ELSEVIER

Trends in Food Science & Technology xx (2012) 1–9

TRENDS IN FOOD SCIENCE & TECHNOLOGY

Technologies for enhanced exploitation of the health-promoting potential of cereals

Jan A. Delcour[a,*], Xavier Rouau[b], Christophe M. Courtin[a], Kaisa Poutanen[c] and Roberto Ranieri[d,1]

[a]Laboratory of Food Chemistry and Biochemistry and LFoRCe, Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, 3001 Heverlee, Belgium (e-mail: jan.delcour@biw.kuleuven.be) (e-mail: christophe.courtin@biw.kuleuven.be)

[b]INRA, UMR Agropolymer Engineering and Emerging Technologies, 2 place Viala, 34060 Montpellier Cedex 2, France (e-mail: rouau@supagro.inra.fr)

[c]VTT Technical Research Centre of Finland, Tietotie 2, P.O. Box 1000, FI-02044 VTT, Espoo, Finland (e-mail: kaisa.poutanen@vtt.fi)

[d]Barilla, via Mantova, 166 43100 Parma, Italy (e-mail: r.ranieri@openfields.it)

As part of a general trend, the grain processing industry faces the challenge to produce new ingredients and foods with added value for consumer health. In this context, the EU 6th Framework Integrated Project HEALTHGRAIN, as part of its overall goal to provide the scientific basis for increasing the intake of health-promoting compounds in whole grains or their fractions, developed new technologies for cereal ingredient and food manufacture. We here report on the outcome of this work, with a main emphasis on wheat processing. It included revisiting dry milling and exploration of wet enzyme-based fractionation processes as well as fermentation in order to produce food ingredients and/or foods with increased levels of health relevant components and structural features delivering good sensory properties. A novel wheat grain fractionation diagram was developed for incorporating bioactive compounds in flour and removing the parts of the grain detrimental for technological quality and safety. Processing eliminated the pericarp by initial pearling to leave only the crease material attached to the kernel, the resultant grain was milled to eliminate the bran crease material, and the white flour was remixed with the pearling fraction to incorporate as much as possible of the aleurone layer material into the flour. A process for isolating aleurone from wheat bran starts with size reduction of bran particles to favour tissue separation. The aleurone tissue is then mechanically separated from the other seed coats by using impact or shearing forces. After these fragmentations, the resultant blend is mechanically separated. Further purification of aleurone cells can be achieved using electrostatic separation to yield a powder containing about 90% aleurone which has high antioxidant activity and contains significant levels of vitamins, minerals, phytosterols, lignans and other phenolic substances. The size distribution of the particles ground to ultra-fine size was narrower for cryogenic grinding than for ambient grinding. Further work dealt with wet processing. The abundance of arabinoxylan (AX) in bran offered excellent possibilities for manufacturing AX oligosaccharides (AXOS), which meet the criteria for prebiotics. Xylanases release AXOS from AX with a yield which was negatively correlated with the arabinose to xylose ratio of wheat bran AX. In addition, hyperthermophilic xylanases allow producing AXOS *in situ* during bread making without the negative impacts of AX extensive hydrolysis on dough processing that some regular xylanases induce. Bran fermentation with yeast prior to bread making leads to higher bread volume and greater crumb softness. Moreover, bioprocessing of bran by enzyme-aided fermentation increases the content of soluble fibre and the *in vitro* and *in vivo* bioaccessibility of phenolic acids. The quality of gluten-free breads can be improved by using lactic acid bacteria with properties including antifungal activity, or the production of exopolysaccharides or enzymes. Finally, studies and demonstration activities were carried out on laboratory and pilot scale.

* Corresponding author.
[1] Current address: Openfields, strada Consortile 2, 43044 Collecchio PR, Italy.

0924-2244/$ - see front matter
doi:10.1016/j.tifs.2012.01.007

Please cite this article in press as: Delcour, J. A., et al., Technologies for enhanced exploitation of the health-promoting potential of cereals, Trends in Food Science & Technology (2012), doi:10.1016/j.tifs.2012.01.007

Introduction

The HEALTHGRAIN Integrated Project was supported for a five year period beginning in 2005 as part of the EU 6th Framework food research programme. It aimed to improve the well-being and to reduce the risk of metabolic syndrome-related diseases in Europe by providing a scientific basis for increasing the intake of protective compounds in whole grains or their fractions. As outlined earlier (Poutanen *et al*., 2008, 2010), HEALTHGRAIN was an integrated, multi-disciplinary effort establishing the variation, process-induced changes and human metabolism of bioactive compounds of the major European bread grain wheat, and also of rye. The work carried out ranged from consumer studies to crop improvement, food technology, bioprocessing, and nutrition. HEALTHGRAIN also included a comprehensive programme to interact with various stakeholders.

We here focus on the technology work in the project. Indeed, the production of healthier cereal foods relies on new ingredients, new processing tools, and knowledge of process-induced changes in cereal matrices. The work not only revisited dry milling, but also explored the potential of wet fractionation processes, enzymatic processing and fermentation for producing food ingredients and foods with increased levels of health-related components and structural features delivering good sensory properties. In particular, it concentrated on developing new bioactive food ingredients by isolating and/or processing cereal fractions using economically viable technologies. Cereal foods of high nutritional impact were developed and process-induced changes in bioactive compounds in European grains were studied. The emphasis was clearly on bread systems. Bread is a staple food, contributing an important part of the human diet, and hence is an excellent carrier of health-promoting components. Technologies for producing gluten-free foods suitable for coeliac patients were investigated. Last but not least, the work included pilot scale and demonstration activities.

Novel technologies for whole grain dry fractionation

Dry milling transforms cereals into more palatable and, hence, more desirable food raw materials. It separates the anatomical parts of the grain as cleanly as possible. Subsequently, some of the parts are reduced in particle size. Milling involves recovery of the main tissue (*i.e*. the starchy endosperm or flour) and the concomitant removal of the material the miller calls “bran”. While botanists define the bran as consisting of the pericarp, the testa (seed coat) and the nucellar epidermis of a wheat kernel, miller’s bran consists of the pericarp, the testa (seed coat), the nucellar epidermis and the aleurone layer as it is separated as such in the milling process. In addition, during milling, the germ is usually removed from the endosperm. The bran and germ are relatively rich in protein, dietary fibre, B vitamins, minerals, and lipids, and the separated endosperm is therefore lower in these components than the original grain (Delcour & Hoseney, 2010).

