PROPERTIES OF WHEAT FLOUR PROTEINASES[1]

C. E. McDONALD[2] AND LORA L. CHEN

ABSTRACT

Much of the proteolytically active material in wheat flours is extracted by water adjusted to pH 8 or by acetate buffer at pH 3.8. The activity optimum on hemoglobin substrate is pH 3.8 for extracted enzyme. Unextracted proteinase has an activity optimum at pH 4.4. This insoluble material was not extractable in active form by acetate buffers (0.1–0.4*M*, pH 4.6, or 0.1*M*, pH 3.8), 0.2*M* NaCl at pH 7.5, borate-acetate buffer (pH 8.2), or 10% sodium chloride (pH 5.8). Self-digestion of flour showed a pH 4.0 optimum, with activity much lower than that observed with hemoglobin as substrate. Both dough-mixing and the presence of sodium chloride substantially reduced the proteolytic activity of flour. Experiments with sulfhydryl-blocking reagents indicate that papainase-type enzymes account for only part of the proteolytic activity of flour. Several trypsin or chymotrypsin inhibitors were ineffective against the flour proteinases.

Although a number of papers have been published in the last 30 years on the proteolytic effects produced in bread dough by oxidizing agents, reducing agents, and added proteinases, only a few studies have been reported on the properties of wheat flour proteinases themselves. Some workers (1,2) have theorized that oxidizing and reducing agents affect dough properties by inhibiting and activating, respectively, a natural papainase-type enzyme in flour. A papainase type of enzymatic material has been prepared from wheat bran and flour (3,4). However, the presence in flour of proteinases other than the papainase type has also been reported (5). These reports indicate that both types of enzymes are probably present.

Whatever the nature of the proteinases in wheat flour, their action could be very important for proper dough development during mixing and fermentation. Enzymatic splitting of only a few strategic peptide bonds of flour proteins might significantly alter the ability of the flour to produce normal doughs. The proteolysis might directly affect the physical properties or might allow sulfhydryl and disulfide groups along the split peptide chains to come together more freely for oxidation and interchange reactions to produce a protein network throughout the dough. More knowledge of the properties of flour proteinases would be valuable for ascertaining their effects on normal dough de-

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[2]Present address: North Dakota State University, Fargo.

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velopment. This paper presents results of a study on the proteolytic activities of wheat flour and its extracts.

Materials

Flours. Unbleached hard red winter (HRW) and hard red spring (HRS) patent flours, a soft commercial whole-wheat flour, and a ground hard red winter wheat were studied. All samples were stored at −18°C. or 1°C. prior to use. Sample designations and protein contents are in Table I.

TABLE I
FLOUR SAMPLES USED

TYPE OF WHEAT	CROP YEAR	PROTEIN (N × 5.7) AT 14% MOISTURE
		%
Commercial HRS-56	1956	15.1
Commercial HRS-58	1958	13.4
Commercial HRS-62	1962	14.6
Kansas 322, HRW (Kaw)[a]	1962	10.3
Texas 327, HRW (unnamed)[a]	1962	13.5
Commercial HRW-63	1963	10.7
Commercial soft whole wheat	...	10.3
Commercial HRW whole wheat[b]	1962	12.2

[a] Hard Red Wheat Quality Council samples.
[b] Ground through Wiley mill to pass 40-mesh.

Chemicals. Sodium *p*-chloromercuribenzoate and *p*-chloromercuriphenyl sulfonic acid were obtained from Sigma Chemical Co.; *p*-chloromercuric benzoic acid from Aldrich Chemical Co., Inc.; and N-ethylmaleimide from Schwarz Bioresearch, Inc. Iodoacetamide was a laboratory preparation (m.p. 94°C.) that was made available to us. *p*-Nitrophenyl trimethylacetate was synthesized (6). Hemoglobin was purchased from Difco Lab.; phenol (Folin) reagent from Van Waters and Rogers, Inc.

Natural Inhibitors and Enzymes. Crystalline soybean trypsin inhibitor and ovomucoid were purchased from Worthington Biochemical Corp.; lima-bean trypsin inhibitor from Sigma Chemical Co. Whole-wheat trypsin inhibitor was prepared according to the method of Shyamala *et al.* (7). Crystalline chymotrypsin inhibtor from potatoes was prepared by Ryan and Balls (8). Twice-crystallized pepsin was obtained from Worthington Biochemical Corp.

