Note on the Effects of Protease from *Saccharomyces carlsbergensis* on Dough Strength

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Cereal Chem. 57(4):290-293

Proteases are added to bread doughs to help control bread texture and assure dough uniformity. Protease addition improves dough handling properties, gluten elasticity, and texture; shortens mixing time; and increases loaf volume and crust browning (Barrett 1975, El-Dash and Johnson 1967, Johnson and Miller 1949, Pomeranz 1966, Waldt 1965). For this reason two-thirds of the white bread produced in the United States is made from dough to which protease, from the fungus *Aspergillus oryzae*, has been added (Barrett 1975). Close control of protease is important because too much protease gives a slack, sticky, gummy dough and a coarse, harsh texture in the finished bread (Barrett 1975, Conn et al 1950, Johnson and Miller 1949, Waldt 1965).

Only certain proteases are suitable for use in doughs. For example, wheat flour contains trypsin and papain inhibitors that limit the use of these enzymes (Barrett 1975). The amount of papain

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0009-0352/80/04029004/$03.00/0

required to overcome this inhibition has deleterious effects on loaf volume (El-Dash and Johnson 1967). The baking industry uses, almost exclusively, protease preparations from *A. oryzae* (Matz and Matz 1978), although the Standards of Identity of White Bread permit other fungal proteases, bromelain, and papain (Barrett 1975). Bacterial proteases have been successfully used experimentally (Barrett 1975).

Brewer's yeast (*Saccharomyces carlsbergensis*) offers a plentiful and reliable source of enzymes, as well as a potential source of protein for human consumption (Kinsella and Shetty 1978, Mateles and Tannenbaum 1968). A procedure for partial purification of proteases from brewer's yeast and some enzymatic characteristics of these proteases have been described.[2]

The behavior of the protease from *S. carlsbergensis* in bread dough was examined in the present study.

[2]F. C. Woods and J. E. Kinsella. 1980. Isolation and some properties of proteases from *Saccharomyces carlsbergensis*. Unpublished.

MATERIALS AND METHODS

Protease Preparation

Washed and lyophilized cells of *S. carlsbergensis* were autolyzed at pH 7 with chloroform.[2] The proteolytic activity (pH optimum at pH 6) in the crude cell-free extract was then purified threefold by precipitation at 60% ammonium sulfate saturation. This crude preparation (protein 1.6 mg/ml) was frozen in 1-ml aliquots.

A modification of the method of Kunitz was used to measure general proteolytic activity (Kunimitsu and Yasunobu 1970, Kunitz 1947). With Hammersten casein as the substrate, 1 ml of the protease preparation caused a change of 0.3 in the absorbance at 280 nm after 20 min of incubation at 37°C and pH 6. The crude

TABLE I
Effects of Increasing Concentrations of Protease from *Saccaromyces carlsbergensis* on Mixogram Peak Heights of Experimental Bread Dough

Protease (mg)	Mixogram Peak Height (mm)
0	56.0
0.4	53.0
0.8	51.0
1.6	47.3
3.2	42.8

