

PROPERTIES OF PURIFIED ALPHA-AMYLASE FROM
GERMINATED PEARL MILLET

by

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INTRODUCTION

Pearl millet (Pennisetum americanum (L.) Leeke) is one of the most drought tolerant of man's food crops. It is grown in the dry Sahel Zone across Africa and in the semiarid areas of India. It is consumed directly as human food with only minor amounts being fed to animals.

Germinated pearl millet is used for preparing processed foods for children, sweet goods, malt vinegar, and beer (Asha et al., 1976a; Vogel and Graham, 1978). During grain germination, the alpha-amylase activity increases dramatically, as much as 1000-fold for some grains (Barrett, 1975). As with other cereal grains, it is quite likely that alpha-amylase plays an important role in determining the quality of millet-based products. Therefore, the objectives of this study were (1) to isolate and purify alpha-amylase from germinated pearl millet, and (2) to determine the molecular size of millet starch, and starch fractions after hydrolysis by purified millet alpha-amylase.

REVIEW OF LITERATURE

Methods used to purify and characterize alpha-amylases from cereal grains, the nature of the products obtained by alpha-amylolytic action on solubilized starch and starch fractions, and the action of cereal alpha-amylases on raw starch granules has been thoroughly reviewed by Beleia (1980). Therefore, only a brief synopsis of that literature will be given.

Germination and Alpha-amylase Activity

Seed germination is the resumption of active growth of the embryo that results in the rupture of the seed coat and the emergence of the young plant. This definition presumes that the seed has been in a state of rest after its formation and development (Copeland, 1976).

It is well-established that diastatic power increases during cereal grain germination (Akazawa, 1976). Several authors have found an increase in beta- and alpha-amylase activity in germinated sorghum and pearl millet (Dyer and Novellie, 1966; Jain and Date, 1975; Asha et al., 1976a, b). Jain and Date (1975) have shown that after a 72 hr. germination period, the diastatic power of pearl millet was essentially equivalent to that of barley. Diastatic power is affected by germination temperature. As the germination temperature for pearl millet is decreased from 35°C to 25°C, there is a significant increase in total amylase activity (Asha et al., 1976b).

Characteristics of Cereal Alpha-Amylases

Alpha-amylase (alpha, 1-4 glucan 4-glucanohydrolase, E.C. 3.2 1:1) hydrolyzes alpha 1 → 4 linked glucose polymers. The endoenzyme is named alpha-amylase because the hydrolysis products have the alpha configuration.

Alpha-amylase functions as a hydrolase catalyzing the breakdown of reserve starch in the endosperm (Paleg, 1965; Guardiola and Sutcliffe, 1971). It is active both in vivo and in vitro (Dunn, 1974). Alpha-amylase is considered to be unique in that it is the only plant enzyme capable of degrading native starch granules.

Alpha-amylase has been isolated from immature grains (Marchylo et al., 1976; MacGregor et al., 1974; Kruger, 1972a), mature grains (Maeda et al., 1978; Beleia and Varriano-Marston, 1981; Warchalewski and Tkachuk, 1978), and malted grains (Mitchell, 1972; Botes et al., 1967; Kruger and Tkachuk, 1969; MacGregor, 1977; Kruger, 1972b). No work has been reported on the characteristics of alpha-amylase from germinated pearl millet.

Alpha-amylase from various cereal sources show similar physical and chemical characteristics. The optimum pH for most cereal alpha-amylases is about 4.5 at 30°C (Kneen, 1945; Dube and Nordin, 1961; Kulp, 1975). Molecular weights of purified wheat, barley, and sorghum alpha-amylase range from 20,000 to 52,000 (Botes et al., 1967; Takachuk and Kruger, 1974; Greenwood and Milne, 1968a, b).

Beleia and Varriano-Marston (1981) purified alpha-amylases from mature pearl millet grain and found that the molecular weights, determined by SDS gel electrophoresis, ranged from 22,000 to 53,000. The pH optimum for the enzyme was between 4.4 and 4.8; the temperature optimum was 55°C.

Degradation of Starch by Cereal Alpha-Amylases

In vivo studies on morphological changes in starch granules in germinating barley and wheat have been reported by Palmer (1972) and Bathage and Palmer (1972). Alpha-amylolytic attack on small starch granules was slower than that on larger granules. The differences in susceptibility of intact granules was thought to be dependent on the genetically inherited

degree of molecular association within the granules.

Lineback and Ponpipon (1977) germinated wheat, oats, and pearl millet and studied the morphological changes in the granules as a result of alpha-amylase attack. Alpha-amylase action was observed as channels or pits on the surface of the granules. Millet starch was more extensively degraded than wheat or oat starches. Others have shown that millet alpha-amylase preferentially attacks the center of the granules leaving an outer starch shell (Beleia and Varriano-Marston, 1981; Sullins and Rooney, 1977).

Hydrolysis of raw millet starch by pearl millet alpha-amylase produces glucose and maltose during the first 5 hr. of reaction (Beleia and Varriano-Marston, 1981). After 8 hr. maltotetraose can be detected. The larger molecular weight products formed during degradation have not been characterized.

MATERIALS AND METHODS

The pearl millet used in this study was HMP 700 (Hays millet population) grown in Hays, Kansas, in 1980.

Germination

Pearl millet (120-150 g) was cleaned and sieved (40 wire mesh) to obtain uniform kernel size. Broken kernels were removed by hand. Sound grains were steeped for 12-14 hr. at room temperature in deionized water containing 0.3% chlorox to prevent microbial growth. The chlorox solution was changed three times during the steeping stage. Samples (30 g) were placed in Pyrex test tubes (6" x 1") and stoppered with a cork having a 3/16" diameter hole, or 80 to 100 g samples were placed on wet filter paper in large trays. The grains were germinated at room temperature until sprouts were visible (1-3 days). After sprouting, samples were frozen and freeze-dried. Rootlets were removed from the dry samples by rubbing the sprouted grains between the hands and sifting the material through a 40 wire mesh screen. Samples were then milled on a UDY Cyclone mill.

Alpha-Amylase Activity

Phadebas method. A modification of the Barnes and Blakeney (1974) method was used to determine alpha-amylase activity in crude and purified extracts. The commercial Phadebas tablets were not used. Forty mg of cross-linked potato amylose covalently labelled with Cibachron Blue (gift from Pharmacia, Piscataway, NJ) was added to 5 ml of enzyme-buffer solution (0.05 M acetate, pH 5.0, containing 0.01 M CaCl_2) held at 35°C. The digest was incubated for 15 min., shaking every 5 min., and then 1 ml of 0.5 N NaOH was added to terminate the reaction. The volume of the digest was made to 10 ml with water, filtered (Whatman No. 4), and the absorbance was read

at 620 nm. The absorbance reading is converted to alpha-amylase activity units by use of a standard curve prepared from data obtained by the reaction of various concentrations of purified millet alpha-amylase with the amylose-Cibachron Blue substrate under the conditions described above. Enzyme activity (units of activity) was expressed as μ moles of apparent maltose produced per ml of alpha-amylase solution per minute.

Reducing power method. The activity of alpha-amylase purified by glycogen complex formation was determined by the method of Robyt and Whelan (1968). Reducing sugars were determined by Nelson's colorimetric copper method (1944).

Specific activity. Specific activity was expressed as Units (U) of alpha-amylase per mg of protein. Protein content of the enzyme extracts was determined by Miller's (1959) method.

Purification of Glycogen

Protein contaminating shellfish glycogen solutions was removed by the method of Loyter and Schram (1962). The reagent was kept frozen until used.

