CEREAL SIDE-STREAMS AS ALTERNATIVE PROTEIN SOURCES

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Why Develop Cereal Side-Streams as New Protein Sources?
There is a global need to increase dietary intake of plant protein to gain sustainability, food security, and nutritional benefits. The side-streams from cereal grain processing offer large quantities of underexploited raw materials with the potential for use in new plant-based food ingredients. Efficient use of these side-streams as food ingredients would improve processing and raw material efficiency. Moreover, the availability of new protein ingredients will create business opportunities for new plant-based food and protein product concepts and diversify consumer choices by providing new healthy food options.

To harness the potential of cereal side-streams, technologically and economically feasible and sustainable technologies need to be developed for the concentration of proteins from solid and liquid material streams. Protein functionality must also be improved to develop new food concepts that meet consumer demands. New technologies are required at different processing stages. First, feasible and sustainable processes are needed to fractionate, concentrate, and isolate protein from solid and liquid cereal side-streams. Second, the technological functionality and sensory properties (e.g., solubility, emulsifying, and foaming ability) of protein-rich fractions need to be improved, and food concepts in which new protein ingredients are utilized either as performance proteins or as sources of dietary protein must be developed. At the same time, we must assess process scenarios in which protein ingredients are developed sustainably, and the basic socioeconomic performance of the product system must be evaluated. Finally, for the new business ecosystem to be viable, it is important to establish business cases, including new value chains in the food industry, for resource efficiency and sustainable plant-based protein ingredients.

To realize these objectives, close collaboration throughout the value chain and new partnerships within the industry are needed. Companies from both the ingredient and food production sectors and research partners covering technology, research, and analytics should collaborate to generate novel technological concepts that will enable new business development.

Potential of Cereal Brans as Protein Sources
Wheat and rice are the largest cereal crops produced after corn. They serve as staple foods for the majority of the world’s population, but also have important industrial uses. Milling and starch or bioethanol production generate substantial volumes of protein-rich wheat and rice side-streams that generally are used for feed or incinerated. The global annual production volumes for wheat and rice bran are 167 and 27 million tons, respectively, of which on average 15% is protein. This adds up to around 30 million tons of bran protein in side-streams.

The bran fraction of wheat constitutes 14–19% of the whole kernel (42,47). Dietary fiber makes up more than half of the bran (56), and 8.3–19.3% of the bran is protein (47). The bran proteins are composed primarily of albumins (23.5%), globulins (15.5%), prolamins (18.5%), and glutelins 25.5% (28). The soluble bran proteins are mainly albumins and globulins. Wheat bran amino-acid composition is superior compared with endosperm flour: bran protein contains more lysine, arginine, alanine, asparagine, and glycine and less glutamine, proline, phenylalanine, and sulfur amino acids (15). According to Meziani et al. (36), 66% of the aleurone layer protein in wheat is composed of different types of globulins. The cell walls of the aleurone contain twofold the amount of protein, with similar amino-acid composition, but more glycine and less glutamine/glutamic acid compared with the cell walls of the endosperm. The digestibility of bran protein is hindered by the aleurone cell walls, which act as a barrier to digestive enzymes. Only 50% of the protein from wheat bran is digestible, and a significant amount of aleurone cells is left intact with incomplete protein digestion (2). On the other hand, there are only a few published studies on the technological functionality of wheat bran proteins (6,28).

The bran fraction of rice constitutes around 10% of the dehulled rice kernel and is obtained by polishing brown rice. Rice bran contains 12% (native bran) to 20% (defatted bran) protein (1). In rice bran, water-soluble albumins comprise the major protein fraction (37% of bran proteins), together with globulins (36% of bran proteins), followed by glutelins and prolamins (20), which render good functional properties for rice bran proteins compared with rice endosperm proteins and other cereal proteins (8,60). Rice bran protein is hypoallergenic, enabling its use in infant foods, and has a higher lysine content than rice endosperm protein (32). Indeed, among cereals, rice bran protein is claimed to have the highest nutritional value due to its high content of essential amino acids, especially lysine, as well as histidine, arginine, threonine, glycine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine. The nutritional status of rice bran protein is also reported to be much better compared with that of rice endosperm protein, and rice bran proteins are easily digestible (1). Rice bran is not consumed as a food, however, mainly because of its high fiber content, possible hull contamination, and rancidity due to its high lipid content and lipase activity (21).

Fractionation and Extraction of Proteins from Cereal Side-Streams
Extraction and concentration of proteins from any cereal side-stream are often challenging because the proteins are en-
trapped within the complex cereal cell wall matrices. Wheat bran contains subaleurone cells with protein and starch trapped inside, whereas rice bran contains elongated pericarp structures (>500 µm) with loose protein bodies packed among the broken aleurone layers and pieces of cell walls (Fig. 1).

