Relationship between common wheat (*Triticum aestivum* L.) gluten proteins and dough rheological properties

Gluten proteins and rheological properties in wheat

Elpidio Peña¹, Angeles Bernardo¹, Consuelo Soler² & Nicolás Jouvé¹,*

¹Department Biología Celular y Genética, University Alcalá, Campus Universitario, 28871 Alcalá de Henares, Madrid, Spain; ²Plant Breeding Unit, C.I.T., Instituto Nacional de Investigaciones Agrarias, I.N.A., La Canaleja, Alcalá de Henares, Madrid, Spain; (*author for correspondence: e-mail: nicolas.jouve@uah.es)

Received 10 October 2004; accepted 4 March 2005

Key words: gliadin, gluten, glutenin, *Triticum aestivum*, wheat, wheat quality

Summary

This paper reports the correlation between the rheological properties of bread wheat dough and the types and quantities of endosperm proteins in 28 common wheat cultivars. Different methods were used to analyse the allelic composition of these cultivars and the relative quantities of the different proteins contributing to the gluten structure. Neither dough strength (W) nor tenacity/extensibility (P/L) correlated with allelic composition. Different wheats with the same allelic composition (i.e., with respect to glutenins) showed different rheological properties. The glutenins were the most influential components of W and P/L, especially the high molecular weight (HMW) glutenin subunits and in particular the type x form. These proteins seem to increase W and are the main constituents of the gluten network. The gliadins and low molecular weight (LMW) glutenin subunits appear to act as a “solvent”, and thus modify the rheological properties of the dough by either interfering with the polymerisation of the HMW glutenin subunits, or by altering the relative amounts of the different types of glutenin available. Thus, the protein subunits coded for by the alleles *Glu-B1x7* and *Glu-D1x5* stabilised the gluten network, whereas those coded for by *Glu-B1x17* and *Glu-D1x2* had the opposite effect. Dough properties therefore appear to depend on the glutenin/gliadins balance, and on the ratio of the type x and type y HMW proteins. The influence of external factors seems to depend on the allelic composition of each cultivar.

Introduction

Wheat is currently the most important crop in the world. It is unique because of the special properties of its flour, which forms a cohesive mass – gluten or dough – which is useful in baking. The properties of wheat flour reside primarily in the types and quantities of gluten proteins it contains, the glutenins and gliadins being the most important in the formation of the gluten network. Glutenins are composed of two types of subunit, one of high molecular weight (HMW), the other of low molecular weight (LMW), coded for by different genes. Many authors have focused their analyses on the different HMW subunits because of the influence they appear to have on the rheological properties of the dough (Payne, 1987; Shewry et al., 2001).

It has been shown, however, that LMW glutenin subunits influence the quality of durum wheats, and the question arises as to whether they may do the same in bread-making flour. Gupta et al. (1991) produced a list of the LMW alleles in bread wheats according to the extensographic behaviour of their corresponding doughs. D’Ovidio (1993) and Masci et al. (2000) characterised the LMW glutenin subunits associated with quality in durum wheats that showed similarity to subunits present in bread wheats. However, the influence of the relative amount of LMW subunits on the rheological properties of bread wheat doughs has not been...
analysed. The gliadins would appear to be less important in determining bread quality, yet the addition of gliadins or the over-expression of certain gliadins reduces dough strength (Fido et al., 1997; Metakovsky et al., 1990). Their relationship with dough extensibility has also been speculated (Branlard & Dardevet, 1985).

The influence of the different gluten protein fractions on the rheological properties of the dough, and consequently on bread quality, is influenced greatly not only by cultivar genotype but also by the environmental conditions faced by the plants during grain ripening. The aim of the present work was to characterise the allelic composition of a number of Spanish wheat cultivars cultivated under the same environmental conditions, and to quantify the proteins of their corresponding glutens. The types of protein found plus their absolute and relative quantities were correlated with the main variables thought to determine the rheological properties of dough.

Materials and methods

Flours

Flour was obtained from the grain of 12 selected wheat lines (all in an advanced state of improvement) and from 16 varieties (the most commonly cultivated in Spain). The plant material was grown following a randomised complete block design with three replications at the La Canaleja, experimental field station (National Institute of Agricultural Research [I.N.I.A.], Alcalá de Henares, Madrid, Spain). The milling was carried out on 500 g of grain of wheat cleaned and conditioned for humidification or for desiccation. The grain was cleaned, scoured and tempered overnight to optimum moisture of 16.5%. A laboratory mill (Chopin CD1) for the milling was used. Two parts of milling were obtained, the flour and the “rest”. If it was necessary, the rest was returned to the mill to obtain a minimal quantity of flour of 255 g: 250 g for the assay and 5 g to determine the dampness of the flour.

Alveographic determinations

The rheological properties of the different doughs (strength [\(W\)], tenacity [\(P\)], extensibility [\(L\)] and the ratio \(P/L\)) were determined following the method of Faridi and Raspe (1987) (800 g of flour, 2.5% (w/v) \(\text{NaCl} \text{ [water constant]})).

Protein extraction

Samples of seeds from each line were crushed and the proteins extracted in accordance with the method of Sing and Shepherd (1991). The HMW glutenin subunits were obtained using the method of Melas et al. (1994).

SDS-PAGE analysis

SDS-PAGE was used to identify the alleles involved in glutenin production in each cultivar. An amount of 20 ml of the above extracts were loaded onto 12% acrylamide gels for analysis. All gels were stained overnight with Coomassie Brilliant Blue R-250.

DNA extraction and AS-PCR

Genomic DNA was isolated using the method of Benito et al. (1993). AS-PCR molecular markers were used to identify any alleles that presented difficulties in SDS-PAGE analysis. The PCR conditions and primers used for the HMW subunits were those described by De Bustos et al. (2000, 2001) and De Bustos and Jouve (2003); those used for the LMW subunits were previously described by D’Ovidio (1993) and D’Ovidio and Porceddu (1996).

RP-HPLC

RP-HPLC was used to quantify the different protein fractions in each type of flour, and to establish the alleles of the main genes involved in glutenin synthesis. All analyses were repeated three times. The proteins were extracted according to the method of Singh and Shepherd (1991) with the variation of using 70% ethanol instead of propanol-1. For the analysis of the gliadins, after centrifugation of the first step, the supernatant was injected directly into the HPLC after passing through a 0.45 \(\mu\)m PVDF filter. The glutenins were separated using acetone as a solvent according to the method of Melas et al. (1994). Aliquots of the different extracts were analysed using two different systems: a Hewlett Packard 1100 apparatus with a Vydac C18 column, and a Beckman System Gold machine with a Zorbax 300JB-C18 column. The proteins were separated on a linear acetonitrile gradient containing 0.05% trifluoroacetic and Milli-Q water with 0.07% TFA at a flow rate of 0.8 ml/min. The time lapse between injection and recording of the result was 35 min. The column temperature was 60 °C. The eluent was monitored at a detection wavelength of 210 nm.