Comparison of Proteolysis in Five Malting Barley Varieties

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ABSTRACT

Proteolytic activity of five malting barley varieties, as measured by the release of formol and nonprotein nitrogen from grain autolyzed with water at 40°C., showed no significant varietal differences. The varieties, therefore, could not be selected on the basis of proteolytic activity. Proteolysis in barley and malt was inhibited by phenylmercuryacetate and activated by thioglycollic acid, indicating the presence in the autolysates of sulfhydryl-type enzymes.

At present the brewing quality of barley is assessed mainly on the results of laboratory analysis of malt; one of the major criterion of such an assessment is the index of nitrogen modification. A good malting barley is considered to produce malt having a high content of wort nitrogen. Wort nitrogen, however, depends on several factors, the most important according to Sandegren (1) are: (a) the quantity of salt-soluble nitrogen compounds of the barley, (b) the proteolytic enzymes, and (c) the cell-wall degrading enzymes. Results of malt analysis may not, however, always correspond to those of barley because of the variations introduced in the malting process. Sandegren and Klang (2), therefore, suggested that determination of proteolytic activity of barley, in conjunction with other analyses, may give a better indication of the suitability of barley varieties for brewing purposes.

It is generally believed that proteolytic enzymes of barley and malt are of the papain-type and are, therefore, inhibited by oxidizing agents and activated by reducing agents (3). Enari et al. (4) had reported that barley and malt contained, in addition to four papain-types of enzymes, an enzyme which resembled animal trypsin in its substrate specificity and active center. Later, Bhatty (5) showed, by use of specific enzyme inhibitors, this enzyme to be another of the sulfhydryl-type of plant proteolytic enzyme.

This report describes a preliminary study conducted to distinguish a number of malting barley varieties on the basis of proteolysis, measured by the release of formol and nonprotein nitrogen from grain autolyzed with water. In addition, the effects of phenylmercuryacetate, an SH-enzyme inhibitor, and thioglycollic acid, an SH-enzyme activator, on proteolysis in barley and malt were studied to show the development of proteolytic enzymes under the experimental conditions described.

MATERIALS AND METHODS

Materials

Five malting barley varieties, Hunter, Mentor, Proctor, Swallow, and Ymer, all

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of the 1965 harvest, and malt prepared from Proctor barley were used in the study. The grain was supplied by a local brewery.

Methods

(a) *Total nitrogen.* Total nitrogen content of the grain was determined by the macro-Kjeldahl method (6).

(b) *Autolysis of grain.* The equivalent of 25 g. dry weight of barley or malt, finely ground by hand in a coffee mill, was mixed in 250-ml. centrifuge bottles with 100 ml. of distilled water heated to 40°C. The mixture was shaken to ensure complete wetting of the ground grain and autolyzed in a constant temperature water bath at 40°C. for t minus 11 min., where t was 30, 60, 120, 240 min. and 11 min. was the time used to centrifuge the mixture. After appropriate time intervals, the bottles were removed from the water bath and centrifuged at 2,500 × g to remove insoluble materials. The supernatant fraction was filtered through glass wool to remove hull fragments. Suitable aliquots of the filtrate (autolysate) were taken in duplicate to determine water-soluble, formol, and nonprotein nitrogen as described below. When the effect of inhibitor or an activator on proteolysis was studied, the grain was mixed with a solution (pH 7.0) of appropriate concentrations of phenylmercuryacetate or thioglycollic acid and the mixture autolyzed as usual. For control, the grain was autolyzed with distilled water.

Water-soluble nitrogen. Water-soluble nitrogen was determined on aliquots of the autolysate by the conventional micro-Kjeldahl method (6).

Formol nitrogen. Fifty milliliters of the autolysate was titrated potentiometrically to pH 8.5 with 0.05N sodium hydroxide and the titer noted (titer a); this titer was taken as the titrable acidity or acidity contributed mainly by carboxyl and other acidic groups present in the autolysate. Then excess (60 ml.) of neutralized formaldehyde (pH 6.8) was mixed in. The addition of formaldehyde caused a fall in the pH of the mixture. After standing 5 min. at room temperature, the mixture was titrated, while being continuously stirred, to pH 8.5 with 0.05N sodium hydroxide. This titer was taken as a measure of the release of amino nitrogen liberated by proteolysis.

