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REVIEW

Enzymatic polishing of cereal grains for improved nutrient retainment

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Abstract Consumer acceptance of food products is largely driven by the dietary and functional quality of their ingredients. Though whole cereal grains are well known for bioactive components, scientists are facing dire need for better technologies to prevent the nutritional losses incurred through the conventional food processing technologies. Application of enzyme for depolymerisation of carbohydrates present in bran layer of grain is becoming an efficient method for phenolic mobilization and dietary fiber solubilisation. The present article emphasizes deep insights about the application of enzyme as an alternative technology for cereal grain processing to improve the product quality while forbidding the nutritional losses in an eco-friendly manner.

Keywords Cereals · Cellulases · Dietary fiber · Enzymatic polishing · Phenolic antioxidants · Xylanases

Introduction

Nutritional diet is one of the major and most important concerns of today's civilized world. In general, dietary choices are primarily influenced by the demographic life style and geographic location. But natural and organic healthier foods are universally accepted and demanded by consumers worldwide. The expansion of trade and publicity has shifted the trends for healthier foods. To cope up with shifting preferences of consumers, food industries are involving technological changes in production and processing.

Recent studies show that food dietary intake has direct or indirect influences on consumer health (Aldana et al. 2005; Beydoun and Wang 2009; Park et al. 2009). The healthy diet in terms of dietary quality can be described as diet providing basic nutrition and fledged with bioactive components known for lowering the risk of chronic diseases. In context to the nutritional profile, fruit and vegetables have been highly preferred to be part of healthy diet (Bertsias et al. 2005), while cereals still being the cheapest staple food and occupying the centre position in the diet. Cereals are consumed in bulk either as cooked refined grains after dehulling/milling/refining or continuated grits/flour. Storage proteins account for about 50 % of the total protein in mature cereal grains and have important impacts on their nutritional quality for humans and livestock and on their functional properties in food processing (Shewry and Halford 2002). The annual production of cereal grains are 2,313 million tonnes in 2011, which is around 3.3 % higher than 2010 (FAO 2011).

Cereal crops are grasses belonging to monocot family of Poaceae or Gramineae. These grasses are cultivated to produce single seeded fruit, caryopsis which are commonly termed as grains. Structurally, the caryopsis consists of three main parts including the endosperm, embryo, and bran (Evers and Millar 2002). Embryo or germ is diploid formed by the fertilization of male and female gametes, which is rich source of unsaturated fat, vitamins (E and B), protein and minerals. The endosperm, triploid in nature is formed by a second fertilization and consists of two distinct tissues, starchy endosperm and the aleurone layer. Starchy endosperm has starch granules and protein bodies embedded in protein matrix while aleurone coating is of 1–3 layers thick. Bran makes up 5 % of total kernel and composed of valuable components like dietary fiber, vitamins, phytochemicals etc., (Izydorczyk and

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Biliaderis 1994, 1995). It has been reported that the whole cereal grain consisting of phytochemicals work synergistically to protect body against cardiovascular diseases (Katcher et al. 2008), cancer (Slavin et al. 1997, 1999, 2001; Slavin 2003; Flight and Clifton 2006) and diabetes (Venn and Mann 2004; Bays et al. 2011; Youn et al. 2011). The primary problem associated with whole cereal grain consumption lies in the limited accessibility of digestive enzymes for starch hydrolysis, primarily by outer protective layer of kernel. The heterogeneity and complex chemical structure of cereal cell walls, polysaccharides such as arabinoxylan (Knudsen and Lærke 2010; Zheng et al. 2011), β-glucan, and cellulose along with associated phenolics act as barriers for digestive enzymes (Cui and Wang 2009).

