Factors Affecting the Viscosity of Flour-Water Extracts[1]

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ABSTRACT

Allowing flour and water to remain together for periods of time increases the viscosity of the water-soluble extract. On the other hand, if the extract is held after centrifugation, the viscosity of the extract decreases. This could be explained by the action of an endopentosanase; however, such enzymes have not been demonstrated to occur in flour. If the phenomenon were caused by an enzyme, it should have a well-defined pH optimum. No pH optimum was found. Instead, the loss of viscosity appeared to be more rapid at low pH. We concluded that there was no significant endopentosanase activity. The increase in viscosity of a flour-water mixture as a function of time resulted from increased solubility. The more rapid decrease in viscosity of the centrifuged extract at low pH was speculated to result from hydrolysis of the arabinose side chains followed by precipitation of the xylan chains.

Wheat flour contains both water-soluble and water-insoluble pentosans. The water-soluble fraction usually has been isolated using a single extraction of flour at a ratio of flour to water between 1:2 and 1:4 (Baker et al 1943, Perlin 1951a, Montgomery and Smith 1955, Kuendig et al 1961, Medcalf et al 1968, Painter and Neukom 1968, Lineback et al 1977, Yeh et al 1980). Extraction of flour with a 1:2 ratio of flour to water yields approximately 0.8% water-soluble pentosans, based on flour weight (Baker et al 1945). Pentosans form viscous solutions in water. The viscosity of aqueous wheat flour extracts is concentration dependent (Neukom et al 1967).

Variability in the initial viscosity of water-solubles results from solubility differences (Hoseney et al 1969, Markwalder 1975). It has been suggested that enzyme activity was responsible for variations in the initial viscosity of water solubles, the gradual decrease in the viscosity of water solubles with time, and the liquification of purified gels (Durham 1925, Baker et al 1943, Neukom and Markwalder 1978, Martinez-Munoz 1985). Elevated temperatures purportedly affect polymer solubility by increasing enzymatic degradation of water-insoluble pentosans to a water-soluble form via transarabinosylation (Preece and Hobkirk 1955, D'Appolonia and Kim 1976). Decreases in the viscosity of aqueous dispersions as well as the liquification of gels may result from naturally occurring enzymes believed to attack internal glycosidic linkages in the pentosan molecule (Preece and MacDougall 1958). Regions of approximately five unbranched xylose units are suspected to be susceptible to such enzymatic attack. To avoid enzymatic changes, aqueous extracts are heated or the flour is extracted with hot 70–80% ethanol (Baker et al 1943, Pence et al 1950, Simpson 1954, Montgomery and Smith 1955, Preece and MacDougall 1958).

Current evidence suggests that the enzyme systems that degrade pentosans are not found at substantial levels in wheat. Wheat flour is deficient in endoxylanase (Preece and MacDougall 1958), the enzyme responsible for the rapid decrease in viscosity. Frequently cited studies on the enzymolysis of wheat pentosans utilized exogenous enzyme sources (Simpson 1954, Mauritzen and Stewart 1965, Wrench 1965, Kulp 1968).

The purpose of this study was to determine if the changes in the solubility and viscosity of aqueous wheat flour extracts over time are caused by enzyme action or by other mechanisms.

MATERIALS AND METHODS

Materials

Chemicals. Sodium hydroxide, phenol, xylose (Fisher Scientific Co., Fair Lawn, NJ), sulfuric acid, and hydrochloric acid (Sigma Chemical Co., St. Louis, MO) were reagent grade.

Flour. Commercial straight-grade malted flour containing 13.0% protein, 13.4% moisture, and 0.47% ash was used (Ross Mills, Wichita, KS).

Methods

Preparation of flour extracts. Flour and water were combined at a ratio of 1:5 (w/v). The resultant slurries were stirred for specific time intervals (0, 30, 60, or 90 min) and then centrifuged 15 min (360 × g). The supernatant was decanted, and the precipitate was discarded. The supernatant was recentrifuged for 10 min (1,000 × g). The resultant aqueous extracts were used without further purification.

The time that flour and water remained in contact prior to centrifugation is referred to as time before centrifugation. A no-time extraction refers to centrifugation immediately after uniform slurry preparation. Samples having time before centrifugation greater than zero were stirred every 5 min until centrifugation. In certain experiments, the pH of the slurries was adjusted using either 0.1N NaOH or 0.1N hydrochloric acid.

