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Optimization of a micro-scale extension test for rehydrated vital wheat gluten

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ABSTRACT

Micro-scale extension tests are useful to assess the viscoelastic properties of rehydrated vital wheat gluten, but currently there is no standardized procedure. Therefore, a default experimental setup was optimized by systematically varying one parameter (hydration time, type of centrifuge, centrifugation speed, centrifugation time, rest period, and test speed) at a time. After data analysis and statistical evaluation taking reproducibility, discrimination of different samples, and duration of analysis into account, the optimized parameters were a hydration time of 5 min, centrifugation (3060× *g*, 10 min, 22 °C, swinging-bucket rotor) in a custom-made Teflon mold, a rest period of 15 min and measurement at a test speed of 3.3 mm/s with the SMS/Kieffer Dough and Gluten Extensibility Rig fitted to a texture analyzer. This optimized method may be used for an international collaborative study to have its performance validated and allow its approval by international standardization organizations.

1. Introduction

Gluten, derived from the Latin word for “glue”, may be defined as the “rubber-like proteinaceous mass that remains, when wheat dough is washed with water or salt solution to remove soluble constituents and starch granules”. This procedure of wheat gluten preparation was first described in 1728 by an Italian chemist named Beccari (Bailey, 1941) and is essentially still the basis for the modern, commercial process of gluten-starch separation. Composed of hundreds of single proteins, gluten is formed by the storage proteins (alcohol-soluble gliadins and alcohol-insoluble glutenins) located in the starchy endosperm of wheat grains (Wieser et al., 2014). The unique features of wheat gluten are its rheological properties, such as cohesivity, elasticity, viscosity, and extensibility, which allow the formation of a hydrated gluten network upon water addition to wheat flour. This network is capable of retaining gas produced during dough leavening and of stabilizing the characteristic, foam-like structure of wheat bread crumb (Wieser, 2007). Due to these unique techno-functional properties, wheat flour is used for a great diversity of food products from breads to noodles, cookies, cakes, and many other foods (Shewry, 2009). The global importance of wheat and wheat gluten is also reflected by the worldwide production of 729 million metric tons of wheat grains in 2014, which equals approximately 70 million tons of gluten. This makes wheat the fourth most important commodity in terms of production yield after sugar cane, corn, and rice (FAO). Beside its direct use for food and feed, wheat flour is utilized as a source of gluten and starch, separated by the Martin process or the Batter process or some variation of either process. After drying, typically in a flash or ring drier at low temperatures to retain the functional properties (Van der Borght et al., 2005), vital wheat gluten is obtained as a free-flowing powder with at least 80% crude protein (N × 6.25, dry weight basis (dw)), ≤ 10% moisture, ≤ 2% ash (dw), ≤ 1.5% crude fiber (dw), and varying amounts of starch and lipids (Codex Stan 163-1987). Upon rehydration of the powder, vital wheat gluten partly regains the unique viscoelastic properties of freshly prepared wet gluten. The most common application for vital wheat gluten is its addition to baked goods to fortify flours with low protein contents to improve dough strength, mixing tolerance, gas retention, volume, and textural properties. Due to its ability to absorb fat and water, vital wheat gluten may be used in meat and fish products as a binder and structuring agent. It also serves as a meat replacement in vegetarian foods, as a basis for synthetic cheese, seafood analogues, and the production of monosodium glutamate, or as nutritious, texturizing ingredient in extruded snacks (Day et al., 2006). Non-food uses of vital wheat gluten

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include cosmetics, pet food, and the production of natural adhesives or films. Vital wheat gluten is approved by the U.S. Food & Drug Administration as Generally Recognized as Safe (GRAS) under 21 C.F.R. §184.1322 for technological uses at levels not exceeding good manufacturing practice.

Uniaxial micro-scale extension measurements using the Stable Micro Systems (SMS)/Kieffer Dough and Gluten Extensibility Rig provide useful information on the rheological properties, such as resistance to extension and extensibility of rehydrated vital wheat gluten (hvw gluten) (Hartmann and Koehler, 2008; Kieffer et al., 1981a, 2007; Roccia et al., 2009; Sliwinski et al., 2004). In these tests force-distance curves were obtained, and the area under the curve was related to the protein quality of gluten (Tronsmo et al., 2003). The maximum resistance to extension of gluten was also highly correlated with the concentration of glutenins, especially high-molecular-weight glutenin subunits (Wieser and Kieffer, 2001). The experimental setup consists of the following steps: rehydration of vital wheat gluten, centrifugation in custom-made molds, pressing and resting in a fluted panel, and the extension measurement itself at a defined test speed. The devices for preparing hvw gluten for extension measurements are so far not commercially available and individual solutions are reported, especially for the centrifugation step (Koehler and Grosch, 1999; Tronsmo et al., 2003). In addition, there is no standardized experimental setup available with defined parameters for each step, which would allow the comparison of data between different laboratories. Taking the rest period in the fluted panel as an example, various rest periods at different temperatures have been reported ranging from 30 min at 22 °C (Hartmann and Koehler, 2008; Kieffer et al., 2007) to 40 min at 30 °C (Grausgruber et al., 2002; Roccia et al., 2009; Tronsmo et al., 2003) and 60 min at 30 °C (Wang et al., 2003a, 2003b). Additionally, the influence of varying parameters for each step of sample preparation on micro-scale extension test curves of hvw gluten has not been systematically studied so far. Each step has a substantial influence on the rheological properties, so that different procedures will result in variable results, which may or may not correlate to other parameters. This influence is also different depending on the specific inherent properties of the gluten sample, so that a standardized procedure is needed to get consistent results that may be reproducibly correlated to the results of other types of measurement. Unless the impact of the single steps on the results is understood in-depth, the results will always be variable just because of the methodological setup per se. Consistent and reproducible micro-scale extensibility results can only be obtained with an improved standardized procedure that is built upon the best parameters for each step of sample preparation.

