

Isolation and Identification of a Wheat Flour Compound Causing Sticky Dough¹

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ABSTRACT

Cereal Chem. 76(2):276–281

Fractionation and reconstitution studies of a flour from 1B/1R wheat showed that the factor causing sticky dough was water soluble. In addition, these studies showed that enzymes and lipids in the flour were not responsible for producing sticky dough. Dialysis experiments showed that the active component was nondialyzable. Gel-filtration chromatography of the retentate fraction showed that the substance causing sticky dough contained both a carbohydrate and a UV-absorbing material. Treatment of the active fraction with base caused the fraction to lose its ability to

cause stickiness. The UV-absorbing material and the carbohydrate fraction had to be covalently linked for the compound to be active. Gas chromatography-mass spectrometry and HPLC analysis showed that the UV-absorbing moiety was predominantly *trans*-ferulic acid, and the carbohydrate part was a glucose polymer. The glucose polymer was not degraded by α -amylase but was degraded by lichenase, suggesting that the glucose polymer was a mixed-linkage β -glucan.

The 1B/1R chromosome translocation, in which part of the short arm of the 1B chromosome of wheat has been replaced by part of the short arm of the 1R chromosome of rye, is being used in breeding programs for both winter and spring wheats around the world. For example in Germany, 30% of the breeding lines were reported to contain the 1B/1R translocation (Metin and Bluthner 1984), whereas \approx 45% of advanced lines in the CIMMYT program carry this chromosome (Dhaliwal et al 1988).

However, in contrast to substantial agronomic improvements, many wheat cultivars containing the 1B/1R translocation have a number of quality deficiencies. The most serious consequence is dough that is sticky (Law and Payne 1983, Moonen and Zeven 1984, Martin and Stewart 1986). This is of particular concern in modern mechanized bakeries where hundreds or thousands of doughs may be produced in a short time. Sticky dough can cause costly disruptions to production schedules and loss of product quality.

Some wheat flours produced from wheats that do not have the 1B/1R translocation will also produce sticky doughs. In addition, sticky doughs can be created by overmixing or the use of excess water, which can be avoided by careful control of those factors. However, sticky dough caused by the flour cannot be controlled in the bakery (Law and Payne 1983, Moonen and Zeven 1984, Martin and Stewart 1986). This has led to numerous studies of the cause of sticky dough.

Factors such as proteolytic enzyme activity (Hwang and Bushuk 1973); increased water absorption and amount of water-soluble pentosans (Zeller et al 1982); α -amylase activity (Ibrahim and D'Appolonia 1979, Hosene et al 1990); and differences in protein composition (Dhaliwal et al 1988) have been studied in relation to flour dough stickiness.

Stickiness of dough involves quite different and variable factors. Contact of a dough with a surface causes a force of adhesion (Saunders et al 1992) which may vary from flour to flour. Variation in the rheological properties of the dough also plays a role. If the dough is strong and elastic, the adhesion force is overcome, and the dough will separate from the surface (i.e., the dough is not sticky). If the dough is viscous, it will flow and not overcome the adhesive force (i.e., the dough is sticky). Chen and Hosene (1995a) developed a procedure that was designed to measure the adhesive force independently of the rheological properties of the dough. The objec-

tives of this study were to isolate and identify the wheat flour compounds that cause the adhesiveness of doughs to solid surfaces.

MATERIALS AND METHODS

Samples

A commercial bread flour donated by Cargill was designated as the nonsticky dough flour (NSD). The flour sample from 1B/1R translocation wheat that produced sticky doughs was designated as the sticky dough flour (SD) and was a blend of the Siouland samples described previously (Chen and Hosene 1995a). The moisture contents were 11.4% for NSD flour and 11.5% for SD flour. Flours were stored in double-layered plastic bags at 4°C. All chemicals used in the study were reagent grade.

Improved Dough Stickiness Cell

The original dough stickiness cell (Chen and Hosene 1995a) consisted of six pieces. Although the cell performed satisfactorily, it had several shortcomings, including the propensity to break and to produce a torsional stress that interfered with the stickiness measurement.

To overcome those disadvantages, we used a new dough stickiness cell produced by Stable Micro Systems Ltd. (Godalming, Surrey, England). It is made of aluminum and has two parts: a lid with 37–1.5 mm openings around its center and a chamber structure (Fig. 1). Because it is easily cleaned, samples can be measured rapidly. Most importantly, the torsional stress applied to the dough is reduced to zero, resulting in greater reproducibility in measurements. With the new cell, the extrusion of the dough is accomplished by rotating an internal screw to move a piston and the dough upwards and through the holes in the lid.