Gel Filtration Chromatography

Columns (2.6 x 76 cm) were packed with Sepharose 2B Cl, 4B Cl, or Biogel P10 (Pharmacia, Uppsala, Sweden) in 0.01 N NaOH containing 0.02% sodium azide. Ten mg of carbohydrate was loaded onto the column, the column was eluted in an ascending direction with 0.01N NaOH containing 0.02% sodium azide, and 5 ml fractions were collected at a flow rate of 25 ml/hr. Total carbohydrate in the fractions was determined by the phenol-sulfuric acid method (Dubois et al., 1956).

SDS Gel Electrophoresis

The method of Weber and Osborn (1969) was used to determine the molecular weight of purified alpha-amylase.

pH and Temperature Optima

Optimum pH for activity of purified alpha-amylases from millet was determined using a soluble starch substrate and acetate or tris buffers (containing 0.01 M CaCl_2) in a pH range from 3.0 to 7.2. The method of Robyt and Whelan (1968) was used except the temperature was maintained at 35°C. The temperature optimum was determined at the optimum pH using 0.02 M acetate buffer containing 0.01 M CaCl_2 .

Starch Isolation and Fractionation

Starch was isolated from pearl millet as described by Beleia et al. (1980). Millet starch was fractionated into amylose and amylopectin by the methods of Montgomery and Senti (1958).

Hydrolysis of Starch and Starch Fractions

Raw starch. Fifty mg of defatted millet starch was suspended in 5 ml of 0.05 M acetate buffer, pH 5.0, containing 0.01 M CaCl_2 . Purified alpha-amylase (270 U or 540 U) was added and the suspension was incubated for 24 hr. at 35°C. After incubation the suspension was centrifuged at 5,000 rpm for 10 min., the supernatant was poured off, the starch pellet was dissolved in 3 ml of 1 N NaOH, and an aliquot containing 10 mg/ml was applied to a Sepharose 2B Cl and eluted as described above.

The supernatant was filtered through a 0.45 μm filter, boiled to inactivate the enzyme, and reducing sugars were determined in the filtrate by Nelson's (1944) method. Percent hydrolyses was calculated as follows:

$$\% \text{ Hydrolysis} = \frac{\text{Reducing sugars (maltose equiv.) in digest}}{\text{Total CHO (maltose equiv.) in digest}} \times 100$$

Amylopectin and amylose. Millet starch or its fractions (20 mg) were dissolved in 1 N NaOH, and the pH of the solutions were adjusted to 6.0

with 1 N HCl. Buffer (0.05 M acetate, pH 5.0, 0.01 M CaCl_2) was added to make a total volume of 5 ml, purified millet alpha-amylase was added (27 U), and the mixture was incubated for 5 to 15 min. at 35°C. After incubation, the mixture was boiled to inactivate the enzyme, and an aliquot containing 10 mg was applied to a 4B-C1 column (amylose), a 2B C1 column (amylopectin), or Biogel P10 (amylopectin).

RESULTS AND DISCUSSION

Isolation of Alpha-Amylase from Germinated Pearl Millet

Alpha-amylase was extracted from germinated millet using 0.05 M sodium acetate buffer, pH 5.0, containing 0.01 M CaCl_2 . The ratio of buffer to meal was 2.5:1; extraction time was 2 hr. The slurry was centrifuged for 15 min. at 4,000 rpm and the precipitate was discarded.

The filtered supernatant was made to 0.2% with calcium acetate and the pH was adjusted to 6.0. The extract was divided into 15 ml fractions and heated at 70°C for 15 min. to inactivate beta-amylase, followed by rapid cooling in an ice bath and filtration. The filtrate was dialyzed overnight at 4°C against 0.2% calcium acetate, the insoluble material was removed by filtration, and the filtrate was freeze-dried.

The freeze-dried sample was dissolved in the same acetate buffer that was used in the extraction step. Purification of alpha-amylase was completed by forming an amylase-glycogen complex according to the method of Schramm and Loyter (1966). Details of the isolation procedure are given in Figure 1.

Enzyme activity was determined at each step in the purification process and the data are shown in Table 1. There was a substantial reduction in the alpha-amylase activity of the extract as a result of the heat-treatment; only 57% of the activity was recovered. At the glycogen complex stage, 11% of the enzyme was recovered with about a 17 fold increase in specific activity. Germination caused a 120-fold increase in specific activity over what was observed for the purification of alpha-amylase from mature pearl millet (Beleia and Varriano-Marston, 1981).

Figure 1. Alpha-amylase isolation procedure.

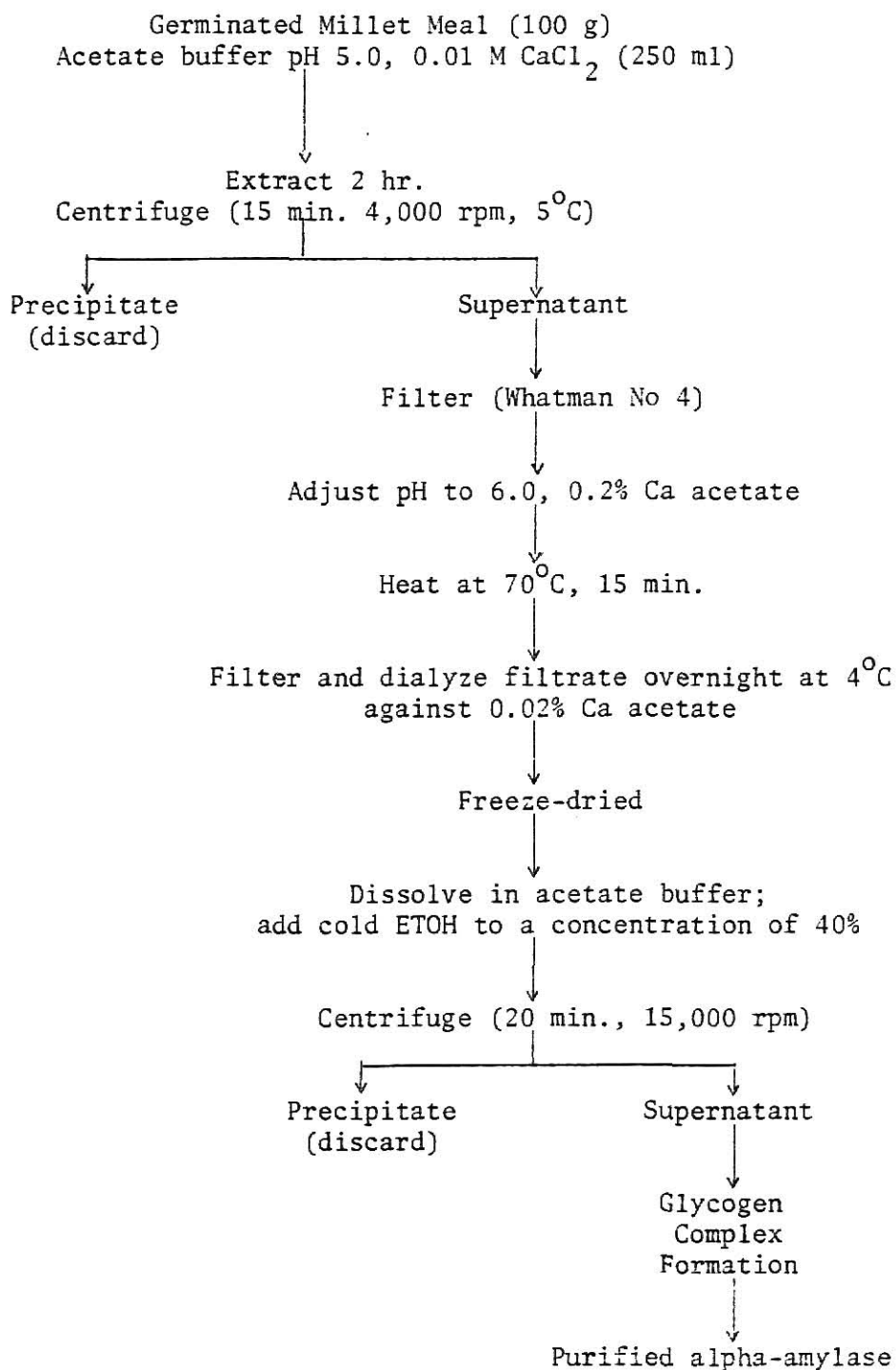


Table I. Purification of alpha-amylase from germinated pearl millet.

