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# Improving wheat to remove coeliac epitopes but retain functionality

Peter R. Shewry [a, b, *], Arthur S. Tatham [c]

[a] Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK
[b] University of Reading, Whiteknights, Reading, Berkshire RG6 6AH, UK
[c] Cardiff School of Heath Sciences, Cardiff Metropolitan University, Llandaff Campus, Western Avenue, Cardiff CF5 2YB, UK

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ABSTRACT

Coeliac disease is an intolerance triggered by the ingestion of wheat gluten proteins. It is of increasing concern to consumers and health professionals as its incidence appears to be increasing. The amino acid sequences in gluten proteins that are responsible for triggering responses in sensitive individuals have been identified showing that they vary in distribution among and between different groups of gluten proteins. Conventional breeding may therefore be used to select for gluten protein fractions with lower contents of coeliac epitopes. Molecular breeding approaches can also be used to specifically down-regulate coeliac-toxic proteins or mutate coeliac epitopes within individual proteins. A combination of these approaches may therefore be used to develop a "coeliac-safe" wheat. However, this remains a formidable challenge due to the complex multigenic control of gluten protein composition. Furthermore, any modified wheats must retain acceptable properties for making bread and other processed foods. Not surprisingly, such coeliac-safe wheats have not yet been developed despite over a decade of research.



## 1. Introduction

### 1.1. The importance of wheat in human nutrition and health

Cereals are the most widely grown and consumed staple foods in the world, with three species alone (maize, rice and wheat) accounting for about 90% of the total production. Although wheat is third in order of total production, with about 713 m tonnes grown in 2013 compared with 745 m tonnes of rice and 1017 m tonnes of maize (http://faostat.fao.org/site/339/default.aspx), it has the widest geographical distribution, being grown and consumed as a staple food between 67°N in Scandinavia and 45°S Argentina (Feldman, 1995), and in both highly industrialised western economies (Western Europe, North America) and in developing economies (China, Brazil, India). Hence it can be argued that wheat is the most important crop in the world in its global impact on human nutrition. Wheat consumption is also increasing globally, For example, the availability of wheat as a %age of total kCal in food increased from 11.85 in 1961 to 24.41% in 2011 in India, and from 12.20% to 17.83% in China (FAO Food Balance Sheets http://faostat3.fao.org/faostat-gateway/go/to/download/FB/FBS/E). Furthermore, demand is increasing dramatically in industrialising countries in which the production of wheat is limited by climatic conditions, such as West Africa (e.g. an increase from 0.89% to 6.64% of the total available kCal in Nigeria between 1961 and 2011).

Within these countries wheat makes important contributions to diet and health, particularly the provision of dietary fibre, B vitamins (notably vitamins B1, B2, B3, B6 and B9 (folates)) and mineral micronutrients (notably Fe, Zn, Se). This contribution is most readily demonstrated for developed economies where accurate data are available on food intakes. For example, the UK National Diet and Nutrition Survey (NDNS) showed that cereals account for 31% (and breads for 10–12%) of the total daily intake of energy in adults between the ages of 19 and 64, 23% (breads for 10–11%) of the total daily intake of protein, 37–40% (breads for 18–21%) of the total daily intake of non-starch polysaccharides (ie dietary fibre), 38–40% (breads for 15–16%) of the total daily intake of Fe and 27% (breads 12%) of the total daily intake of folates (https://www.gov.uk/government/publications/national-diet-and-nutrition-survey-results-from-years-1-to-4-combined-of-the-rolling-programme-for-2008-and-2009-to-2011-and-2012).

Vitamins and minerals have long been known to be essential for human health, while cereal dietary fibre has been shown to reduce the risk of a range of chronic diseases (including cardio-vascular disease, type 2 diabetes and colo-rectal cancer (Topping, 2007;

* Corresponding author. Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK.
*E-mail address:* peter.shewry@rothamsted.ac.uk (P.R. Shewry).

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Buttriss and Stokes, 2008; Anderson et al., 2009a,b; Aune et al., 2011; Threapleton et al., 2013; Lafiandra et al., 2014; SACN, 2014). In addition, wheat is rich in a range of phytochemicals, notably phenolic acids and other phenolic compounds, which have been reported (but not conclusively established) to have benefits in reducing the risk of chronic diseases. Hence, restricting the intake of wheat in the diet can have serious consequences for the intake of essential nutrients and other beneficial components unless equivalent sources of these are provided.

Almost all of the wheat consumed by humans undergoes extensive processing before consumption. This usually comprises two stages. Firstly, the grain is milled to give fine particles and, in most cases, to separate the starch-rich endosperm (which is the origin of white flour) from the outer layers (aleurone, pericarp and testa) and germ (which together form the bran). Secondly, the flour is processed into various foods, most commonly bread but also other baked goods (cakes, biscuits), noodles (bread wheat) and pasta (durum wheat) and breakfast cereals. Wheat flour, and gluten (see below), are also widely used as ingredients in the food industry.