Dough Stickiness

Dough stickiness was measured using the new dough stickiness cell and an improved testing procedure (Huang 1997). A dough prepared from 10 g of flour (14% moisture basis) and optimum water (Finney and Shogren 1972) was mixed to optimum (peak) in a 10-g mixograph and transferred into the chamber. The cell lid then was screwed on the chamber. The internal screw was rotated to extrude a small amount of dough through the openings, and this first extrusion was wiped off the lid surface with a blade. The screw was turned once again sufficient to extrude a dough sample 1-mm high and turned backwards slightly to reduce the pressure and ensure that dough was not extruded further. A moist piece of cloth was placed over (but not in contact with) the exposed dough surface to minimize moisture loss, and the prepared dough surface was allowed to rest 15 sec. Then, the cover was removed and the dough stickiness cell was placed directly under the texture analyzer probe where the test was run. The force

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required for separating the probe from the dough surface was recorded. The dough then was removed from the lid surface and extruded again to repeat the test using the same procedure. Triplicate determinations were performed and averaged as dough stickiness. Standard deviation of the determination was ≈ 1.2 g-force.

Fractionation and Reconstitution Study

The NSD and SD flours were dispersed in distilled water at ambient temperature (1:4 flour-to-water ratio). Each mixture was stirred on a stir plate at moderate speed for 20 min and centrifuged (CU-5000 Damon/IEC centrifuge) at $3,000 \times g$ for 30 min. The water-soluble supernatant and insoluble residue fractions were frozen and lyophilized. Doughs were prepared with the mixograph using four different combinations as detailed in Table I. Optimum mixing time and water absorption of doughs made with each combination were determined using a mixograph (Finney and Shogren 1972).

Treatment of Water-Soluble Fractions

The water-soluble fractions isolated from SD flour were heated on a stir plate to boiling, held for 15 min, cooled, and lyophilized. The dried water-soluble fraction was mixed with the dried insoluble fraction from the same flour to produce a reconstituted flour. Stickiness of the dough from that flour was measured at optimum mixing time and water absorption.

TABLE I
Effect of Water-Soluble Fractions on Dough Stickiness^a

Unfractionated Flour	Water Insoluble	Water Soluble	Dough Stickiness ^b
NSD	30.2 ± 1.0
SD	70.9 ± 1.8
	SD	SD	66.3 ± 1.8
	SD	NSD	40.9 ± 1.0
	NSD	NSD	32.7 ± 1.0
	NSD	SD	58.1 ± 1.5

^a Nonsticky dough flour (NSD), sticky dough flour (SD).

^b Measured as g-force ± standard deviation.

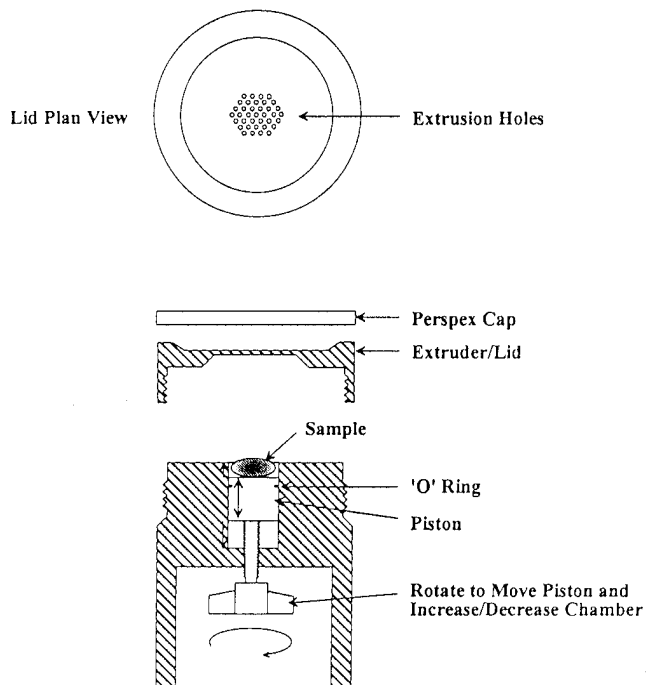


Fig. 1. Diagram of the new dough stickiness cell.

Defatting of Flour

SD flour was extracted with petroleum ether (bp 40–60°C) in a Soxhlet apparatus. The defatted flour was exposed to air and allowed to stand at room temperature for 48 hr to remove the solvent. Doughs made with the defatted flour were mixed to optimum, and stickiness was measured.

