Dried Japanese Noodles. II. Effect of Amylase, Protease, Salts, and pH on Noodle Doughs[1]

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ABSTRACT

Malted wheat flour extracts, treated to inactivate amylase or protease, were added to Japanese-type noodle doughs. Both α-amylase and protease-active fractions produced sticky and too-extensible doughs. Heat-stable water-solubles from malted wheat flour also contributed to dough softening. However, one wheat α-amylase fraction isolated from malted wheat flour did not modify raw noodle doughs, in contrast to its activity on gelatinized and soluble starch. Of several improving agents, the more effective suppressed protease activity and/or strengthened dough structure. Sodium chloride and trisodium phosphate, both in use in Japan in various noodle products, restored dough properties nearly to normal when sprout damage approximated the effects of adding 0.5% malted wheat flour (falling number 180). Disodium phosphate, permitted in macaroni products in the U.S., also improved noodle dough properties. However, amylograph hot-paste viscosities were increased relatively little by these salts at concentrations below 3%, and most of the increases were accounted for by their effects on pH. Sodium phytate likewise had a small effect. Their improving effects on noodle dough properties thus could not be attributed to inhibition of α-amylase. Conversely, the calcium chelating agent, disodium EDTA, was an effective amylase inhibitor but required high levels to modify noodle dough properties. Increased α-amylase activity is associated with sprouted and otherwise field-damaged wheats, but the principal deleterious effects on noodle dough properties may arise from changes in other components.

Problems in the use of sprout-damaged wheats are generally attributed to excessive α-amylase activity during processing of the flour into a finished product. With most products, starch gelatinized during baking is susceptible to amylase attack until the enzyme is heat-inactivated. In the processing of raw Japanese

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[1]Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

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noodle doughs, amylase activity must be limited by the small amount of mechanically damaged starch available as a substrate and the relatively short time needed to process and dry the raw dough. Minor effects of α-amylase on dough properties might therefore be expected.

In part I of this report (1), flours with relatively low levels of amylase activity were nevertheless shown to cause problems in the processing of noodle doughs. Several observations suggested that protease activity may also be involved. These include the stretchy quality of the doughs made from sprout-damaged wheat and the apparent improvement in dough properties when salt level is increased. The relative effects of amylase and protease on noodle doughs and ways to inhibit or counteract their activity are considered in this paper.

MATERIALS AND METHODS

The commercially milled flour and field-damaged Burt sample were described in part I (1), as were noodle preparation and amylase activity determinations.

Enzyme Preparations

Protease or amylase in a malted wheat flour extract was differentially inactivated by the procedures of Miller and Johnson (2). Protease was determined by a cathepsin method (3). Extract A (Table I) was an unheated 0.01M calcium acetate extract. The amylase-active extract B is a portion of extract A adjusted to pH 9.4, heated 30 min. at 50°C. to inactivate protease, then restored to pH 5.7. The protease-active extract C was a water extract of malted wheat flour, adjusted to pH 3.9, heated 30 min. at 50°C., then restored to pH 5.7. These extracts were added as part of the absorption water to noodle doughs at a level equivalent to the addition of 0.5% malted wheat flour (noodle flour basis).

A purified α-amylase fraction was also prepared from malted wheat flour. Extraction, heating, and ion-exchange separation on DEAE cellulose were done

TABLE I. EFFECT OF SELECTED ENZYME FRACTIONS ON
NOODLE DOUGH PROPERTIES

Major Activity	SKB units	SKB in Flour units	Falling Number sec.	Relative Protease Activity %	Noodle Dough Properties Handling	No. fell[a]	Sag in.
A. Differential Inactivation							
Malted Wheat Flour Extract[b]							
A Unheated	70	0.35	180	100	Soft, sticky	11	3
B Amylase-active	41	0.2	220	10	Soft, sticky	12	2.5
C Protease-active	---	---	455	65	Slightly soft	6	2
B. Isolated Fraction							
Isolated amylase fraction		0.45	196		Pliable Similar to sound control	0	1.5
Sound control		---	452		Pliable	0	1.5

[a]Of 12 total.
[b]Equivalent to that extracted from 0.5% malted wheat flour.

according to the method of Kruger and Tkachuk (4). Then gel-permeation chromatography on Sephadex G-50 and G-100 was used to separate the most active amylase fraction. It was added to flour at a level (0.45 SKB units) that gave a falling number of 196.