Methods

Extraction of Proteolytic Activity. Extracts of flour were prepared by sieving flour into well-stirred extractants (20 g. flour to 80 ml. extractant) and stirring the mixture for 30 min. Extractants used were

water at pH 5.8 and 8.0, various acetate buffers, and 10% sodium chloride solution. For extractions at pH 8, a mixture of distilled water and flour was adjusted to pH 8.0 by addition of 0.1*N* sodium hydroxide before extraction by stirring was continued. At pH 5.8 no adjustment of pH was necessary. The extraction mixtures were centrifuged 20 min. at 1,000 × *g* to remove insoluble material. When sodium chloride solutions or borate-acetate buffer (pH 8.2) was the extractant, the salts were removed from the extract by dialysis (1°C.) before the extract was assayed.

Flour residues after extraction of soluble enzyme were prepared for assay of proteolytic activity by freeze-drying and grinding in a Wiley mill to pass a 40-mesh sieve or by simply homogenizing the wet residue in a Servall Omni-mixer.

Proteinase Assay. Enzyme activities on hemoglobin substrate in 0.10*M* sodium acetate buffers were determined by a modification of the Ayre-Anderson method (9) which was shortened in the following ways. Digestions were conducted for 2 instead of 4 hr.; instead of Kjeldahl nitrogen analyses for digested material made soluble in trichloroacetic acid by proteolysis, the colorimetric Folin method of Lowry *et al.* (10), as described by Cowgill and Pardee (11), was used after 20-fold dilution of digestion filtrates. In addition to producing color from tyrosine and tryptophan residues, this reagent produces color from peptides that do not contain these residues. It was found necessary to allow the sample and the Folin reagent to develop for 1 hr. to obtain maximum color at room temperature. When trichloroacetic acid filtrates from digestions were cloudy, they were clarified by heating to boiling. The digestion mixtures contained $10^{-3}M$ cysteine and $10^{-4}M$ versene to activate papainase-type enzymes. The presence of more cysteine or none at all made very little difference in the activity of several wheat flours tested.

Activity of flour proteinases against flour proteins themselves (self-digestion) was determined as described above, but in the absence of hemoglobin substrate. Because of low activity, however, 4-hr. digestions were necessary.

The activity of crystalline pepsin was also determined by our modified Ayre-Anderson method, but hemoglobin substrate acidified with hydrochloric acid to pH 1.8 (the optimum pH for pepsin activity) and a 20-min. digestion period were used.

Units of proteinase activity were calculated from a standard tyrosine curve (0.02–0.24 μmoles). The results of the Folin analysis of trichloroacetic acid filtrates were first expressed as total increase in tyrosine resulting from enzyme activity. This increase in tyrosine is linearly

related to quantity of enzyme when pepsin activity is determined, but not when flour proteinases are assayed. If these data with flour proteinases are raised to the $\frac{3}{2}$ power, as suggested by Miller and Johnson (12), the activity can be related to enzyme quantity in a linear manner. Proteinase units for flour enzymes are then calculated as the number of $\frac{3}{2}$-transformed micromoles of tyrosine that are formed per min. With pepsin, the units are simply expressed as the number of μmoles of tyrosine formed per min. This unit of proteinase activity agrees with that recommended in the report of the 1961 Commission on Enzymes of the International Union of Biochemistry (13).

Inhibitor Studies. For the sulfhydryl-blocking experiments on flours, a mixture containing 5 g. flour, 0.7 g. hemoglobin, and 3 g. pumice (and 0.23 meq. acetic acid in the case of experiments conducted at pH 6.2) was mixed well with 22 ml. of inhibitor solution. The mixture was allowed to stand at room temperature for 30 min., with swirling every 10 min. In studies using flour improvers and inhibitors of trypsin and chymotrypsin (at pH 5.8), 22 ml. of inhibitor solution was allowed to stand with flour only. Then 0.24 meq. of acetic acid (to prevent a rise in pH on addition of hemoglobin) and a mixture of 0.7 g. hemoglobin and 3 g. pumice were added just before the assay was started. After this mixture had been warmed to 40°C. in a water bath, the assay was started: 2.5 ml. of 1.0*M* sodium acetate was added to adjust the pH to 3.8 or 4.6 (whichever is optimum for activity of the flour proteinases being assayed).

