Research Questions

Predicting flour performance and consistency remains a challenge for processors, mainly due to the issue that the nature of interactions between the protein sub-units is different in different types of wheat. Wheat protein quality is being recognized as more important than protein quantity. In recent work in our laboratory, we showed that flour performance is in part related to the kinetics of gluten aggregation as measured by using a Glutopeak tester - a new high shear based procedure developed by Brabender. Furthermore, it has been shown that the nature of gluten aggregation is dependent on the type of protein-protein interaction – either hydrophobic interaction or disulphide interaction (Huschka et al., 2012). Front-face fluorescence is a well established technique to observe protein conformational changes in protein-based matrices during processing and to assess structural features of proteins in wheat flour dough. The acquisition of Front face fluorescence spectrophotometer is critical to analyzing the nature of interactions in wheat varieties grown in different years and locations.

Results

Fluorescence spectroscopy is a very powerful tool for studying protein structural changes in complex matrices, such as food products. The fluorescence spectrum is determined by the chemical environment of a fluorescent component (in proteins, it is usually the fluorescent amino acid tryptophan), and therefore, changes in the emission spectra of tryptophan often occur in response to conformational transitions, subunit association, substrate binding, or denaturation of the proteins present in the sample. There is a shift of the maximum of emission to higher wavelength when tryptophan moves from a hydrophobic surrounding to a hydrophilic one (e.g. from the protein interior to the aqueous media). Also fluorescence intensity can change: some molecules can work as quenchers and adsorb the emitted light (Bonomi et al., 2004).

Traditionally, fluorescence studies have been performed on clear solutions. Cereal matrices are characterized by a dominant presence of proteins - gliadins and glutelins - that cannot be brought into solution without significant alteration of their structural features. Solid-state Fluorescence (also known as front-face fluorescence) represents a useful tool for extending studies on reporter molecules to insoluble proteins in semisolid systems, such as those often found in foods.

Information about protein structural organization may be gathered also by using fluorescent dyes. One of the most popular probes is 1-anilinonaphthalene-8-sulfonate (ANS), that becomes fluorescent when interacting with a hydrophobic region, making it possible to study protein structural changes in complex systems after a specific treatment (Bonomi et al., 2004). Titration with ANS may provide information on both the number of surface hydrophobic sites and their average affinity towards the probe. Differences observed at high ANS concentration are more likely to result from changes in the number of probe binding sites, whereas changes observed at low concentrations of ANS would be an indication of changes in the affinity of hydrophobic moieties to the fluorescent probe (Bonomi et al., 2004). Previous studies demonstrated that probe concentrations around 0.2 mM at 40% moisture would be best suited for investigating the behaviour of wheat flours (Huschka et al., 2012). For this reason, in this study, a probe concentration of 0.2 mM was used.

The wavelength of maximum fluorescence emission provides information about the hydrophobic properties of the dough, as it relates to alterations in the chemical environment that surrounds hydrophobic “reporters” (amino acids residues such as tryptophan or non covalently bound probes, such as ANS in this study). In this study, the maximum intensity of ANS emission remained at 470 nm for all the samples, indicating a stable environment for the bound probe (see Figure 1).

Differences in intensity values among the samples suggest different exposure of hydrophobic sites on the protein surface (see Figure 2). In particular, low intensity suggests high hydrophobic interactions between proteins. In other words, hydrophobic regions involved in protein-protein interactions were no longer available for binding of the probe and therefore decreased fluorescence intensity was observed. The effect of the location on hydrophobic interactions in wheat doughs seems to be predominant to the effect of the year. Indeed, dough samples can be clearly distinguished into two groups: the varieties cultivated in Roseau and those cultivated in Oklee exhibited high intensity, while samples cultivated in Stephen in 2012 and 2013 were characterized by low maximum intensity values, indicating a more compact network structure as a result of either low exposure (intramolecular hydrophobic interactions) or increased intermolecular hydrophobic interactions.
The emission fluorescence spectra in Figure 1 provide evidence for the appearance of a red-shifted shoulder in the emission fluorescence spectrum (especially for dough samples prepared from Knudson, Marshall, and Samson). Indeed, the extent of probe exposure may be inferred by the ratio of fluorescence intensity at emission wavelengths typical of each species (Caldinelli et al., 2004). The shift toward 505 nm with ANS represents a re-orientation of the probe in the hydrophobic patches of the protein from a mostly buried location to a partially exposed one, which leads to a red-shift in fluorescence. Figure 3 shows the ratio between ANS fluorescence intensities at 505 and 470 nm. This ratio provides an estimate of the “red shift” in the fluorescence emission of protein-bound ANS. In general, Knudson and Samson exhibited an important shift towards 505 nm of the fluorescence emission maximum compared to Marshall and Prosper. Depending on the variety, location and year increased or decreased the exposure of ANS but a clear trend was not observed (see Figure 3).