Fraction	U ml ⁻¹ ^a	Protein mg ml ⁻¹	Specific activity U/mg	Volume (ml)	Recovery ^b %
Buffer extract	1235	10.8	114	123	100
Heat treated extract	1025	9.4	109	85	57
Glycogen Complex	1359	0.7	1928	12	11

$$^a U = \frac{\mu\text{g maltose/ml/min}}{342} \times 2$$

$$^b \frac{U \text{ ml}^{-1} \times \text{volume}}{\text{Total activity of buffer extract}} \times 100$$

Properties of Purified Alpha-Amylase

SDS gels of alpha-amylase from germinated pearl millet purified by glycogen complex formation showed one protein band at a molecular weight of 48,000 (Fig. 2). That value falls within the range reported for alpha-amylases from malted grains (Greenwood and Milne, 1968; MacGregor, 1978; Tkachuk and Kruger, 1974).

Data on optimum pH of purified alpha-amylase recorded as a percentage of maximum activity, are presented in Figure 3. Optimum pH for alpha-amylase from malted millet was 4.0 to 4.5 which is slightly lower than the 4.4 to 4.8 range reported for alpha-amylases from mature millet (Beleia and Varriano-Marston, 1981). The acid tolerance of millet alpha-amylase may be important in preparing some fermented millet foods, e.g. kisra, where the pH drops to about 4 in 18 hr. (El Tinay, 1979).

Temperature optimum for alpha-amylase from malted millet was determined at pH 4.2 with 0.02M acetate buffer containing 0.01M CaCl_2 . Maximum activity of the enzyme was obtained at 45 to 55°C (Fig. 4). Only 75% of the activity remained after heating to 65°C which explains the reduction in alpha-amylase activity that occurs during the heat-treatment step in the purification procedure.

Alpha-Amylolysis of Millet Starch and Starch Fractions

Raw starch. The molecular weight of millet starch (dissolved in 1N-NaOH) is so large ($> 20 \times 10^6$) that most of the sample elutes at the void volume (V_0) of the sepharose 2B Cl column (Fig. 5). That elution pattern was altered by the 24 hr. hydrolysis of raw starch by millet alpha-amylase. The number of starch fragments having molecular weights between 70,000 and 500,000, increased. The column was calibrated with standard dextrans (Fig. 6). In

Figure 2. Molecular weight of purified alpha-amylase as determined by SDS gel electrophoresis (designated by an arrow).

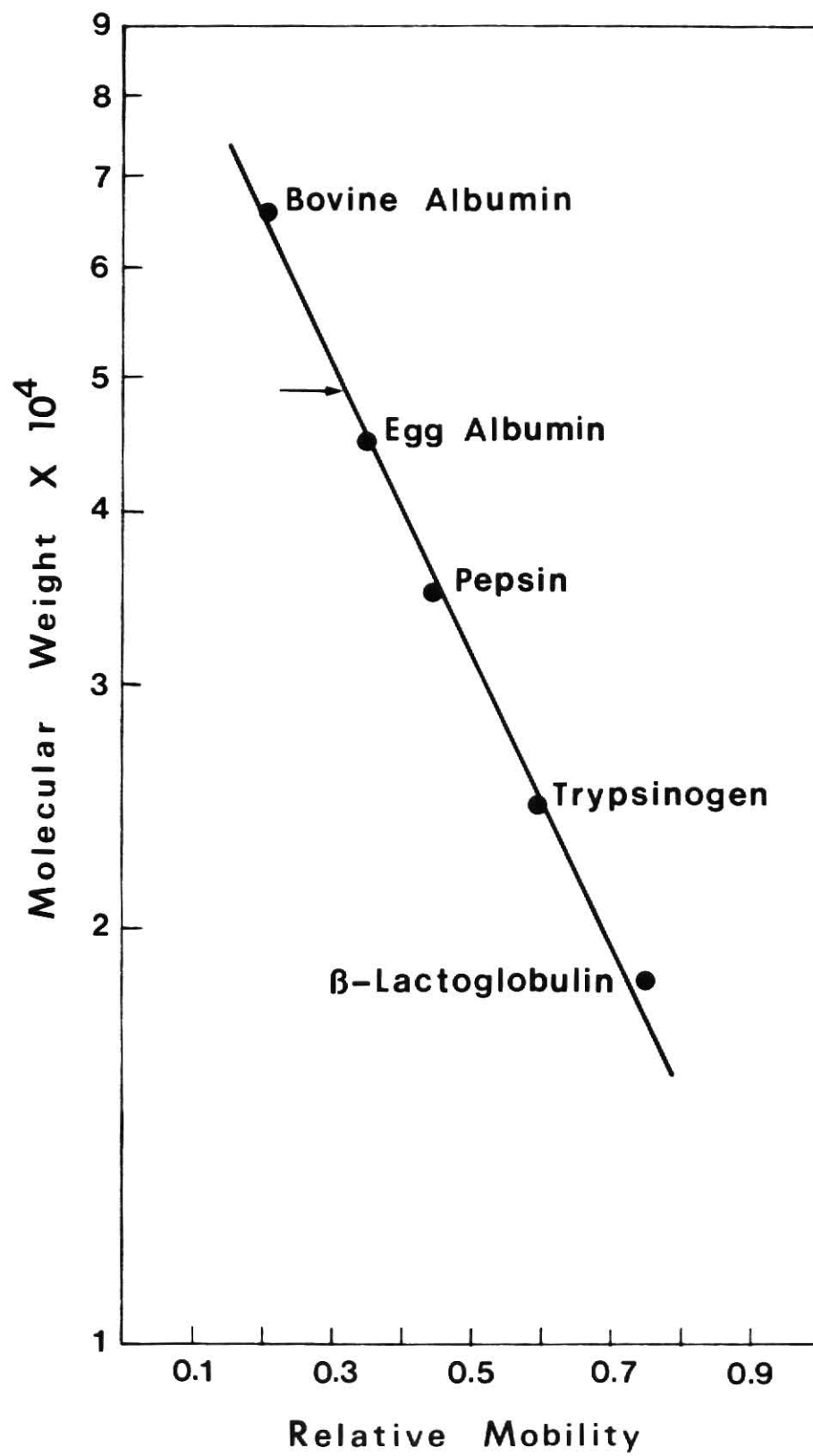


Figure 3. pH optima for alpha-amylase.

