The Effect of Bran Reduction on Protein Secondary Structure in Intermediate Wheatgrass (*Thinopyrum intermedium*) Dough

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Thinopyrum intermedium, commonly known as intermediate wheatgrass (IWG) is a perennial crop that provides sustainable environmental benefits and shows a great potential to be developed as a grain crop [1]. Compared to wheat, IWG is generally higher in protein, fiber and antioxidants, but lower in total starch [2]. While higher in protein content, we found that the protein distribution/profile of IWG is considerably different than that of common wheat. The protein component in IWG consists mainly of gliadins and low molecular weight glutenins (LMWG), and is deficient in high molecular weight glutenins (HMWG), which are important for dough strength in bread applications.

Fiber content in wheat flour negatively impacts gluten network formation and hence bread quality [3]. Fiber causes water redistribution in a dough system, thus altering the protein confirmation from β spiral (consecutive β turns) structure to β sheet structure [4]. This main structural change in the gluten network due to fiber presence may result in physical disruption of gas cells producing poor quality bread. The fiber content is higher in whole IWG flour than in whole wheat flour due to higher ratio of bran to endosperm [5]. It is, therefore, hypothesized that the fiber interference would have a major impact on protein network formation in IWG dough system. With the fiber interference in mind, it is essential to optimize the mixing conditions (temperature and time) along with the flour refinement for desired protein network formation, as recently shown [6] for both strong and weak common wheat flours.

Therefore, the objective of this study was to determine the effect of bran reduction along with various mixing conditions on protein network formation by monitoring changes in the protein's secondary structure and state of water in IWG dough using attenuated total reflectance (ATR) fourier transform infrared (FTIR) spectroscopy.

IWG grains sample was milled a Quadrumat Junior flour mill (Brabender Inc.) and bran was separated. Bran was added back to refined IWG flour at 100%, 75%, 50%, 25% and 0% of original bran content. Different flour samples were evaluated for dough strength using farinograph following the constant flour weight procedure according to AACC method 54-21.02. Dough samples were collected at different time points during mixing: dough development time (DDT), stability departure, and over-mixing at two temperatures (21°C and 30°C). The infrared spectra of the dough samples were recorded using an ATR-FTIR spectrophotometer. Spectra of reference H_2O/D_2O mixtures, matched to the moisture content of the dough samples, were collected and used for subtraction of water contributions in the amide I region (1600-1700 cm^{-1}) of the vector-normalized spectra. The quantitative estimation of protein secondary structure in the amide I region of dough was assigned as 1620-1644 cm^{-1} for β-sheets, 1644-1652 cm^{-1} for random coil, 1652-1660 cm^{-1} for α-helix, and 1660-1685 cm^{-1} for β-turn structures.

Regardless of the mixing temperature, as the level of refinement increased, water absorption decreased significantly ($P \leq 0.05$) for both hard red winter wheat (HRWW) and IWG reconstituted flour systems. As the refinement level decreased, the dough development time significantly ($P \leq 0.05$) increased, an

observation attributed to the protein fractions being diluted from addition of bran, causing interference in the development of the gluten network. The dough stability increased as the refinement level decreased for IWG because of the ability of the fiber to assume a rigid conformation, improving the strength of the dough.

With regards to the state of water, significant changes in the frequency of OH stretch band of water were seen upon decreasing the refinement level implying alterations in the relative hydrogen bonding properties and possible restructuring of water. In IWG dough prepared at DDT at 30°C, as the level of flour refinement decreased, peak at 3600 cm^{-1} shifted to significantly ($P \leq 0.05$) higher frequencies, suggesting the presence of more free water. This observation revealed that the water absorbed by bran may not be completely bound to dough components (mainly starch and gluten) but structured around them as free water. However, bran addition had no significant effect on the water hydrogen-bonded to the polymer matrix as demonstrated by the lack of shift in the peak at 3160 cm^{-1}, indicating that the overall energy state of this population of water was not affected by the bran content of the dough.

In terms of protein secondary structure, IWG dough systems made at the DDT followed the order of β-turn > β-sheet > random coil > α-helix regardless of the refinement level and mixing temperature. The relative amount of secondary structures in control wheat flour dough systems followed the order β-sheet > β-turn > random coil > α-helix, confirming that gluten primarily forms β-sheet structures in developed dough [7]. Only β-sheet content increased significantly as the amount of bran increased ($P \leq 0.05$) while β-turns, random structures and α-helix content remained unchanged in IWG dough made at DDT at 30°C. As the mixing time increased for IWG dough made at 30°C, a significant ($P \leq 0.05$) increase in β-sheets at the cost of β-turns was seen for 0%bran_IWG, 25%bran_IWG, and 50%bran_IWG. On the other hand, β-turns content in 75%bran_IWG and 100%bran_IWG doughs did not change significantly. The content of α-helix and random structures did not change for all the refinement levels in IWG as the mixing time increased.

In conclusion, the refinement level, mixing temperature, and mixing time greatly affected the structure of gluten forming proteins. β-turn represented the predominant secondary structure in IWG dough systems compared to β-sheets in control wheat dough. However, water redistribution in the dough upon bran addition altered the conformation of the gluten network resulting in more β-sheets. Moreover, Mixing at 21°C improved the mixing properties and dough rheology of IWG flours. 75% bran enrichment showed the best β-sheets/β-turns ratio at 21°C suggesting a good compromise between dough extensibility and elasticity. Determining differences in protein secondary structure as affected by bran content during dough formation provides insights to gluten network formation and stability during dough mixing and baking. This information may lead to expanding the market potential of IWG.

References:

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