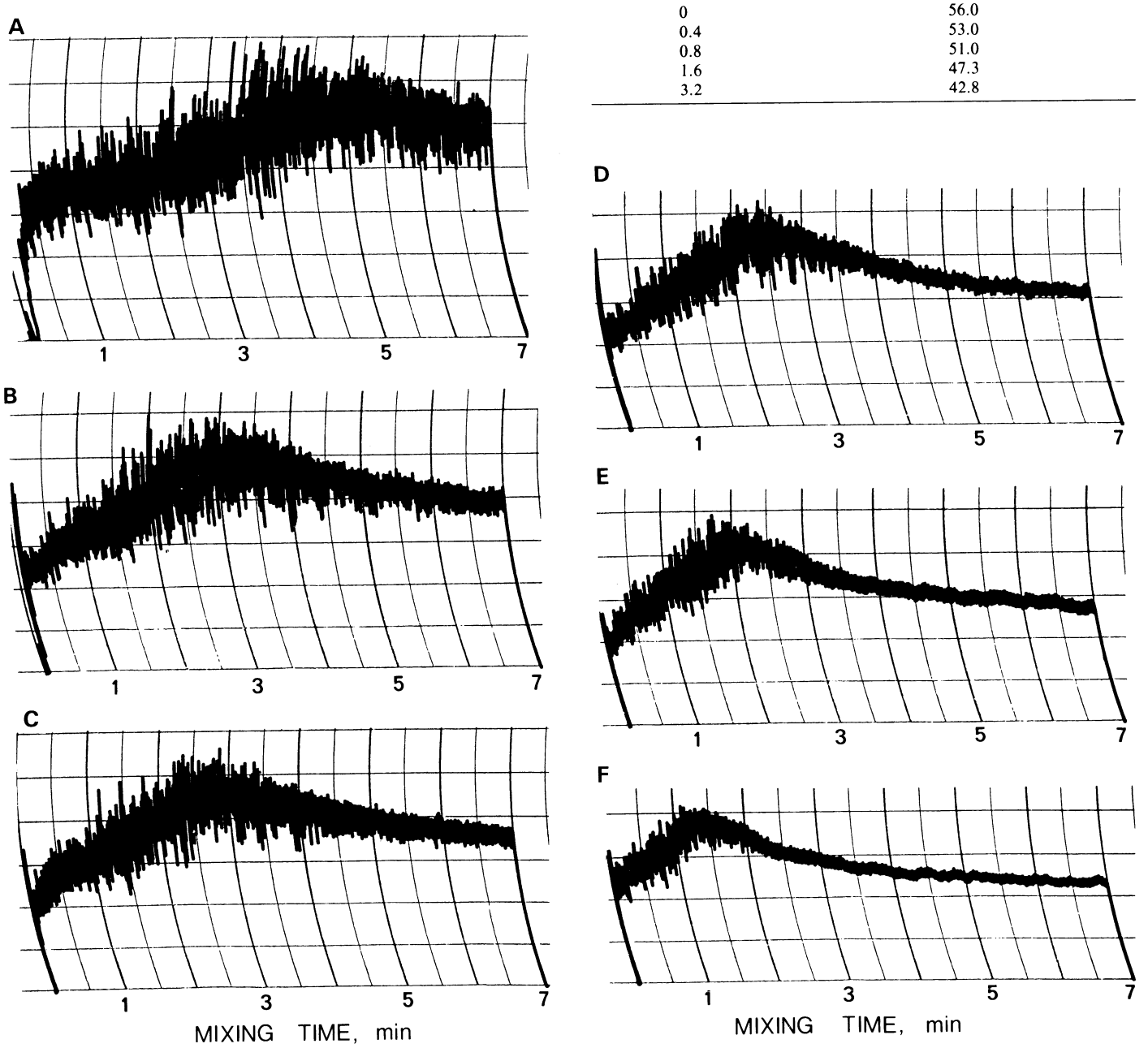

Fig. 1. Mixograms of fermented bread doughs containing increasing concentrations of protease from *Saccharomyces carlsbergensis*. Each dough was fermented for 3 hr (except for the unfermented control) and was then mixed for 7 min in the mixograph. **A,** Standard unfermented dough; **B,** standard dough fermented with no protease; doughs fermented with proteases (mg): **C,** 0.4; **D,** 0.8; **E,** 1.6; **F,** 3.2.

enzyme displayed two pH optima at pH 3 and pH 6–8. At pH 5–6, the normal pH of sponge dough (Johnson and Miller 1953), proteolytic activity was caused by a serine protease.[2] This enzyme displayed optimum activity and stability at pH 6 and 37° C but was unstable at temperatures above 50° C and at pH's above pH 8 and below pH 4. It was inhibited by high concentrations of sodium chloride, was activated by β-mercaptoethanol, and was not affected by ascorbic acid.

Materials

The flour was a commercially milled unbleached, unmalted, medium strength baker's grade bread flour (11.8% protein) purchased from Centennial Flour Mills (Spokane, WA). Compressed yeast (*S. cerevisiae*) was obtained from Fleischmanns (Sumner, WA) and malt from Ross Industries Inc. (Wichita, KS).

Measurement of Activity in a Bread Dough

The effect of proteolysis in doughs may be evaluated by measuring changes in consistency of doughs incubated with proteases (Johnson and Miller 1953), using the Brabender farinograph and mixograph.

A standard method based on Johnson and Miller's (1953) procedure using a 10-g mixograph (Finney and Shogren 1972), was used to assess the activity in bread doughs of protease from *S. carlsbergensis*. Seven grams (70% of total flour, 14% moisture basis) of bread flour was mixed with 2 ml of a 10% yeast suspension, 2 ml of a 10% sucrose solution (0.2 g), 0.5 ml of a 0.5% malt solution (2.5 mg), crude yeast, protease, and enough water to equal 6.8 ml (total liquid). Each dough mixture was stirred for 15 sec to give a uniform slurry. The doughs were then fermented for 3 hr at 30° C and 98% relative humidity. Following fermentation, 3 g (14% moisture basis) of additional flour was added to each dough. The doughs were then transferred to a mixograph bowl and mixed for 7 min.