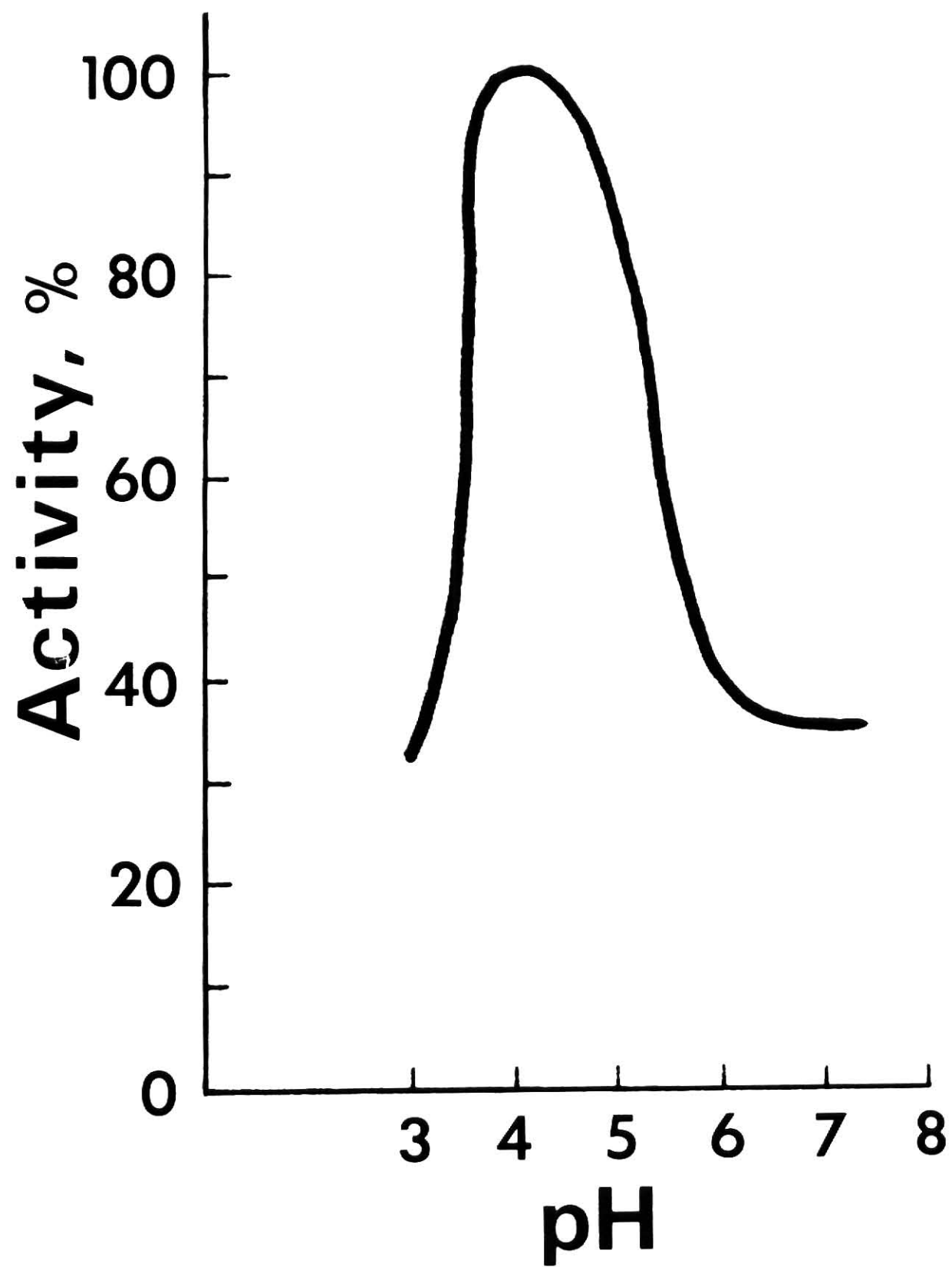


Figure 4. Temperature optima for alpha-amylase.

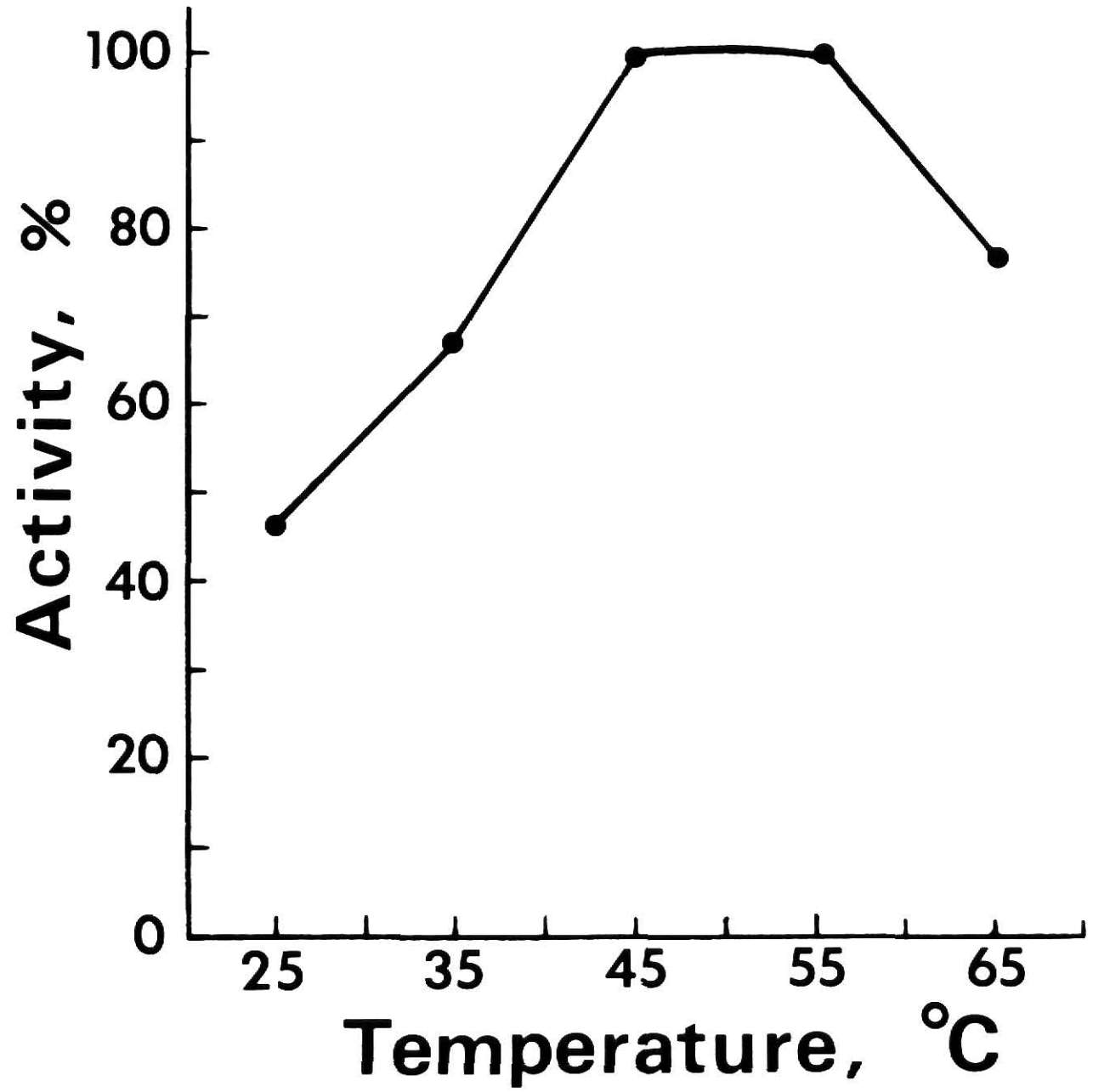


Figure 5. Elution profile of untreated millet starch (—) and starch hydrolyzed (26%) by millet alpha-amylase (----). Sepharose 2B C1 column.