Dialysis of Water-Soluble Fraction

Dialysis (Spectrapor membrane tubing) was used to fractionate the water-soluble fraction from the SD flour. The molecular weight cutoff for the dialysis membrane was 6,000–8,000 Da. The water solubles isolated from SD flour were dialyzed against distilled water at 5°C for 24 hr. Both the retentate and permeate were lyophilized. Doughs were made by optimally mixing the NSD flour supplemented with the recovered levels of either the retentate or permeate, and stickiness was determined.

Treatment with Sodium Hydroxide

Doughs were made with NSD flour supplemented with the retentate fraction from SD flour that had been treated with 0.01N NaOH. Doughs also were made using SD flour but the water was replaced with 0.01N NaOH solution. The doughs were mixed to optimum, and stickiness was measured.

Gel-Filtration Chromatography

Gel-filtration chromatography was used to separate materials based on size. A column 70 cm in length and 2.5 cm in diameter was packed with Bio-gel P-30 (Bio-Rad Laboratories, Richmond, CA). A peristaltic pump p-1 (Pharmacia Fine Chemicals, Uppsala, Sweden) was used to maintain a flow rate of 36 mL/hr. A fraction collector (Frac-100, Pharmacia Fine Chemicals) was used to collect 6-mL samples and a total volume of 570 mL. The retentates from the dialysis experiment that caused sticky dough were used as the starting sample.

Carbohydrate and UV Analysis

The phenol-sulfuric acid method was used for carbohydrate analysis (Dubois et al 1956) with glucose as a standard. Each fraction also was tested for UV absorbance at 280 nm.

TABLE II
Effect of Heat-Treated Water Solubles on Dough Stickiness^a

Water Insoluble	Water Soluble	Boiled	Dough Stickiness ^b
NSD	SD	No	57.5 ± 1.5
NSD	SD	Yes	55.6 ± 2.0
SD	SD	No	67.5 ± 2.0
SD	SD	Yes	68.3 ± 1.8

^a Nonsticky dough flour (NSD), sticky dough flour (SD).

^b Measured as g-force ± standard deviation.

TABLE III
Effect of Defatting of Flour on Dough Stickiness^a

Flour Sample	Treatment	Dough Stickiness ^b
SD	None	70.9 ± 1.8
SD	Defatted	72.5 ± 1.5

^a Sticky dough flour (SD).

^b Measured as g-force ± standard deviation.

TABLE IV
Effect of Dialyzed Fractions on Dough Stickiness^a

Flour Sample	Added Fraction	Dough Stickiness ^b
NSD	None	30.2 ± 1.0
NSD	Permeate	33.5 ± 0.8
NSD	Retentate	55.2 ± 1.8
NSD	Permeate and retentate	56.5 ± 1.8

^a Nonsticky dough flour (NSD).

^b Measured as g-force ± standard deviation.

P-30 Gel Filtration Fractions

The retentate fraction from sticky dough was separated into two carbohydrate-containing peaks by the gel-filtration column. The higher molecular weight peak also contained UV-absorbing material. Both peaks were collected separately and lyophilized. Doughs were prepared by mixing NSD flour individually with each of the two samples, and stickiness was measured.

Bio-Gel P-60 Gel Filtration Chromatography

Because the first peak on Bio-gel P-30 was at, or near, the void volume, the higher molecular weight carbohydrate peak (containing the UV-absorbing material) was fractionated on a Bio-gel P-60 gel-filtration column. Column size and flow rate were as described above. Both the carbohydrate and UV-absorbing material were monitored. The

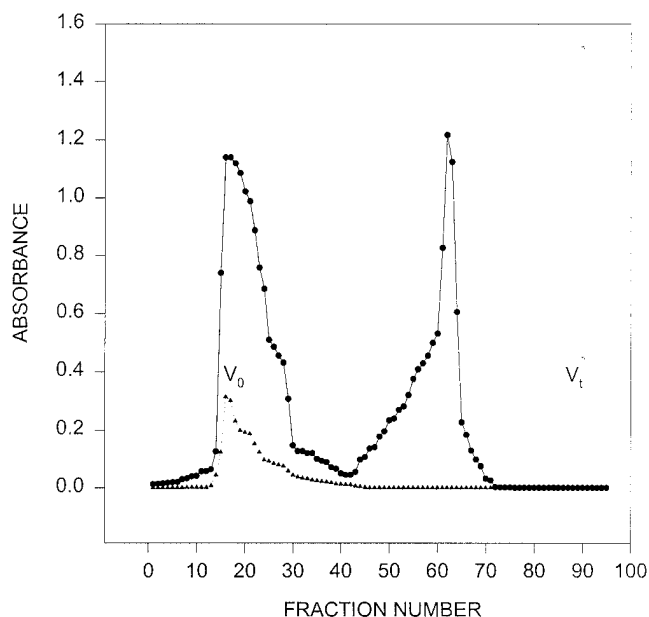


Fig. 2. Gel-filtration chromatogram (Bio-gel P-30) of material containing the compound causing sticky dough. V_0 and V_t = void volume and total volume of column, respectively.