The health benefits of whole grain and grain dietary fibre are well-documented and dietary recommendations worldwide call for an increase in the intake of foods containing more of these healthy ingredients. The intake of both dietary fibre and whole grain foods today is clearly less than recommended. This is in part due to the technological challenge of achieving sensory properties that appeal to consumers. The presence of the hard and strong-tasting outer grain layers (bran) containing most of the health-promoting compounds requires new processing techniques to improve the quality of cereal food. Thus, while milling increases the palatability of cereal products, it decreases the nutritional value of the main product obtained (Delcour & Hoseney, 2010).

The bioactive compounds are indeed mostly concentrated in the grain outer layers. This implies that the levels of bioactive compounds in cereal wholemeal are at least two times higher than those in white flour. However, some of the bioactive compounds in the peripheral layers have low bioaccessibility as they are trapped in strong cell wall structures which resist conventional milling. They can also be localised close to undesirable contaminants such as microorganisms, mycotoxins, pesticide residues and heavy metals.

Therefore, novel technologies were developed for transformation processes that better exploit the cereal nutritional potential and meet food safety requirements.

New tools for grain dry fractionation

As a first step, new tools were developed for monitoring separation processes, based on fresh insights into grain tissue structure, properties and composition. New mechanical devices (Martelli, Barron, Mabille, Rouau, & Sadoudi, 2010) and innovative technologies (Martelli, Brygo, Delaporte, Rouau, & Barron, 2012; Martelli *et al*., 2009) coupled with microscopy and microspectroscopy were developed for determining the local compositions and properties of tissues and their interfaces as a prerequisite for underpinning the development of improved fractionation processes. In particular the effects of temperature and water content on the physical properties of outer layer tissues were investigated (Hemery, Anson *et al*., 2010; Hemery, Mabille *et al*., 2010).

A quantitative method based on biochemical markers was developed for assessing grain tissue proportions in fractions resulting from fractionation processes. The tissues quantified in industrial bran were the outer pericarp, the intermediate layer (including the testa and the hyaline layers), the aleurone cell walls, and the aleurone cell contents. Also quantified were the endosperm and the germ. Selected grain tissues were dissected by hand and analysed (Barron, Surget, & Rouau, 2007). This allowed the establishment of biochemical markers which were ferulic acid trimer (for the outer pericarp), alkylresorcinols (intermediate layer), *p*-coumaric acid (aleurone cell walls), phytic acid (aleurone cell contents), starch (endosperm) and wheat germ agglutinin (germ). The results of quantification of tissues by hand dissection and by calculation based on tissue

Please cite this article in press as: Delcour, J. A., et al., Technologies for enhanced exploitation of the health-promoting potential of cereals, Trends in Food Science & Technology (2012), doi:10.1016/j.tifs.2012.01.007

markers were in good agreement. The efficiency of this method was tested by assessing the grain tissue proportions in fractions with very different compositions such as flour, bran and aleurone-rich fractions obtained from dry grain (conventional milling, debranning process) or bran (production of aleurone-rich fractions) fractionation processes. The composition of the products generated allowed the distribution of the different tissues among fractions resulting from the different processes to be studied and showed the usefulness of this quantitative method for monitoring and improving processes (Hemery, Lullien-Pellerin *et al.*, 2009; Hemery, Rouau *et al.*, 2009). The biochemical marker methodology has further been applied to large sets of grains of different origins for assessing the variability and the applicability of the method (Barron, Samson, Lullien-Pellerin, & Rouau, 2011). Other methods allowing faster determination of the tissue composition of grain milling fractions based on spectroscopy are being developed (Barron & Rouau, 2008).

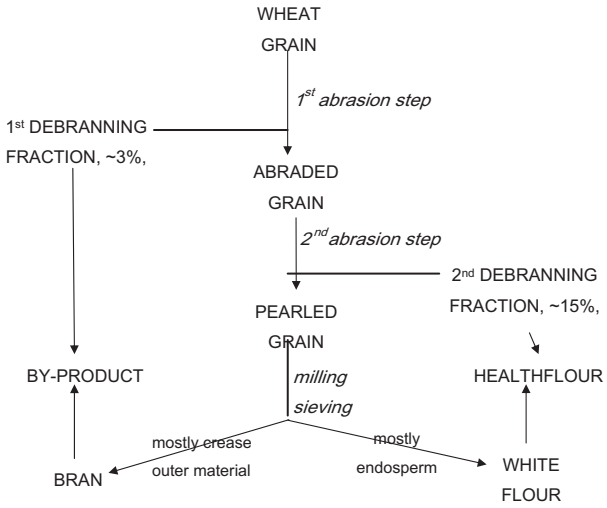

Fig. 1. Diagram of wheat grain dry fractionation for the production of "Healthflour". The first debranning fraction is rich in pericarp, the second one in aleurone.

Flour containing high levels of selected parts of the grain outer layers

A first way to enrich cereal products with bioactive compounds is to manufacture flour with high levels of selected parts of the outer layers. To remove the very outermost layers, partial debranning of grains by friction (*i.e.* peeling) or abrasion (*i.e.* pearling) was combined with milling to produce flour with tailored tissue composition and thus controlled content of bioactive compounds, as monitored by using the above-mentioned marker methodology. Flour from peeled grains, peeled and pearled grains and grains with the outermost layer and crease parts removed had higher contents of bioactive compounds than those of white flours (Hemery, Rouau, Lullien-Pellerin, Barron, & Abecassis, 2007).

A novel wheat grain fractionation procedure diagram (Fig. 1) was developed for incorporating high levels of bioactive compounds in flour while removing the parts of the grain known or suspected to be detrimental for technological quality and safety. This low-pericarp-and-low-crease-material-whole-grain flour was tentatively called "Healthflour", with reference to the HEALTHGRAIN project. The processing was designed to eliminate the outer pericarp by initially debranning and pearling to leave only the crease material attached to the kernel, then milling to eliminate the bran crease material, and finally re-mixing the white flour with the pearling fraction to incorporate as much as possible of the aleurone layer material into the final flour. Analysis of biochemical markers showed that the first step of grain abrasion (~3% off) efficiently removed the outer pericarp material. The second step consisted of further abrasion and yielded a pearling fraction which comprised the material between ~3% and ~15% grain weight. This pearling fraction was re-incorporated into the white flour after elimination of the crease bran by milling. The latter was achieved by grinding the pearled grain and then separating the coarse crease bran particles from the white flour by sieving. In fact, the abrasion of the grain did not progress equally during processing and, due to its rugby ball shape, the grain extremities were first attacked, which led to the presence of some endosperm, aleurone, intermediate and germ material in the first abrasion fractions. While appreciable levels of aleurone material were present in the bran of the ~15% abraded grains, these experiments in which debranning and milling techniques were combined showed the feasibility of designing flour with chosen contents of peripheral tissue, as monitored by tissue markers. Flours from peeled or peeled and pearled kernels or "Healthflour" are attractive alternatives to whole grain flour, in term of tissue composition, and therefore nutritional value, taking into account what is known about the chemical composition of these tissues.