The effects of enzyme inhibitors on proteolytic activities of flour extracts were also studied. In these experiments the inhibiting agent was dissolved in about 45 ml. of extract, and the inhibitor-enzyme solution allowed to stand 30 min. at room temperature. Then 5.2 ml. of 1.0*M* acetate buffer (pH 3.6 or 4.5) was added, and the solution was made up to 52 ml. with water. The solution was warmed to 40°C., and 25-ml. aliquots of it were added to mixtures containing 0.7 g. hemoglobin and 3 g. pumice to begin the proteolytic assay.

When the inhibition by sodium chloride was studied, the salt was simply dissolved in the acetate buffer or extract just before the assay and this was added to the hemoglobin substrate to begin the assay.

The amounts of inhibitors used in the sulfhydryl-blocking experiments represented more than a threefold excess over the total amount calculated to be required to react with all the sulfhydryl groups present in the 5 g. flour (5 μeq. –SH) and 0.7 g. hemoglobin (8 μeq. –SH) used in the assay.

Appropriate controls containing everything but the inhibiting agents were run simultaneously with inhibitor-treated samples to cor-

rect for any loss in activity not due to the inhibitor. When sulfhydryl-blocking reagents were tested, cysteine was added only to the control (containing no inhibitor) just before assay to activate papainase-type enzymes.

Stability Studies. To study flour proteinase stability at 40°C., 5-g. samples of HRS-62 flour were suspended in 22 ml. of distilled water (pH 5.7), 0.0087*M* acetic acid (pH 4.7), or 0.0043*M* sodium hydroxide (pH 7.2). The suspensions were kept at 40°C. in a shaking water bath for 60 min. and then were assayed for proteolytic activity.

For experiments on heated extracts prepared at pH 8, aliquots were adjusted to pH 5.8 or 4.7 with acetic acid. These solutions were heated in Erlenmeyer flasks in a 90°–95°C. bath, held at the desired maximum temperature for either 1 or 4 min., and then quickly cooled in a water-ice bath.

In studies on the stability of proteinases to mixing, the farinograph mixer was used to make flour-water doughs. Mixed doughs were freeze-dried, ground in a Wiley mill to pass through a 40-mesh sieve, and assayed for proteinase activity.

Calculation of Activation Energies. The activation energies for proteolysis were calculated according to the integrated Arrhenius equation:

$$\ln \frac{k_2}{k_1} = \frac{A}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$$

where k_1 and k_2 are reaction rate constants at absolute temperatures T_1 and T_2, A is the activation energy in cal. per mole, and R is the gas constant (1.99 cal. per degree per mole). A was determined from the slope of the line obtained by plotting k against 1/T. The slope is equal to A/2.30R or A/4.58.

Results

Proteolytic Activities of Extracts and Flours. Table II lists the activities extracted from five patent flours with distilled water (pH 5.8), with flour-water mixture adjusted to pH 8.0, and with pH 3.8, 0.10*M* sodium acetate buffer, as well as the activities of the five flours themselves. Assays for proteolytic activity were run at pH 3.8 after that pH was found to be optimum for proteinases in a pH 8 extract of HRS-56 flour with hemoglobin as the substrate. Material extracted by distilled water gave only 11 to 25% of the proteolytic activity of the whole flours. Material extracted by water adjusted to pH 8 or by acetate buffer at pH 3.8 gave 62 to 100% of the whole-flour activity, measured at pH 3.8. Also, 10% sodium chloride ($3\times10^{-4}M$ in cysteine) was found to be a good solvent for extracting proteinase from HRS-62 flour.

TABLE II

PROTEOLYTIC ACTIVITY IN WHOLE FLOUR AND FLOUR EXTRACTS
(Measured at pH 3.8)

SOURCE	WHOLE FLOUR	EXTRACTS PREPARED WITH:		
		Distilled Water, pH 5.8	Water with NaOH, pH 8	Acetate Buffer, pH 3.8
	units[a]	*units*[a]	*units*[a]	*units*[a]
HRS-56	1.8	0.2	2.2	1.8
HRS-58	3.4	0.47	3.1	2.1
HRS-62	4.0	1.0	3.1	3.3
Kansas 322	3.0	0.33	2.3	2.8
Texas 327	3.2	0.43	2.5	2.8

[a] Units of activity per 5 g. flour or 20 ml. extract. (See "Methods" section for definition of "units of activity.") Hemoglobin was the substrate.