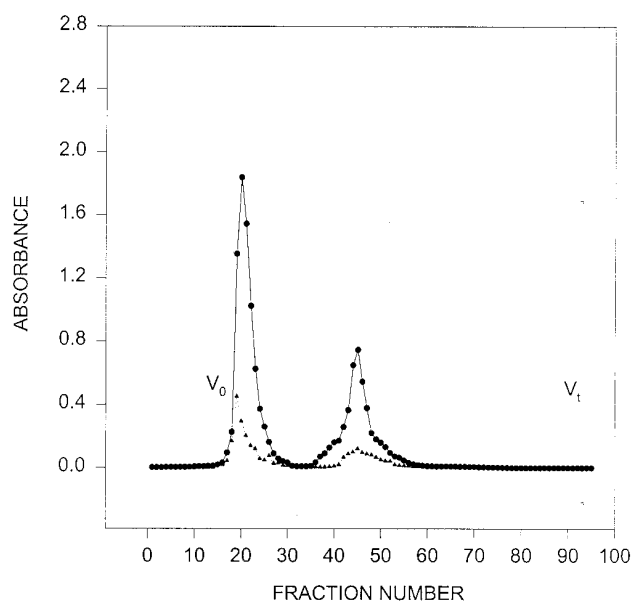


Fig. 3. Gel-filtration chromatogram (Bio-gel P-60) of material containing the compound causing sticky dough. V_0 and V_t = void volume and total volume of column, respectively.

void volume (V_0) and total volume (V_t) of the column were determined using blue dextran and glucose, respectively.

P-60 Gel Filtration Fractions

Two carbohydrate-containing peaks that had UV absorbance were obtained from the P-60 column. The peaks were collected separately and lyophilized. Doughs were made by individually mixing the NSD flour with the two fractions, and stickiness was measured.

NaOH Treatment of Active Peak Materials

The active peak materials obtained from the P-60 column were dissolved in 0.01N NaOH, and the mixture was allowed to stand for 30 min. The treated mixture was separated on the same gel-filtration column used previously (Bio-gel P-60). Again, carbohydrate and UV-absorbing materials were measured. Doughs were prepared by mixing the NSD flour individually with the carbohydrate peak, and the UV-absorbing peak and stickiness were measured.

GC and GC-MS Analysis of UV-Absorbing Peak Material

The UV-absorbing peak released by the NaOH treatment and separated on the P-60 column was collected and subjected to GC analysis with a gas chromatograph (model 5890-II, Hewlett Packard [HP], Englewood, CO) equipped with a 30-m \times 0.25-mm fused silica capillary column. The flow rate of the carrier gas was 40 cm^3/min of nitrogen, and the detector temperature was 270°C. The samples were silylated by slight warming with Tri-Sil/BSA Formula D (Pierce Chemical Co., Rockford, IL) before being introduced into the column. Samples (1 μL) were injected using a splitless injector with the bypass valve open for 1 min. The column oven was programmed for an initial 1-min hold at 60°C, followed by a 20°C/min rise to 270°C, and a final hold at that temperature. Phenolic acids were identified by comparing the retention times of the trimethylsilyl derivatives with those of the trimethylsilyl derivatives of standard acids. The results were authenticated further by mass spectra.

The GC-MS analysis was conducted using an HP 5890-II GC coupled to an HP 5989A mass selective detector (MSD). The MSD

TABLE V
Effect of Sodium Hydroxide on Dough Stickiness^a

Flour Sample	Added Fraction	Dough Stickiness ^b
NSD	Retentate	55.2 \pm 1.8
NSD	Retentate and 0.01N NaOH	31.9 \pm 1.0
SD	None	70.9 \pm 1.8
SD	0.01N NaOH	36.2 \pm 1.0

^a Nonsticky dough flour (NSD), sticky dough flour (SD).

^b Measured as g-force \pm standard deviation.