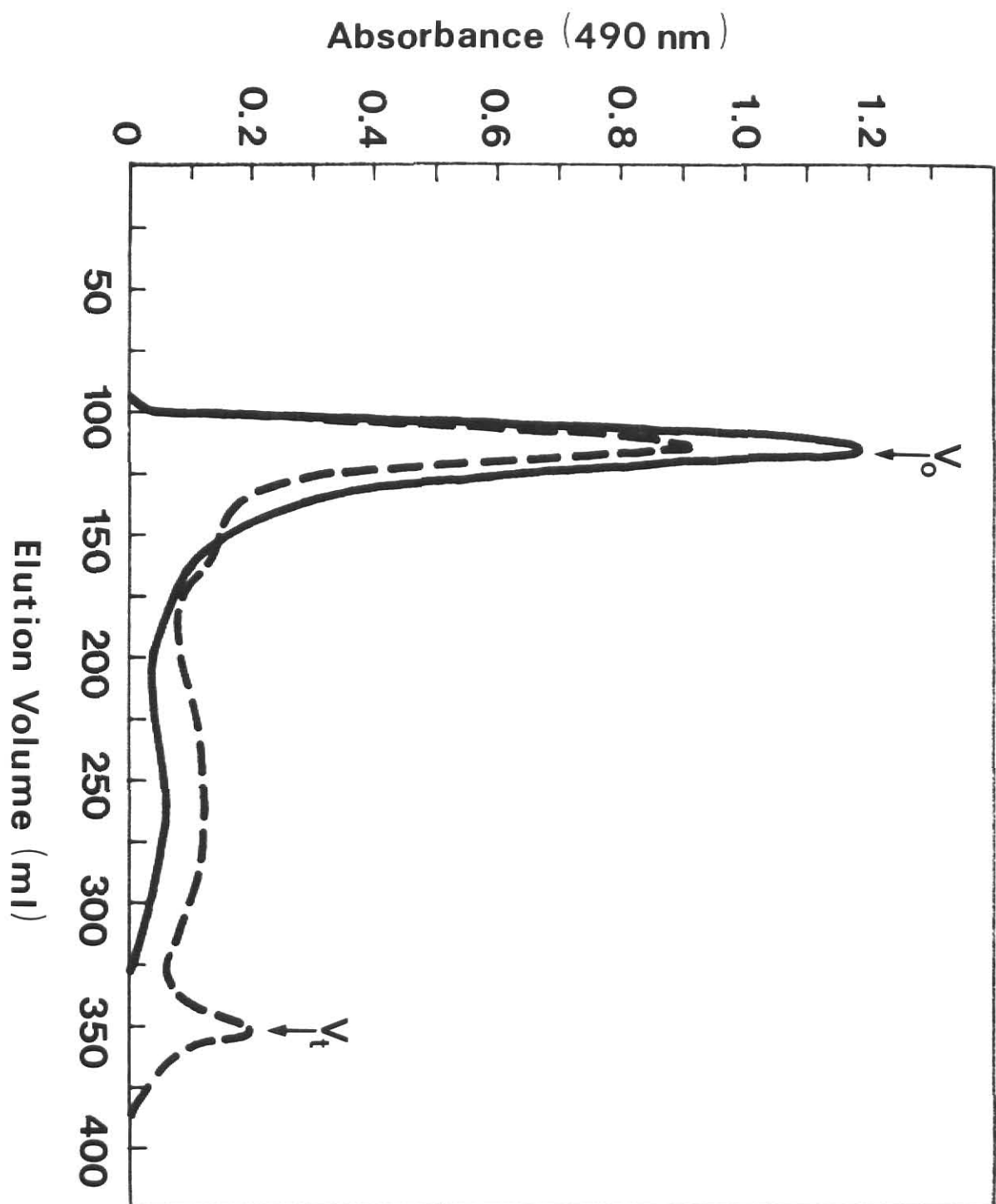
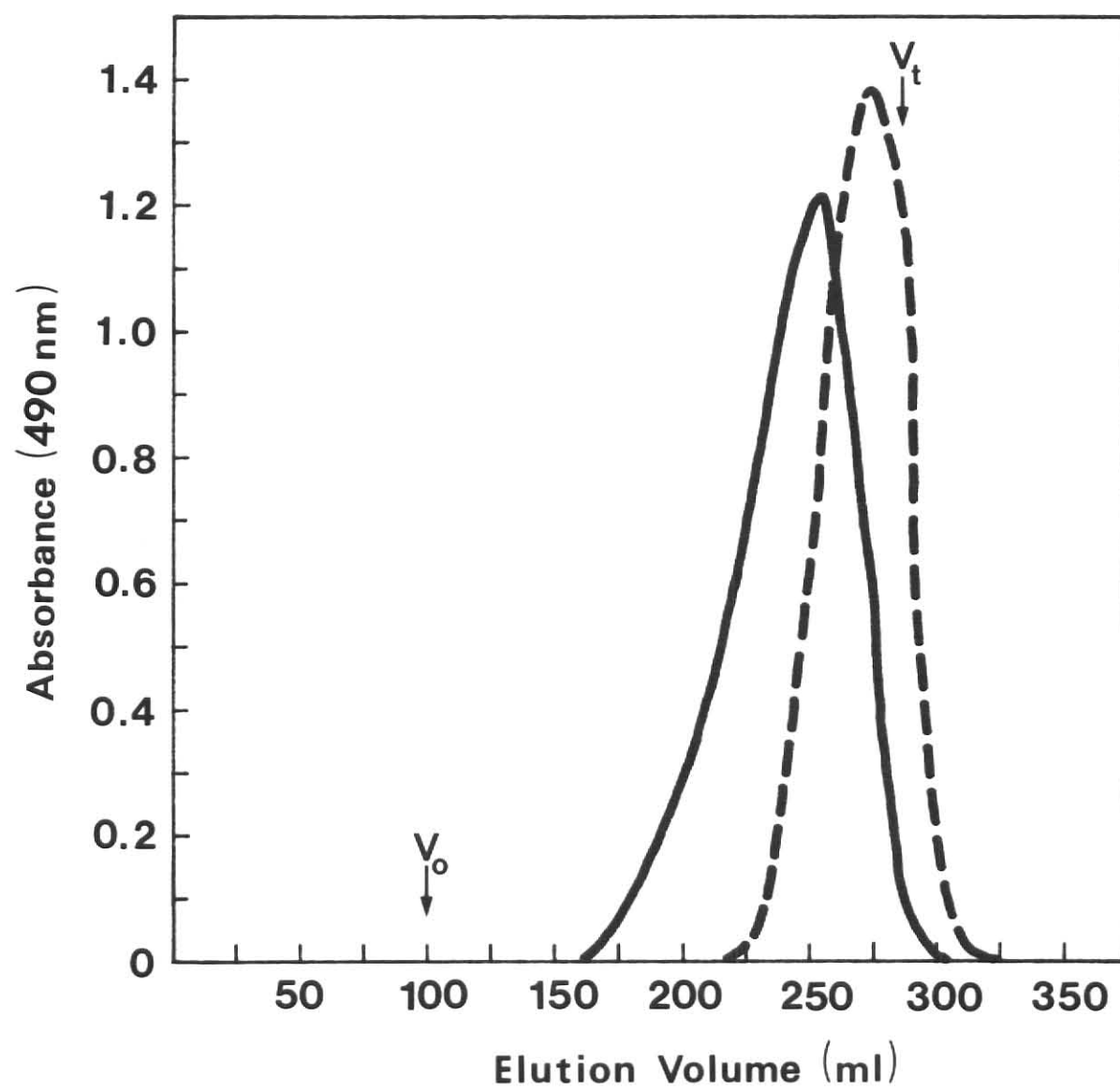


Figure 6. Elution patterns of standard dextrans:
500,000 (—) and 70,000 (---) molecular
weights. Sepharose 2B Cl column.



addition, starch fragments appeared at the V_t (total volume) of the column and probably consist of the lower molecular weight products that are formed during the initial stages of amylolysis. The limited starch hydrolysis (26%) after a 24-hr. digestion period indicates that raw millet starch is quite resistant to degradation by pearl millet alpha-amylase. Beleia and Varriano-Marston (1981) found that raw wheat starch was more easily digested by millet amylases than was raw millet starch suggesting that the latter starch has a more ordered arrangement of molecules.

Amylose. The elution pattern of millet amylose from Sepharose 2B C1 is shown in Figure 7. A portion of the amylose elutes at the V_0 indicating that those particles have molecular weights $> 5 \times 10^6$ (Pharmacia, 1975). Millet amylose may have a limited amount of branching, as has been observed for other starches (Banks and Greenwood, 1966). The beta-amylolysis of millet amylose as determined by Whelan's (1964) method averaged 73.9% compared to 67.2% for a wheat amylose fractionated from wheat starch by the same procedure. The incomplete conversion of amylose to maltose by beta-amylase may be evidence of branching in amylose.

