EFFECT OF MALTING PROCEDURE AND WHEAT STORAGE CONDITIONS ON ALPHA-AMYLASE AND PROTEASE ACTIVITIES[1]

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ABSTRACT

Factors influencing alpha-amylase and protease activities of wheat malted on a laboratory scale were studied. Wheat malts were produced by steeping to 40–46% moisture and germinating at 42° to 68°F. for 1 to 12 days. Germination time was the most important factor influencing enzyme production. The optimum malting conditions appeared to be approximately 42% steep moisture level and 60°F. germination temperature. Kilning temperatures above 110°F. caused loss of both enzymes.

Enzyme activities of freshly harvested wheat did not reach their maximum until after 2 months of storage. Storage at low temperatures (40°F.) favored production of greater alpha-amylase and protease activities.

Numerous studies of the effects of malting conditions on the production of alpha-amylase have been made and the conclusions reached have agreed quite well. Geddes, Hildebrand, and Anderson (5), Kneen, Miller, and Sandstedt (6), Dickson, Olson, and Shands (3), and Dickson and Shands (2) demonstrated that increased steeping moisture, germination time, and germination temperature increased alpha-amylase production.

Relatively little information, however, is available concerning the effect of malting conditions on protease production. It is generally believed that protease activity parallels the alpha-amylase activity of germinated cereals. Geddes *et al.* (5) reported that the autolytic protease activity of malted wheat flour was augmented by longer periods of germination, increased steep levels, and higher protein content. Otis and Olson (10) demonstrated that proteolytic activity of barley malts was enhanced when the steep moisture was increased, but the duration of the germination period was more important than the germination moisture. Mounfield (9) reported that wheats which were stored for 2 years at 18°C. lost much of their ability to produce proteases during germination.

The primary purpose of this investigation was to obtain information concerning the factors influencing protease and alpha-amylase

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production during the malting of wheat. Several hundred malts were produced by employing a wide range of steep moisture levels, germination times, germination temperatures, kilning temperatures and wheat storage conditions. Procedures such as programmed kilning, in which temperatures are increased as the moisture content of the malt is lowered, were not employed in order to simplify operations.

Materials and Methods

Malting Equipment. The design of the laboratory malting equipment was evolved through visits to several wheat-malting laboratories. The steeping, germinating, and kilning of wheats were carried out in parabolic-shaped wire screen baskets holding 1.5 lb. of malted wheat. The steeping bath was an insulated, refrigerated, stainless-steel tank. It was provided with a water circulation pump and intake and overflow pipes to assure continual aeration and exchange of water. The temperature was maintained thermostatically within ±1°F. The germinator was a large commercial refrigerator which was equipped with additional cooling and heating coils and air-circulating and humidifying units. The desired temperature and a saturated atmosphere were maintained by means of thermostatic controls and a Minneapolis-Honeywell temperature and humidity recorder. The wire baskets containing the germinating grain were held by means of a revolving arm assembly which was rotated at 5 revolutions per hour. In this manner the grain was slowly moved to provide for proper aeration and to guard against overheating of portions of the sample. The sprouted wheats were dried in a large forced-air convection-type oven which was equipped with a revolving assembly to facilitate uniform drying of the green malts.

Malting Procedure. The standard wheat was a sample of Pawnee grown at Manhattan, Kansas. One-pound lots were routinely used for the production of each malt. All lots were steeped at 50°F. for the time necessary to bring the moisture content to the 40, 42, 44, and 46% levels. Germination was carried out at 42°, 50°, 60°, and 68°F. for 1 to 12 days. All of the green malts, except those employed in the kilning temperature study, were dried at 104°F. for 24 hours. Rootlets and acrospires were removed by kneading the dried malts in a plastic bag.

In the study of the effects of drying temperatures on enzyme activities, the wheats were steeped to 42% moisture content and germinated at 68°F. for 6 days. Drying temperatures ranged from 100° to 150°F.

To check the precision of the malting equipment and procedures,

a number of portions of wheat were malted for 2, 4, 6, and 8 days at 68°F. The malts were assayed for alpha-amylase and protease activities after drying and cleaning.

To study the effect of the age of wheat and storage temperatures, two samples of hard red winter wheats (Concho and Triumph) each were divided into three lots immediately after harvest. One lot of each wheat was stored at 40°F., 70° to 80°F. (room temperature) and 100°F. One-pound samples of each lot were malted after 1, 2, 4, 6, and 7 months of storage. Germination was carried out at 42% moisture and 68°F. for 8 days. Malts were dried at 104°F. for 24 hours.