The mechanization of cereal processing system came up with the new step 'milling/refining' to make the cereal grain more digestible and appealing. Milling of cereal grains are done in two ways, wet and dry milling. During milling the germ and bran layer are removed from the grain. As a result, the bioactive compounds such as phenolics, vitamins, dietary fiber, protein and minerals of cereals, concentrated in the peripheral layers of the grains are lost (Slavin 1994, 2004, 2010). Recently, attention is being directed towards efforts to enrich the cereal products with dietary fiber and phenolics (Dykes and Rooney 2006; Djordjevic et al. 2010; Dai and Mumper 2010). In order to facilitate production of healthier cereal foods, scientists are facing immense challenge in development of new processing tools to overcome the processing losses of bioactive compounds.

The application of enzymes as an alternative method to the conventional processes is increased in the last few years for improving the processing behaviour or properties of cereal foods (Fernandes 2010). Exogenous enzyme supplementation for proper removal of cell wall polysaccharides without nutrient loss can easily be achieved by means of microbial enzymes. Enzymes used in cereal processing are generally produced from GRAS (Generally regarded as safe) organism and are denatured during subsequent processing steps and absent in the final food product. Complete breakdown of cell wall requires concoction of several hydrolytic enzymes with diverse specificity and modes of action. Hemicellulases and cellulases are the major enzymes involved in the degradation of such complex polysaccharides (Poutanen 1997). The quality of grains can be controlled through optimization of influencing parameters such as dosing, temperature, pH and time.

During enzymatic polishing of grains, easy transformation of insoluble cell wall polysaccharides occurs in shorter time. Exogenous application of enzymes on whole cereal not only eliminates the complicacies of mechanization of the underlying process, but increases nutrient retainment as well. Moreover, enzymatic food processing lowers the required capital venture as compared to the conventional mechanized way of food processing.

The present article covers the grain processing approaches and reveals its effect on nutritional and functional attributes in the prepared grain. This article also throws light on the merits and demerits of the set of methods used to transform raw grains into refined grains for human as well as animal consumption.

Grain processing techniques

Grain processing is defined as the set of operational activities carried out for grain refining and making it consumable as food and feed. Basically, the grains are processed to increase the starch availability and digestibility (Owens et al. 1997; Corona et al. 2005; Gorocica-Buenfil and Loerch 2005). Grain processing methods are employed to make the grain products attractive, more satisfying and easier to digest as well as overcoming the deterioration problem.

Palatability of food products among consumers is highly dependent on the appearance, flavour, texture and nutrition of the product. However, whole cereal grain being one of the most prime sources of bioactive compounds is still unacceptable by major part of the population due to its appearance and digestibility. Conventional processing techniques are one of the prevailing problems in producing nutritionally enriched refined grains as end product. Before being eaten, whole cereal grains need certain refinement to transform them according to the consumer requirement. The trends indicate that refined cereals are now increasingly in high demand due to their texture, high shelf life, appearance and speciality for being used for baked products. Grain processing operations take place in phases and type of processing employed largely depend upon the structure of grain as well as on the physical and mechanical properties of caryopsis.

Conventional grain processing This processing includes mechanical and chemical means to bring together the desirable quality with exclusion of the undesirable ones. Mechanical grain processing is an old age method which includes milling. A grain processing plant or mill receives grain from an elevator and undergoes various mechanized steps with their respective grains. Innovation in the improvement of traditional equipment has led to quality enhancement, higher recovery and energy competent way to minimise the post harvest losses.

Normally, mechanical grain processing starts with the harvesting of crops. After separating the seed head from the stem of the crop, some grains such as oats, barley, and rice need to be dehusked for removal of hulls (Webster 2002) before processing whereas, wheat, rye and millets, were exempted of hull removal being ready for immediate drying and cleaning. Drying is the preliminary common process for all types of grains due to difference in their moisture content (Parde et al. 2003). High moisture content causes both sprouting and microbial spoilage during storage with cracks and fissure in grains which are commonly occurring problems (Kunze 2001), affecting the overall yield of polished grains. Procedures of drying vary from one location to another depending upon the level of mechanization as well as economic viability. Subsequent to drying, cleaning of grain is done for separating the grains primarily based on their size, shape, and weight. The present processing technologies are almost same for all types of grains, while further processing varies depending upon the end products.

