Changes in Beta-Amylase Enzymes of Barley During Malting[1,2]

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ABSTRACT

Three barley cultivars, Bonanza, Diamond, and Klages, were used to study changes in β-amylase enzymes during malting. Freeze-dried samples taken after 24 and 48 hr of steeping and after one, two, three, four, and five days of germination were analyzed by chromatofocusing and polyacrylamide gel isoelectric focusing. The effect of kilning on β-amylase components of malt also was studied. Chromatofocusing analysis indicated that during steeping and early stages of germination, barley β-amylases disappear or decrease to low levels concomitant with the production of at least two new β-amylase components that have higher apparent pI values. Diamond produces primarily β-amylase "A" (apparent pI 6.89), Klages primarily β-amylase "B" (apparent pI 6.68), whereas both enzymes are produced in malts prepared from Bonanza barley. Polyacrylamide gel isoelectric focusing analysis provided evidence that confirmed the changes observed by chromatofocusing. These enzymes also are produced by extracting ground barley directly with papain.

Key words: Barley, β-Amylase, Chromatofocusing, Polyacrylamide gel isoelectric focusing (PAGIEF)

Chromatofocusing and polyacrylamide gel isoelectric focusing (PAGIEF) were used extensively in previous studies to investigate changes in β-amylase enzymes during kernel development of barley (6) and to investigate the heterogeneity of β-amylase enzymes of barley (5). Using the same techniques, this study was designed to investigate changes in β-amylase enzymes that occur when barley is malted.

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EXPERIMENTAL

Three barley cultivars were used to study changes in β-amylase enzymes that occur when barley is converted to malt. These cultivars were known to produce malt β-amylase enzymes that exhibit distinctly different chromatofocusing profiles. The cultivars included Bonanza, a six-rowed malting barley, Diamond, a six-rowed feed barley, and Klages, a two-rowed malting barley. Portions of samples of these cultivars were freeze-dried after 24 and 48 hr of steeping and after one, two, three, four, and five days of germination (7). The remaining portions of these samples were kilned. Kilning was for 24 hr as follows: 12 hr at 50° C, 5 hr at 68° C, 3 hr at 82° C, and 4 hr for temperature transitions during the kilning process.

Extraction of β-Amylase Enzymes

Samples of barley and malt were ground in a Wiley mill equipped with a 1-mm sieve. Ground samples (75 g) were extracted with 200 ml of 0.1 *M* citrate buffer, pH 6, and centrifuged for 15 min at 7,000 × *g*. Supernatant solutions were dialyzed extensively with 0.02 *M* acetate buffer, pH 4.75. Extractions were performed at 4° C, and all buffers contained 10 m*M* monothioglycerol (Sigma Chemical Co., St. Louis, MO).

Isoelectric Focusing Experiments and Assay Procedures

Procedures for chromatofocusing, PAGIEF, and for assaying β-amylase were described previously (5).

Extraction of Barley with Papain

Mature barley of each of the three cultivars was used in experiments that included papain in the extraction medium. Ground barley (15 g) was extracted overnight at room temperature with stirring with 50 ml of 1% aqueous papain (Type II, Sigma Chemical Co., St. Louis, MO). The samples were centrifuged at 10,000 × *g* for 10 min at room temperature.