The level of activity of flour proteinase at pH 3.8 is such that 5 g. of flour gives activity equivalent to 70–130 μg. of crystalline pepsin at its pH optimum of 1.8.

The material extracted from flour by water at pH 8 can be freeze-dried with little loss of proteolytic activity. This preparation is a con-

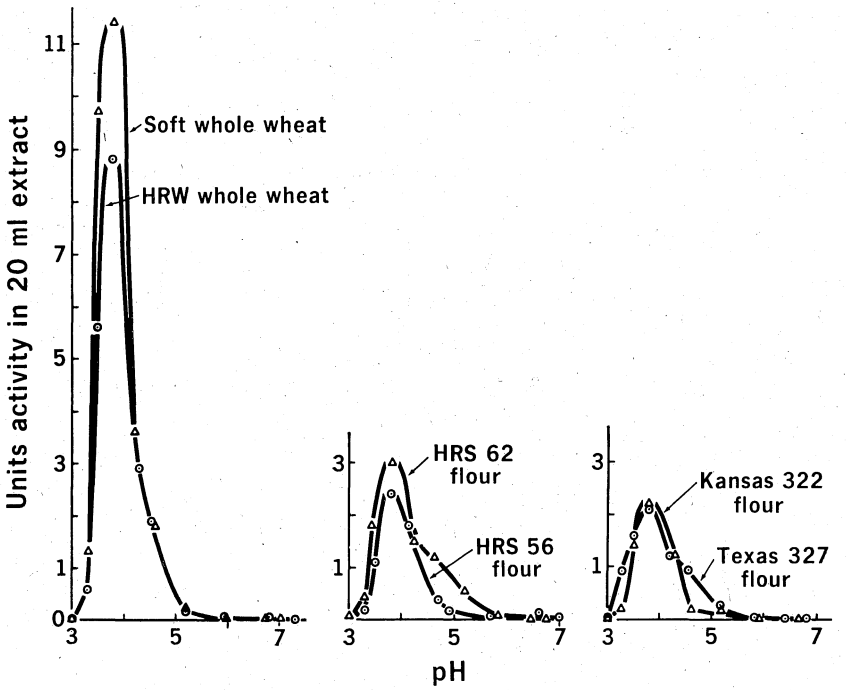

Fig. 1. Proteolytic activity (at various pH levels) of flour extracts (extracted at pH 8). Hemoglobin was the substrate. (See "Methods" section for definition of "unit of activity.")

venient material for use in further studies. Both freshly prepared and freeze-dried extracts were used in our studies.

Activities of flour extracts prepared at pH 8 and measured in acetate buffer at various pH's are shown in Fig. 1. Proteinase activity in the extracts was optimum at pH 3.8: at that pH, extracts of whole wheat (soft and HRW) had threefold to fivefold the activity of extracts of milled patent flours. Shoulders on the activity curves for extracts of HRS-62 and Texas 327 flour suggest the presence of a second proteinase with an activity optimum near pH 4.6.

The effect of pH on self-digestion of two patent flours is shown in Fig. 2. The optimum activity was near pH 4.0 but was only about 17%

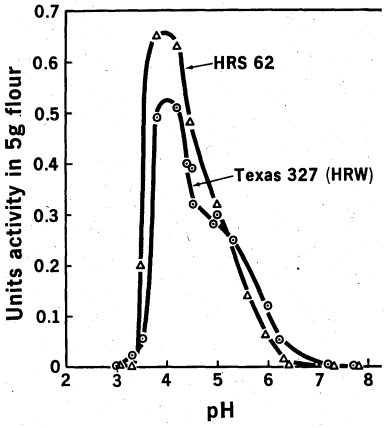

Fig. 2. Self-digestion of flour as a function of pH. (See "Methods" section for definition of "unit of activity.")

of that observed with hemoglobin as substrate (Table II). Above pH 4, activity dropped, and only about one-fifth of the activity observed at pH 4.0 was evident at the natural pH of flour (about pH 5.8).