Bran as a source of healthy ingredients

A further way of exploiting the potential of cereals is to use bran as a source of healthy ingredients. A patented process has been used for recovering aleurone layer tissue or entire aleurone cells as pure as possible. Innovative experimental processes to fractionate ultra-fine bran at the subcellular scale to prepare healthy food ingredients were also developed.

Bühler AG have developed processes for isolating the aleurone layer, which is particularly rich in bioactive compounds, from wheat bran. The process starts with size reduction of bran particles in order to favour the separation of the tissues. The aleurone tissue is then mechanically separated from the other seed coats by using impact or shearing forces in different types of mills. After these fragmentation steps, the blend of aleurone particles and other outer layer particles is subjected to one or several mechanical separation steps. Further purification of aleurone cells can be achieved using electrostatic separation

Please cite this article in press as: Delcour, J. A., et al., Technologies for enhanced exploitation of the health-promoting potential of cereals, Trends in Food Science & Technology (2012), doi:10.1016/j.tifs.2012.01.007

(Bohm & Kratzer, 2010). This sorting process allows the preparation of a practically pure "aleurone powder", that contains about 90% w/w aleurone and is made up of platelet-like clusters of aleurone cells attached to the hyaline layer and to minor amounts of testa, pericarp and endosperm (Fig. 2). This fraction has a high antioxidant activity and contains significant levels of vitamins, minerals and phytochemicals such as phytosterols, lignans and other phenolic substances (von Reding & Spoerndli, 2008).

A different approach applied ultra-fine grinding of bran under ambient or cryogenic conditions to achieve full dissociation of the material at a sub-cellular level. The influence of grinding temperature (ambient or cryogenic grinding) on the size distribution of particles, their composition, and their microstructure was studied, both at the lab and pilot scales (Hemery, Chaurand *et al*., 2011; Hemery, Holopainen *et al*., 2011). The intrinsic characteristics of bran (glass transition within intermediate layers at ~−50 °C) had more influence on its grinding behaviour than the type of grinding device used. However, while the size distribution of the particles was narrow for cryogenic grinding, it was broader for ambient grinding. Temperatures well below 0 °C made the material brittle and favoured fast bran fragmentation. Indeed, while one step of cryogenic grinding allowed a median particle size of nearly 50 μm to be reached, three successive steps of ambient grinding were needed to obtain the same result. However, by enhancing differences in extensibility of the different constituent layers of wheat bran, ambient temperature favoured their dissociation and produced fewer composite particles than cryogenic grinding.

Hemery, Anson *et al*. (2010) and Hemery, Mabille *et al*. (2010) showed that reducing wheat bran particle size (micronization) and using the resultant material in bread making increases the bioaccessibility of ferulic and sinapic acids. Micronized wheat bran is can be used as an ingredient to improve the nutritional potential of the food system it is used in, or as a starting material for other processes, such as extraction of specific compounds or fractionation of bran into specific ingredients. When micronized, bran gives a blend of very fine particles of the different component tissues: pericarp, testa and aleurone. Analysis of the electric behaviour of bran tissues and fine particles (Dascalescu *et al*., 2010; Hemery, Lullien-Pellerin *et al*., 2009; Hemery, Rouau *et al*., 2009) allowed the use of electrostatic separation to classify particles according to their surface composition and to obtain powder fractions with different properties (Hemery, Chaurand *et al*., 2011; Hemery, Holopainen *et al*., 2011). The charge of the particles was influenced by their composition with particles rich in pericarp being separated from particles rich in aleurone cell walls. The most positively charged fraction represented one third of the initial bran, but contained two third of the ferulic acid present in the initial bran. *In vitro* digestion studies of bran-enriched breads have shown that the bioaccessibility of phenolic acids and minerals improves not only with decreasing particle size but also with increasing concentration of micronized aleurone material, as recovered by electrostatic separation (Hemery, Anson *et al*., 2010; Hemery, Mabille *et al*., 2010; Rouau *et al*., 2010).

It is therefore possible to prepare bioactive ingredients with good yields by merely combining dry processing

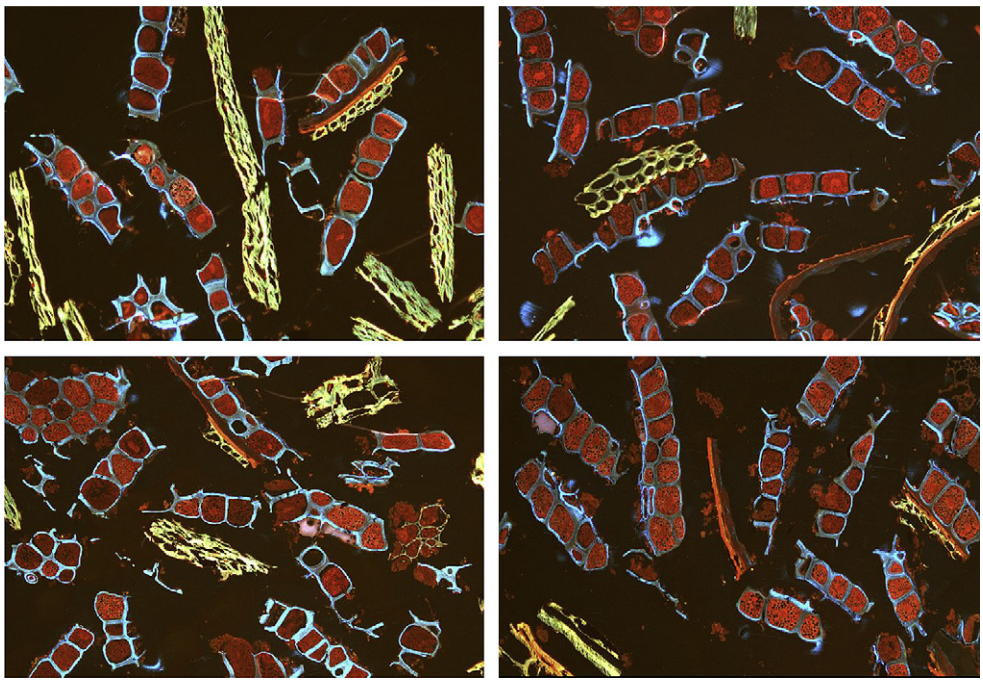

Fig. 2. Micrographs of aleurone tissue samples (courtesy of Ulla Holopainen, VTT Technical Research Centre of Finland, Espoo, Finland).