Aspergillus oryzae (fungal) α-amylase and *Bacillus subtilis* (bacterial) α-amylase were both A-grade products purchased from Cal Biochem Company. Swine pancreatic α-amylase was purchased from Worthington Biochemical Corporation.

Additives were dissolved in the absorption water prior to adding to doughs; pH was measured directly on the noodle doughs, using a Beckman Zeromatic pH meter and glass electrode.

RESULTS AND DISCUSSION

Differential Inactivation

Extracts of malted wheat flour were treated as described to reduce selectively either protease or amylase activity. Pertinent results obtained with these extracts in noodle doughs are summarized in Table I-A. Extract A had significant protease and amylase activity and adversely affected the noodle doughs which contained 1% salt. Extract B, treated to inactivate protease, retained significant amylase activity with only 10% residual protease activity. The SKB value was reduced from 70 to 41 units in the extract. This was equivalent to 0.35 to 0.2 units (flour basis) when added to noodle doughs as described above. This lower activity was sufficient to reduce the falling number to 220 from a sound control of 452. The noodles containing this extract were soft and sticky, similar to those obtained with the unheated extract A.

The protease-active extract C contained 65% residual protease activity and no measurable amylase activity in SKB and falling number tests. Addition of this extract gave doughs less sticky than those containing unheated extract A, but the soft, stretchy quality remained, suggesting some effects of protease.

Inactivating either protease or amylase improved noodle dough properties without completely regaining those of control doughs, so both enzymes may be factors. In addition, all three extracts would contain some non-enzyme solubles. To check the possibility that they may affect dough properties, extracts were heated to boiling, centrifuged, and the supernatant added to dough at the equivalent of 0.5 and 2.5% malted wheat flour levels (one and five times the levels used in A, B, and C). The doughs were somewhat similar to those containing the protease active extract (C), with no change in behavior when five times the extract was used. Thus soluble, heat-stable components of the malted wheat flour may also soften doughs. No further assessment has been made of this factor, since the extracts were prepared from malted wheat flour which may supply an array of solubles not represented in flours milled from slightly sprouted wheats.

To evaluate further the role of α-amylase in the system, results are shown for a purified α-amylase fraction, prepared from malted wheat flour by the Kruger and Tkachuk method (4) with modifications noted earlier (Table I-B). The fraction gave the flour a fallling number of 196, and measured as 0.45 SKB units. It had no effect on noodle doughs. In other words, this fraction could effectively attack soluble starch as in the SKB method, gelatinized starch as in the falling number method, but could not alter the starch in raw dough sufficiently to produce adverse effects in noodles in the short time needed to make and dry them (about 30 min.).

TABLE II. EFFECTS OF PHOSPHATE ON NOODLE DOUGH
PROPERTIES OF A FIELD-DAMAGED WHEAT[a]

Salt Concentration M	% of flour	Noodle Dough Properties			
		pH	Handling	No. fell[b]	Sag in.
Control		6.0	Soft, sticky	11	4
Na_3PO_4					
0.01	0.05	6.6	Somewhat dry	11	3
0.03	0.16	7.4	Somewhat dry	0	2.75
0.05	0.27	7.9	Dry and crumbly	0	1.25
pH 6.0 phosphate buffer					
0.1	0.42	6.1	Soft	9	3
0.3	1.26	6.1	Firm	0	1.875

[a]Burt variety sample B, 8.4% protein, FN = 206.
[b]Of 12 total.

The chromatographic preparation of α-amylase yielded several fractions of differing activities. The fraction used in this study was the most active by the SKB assay. It is possible that other fractions might produce different results that would be more related to the noodle dough problems. However, the differential inactivation experiments do suggest that amylase is only one factor. Other experiments in this laboratory designed to inactivate amylase with classical amylase inhibitors (silver nitrate or mercuric chloride) or phospholipids did not satisfactorily restore the noodle dough properties, suggesting further that amylase activity, per se, was not the only factor. Canadian workers have reported similar findings (5) using isolated amylase preparations in spaghetti flours mixed in the farinograph and tested for cooking quality.