Flour milling, which is commonly done for wheat (Lana et al. 2003), oat and rye (Gómez et al. 2009), initially requires grinding of grains followed by sifting and purifying (Berghofer et al. 2003). With succession of grinding stages, it produces a mixture of coarse, medium and fine fractions including flour. To maintain the uniformity, removal of the bran and germ particles is done using air currents and sieves. Preparation of rice flour can be done by any of the three ways i.e. dry, wet or semi dry milling by using the polished or broken grains, but major difference lies in the physicochemical characteristics of flour (Ngamnikom and Songsermpong 2011). Wet milling is highly preferred one because the output is fine fractions of flour with minimum damaged starch and less amylopectin fragmentation than dry milling (Suksomboon and Naivikul 2006).

Rice milling has always been a thrust area for the food scientists. They are consumed as whole white grain after hull removal and polishing, therefore its appearance after and before cooking is of prime importance (Conway et al. 1991). First of all, dehusked rice is cleaned to remove unwanted matters like mud and stones. This bulk brown rice is then fed to mills where with the help of roller, polishing is done by mild abrasion or friction (Yadav and Jindal 2008). The whiteness of rice is directly proportional to degree of bran removal portion (Park et al. 2001). Head and broken rice, present in milled portion are separated before packaging.

Technology advancement has provided innovative solutions to these mechanized processing units with better equipment and control system facilities for development of number of cereal products, but majorly at the cost of nutrient loss. Above all, these grain processing units need to function with sustaining profitability and require development of a cost effective process which limits huge investment and maintenance capital.

On the other hand chemical processing was noticed in case of enrichment of parboiled rice with thiamine, where rice was soaked in 1 % acetic acid or HCl (Kondo et al. 1951). The drawback of chemically processed parboiled rice was its colour and stickiness. Inspite of better thiamine content in parboiled rice than normally water soaked rice, it was unacceptable to the consumers due to its appearance. Use of chemical wetting agents for enhancing the wetting property of siliceous hull of rice during soaking was also employed. Alkali (Rose et al. 2010; Luh and Mikus 1980) as well as acid (Kondo et al. 1951) both were tried to produce better quality of rice. Higher rate of absorption was noticed in case of alkali than acids, which led to proper solubilisation of matrix, increasing the permeability of hull (Bello et al. 2004). Use of acetic acid during rice cooking causes enhanced transparency, glossiness and stickiness (Kasai et al. 2001; Ohishi et al. 2007) effecting the rice gelatinization. Involvement of chemicals is also noticed in case of wet milling where corns and sorghum are pre-incubated with SO_2 or lactic acid to soften the kernel before milling (Buffo et al. 1997). Even though there are some advantages of the grain processing through chemical agents, but because of the disadvantages, these processes are avoided.

Problems associated with conventional grain processing Processing has always thought to reduce nutritional value (Chowdhury and Punia 1997; Emmons et al. 1999). Mostly, milled fractions are compared with bran portions to evaluate the nutrient loss, because the extent and impact of milling process highly influences the composition and proportions of the nutrient in the bran (Greffeuille et al. 2006). Variation in the bran portion has been noticed which differs with grain type and milling process indicates that the conventional processing is not standardized one. Kamal-Eldine et al. (2009) collected two samples of rye bran from roller mill from Sweden, Denmark and Finland each. The obtained result on these two samples proved that the extent of roller milling was not even, with respect to particle size, colour, dietary fibre and starch. Degree of milling (DOM) is influenced by the grain size, shape and hardness and affects the sensory and textural properties of grain. Optimizing the level of DOM is highly essential to produce nutritious and functional grain products. Inspite of extensive research on the engineering aspect of mills, a simple test for evaluating the degree of milling and consistent performance is still missing. Practically, no systematic research has been done.