Please cite this article in press as: Delcour, J. A., et al., Technologies for enhanced exploitation of the health-promoting potential of cereals, Trends in Food Science & Technology (2012), doi:10.1016/j.tifs.2012.01.007

unit operations. One limitation of the dry fractionation processes is that, in contrast to wet processing, fractionation is not possible at the molecular scale. However, it has the advantage of retaining a matrix effect that may be beneficial for the biological activity of nutritionally important compounds. Also, in terms of process viability, the absence of water consumption and effluent production presents a clear advantage.

Novel wet processing technologies for producing bioactives

Wet processing allows the separation of cereal fractions and/or the manipulation of their structure or those of their constituents to improve their technological or health-promoting properties. It allows the specific targeting or isolation of components of interest by using enzymes as environmentally friendly and efficient tools. However, this requires basic knowledge of the reactions catalysed.

Targeted modification of cereal arabinoxylan using xylanases

AX, the major dietary fibre component of wheat bran, is important from both technological and nutritional points of view. In fact, its abundance in bran offers excellent possibilities for the manufacture of new ingredients and it is a prime target for selective enzymic modification (Fig. 3). The challenge here was to provide means to match the nutritional and technological requirements when manipulating AX structure.

Xylanases specifically hydrolyse β 1,4-bonds between the xylose residues of the AX backbone, which ultimately leads to AX oligosaccharides (AXOS). In addition to in-depth characterization and evaluation of novel and existing xylanases with regard to substrate specificity and other biochemical parameters (Berrin & Juge, 2008; Pollet, Delcour, & Courtin, 2010; Pollet, Lagaert, Kulminskaya, Delcour, & Courtin, 2010; Pollet, Schoepe *et al*., 2010; Verjans, Dornez, Segers *et al*., 2010), molecular engineering resulted in enzymes with improved catalytic efficiency and altered substrate specificity (André-Leroux, Berrin, Géoris, Arnaut, & Juge, 2008; Berrin, Ajandouz, Géoris, Arnaut, & Juge, 2007; Tison *et al*., 2009). The work also provided psychrophilic and (hyper)thermophilic xylanases with optimal activity at low and high temperature, respectively (Dornez, Verjans, Arnaut, Delcour, & Courtin, 2011; Dornez, Verjans, Broekaert *et al*., 2011; Verjans, Dornez, Segers *et al*., 2010).

Arabinoxylan oligosaccharides (AXOS) as prebiotic components

AXOS are considered to meet all of the criteria required to classify them as prebiotics. Prebiotics were first defined as non-digestible food ingredients that selectively stimulate the growth and/or activity of one or a limited number of bacteria in the colon which improve host health (Gibson & Roberfroid, 1995). Essentially, three criteria need to be fulfilled before by candidate prebiotics. These are (i) resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption, (ii) fermentation by intestinal microflora, and (iii) selective stimulation of the growth and/or activity of intestinal bacteria associated with health and well-being (Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004). As extensively reviewed by Broekaert *et al*. (2011), the evidence today for the prebiotic potential of AXOS comes from *in vitro*, animal and clinical studies in which AXOS, enzymatically released from wheat bran AX, are used as a supplement. AXOS are more heat- and acid-stable than fructo-oligosaccharides (Courtin, Swennen, Verjans, & Delcour, 2009), which are generally considered to meet the criteria of prebiotics. AXOS also

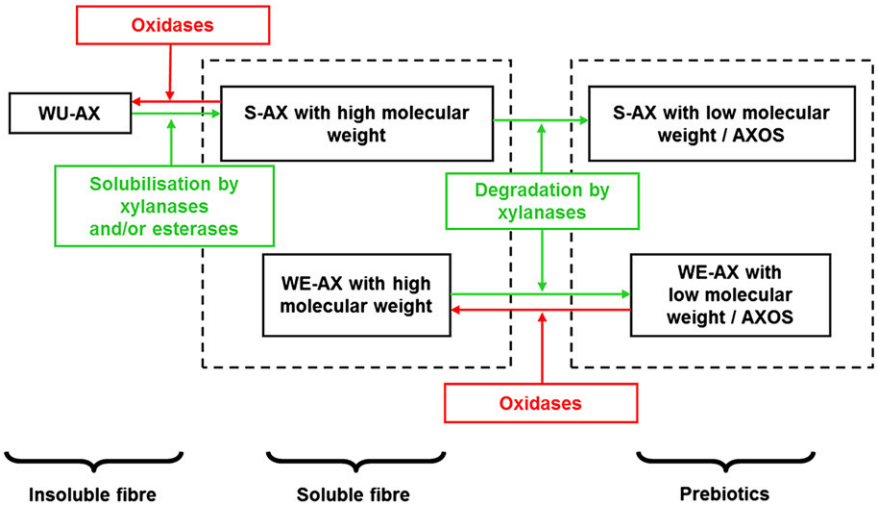

Fig. 3. Schematic representation of the different types of arabinoxylan that can be distinguished in cereal based systems and the possibilities of (inter) conversion between these types using targeted enzymes. WU-AX: water-unextractable arabinoxylan, S-AX: solubilised arabinoxylan; WE-AX: water extractable arabinoxylan; AXOS: arabinoxylan oligosaccharide.

Please cite this article in press as: Delcour, J. A., et al., Technologies for enhanced exploitation of the health-promoting potential of cereals, Trends in Food Science & Technology (2012), doi:10.1016/j.tifs.2012.01.007

resist hydrolysis by mammalian enzymes and gastrointestinal absorption. In the colon, AXOS are fermented to short chain fatty acids in general and butyric acid in particular. Butyric acid is important as fuel for the large intestine mucosa cells and AXOS are also bifidogenic (*i.e.* they promote the growth of bifidobacteria in the gut). The microbial metabolism and prebiotic potency of AXOS in the human intestine has been reviewed by Grootaert *et al.* (2007).