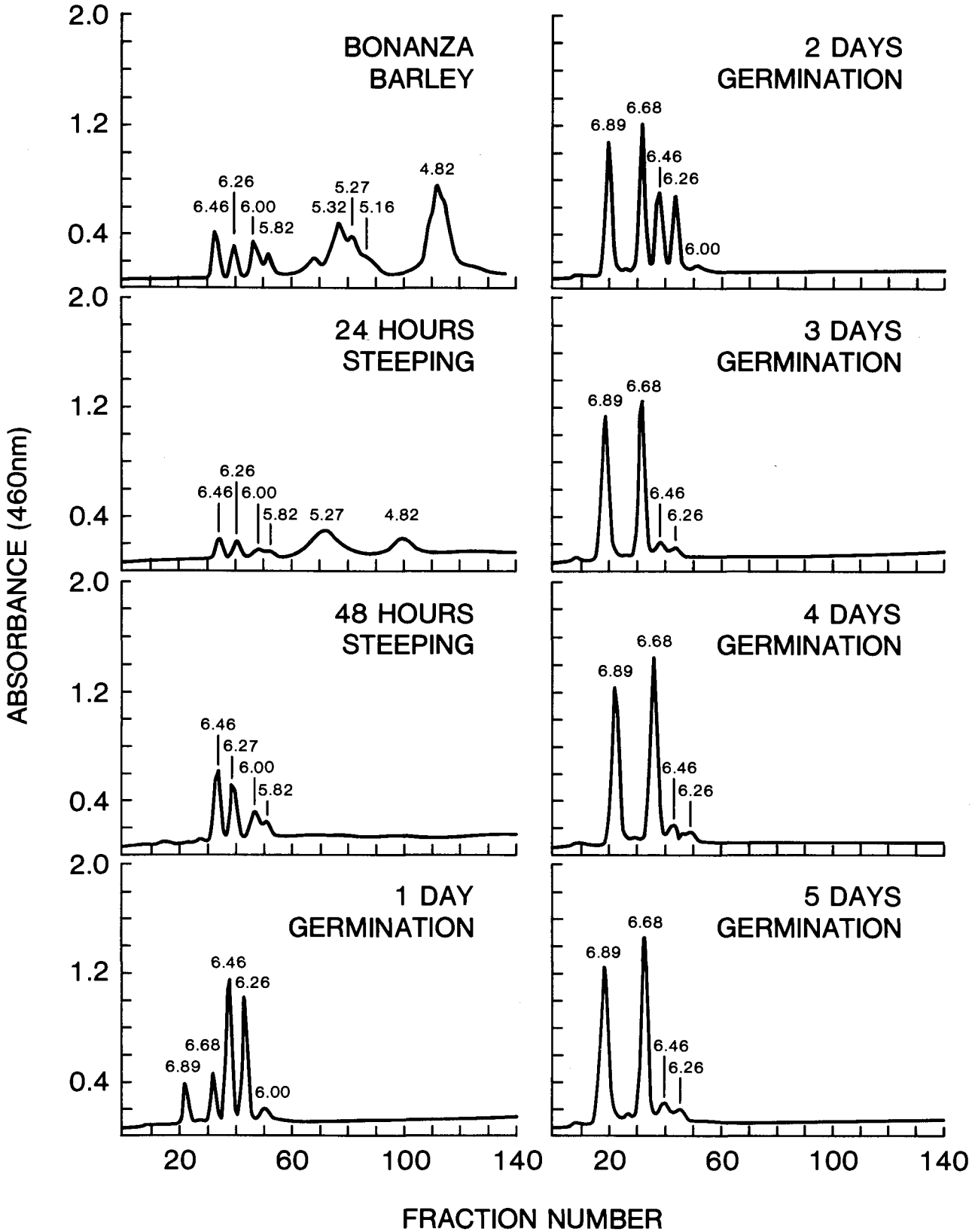

Fig. 1. Chromatofocusing profiles of Bonanza barley and freeze-dried samples taken after 24 and 48 hr of steeping and following one, two, three, four, and five days of germination.