### 1.2. Wheat gluten proteins

The use of wheat for most food products in underpinned by the gluten proteins. These correspond to the major group of grain storage proteins which are deposited in the starchy endosperm cells to support germination and seedling growth. They account for up to 80% of the total grain proteins, which in turn account for between 10 and 15% of the dry weight of grain grown commercially. These proteins form a continuous matrix surrounding the starch granules in the mature starchy endosperm cells, and are brought together to form a continuous network when flour is mixed with water to give dough. This network confers a unique combination of elasticity and viscosity which enable the dough to be processed into the range of products discussed above. Although related proteins are present in other temperate cereals (barley, rye and oats) they do not share the same properties and it is necessary to blend them with wheat flour to make products which are acceptable to most consumers. It is therefore crucial that any modifications that are made to the amount and composition of the gluten proteins should also be considered in relation to their effects on the biophysical and functional properties of dough.

Wheat gluten proteins are traditionally classified into two groups based on their solubility. The gliadins are readily extracted from flour with alcohol:water mixtures, such as 60% (v/v) ethanol or 50% (v/v) propan-1-ol, while the glutenins were traditionally extracted with dilute acid or alkali. However, these fractions contain related proteins and the differences in solubility are determined by their presence as monomers or polymers. Thus, the gliadin fraction comprises mainly proteins which are present as monomers, with small amounts of polymeric components, while the glutenins comprise “subunits” assembled into high molecular mass polymers stabilized principally by inter-chain disulphide bonds. When these disulphide bonds are reduced the monomeric glutenin subunits resemble the gliadins in being soluble in alcohol:water mixtures. Hence, the protein subunits present in both fractions correspond to alcohol-soluble prolamin proteins as defined in the classic studies of Osborne (1924).

Gluten protein fractions comprise many individual components, with a high level of allelic variation in composition between cultivars. The individual components can be classified on the basis of their amino acid compositions and sequences into three families, which have been called the sulphur-rich (S-rich), sulphur-poor (S-poor) and high molecular weight (HMW) prolamins (Shewry et al., 1986).

The gliadins are traditionally divided based on their mobility in electrophoresis at low pH into three groups: the S-rich α-type gliadins and γ-type gliadins (which contain three and four inter-chain disulphide bonds, respectively) and the S-poor ω-gliadins (which lack cysteine residues and hence do not form disulphide bonds) (Fig. 1). Similarly, the glutenin subunits are separated by sodium dodecylsulphate polyacrylamide electrophoresis (SDS-PAGE) into low molecular weight subunits (LMW subunits) and high molecular weight subunits (HMW prolamins) of glutenin (Fig. 1). The LMW and HMW subunits form inter-chain disulphide bonds which stabilise the glutenin polymers, while intra-chain disulphide bonds are also formed by LMW subunits and at least some HMW subunits.

Whereas most of the LMW subunits are S-rich and form a distinct group within the S-rich family (B-type LMW subunits), the fraction also contains small proportions of components that are closely related in sequence to the α-, γ- and ω-gliadins. These components correspond to “mutant” forms of gliadins in which the presence of one or two additional cysteine residues allows their incorporation into polymers. The LMW subunits related to ω-gliadins correspond to one or more bands of slightly higher molecular weight than the B-type components (called D-type LMW subunits) and those related to γ- and ω-gliadins correspond mainly to a group of bands of lower molecular weight (C-type LMW subunits). The B-type LMW subunits can be further sub-divided based on their N-terminal amino acids: M (methionine) or S (serine). There are also clear differences between the amino acid sequences of the ω-gliadins encoded by chromosomes 1A and 1D and those encoded by chromosome 1B (also called ω5-gliadins): these relate to their toxicity in coeliac disease and are discussed below.

The HMW subunits are further divided into x-type and y-type based on their mobility on SDS-PAGE. These two types also differ in their contents and distributions of cysteine residues.

This brief summary is based on a considerable volume of research and the reader is referred to Shewry et al. (1999; 2003a, b; 2009) and Payne (1987) for more detailed discussions and references. This rather complex classification is summarized for clarity in Fig. 2.

### 1.3. Wheat gluten protein genes and expressed proteins

Genetic and molecular analyses indicate that the individual gluten proteins are encoded by multiple genes at complex loci. The classical genetics has been reviewed in detail by Shewry et al. (2003a). The HMW subunits of glutenin are encoded by three loci on the long arms of the group 1 chromosomes (*Glu-A1*, *Glu-B1*, *Glu-D1*), each comprising two genes encoding one x-type and one y-type HMW subunit. Similarly, the α-type gliadins are encoded by

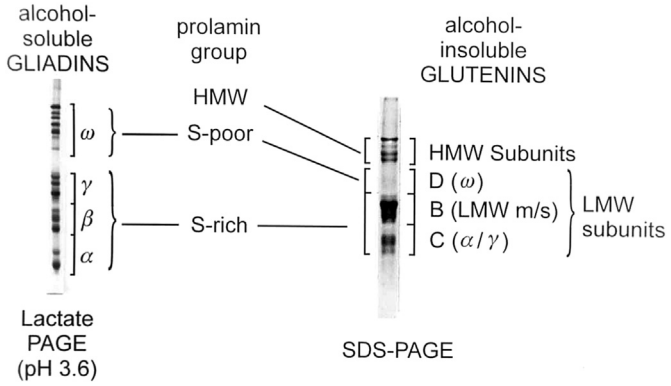


**Fig. 1.** The groups of gliadin and glutenin proteins separated by electrophoresis at low pH and SDS-PAGE, respectively. Taken from Shewry et al. (1999) with permission.