Doughs were tested with several concentrations of yeast protease ranging from 0 to 3.2 mg. The mixograph curve of an unfermented dough containing no protease was also recorded.

The addition of malt to the dough mixtures served as a control for α-amylase, which may have been present in the protease preparation.

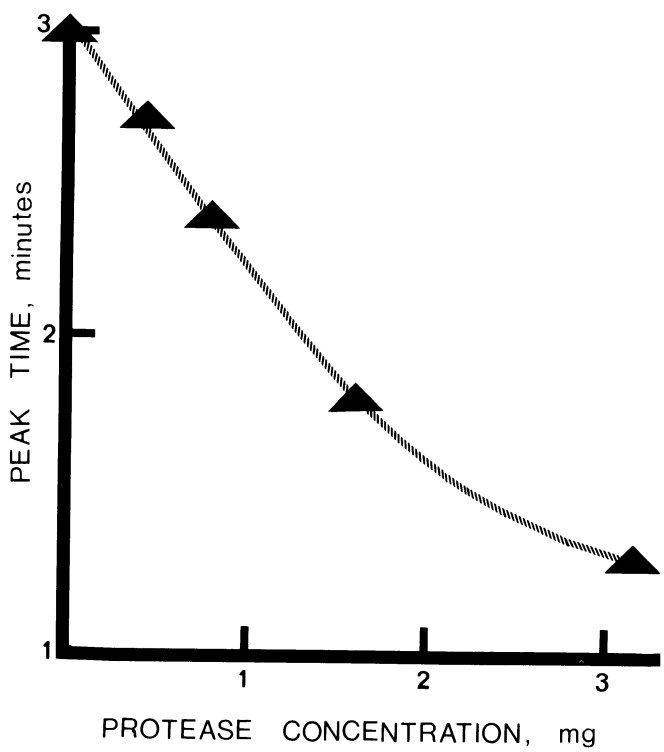

Fig. 2. The effect of protease concentration on time required to attain maximum peak height on mixograph.

RESULTS AND DISCUSSION

The mixograms of doughs fermented with protease from *S. carlsbergensis* are shown in Fig. 1.

The height of a mixograph curve is generally an indication of dough "strength," ie, the ability of its proteins to withstand the stresses of mixing and gas bubble formation during bread fermentation. As increasing concentrations of protease were added to a standard bread dough, mixogram peak height decreased, indicating increased protein breakdown. Peak height decreased 20% when 3.2 mg of crude protease was added to a dough before fermentation (Table I).

The width of a mixograph curve is also an indication of strength and tolerance to mixing. The curve became narrower as protease concentration increased (Fig. 1), reflecting increased protein breakdown.

Peak of mixing time (the time to reach maximum curve height) shortened as the protease concentration was increased, eg, peak time decreased about 60% when 3.2 mg of protease was added to a dough before fermentation (Fig. 2). This factor is important in evaluating the usefulness of a proteolytic enzyme in bread making because proteases are usually added to bread doughs to shorten mixing time (Barrett 1975, Pomeranz 1966, Waldt 1965).

The protease from *S. carlsbergensis* demonstrated vigorous activity in flour doughs, and at high concentrations it turned a typical standard bread wheat flour into a very weak dough. Its behavior resembled that of protease preparations from *A. oryzae* (Johnson and Miller 1953).

A. oryzae preparations contain a mixture of proteases, one of which is a serine protease (Matsubara and Feder 1970, Nakagawa 1970), as is the major protease from *S. carlsbergensis*.[2] Proteolysis in *A. oryzae* preparations is inhibited by the sodium chloride (Miller and Johnson 1948), thus resembling preparations from *S. carlsbergensis*.[2] The serine protease from *A. oryzae* is stable in the pH range 5–8.5 (Nakagawa 1970), whereas the pH stability range of the serine protease from *S. carlsbergensis* falls between pH 4 and 8.[2] Because the enzymes from these two organisms display some similar characteristics, similar behavior in doughs is plausible, and future studies should include a comparison of the relative activities of these two enzymes on dough and bread quality. The availability of large quantities of brewer's yeast may provide an excellent additional source of inexpensive protease for bread making.

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[Received November 15, 1979. Accepted February 4, 1980]