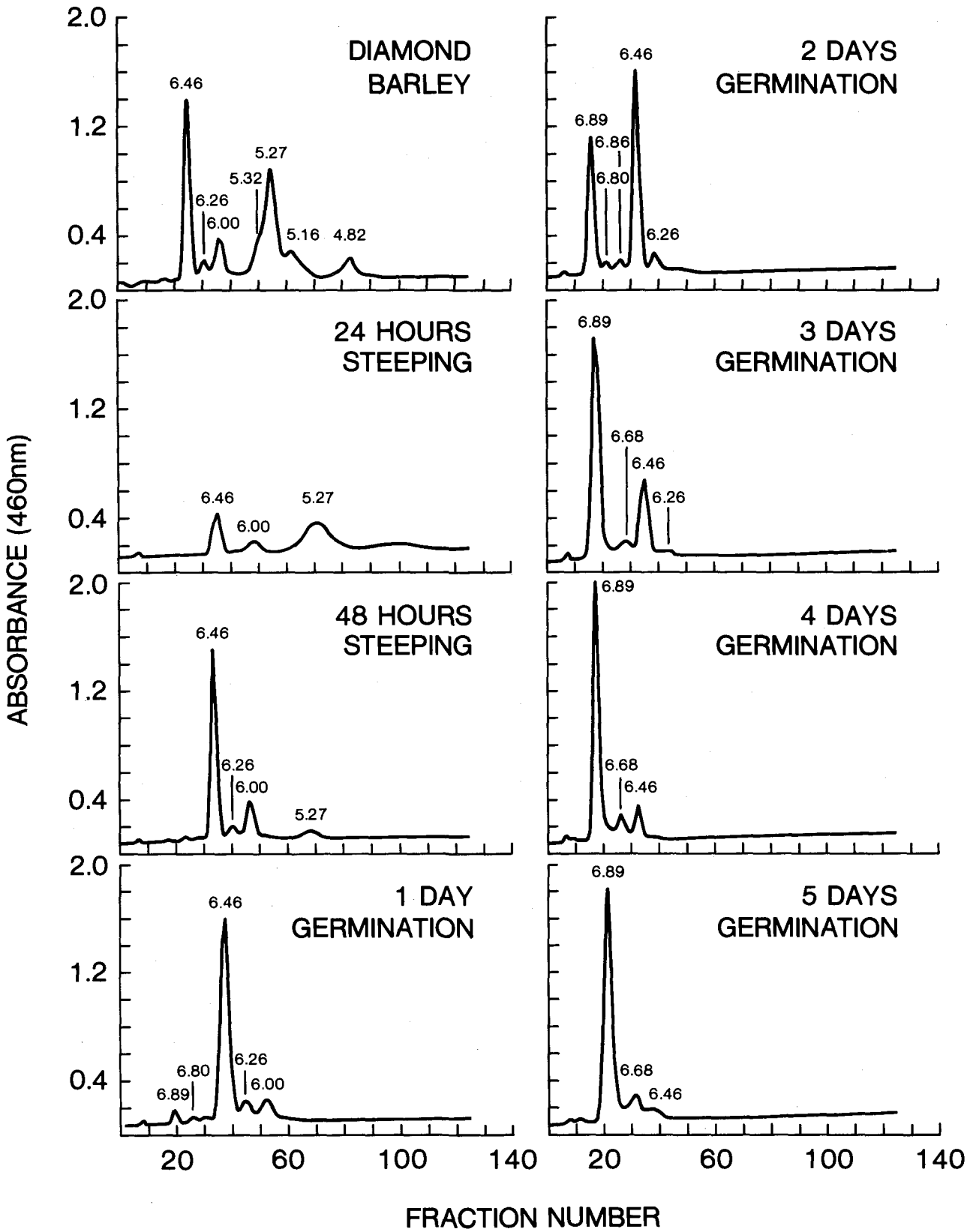

Fig. 2. Chromatofocusing profiles of Diamond barley and freeze-dried samples taken after 24 and 48 hr of steeping and following one, two, three, four, and five days of germination.