The solubilisation and partial hydrolysis of wheat bran AX was studied to produce AXOS as ingredients (Swennen, Courtin, Lindemans, & Delcour, 2006). A number of enzymes were tested on different bran materials showing that the yield of extracted AXOS was negatively correlated with the arabinose to xylose ratio of the wheat bran AX (Van Craeyveld *et al.*, 2010). Ball milling was an alternative method for producing AXOS from wheat and rye bran (Van Craeyveld *et al.*, 2009).

Impact of alkylresorcinols on bread making

Alkylresorcinols are amphiphilic phenolic compounds located in the bran of wheat, durum wheat and rye. They may provide health benefits (Ross, Kamal-Eldin, & Aman, 2004), and have potential as biomarkers of intake of whole grain wheat and rye foods (Landberg, Kamal-Eldin, Andersson, Vessby, & Aman, 2008). When added as isolated extracts at high levels in a bread making recipe, they reduce bread volume because they negatively impact yeast activity. However, naturally occurring alkylresorcinols do not affect bread volume and crumb porosity (Andersson *et al.*, 2011).

Technologies for producing nutritionally and organoleptically optimised breads

Slow digestibility of starch in cereal foods is considered to protect against chronic diseases (Livesey, Taylor, Hulshof, & Howlett, 2008) and several factors modulate starch digestibility (Singh, Dartois, & Kaur, 2010). However, the glycaemic responses to most conventional breads are high, including those of breads made from wholemeal flour; hence the importance of efforts targeting to tailor starch digestibility in bread. In general, nutritionally-optimised breads can be produced by using either enzyme technology, fermentation technology or a combination of both. An attractive approach is to produce AXOS *in situ* in the bread making process, taking advantage of the presence of AX in (bran-enriched) bread recipes, and the optimal conditions for action by xylanases. In the case of bran, a range of can be obtained by fermentation, mainly by controlling the pH which in turn affects the action of endogenous (and also added) enzymes (Poutanen, Flander, & Katina, 2009).

Tailoring starch digestibility in breads

It was shown that the AX population in bread impacts the digestibility of its starch. Wheat breads were made from wholemeal from wheat kernels slightly peeled to improve product palatability and safety sourdough protocols with or without added *Bacillus subtilis* xylanase in the dough recipes. It was found that the sourdough wholemeal wheat bread that had no added xylanase in its recipe resulted in the lowest post-prandial glucose and insulin responses in insulin-resistant subjects. While the breads differed in solubility and depolymerisation of protein and AX, this did not fully explain the *in vivo* findings (Lappi *et al.*, 2010).

AXOS enriched and high molecular weight soluble fibre enriched breads

(Hyper)thermophilic xylanases allow the production of AXOS *in situ* during bread making by extensively degrading part of the AX fraction during baking. In this way, the negative impacts of such extensive hydrolysis on dough processing are avoided (Dornez, Verjans, Broekaert *et al.*, 2011). As the level of AXOS that can be obtained in white wheat flour-based bread using (hyper)thermophilic xylanases is probably insufficient to confer physiological effects, AX-rich fractions or bran can be included in bread recipes to produce AXOS-rich bread (Damen *et al.*, 2011).

The identification of acidophilic and psychrophilic xylanases with high substrate specificity and substrate selectivity allowed the selective conversion of water-unextractable AX to high molecular weight solubilised AX during bread making (Dornez, Verjans, Arnaut *et al.*, 2011; Verjans, Dornez, Delcour, & Courtin, 2010). While this significantly improves product quality, the possible enhancement of a particular bioactivity of the fibre fraction by this treatment remains to be demonstrated.

Enzyme and fermentation-assisted wet processing of bran for bread making

The harsh and strong taste of the bran layers limit their use as a food ingredient in general and in bread making in particular. One option is to pre-treat rye or wheat bran using bioprocessing techniques such as fermentation using specific yeast and lactic acid starter cultures and/or enzymatic treatments using different cell wall degrading enzymes. Bran fermented with yeast prior to bread making is a better ingredient for bread than its unfermented counterpart because it leads to higher bread volume and greater crumb softness than the latter. Moreover, the bioprocessing of bran by enzyme-aided fermentation increases the content of soluble fibre and the concentrations of some potentially bioactive compounds (Katina *et al.*, 2007). Furthermore, this type of processing increases the *in vitro* and *in vivo* bioaccessibility of phenolic acids, resulting in increased levels of human metabolites possessing anti-inflammatory properties as measured *ex vivo* (Anson *et al.*, 2011, 2009).

Technologies for producing nutritionally-optimised gluten-free breads

Recent epidemiological studies show that about 1% of the population worldwide suffer from coeliac disease. Such a rate establishes it as one of the most common food intolerances. Coeliac patients who eat wheat or related

Please cite this article in press as: Delcour, J. A., et al., Technologies for enhanced exploitation of the health-promoting potential of cereals, Trends in Food Science & Technology (2012), doi:10.1016/j.tifs.2012.01.007

grain prolamins such as hordeins (barley) or secalins (rye) undergo a T cell-mediated autoimmune response, localized in the small intestine, which destroys mature absorptive epithelial cells on the surface of the small intestine. Currently, the only way that coeliac disease can be treated is by a total lifelong avoidance of gluten ingestion. Due to the unique properties of gluten (Delcour & Hoseney, 2010), it is a major challenge to produce diverse gluten-free products of high eating quality.

Novel methods to improve the quality of gluten-free breads were developed, including the use of lactic acid bacteria with properties including antifungal activity, or the production of exopolysaccharides or enzymes. The use of selected lactic acid bacteria significantly improves the quality and shelf-life of gluten-free breads (Arendt, Moore, Schober, & Ulmer, 2006; Moore, Dal Bello, & Arendt, 2008; Moroni, Dal Bello, & Arendt, 2009; Moore, Juga, Schober, & Arendt, 2007). As oats are recommended for coeliac patients in some countries, part of the work was devoted to improving the texture in oat bread (Hüttner & Arendt, 2010; Hüttner, Dal Bello, & Arendt, 2010).

One of the major problems associated with gluten-free products is their texture. Enzymes such as transglutaminase, glucose oxidase and protease (Renzetti, Courtin, Delcour, & Arendt, 2010) can improve the structure of gluten-free bread, but the impact of the enzymes varies with the gluten-free material used, such as shown for teff, buckwheat and brown rice (Renzetti & Arendt, 2009). High pressure processing can also be used to create ingredients for gluten-free cereal products (Hüttner, Dal Bello, Poutanen, & Arendt, 2009; Vallons, Ryan, & Arendt, 2011).