Analytical Methods. The rate of water absorption for each variety of wheat was determined by a modification of the method of Banasik *et al.* (1). Twenty-five grams of wheat were steeped in perforated polyethylene bags for 16 to 96 hours. Samples were transferred to tared, perforated, plastic centrifuge tubes and centrifuged for 5 minutes at 1,800 r.p.m. The gain in weight was then used to calculate the steep moisture level attained. When steeping was performed in perforated plastic centrifuge tubes in accordance with the original procedure (1) used for barley, the swelling of the wheat mass was restricted and abnormal water absorption values were obtained.

Alpha-amylase activity was determined by the method of Sandstedt, Kneen, and Blish (12) as modified by Redfern (11). Protease activities were determined by the method of Miller and Johnson (8) and the gelatin viscosity technique of Koch and Ferrari (7). Malting losses were determined by comparing the dry weights of 1,000 kernels of the original and malted wheats. All of the enzyme activities are those found in the finished malt and expressed on a dry matter basis.

Results and Discussion

Data concerning the replicability of the malting procedure are given in Table I. The small standard deviation of each set of replicates established the dependability of the malting procedures and equipment.

The two procedures used to determine protease activities were highly correlated ($r = 0.993$, 31 d.f.). It was decided, therefore, to use the Miller-Johnson method (8) for routine protease determinations.

Effect of Germination Temperature. Enzyme production (Fig. 1, graphs A and C) was sharply curtailed in malts germinated at 42°F. or 50°F. compared with those germinated at 60°F. or above. Relatively small increases in activity were noted when the temperature was raised from 60° to 68°F. For further routine work, 60°F. was selected for

TABLE I

REPLICABILITY OF THE MALTING PROCEDURE AS MEASURED BY ALPHA-AMYLASE ACTIVITY, PROTEASE ACTIVITY, AND MALT YIELD[a]

Replication No.	Germination Time (Days)								
	2	4	8	2	4	8	2	4	8
	Alpha-Amylase Activity			Protease Activity			Malt Yield		
	SKB units/g	*SKB units/g*	*SKB units/g*	*HU/g*	*HU/g*	*HU/g*	%	%	%
1		209	363		84.1	101.4		92.3	87.6
2		218	378		87.5	98.7		91.7	86.4
3	84	200	361	61.5	81.9	102.0	96.5	92.5	87.1
4	65	202	354	62.8	84.5	106.4	96.1	91.8	85.9
5	79	219	374	59.7	84.9	101.7	97.2	91.3	87.3
6	80	209	360	60.7	84.1	102.5	96.7	92.3	86.6
Mean	77	210	365	61.2	84.5	102.2	96.6	92.0	86.8
Std. Dev.	8.3	7.8	9.1	1.3	1.8	2.4	0.70	0.46	0.63

[a] Conditions: Pawnee wheat steeped to 42% moisture, germinated at 68°F., and kilned at 104°F. for 24 hours.

the germination temperature.

Effect of Steep Moisture. Figure 1, graphs B and D, shows that enzyme activities were increased with each increment in the steep moisture content. Additional data (unpublished), obtained with lower steep levels, indicated that 40% was the lowest practical level for acceptable response. Singruen (13) stated that the moisture content should be optimal at the initiation of germination, since modification of other conditions would not compensate for inadequate moisture. The indicated preference for steep moisture levels in the 40–42% range confirms the earlier work of Geddes *et al.* (5) and Dickson and Geddes (4). Steep moisture levels higher than 40–42% increased the enzyme production only slightly. For further routine work a steep moisture level of 42% was adopted.

Effect of Germination Temperature and Steep Moisture Level on Malt Yield. The effect of germination temperature and time on malt yield is shown in Fig. 2, A. Increasing germination temperatures not only decreased the malt yield but created a mold growth problem at the highest temperature for certain wheat samples. Germination time ranging from 1 to 12 days decreased the malt yield approximately 10%.

Increasing the steep moisture level (Fig. 2, B) decreased the malting yield. When steep moisture level was increased from 40 to 46%, the steep time was increased 20–30 hours, depending on the sample of wheat. Decrease in yield during 12 days of germination amounted to approximately 10% depending on the steep moisture level. The decrease during steeping, germination, and drying reflected losses due to leaching, respiration, and root and shoot growth.