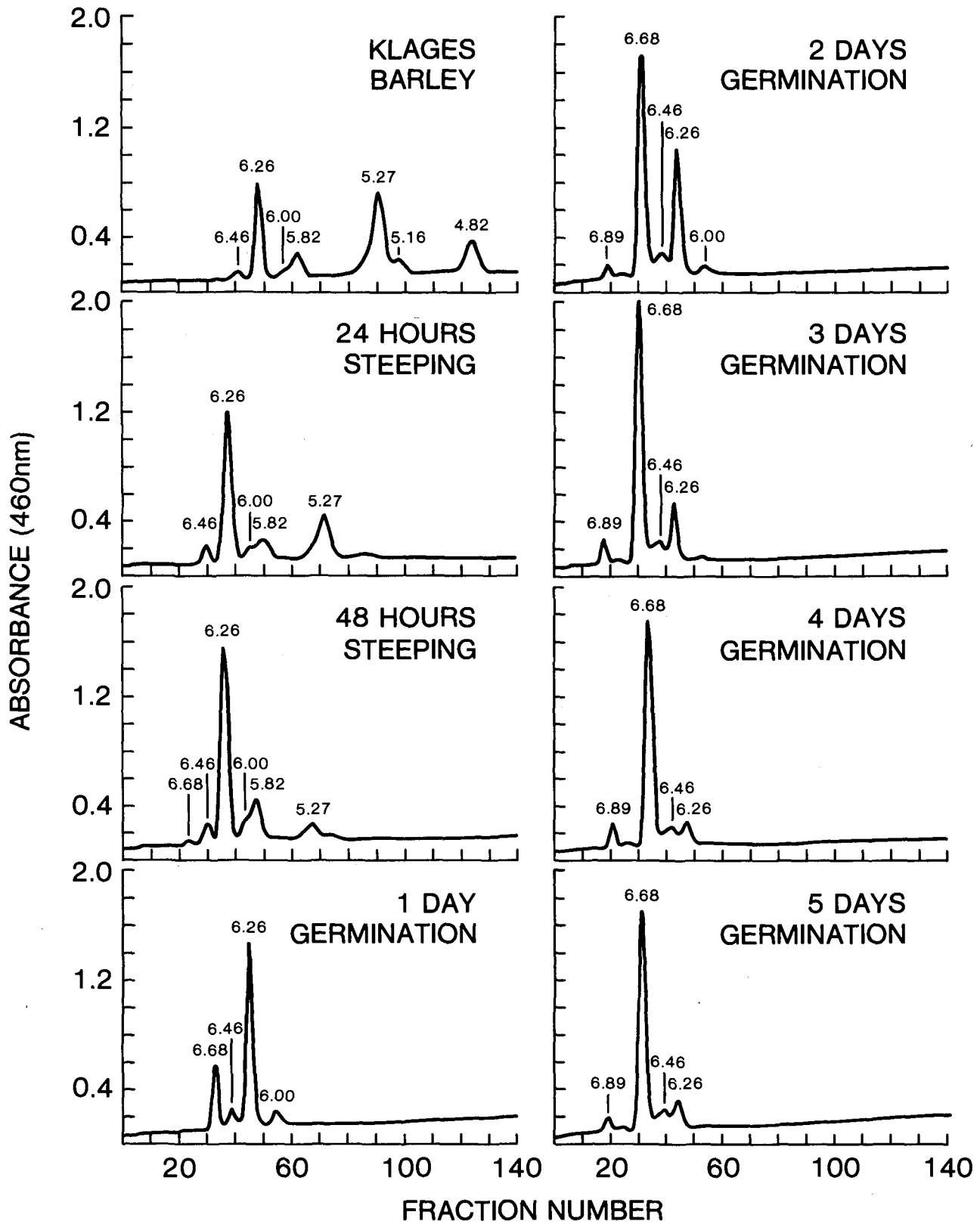

Fig. 3. Chromatofocusing profiles of Klages barley and freeze-dried samples taken after 24 and 48 hr of steeping and following one, two, three, four, and five days of germination.

RESULTS

Chromatofocusing Experiments with Barley and Freeze-Dried Malt Samples

Changes in elution profiles obtained by chromatofocusing of β-amylase components of Bonanza barley during various stages of malting are shown in Figure 1. Previous studies demonstrated that Bonanza barley has eight major and several minor β-amylase components with distinctive apparent pI values (5), and this is demonstrated again in the top left chromatogram. To facilitate discussion of the properties of these components, the major β-amylase enzymes were arbitrarily identified with Roman numerals I to VIII in order of elution from chromatofocusing columns (5).

During steeping, there is an apparent decrease in the amount of β-amylase that is detected, as well as disappearance of the four β-amylase components with lower apparent pI values. The phenomenon of reduced β-amylase activity detectable during steeping of barley was noted previously (1).