Though the refined grains were highly acceptable among consumers but epidemiological studies conducted on various populations proved that refined grains are not the right choice (Table 1). Cross-sectional study by Radhika et al. (2009) on the 2042 individuals population size showed that the higher refined grain intake was also significantly associated with higher body weight, waist circumference, serum fasting plasma glucose, triglyceride levels and blood pressure. The meta-analysis reported by Mikušová et al. (2011) and Liu et al. (2003) also supported that intake of refined grains leads to weight gain. Study conducted by Bazzano et al. (2005) on 22,071 U.S. male physicians failed to correlate the refined grain consumption with the increase in weight gain. Their study showed no major difference on the weight gain while consuming whole grain or refined grain. Consumption of whole grain and its antioxidative

Table 1 Comparative epidemiological studies between whole grain and refined

Physiological effects	Population size	Outcome	Author's
Cardiovascular diseases	17 participants	Administration of whole grain diet decreased plasma total and LDL-cholesterol, in comparison to the refined grain diet	Ross et al. 2011
Body fat distribution	2,834 participants	Lowering of visceral adipose tissue (VAT) was noticed with increased whole-grain intake whereas higher intakes of refined grains are associated with higher VAT	McKeown et al. 2010
Inflammation	1,625 participants	Whole grain intake resulted in lowered plasma inflammatory protein (PAI-1 and CRP) while refined grains showed proinflammatory responses only	Masters et al. 2010
Antioxidative properties	20 participants	No significant difference in antioxidant activity was noticed with intake of whole grain and refined grain intake	Enright and Slavin 2010
Weight gain and plasma lipids	1,516 participants	Whole grain intake have lower prevalence of overweight while refined grain were not significantly associated with any other risk factor except having positive relation with fasting glucose only in case of women	Newby et al. 2007
Cardiovascular disease	11,940 participants	Whole grain resulted in a 23–28 % lower risk of total mortality and coronary diseases while refined-grain intake, had a 34 % higher risk of total mortality and a 54 % higher risk of coronary disease	Steffen et al. 2003

property with respect to refined grains was supported by Baublis et al. (2000). Whole grain and refined grain were processed to develop wheat-based ready-to-eat (RTE) breakfast product (Bodinham et al. 2011). The antioxidant activity of the whole grain was more than the refined wheat (Revanappa and Salimath 2011; Neyrinck et al. 2011; Jonnalagadda et al. 2011). Maki et al. (2010) reported that whole grain oats based RTE food was capable to reduce the waist circumference and cholesterol level while studying the population size of 204. Health benefits of whole cereal grain consumption has been given in Fig. 1.