Viscosity measurements. The relative viscosity of aqueous flour extracts was recorded using Cannon-Fenske (size 50) capillary viscometers held in a constant-temperature water bath (30 ± 0.1°C). The tubes were calibrated with distilled water. Each measurement consisted of pipetting 5 ml of solution into the tube and allowing a 5-min temperature equilibration period. The time required for a constant volume of solution to flow through the capillary was recorded, and relative viscosity was calculated as:

$$\text{Relative viscosity} = \frac{\text{flow time of the aqueous extract}}{\text{flow time of distilled water}}$$

Determination of pH optima. After centrifugation, the pH of aqueous flour extracts (1:5) was adjusted with either 0.1N HCl or 0.1N NaOH. The pH range evaluated was pH 2.0–8.5 in increments of 0.5. Relative viscosity of aqueous extracts was measured at 0, 2, 4, or 6 hr after centrifugation.

Statistical analysis. SAS, a statistical analysis system (SAS Institute, Cary, NC), was used to analyze the relative viscosity of aqueous extracts at all combinations of time before and after centrifugation. A backwards, step-wise elimination procedure provided a quadratic viscosity prediction model for the system ($R^2 = 0.90$, $\alpha = 0.1$). Regression analyses were calculated to ensure that the model gave a normalized distribution.

Carbohydrate quantitation. Total carbohydrate content of 1:5 aqueous extracts was estimated using a modified phenol sulfuric procedure (Southgate 1976). A stock solution of 70 μg D-xylose per milliliter was used as a standard. The supernatant was diluted 1:500 prior to analysis. Absorbance was measured at 480 nm.

Effect of TBC. The relative viscosity of aqueous extracts increased with the time the flour and water remained in contact prior to centrifugation (Fig. 1). The concentration of carbo-

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TABLE I
**Effect of Increasing Time Before Centrifugation
on the Concentration of Aqueous Extracts**

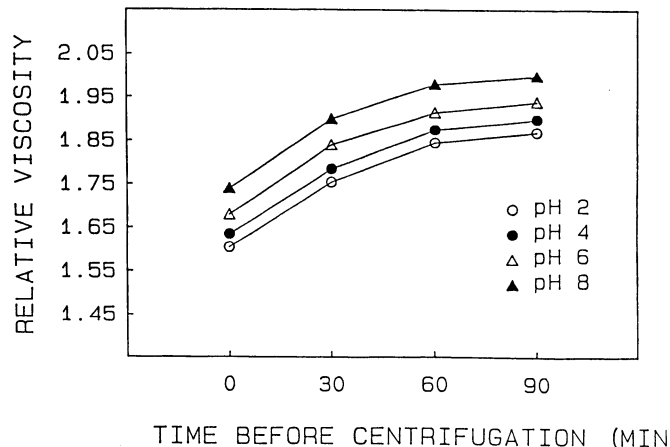| Time Before Centrifugation (min)	Xylose Equivalents (mg/ml)
0	7.5
30	11.5
60	14.1
90	19.4

Fig. 1. Increase in relative viscosity with the time that flour and water remain in contact before centrifugation. Time after centrifugation was zero.

Fig. 2. Effect of pH at 0 min after centrifugation on the relative viscosity of aqueous extracts.

Fig. 3. Effect of pH at 90 min after centrifugation on the relative viscosity of aqueous extracts.

hydrates present in the extracts also increased with the length of time before centrifugation (Table I). The increase in viscosity was curvilinear. This illustrates the characteristic nonlinear relationship between concentration of a solute and viscosity at a constant temperature (Charm 1981). Taken together, the data in Table I and Figure 1 suggest that some of the flour components, presumably the water-insoluble pentosans, were becoming soluble with time.

Based on other studies (Preece and Hobkirk 1955, Martinez-Munoz 1985), it was presumed that an enzyme was acting on the insoluble material and making it soluble. It also was presumed that once the insoluble substrate was removed by centrifugation, the enzyme proceeded to degrade the soluble material. If an enzyme were responsible for changes in solubility and viscosity of aqueous extracts, then it should be possible to determine the pH optimum for that enzyme.

Adjustment of the pH of aqueous extracts directly affected the initial viscosity (Fig. 1). The higher pH values gave higher relative viscosities.

Effect of time after centrifugation. The relative viscosities of aqueous extracts decreased as the time after centrifugation increased (Figs. 2 and 3). Such a change in relative viscosity suggests a decrease either in molecular weight, concentration, or an aggregation of the polymers.

