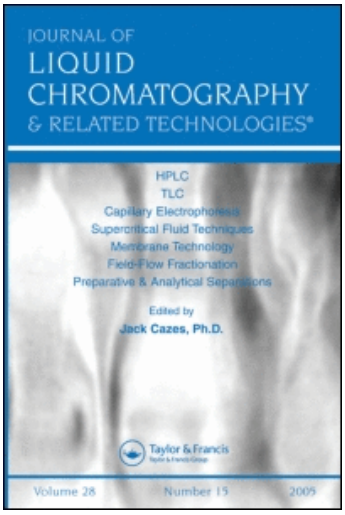

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EFFECTS OF SURFACTANTS ON WHEAT PROTEIN FRACTIONATION BY FLOW FIELD-FLOW FRACTIONATION†

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ABSTRACT

The effects of different nonionic (Brij 35, FL-70, Triton X-100, Tween 20 and Tween 80), cationic (CTAB) and anionic (SDS) surfactants in 0.05M acetic acid, at several concentrations, on flow field-flow fractograms of three wheat gluten fractions were assessed. Alcohol soluble gliadins, acetic acid soluble glutenins and sonicated acetic acid insoluble glutenin fractions all showed optimum resolution and reproducibility with FL-70, Brij 35 or Triton X-100. Fl-70 proved superior compared to the other two surfactants due to its lower tendency to foam, making buffer preparation much easier. Fractograms obtained in the presence of low concentration of other nonionic surfactants (Tween 20 and 80) and the cationic surfactant, CTAB, gave similar fractograms but resolution was inferior. At higher concentration, above the critical micelle limit, CTAB gave poor resolution and reproducibility.

Low concentrations of SDS caused protein precipitation. At a higher concentration of SDS, also above the critical micelle concentration, reproducibility was also a problem. However,

results indicated that SDS was able to release lower molecular weight proteins from the larger polymeric insoluble gluten fraction which remained bound with nonionic detergents.

INTRODUCTION

The proteins of wheat endosperm show a wide molecular weight range, ranging from less than 15,000 to values of 10 million daltons or more.[1,2] The lower molecular weight (<100,000 daltons) proteins consist of water soluble albumins, salt (0.5% NaCl) soluble globulins and alcohol (70% ethanol) soluble gliadins. The higher molecular weight insoluble glutenin proteins are polymeric in nature, consisting of mixtures of disulphide bonded high molecular weight (HMW) and low molecular weight (LMW) subunits. The size of these polymeric proteins is dependent on the subunit composition which is genetically determined. [3-6]

A strong positive relationship has been established between dough processing properties (dough strength) and baking quality, and the average molecular size of the gluten proteins (gliadin and glutenin).[7] The size distribution of the polymeric glutenin proteins, especially the larger polymers, plays a particularly important role in this relationship.[3,5, 8-10]

Previous studies in our laboratory have shown that symmetrical flow field-flow fractionation (FFF) provides good resolution of wheat protein fractions.[11] Similar results have been obtained by Wahlund and co-workers using asymmetrical flow FFF.[12] Its use for studying the larger polymeric wheat proteins may be particularly advantageous since, unlike gel filtration, electrophoresis, and size-exclusion chromatography, resolution is not impeded by an exclusion limit.[13]

To optimize resolution in flow FFF, the channel manufacturer recommends addition of surfactant to condition the membrane. Although this conditioning process is not well understood, it is likely related to binding of surfactant to the membrane which reduces interactions between the membrane and the components being fractionated. Using gravitational FFF for fractionation of latex particles, Pazourek and Chmelík[14] demonstrated that the type and concentration of surfactant could strongly influence resolution. They attributed these differences to the effects of the surfactants on particle - particle, particle - eluent and particle - cell surface interactions. Although FL-70 has been the most widely used surfactant for flow FFF, other nonionic surfactants, such as Triton X-100, and charged surfactants, such as sodium dodecyl sulfate (SDS), have also been used.[12-13,15-16] At present, no information has been

published on the relative effects of these surfactants on wheat protein fractograms. In the present study, a number of nonionic, anionic and cationic surfactants in 0.05 M acetic acid (HAc) at different concentrations have been used to investigate their influence on the resolution of flow FFF fractograms obtained with the major wheat gluten protein fractions.