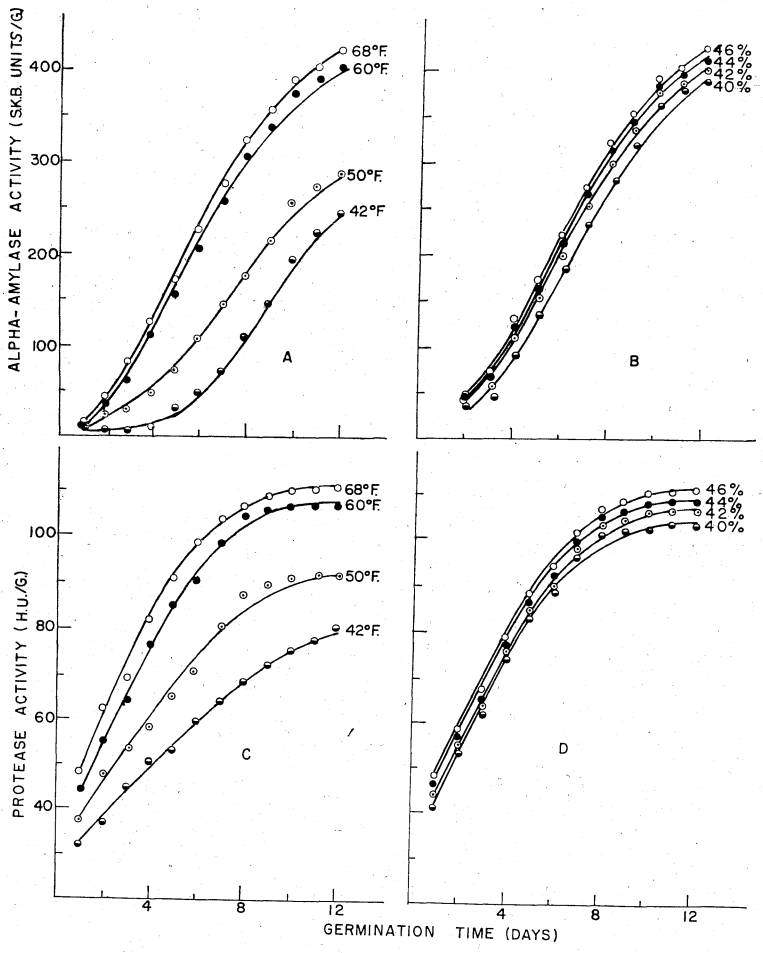

Fig. 1. Influence of germination temperature and steeping moisture level on alpha-amylase and protease activities of malted wheat.

Effect of Kilning Temperature. The influence of drying temperatures applied throughout the whole period of kilning on enzyme activities is shown in Fig. 3. A gradual decrease took place over the full range of the temperatures studied. The lowest practicable temperature that could be maintained under the conditions of these experiments was 104°F. While gross differences in both protease and alpha-amylase activities are reflected in the different varieties and classes of wheat, the effect of drying temperature, except for the protease of Pawnee, is essentially uniform.

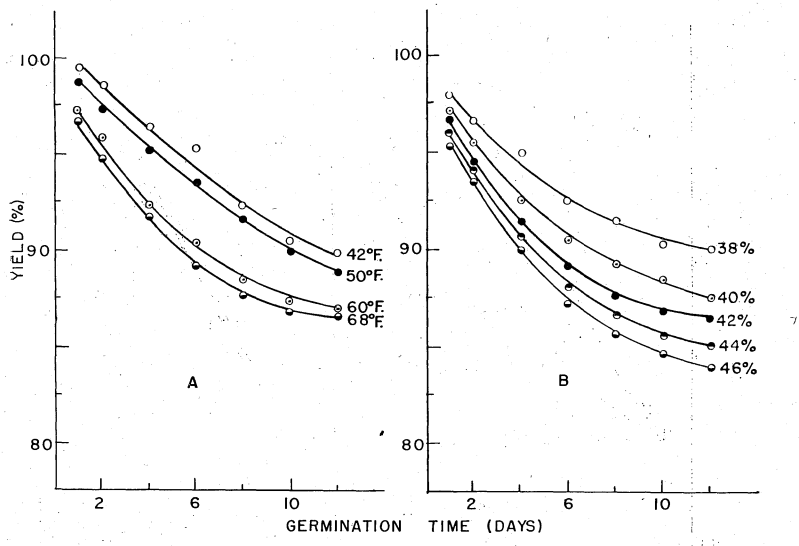

Fig. 2. Influence of germinating temperature and steeping moisture level on malt yield.