TABLE VI
Effect of Peak Materials from Bio-gel P-30 on Dough Stickiness^a

Flour Sample	Added Fraction	Dough Stickiness ^b
NSD	None	30.2 \pm 1.0
NSD	First peak materials	58.6 \pm 1.8
NSD	Second peak material	31.5 \pm 1.0
NSD	First and second peaks	60.5 \pm 2.0

^a Nonsticky dough flour (NSD).

^b Measured as g-force \pm standard deviation.

TABLE VII
Effect of Peak Materials from Bio-gel P-60 on Dough Stickiness^a

Flour Sample	Added Fraction	Dough Stickiness ^b
NSD	None	30.2 \pm 1.0
NSD	First peak materials	34.5 \pm 1.0
NSD	Second peak material	60.1 \pm 2.0
NSD	First and second peaks	57.8 \pm 1.8

^a Nonsticky dough flour (NSD).

^b Measured as g-force \pm standard deviation.

was optimized using HP software under Autotune conditions. The GC-MS conditions were ion source pressure of 4×10^{-6} torr and ion source temperature of 200°C. Mass spectra were acquired at an ionization energy of 70 eV and at a scan rate of 2.5 sec from mass 50 to mass 500 amu. Mass spectra of the UV-absorbing peak material and the standard phenolic acids were compared.

Acid Hydrolysis and HPLC-PAD Analysis of Carbohydrate Peak Material

The carbohydrate peak material obtained from Bio-Gel P-60 after base treatment was hydrolyzed. Sulfuric acid (9 mL, 1N) was added to 1 mg of the carbohydrate peak material. The mixture was held for 5 hr in a boiling water bath. After hydrolysis, the solution was cooled to room temperature, neutralized to pH 7.0 with barium hydroxide, and then filtered with glass fiber filter pad (Grade 984H, Whatman).

The HPLC identification of monosaccharide composition of the hydrolysate from the carbohydrate peak material was performed on a Dionex system (Dionex Corp., Marlton, NJ) with a pulsed amperometric detector (HPLC-PAD). A CarboPac PA1 analytical column (4 × 250 mm) with a CarboPac PA Guard column (3 × 25 mm) (Dionex) was used. The column was maintained at 25°C and developed with a mixture of 24 mM sodium hydroxide and 1.6 mM sodium acetate at a flow rate of 1.0 mL/min. Pulse potentials (volts) and duration (sec) on the PAD were: E1 = 0.05 (T1 = 0); E2 = 0.05 (T2 = 0.50); E3 = 0.60 (T3 = 0.51); E4 = 0.60 (T4 = 0.59); E5 = -0.60 (T5 = 0.60); and E6 = -0.06 (T6 = 0.65). Samples were introduced through IR-120, filtered with syringe filters (Super Acrodisc, pore size 0.45 μm) and then injected through a 20-μL injection loop. The HPLC chromatograms were recorded on a Chromatopac LR 601 digital integrator. Two standard solutions were used: 1) a mixture of D-galactose, D-glucose, D-arabinose, and D-xylose, and 2) D-glucose.

Determination of Degree of Polymerization (DP) and Reducing Power of Carbohydrate Peak Material

The average DP was determined for a sample of the carbohydrate peak material sample by using the modified Park-Johnson method as described by Hizukuri et al (1981). A 1.0- mL sample containing a maximum of 5 μg of glucose was added to 0.5 mL of

sodium carbonate-carbonic acid buffer containing potassium cyanide (4.8 g of Na₂CO₃, 9.2 g of NaHCO₃, and 0.65 g of KCN/L). Potassium ferricyanide (0.5 mL) solution (0.5 g K₃Fe(CN)₆/L), was added, and the mixture was heated for exactly 15 min in a vigorously boiling water bath. After the mixture was cooled for 10 min in running tap water, 2.5 mL of ferric ammonium sulphate solution (3g/L of 50 mM H₂SO₄) was added, and the mixture was kept in a fume hood for 20 min at room temperature. The absorbance of the resulting solution was then determined at 715 nm, and the reducing values and the average DP (DP_n) were calculated from a standard curve using D-glucose as a reference.