MATERIALS AND METHODS

Three wheat gluten protein fractions: gliadins (soluble in 70% ethanol); HAc soluble glutenins (soluble in 0.05M HAc), and sonicated HAc insoluble glutenins (insoluble in 0.05M HAc, but "soluble" following 30 sec. sonication) were extracted from defatted Katepwa red spring as previously described.[11] Flow FFF was run on a Model #F100 fractionator (FFFractionation Inc., Salt Lake City, UT) with nominal dimensions of: length-28.5 cm (tip to tip), breadth-2.0 cm and thickness-0.025 cm fitted with a YM-10 membrane. Protein fractions (approximately 1 μg protein in 20 μL in carrier fluid under examination) were injected into the channel, relaxed for 16 sec, and eluted at 2 mL/min with a cross flow of 5.0 mL/min using degassed 0.05 M acetic acid (HAc) containing surfactant as carrier fluid. A Shimadzu detector SPD-10AV, connected to the outlet of the FFF unit, was used to monitor the absorbance of the sample fractions at 210 nm. A formula for calculating Stokes diameters (d) based on retention time was derived in the same manner as previously described for the thin channel.[11] A calibration curve was obtained by plotting d against peak retention times (t_r) as recommended by the manufacturer (FFFractionation Inc.).

Proteins used for the calibration curve (values for MW in daltons, D in Ficks and d in nm, respectively, given in brackets) were cytochrome c (12,500, 13.0, 3.3), chymotrypsinogen A (25,000, 9.5, 4.5), hen egg albumin (45,000, 7.8, 5.5), bovine serum albumin (BSA)(67,000, 5.9, 7.3), aldolase (158,000, 4.6, 9.3), catalase (240,000, 4.1, 10.5) and ferritin (450,000, 3.6, 11.9). The linear curve, $d = 0.725+1.019\ t_r$ ($r = 0.93$), was then used to calculate corresponding values for wheat protein fractions based upon retention times. Cytochrome c (1/10 dilution of 0.001g/mL stock solution) and ferritin (1/2 dilution of 0.01mL/mL stock solution) were diluted with 0.05M HAc and run as markers at the beginning of each sample set. Three runs of samples and standards were done for each type and level of surfactant.

Surfactants were diluted in 0.05M acetic acid (HAc) to levels indicated in brackets to provide carrier fluids for this study: Surfactants included Brij 35 (0.002%, 0.001%; v/v), cetyl trimethylammonium bromide (CTAB) (0.36%, 0.002%, 0.001%, w/v), FL-70 (0.004%, 0.002%, 0.001%; v/v), SDS (0.17%,