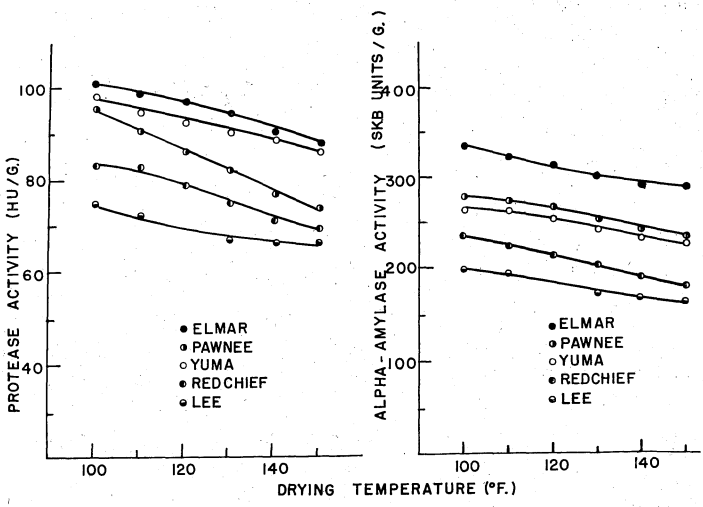

Fig. 3. Influence of drying temperature on alpha-amylase and protease activities of malted wheat of various varieties.

Witt (14) demonstrated that malt enzymes were more susceptible to heat when the moisture content of the malt was high. Since only one temperature was used in the routine kilning of the present samples, the data may not be interpretable to commercial malting opera-

tions which generally employ higher kilning temperatures after most of the moisture has been removed at lower temperatures.

Effect of Storage Temperature and Time on Malting Properties of Wheats. The effect of wheat storage at different temperatures is shown in Fig. 4. The freshly harvested wheats germinated poorly because of the temporary dormancy of the seeds at this stage of maturation. The percentage of seeds that germinated, the acrospire lengths, and the alpha-amylase and protease activities all increased appreciably during the first 2 months of storage. The enzyme activities of wheats stored at 80° or 100°F. did not reach the levels of those stored at 40°F. Viability of seeds stored at 100°F. was reduced during the storage period, but not to the same extent as was the production of alpha-amylase and protease enzymes. The data thus indicate that the con-

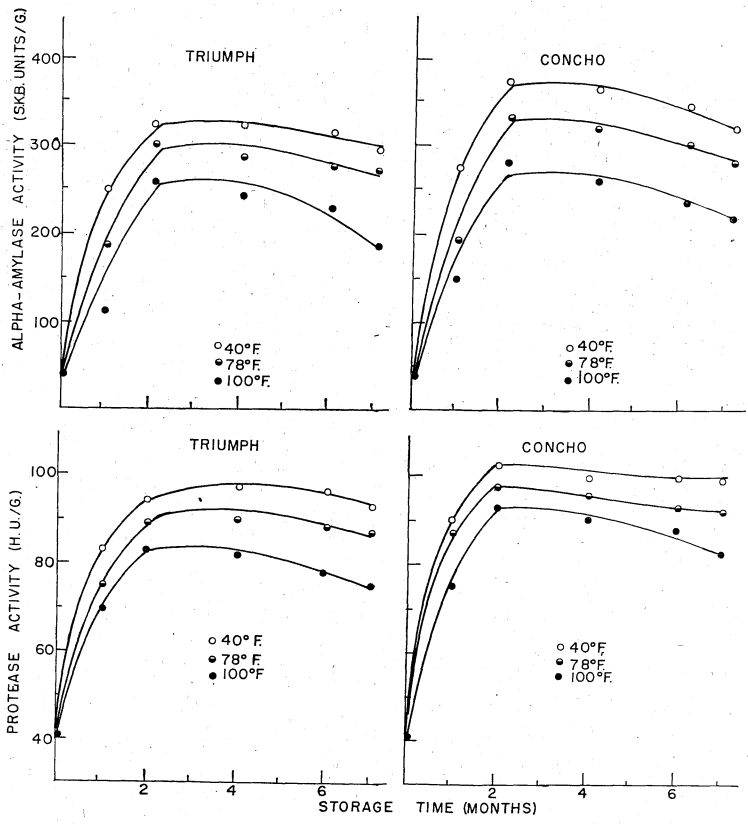

Fig. 4. Influence of wheat storage conditions on alpha-amylase and protease activities of malted wheat of two varieties.

ditions under which wheat is stored prior to malting are extremely important.

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