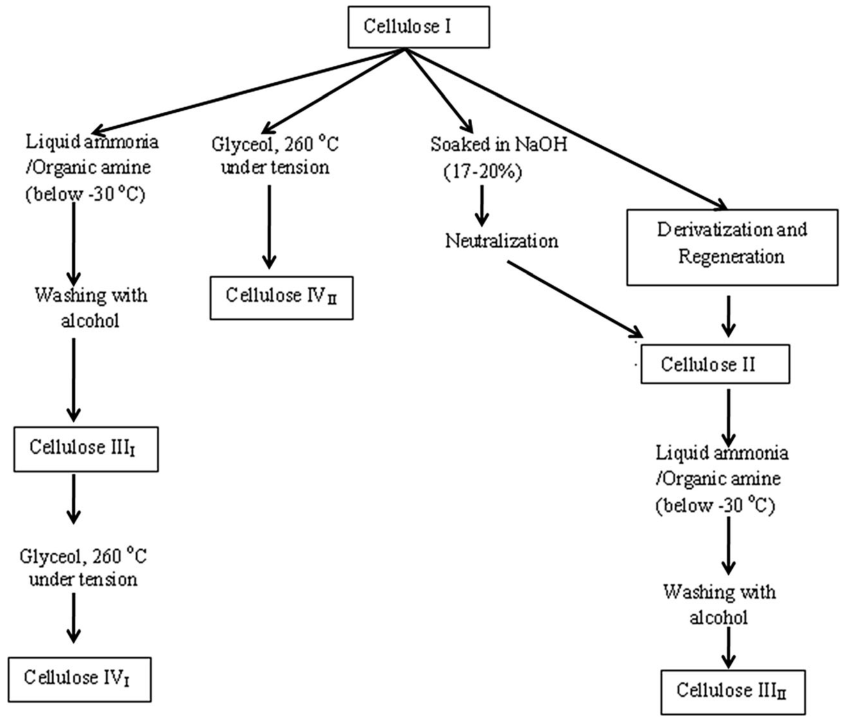
During the milling, the bran and germ layer of the grains are removed, resulting in overall loss of nutrients (dietary fiber, minerals, vitamins, lignans, phytoestrogens and phenolic compounds). McMurrough et al. (1983) examined that milled fractions obtained from coarse milling of hulled barley have less phenolic content than the coarsest fraction. Various workers utilized the bran for extraction of phenolics and reported that major phenolics reside in bran after milling (Okarter and Liu 2010). In case of sorghum, bran enriched fraction have higher phenolics than the mechanically milled fraction (Beta et al. 2000). Not only bran and germ are removed during milling but it was also found that the outer endospermic tissue, aleurone layer composed of biofunctional molecules also get removed during roller milling. Conventional processing also has negative impact on the mineral content (Robberecht et al. 1990; Ahmad et al. 1994) since they are more concentrated in germ and aleurone layer (Guttieri et al. 2006; Ŝramková et al. 2009). The mills are going for modern processing technology to boost up quality production but at the same time loss of nutrients can not be minimized.

Apart from nutrient loss, another major problem associated with the mechanical milling process is broken grains. During the mechanical polishing of rice major portion come up with broken rice which is considered to be a waste or to be sold at reduced price (Das et al. 2008a). Majority of the mills are continuously confronting the problems of low capacity and working capital limitations due to which it is becoming time consuming process. It has been also observed that once a processing method is selected for a particular grain, investments are too huge and a simple modulation in the equipment becomes difficult and costly. It is also noticed that the storage of the grains before milling is a tedious process, as these bulk masses when stored, nutrient losses take place. The inertia of these negative factors of conventional methods requires a cost effective and eco-friendly process to conquer the drawbacks of mechanical processing.

Enzymatic polishing

Knocking down the demerits of both chemical and mechanical processes, biotechnological approaches came up as

Fig 1 Health benefits associated with consumption of whole cereal grains



emerging tool to the food processing system in terms of human health. Biotechnological concepts have also been applied to initial grain processing scheme by making use of cell wall degrading enzymes to modulate the grain properties such as the taste, texture, shelf-life, with limited detriment in nutritional value. Liberation of cell bound nutrients and fibres from bran without removing the germ and bran layer was possible only by the help of cell wall hydrolyzing enzymes. A US patent was granted on 17th Nov, 1964, to Allen and Thompson (1964) for their innovative application of proteolytic enzymes for enzymatic treatment of grain. Another patent was granted to Blanchon (1966), where the inventor used pectinases to dissolve the pectin sheath enclosing the cellulose to release the nutrient and bioactive elements. Tobey et al. (1997) and his team invented the process of grain conditioning by using multienzymes which was patented on 2nd September, 1997. During the conditioning, concoctions of carbohydrate cleaving enzymes were used in two grain conditioners. Hemicellulases, pectinases, β-glucanase and amylase were used for easier-to-digest grains, while pectinase, protease, beta-glucanase and amylase were included in another grain conditioner which was applicable and effective for all grain with at least 30 min contact time. Recently, on 3rd August, 2007 (WO/2007/051091), an exogenous enzyme treatment process was patented by Jeffery (2007), for breaking the one layer from the endosperm core without mechanically damaging the cereal grain. Such result signifies the applicability and effectiveness of enzymatic methods but still the most efficacious conditions for the application of enzymes on grains have yet to be defined.