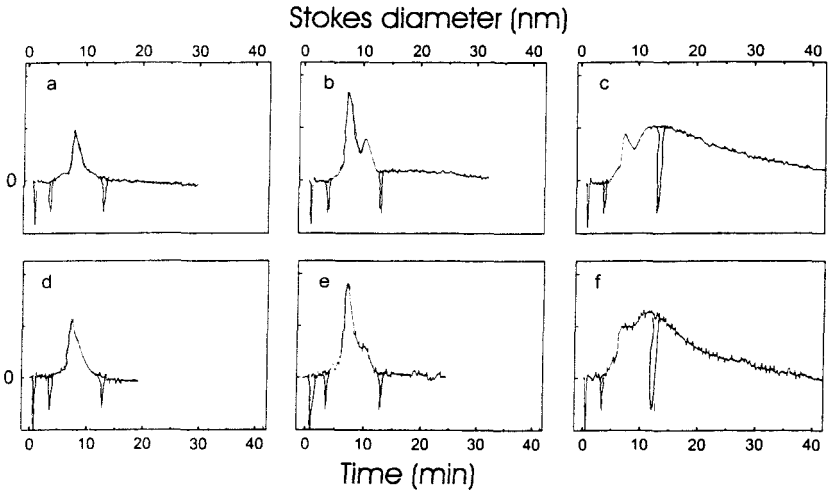

Figure 1. Representative fractograms for wheat protein fractions run in nonionic surfactants. a) gliadin, b) HAc soluble glutenin, c) HAc insoluble sonicated glutenin fractions in 0.002% FL-70, d) gliadin, e) HAc soluble glutenin, and f) HAc insoluble sonicated glutenin fractions in 0.002% Tween 80.

0.06%; w/v), Triton X-100 (0.002%,0.001%; v/v), Tween 20 (0.002%,0.001%; v/v) and Tween 80 (0.002%,0.001%; v/v). With the exception of FL-70, all the nonionic surfactants formed stable foams during buffer preparation which made mixing and vacuum filtering difficult. Three runs of samples and standards were also done using 0.05M HAc, without surfactant added, following the third run at 0.001% FL-70 in HAc.

RESULTS AND DISCUSSION

Fractograms are shown in Figure 1 for gliadin, HAc soluble glutenin, and HAc insoluble glutenin solubilized by sonication (HAc insoluble sonicated) fractions obtained with different surfactants. The location of the elution peak for cytochrome c (d=4.0) and ferritin (d =13.2) are indicated by bold lines for reference. Fractograms obtained for the three protein fractions, using 0.002% FL-70 (Fig. 1a,b and c), were very similar to those obtained for the same fractions in a previous study using the same concentration of surfactant under similar running conditions.[11] The gliadin fraction showed a single major peak with an estimated Stokes diameter (d) of 7.9nm while two peaks (d=7.2 and

10.5nm) were evident for the HAc soluble glutenin fraction. The HAc insoluble, sonicated glutenin fraction showed a peak at 6.8 min (d=7.6nm) and a broad peak with a maximum at approximately 12.2 min. (d=13.2nm).

Differences were not observed in fractograms for these protein fractions when the FL-70 concentration was reduced to 0.001% or increased to 0.004% (data not shown). Resolution was also not affected when FL-70 was replaced with the nonionic surfactants, Brij 35 and Triton X-100, at two different concentrations (0.001% or 0.002%; v/v). Fractograms for the nonionic surfactants, Tween 20 and Tween 80, were very similar to each other for both levels of surfactant (0.001% and 0.002%;v/v) tested and, in general, similar to those obtained with the other three nonionic surfactants, as shown in Figure 1d, 1e, and 1f. Peak resolution with Tween 20 and 80, however, was clearly inferior to that obtained with Brij 35, Triton X-100 or Fl-70, particularly for the two glutenin fractions.

Removal of FL-70 from treated channels by flushing and running fractions in 0.05M HAc resulted in fractograms equivalent to those obtained in the presence of FL-70 (data not shown). This procedure allows isolation of protein peaks with minimal levels of surfactant contamination. These results also suggest that surfactant interactions with the proteins were not required to optimize resolution. Sufficient FL-70 presumably remained bound to the membrane to ensure optimum conditioning.

At lower concentrations (0.001% and 0.002%; w/v), the cationic surfactant, CTAB, produced separations similar to those obtained with the nonionic surfactants Tween 20 and Tween 80 (Fig. 2a, b and c), but inferior to FL-70. Low concentrations of the anionic surfactant, SDS (0.001M), caused precipitation of the proteins. The negative charge on the SDS probably neutralizes the positively charged gluten proteins resulting in aggregation. Low concentration of salts show a similar effect.[17]