The principal β-amylase components of Bonanza after 48 hr of steeping and one day of germination are β-amylases I and II, with apparent pI values of 6.46 and 6.26, respectively (Fig. 1). These enzymes appear to increase in amount after one day of germination. These increases are related to molecular rearrangements of β-amylase enzymes noted earlier (5). As the malting process continues, β-amylases I and II decrease to low levels, and two β-amylase enzymes with apparent pI values of 6.89 and 6.68 become the major β-amylase enzymes found in malt. These malt enzymes are arbitrarily identified as "A" and "B", respectively, to clearly distinguish them from the major β-amylase components detected in Bonanza barley.

Very similar phenomena were observed by chromatofocusing when Diamond barley was malted (Fig. 2). During steeping, there was an apparent decrease in total β-amylase activity. After 48 hr of steeping and one day of germination, β-amylase enzymes with lower apparent pI values declined or disappeared, and the principal enzyme detected was β-amylase I (5) with an apparent pI of 6.46. This enzyme also declined during continued malting to very low levels, and a single enzyme, malt β-amylase A with an apparent pI of 6.89, was predominant in three-to-five day green malts of Diamond barley.

Similar changes of β-amylase enzymes were observed when Klages barley was malted (Fig. 3). Following 48 hr of steeping and one day of germination, β-amylase II with an apparent pI of 6.26 was the predominant barley enzyme (5). In fully modified malts of Klages barley, the predominant enzyme was malt β-amylase B with an apparent pI of 6.68.

These studies demonstrated that although there are similarities in the changes in β-amylase enzymes during malting, there also are

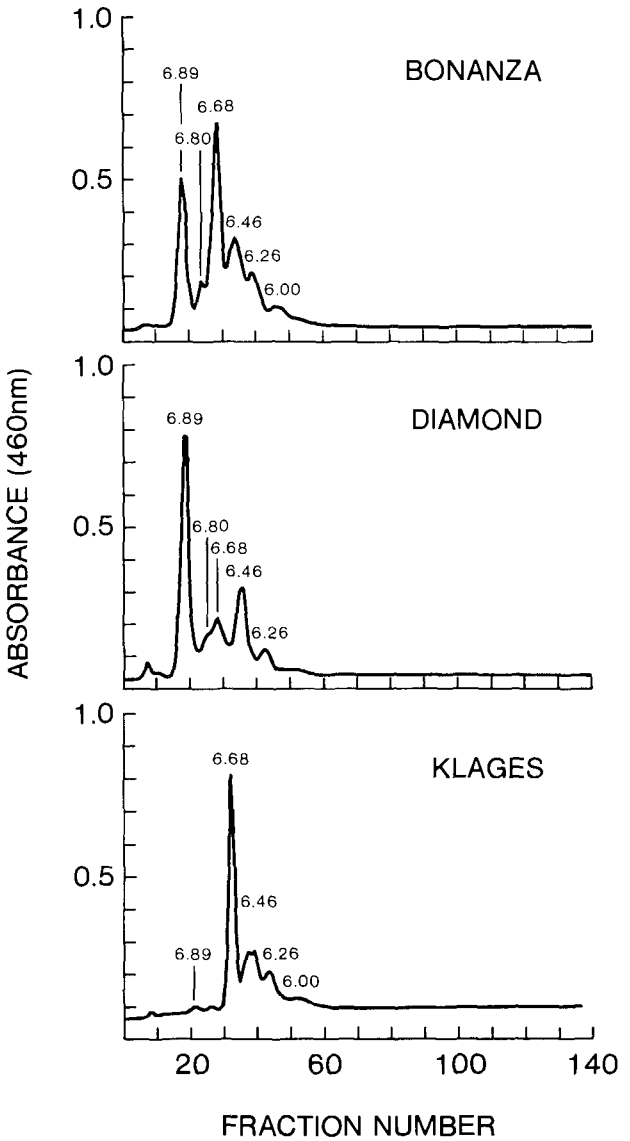

Fig. 4. Chromatofocusing profiles of Bonanza, Diamond, and Klages malts kilned after five days of germination.