In addition to the protein bodies trapped within the cereal cell wall matrices, there are also several components that adversely affect sensory and nutritional qualities, limiting the food ingredient applications for cereal side-streams. Sustainable and feasible fractionation technologies are necessary to enrich the protein contents from cereal matrices and enable their use as food ingredients after suitable postprocessing. Feasible fractionation and extraction methods, especially for bran proteins, are not available in the food industry today. However, combinations of wet/dry fractionation and supercritical carbon dioxide (SC-CO₂) extraction (of lipids) with bioprocessing (e.g., enzymes and fermentation) and thermomechanical treatments (e.g., extrusion) have been elucidated to develop innovative and sustainable fractionation and extraction methods for obtaining protein-rich fractions from cereal side-streams (Fig. 2).

Current industrial plant protein extraction technologies include wet extraction, either in alkaline or saline conditions (12,60). The extraction and precipitation pH affects not only the yield, but also the functional properties of the recovered proteins, and thus, protein solubility may be reduced due to partial denaturation. Degradation, cross-linking, or racemization of amino acids may also occur. Connor et al. (12) produced rice bran protein concentrates (33–38% protein) using wet alkaline extraction. Although there was a lot of fat in the protein concentrate (49–55%), when the fat was removed prior to protein extraction the protein content was higher (34–62%). Wang et al. (60) prepared rice bran protein isolates with up to 90% protein.

Fig. 1. Micrographs of wheat bran (15–20% protein) (A) and rice bran (11–17% protein) (B) stained with acid fuchsin and Calcofluor to show proteins (red) and cell walls (light blue).

Fig. 2. Possible processing steps involved in upgrading cereal side-streams for use as food ingredients.
content. However, their method was based on a complicated extraction procedure that included enzyme treatment, inactivation of enzymes by alkali, centrifugation, acidification (pH 4), another centrifugation step, neutralization (pH 7), freeze-drying, and storage at 5°C. In terms of hydrothermal pretreatment, van den Borne et al. (58) studied the acidic treatment for disintegration of wheat bran in connection with protein properties. Acidic conditions increased the digestibility of the residual protein in vitro without impairing the quality of protein for animal feeding. Reisinger et al. (45) studied different hydrothermal conditions in a biorefinery and revealed that the highest protein yield (73%) from wheat bran was achieved at 180°C (20 min). Celiktas et al. (7) investigated protein extraction from wheat bran using a biofinery approach and obtained high yields (up to 92%); however, they used harsh conditions (pH > 8 and temperature > 80°C). The current wet extraction and concentration processes are harsh and water-intensive. Therefore, future research and development in this area should focus on high-consistency extraction combined with gentle bioprocessing conditions, which could enable efficient, sustainable protein extraction with improved technological functionality and sensory properties.

Bioprocessing with enzymes or microbes can be utilized to modify bran structure through the action of both endogenous and microbial enzymes, thereby affecting the technological functionality, bioactivity, and bioavailability of bran components (4,5,38,41,50). Treatment of wheat bran with carbohydrases and proteases resulted in increased protein solubilization of up to 58% (4,5), and activation of bran endogenous enzymes resulted in increased solubility of up to 75%. Enzymatic processing still needs to be improved by, for example, decreasing the treatment time using new or current enzymes. In enzymatic treatment of wheat bran with xylanase (a cell wall-degrading enzyme) at high consistency, we showed that the proteins from the bran aleurone cells were released; however, their solubility was reduced (49). By releasing the proteins from the bran matrix, the bioavailability of the proteins can be improved. In enzyme-aided fermentation, the addition of exogenous enzymes before or together with microbes degraded cell walls and released protein from the rye bran aleurone, which in the end improved protein digestibility (38). Enzymatic treatments have also been shown to have a positive impact on protein extraction from rice bran. Carbohydrases active on cell wall components can increase protein yield by liberating more protein from the polysaccharide matrix of the bran (3,57,60). Other enzymes, such as α-amylase and phytase, have been shown to enhance protein extraction by inhibiting the interaction of proteins with starch and phytate in the bran (54,57,60).

Thermomechanical processing can be used to modify the structure of plant materials to obtain better separation or extractability of proteins (11,23,35). There are not many published studies on the effects of thermomechanical treatment of wheat and rice brans. However, the solubilization of protein has been shown to increase when treating wheat bran at 40% water content, with or without xylanase enzyme (49). Processing at low water content can be carried out in high-shear mixers, such as a farinograph or extruder (49,50). Jacquemin et al. (29) studied the thermomechanical treatment of wheat bran and wheat straw mixtures to extract hemicelluloses by adding NaOH during the extrusion. They showed how proteins were coextracted with hydrolyzed hemicelluloses to obtain food-grade protein concentrates.