Therefore, the aim of this investigation was to optimize and standardize a method for micro-scale extension testing of hvw gluten. Each step of the experimental setup was systematically varied and the impact on reproducibility, discrimination of different samples, and duration of analysis was evaluated statistically. In total, 15 variations of the experimental setup were studied to identify the method that offered the most reliable performance characteristics along with the shortest possible duration of analysis. This standardized method is intended to be used for validation in international collaborative studies to get subsequent approval as a standard method with the International Association for Cereal Science and Technology (ICC) and the American Association of Cereal Chemists International (AACCI).

2. Materials and methods

2.1. Vital wheat gluten samples and additives

Four samples of vital wheat gluten, Amygluten 150 (G1), Amygluten 120 (G2), Amygluten 160 (G4), and Amygluten 110 (G5) were provided by Tereos Syral (Aalst, Belgium). Two samples of wet wheat gluten (G3, G6) from Hermann Kröner (Ibbenbüren, Germany) were lyophilized and carefully ground after shock-freezing with liquid nitrogen in an ultracentrifugal mill ZM 200 (Retsch, Haan, Germany) using a 0.2 mm sieve. Samples G1, G2, and G3 were used for the entire study, samples G4, G5, and G6 were used as additional samples to assess the influence of the test speed on the results (see 2.3.5). Sodium chloride was from Merck (Darmstadt, Germany).

2.2. Sample preparation and micro-scale extension tests of hydrated vital wheat gluten

All samples were prepared under controlled environmental conditions (temperature 22 ± 2 °C, relative humidity ≥ 60%).

2.2.1. Hydration

1.5 g vital wheat gluten was weighed into a 50 mL beaker (diameter: 4.5 cm). The beaker was carefully knocked against the work surface at an angle of 30–45° to accumulate the material on one side of the bottom of the beaker. Salt solution (5 mL; 2% NaCl, w/w) was added to the side of the beaker without the gluten to avoid preliminary mixing of gluten and salt solution. Then the suspension was manually mixed with a metal spatula for 7 s as rapidly as possible (approximately 60 rpm), so that no residual gluten powder was visible anymore. The gluten/water mixture was left for 5 min to allow hydration of the gluten.

2.2.2. Sedimentation (centrifugation)

The hvw gluten was transferred into a specially notched Teflon mold and a second, smooth Teflon mold was placed on top. The assembled molds were placed in the cylindrical centrifuge insert (Fig. 1 A–C) and centrifuged (3060× *g*, 10 min, 22 °C) in a Heraeus Labofuge 400 R (Thermo Fisher Scientific, Osterode, Germany).

2.2.3. Micro-scale extension test of hydrated vital wheat gluten

The pre-shaped gluten strands were precisely laid onto a fluted Teflon panel (Kieffer et al., 1981b) with trapezoid grooves (height: 4.5 mm, length: 45 mm, lower parallel side: 3.5 mm, upper parallel

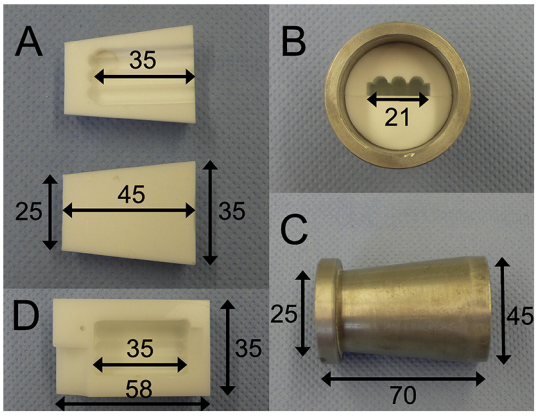

Fig. 1. Teflon molds, notched and smooth (A), and cylindrical centrifuge insert (B, C) used for centrifugation of hydrated vital wheat gluten in the Heraeus Labofuge. Teflon mold (D) for the Gluten-Index centrifuge. All dimensions in [mm].