Consequently, it was also observed that the improvements in initial processing with the introduction of newer, efficient and eco-friendly 'enzyme' technologies will help to improve the dietary quality and overall performance of the industry by controlled polishing. Application of specific enzymes is a novel approach for the enrichment of grains with bioactive nutrients as transformation can occur at ambient temperature in an eco-friendly manner. Though, the use of enzyme in food sector is an age old process, but the particular innovative strategies developed works on the concept of zero waste.

To breach the plant cell wall and its use as a source of nutrients, cell wall degrading enzymes are essential. These enzymes are produced by several microbes including both bacteria and fungi under proper environmental conditions. Among food grade biocatalysts, fungal enzymes have acquired a special place in comparison to bacterial enzymes. Application of enzyme in any industrial sector is highly dependent upon the nature of microbes and requires the evaluation of the production strain for the enzyme safety (Pariza and Johnson 2001). GRAS organisms are preferred choice for enzyme production and its application in food sector due to the safety issues (Gaynor 2006; Olempska-Beer et al. 2006).

Maeda and Morita (2003) studied the effect of enzymatically polished wheat on bread making. Combination of traditional food-processing and enzymatic approach has also been studied to improve the content and bioavailability of dietary bioactive component (Rahman et al. 2005; Arora et al. 2007). Ramírez et al. (2009) developed enzymatic corn wet milling (E-milling) process for the recovery of starch using proteases, eliminating the need of sulfites for steeping process. The complexity of cell wall polysaccharides is one factor which contributes to improper digestion and accessibility of starch from whole cereal grain. Effect of a cell wall degrading enzymes on sorghum and maize were studied by Serna-Saldívar and Mezo-Villanueva (2003). It was found that after the enzymatic hydrolysis the yield of starch and protein content were improved.

Enzymes for biopolishing

Carbohydrate cleaving enzymes During the polishing, bran and germ layers are removed, thus cell wall degrading enzymes emerged as a tool to degrade the polysaccharide linkages for enrichment. Common carbohydrate cleaving enzymes used for cereal processing are as follows:

Cellulase Cellulases are one of the potential biocatalysts used in fruit juice and animal feed sector (Bhat 2000; Kuhad et al. 1997). Inclusion of cellulase in combination with other cell wall depolymerising enzymes has proved to be innovative way of cereal grain enrichment. Cellulase consists of three classes of enzymes: endoglucanase, exoglucanase and beta-glucosidases, of which endoglucanase-(EC 3.2.1.4; 1,4-β-D-glucanglucanohydrolase) acts on carboxy methyl cellulose, causing random scission of cellulose chains in cereal cell wall yielding glucose and cello-oligosaccharides (Ciolacu et al. 2011). Exoglucanase-(EC 3.2.1.91; 1, 4-β-D-glucancellobiohydrolase) acts on microcrystalline cellulose (avicel), imparting an exo-attack on the non-reducing end of cellulose, liberating cellobiose as the primary product and beta-glucosidases (EC 3.2.1.21) facilitates the hydrolysis of cellobiose to glucose. Das et al. (2008b) reported cellulase mediated treatment resulted in overall increase in antioxidant activity, reducing power, free amino acids, proteins, crude fiber, ash, oil, and phenolic content in the order of brown rice>enzyme treated rice>milled rice. Arora et al. (2007) reported increase in nutritional quality of cellulase treated basmati rice. Mostly, blend of enzymes have been used for the polishing of brown rice. Sarao et al. (2011) treated brown rice with fungal enzymes (cellulase, xylanase and protease) prior to milling, to increase the head rice yield and cooking quality. Cellulases are less explored for the cell wall degradability during the grain processing, but its potential for breaching the cell wall unlocks a novel mode for bran removal and nutritional retainment.

