Journal of Cereal Science 73 (2017) 122–129

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Polymerisation of gluten proteins in developing wheat grain as affected by desiccation

S. Koga [a, b, *], U. Böcker [a], H. Wieser [c], P. Koehler [c], A.K. Uhlen [a, b], A. Moldestad [a, 1]

[a] Nofima, Osloveien 1, NO-1430 Ås, Norway
[b] Department of Plant Sciences, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432 Ås, Norway
[c] Deutsche Forschungsanstalt für Lebensmittelchemie, Leibniz Institut, Lise-Meitner-Straße 34, 85354 Freising, Germany

ARTICLE INFO

Article history:
Received 18 December 2015
Received in revised form
12 December 2016
Accepted 15 December 2016
Available online 21 December 2016

Keywords:
Gluten proteins
Polymerisation
Breadmaking quality
Artificial drying

ABSTRACT

The breadmaking quality of wheat is affected by the composition of gluten proteins and the polymerisation of subunits that are synthesised and accumulated in developing wheat grain. The biological mechanisms and time course of these events during grain development are documented, but not widely confirmed. Therefore, the aim of this study was to monitor the accumulation of gluten protein subunits and the size distribution of protein aggregates during grain development. The effect of desiccation on the polymerisation of gluten proteins and the functional properties of gluten were also studied. The results showed that the size of glutenin polymers remained consistently low until yellow ripeness (YR), while it increased during grain desiccation after YR. Hence, this polymerisation process was presumed to be initiated by desiccation. A similar polymerisation event was also observed when premature grains were dried artificially. The composition of gluten proteins, the ratios of glutenin to gliadin and high molecular weight-glutenin subunits to low molecular weight-glutenin subunits, in premature grain after artificial desiccation showed close association with the size of glutenin polymers in artificially dried grain. Functional properties of gluten in these samples were also associated with polymer size after artificial desiccation.

1. Introduction

Wheat gluten has the unique ability to form a viscoelastic network that gives wheat dough the capability to retain gas produced by yeast and to provide leavened bread with porous crumb structure after baking. The physical properties of the gluten network are primarily determined by the composition of gluten proteins that are synthesised in the endosperm cells during grain development. Gliadins (mainly monomeric proteins) and glutenins (polymeric proteins) are two major types of gluten proteins that account for about 60–70% and 30–40% of total gluten proteins, respectively (Wieser et al., 2004; Wieser and Seilmeier, 1998). Gliadins contribute to the viscosity, while glutenin polymers contribute to the elasticity of wheat dough. Tosi et al. (2009) observed gluten protein bodies in developing grain as early as 8 days after anthesis (DAA). Shewry et al. (2009) reported that low molecular weight-glutenin subunits (LMW-GS) and gliadins were synthesised and accumulated most rapidly between 12 DAA and 35 DAA, while high molecular weight-glutenin subunits (HMW-GS) accumulated more slowly but for a longer period during grain filling. Glutenin subunits are linked by intermolecular disulphide bonds and form large glutenin polymers with molecular weights ranging from about 500,000 to more than 10 million (Shewry and Tatham, 1997; Wahlund et al., 1996). The proportion between glutenins and gliadins, as well as the allelic variations of both HMW-GS and LMW-GS in glutenins, have been shown to influence the technological properties of gluten, in particular breadmaking quality (Flaete and Uhlen, 2003; Gupta et al., 1994; Payne et al., 1979, 1983; Uthayakumaran et al., 1999). Moreover, the proportion of the largest glutenin polymers, known as sodium

Abbreviations used: ADG, artificially dried grain; DAA, days after anthesis; FDG, freeze-dried grain; GMP, glutenin macropolymer; HMW-GS, high molecular weight-glutenin subunits; LMW-GS, low molecular weight-glutenin subunits; R_{max}, maximum resistance to extension; RP-HPLC, reversed-phase high-performance liquid chromatography; SDS, sodium dodecyl sulphate; SE-FPLC, size-exclusion fast performance liquid chromatography; TFA, trifluoroacetic acid; UPP, SDS-unextractable polymeric proteins; %UPP, the proportion of SDS-unextractable polymeric protein in total polymeric proteins; YR, yellow ripeness.

* Corresponding author. Nofima, Osloveien 1, NO-1430 Ås, Norway.
E-mail address: shiori.koga@nofima.no (S. Koga).
[1] Present address: Orkla Foods Norge, Lienga 6, NO-1414 Trollåsen, Norway.

http://dx.doi.org/10.1016/j.jcs.2016.12.003
0733-5210/

dodecylsulphate- (SDS) unextractable polymeric proteins (UPP) or glutenin macropolymer (GMP), are associated with the strength and the elasticity of dough (Don et al., 2003; Gupta et al., 1993). The size of these glutenin polymers is controlled to a large extent by genotype (the composition of HMW-GS as well as LMW-GS) but also by environmental effects (Gupta and MacRitchie, 1994; Moldestad et al., 2014).

The initial assembly of glutenin subunits into glutenin polymers occurs shortly after synthesis, while the rapid increase in the molecular weight of glutenin polymers occurs during the desiccation/maturation phase at the end of grain development (Carceller and Aussenac, 1999, 2001; Daniel and Triboi, 2002; Shewry et al., 2009). Carceller and Aussenac (2001) demonstrated the close relationship between the composition of glutenin polymers, particularly the ratio of HMW-GS to LMW-GS, and the polymerisation index (SDS-insoluble polymers/total polymers), when premature grains were desiccated during the cell enlargement phase. In their study, the amount of GMP in desiccated grains increased from 15 DAA to 32 DAA during grain development, and plateaued from 32 DAA to maturity (53 DAA) (Carceller and Aussenac, 1999). Daniel and Triboi (2002) evaluated the effects of environmental factors, particularly temperature and drought, on the aggregation of gluten proteins and showed that drought caused the early onset of the rapid polymerisation of gluten proteins leading to poor solubility. From these results, Shewry et al. (2009) suggested that grain desiccation at the end of grain development drives protein polymerisation, which leads to an increase in the molecular weight of glutenin polymers. However, the number of publications reporting the time course of the accumulation of gluten proteins as well as the polymerisation of glutenin subunits during grain development and desiccation, is still limited.