When millet amylose was incubated for 10 min. at 35°C with 27 U of pearl millet alpha-amylase, 36% of the amylose was hydrolyzed. Fragments having molecular sizes ranging from 10,000 to 40,000 (Fig. 7) were produced with the majority having molecular weights of about 10,000. The sepharose 4B C1 column was calibrated with standard dextrans (Fig. 8).

Amylopectin. Millet amylopectin had an average chain length ($\overline{C.L.}$) of 28 as determined by the method of Gunja-Smith (1971). This is larger than a $\overline{C.L.}$ of 17 we obtained for wheat amylopectin prepared by the method of Montgomery and Senti (1958).

Figure 7. Sepharose 4B C1 elution profile of millet amylose before (—) and after hydrolysis by millet alpha-amylase (----).

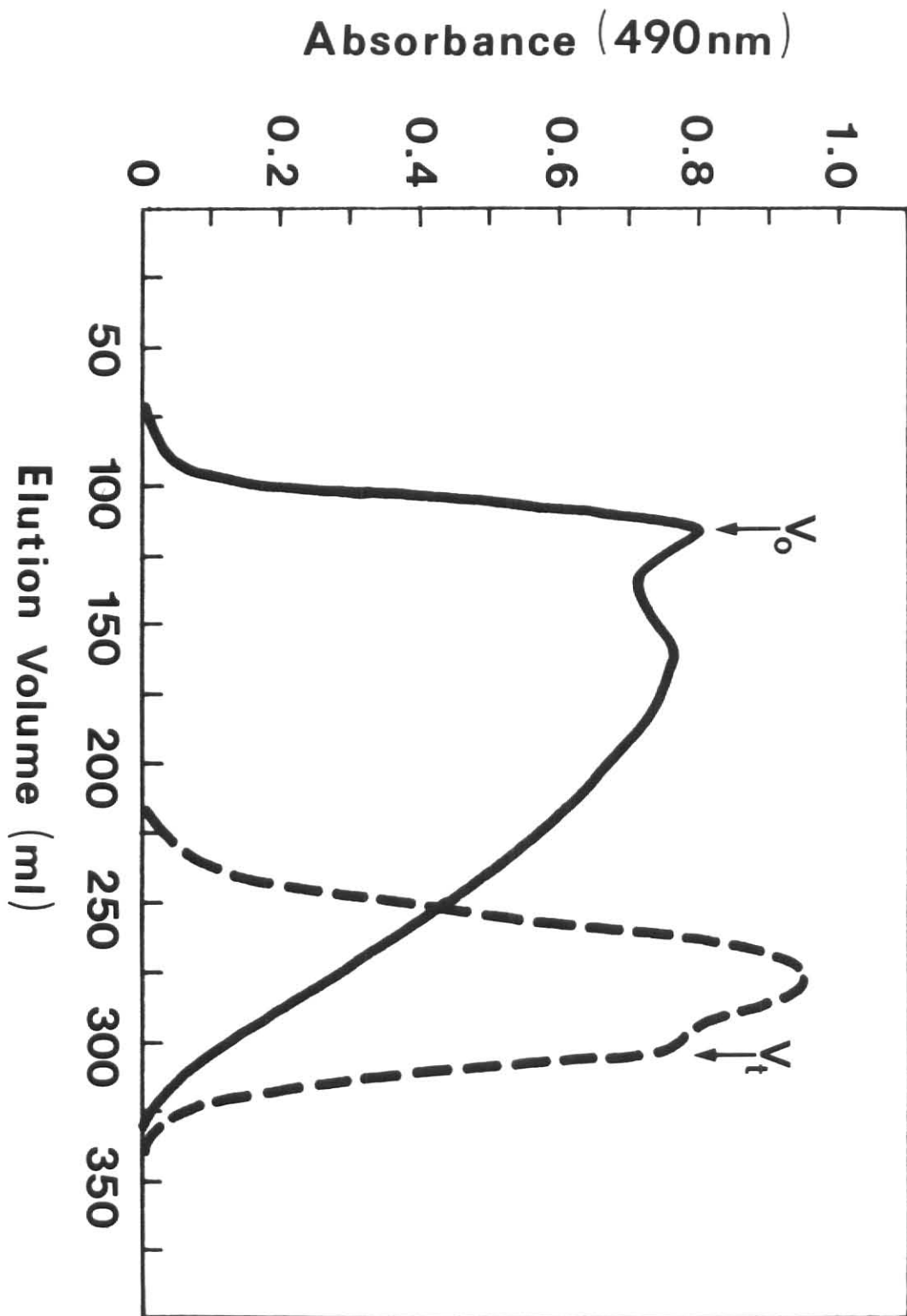
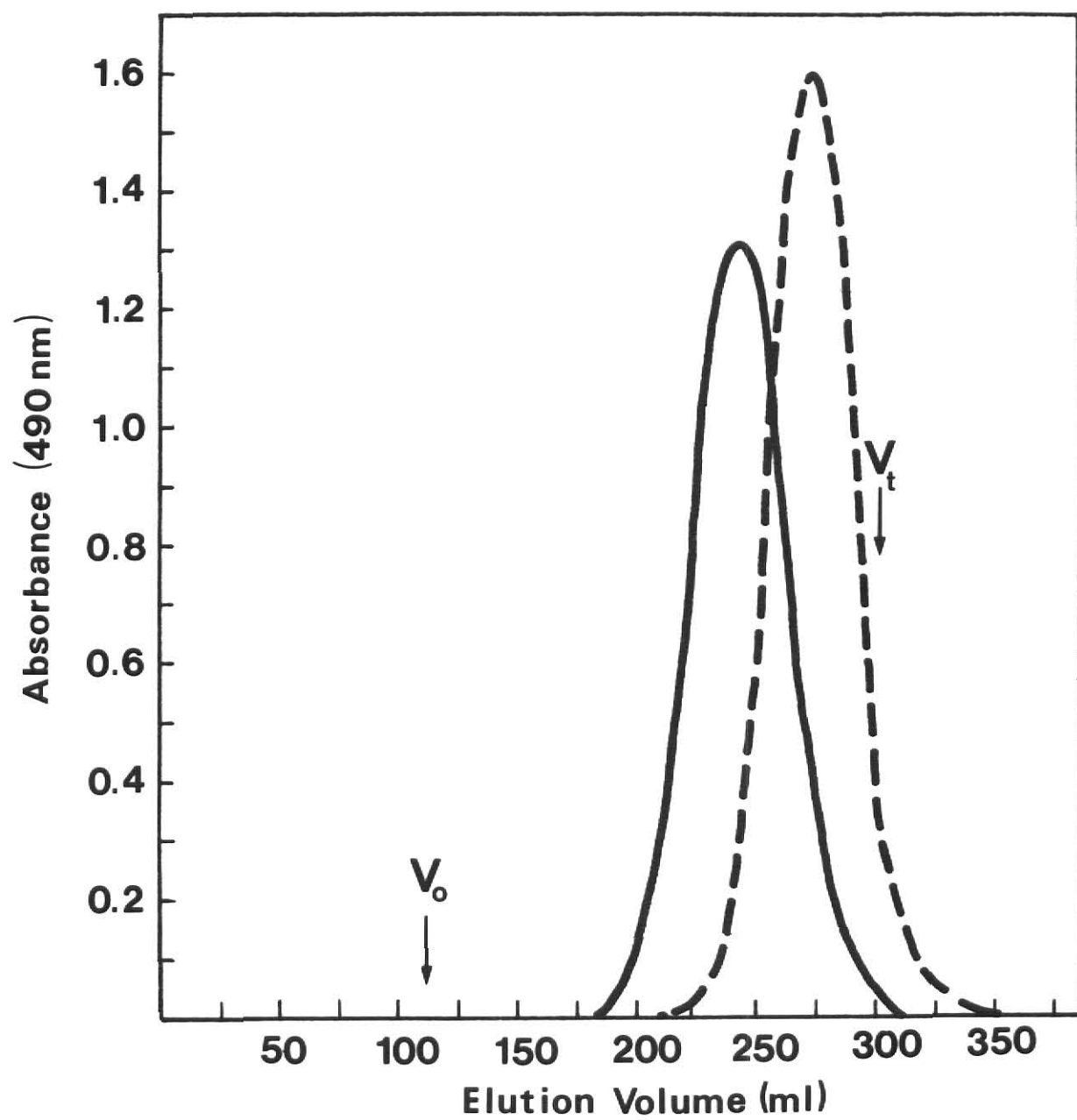


Figure 8. Sepharose 4B Cl elution profiles of
standard dextrans: 10,000 (----)
and 40,000 (—) molecular weights.