Pilot and industrial scale and demonstration activities

Feasibility analyses were also carried out both for the production of 'Healthflour' and for the production of aleurone tissue.

'Healthflour'

Pilot and industrial scale tests and a feasibility analysis of 'Healthflour' (see above) production revealed that 'Healthflour' has less potential food safety issues than whole wheat flour and that the use of 'Healthflour' rather than its control counterpart in bread recipes significantly improves the taste of end products. The production costs calculated for a mill with a capacity of 15 tonnes/h of 'Healthflour' is about 4% higher than the production cost of regular whole wheat flour. The main components of this cost are depreciation, maintenance and energy.

Aleurone tissue

The process for producing aleurone-rich tissue consists of several steps. The first unit operation is ultra-fine milling either by cryogenic grinding or by ambient ultra-fine milling (*e.g.* with a jet mill). The ground bran is then submitted to a three step electrostatic separation in which positively charged particles are collected. It was estimated that the production cost of the electrostatic positively charged fraction, rich in aleurone, is in line with those for the production of other products rich in micronutrients.

Conclusions

Wheat and, to a lesser extent rye, are important raw materials for the production of the staple food bread. It is clear from a body of evidence that a high intake of protective compounds in whole grains or their fractions can reduce the risk of diseases related to the metabolic syndrome. Re-examination of the classical conversion chain from cereal to bread, shows that new technologies can contribute to the production of ingredients, cereal fractions and/or final products that can be used to increase the intake of bioactive components and products. The first achievement of the technological studies in HEALTHGRAIN was therefore to revisit the milling process and to provide a scientific basis for producing cereal fractions with increased safety and palatability, for enriching health-promoting constituents in particular fractions and for launching the concept of "Healthflour". A second achievement is that the project contributed to the development of enzyme technologies for extracting prebiotic constituents from bran, and for raising the levels of such constituents in bread systems *in situ*. A third achievement is that the project established fermentation and/or enzymatic technologies to improve the palatability of bran-containing breads and the bioaccessibility of health-promoting components in these. Last but not least, significant progress has been made in the development of gluten-free bread systems.

Acknowledgements

This publication is financially supported by the European Commission in the Community 6th Framework Programme, Project HEALTHGRAIN (FOOD-CT-2005-514008). It reflects the authors' views and the Community is not liable for any use that may be made of the information contained in this publication. Dr. Inge Celus at the Katholieke Universiteit Leuven is thanked for editorial work. Jan A. Delcour is W.K. Kellogg Chair in Cereal Science and Nutrition at the Katholieke Universiteit Leuven.

References

Andersson, A., Landberg, R., Söderman, T., Hedkvist, S., Katina, K., Juvonen, R., et al. (2011). Effects of alkylresorcinols on volume and structure of yeast-leavened bread. *Journal of the Science of Food and Agriculture, 91*, 226–232.

André-Leroux, G., Berrin, J. G., Géoris, J., Arnaut, F., & Juge, N. (2008). Structure-based mutagenesis of *Penicillium griseofulvum* xylanase using computational design. *Proteins-Structure Function and Bioinformatics, 72*, 1298–1307.

Anson, N. M., Aura, A. M., Selinheimo, E., Mattila, I., Poutanen, K., van den Berg, R., et al. (2011). Bioprocessing of wheat bran in whole wheat bread increases the bioavailability of phenolic acids in men and exerts anti-inflammatory effects ex vivo. *Journal of Nutrition, 141*, 137–143.

Please cite this article in press as: Delcour, J. A., et al., Technologies for enhanced exploitation of the health-promoting potential of cereals, Trends in Food Science & Technology (2012), doi:10.1016/j.tifs.2012.01.007

Anson, N. M., Selinheimo, E., Havenaar, R., Aura, A. M., Mattila, I., Lehtinen, P., et al. (2009). Bioprocessing of wheat bran improves in vitro bioaccessibility and colonic metabolism of phenolic compounds. *Journal of Agricultural and Food Chemistry, 57*, 6148–6155.

Arendt, E. K., Moore, M. M., Schober, T., & Ulmer, H. (2006). Sourdough fermented by *Lactobaccillus plantarum* FST 1.7 improves the quality and shelf life of gluten-free bread. *European Food and Research Journal, 226*, 1309–1316.

Barron, C., & Rouau, X. (2008). FTIR and Raman signatures of wheat grain peripheral tissues. *Cereal Chemistry, 85*, 619–625.

Barron, C., Samson, M.-F., Lullien-Pellerin, V., & Rouau, X. (2011). Wheat grain tissue proportions in milling fractions using biochemical marker measurements: application to different wheat cultivars. *Journal of Cereal Science, 53*, 306–311.

Barron, C., Surget, A., & Rouau, X. (2007). Relative amounts of tissues in mature wheat (*Triticum aestivum* L.) grain and their carbohydrate and phenolic acid composition. *Journal of Cereal Science, 45*, 88–96.

Berrin, J. G., Ajandouz, E. H., Géoris, J., Arnaut, F., & Juge, N. (2007). Substrate and product hydrolysis specificity in family 11 glycoside hydrolases: an analysis of *Penicillium funiculosum* and *Penicillium griseofulvum* xylanases. *Applied Microbiology and Biotechnology, 74*, 1001–1010.

Berrin, J. G., & Juge, N. (2008). Factors affecting xylanase functionality in the degradation of arabinoxylans. *Biotechnology Letters, 30*, 1139–1150.

Bohm, A., & Kratzer, A. (2010). *Method for isolating aleurone particles*. In S. Bühler (Ed.), US patent (Vol. US7780101–B2, DE 10154462(20011108)).

Broekaert, W. F., Courtin, C. M., Verbeke, K., Van de Wiele, T., Verstraete, W., & Delcour, J. A. (2011). Prebiotic and other health-related effects of cereal-derived arabinoxylans, arabinoxylan-oligosaccharides and xylooligosaccharides. *Critical Reviews in Food Science and Nutrition, 51*, 178–194.

Courtin, C. M., Swennen, K., Verjans, P., & Delcour, J. A. (2009). Heat and pH stability of prebiotic arabinoxylooligosaccharides, xylooligosaccharides and fructooligosaccharides. *Food Chemistry, 112*, 831–837.