Commercial α-amylase of fungal, bacterial, and pancreatic origin gave noodle dough properties similar to those obtained with the malted wheat flour amylase-active extracts (A and B in Table I). All three commercial preparations were found to contain protease activity sufficient to contribute to noodle dough behavior problems.

Improvement of Doughs with Additives

It was noted in part I (1) that Japanese noodle-makers often used 2 to 3% salt in noodle doughs and these amounts appear to control problems related to low levels of enzyme activity or sprout damage. They also use trisodium phosphate or a solution of potassium and sodium carbonate in the preparation of certain types of noodles that are sold without drying or are cooked immediately after cutting. These compounds produce a high pH (7.0 to 7.5), a slightly yellow color, and a flavor desired by consumers. It has been reported that trisodium phosphate is sometimes added to dried noodles along with the salt, when the white color is not essential.

We had attempted to inhibit α-amylase activity by forming phospholipid-metal complexes which had been shown in model experiments to reduce α-amylase activity on soluble starch. The inhibition was greater at pH 8 than at natural dough pH (6). However, in the noodle dough system, when the pH was raised above 7.0 with a phosphate buffer, noodle dough properties were improved with or without added phospholipid. When trisodium phosphate was used to raise the dough pH, very

small amounts reduced the amount of sag and eliminated falling. Table II gives results using the flour from Burt variety field-damaged wheat described in part I (1). A 0.03M solution contributed about 0.16% trisodium phosphate (flour basis) and raised the dough pH to 7.4. The doughs felt drier at the high pH and thus easier to handle than the control.

Disodium phosphate was also used to raise the dough pH. Results, not shown, indicated that this compound corrected properties of noodle doughs made from the Burt sample when it was added at levels between 0.5 and 1%. It should be noted that this compound is allowed in U.S. macaroni products at the levels reported here (7).

To test the phosphate effect at a pH closer to flour, a pH 6.0 buffer was used. A 0.3M solution contributes approximately 1.26% phosphate salt and effectively reduced the stretchy and sticky qualities of the dough and eliminated falling (Table II). The sticky quality of the doughs was eliminated with the slightest phosphate addition before the stretchy qualities were corrected.

Both sodium phytate and ethylenediaminetetraacetic acid (EDTA) were found to improve noodle dough properties moderately but at levels (0.5 to 1%) too high to be acceptable in products consumed daily as a major part of the food supply.

Additives as α-Amylase Inhibitors

The improving effect of salts, especially phosphates, on noodle doughs, indicates that protease inhibition and modification of the properties of the flour protein
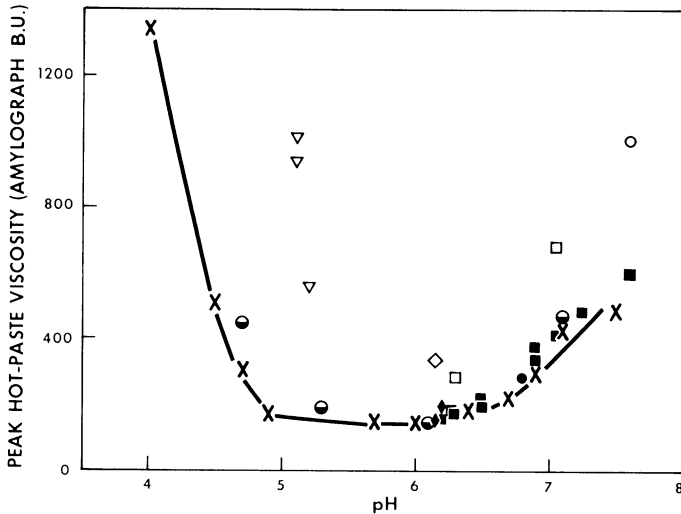