Millet amylopectin eluted as a single peak at the V_0 of a Sepharose 2B C1 column (Fig. 9). Hydrolysis by pearl millet alpha-amylase (27 U, 15 min.) produced particle sizes ranging from 40,000 to 500,000. Longer digestion (35%) produced one peak eluting at the V_t of the column (Fig. 9). The fractions in that peak were pooled and applied to a Bio Gel P10 column (Fig. 10). Part of the millet amylopectin was resistant to amylolysis because there were still some large molecular weight products eluting at the V_0 of the column ($> 70,000$ MW). The majority of fragments had molecular weights ranging from 5,000 to 10,000. Digestion to 70% gave much smaller fragments (Fig. 11). Thus it appears that the resistant bonds in millet amylopectin are broken with longer digestion.

Figure 9. Sepharose 2B Cl elution patterns of millet amylopectin before (—) and after hydrolyzed 12% (---) and 35% (— —) by millet alpha-amylase.

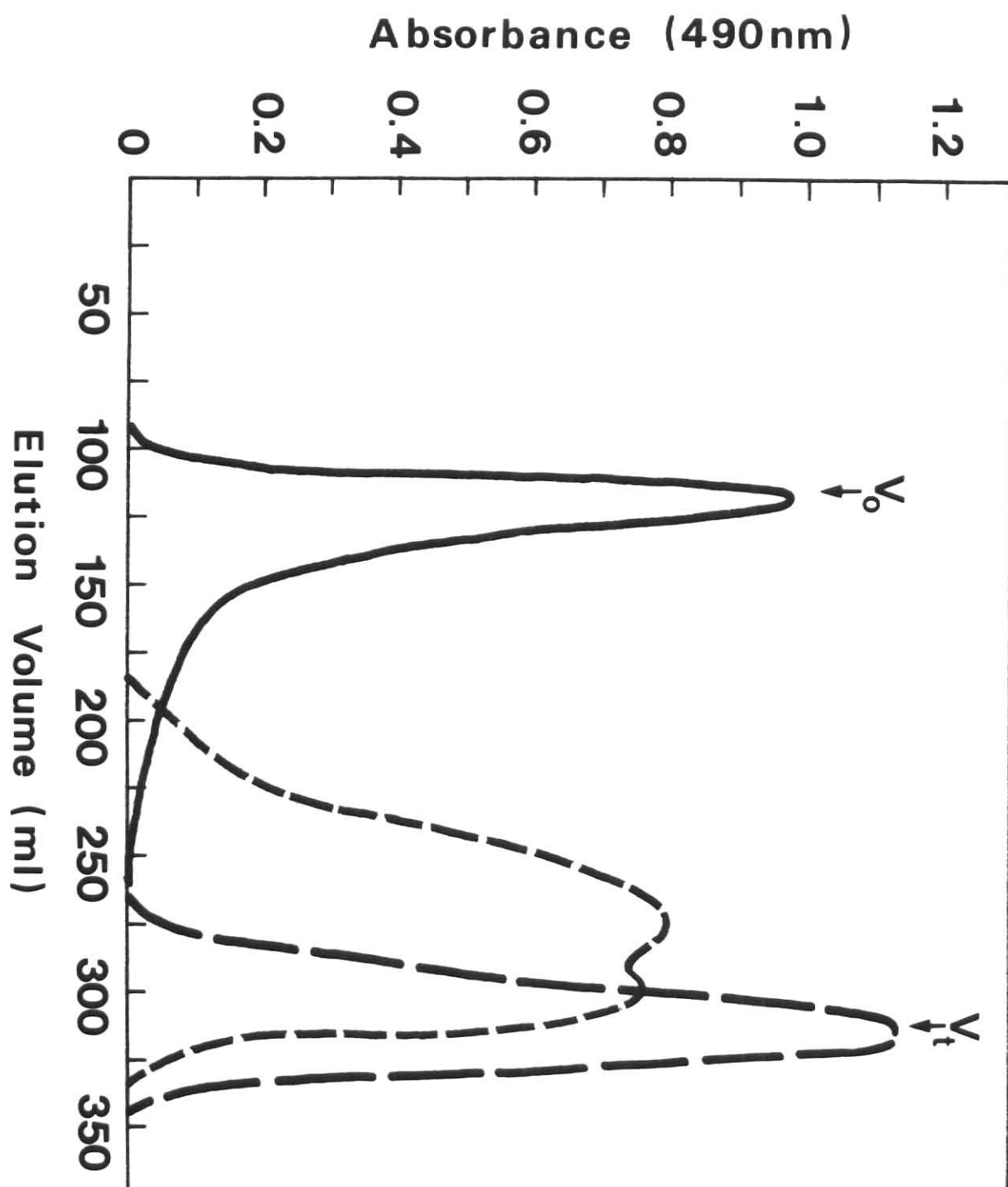


Figure 10. Bio-Gel P 10 elution profile of millet amylopectin hydrolyzed 35% by millet alpha-amylase.