Damen, B., Pollet, A., Broekaert, W. F., Van Haesendonck, I., Trogh, I., Arnaut, F., et al. (2011). Xylanase-mediated *in situ* production of arabinoxylan oligosaccharides with prebiotic potential in whole meal breads and breads enriched with arabinoxylan rich materials. *Food Chemistry, 131*, 111–118.

Dascalescu, L., Dragan, C., Bilici, M., Beleca, R., Hemery, Y., & Rouau, X. (2010). Electrostatic basis for separation of wheat bran tissues. *IEEE Transactions on Industry Applications, 46*, 659–665.

Delcour, J. A., & Hoseney, R. C. (2010). *Principles of cereal science and technology*. St. Paul, MN, USA: AACC International.

Dornez, E., Verjans, P., Arnaut, F., Delcour, J. A., & Courtin, C. M. (2011). Use of psychrophilic xylanases provides insight into the xylanase functionality in bread making. *Journal of Agricultural and Food Chemistry, 59*, 9553–9562.

Dornez, E., Verjans, P., Broekaert, W. F., Cappuyns, A., Van Impe, J. F., Arnaut, F., et al. (2011). *In situ* production of prebiotic AXOS by hyperthermophilic xylanase B from *Thermotoga maritima* in high-quality bread. *Cereal Chemistry, 88*, 124–129.

Gibson, G. R., Probert, H. M., Van Loo, J., Rastall, R. A., & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition Research Reviews, 17*, 259–275.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota – introducing the concept of prebiotics. *Journal of Nutrition, 125*, 1401–1412.

Grootaert, C., Delcour, J. A., Courtin, C. M., Broekaert, W. F., Verstraete, W., & Van de Wiele, T. (2007). Microbial metabolism and prebiotic potency of arabinoxylan oligosaccharides in the human intestine. *Trends in Food Science & Technology, 18*, 64–71.

Hüttner, E. K., & Arendt, E. K. (2010). Recent advances in gluten-freegluten-free baking and the current status of oats. *Trends in Food Science & Technology, 21*, 303–312.

Hüttner, E. K., Dal Bello, F., & Arendt, E. K. (2010). Identification of lactic acid bacteria isolated from oat sourdough and investigation of their potential for the improvement of oat bread quality. *European Food Research and Technology, 230*, 849–857.

Hüttner, E. K., Dal Bello, F., Poutanen, K., & Arendt, E. K. (2009). Fundamental evaluation of the impact of high hydrostatic pressure on oat batters. *Journal of Cereal Science, 49*, 363–370.

Hemery, Y., Anson, N. M., Havenaar, R., Haenen, G. R. M. M., Noort, M. W. J., & Rouau, X. (2010). Dry-fractionation of wheat bran increases the bioaccessibility of phenolic acids in breads made from processed bran fractions. *Food Research International, 43*, 1429–1438.

Hemery, Y., Chaurand, M., Holopainen, U., Lampi, A. M., Lehtinen, P., Piironen, V., et al. (2011). Potential of dry fractionation of wheat bran for the development of food ingredients, Part I: influence of ultra-fine grinding. *Journal of Cereal Science, 53*, 1–8.

Hemery, Y., Holopainen, U., Lampi, A. M., Lehtinen, P., Nurmi, T., Piironen, V., et al. (2011). Potential of dry fractionation of wheat bran for the development of food ingredients, Part II: electrostatic separation of particles. *Journal of Cereal Science, 53*, 9–18.

Hemery, Y., Lullien-Pellerin, V., Rouau, X., Abecassis, J., Samson, M. F., Aman, P., et al. (2009). Biochemical markers: efficient tools for the assessment of wheat grain tissue proportions in milling fractions. *Journal of Cereal Science, 49*, 55–64.

Hemery, Y., Mabille, F., Martelli, M. R., & Rouau, X. (2010). Influence of water content and negative temperatures on the mechanical properties of wheat bran and its constitutive layers. *Journal of Food Engineering, 98*, 360–369.

Hemery, Y., Rouau, X., Dragan, C., Bilici, M., Beleca, R., & Dascalescu, L. (2009). Electrostatic properties of wheat bran and its constitutive layers: influence of particle size, composition, and moisture content. *Journal of Food Engineering, 93*, 114–124.

Hemery, Y., Rouau, X., Lullien-Pellerin, V., Barron, C., & Abecassis, J. (2007). Dry processes to develop wheat fractions and products with enhanced nutritional quality. *Journal of Cereal Science, 46*, 327–347.

Katina, K., Laitila, A., Juvonen, R., Liukkonen, K. H., Kariluoto, S., Piironen, V., et al. (2007). Bran fermentation as a means to enhance technological properties and bioactivity of rye. *Food Microbiology, 24*, 175–186.

Landberg, R., Kamal-Eldin, A., Andersson, A., Vessby, B., & Aman, P. (2008). Alkylresorcinols as biomarkers of whole-grain wheat and rye intake: plasma concentration and intake estimated from dietary records. *American Journal of Clinical Nutrition, 87*, 832–838.

Lappi, J., Selinheimo, E., Schwab, U., Katina, K., Lehtinen, P., Mykkanen, H., et al. (2010). Sourdough fermentation of wholemeal wheat bread increases solubility of arabinoxylan and protein and decreases postprandial glucose and insulin responses. *Journal of Cereal Science, 51*, 152–158.

Livesey, G., Taylor, R., Hulshof, T., & Howlett, J. (2008). Glycemic response and health – a systematic review and meta-analysis: relations between dietary glycemic properties and health outcomes. *American Journal of Clinical Nutrition, 87*, 258S–268S.

Martelli, M. R., Barron, C., Delaporte, P., Viennois, G., Rouau, X., & Sadoudi, A. (2009). Pulsed laser ablation: a new approach to reveal wheat outer layer properties. *Journal of Cereal Science, 49*, 354–362.

Martelli, M. R., Barron, C., Mabille, F., Rouau, X., & Sadoudi, A. (2010). Adherence within biological multilayered systems: development and application of a peel test on wheat grain peripheral tissues. *Journal of Cereal Science, 52*, 83–89.

Martelli, M. R., Brygo, F., Delaporte, P., Rouau, X., & Barron, C. (2012). Estimation of wheat tissue cohesion via laser induced

Please cite this article in press as: Delcour, J. A., et al., Technologies for enhanced exploitation of the health-promoting potential of cereals, Trends in Food Science & Technology (2012), doi:10.1016/j.tifs.2012.01.007

breakdown spectroscopy. *Food Biophysics*. doi:10.1007/s11483.11011-19222-11483.