The pH optimum for pentosanases has been reported to be between pH 4.5 and 5.0 (Preece and MacDougall 1958). Thus, the most rapid decrease in viscosity would be expected at that pH range. The decreases in relative viscosity for all pHs evaluated (pH 2–8.5) gave parallel curves. The relative viscosity of aqueous extracts at pH 4.0, 4.5, and 5.0 did not decrease more rapidly than those at the other pHs tested; only four curves are shown in Figures 2 and 3 to avoid confusion.

The decrease in viscosity was pH dependent. A quadratic viscosity prediction model was fitted to the data and showed that this change in viscosity differed with pH. The relative viscosity of aqueous extracts at low pH values continued to decrease as time after centrifugation increased. At high pH, there was a plateau in the decrease of relative viscosity beyond 4 hr after
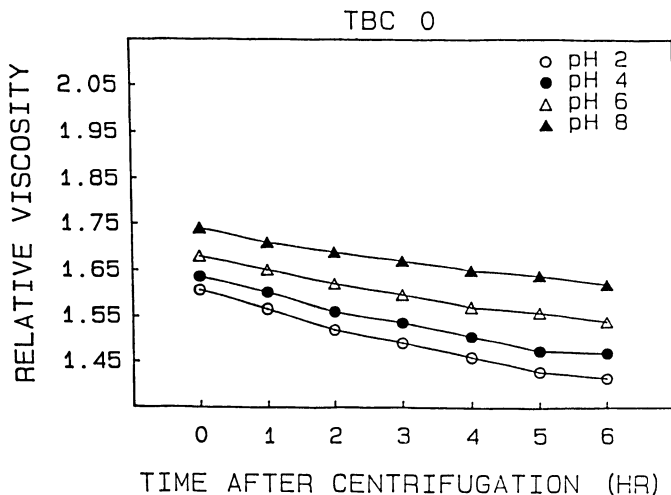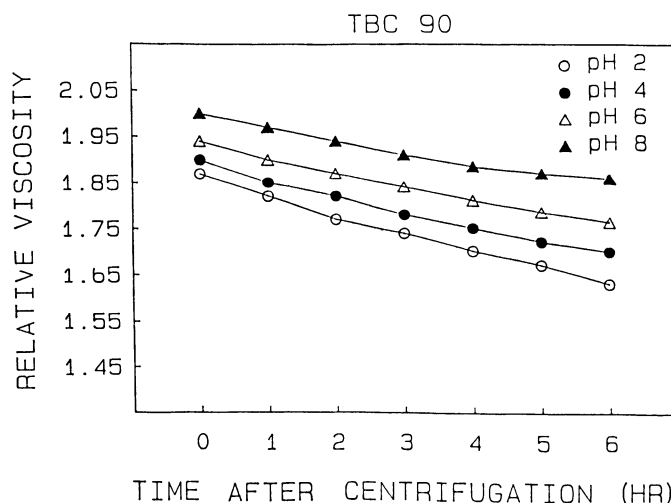
centrifugation. The pronounced decrease in viscosity at pH 2 may be attributed to the acid lability of the hemiacetal bond of arabinose (Fincher et al 1974). Cleavage of the arabinose side chains may allow the xylan chain to associate and precipitate from solution, resulting in decreased viscosity.

The absence of a pH optimum for the decrease in viscosity suggested that an enzyme was not responsible for either the increase or the decrease in viscosity with time. This finding is consistent with reports that wheat is deficient in enzymes needed to degrade pentosans (Preece and Hobkirk 1955).

SUMMARY

Inability to locate a pH optimum for the decrease in viscosity indicated that an enzyme is not responsible for the physical changes that occur with time. Therefore, we suggest that the increase in viscosity with time before centrifugation is the result of the pentosan slowly solubilizing. The decrease in viscosity during time after centrifugation is presumed to be caused by acid hydrolysis of the arabinose side chains and precipitation of the resulting xylan chains.

LITERATURE CITED

BAKER, J. C., PARKER, H. K., and MIZE, M. D. 1943. The pentosans of wheat flour. Cereal Chem. 20:267.
BAKER, J. C., PARKER, H. K., and MIZE, M. D. 1945. Super-

centrifugates from dough. Cereal Chem. 23:16.

CHARM, S. E. 1981. Extraction. Page 464 in: Fundamentals of Food Engineering, 3rd ed. Avi Publ. Co.: Westport, CT.

D'APPOLONIA, B. L., and KIM, S. K. 1976. Recent developments on wheat flour pentosans. Baker's Dig. 50(3):45.