Dry processing through a series of milling and subsequent fractionation steps can be utilized to valorize cereal side-streams and is often considered a more feasible method than wet processing, because no drying step is required in downstream processing. The separation of protein using dry fractionation techniques depends largely on the interactions of cell walls and protein and starch components in the plant cell matrix. The yield and purity of protein-rich fractions resulting from the separation of protein from the other cell components depends strongly on the milling operation and milling quality. Wheat bran separation has been studied using different dry fractionation methods (24–26). The fractionated aleurone layer can contain twice as much protein (up to 21%) compared with wheat bran (10% protein) (26). However, the separation of the aleurone layer requires a specific method called electrostatic separation, which is still considered too expensive for processes with high throughput and relatively low-value end products.

Air classification is a potential fractionation method (52) that thus far has only been studied with untreated wheat bran (43). We previously demonstrated that air classification could offer new possibilities for protein separation when combined with pretreatments such as SC-CO2 extraction (55) or thermomechanical bioprocessing (50). Defatting by SC-CO2 can be used to enhance the dissociation of fiber, starch, and protein particles, providing advantages for enrichment of protein through dry grinding and dry fractionation (55). At the same time, defatting by SC-CO2 can stabilize the quality of side-stream-based ingredients, especially in the case of rice bran, because it has a high content of lipids, which are prone to oxidation and can cause rancidity (9,34). Traditional defatting using nonpolar solvents (e.g., hexane) and/or heat stabilization can negatively affect protein quality. Due to the low lipids content in wheat bran, SC-CO2 extraction has been shown to have a fast and linear initial extraction period followed by a slower extraction period (44). The extraction time has a direct influence on the overall feasibility of a process, and thus, optimization would be needed to obtain a sufficiently low fat content with a short processing time (17). The process for wheat has been shown to be faster than that for rice bran defatting (9,34), implying greater feasibility for industrial production with wheat. Air classification of defatted rice bran has also shown the potential to provide slightly enriched protein fractions (27,30,48,51).

**Applicability of Cereal Proteins in Foods**

The physicochemical properties of cereal proteins (e.g., their mostly insoluble nature) restrict full utilization of these ingredients in different foods. Moreover, the functional properties of wheat bran proteins in particular are not yet well characterized, and very little is known about their potential food applications. Neither the functional properties of the endosperm protein fractions of wheat nor those of rice are comparable to animal-based proteins. When attempting to increase the amount of plant proteins in the daily diet and/or when replacing animal-based performance proteins with plant-based alternatives, sophisticated protein and food modification tools are required to improve their technological, nutritional, and sensory functionality. Another important challenge, which may actually be converted into an advantage, is the low degree of purity, i.e., the presence of other components such as starch, dietary fiber, lipids, etc. in protein-enriched fractions from wheat and rice dry processing. To maximize cost efficiency, extensive purification of plant proteins should be minimized, and the functional benefits or disadvantages of other compounds that are coextracted or coenriched together with proteins should be thoroughly
evaluated. Selective refining will enable the industry to optimize the functionality, feasibility, and sustainability of cereal proteins. Multifunctional ingredients, thus, can be obtained in economically feasible and sustainable ways and find use in conventional as well as new food concepts.

**Liquid and Semisolid Food Applications**

Solubility or dispersability in the form of stable colloidal aggregates is one of the main requirements for the functionality of proteins as emulsifying, foaming, or gel-forming agents. Cereal proteins generally show low solubility/dispersability at the pH values that are common in most foods. Furthermore, processes such as oil extraction or high temperature treatments for enzyme inactivation may induce denaturation, which further decreases solubility. Despite the low solubility of cereal proteins, the so-called “soluble aggregates” (colloidal particles) under certain environmental conditions (e.g., pH and ionic environment) have shown good surface-active properties. We have shown that soluble aggregates from oat globulins lowered surface tension at the air–water interface, which was comparable to milk proteins (18). Colloidal zein particles (~70 nm) were reported to have good wetting properties at the oil–water interface under various environmental conditions, resulting in good inherent surface activity (13). In fact, the notable ability of particles to stabilize foams and emulsions (Pickering emulsions) has been well documented (14,59). Thus, cereal proteins, despite their low solubility, may find applications in the structuring of food colloids, either in already existing products or in the formulation of novel food structures.