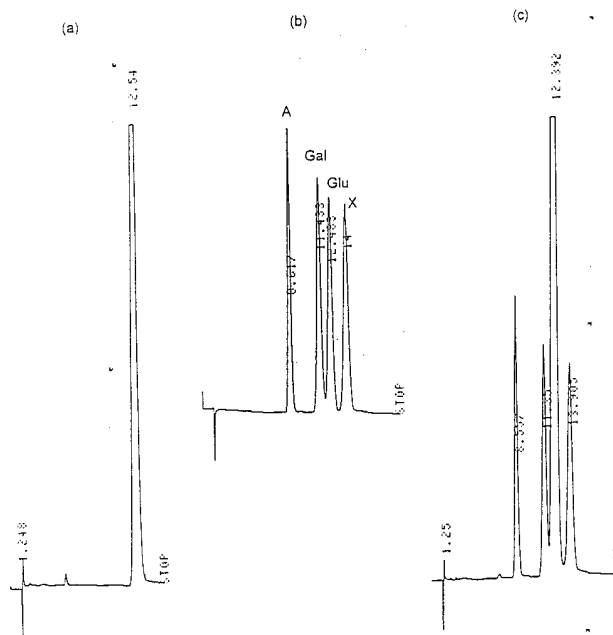


Fig. 5. HPLC traces of unknown carbohydrate (a); standards, A = arabinose, Gal = galactose, Glu = glucose, and X = xylose (b); and standards spiked with the unknown sample (c).

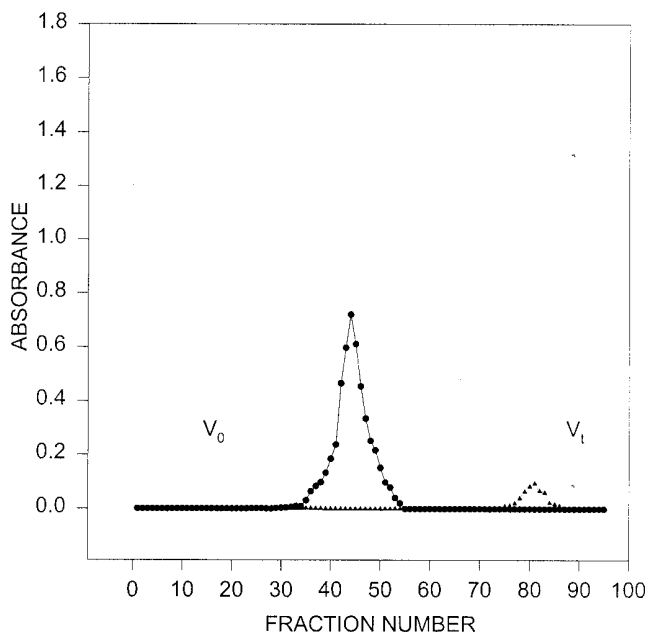


Fig. 4. Gel-filtration chromatogram (Bio-gel P-60) of material containing the compound causing sticky dough after sodium hydroxide treatment. V₀ and V_t = void volume and total volume of column, respectively.

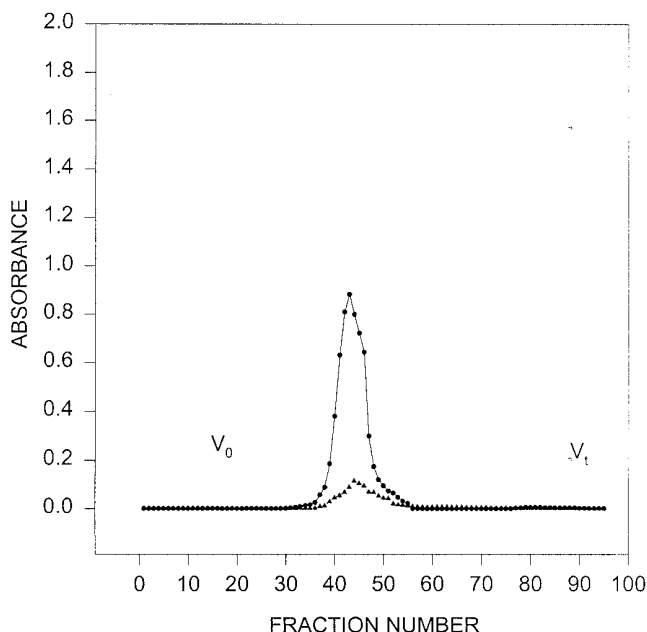


Fig. 6. Gel-filtration chromatogram (Bio-gel P-60) of material containing the compound causing sticky dough after α-amylase treatment. V₀ and V_t = void volume and total volume of column, respectively.

Enzyme Treatments of Peak Materials

Two enzymes, lichenase (endo-1, 3(4)- β -glucanase, EC 3.2.1.73, from *Bacillus subtilis*, Megazyme International Ireland Ltd. [Wicklow, Ireland]) and α -amylase (EC 3.2.1.1, from *Bacillus licheniformis*, Sigma Chemical Co. [St Louis, MO]), were used separately to treat the active peak materials isolated from Bio-gel P-60. The treated mixture then was applied to the gel-filtration column as described above. Carbohydrate and UV-absorbing materials were measured again.