Moore, M. M., Dal Bello, F., & Arendt, E. K. (2008). Sourdough fermented by *Lactobacillus plantarum* FST 1.7 improves the quality and shelf life of gluten-freegluten-free bread. *European Food Research and Technology*, *226*, 1309–1316.

Moore, M. M., Juga, B., Schober, T. J., & Arendt, E. K. (2007). Effect of lactic acid bacteria on properties of gluten-free sourdoughs, batters, and quality and ultrastructure of gluten-free bread. *Cereal Chemistry*, *84*, 357–364.

Moroni, A. V., Dal Bello, F., & Arendt, E. K. (2009). Sourdough in gluten-free bread making: an ancient technology to solve a novel issue? *Journal of Food Microbiology*, *26*, 676–684.

Pollet, A., Delcour, J. A., & Courtin, C. M. (2010). Structural determinants of the substrate specificities of xylanases from different glycoside hydrolase families. *Critical Reviews in Biotechnology*, *30*, 176–191.

Pollet, A., Lagaert, S., Kulminskaya, A., Delcour, J. A., & Courtin, C. M. (2010). Mutagenesis and subsite mapping underpin the importance for substrate specificity of the aglycon subsites of glycoside hydrolase family 11 xylanases. *Biochimica et Biophysica Acta – Proteins and Proteomics*, *1804*, 977–985.

Pollet, A., Schoepe, J., Dornez, E., Strelkov, S. V., Delcour, J. A., & Courtin, C. M. (2010). Functional analysis of glycoside hydrolase family 8 xylanases shows narrow but distinct substrate specificities and biotechnological potential. *Applied Microbiology and Biotechnology*, *87*, 2125–2135.

Poutanen, K., Flander, L., & Katina, K. (2009). Sourdough and cereal fermentation in a nutritional perspective. *Food Microbiology*, *26*, 693–699.

Poutanen, K., Shepherd, R., Shewry, P. R., Delcour, J. A., Björck, I., & van der Kamp, J. W. (2008). Beyond whole grain: the European HEALTHGRAIN project aims at healthier cereal foods. *Cereal Foods World*, *53*, 32–35.

Poutanen, K., Shepherd, R., Shewry, P. R., Delcour, J. A., Björck, I., van der Kamp, J. W., et al. (2010). More of the grain – progress in the HEALTHGRAIN project for healthy cereal foods. *Cereal Foods World*, *55*, 79–84.

von Reding, W., & Spoerndli, C. (2008). Isolation and characterization of aleurone from wheat bran. In P. L. Weegels, C. M. Courtin, A. Arrachid, & M. Silvestri & (Eds.), *Consumer driven cereal innovation: Where science meets industry*. *First cereal & Europe spring meeting* (pp. 173–176), Montpellier, France.

Renzetti, S., & Arendt, E. K. (2009). Effect of protease treatment on the baking quality of brown rice bread: from textural and rheological properties to biochemistry and microstructure. *Journal of Cereal Science*, *50*, 22–28.

Renzetti, S., Courtin, C. M., Delcour, J. A., & Arendt, E. K. (2010). Oxidative and proteolytic enzyme preparations as promising improvers for oat bread formulations: rheological, biochemical and microstructural background. *Food Chemistry*, *119*, 1465–1473.

Ross, A. B., Kamal-Eldin, A., & Aman, P. (2004). Dietary alkylresorcinols: absorption, bioactivities, and possible use as biomarkers of whole-grain wheat-and rye-rich foods. *Nutrition Reviews*, *62*, 81–95.

Rouau, X., Anson, N. M., Barron, C., Chaurand, M., Lullien-Pellerin, V., Mabille, F., et al. (2010). Effect of fractionation processes on composition and some nutritional properties of cereal products. *Cahiers de Nutrition et de Diététique*, *45*, 230–236.

Singh, J., Dartois, A., & Kaur, L. (2010). Starch digestibility in food matrix: a review. *Trends in Food Science & Technology*, *21*, 168–180.

Swennen, K., Courtin, C. M., Lindemans, G. C. J. E., & Delcour, J. A. (2006). Large-scale production and characterisation of wheat bran arabinoxylooligosaccharides. *Journal of the Science of Food and Agriculture*, *86*, 1722–1731.

Tison, M. C., André-Leroux, G., Lafond, M., Géoris, J., Juge, N., & Berrin, J. G. (2009). Molecular determinants of substrate and inhibitor specificities of the *Penicillium griseofulvum* family 11 xylanases. *Biochimica Et Biophysica Acta-Proteins and Proteomics*, *1794*, 438–445.

Vallons, K. J. R., Ryan, L. A. M., & Arendt, E. K. (2011). Promoting structure formation by high pressure in gluten-freegluten-free flours. *Lwt – Food Science and Technology*, *44*, 1672–1680.

Van Craeyveld, V., Dornez, E., Holopainen, U., Selinheimo, E., Poutanen, K., Delcour, J. A., et al. (2010). Wheat bran AX properties and choice of xylanase affect enzymic production of wheat bran-derived arabinoxylan-oligosaccharides. *Cereal Chemistry*, *87*, 283–291.

Van Craeyveld, V., Holopainen, U., Selinheimo, E., Poutanen, K., Delcour, J. A., & Courtin, C. M. (2009). Extensive dry ball milling of wheat and rye bran leads to in situ production of arabinoxylan oligosaccharides through nanoscale fragmentation. *Journal of Agricultural and Food Chemistry*, *57*, 8467–8473.

Verjans, P., Dornez, E., Delcour, J. A., & Courtin, C. M. (2010). Selectivity for water-unextractable arabinoxylan and inhibition sensitivity govern the strong bread improving potential of an acidophilic GH11 *Aureobasidium pullulans* xylanase. *Food Chemistry*, *123*, 331–337.

Verjans, P., Dornez, E., Segers, M., Van Campenhout, S., Bernaerts, K., Belien, T., et al. (2010). Truncated derivatives of a multidomain thermophilic glycosyl hydrolase family 10 xylanase from *Thermotoga maritima* reveal structure related activity profiles and substrate hydrolysis patterns. *Journal of Biotechnology*, *145*, 160–167.

Please cite this article in press as: Delcour, J. A., et al., Technologies for enhanced exploitation of the health-promoting potential of cereals, Trends in Food Science & Technology (2012), doi:10.1016/j.tifs.2012.01.007