Nonprotein nitrogen Twenty-five milliliters of the autolysate was precipitated in a conical flask by adding 2 ml. each of 50% (v./v.) sulfuric acid and 50% (w./v.) sodium molybdate, the precipitating agent being phosphomolybdic acid. After allowing to stand at room temperature for 10 min. to ensure a thorough precipitation of the proteins, the contents of the flask were filtered through Whatman No. 1 filter paper. An aliquot of the filtrate was taken to determine total nitrogen by the micro-Kjeldahl distillation method (6).

RESULTS

Total Nitrogen Content of the Grain

The total nitrogen content of five barley varieties varied from a lowest of 1.29% for Ymer barley to a highest of 1.65% for Swallow barley (Table I, where the nitrogen content of the varieties, tabulated in order of increasing nitrogen content, is reported as mg. per 100 g. dry barley). On the basis of the nitrogen content of the grain, the varieties may be divided into those having a low nitrogen content, i.e., Proctor and Ymer, and those having a high nitrogen content, i.e., Hunter, Mentor, and Swallow.

TABLE I. RELEASE OF WATER-SOLUBLE, FORMOL, AND NONPROTEIN NITROGEN IN FIVE BARLEY VARIETIES AUTOLYZED WITH WATER FOR 30, 60, 120, AND 240 MIN.

Barley Variety	Autolysis Time (min.)	mg. per 100 g. dry Barley				WSN X 100 / TN	FN X 100 / WSN	NPN X 100 / WSN
		TN[a]	WSN[a]	FN[a]	NPN[a]			
Ymer	30	1,290	149.0	19.4	36.6	10.8	14.0	26.3
	60		180.3	33.6	47.3	14.0	20.3	26.2
	120		227.7	40.1	68.6	17.3	16.3	30.1
	240		268.0	49.1	83.4	20.8	18.3	34.8
Proctor	30	1,360	150.1	20.0	39.6	11.1	13.2	26.2
	60		186.7	30.2	49.6	13.7	18.3	26.5
	120		233.8	35.4	59.7	17.2	15.1	25.5
	240		272.1	49.1	80.4	20.0	14.3	29.5
Hunter	30	1,500	157.6	18.2	39.0	10.5	11.6	24.7
	60		205.1	29.7	50.2	13.7	14.5	24.5
	120		253.7	34.8	67.4	16.9	13.7	26.6
	240		306.3	54.3	90.4	20.4	17.7	29.5
Mentor	30	1,550	180.3	22.7	47.9	11.0	13.3	28.1
	60		219.2	36.2	57.9	14.1	16.5	26.4
	120		262.8	39.4	79.8	17.0	15.0	20.4
	240		338.3	55.0	102.8	21.8	16.3	30.4
Swallow	30	1,560	170.1	23.2	45.5	10.3	13.6	26.7
	60		210.9	34.6	58.5	12.8	16.4	27.7
	120		297.9	39.4	81.0	18.0	13.2	27.5
	240		333.7	59.8	108.2	20.2	17.9	32.4

[a]TN = total nitrogen; WSN = water-soluble nitrogen; FN = formol nitrogen; NPN = nonprotein nitrogen.

TABLE II. EFFECT OF VARIOUS CONCENTRATIONS OF PHENYLMERCURYACETATE AND THIOGLYCOLLIC ACID ON THE RELEASE OF NONPROTEIN AND FORMOL NITROGEN IN BARLEY AND MALT AUTOLYZED FOR 240 MIN.

Reagents	Concentration		Nonprotein Nitrogen[a]		Formol Nitrogen[a]	
	mg./liter	mM	Barley	Malt	Barley	Malt
Water (control)	...	...	88.2	435.5	42.6	185.9
Phenylmercury-acetate	125	0.4	86.2	415.3	40.3	180.3
	500	1.6	81.5	364.6	37.5	142.2
	2,000	6.4	70.3	342.0	29.7	136.6
Thioglycollic acid	125	1.1	92.8	450.8	43.1	201.6
	500	4.4	95.5	459.7	47.3	219.2
	2,000	17.6	99.5	474.7	50.9	229.3

[a]Expressed as mg./100 g. dry barley or malt.