RESULTS AND DISCUSSION

Fractionation and Reconstitution

Sticky doughs were produced by mixing the water solubles isolated from SD flour with either its own water insolubles or with water insolubles of NSD flour (Table I). Nonsticky doughs were produced when doughs were prepared from water solubles of NSD flour mixed with its water insolubles or with the water insolubles of SD flour. This indicated that, to produce sticky dough, the water solubles of SD flour had to be present. Based on this, we concluded that something in the water-soluble fraction of SD flour was responsible for producing sticky doughs.

Heat-Treated Water Solubles

The water-soluble fraction of wheat flour contains proteins, starch, nonstarchy carbohydrates, enzymes, and many other substances. The water solubles from the SD flour were boiled to denature enzymes and then added back to NSD flour to make doughs. The resulting dough was still sticky (Table II). This indicated that the substance causing sticky dough was not an enzyme.

Defatting of Flour

No literature report was found on the effect of different quantities or types of lipids on dough stickiness. To eliminate the possibility of lipids causing stickiness, the SD flour was defatted.

The data in Table III show that doughs made using defatted SD flour were still sticky, indicating that petroleum ether extractable lipids were not involved in stickiness.

TABLE VIII
Effect of Peak Materials from Bio-gel P-60
After NaOH Treatment on Dough Stickiness^a

Flour Sample	Added Fraction	Dough Stickiness ^b
NSD	None	30.2 \pm 1.0
NSD	Carbohydrate peak	35.7 \pm 0.8
NSD	UV-absorbing peak	32.5 \pm 0.8
NSD	Carbohydrate and UV-peaks	34.5 \pm 1.0

^a Nonsticky dough flour (NSD).

^b Measured as g-force \pm standard deviation.

TABLE IX
Characteristic Fragment Ions Obtained by Mass Spectrometry from
Major UV-Absorbing Peak Material and Standard *trans*-Ferulic Acid

Unknown		<i>trans</i> -Ferulic Acid	
m/e ^a	Relative Abundance (%)	m/e ^a	Relative Abundance (%)
203	8.41	203	7.87
219	21.84	219	22.05
233	4.85	233	4.72
249	65.53	249	62.68
264	3.40	264	3.15
279	19.58	279	18.74
293	58.52	293	54.33
308	70.01	308	67.72
323	75.73	323	74.02
338	100	338	100

^a Mass-to-charge ratio.

Dialyzed Fractions

Most industrial adhesives, glues for instance, are composed of large polymers. This raised a question of whether the substance responsible for producing sticky doughs was also a polymer. To answer this question, a dialysis technique was used to fractionate the water-soluble material from the SD flour. When a dough was made using NSD flour with the permeate fraction, it was not sticky. However, a sticky dough was produced when the retentate fraction was mixed with the NSD flour. Sticky dough also was produced when both the permeate and retentate fractions were mixed with NSD flour (Table IV). Thus, the substance causing sticky dough was in the retentate. This is in contrast to the report of Chen and Hosney (1995b) that the active substance was in the permeate. Small differences in the dialysis procedure may account for the different results as the compound in question is reasonably near the cut-off size of the dialysis tubing.

Effect of Sodium Hydroxide

A previous study (Chen and Hosney 1995b) suggested that sodium hydroxide was effective in reducing stickiness. To test the effect of NaOH, doughs were prepared from NSD flour and the retentate fraction was treated with 0.01N NaOH. Doughs also were prepared with SD flour and 0.01N NaOH. The dough stickiness (Table V) was reduced sharply by the NaOH.

Gel-Filtration Chromatography (Bio-Gel P-30)

Size-exclusion gel filtration was used to fractionate the water-soluble fraction based on molecular size. Two peaks containing carbohydrate were detected (Fig. 2). In addition, a UV-absorbing (280 nm) material co-eluted with the first carbohydrate peak. When the material from the first carbohydrate peak was mixed with NSD flour, sticky doughs were produced (Table VI).

P-60 Gel-Filtration Experiment

Because the first peak eluted from Bio-gel P-30 was at, or near, the void volume, the peak material was collected and fractionated with a P-60 Bio-gel column. Two peaks both containing carbohydrate and UV-absorbing material were obtained (Fig. 3). Doughs were made by mixing NSD flour with the material collected from those peaks. Dough stickiness data showed that only the second carbohydrate and UV peak produced sticky dough (Table VII).

