ELSEVIER

Journal of Cereal Science 45 (2007) 285–292

Journal of CEREAL SCIENCE

www.elsevier.com/locate/yjcrs

Effect of hydrostatic pressure and temperature on the chemical and functional properties of wheat gluten: Studies on gluten, gliadin and glutenin

R. Kieffer, F. Schurer, P. Köhler, H. Wieser*

German Research Centre for Food Chemistry and Hans-Dieter-Belitz-Institute for Cereal Grain Research, Lichtenbergstraße 4, D-85748 Garching, Germany

Received 24 February 2006; received in revised form 14 August 2006; accepted 12 September 2006

Abstract

The effect of hydrostatic pressure (0.1–800 MPa) in combination with various temperatures (30–80 °C) on the chemical and physical properties of wheat gluten, gliadin and glutenin was studied. Chemical changes of proteins were determined by extraction, reversed-phase high-performance liquid chromatography (HPLC), sodium dodecylsulphate (SDS) polyacrylamide gel electrophoresis (PAGE), circular dichroism (CD) spectroscopy, thiol measurement and studies on disulphide bonds. Rheological changes were measured by extension tests and dynamic stress rheometry. Treatment of gluten with low pressure (200 MPa) and temperature (30 °C) increased the proportion of the ethanol-soluble fraction (ESF) and decreased gluten strength. The enhancement of both pressure and temperature provoked a strong reduction of the ESF and the thiol content of gluten. Within gliadin types, cysteine containing α- and γ-gliadins, but not cysteine-free ω-gliadins were sensitive to pressure and were transferred to the ethanol-insoluble fraction. Disulphide peptides isolated from treated gluten confirmed that cleavage and rearrangement of disulphide bonds were involved in pressure-induced reactions. Increased pressure and temperature induced a significant strengthening of gluten, and under extreme conditions (e.g. 800 MPa, 60 °C), gluten cohesivity was lost. Isolated gliadin and glutenin reacted differently: solubility, HPLC and SDS-PAGE patterns of gliadin having a very low thiol content were not influenced by pressure and heat treatment; only conformational changes were detected by CD spectroscopy. In contrast, the properties of isolated glutenin having a relatively high thiol content were strongly affected by high pressure and temperature, similar to the effects on total gluten.

Keywords: Wheat gluten; Hydrostatic pressure; Protein solubility; Rheological properties

1. Introduction

Structural and functional properties of proteins can be irreversibly changed by treatment at high hydrostatic pressures (HP) (for reviews see Pfister et al., 2001; Winter, 2003). The sensitivity of protein structure to high HP is mostly due to weakening of electrostatic and hydrophobic bonds. Under such conditions, protein aggregates, e.g. casein micelles, dissociate into their subunits even at relatively low pressure ($\approx$150 MPa). Secondary and tertiary protein structures are usually influenced by HP > 700 and > 200 MPa, respectively (Pfister et al., 2001); α-helices are less stable than β-sheet structures. Depending on the nature of protein and the intensity of HP applied, denaturation may be reversible or irreversible. New tertiary structures and aggregates may develop due to the formation of new non-covalent bonds. Up to 1000 MPa covalent bonds remain unaffected by high HP except disulphide bonds that can be cleaved and rearranged by thiol/disulphide exchange reactions. Important factors

Abbreviations: AST, Astron; CON, Contra; DTNB, 5, 5′-dithiobis-(2-nitrobenzoic acid); EIF, ethanol-insoluble fraction; ESF, ethanol-soluble fraction; EX; extensibility; HP, hydrostatic pressure; HPLC, high-performance liquid chromatography; PAGE, polyacrylamide gel electrophoresis; RE, maximum resistance to extension; RP, reversed phase; SDS, sodium dodecylsulphate.

*Corresponding author. Deutsche Forschungsanstalt für Lebensmittelchemie, D-85748 Garching, Germany. Tel.: +89 289 14170; fax: +89 289 14183.

E-mail address: h.wieser@lrz.tum.de (H. Wieser).

0733-5210/$ - see front matter
doi:10.1016/j.jcs.2006.09.008

influencing protein changes at high HP are temperature, time of treatment, solvent, additives, pH value and protein concentration. Due to changes in protein properties, e.g. water binding capacity, viscosity, elasticity, gel formation, solubility and enzymatic activity, interest in HP treatment has become widespread in the field of protein-rich foods (Pfister et al., 2001). After its first use by Hite (1899) for the treatment of milk, pasteurisation of foods and beverages by high HP has been the major application. Improvement of protein texture and gel formation, e.g. in egg, fish, meat and milk products are additional applications.

