Demonstration of the 2-Gram Mixograph as a Research Tool

To the Editor:

While the effects of lipids on breadmaking potential are well known, the relationship between mixing behavior and lipid composition of the flour is little known.

Several lines of indirect evidence indicate that lipid-binding proteins play a part in the effect that hexane-extractable ("free") lipids have on breadmaking properties of wheat flours (Bekes et al 1986, Lasztity et al 1988, MacRitchie et al 1989). In a recent study of Australian wheat varieties, the amount of ethanol-extractable lipid-mediated aggregates (LMA) isolated from gluten was closely related to loaf volume (Bekes et al 1991). These results are consistent with those of Nierle (1988), who compared the effects of the addition of lipids or lipid-gliadin complex on baking performance.

Until now, direct observation of the effects of highly purified protein and lipoprotein components on dough properties has been restricted by the large quantities of material required. The development of a recording dough mixer using only 2 g of flour (Rath et al 1990) has greatly reduced the amount of material required for such tests. We report here the direct observation of the effects that as little as 5 mg of purified protein or lipoprotein fractions had on the mixing properties of flour by increasing the relative proportions of the particular fractions in the parent flour.

Flour (cv. Timgalen, protein content 12.7%, N × 5.7, as-is basis) was used for mixing studies and for the isolation of four specific protein fractions. Purothionin was isolated and purified (Bekes 1976) from the petroleum ether extract of flour. Polyacrylamide gel electrophoresis of the purified isolate at pH 3.1 gave the characteristic "fast-moving globulin doublet" (Redman and Fisher 1968) without any evidence of contamination by other proteins. Purified S-protein (Zawistowska et al 1985) contained six major components on reversed-phase high-performance liquid chromatography (Bekes et al 1991). LMA and low molecular weight glutenin (LMWG) were isolated and characterized (Bekes et al 1991) from the excluded fractions of size-exclusion high-performance liquid chromatographic separations of 70% ethanol extracts of untreated and defatted gluten samples derived from flour.

Mixing properties were obtained using a 2-g mixograph (TMCO, Lincoln, NE) and a base flour (2 g of flour, 14% moisture basis, 1.20 ml of water) with additions of 0 (control), 5, and 10 mg each of purothionin, S-protein, LMWG, or LMA. All results quoted are the means from duplicate mixings. Mixing parameters were obtained by automated interpretation (Gras et al 1990). Peak resistance was calculated as a percentage of full-scale deflection, using the known relation between peak resistance on a 35-g mixograph and peak resistance of corresponding samples on the 2-g mixograph (Rath et al 1990). Tolerance to mixing is defined as the dough resistance 3 min after peak dough resistance (mixing time), expressed as a percentage of peak dough resistance. Statistical analyses were performed by standard procedures.

The nature of the structural interactions among the components of protein lipid aggregates of wheat flour and gluten is unknown. The common feature of the lipid-binding proteins discussed here is the homology of the amino acid sequences (and the charge and polarity distributions [Bekes et al 1991, MacRitchie et al 1989]) of the N-terminal end of LMWG (Shewry et al 1983) and one of the lipid-binding regions of purothionin (Bekes and Smied 1981). Globulinlike components of LMA have shown similarities in chemical composition and characteristics with lipid-binding wheat proteins (CM-proteins and S-protein, Zawistowska et al 1985).

Additions of small amounts of protein or lipoprotein fractions to the parent flour had significant effects on dough mixing parameters (Table I). The effects were not consistent from fraction to fraction. Purothionin decreased mixing time, but it increased peak dough resistance and tolerance to overmixing. S-protein increased the mixing time, peak dough resistance, and tolerance to overmixing. LMWG increased the mixing requirement and tolerance to overmixing but decreased the peak dough resistance. LMA (which are essentially aggregates of LMWG, S-protein, and lipid with some gliadin admixture) had no effect on mixing time or tolerance to overmixing, but they reduced the peak dough resistance.

These observations demonstrate significant differences between the effects of particular wheat proteins (S-protein and LMWG) and their aggregates with lipids. This may imply that the effects on mixing behavior mirror changes in protein-protein associations formed during wetting and dough mixing. These effects can be greatly altered by mediation with grain lipids, as in the case of LMA isolated from gluten.

In many of these cases, the addition of less than 2% of the total protein content of the flour, that is, 5 mg of the specific fraction, had a statistically significant effect on the whole range of mixing parameters. This quantity of specific wheat proteins is now accessible by a range of modern techniques of protein preparation, isolation, and purification.

Thus, these results show the value of very small-scale mixing studies as a tool for the determination of the role that specific

TABLE I
Effect of Addition of Lipid-Binding Proteins and Lipid-Mediated Aggregates on the Mixing Properties of Wheat Flour Determined on a Prototype 2-Gram Mixograph

	Quantity			
Mixing Parameter	**0 mg**	**5 mg**	**10 mg**	**LSD[a]**
Purothionin				
Development time, sec	161 a[b]	153 b	146 c	3.9
Peak resistance, % fsd[c]	64 a	67 b	68 b	2.1
Tolerance to overmixing, %	66 a	72 b	85 c	2.4
S-protein				
Development time, sec	161 a	185 b	185 b	4.9
Peak resistance, % fsd	64 a	66 a	67 a	NS[d]
Tolerance to overmixing, %	66 a	71 b	75 c	2.4
Low molecular weight glutenin				
Development time, sec	161 a	166 b	176 c	4.4
Peak resistance, % fsd	64 a	53 b	52 b	2.0
Tolerance to overmixing, %	66 a	72 a	85 b	10.3
Lipid-mediated aggregates				
Development time, sec	161 a	158 a	152 a	NS
Peak resistance, % fsd	64 a	55 b	51 c	2.1
Tolerance to overmixing, %	66 a	64 a	69 a	NS

[a] Least significant difference.
[b] Means within the same column that are followed by a common letter are not significantly different.
[c] Full-scale deflection on a standard mixograph.
[d] Not significant.

protein and/or lipoprotein fractions play in dough mixing behavior.

F. BEKES and P. W. GRAS
Grain Quality Research Laboratory
Commonwealth Scientific and Industrial Research Organisation
Division of Plant Industry
P.O. Box 7
North Ryde, NSW 2113, Australia

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