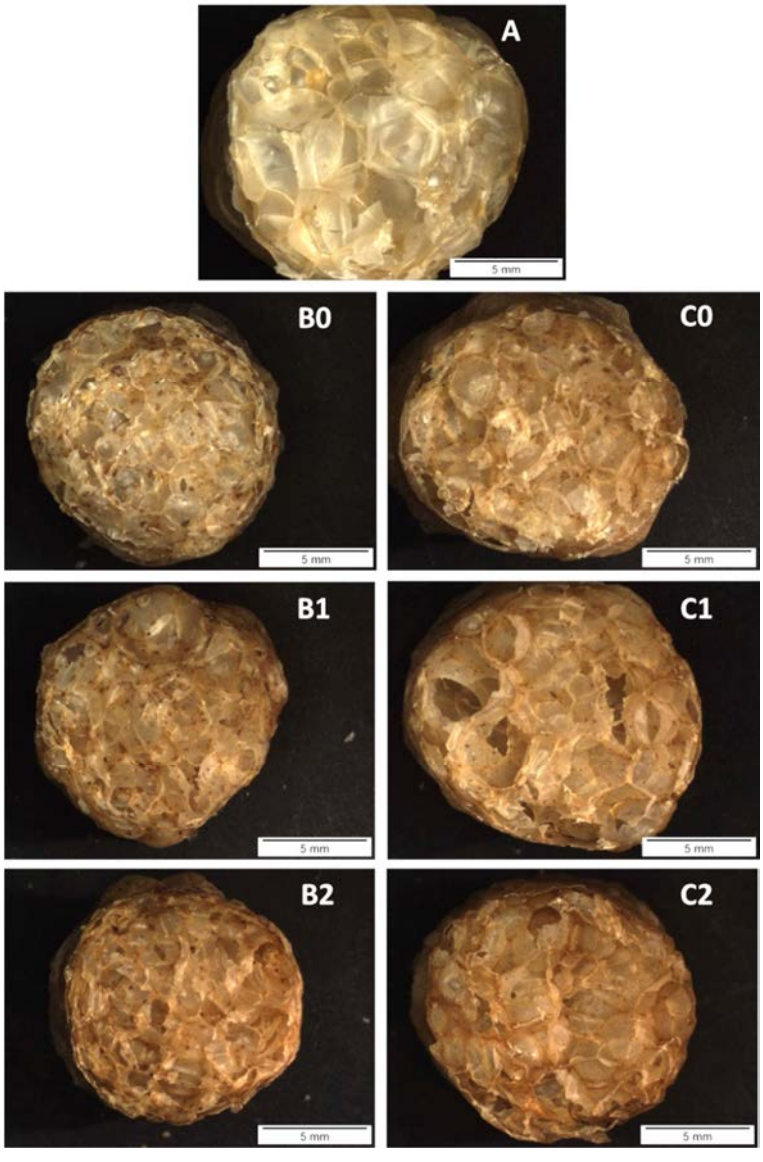

Figure 10. Stereomicroscope images of radial sections of the extrudates. Control extrudate with no added bran (A), extrudates supplemented with 20% of untreated coarse bran (B0), coarse bran treated with no added enzymes and direct oven drying (B1), coarse bran incubated 4 h with Depol 716P enzyme preparation (B2), untreated ultrafine bran (C0), ultrafine bran treated with no added enzymes and direct oven drying (C1), ultrafine bran incubated 4 h with Depol 716P enzyme preparation (C2) (Publication IV).

5. Conclusions

Enzymatic modification of plant-based materials at reduced water content could offer several advantages over processing at high water content, including reduced processing volumes and reduced downstream processing costs. In this work, the impact of water content on enzymatic modification of wheat bran was examined with the aim of increasing the technological functionality of wheat bran in food applications.