At higher concentrations, above their critical micelle concentration,[18] both CTAB and SDS have proven to be useful in promoting extractability of gluten protein, particularly the larger polymeric glutenin, due to their ability to decrease hydrophobic inter-protein interactions.[7,19] Poorly resolved, non-reproducible fractograms were obtained with CTAB, at a higher concentration of 0.36% (the concentration recommended by Meredith and Wren[19]). The buffer became viscous and maintenance of balanced channel and cross flows was very difficult. The interaction of CTAB micelles with the protein components may also have influenced resolution. SDS (0.05M in phosphate buffer) has been used by Wahlund and co-workers[12] to fractionate gluten proteins by asymmetrical flow FFF with good resolution. An SDS

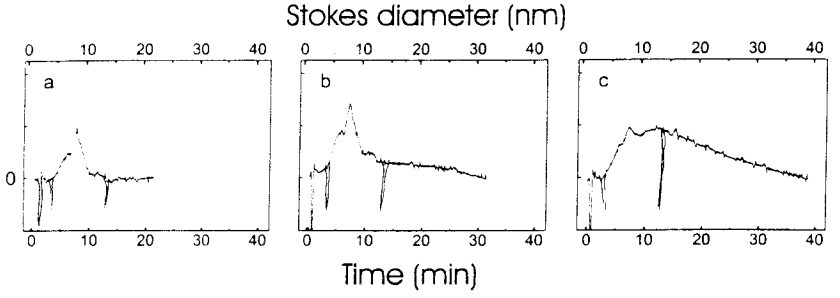

Figure 2. Representative fractograms for a) gliadin, b) HAc soluble glutenin, and c) HAc insoluble sonicated glutenin fractions run in cationic surfactant 0.002% CTAB.

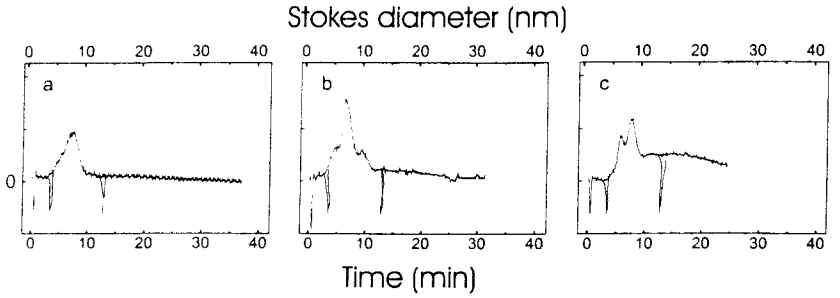

Figure 3. Representative fractograms for a) gliadin, b) HAc soluble glutenin, and c) HAc insoluble sonicated glutenin fractions run in anionic surfactant 0.17% SDS.

concentration of 0.17 % w/v was chosen to minimize buffer viscosity (reduce back pressure) while maintaining protein solubility. Some difficulty, however, was experienced with reproducibility of fractograms under these conditions. Lack of reproducibility may have been related to difficulties in balancing channel and cross flows due to the higher viscosity of the solution and to the tendency of proteins to absorb onto the membrane. It is possible that SDS is not as good a surfactant for membrane conditioning as some of the others used. Gliadin and soluble glutenin fractograms were generally similar to those obtained with nonionic surfactants, but resolution was inferior to that obtained with FL-70, Brij 35 and Triton X-100 (Fig. 3a and b). A second, earlier, peak at 5.6 min (d =6.4nm) was evident for the insoluble glutenin fraction. This peak was not present when nonionic surfactants were used. The amount of higher molecular size glutenin also was reduced (Fig. 3c). This result indicates

that SDS releases tightly bound lower molecular weight protein from the large polymeric glutenin. The presence of lower molecular weight proteins, tightly bound to high molecular weight polymeric glutenin, has also been demonstrated by gel filtration using chaotropic agents.[20]

In conclusion, this study demonstrates that optimum resolution and reproducibility of wheat protein flow FFF fractograms are obtained with nonionic surfactants. Of those studied, FL-70 is the best choice due to minimum viscosity and lack of foaming during buffer preparation. Different results may be obtained with base buffers other than dilute acetic acid. However, acetic and other dilute acids are preferable as solvents for wheat proteins since they provide good solubility and have minimal effects upon gluten structural and functional properties.[7,21]

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