Xylanases Xylanases have wide use in baking industries to improve the texture related properties of dough, bread, biscuits and cakes (Courtin and Delcour 2002; Poutanen 1997). Addition of exogenous xylanase for cereal processing is emerging as new technique to enrich the grains with bioactive compounds. In the majority of cases, it was found that the enzymatic modification of bran with xylanases, resulted in enhanced soluble dietary fiber content (Bednar et al. 2001; Figueroa-Espinoza et al. 2004; Napolitano et al. 2006; Santala et al. 2011). Several models have been proposed to explain the mechanism of xylanase action (Xu et al. 1998; Bolam et al. 2001). Xylanase activity leads to the hydrolysis of xylan. Generally, hydrolysis may result either in the retention or inversion of the anomeric centre of the reducing sugar monomer of the carbohydrate. This suggests the involvement of one or two chemical transition states. Glycosyl transferase usually result in nucleophilic substitution at the saturated carbon of the anomeric centre and take place with either retention or inversion of the anomeric configuration. This cell wall degrading enzyme has also known to release the phenolics from the complex wall structure of cereal brans (Anson et al. 2009). Bartolomé and Gòmez-Cordovés (1999) found that the ferulic acid release was much enhanced when ferulic acid esterase and xylanase were used in concoction. Moore et al. (2006) used cellulase, xylanase and β-glucanases concoction to increase the antioxidant properties of wheat bran. Recently, xylanase tagged with fluorescent probe has been used for visualization of arabinoxylans (AXs) in cereal grain (Bengtsson and Åman 1990; Dornez et al. 2011). Xylanases have also been used to modify the feeding value. In case of broiler chickens exogenous xylanase supplementation on wheat diet led to enhanced starch digestibility (Bergmans et al. 1996; Choct et al. 1995)

Major problem associated with xylanase treatment is the presence of xylanase inhibitor proteins (XIPs) in some grain, which inhibits the hydrolysis. These XIPs block the endo-1, 4-β-D-xylanase and has been reported in barley and wheat (Elliott et al. 2003). Genetic engineering concept can be a solution for producing the transgenic strains which encode genes for inhibitors and can be either removed or silenced.

β-glucanases β-glucanases (EC 3.2.1.73) hydrolyse the 1, 4 or 1, 3 linkages found in mixed-linked glucans disrupting the integrity of endospermic cell wall (Georg-Kraemer et al. 2004; Jin et al. 2004). The present biocatalyst has been mainly used as feed enzyme to modify the non-starchy polysaccharide components of cereal for improving digestibility and feed efficiency (Li et al. 1996; Sieo et al. 2005; Samuelsen et al. 2011). The digestion of cereal grains with β-glucanase improved the illeal digestibility of the cereal protein (Ji et al. 2008). β-glucanases supplementation on barley and oat based diet resulted in better feed with good amount of dietary fiber and improved weight of broiler chicken (Józefiak et al. 2006; Kováčová and Eva 2007; Papathanasopoulos and Camilleri 2010).

Esterases Apart from carbohydrate degrading enzymes, esterases have also shown equal potential during enzymatic processing for nutrient recovery. Cross-linking of ferulic acid is among the main factors which inhibits the release of fermentable carbohydrates and phenolics from grains (Garcia-Conesa et al. 1997). Xylanases and ferulic esterase, these two enzymatic systems are known to work in synergy (Faulds et al. 2006). Incubation of barley and wheat grains with recombinant xylanases and ferulic esterase (*Aspergillus niger*) resulted in diferulic acid release from the xylanase treated fraction (Bartolomé et al. 1997; Sancho et al. 2001). Hence, it can be said that the ferulic esterases help in release of phenolic acids.

Recent studies on production of transgenic grains showed that the enzymatic processing results are encouraging for nutritional retainment (Kimura et al. 2003). Two important enzymes xylanases and ferulic acid esterase were expressed in wheat resulting in 15–40 % increase in water-unextractable arabinoxylan (Hoffmann et al. 1992; Harholt et al. 2010). Tall Fescue the plant belonging to grass family was chosen for expression of fungal ferulic esterase in golgi or apoplast which has significant application for fodder (Buanafina et al. 2010).

