IMPROVING THE PROTEIN CONTENT AND QUALITY OF TEMPERATE CEREALS: WHEAT, BARLEY AND RYE

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Summary

Wheat, barley and rye are major sources of protein for the nutrition of humans and livestock, but are deficient in certain essential amino acids when used as food for monogastic animals. In particular, they contain low levels of lysine (the first limiting amino acid) and to a lesser extent, threonine (the second limiting amino acid) resulting from deficiencies of these amino acids in the prolamin storage proteins, which account for about half of the total nitrogen in the mature grains. The total protein content of the grain can be manipulated by adding fertilizer nitrogen but “genes” conferring high grain protein have been identified in wild tetraploid wheats. However, these have not yet been exploited to increase the protein content of cultivated wheat. Similarly, mutant genes have been identified, which confer the high lysine phenotype to barley grain, resulting from decreases in the proportion of lysine-poor prolamins and/or increases in specific lysine-rich proteins. However, these genes are also associated with low yields, and have not been successful when incorporated into cultivated lines. Genetic engineering has great potential for increasing the essential amino acid content of temperate cereals, using one of two strategies. Transformation with bacterial genes encoding feedback insensitive enzymes of lysine biosynthesis may lead to increased accumulation of lysine as free amino acid. Alternatively, expression of additional genes for lysine-rich proteins may lead to increased accumulation of lysine in proteins. These two strategies may also be combined.

1. Introduction

Barley, wheat and rye are related members of the Tribe Triticeae and consequently share many genetic and biochemical characteristics. Cultivated wheat exists in diploid, tetraploid and hexaploid forms but the vast majority of the wheat grown worldwide is of two types – hexaploid *Triticum aestivum* var *aestivum* (genome constitution AABBDD) (bread wheat) and tetraploid *T. turgidum* var *durum* (genome constitution AABB) (durum or pasta wheat). However, other varieties of these two species have been cultivated historically and are still grown in small amounts in some parts of the world, as are other primitive diploid and tetraploid species. In addition, a number of wild diploid species related to the progenitors of the A, B and D genomes of cultivated wheats and wild *T. turgidum* var. *dicoccoides* also occur, providing a reservoir of valuable variation for plant breeding.

In contrast to wheat, cultivated barley comprises only one species, *Hordeum vulgare*, which is diploid and crosses readily with the wild form *H. spontaneum*, which is now generally considered to belong to the same species. Whereas barley and wheat are inbreeding, rye (*Secale cereale*) is an out-breeding diploid. A number of wild species occur, but cross-fertility between these may be limited by the presence of chromosome translocations.

Wheat, barley and rye are important sources of protein for the nutrition of humankind and of livestock, which is in turn used to provide dietary protein. The content and nutritional quality of the grain proteins is therefore an important consideration (see *Plant Based Sources of Proteins and Amino Acids in Relation to Human Health*). However, it is also important to bear in mind the impact of the grain proteins on the processing properties, with most cereals being consumed by humans after processing into food or beverages rather than as whole or milled grain.

When used for animal feed, the quality requirements depend on the animal in question (particularly whether ruminant or non-ruminant) and the stage of development. In the case of ruminants, the microflora present in the rumen can synthesize all twenty protein amino acids, which is sufficient to provide for the dietary requirements of the animal, except under high production conditions. For non-ruminants, about half of these amino acids are essential, in that they must be provided in the diet. Thus, if even one essential amino acid is present in insufficient amounts, the remaining amino acids will be broken down, leading to nitrogen loss (and subsequent environmental pollution in high production conditions) and poor growth. When compared with the WHO requirements of essential amino acids for humans, wheat, barley and rye are seen to be deficient in lysine, with threonine being the second limiting amino acid (Table 1).

Amino acids	Wheat	Rye	Barley	WHO Recommended levels
Cysteine	2.6	2.9	2.9	3.5 (Cysteine + Methionine)
Methionine	1.3	1.7	1.7	
Lysine	2.0	3.3	3.1	5.5
Isoleucine	3.6	3.6	3.6	4.0

Leucine	6.7	6.7	7.2	7.0
Phenylalanine	5.1	4.9	5.5	6.0
Tyrosine	2.6	2.1	2.7	
Threonine	2.7	3.4	3.3	4.0
Tryptophan	1.1	1.8	2.0	1.0
Valine	3.7	4.4	4.6	5.0
Histidine	2.2	2.1	1.9	-

Amounts are expressed as g/100g protein. Cysteine and tyrosine are not truly essential as they can be synthesised from methionine and phenylalanine, respectively. Hence combined values for cysteine + methionine and phenylalanine + tyrosine are given by WHO. Similarly, histidine is essential for human children but not adults.

Data taken from J.A.D. Ewart (1967) Amino acid analyses of cereal flour proteins. *Journal of the Science of Food and Agriculture* **18**, 548-552 and FAO (1973) Energy and Protein Requirements, FAO Nutritional Meeting Report, Series No.52, WHO Technical Report Series No.552, Rome.

Table 1**.** Contents of essential amino acids in grain of wheat, rye and barley compared with the WHO recommended levels.