Rice bran protein isolates (prepared by enzyme- and/or alkaline-assisted extraction and isoelectric precipitation) were tested for their foaming and emulsifying activity and stability, as well as their water- or fat-binding abilities in several reports (8,39,60,62). The outcomes of these studies vary considerably depending on the environmental conditions (e.g., pH, ionic environment, salt or sugar content, etc.) and grain/bran pretreatments and extraction conditions (e.g., heat stabilization, enzymatic hydrolysis of cell walls, enzymatic or acid hydrolysis of proteins, etc.), making it difficult to draw comparisons and identify the most promising processes. If a general conclusion can be drawn, it could be stated that formation of aerated or emulsified structures may be possible using rice protein isolates and their hydrolysates, for example; however, rendering them with high stability (comparable to that of dairy or egg proteins) is challenging. A systematic approach that takes food-relevant processing conditions into consideration together with other bulk ingredients is vital to develop the market value of such protein fractions.

Potential food-compatible technologies for improving the functionality of cereal proteins include protein cross-linking by enzymatic means (18,37,40); shear-induced processing (e.g., microfluidization) (16); complexation with polysaccharides (19,21,33,53) or other proteins (22); and complete or limited hydrolysis by enzymatic means (62). The major challenges associated with hydrolysis methods is the off-flavor formation generated by peptides, and therefore, limited/controlled hydrolysis should be favored as an approach in many cases.

**Solid Food Applications**

Animal-based (i.e., egg and dairy) proteins are important performance ingredients in bakery products (e.g., cakes and muffins) in which the airy structure of the product is developed through the emulsifying and foaming ability of egg proteins during mixing. Egg proteins enable the dispersion of large volumes of air into the batter during mixing, and during the baking process the formed air bubbles expand and protein coagulation stabilizes the structure. Dairy proteins create a strong batter that will ultimately provide an increase in loaf volume and a soft/tender crumb. There are several reports on successful replacement of animal-based performance proteins with legume plant proteins. However, information related to the functionality of rice bran protein is quite limited. Wheat bread enriched with alkaline-extracted rice bran protein concentrate (69% protein content) had lower dough weight and specific volume of bread compared with bread made with white wheat flour (31). Addition of more than 1% rice bran protein concentrate with a final protein concentration of 10% in bread decreased the scores for color, taste, odor, texture, and overall liking of the bread (31). Defatted, alkaline-extracted rice bran protein concentrate (36.5% protein content) was also used for enrichment of wheat bread (46). Bread prepared with refined wheat flour replacement of up to 5% (final protein concentration in bread was 12%) was comparable in sensory attributes to bread prepared with refined wheat flour (46). The addition of prefermented rice bran protein up to 10% resulted in breads that were not different from the control white wheat bread in terms of texture and volume (10). In biscuits, the addition of rice bran protein concentrate (5–10%) affected the physical and texture characteristics of biscuits by decreasing the diameter andspread ratio and increasing the fracture hardness (61). Sensory evaluation showed that the increase in rice bran protein level lowered the scores for the color, texture, appearance, and flavor intensity of the biscuits (61).

The challenges related to development of high-protein or protein-rich solid foods (e.g., biscuits and pasta) could be tackled using bioprocessing (e.g., enzymes and microbes) and thermomechanical technologies, with an attempt to improve the functionality of the protein ingredients in terms of technological, sensory, and nutritional qualities.

**Ongoing Research**

The potential of cereal side-streams as sources of protein is currently being studied in the EU BBI project PROMINENT (Protein Mining of Cereal Side-Streams Exploring Novel Technological Concepts [www.prominent-protein.eu]). The three year project aims to develop technologically, economically, and environmentally viable protein ingredients and foods from wheat and rice side-streams. The VTT-coordinated consortium members include Südzucker AG, AB Enzymes, Upfront, United Biscuits, Barilla, Olvi, Natural Resources Institute Finland, and Bridge2Food. A strong focus, in addition to developing novel fractionation methods, is the use of enzymatic and thermomechanical techniques to improve the technological functionality and sensory properties of protein ingredients to achieve desirable taste and texture in food applications such as pastas, biscuits, cakes, and beverages.

**Conclusions**

Side-streams in grain processing (e.g., wheat and rice brans) offer extensive underexploited potential raw materials for new plant-based food ingredients. Valorization of such streams to be used as food ingredients would contribute to sustainable production of new alternative protein sources and multifunctional food ingredients. To date, bran proteins have been relatively poorly studied in terms of their technological functionality and nutritional properties. Dry fractionation is a sustainable, non–water-
intensive process, but it delivers protein concentrates with limited purity, which may require modification or functionalization of both the protein and the other biopolymeric components. Technological functionality of cereal proteins is generally limited, and thus, enzymatic or other modification methods need to be developed. The nutritional value of these proteins needs to be further investigated considering their restricted bioavailability due to the plant matrix.

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