DURHAM, R. K. 1925. Effect of hydrogen peroxide on relative viscosity measurements of wheat and flour suspensions. Cereal Chem. 2:303.

FINCHER, G. B., SAWYER, W. H., and STONE, B. A. 1974. Physical properties of an arabinogalactan-peptide from wheat endosperm. Biochem. J. 139:535.

HOSENEY, R. C., FINNEY, K. R., SHOGREN, M. D., and POMERANZ, Y. 1969. Functional (breadmaking) and biochemical properties of wheat flour components. II. Role of water-solubles. Cereal Chem. 46:117.

KUENDIG, W., NEUKOM, H., and DEUEL, H. 1961. Untersuchungen uber Getreideschleimstoffe. I. Chromatographische fraktionierung von Wasserloeslichen Weizenmehlpentosanen an Diaethylaminoaethyl-cellulose. Helv. Chim. Acta. 44:823.

KULP, K. 1968. Enzymolysis of pentosans of wheat flour. Cereal Chem. 45:339.

LINEBACK, D. R., SOMNAPAN, K. N., and TSEN, C.C. 1977. Carbohydrate composition of water-soluble pentosans from different types of wheat flours. J. Food Sci. 42:46.

MARKWALDER, H. 1975. Isolierung und Charakterisierung der nichtstarkeartigen Polysaccharide im Weizenmehl. Swiss Federal Institute of Technology, Zurich. Dissertation Nr. 5497.

MARTINEZ-MUNOZ, I. M. 1985. Studies on the oxidative gelation mechanism: Effect of inhibitors, time, and concentration of water solubles on the relative viscosity of wheat flour soluble pentosans. M.S. thesis. Kansas State University, Manhattan, KS.

MAURITZEN, C. M., and STEWART, P. R. 1965. The ultra-centrifugation of doughs made from wheat flour. Aust. J. Biol. Sci. 18:173.

MEDCALF, D. C., D'APPOLONIA, B.L., and GILLES, K.A. 1968. Comparison of chemical composition and properties between hard red spring and durum wheat endosperm pentosans. Cereal Chem. 45:539.

MEDCALF, D. C., and GILLES, K. A. 1968. Structural characterization of a pentosan from the water-insoluble portion of durum wheat endosperm. Cereal Chem. 45:550.

MONTGOMERY, R., and SMITH, F. 1955. The carbohydrate of the Gramineae. VII. The constitution of a water-soluble hemicellulose of the endosperm of wheat (*Triticum vulgare*). J. Am. Chem. Soc. 77:3325.

NEUKOM, H., and MARKWALDER, H. U. 1978. Oxidative gelation of wheat flour pentosans: A new way of cross-linking polymers. Cereal Foods World 23:374.

NEUKOM, H., GEISSMANN, T., and PAINTER, T. J. 1967. New aspects of the functions and properties of the soluble wheat-flour pentosans. Baker's Dig. 41(5):52.

PAINTER, T. J., and NEUKOM, H. 1968. The mechanism of oxidative gelation of a glycoprotein from wheat flour. Biochem. Biophys. Acta. 158:363.

PENCE, J. W., ELDER, A. H., and HECHAM, D. K. 1950. Preparation of wheat flour pentosans for use in reconstituted doughs. Cereal Chem. 27:60.

PERLIN, A.S. 1951a. Isolation and composition of the soluble pentosans of wheat flour. Cereal Chem. 28:370.

PREECE, I. A., and HOBKIRK, R. 1955. Non-starchy polysaccharides of cereal grains. VII. Preliminary study of pentosan enzymolysis. J. Inst. Brew. 61:393.

PREECE, I. A., and MACDOUGALL, M. 1958. Enzymic degradation of cereal hemicelluloses. II. Pattern of pentosan degradation. J. Inst. Brew. 64:489.

SIMPSON, F. J. 1954. Microbial pentosanases. I. A survey of micro-organisms for the production of enzymes that attack the pentosans of wheat flour. Can. J. Microbiol. 1:131.

SOUTHGATE, D. A. T. 1976. Phenol sulfuric method for total carbohydrates. Page 108 in: Determination of Food Carbohydrates. London: Applied Science Publishers.

WRENCH, P. M. 1965. The role of wheat flour pentosans in baking. III. Enzymic degradation of pentosan fractions. J. Sci. Food Agric. 16:51.

YEH, Y. F., HOSENEY, R. C., and LINEBACK, D. R. 1980. Changes in wheat flour as a result of dough mixing and oxidation. Cereal Chem. 57:144.