Much of the wheat consumed by humans is in the form of bread, which occurs in a vast range of forms in different cultures, including leavened pan and hearth-baked breads, flat and pocket breads and steamed breads, with numerous variants within these different types. In addition, a range of types of noodles (made from bread wheat) and pasta (from durum wheat) and other baked products (cakes, cookies etc.) are made. Consequently, it is important to maintain the quality for specific end uses when attempts are made to improve wheat protein quality – failure to do this may lead to lack of acceptance irrespective of any improvement in nutritional quality.

Barley and rye are consumed less frequently in processed foods than wheat, with rye flour often being blended with wheat flour to improve its functional properties. The major "food" use of barley in developed countries is for malting, brewing and distilling. In this case, processing quality is the overwhelming consideration. However, barley is still a staple food in some other parts of the world and nutritional quality is, therefore, important.

1.1. Cereal Grain Proteins

The systematic study of plant proteins, including those of cereal grains, is founded on the work of T.B. Osborne, working at the Connecticut Agricultural Research Station, from 1886 until 1928. He classified cereal proteins into four groups, based on their sequential extraction in a series of solvents. Thus, the albumins were extracted in water followed by the globulins in sodium chloride solution, the prolamins in aqueous alcohols and the glutelins in dilute acid or alkali. This system has proved remarkably durable and still provides a framework for modern cereal chemistry. However, it is inevitable that the extraction procedures have been modified in the light of our improved knowledge of the structures, functions and genetics of the proteins present within the fractions.

We now know that the albumin and globulin fractions of cereals contain predominantly structural, metabolic and protective proteins, although 7S storage globulins are present in the aleurone layer of the endosperm and the scutellum of the embryo. In contrast, the prolamins comprise the major grain storage proteins and are located in the starchy endosperm cells. However, whereas prolamins were classically extracted with aqueous (60-70 per cent (v/v)) ethanol, it is now usual to use other alcohols which give more efficient extraction (often 50 per cent (v/v) propan-l-ol) and to include a reducing agent (2-mercaptoethanol or dithiothreitol) to extract prolamins which are not soluble in aqueous alcohols due to their assembly into high M_r polymers stabilized by inter-chain disulphide bonds. Once these proteins are removed, the residual glutelin proteins, which probably include insoluble cell wall proteins and other structural proteins, are usually extracted using a solvent such as sodium dodecylsulphate (SDS) rather than acid or alkali, which may result in some degradation. Finally, even after the extraction of glutelins, a small proportion of the total proteins remains in the residual meal.

When extracted efficiently, the prolamin fractions of wheat, rye and barley may account for 50 per cent or more of the total grain nitrogen, as discussed below. However, these fractions contain only about 1 mol percent or less lysine compared with about 4-5 mol percent lysine in the albumin, globulin, glutelin and residual fractions (Table 2). Similarly, the prolamin fractions are also low in threonine. Consequently, it can be concluded that the poor nutritional quality of the whole grain proteins results from deficiency of essential amino acids in the prolamin storage proteins. It is, therefore, necessary to briefly describe the characteristics of the prolamins before discussing strategies to manipulate their amount and composition.

Amino acid	Barley	Wheat	Rye
Lysine	1.0	0.9	0.9
Histidine	1.2	1.7	1.3
Arginine	2.6	2.1	1.5
Aspartate[a]	1.9	3.0	2.1
Threonine	2.5	2.7	2.5
Serine	2.9	5.4	5.8
Glutamate[a]	31.8	32.6	35.8
Proline	20.1	17.3	20.2
Cysteine	2.9	1.9	2.5
Glycine	3.2	5.6	4.2
Alanine	3.0	3.7	2.7
Valine	5.1	3.8	4.4
Methionine	0.6	1.2	1.1
Isoleucine	4.1	3.7	3.0
Leucine	7.4	7.1	5.7
Tyrosine	2.6	2.6	1.7
Phenylalanine	5.2	4.5	4.6

[a]Values reported for aspartate and glutamate include asparagine and glutamine, respectively.
Tryptophan was not determined.

Data taken from Kreis, M., Shewry, P.E., Forde, B.G., Rahman, S., Bahramian, M.B. and Miflin, B.J. (1984) Molecular analysis of the effects of the mutant *lys 3a* gene on the expression of *Hor* loci in developing endosperms of barley (*Hordeum vulgare*). *Biochemical Genetics* **22**, 231. Byers M., Miflin, B.J. and Smith, S.J. (1983) A quantitative comparison of the extraction of protein fractions from wheat grain by different solvents, and of the polypeptide and amino acid composition of the alcohol-soluble proteins. *Journal of the Science of Food and Agriculture* **34**, 447-462 and Bright, S.W.J and Shewry, P.R. (1983) Improvement of protein quality in cereals. *CRC Critical Reviews in Plant Sciences* **1**, 49-93.

Table 2**.** Amino acid compositions (mol per cent) of total prolamin fractions from barley, wheat and rye.

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Biographical Sketch

Peter Shewry is an authority on the structures and properties of plant proteins, and in particular the seed storage proteins of cereals. He has published extensively on the proteins of barley, wheat and rye grains over a period of 25 years and contributed to many collaborative research programmes and to international conferences and workshops. In 2000 he was awarded the Thomas Burr Osborne medal by the American Association of Cereal Chemists. He is currently Associate Director and Head of the Crop Performance and Improvement Division at Rothamsted Research. He is also Professor of Agricultural Sciences in the University of Bristol.