Unextracted Proteolytic Activity. In other studies with hemoglobin as substrate, an unexpected high activity was observed at pH 4.6 for HRS-62 flour itself (pH-activity curve, Fig. 3). The pH optimum was 4.4 instead of the pH 3.8 optimum observed for extracts. The activity was five times that in extracts of HRS-62 flour assayed at pH 4.4 and twice that of the extract assayed at its pH optimum, 3.8. Obviously, extracting at pH 8 either failed to solubilize or else resulted in the denaturation of a considerable amount of flour proteinase in HRS-62 flour. For convenience this will be referred to as unextracted enzyme. A hypothetical curve for unextracted enzyme (Fig. 3) was obtained by subtracting the extract curve from the flour curve. The pH optimum for unextracted enzyme, as for flour, was 4.4.

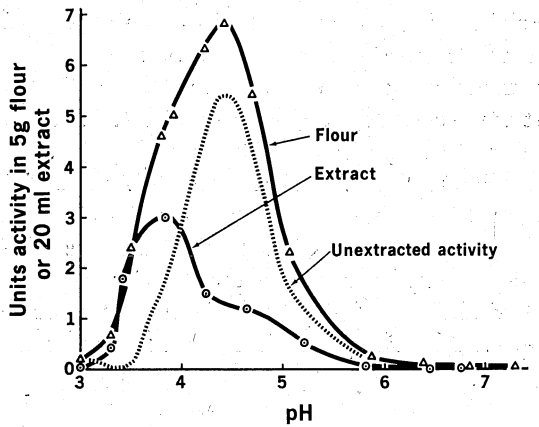

Fig. 3. Effect of pH on activity of HRS-62 flour, its pH 8 extract, and unextracted proteinase. (See "Methods" section for definition of "unit of activity.")

The presence of unextracted enzyme in other flours is indicated by data in Table III. Whole-flour activity (measured at pH 4.6) in excess

TABLE III
PROTEOLYTIC ACTIVITY IN WHOLE FLOUR AND FLOUR EXTRACTS
(Measured at pH 4.6)

SOURCE	WHOLE FLOUR	pH 8 EXTRACT
	units [a]	*units* [a]
HRS-56	3.1	0.6
HRS-62	5.8	1.2
Kansas 322	3.3	0.3
Texas 327	2.9	0.9
HRW-63	2.4	0.5
Soft whole wheat	7.1	1.7
HRW whole wheat	4.7	1.7

[a] Units of activity per g. flour or 20 ml. extract. (See "Methods" section for definition of "unit of activity.")

of that observed for extract is assumed to be the activity of unextracted enzyme and represents 70 to 90% of the total. Only the soft wheat-flour showed more unextracted enzyme than did HRS-62 flour.

Attempts to solubilize the unextracted enzyme in HRS-62 flour were unsuccessful, either with acetate buffer (0.1–0.4*M*, pH 4.6; 0.1*M*, pH 3.8; and 0.4*M*, pH 7.5) or with cold (1°C.) 10% sodium chloride (pH 5.8, $10^{-4}M$ cysteine). Removal of lipids from the flour with ethyl ether or n-butanol to help free any bound enzymes did not aid in solubilizing this enzyme. The sodium chloride (0.2*M*, pH 7.5) or borate-acetate buffer (pH 8.2), used by MacDonnell *et al.* (14) for extracting cell-wall-bound pectinesterase of orange, did not release active proteinase.

Extraction of soluble enzyme of HRS-62 flour with water adjusted to pH 8 or pH 3.8, 0.10*M* acetate buffer resulted in flour residues that exhibited no unextracted activity. This effect was observed in both wet and freeze-dried residues. Adding the extracts back to the flour residues restored a small portion of the activity of the unextracted proteinase. Freeze-drying of flour residues before assay does not appear to cause this loss, since freeze-drying of HRS-62 flour that was completely wetted with water resulted in only a small loss of extractable activity, and undried wet residues already showed the loss. Other methods for extraction of this activity must be sought.

Effect of Temperature and Dough Mixing on Activity. The curves in Fig. 4 indicate the stability of proteinases in HRS-62 flour and extract under the conditions used in our Ayre-Anderson assay. Proteinases of flour assayed at pH 4.6 (80% unextractable enzyme) appear to be denatured above 42°C.; and those of the extract assayed at pH 3.8, above 45°C.