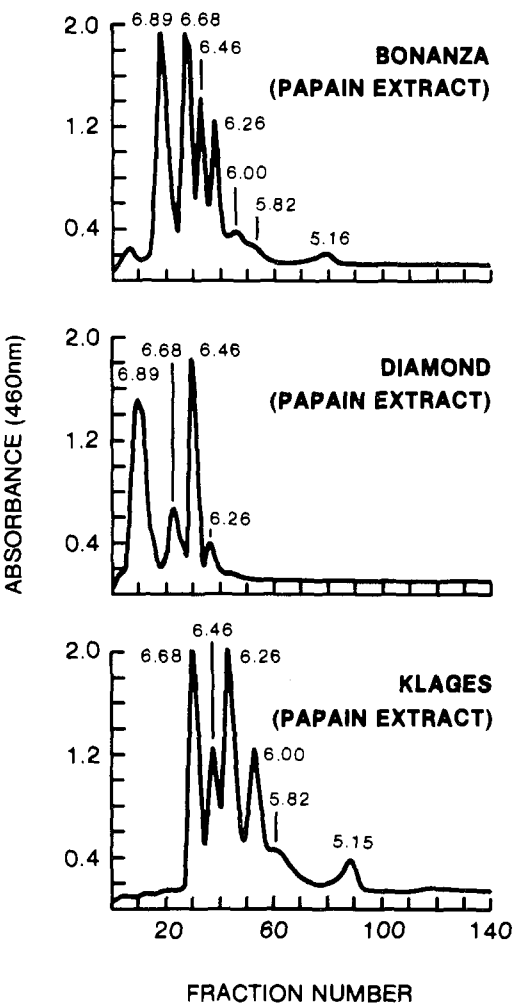

Fig. 5. Chromatofocusing profiles of Bonanza, Diamond, and Klages following extraction of barley grist with papain.

major differences for the three barley cultivars. The principal β-amylolytic enzyme of Diamond barley is β-amylase I, whereas well-modified malt of Diamond barley contains primarily β-amylase A. The major enzyme of Klages barley is β-amylase II, and during malting the principal enzyme becomes β-amylase B. By contrast, β-amylases I and II are the principal β-amylolytic enzymes of Bonanza barley, and β-amylases A and B are present in malts of Bonanza barley. Further studies with Argyle, a newly registered six-rowed malting cultivar, demonstrated another malt β-amylase "C" with an apparent pI of 6.80 (*unpublished*).

Effect of Kilning on β-Amylase Activity

The previous chromatofocusing studies were performed on freeze-dried samples of malt. The effects of kilning malt after five days of germination are shown in Figure 4. β-Amylase is quite heat-labile and some activity is lost during kilning. There is a very substantial decrease in the proportion of malt enzymes A and B (apparent pI's of 6.89 and 6.68, respectively) relative to the residual "barley" β-amylase components present in malt (Fig. 4). This suggests that "malt" β-amylase enzymes are more heat-labile than barley enzymes.

Extraction of Barley with Papain

From one-third to two-thirds of malt β-amylase occurs as a result of release of the "bound" β-amylase present in barley (4). Much of the remaining malt β-amylase forms as a result of conversion of barley β-amylase to malt β-amylase (6). This conversion appears to be mediated by proteolysis. A small proportion of β-amylolytic activity of malt is due to residual barley-type β-amylases still present in malt (Figs. 1–4).

It is well known that bound β-amylase in barley can be released by treatment of ground barley with papain (3,6) or with reducing agents, particularly thiolated compounds (5,6,8). β-Amylase released by reducing agents invariably gives rise to barley-type β-amylases, whereas treatment of barley-type or bound β-amylase with papain gives rise to malt-type β-amylase enzymes. Although either method is effective to extract bound β-amylase from barley, producing "free" β-amylases, the reactions are not identical. Presumably, reducing agents cleave disulfide bonds to produce barley-type β-amylase enzymes, whereas papain cleaves peptide bonds to produce malt-type enzymes. The reduction reaction may be mediated by protein disulfide reductase that is synthesized de novo during malting (10), whereas the latter reaction would be catalyzed by an unidentified protease or proteases. Because treatment of barley-type β-amylase with papain produces malt-type β-amylase, the malt enzymes must have lower molecular weights than barley-type β-amylase enzymes.