The minimum required water content for the action of xylanase on wheat bran, indicated by AX solubilisation, during continuous mixing was between water contents of 20 and 30%, corresponding to a_w of 0.83–0.89. Xylanase action was significantly enhanced at a water content of 40% (a_w 0.93), at which the granular material was transformed to a continuous paste. Furthermore, it was shown that the use of an extruder for pre-mixing and forming of bran-water mixture increased the action of xylanase during stationary incubation at a water content of ≤54%, as compared to pre-mixing with a blade-mixer. The results indicated that the formation of a continuous paste is important for efficient enzyme action at low water content, probably due to improved diffusion, and that it is possible to increase the enzyme action by changing the granular structure of the material to a continuous paste using an extruder, without increasing the water content. The results also showed that without added enzyme, the solubilisation of AX was higher at low (40–60%) than at high (90–92%) water content, suggesting that low water content may be favourable for the action of endogenous bran hydrolytic enzymes. Due to the higher shear exerted on the bran at low water content and the possible solubilisation of AX by mechanical mechanisms, the effect of water content on the action of endogenous enzymes needs to be further confirmed.

The mode of action of xylanase was examined by analysing the apparent MW of WEAX. During the treatment with xylanase, the MW distribution of WEAX precipitated with 65% EtOH was not affected by the water content or processing method at a water content of > 40%. However, since oligosaccharides were not included in the analysis, the impact of water content on their amount and MW needs to be further studied. When bran was treated with continuous mixing, the A/X ratio of the bran water extract decreased similarly at both water contents of 40% and 90%, suggesting that AX was solubilised from the same bran tissues regardless of the processing conditions studied.

The results showed that reduction of particle size, either prior to the treatment by grinding or during the treatment by intensive mixing and shear, may be used as a means to enhance enzyme action and AX solubilisation. At reduced water content, the shear exerted on the bran-water mixture caused reduction of bran particle size and might have enhanced AX solubilisation by mechanical mechanisms. However, it was also shown that reduction of bran particle size by grinding enhanced the action of xylanase, presumably due to improved substrate availability as a result of increased surface area, and the effect was rather similar at all water contents and in all process conditions studied. Furthermore, small particle size favoured the transformation of the bran-water mixture from granular mass to a continuous paste, which also enhanced enzyme action.

The study showed that both structural and physicochemical properties of bran were affected by the treatment water content. When processed with continuous mixing, the bran treated at a water content of 40% was characterized by higher solubilisation of DF polysaccharides, smaller average particle size, lower WHC and more changes in bran proteins than the treatment at a water content of 90%. The more intensive changes in the properties of bran treated at low water content with or without enzyme were related to the compact consistency and thus higher impacts of shear exerted on the bran-water mixture. Solubility of DF, bran particle size and its hydration properties are amongst the most important properties of bran affecting its technological functionality in food applications. In this work, the technological functionality of modified bran was demonstrated in bran-supplemented expanded extrudates. Modification of bran by hydrolytic enzymes by a low-moisture process increased the crispiness and reduced the hardness and piece density of the bran-enriched expanded extrudates. The improvements in extrudate properties were attributed to the increased WEAX content and decreased WHC of the modified brans, since the mechanical properties analysed significantly correlated with these properties.

The results of the work showed that enzymatic solubilisation of bran AX and improved technological functionality of bran can be achieved by enzymatic modification at a water content of 40–50%, which is well below the point of absence of free bulk water (70–80%). The consistency of the reaction mixture, mixing method and bran particle size were found to be important factors affecting the intensity of the modification process at reduced water content. It was shown that the use of an extruder-aided pre-mixing process enabled efficient xylanase action on wheat bran at low water content without the requirement for continuous mixing, and the processing method may also be applicable to other biomass sources. The results encourage further development of processes at reduced water content for the enzymatic modification of plant raw materials. The role of water in enzymatic processing is however complex, and numerous aspects of process economy need to be further elucidated in order to develop overall industrially feasible low-water processes.