Pros and cons of enzymatic polishing of grain Bio-polishing, a novel processing technology which protects sensitive nutrients from thermal degradation and crack as grain processing takes place at normal room temperature and pressure in reaction vessels. Products obtained, therefore, are nutritionally more enriched than the ones obtained by alternative mechanical and chemical means because both processes tend to be unspecific and consequently generate several by-products. Handling and production of enzyme is safe for the operator and the environment. Most of the enzymes used in polishing are of food grade produced from GRAS organisms. Biodegradable nature of enzymes removes the associated waste disposal problems since they mostly get destroyed during processing.

Because of the potential of cell wall degrading enzymes as suitable candidate for polishing, there has been a rapid increase in the amount of research in this area, but still its implementation at large scale is lacking. One of the major problems associated with enzymatic processing is cost associated with production, storage and transportation of enzymes. Patel et al. (2000) used grain as enzyme producing system, by expressing xylanase gene (*xyn*A) from the rumen fungus, *Neocallimastix patriciarum* in barley endosperm to minimise the expenditure of production and storage as enzyme was stably stored in the matured grain. Use of microbial enzymes sometimes can lead to contamination of the food products. Thus, proper management of safety issues are highly required. The applications of genomic and metagenomic techniques have provided a deep knowledge about the cereal cell wall architecture. Despite, this mechanism of release of phenolics and dietary fiber by cell wall depolymerising microbial enzyme in cereal matrix is still not clear.

Comparison of conventional and enzymatic techniques with reference to rice grain

Rice is a staple diet of half of the world population. Starchy endosperm of rice is protected from external environment by means of hard siliceous husk and resistant impervious bran layer. In case of brown rice the tough bran layer makes the grain unpalatable while the rancidity is developed upon storage due to increase in fatty acid by action of lipolytic enzyme on the oil present in bran layer results in deterioration. The appearance of rice is also an important factor which governs its market value; more whiter the rice, more is its price value. Hence, rice processing is essential to keep up the quality of rice.

The present problem was surmounting by one of the conventional cereal processing method i.e. milling. During milling the starchy endosperm is exposed by the removal of the germ and bran which are 2–3 % and 5–8 % of brown rice weight, respectively (Lamberts et al. 2007). Itani et al. (2002) reported that mineral and protein content was less after milling in exposed endosperm. Park et al. (2001) emphasized on the sensory and textural properties of rice and reported that with the increase in milling, the properties of cooked rice such as agglomeration, adhesiveness, cohesiveness get enhanced while hardness and chewiness decreases.

The mechanized milling process has got direct impact on the nutritional quality as well as on percentage of broken rice generation. Consequently, a new alternative polishing technique has been attempted in the laboratory of authors (Das et al. 2008a) for improved rice polishing. In this process cellulase, xylanase and protease were selected during rice polishing. Enhanced content of phenolics was found in the enzymatically degraded rice bran (Das et al. 2008b). Because of germination during polishing, percentage of GABA (Gamma Amino Butyric Acid) increased in enzyme treated rice. Loss of vitamin and minerals were considerably reduced in comparison to mechanical milling. In case of brown rice, enzymatic processing appeared as better alternative to mechanical milling.

Conclusion

Inclusion of cereals in functional food is increasing, as whole grains are good source of phytochemicals. The nutrient profile of cereals is highly influenced by the processing techniques for polishing or refining, which limits the application of cereals as food additive or as nutraceuticals. A prior knowledge and understanding of the cereal cell wall architecture is necessary for achieving efficient depolymerisation of cell wall polysaccharides during polishing. Among several biotechnological approaches, employment of enzymes for grain processing is becoming an alternative tool for endosperm concentration and enrichment of cereals with bioactive component known for exerting positive effects on human health. Although, exogenous enzymatic processing is an age old process, but implementation of these techniques in grain polishing is now a days gaining popularity. Still, rigorous studies and further research are required for selection and blending of appropriate enzymes for particular grains with their proper kinetic activities for optimum functionality and productivity.

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