Fig. 1. The effect of pH and various additives on amylograph viscosities (peak hot-paste viscosity) of a flour from field-damaged wheat (Burt, sample B; 65 g. flour, 460 ml. water or solution). The curved line shows the changes produced by adjustment of pH with HCl or NaOH (experimental points X). The effects of low levels of additives are largely accounted for by pH. Major deviations from the line (open symbols) occur only with high levels of additives and the chelating agent EDTA. Identity of additives and level (as % of flour) are as follows: ● = 0.5% sodium phytate; ◉ = 0.7% sodium phytate (pH 7.1, adjusted with HCl); ○ = 1.0% sodium phytate; ■ = <1.0% sodium phosphate (tri, di, or buffer); □ = >8.0% phosphate buffer; ◆ = 1.0 or 3.0% sodium chloride; ▽ = 10% sodium chloride; △ = 0.5, 1.0, and 1.5% EDTA (in order of increasing peak viscosity).

systems were responsible. The effectiveness of phosphates in lowering gluten solubility was observed at least as long ago as 1924 (8), and has been used to precipitate gluten from solution for quantitative determinations (9). In contrast, EDTA had relatively less effect on dough properties although it would be expected to inhibit α-amylase effectively because of its calcium-chelating action (10).

These conclusions are reinforced by observations with the amylograph which indicate that α-amylase inhibition does not account for much of the improving effect of the additives most effective in noodle doughs. Results are shown in Fig. 1 for flours from one of the field-damaged wheats. The curve shows the effect of pH adjustment with HCl or NaOH on peak viscosity. The rapid increase below pH 5 and the more gradual increase above pH 6 are consistent with the measurements of Miller and Johnson (2) of the effect of pH on α-amylase stability in malted wheat flour suspensions, although their temperature and time conditions (20 hr. at 5°C.) were quite different.

The effects of inorganic salts, both sodium chloride and the phosphates, are minor except for those which can be attributed to shifts in pH by the phosphates. Where larger effects are noted the salt concentrations are too high to be of any significance or usefulness in food applications. Even sodium phytate, with adjustments of pH with HCl, gave relatively minor effects, although it was expected to bind calcium. In contrast to all the other additives, EDTA produced a marked increase in viscosity at lower concentrations (whether expressed in percentage or molarity) than the inorganic salts, indicative of strong α-amylase inhibition. However, its effects on noodle doughs was significantly less than those of the inorganic salts.

Comparison of effects in noodle doughs with those in the amylograph is complicated by the wide difference in water content and the resultant changes in additive concentrations or in additive-to-flour ratios. Thus 0.03M Na_3PO_4 used to make up a noodle dough gave a pH of 7.4, with the phosphate amounting to 0.16% of the flour weight. In the amylograph, Na_3PO_4 added at 0.16% of the flour weight would give a concentration, roughly, of only 0.0015M and a pH between 6.9 (obtained with 0.001M) and 7.6 (obtained with 0.002M). However, the lack of marked effect of these concentrations of phosphates in the amylograph then only emphasizes the difficulty of explaining the effects of phosphates on noodle dough properties by α-amylase inhibition.

The reverse situation occurs with EDTA as the additive. Relatively low concentrations of EDTA in the amylograph are effective, while much higher concentrations are needed to affect noodle dough properties—much greater than are needed to chelate all the calcium present.

DISCUSSION

The results with raw noodle doughs show that the factors responsible for defects associated with field-damaged wheat can largely be corrected by salt and pH adjustments. Similar results were found with flours from laboratory-sprouted wheats and with flours containing malted wheat flour. The effects on the raw doughs appear to result from inhibition of proteolytic enzymes, as well as physical modifications of dough components. In noodle doughs, α-amylase activity is so limited that its control has little effect on dough properties. This contrasts with cooked products in which α-amylase activity may be a dominant factor because

gelatinized starch becomes available as a substrate during cooking while α-amylase is highly active. Limited testing of cooked noodles indicated that amylase was not a significant problem unless high levels were present and problems associated with such levels were not counteracted during the noodle-making process e.g., salt or phosphate added. Both improved the cooked noodles made from amylase-active flours. However, in cooked products such as thickened soups and sponge cakes amylase activity from sprout-damaged wheat is known to be a problem. Products, cooked at a pH above 7.0, e.g., layer cakes and some cookies, can be successfully made with slightly sprouted wheat.

Acknowledgments

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