Treatment of ground barley of Bonanza, Diamond, and Klages directly with crude papain produced the chromatofocusing results shown in Figure 5. The malt-type β-amylases observed for each of these cultivars are similar to those observed in malts for each of these cultivars (Figs. 1–3). Variations of pH gradients affect the elution volume or fraction number at which β-amylase components elute, but the apparent pI or elution pH of the various β-amylase components are not affected (5). Release of malt-type β-amylase directly from ground barley is due to proteolysis and is not caused by reducing activity of the papaya extract from which papain is derived.

Several workers have found that boiled preparations of papain were as active as unboiled solutions for liberating bound β-amylase from barley (2,3,9). As a result, considerable confusion remains whether the liberating effect of papain is due to proteolysis or to reducing activity. In fact, crude papain has both types of activity, and each effectively liberates bound β-amylase by a combination of reducing activity and proteolysis (*unpublished*).

PAGIEF

Changes in the β-amylase enzymes of Bonanza as determined by PAGIEF during various stages of malting are shown in Figure 6. The banding patterns on the zymogram are complex, but the major

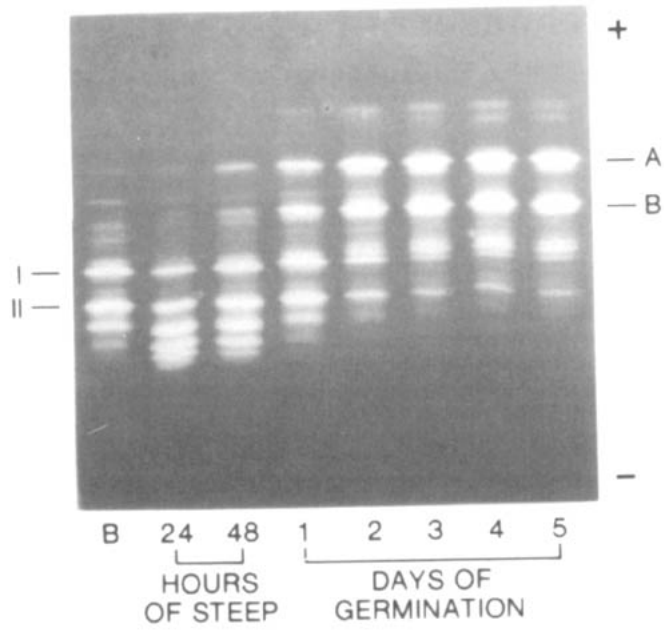

Fig. 6. Polyacrylamide gel isoelectric focusing of extracts of Bonanza barley (**B,** first column) and of samples taken during various stages of malting. The pH profile is from pH 3.5 (top) to pH 9.5 (bottom). The principal barley β-amylase enzymes (**I** and **II**) are identified as are the principal malt enzymes (**A** and **B**).

β-amylase enzymes in barley are identified as I and II and those in malt as A and B. The enzymes eluted during chromatofocusing with lower apparent pI values (III to VIII) are present in barley and steeped barley in the region between β-amylase II and the cathode. These enzymes disappear after two days of germination.

Comparison of starch and β-limit dextrin zymograms indicates that α-amylase isoenzymes are not producing spurious results in Figure 6. Thus, the α-amylase III complex that is produced during malting (11) occurs in the region between β-amylase I and β-amylase B in malt samples. Two bands of α-amylase II appear in the region between malt β-amylase A and the anode (11).

There is a preponderance of A and B in finished malts of Bonanza with minor quantities of I and II remaining. Although the results for Diamond and Klages are not shown, zymograms of samples of these cultivars confirmed the changes observed by chromatofocusing.

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