Though gluten proteins are largely responsible for the unique rheological properties of wheat dough and, consequently, for the baking quality of wheat, only few studies have been reported on the effect of HP on gluten protein characteristics. γ46 Gliadin was used to study the effects of HP and temperature on protein conformation (Lullien-Pellerin et al., 2001). HP above 400 MPa induced a change in conformation that resulted in a decrease of the polarity of the environment of aromatic amino acids combined with an increase in the hydrophobicity of the gliadin surface. The changes observed were found to be reversible. (Apichartsrangkoon et al., 1998, 1999) treated wheat gluten samples at 200–800 MPa and 20–60 °C for 25 or 50 min and analysed the products for structural modification by texture profile analysis, stress rheometry and solubility in sodium dodecylsulphate (SDS) solutions. Significant effects on rheological properties were observed at high HP and temperature. High HP could alter gluten solubility even at low temperatures, but disulphide crosslinking only became significant, when samples were held at 800 MPa for 50 min. The degree of crosslinking strongly increased at the highest temperature (60 °C) in the HP range of 400–800 MPa. The aim of the present work was to obtain a more detailed insight into the changes of structural and rheological properties of gluten treated with HP in combination with varying temperatures. In addition to gluten, the effect of HP on the separated protein fractions, gliadin and glutenin was studied.

2. Material and methods

2.1. Preparation of gluten, gliadin and glutenin

Kernels of the wheat cultivars ‘Astron’ (AST) and ‘Contra’ (CON) were milled into white flour (ash content 0.55%) with a Quadrumat Junior mill (Brabender, Duisburg, Germany) and passed through a 0.2 mm sieve (Retsch, Haan, Germany). Flour (300 g) and a NaCl solution (0.4 mol/l, 165 ml) were mixed in a Farinograph (Brabender) for 2 min at 22 °C. After 10 min resting time, the dough was washed by hand with an NaCl solution (0.4 mol/l, 2 l) until a cohesive mass (gluten) was obtained. Portions of gluten (15 g) were then washed in a Glutomatic (Perten Instruments, Huddinge, Sweden) with a NaCl solution (0.4 mol/l, 270 ml) for 5 min and centrifuged (4000 *g*, 10 min, 22 °C). Wet gluten was either shrink wrapped and stored in ice water or freeze dried and milled. Portions of dried gluten were defatted with light petroleum (boiling temperature 40–60 °C) or were rehydrated and preformed by centrifugation. For the separation of gliadin and glutenin, non-defatted dried gluten (5 g) was successively extracted with 60% (v/v) aqueous ethanol (6×375 ml) by means of an Ultra Turrax (IKA, Staufen, Germany) homogeniser (2 min) and magnetic stirring (15 min) at $\approx$20 °C. The suspensions were then centrifuged at 2700*g* and $\approx$20 °C for 15 min. Combined supernatants (gliadin) and sediment (glutenin) were freeze-dried and milled.

2.2. Pressure treatment

Dried gluten, gliadin and glutenin were rehydrated with an excess of distilled water at $\approx$20 °C for 15 min and centrifuged (10 min, 5000*g*, $\approx$20 °C). Wet gluten, rehydrated gluten, gliadin or glutenin were transferred into Teflon tubes ($2.5 \times 5\,cm^2$) which then were filled with distilled water avoiding air bubbles. Gluten samples were subjected to HP of 0.1–800 MPa at a temperature range of 30–80 °C for a period of 5, 10, 20 or 30 min using a Sitec instrument (Maur/Zürich, Switzerland). The pressure medium was glycol and the temperature was adjusted by an internal thermostat. Pressure increase was nearly linear reaching 200 MPa in 3 min and 800 MPa in 7 min. The selected end temperature was reached within 3 min. Gliadin and glutenin samples were subjected to HP (0.1, 300, 500, 800 MPa) and heat (30, 50, 70 °C) for 10 min using an FPG 10220 instrument (Stansted Fluid Power Inc., Stansted, UK). The pressure medium was ethanol/castor oil and the autoclave was heated by circulating water. The rate of pressure increase was 150 MPa/min. Actual HP and temperature were continuously recorded by computers linked to the instrument.

2.3. Protein analysis

The N contents of freeze-dried gluten, gliadin and glutenin were determined using a Dumas combustion method (FP-328, Leco Instruments, Mönchengladbach, Germany). For the quantification of gluten proteins, a combined extraction/high-performance liquid chromatography (HPLC) procedure previously developed for flour analysis (Wieser et al., 1998) was adapted for gluten analysis. Freeze-dried gluten (20 mg) was extracted stepwise with 3×1.5 ml of 60% (v + v) aqueous ethanol by magnetic stirring at $\approx$20 °C for 15 min. After centrifugation (1600*g*, $\approx$20 °C, 15 min), the supernatants were combined and diluted to 5 ml with 60% (v + v) ethanol (= ethanol-soluble fraction (ESF)). The sediment was extracted under reducing conditions with 3×1.5 ml of 50% (v/v) 1-propanol containing Tris-HCl (0.05 mol/l, pH 7.5), urea (2 mol/l) and dithioerythritol (1%, w/v) under nitrogen at 60 °C for 20 min. After centrifugation (see above) the supernatants were combined and diluted to 5 ml