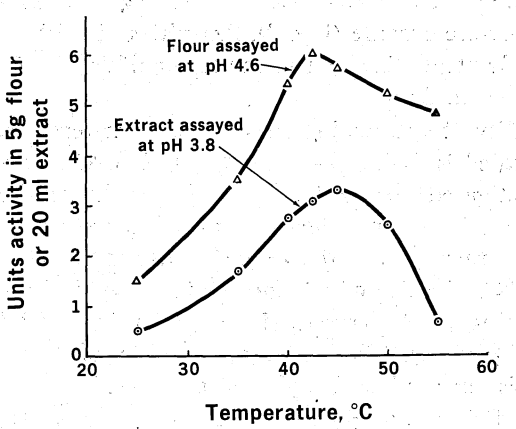

Fig. 4. Activity of HRS-62 flour and extract as a function of temperature (hemoglobin as substrate).

The data obtained at 25° to 40°C. were applied in the Arrhenius equation to calculate the activation energies for the proteolysis reactions catalyzed by the flour proteinases. A value of 12,000 cal. per mole was found for the proteinases of the flour assayed at pH 4.6, and 14,000 cal. per mole for the extract assayed at pH 3.8. Johnson *et al.* (15) have reported 10,700 cal. per mole as the activation energy for proteinases of patent flour assayed at pH 4.7 by an Ayre-Anderson method.

The stability of proteinases in HRS-62 flour suspended in water 60 min. at 40°C. is indicated by the data of Table IV. This temperature is somewhat higher than that customarily used in dough fermen-

TABLE IV

INACTIVATION OF PROTEOLYTIC ACTIVITY IN HRS-62 FLOUR-WATER SUSPENSIONS HELD 60 MIN. AT 40°C.

pH OF SUSPENSION	INACTIVATION	
	At pH 3.8	At pH 4.5
	%	%
4.7	11	25
5.7	14	28
7.2	29	48

tation (36°C.) but not high enough to cause gelatinization of the starch in flour. The activity at pH 4.5, which is predominantly from unextracted enzyme (Table III), was inactivated to about twice the extent that the pH 3.8 activity was. About 65% of the latter is attributable to extractable enzyme (Fig. 3). Activities of both types of enzyme were less stable at pH 7.2 than at the lower pH's of 5.7 and 4.7, which are encountered in doughs.

The stability of extractable enzymes at high temperature — such as that encountered in baking — was also studied. Unextractable enzyme was not investigated because flour must be used, and the thick gelatinized starch that forms upon heating interferes with the assay. Heating aliquots of extract (pH 8) that were adjusted to pH 5.8 and 4.7 for 4 min. at 70°C. inactivated 34 and 39%, respectively, of the enzymes present. Heating an aliquot at pH 4.7 for 1 min. at 90°C. caused complete loss of activity. Thus, the extractable proteinases show only moderate resistance to heat-inactivation.

Stability of flour proteinases in HRS-62 flour-water dough during mixing was investigated. Mixing in a farinograph for 2 and 15 min. past peak time (6 min.) caused, respectively, 18 and 22% loss, of the activity measured at pH 3.8, and 26 and 32% loss of activity measured at pH 4.5.

Enzyme Inhibitor Studies. Experiments were conducted to determine the extent of inhibition of flour proteinases by chemical agents and natural proteinase inhibitors. To detect the presence of papainase-type enzymes, several flours and extracts were treated with sulfhydryl-blocking reagents, which inactivate these enzymes (results, Table V). Of the reagents tested, *p*-chloromercuribenzoate at pH 7.7 and *p*-chloromercuriphenyl sulfonic acid at pH 6.2 produced the most inhibition. The benzoate could not be tested at pH 6.2 because it is not soluble at that pH. N-Ethylmaleimide and iodoacetamide caused much less inhibition of proteinase activity, having almost no effect on activity measured at pH 3.8. Whole-wheat flours, in comparison with milled flours, did not show a markedly greater amount of inhibition, as might

TABLE V
INHIBITION BY SULFHYDRYL-BLOCKING REAGENTS

INHIBITOR PER 5 G. FLOUR OR 20 ML. EXTRACT			ENZYME SOURCE	INHIBITION	
				At pH 3.8	At pH 4.5
	pH	*μmoles*		%	%
p-Chloromercuribenzoate	7.7	47	HRS-62, flour	29	43
			HRS-62, extract	24	..
			HRW-63, flour	18	29
			HRW-63, extract	32	..
p-Chloromercuriphenyl sulfonic acid	6.2	47	HRS-62, flour	7	34
			HRW-63, flour	19	25
			Whole wheat HRW, flour	28	20
N-Ethylmaleimide	6.2	47	HRS-62, flour	0	9
			HRS-62, extract	0	..
			HRW-63, flour	5	18
			Whole wheat HRW, extract	4	..
Iodoacetamide	6.2	96	HRS-62, flour	0	15
			HRW-63, flour	0	4

be expected if the proteinases of the germ and bran were completely of the papainase type.