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Title	**Impact of water content on enzymatic modification of wheat bran**
Author(s)	Outi Santala
Abstract	Enzymatic conversions have a fundamental role in several industrial food manufacturing processes and in the upgrading of agro-industrial residues. Enzymatic reactions are typically conducted in excess water because reduction of water content usually decreases enzymatic conversion. Processing at high solids content could, however, offer several economical advantages. Wheat bran is one of the most important by-products of the cereal industry. Bran is a good source of dietary fibre, protein and health-beneficial compounds, but its use in food applications is limited because unprocessed bran is usually detrimental to product quality. The present work aimed to examine and develop techniques to utilize hydrolytic enzymes, especially xylanase, at reduced water content in order to increase the technological functionality of wheat bran in food applications. The applicability of the modified bran was demonstrated in extruded cereal-based snacks. The results showed that technological functionality of bran can be improved by enzymatic modification at a low water content of 40–50%. Consistency of the reaction mixture, mixing method and bran particle size were important factors affecting the intensity of the modification process at reduced water content. It was possible to increase the enzyme action by changing the granular structure of the material to a continuous paste using an extruder, without increasing the water content. Modification of bran by hydrolytic enzymes by a low-moisture process increased the crispiness and reduced the hardness and piece density of bran-enriched puffed snacks. The results can be utilized for improving the technological functionality of bran in food applications and for developing new processes for the enzymatic modification of plant raw materials at reduced water content.
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Nimeke	**Vesipitoisuuden vaikutus vehnäleseen entsymaattisessa muokkauksessa**
Tekijä(t)	Outi Santala
Tiivistelmä	Entsyymejä hyödynnetään laajasti elintarviketeollisuudessa raaka-aineiden ja lopputuotteiden muokkauksessa sekä maatalouden ja metsäteollisuuden sivutuotteiden prosessoinnissa. Entsyymireaktiot tehdään yleensä suuressa vesimäärässä, koska vesipitoisuuden vähentäminen useimmiten heikentää entsyymien toimintaa. Teollisissa prosesseissa vesipitoisuuden vähentäminen toisi kuitenkin taloudellisia hyötyjä. Vehnälese, joka on yksi viljateollisuuden tärkeimmistä sivutuotteista, on hyvä ravintokuidun, proteiinin ja terveyttä edistävien pienyhdisteiden lähde ja siksi kiinnostava raaka-aine elintarviketeollisuudelle. Käsittelemättömän leseen lisääminen kuitenkin heikentää yleensä elintarvikkeen laatua. Työn tarkoituksena oli tutkia ja kehittää menetelmiä hydrolyyttisten entsyymien, erityisesti ksylanaasien, käyttämiseen matalassa vesipitoisuudessa vehnäleseen ominaisuuksien parantamiseksi elintarvikesovelluksissa. Muokatun leseen teknologinen toimivuus testattiin ekstruusiolla valmistetuissa puffatuissa lesenaksuissa. Tutkimus osoitti, että leseen ominaisuuksia voidaan parantaa entsymaattisella muokkauksella matalassa 40–50 %:n vesipitoisuudessa. Reaktioseoksen fysikaalinen koostumus, sekoitusmenetelmä ja leseen partikkelikoko todettiin tärkeiksi tekijöiksi, jotka vaikuttavat muokkausprosessin tehokkuuteen matalassa vesipitoisuudessa. Työssä havaittiin, että entsyymien toimintaa on mahdollista tehostaa nostamatta vesipitoisuutta muuttamalla lese-vesiseos rakeisesta yhtenäiseksi massaksi ruuvisekoittimen avulla. Matalassa vesipitoisuudessa tehty entsymaattinen leseen muokkaus paransi korkeakuituisten lesenaksujen rapeutta ja vähensi niiden kovuutta ja tiheyttä. Tutkimuksen tuloksia voidaan hyödyntää leseen teknologisen toimivuuden parantamiseen elintarvikesovelluksissa. Tulosten perusteella voidaan myös kehittää uusia entsymaattisia prosesseja kasvimateriaalien muokkaamiseksi matalassa vesipitoisuudessa.
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The results showed that technological functionality of bran can be improved by enzymatic modification at a low water content of 40–50%. Consistency of the reaction mixture, mixing method and bran particle size were important factors affecting the intensity of the modification process at reduced water content. It was possible to increase the enzyme action by changing the granular structure of the material to a continuous paste using an extruder, without increasing the water content. Modification of bran by hydrolytic enzymes by a low-moisture process increased the crispiness and reduced the hardness and piece density of bran-enriched puffed snacks. The results can be utilized for improving the technological functionality of bran in food applications and for developing new processes for the enzymatic modification of plant raw materials at reduced water content.

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