Water-Soluble, Formol, and Nonprotein Nitrogen

Table I shows the release of the water-soluble, formol, and nonprotein nitrogen in the five barley varieties autolyzed for 30 min. to 4 hr. These results show that the differences in the release of the three nitrogen fractions were generally small or even negligible in spite of a significant variation in the nitrogen content of the varieties. It was noted, however, that the release of the water-soluble, formol, and

nonprotein nitrogen, in general, increased with an increase in the nitrogen content of the grain. A close similarity of the varieties was apparent when the formol or nonprotein nitrogen was expressed as percent of water-soluble nitrogen. The water-soluble nitrogen constituted between 10 and 21% of the total nitrogen for autolysis times of 30 to 240 min. and was substantially the same in each variety.

A comparison of the release of formol and nonprotein nitrogen shows that the nonprotein nitrogen was much higher and, in most cases, twice the formol nitrogen in all five varieties. This was so because the formal nitrogen estimated the amino nitrogen, whereas the nonprotein nitrogen contained, in addition to amino acids and low-molecular-weight peptides, nitrogenous compounds present in the autolysate which were not precipitated by phosphomolybdic acid.

Effect of Phenylmercuryacetate and Thioglycollic Acid on Proteolysis in Barley and Malt

Table II shows the effect of three concentrations each of phenylmercuryacetate and thioglycollic acid on the production of nonprotein and formol nitrogen in barley and malt autolyzed for 4 hr. In both barley and malt, phenylmercuryacetate inhibited and thioglycollic acid activated proteolysis. Although the inhibition or activation of proteolysis by these reagents was small, nevertheless, it was consistent and increased with increase in concentrations of the inhibitor or the activator.

DISCUSSION

The autolysis technique as described by Preece and Aitken (7) was employed to compare proteolytic activities of five malting barley varieties of different nitrogen content. This technique was preferred to assay methods which employ artificial substrates, as it provides a system in which the substrate for proteolytic action is provided by the native proteins of the grain. Autolysis technique has been previously employed to measure the proteolytic activity of grain by Ayer and Anderson (8), Miller (9), Zoch and Olson (10), and more recently by Preece (11), although the procedure of Miller is essentially semi-autolytic as the substrate is supplemented with haemoglobin.

The results obtained indicated that the differences in the production of water-soluble, formol, or nonprotein nitrogen were not sufficiently large to suggest any definite varietal trends. The proteolytic activity of the varieties varied with the protein content of the grain. In general, a high protein content was associated with a high proteolytic activity. Similar results have been reported previously by Sandegren (1). The similarity in the proteolytic activity of the varieties became obvious when the water-soluble nitrogen was expressed as percent of total nitrogen and formol or nonprotein nitrogen was expressed as percent of water-soluble nitrogen (Table I). From the point of view of revealing a different method for assessing barley characteristics under routine experimental conditions, the present results may be considered negative. But it may be argued that such a pattern of results was obtained because the varieties compared in the present study were all of good malting quality. Comparison of these varieties with varieties of relatively poor malting quality might have indicated some discernible trends in the proteolytic activity of the varieties. Such an approach might be attempted in a future study to obtain some indications of the suitability of newer barley varieties which may be

developed for malting. Any such future study should include varieties grown on different locations and in different seasons, and having a wider range in protein content, since environmental variations may affect protein content of the grain and possibly its proteolytic activity. Zoch and Olson (10) reported the effect of location of growth on the proteolytic activity of ten varieties of barley grown at ten stations, and malts prepared from these varieties. The barleys from five of the stations gave malts of higher proteolytic activity than those from the other five stations. The proteolytic activity of the barley varieties was, however, essentially the same.

The results obtained with the use of inhibitor and of activator (Table II) suggested that sulfhydryl-dependent enzymes were present in the autolysates of both barley and malt. However, in a system such as autolysis, the degree of activation or inhibition achieved will depend on the penetration of the solvent containing the compounds to the actual site of the enzyme. Furthermore, there are possibilities that the inhibitor or activator was used up in attacking S-S or SH bonds belonging to general protein material rather than at the active center of the enzymes or that they were simply adsorbed to the grain materials. It is probably because of some or all of these reasons that the activation or inhibition of proteolysis achieved was small or even negligible in relation to the concentration, particularly at the highest level, of the reagents employed.

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[Received March 16, 1970. Accepted August 10, 1970]