Finally, comparing the results obtained from ANS fluorescence and those from the Glutopeak test - a new high shear based test that measures the aggregation properties of gluten in wheat flour - a significant negative correlation between the maximum torque (that corresponds to the peak occurring as gluten aggregates) and the fluorescence intensity ($r= -0.71$; $p<0.01$) suggesting that flours with greater number of hydrophobic patches on the protein surface (great fluorescence intensity) create a weak gluten network during aggregation.

**Application/Use**

The approach here proposed is a rapid technique that requires small amount of sample (10 g for dough preparation; ~1g for analysis). For this reason, it can be used as a routine part of the research working with plant breeders to assess different wheat varieties. In addition, understanding the hydrophobic interactions in wheat dough complements the research capabilities and leverage synergies to both assess quality and understand the reasons for wheat variability. Moreover, the quantitation of protein hydrophobicity can be an essential step for prediction of protein functionality.

**Material and Methods**

Thirty-two varieties of spring wheat were grown in different locations (Roseau, Oklee, and Stephen) in 2012-2013, for a total of 128 samples were characterized using a new approach that measures the aggregation properties of wheat gluten. On the basis of the results, 4 varieties (Knudson, Marshall, Prosper, Samson) were chosen for investigating the hydrophobic interactions during dough-making.

Rearrangement of hydrophobic patches on the protein surface of selected samples were measured by spectrofluorometric technique, monitoring the chemical environment of extrinsic fluorophore 1,8-anisilinonaphthalene sulfonate (ANS), as described by Bonomi et al. (2004). A Farinograph-AT (C.W. Brabender Inc., South Hackensack, NJ, USA) equipped with a 10 g mixing bowl was used for preparing the dough (at 30°C and 63 rpm). All the dough samples were prepared at a constant water absorption level (60%). ANS (0.2 mM) was added to the water used for making the dough. Dough samples for analyses were collected at the dough development time, corresponding to the time from first addition of water to the point of maximum consistency range. Fresh samples were pulled from the farinograph with minimal additional physical manipulation, transferred to a fluorescence cell (quartz-windowed standard surface) and analyzed within 3 min. A fluorometer (LS 55, Perkin Elmer, Waltham, MA, USA) was used to measure emission spectra from 400-600 nm with excitation at 390 nm.

**References**


**Related Research**

Previously Dr. Seetharaman has published a study on the effect of differences in flour type relating to their protein quantity and quality on their tryptophan solvation behavior as well as their affinity for hydrophobic probes. The project is a collaboration with Dr. Jim Anderson, Wheat Breeder, University of Minnesota and used wheat varieties developed by other scientists in the region.

**Recommended Future Research**

- Relating gluten aggregation properties and hydrophobic interactions testing a larger number of varieties, years and locations
- Applying proteomics and thiolomics approach to understand variability in flour performance from wheats grown in different environmental conditions.
Figure 1. Fluorescence emission spectra of dough samples collected at the dough development time
Figure 2. Fluorescence intensity of dough samples corrected for the protein content. Lower values indicate better quality.

Figure 3. Ratio between ANS fluorescence emission intensities at 505 nm and 470 nm. Higher values indicate better quality.