The aim of the present work was to study the accumulation of gluten proteins and the polymerisation of glutenin subunits in developing wheat grain during maturation/desiccation and to demonstrate the effect on the functional properties. For this purpose, we monitored the synthesis, the accumulation and the polymerisation of proteins in both freeze-dried and artificially dried wheat grain harvested from 10 DAA to 40 DAA during grain development. The effects that occurred on the molecular level were verified by analysing the functional properties of gluten from artificially dried premature grains by a rheological method.

2. Material and methods

2.1. Wheat material

The Norwegian spring wheat cultivar, Bjarne (HMW-GS; 1Ax2*, 1Bx6+1By8, 1Dx5+1Dy10) was grown in field at Vollebekk experimental farm, Norwegian University of Life Science, Ås, Norway in the 2009 and 2010 seasons. The experiments followed a block design with two replicates, and time-point of harvest during grain development was the experimental factor. One whole plot was harvested randomly at each harvest within each block. Size of the harvested plot was 1.5 × 5 m. The first harvest was performed at 10 DAA, and carried out with five days intervals until 35 DAA, which was morphological YR in 2009, while an extra harvesting was performed after YR at 40 DAA in 2010. Additionally, one plot was harvested with a combiner at maturity. A supplemental field experiment was carried out with the same experimental design in the 2011 season. The harvest regime was the same as in the 2010 season, while harvesting continued after 40 DAA with two days intervals until 50 DAA to follow the polymerisation of glutenin polymers during whole grain desiccation period. Nitrogen was applied at optimal level (120 kgN/ha) as NPK (Yara 22-2-10) at sowing. The date of anthesis was recorded on plot basis, when anther extrusion was seen in 50% of the spikes. In addition, 20–25 ears were randomly selected and tagged with the exact date of anthesis for each plot. At each harvest, 10 of the tagged spikes were taken and grains were collected from well-developed spikelets in the middle of the spikes, which resulted in about 13 grains per spike. Harvested grains were immediately frozen in liquid N_2 and freeze-dried (freeze-dried grain - FDG). The rest of the plot was hand-harvested with a sickle and the spikes were dried in a drying cabinet at 25 °C until the moisture content was below 15% and threshed (artificially dried grain - ADG). This method was chosen to obtain the amounts of grain necessary for milling and a rheological measurement.

2.2. Milling

ADG and mature grains were cleaned and milled into wholemeal flour on a Falling number 3100 hammer mill with 0.8 mm sieve. Due to the small sample size, the FDG samples were milled into wholemeal flour on a Retsch mill with a 0.5 mm sieve.

2.3. Protein content

Protein content was determined in the artificially dried grains with the combustion method according to Dumas using a Leco CHN 1000 Elemental Analyzser (Leco corporation, MI, USA). The factor of 5.7 was applied for protein calculation from the nitrogen content.

2.4. Large deformation rheology

The rheological analysis was performed on artificially dried grain harvested in 2009 and 2010 with the SMS/Kieffer Dough and Gluten Extensibility Rig (Kieffer et al., 1998). Gluten was prepared in a Glutomatic 2200 (Perten AB, Huddinge, Sweden) from wholemeal flour. A 2% (w/v) NaCl solution was used for mixing the dough and washing out starch, bran particles and the salt soluble components. The dough was mixed for 1 min before 10 min of washing. Two types of filter were used for gluten preparation; the filter was changed from 88 μm to 840 μm after 2 min of washing. The gluten was centrifuged in a special centrifuge mould in a swing-out rotor (Rotor 5.51) at 3000×*g* for 10 min at 20 °C, pressed in a standard mould, and rested for 45 min at 30 °C for further analysis. Three pieces of gluten from each gluten preparation were stretched with the Kieffer rig until they disrupted. The maximum resistance to extension (R_{max}) was recorded by a TA.XT *plus* Texture Analyser (Stable Micro Systems, Godalming, UK).

2.5. Quantitation of SDS-unextractable polymeric protein

Both FDG and ADG samples were analysed by extraction/size exclusion fast performance liquid chromatography (SE-FPLC) (data from ADG harvested at 35 DAA is missing in 2010). Flour samples of 240 mg were extracted sequentially to obtain two extracts (SDS-extractable and SDS-unextractable) according to the method of Morel et al. (2000) with modifications described by Tronsmo et al. (2002). The sonication was done by a Sonics VC130 (Sonics and Materials, CT, USA) for 3 min with 70% automatic amplitude compensation. 100 μL of each extract were separated on Superose® 12HR 10/300 (GE Healthcare, Little Chalfont, UK) connected to an ÄKTA SE-FPLC (GE Healthcare) with 0.1% SDS, 0.08 mol/L NaCl, 0.05 mol/L sodium phosphate elution buffer (pH 6.9) at a flow rate of 0.4 mL/min. The effluent was monitored by UV-absorbance at 210 nm.

The SDS-unextractable fraction gave one main peak, denoted F1*, and contained the largest polymers. The SDS-extractable protein fraction gave four main peaks, with F1 – F2 consisting of