The effect of the flour improvers potassium bromate and iodate on proteolytic activity was also studied. These agents should inactivate papainase-type enzymes by oxidation of essential sulfhydryl groups present in the enzyme molecule. Treatment of HRS-62 and HRW-63 patent flours with 0.010*M* potassium bromate for 30 min. at room temperature caused no loss of proteolytic activity measured at pH 3.8 or 4.5. On the other hand, potassium iodate caused 9% loss of pH 3.8 activity in HRW-63 flour under identical conditions, no loss of activity at pH 3.8 in HRS-62 flours, and 20% loss of activity at pH 4.5 in both flours.

Trypsin and chymotrypsin inhibitors were not effective against flour proteinases. Trypsin inhibitors tested were those from soybean, lima bean, whole wheat, egg (ovomucoid), and potato. *p*-Nitrophenyl trimethylacetate was also tested. The last two inhibit both trypsin and chymotrypsin.

Sodium chloride has been reported to inhibit flour proteolytic activity (9). It is, of course, a common ingredient in dough. With salt at the concentration normally used in dough (0.05*M*) and with the buffers used for proteolytic assays, 88% of the activity (pH 3.8) in an HRS-62 extract was lost. With whole flour and with salt at the same concentration, 74% of the activity was lost at pH 3.8 and 32% at pH 4.5. Thus, salt shows marked inhibition of flour proteinases.

Discussion

A large portion of the material in wheat flour that exhibits maximum proteolytic activity at pH 3.8 is readily extracted by water adjusted to pH 8 or by pH 3.8 acetate buffer, but not by distilled water (at pH 5.8). The active materials extracted both from patent flours and from whole-wheat flours show an activity optimum at pH 3.8. The whole-wheat flours, which contain bran and germ, have much greater activity at pH 3.8 than have the patent flours. Thus, enzymes from the bran and germ should also have a pH 3.8 optimum.

Flour also contains proteolytically active material that was not extracted in active form by any of the methods used in this study. This activity is optimum at pH 4.4, and the total activity is usually as great as, or greater than, that of the extractable material. This enzyme, which we have called unextracted proteinase, differs from that of the extractable material in the following ways. It is less stable at 40°C. It is, in most cases, more completely inhibited by sulfhydryl-blocking reagents. It is less completely inhibited by sodium chloride. The activity of the unextractable material is not detectable in the flour residue after removal of extractable enzyme, but this loss of activity does not seem to be due to the removal of a cofactor that might be required for activity. So far as we know, the presence in wheat flour of extractable and unextractable types of proteinases has not been reported previously.

The action of mercury-type sulfhydryl-blocking reagents indicates that a papainase-type enzyme is present in both extractable and unextractable forms. However, results with the sulfhydryl-blocking reagents N-ethylmaleimide and iodoacetamide indicate that only the unextractable enzymes include any of the papainase type. Potassium bromate and iodate gave different results in that bromate caused no inhibition, whereas iodate caused some inhibition in both extractable and unextractable enzyme. This difference may be due to the fact that iodate reacts rapidly in flour, whereas bromate reacts slowly (16,17). Conflicting data with sulfhydryl-blocking reagents and flour improvers may also be due (at least in part) to loss of activity due to changes in the tertiary structure of the enzyme molecules resulting from reactions that take place with sulfhydryl groups in general. Such a phenomenon has been reported with phosphorylase (18,19) when *p*-chloromercuribenzoate, methyl mercuric salts, and iodoacetamide were used as inhibitors.

Self-digestion of flour shows an activity optimum at pH 4.0, indicating that proteolysis by the natural enzymes would occur more rapidly after the pH of dough has been lowered by fermentation than

during mixing. On the other hand, inhibition caused by the presence of salt and loss of activity due to mixing would tend to reduce the extent of proteolysis in doughs.

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