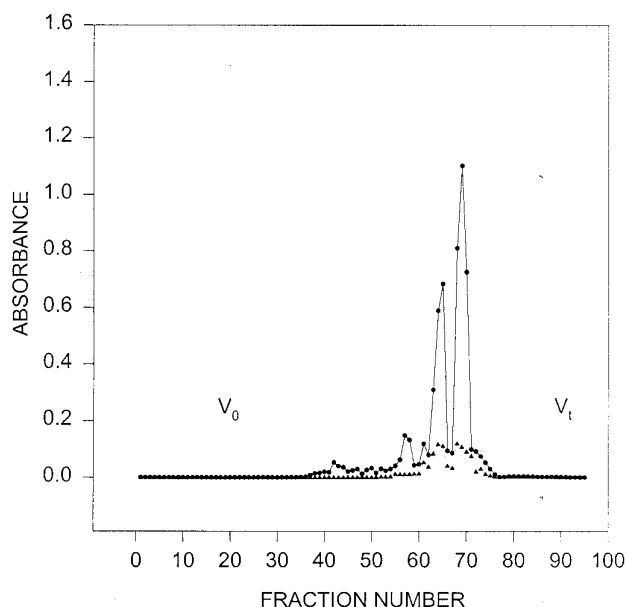


Fig. 7. Gel-filtration chromatogram (Bio-gel P-60) of the compound causing sticky dough after lichenase treatment. V_0 and V_t = void volume and total volume of column, respectively.

NaOH Treatment of Active Peak Materials

The active peak (second peak from Bio-gel P-60) was treated with 0.01N NaOH, and the material was passed again through the same column. The UV-absorbing peak (Fig. 4) was separated from the carbohydrate peak after the NaOH treatment. Thus, the NaOH had cleaved the bond between the two components. Doughs were prepared by mixing NSD flour with materials from either the carbohydrate or UV-absorbing peak. Neither fraction produced a sticky dough (Table VIII). This indicated that the carbohydrate material and the UV-absorbing material had to be linked together to cause sticky dough.

Chemical Composition of UV-Absorbing Peak Material

A GC chromatogram of trimethylsilyl derivatives from the UV-absorbing peak material showed one major peak and two minor peaks. The retention time of the major peak (12.19 min) was essentially the same as that for standard *trans*-ferulic acid (12.04 min). Furthermore, the mass spectra data of the trimethylsilyl derivatives of this compound and authentic *trans*-ferulic acid gave the same molecular mass (338) and the same major fragmentation pattern with essentially the same relative abundance of ions (Table IX). The results indicated that the peak was derived from *trans*-ferulic acid, and that the UV-absorbing peak material was primarily *trans*-ferulic acid.

Isomerization of *trans*-Ferulic Acid to *cis*-Ferulic Acid

Ferulic acid occurs naturally in the more stable *trans* form, but it can be converted partially to the *cis* form by the action of light, especially UV light (Ribereau-Gayon 1972). The two small peaks were identified by GC-mass spectra to be *cis*-ferulic acid and 3-hydroxy-4-methoxycinnamic acid, both isomers of *trans*-ferulic acid.

Carbohydrate Peak Material

The HPLC chromatography of the acid hydrolysate of the carbohydrate peak material and the glucose standard showed similar retention times. The sample peak increased when spiked with standard glucose (data not shown). A similar increase in the standard glucose peak occurred when the sample was incorporated into a standard solution of galactose, glucose, arabinose, and xylose (Fig. 5). The results showed that glucose was the repeating unit of the carbohydrate peak material.

Enzyme Treatments of Active Peak Materials

α -Amylase and lichenase treatments of the active peak materials showed that the glucose polymer was not degraded by α -amylase (Fig. 6) but was degraded by lichenase (Fig. 7). The experiments showed that the glucose polymer was a mixed-linkage (1 \rightarrow 3)(1 \rightarrow 4)- β -D-glucan. Based on the reducing power, the carbohydrate chain length was estimated to be \approx 71 or a molecular mass of \approx 11,500D.

CONCLUSIONS

Two flours, one giving a sticky dough and the other a nonsticky dough when both were mixed under optimum conditions, were studied to identify the factor causing the stickiness. The compound

causing sticky dough was a water-soluble material containing both a carbohydrate and UV-absorbing material. It was nondialyzable and susceptible to sodium hydroxide. We propose that the compound responsible for sticky dough is a *trans*-ferulic acid moiety attached to a mixed-linkage β -glucan chain of \approx 71 anhydroglucose units.

ACKNOWLEDGMENTS

We are very grateful to Bruce Plashko for his assistance with the GC and GC-MS analyses.

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[Received June 1, 1998. Accepted November 24, 1998.]