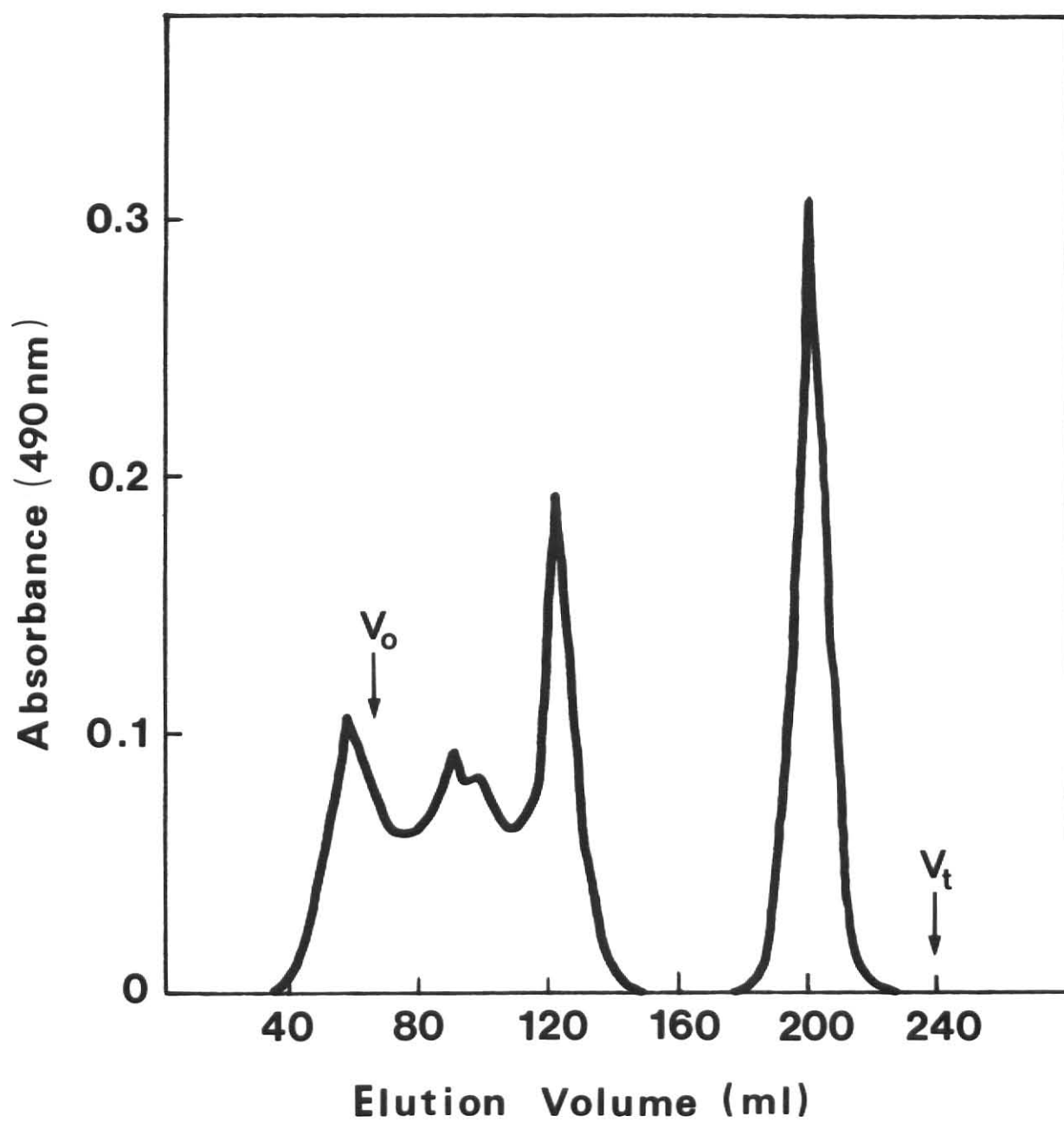
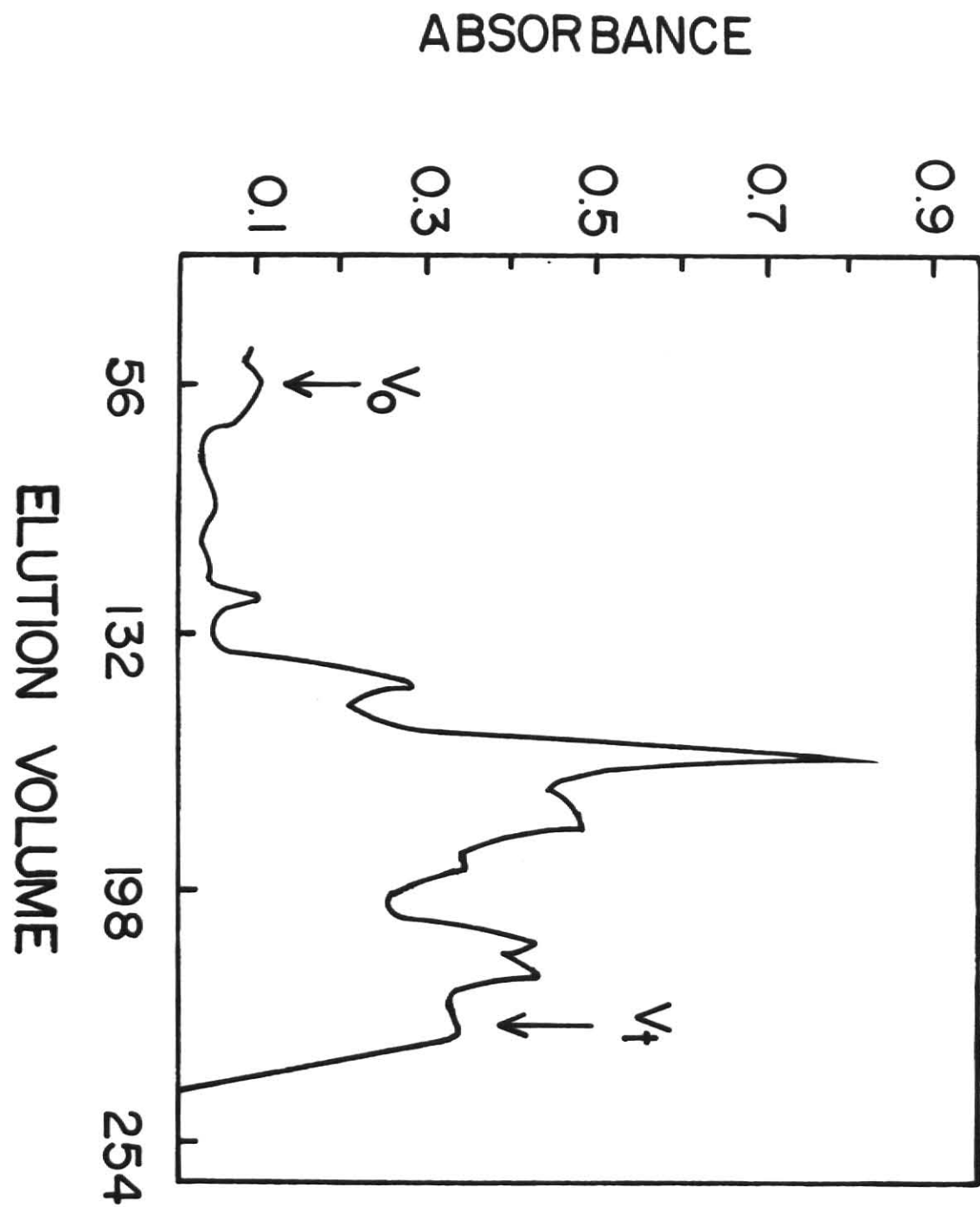


Figure 11. Bio-Gel P 10 elution profile of millet amylopectin hydrolyzed 70% by millet alpha-amylase.



LITERATURE CITED

- Akazawa, T. 1976. Polysaccharides, In "Plant Biochemistry," ed. by J. Bonner and J. E. Varner. Chap. 12, Academic Press, Inc., N.Y.
- Asha, Bal, Wagle, D. S., and Sheorain, V. S. 1976a. "Some enzymatic studies on Bajra "Pennisetum typhoides" and Barley (Hordeum Vulgare) during malting." J. Food Sci. and Tech. 13: 75.
- Asha, Bal, Wagle, D. S., and Scheorain, V. S. 1976b. "Effect of germ temperature on amylolytic and proteolytic activities of Bajra and barley during germination." J. Food Sci. and Tech. 13: 253.
- Banks, W., and Greenwood, C. T. 1966. The fine structure of amylose. The action of pullulanase as evidence of branching. Arch. Biochem. and Biophys. 117: 674.
- Barnes, W. C., and Blakeney, A. B. 1974. Determination of alpha-amylase using a commercially available dye-labelled substrate. Die Stärke 26: 193.
- Barrett, F. F. 1975. Enzyme uses in the milling and baking industries. In "Enzyme in Food Processing," ed. by G. Reed. p. 301. Academic Press, Inc., N.Y.
- Bathgate, G. N., and Palmer, G. H. 1972. A reassessment of the chemical structure of barley and wheat starch granules. Die Stärke 24: 336.
- Beleia, A. 1980. Characterization of starch and alpha-amylase. Ph.D. thesis, Kansas State University, Manhattan, Kansas.
- Beleia, A., Varriano-Marston, E., and Hoseney, R. C. 1980. Characterization of starch from pearl millet. Cereal Chem. 57: 300.
- Beleia, A., and Varriano-Marston, E. 1981. Pearl millet alpha-amylases. Cereal Chem. (In press).

- Botes, D. P., Joubert, F. J., and Novellie, L. 1967. Kaffircorn malting and brewing studies. *J. Fd. Agric.* 18: 409.
- Copland, L. O. 1976. Principles of seed science and technology. Chap. 4. Burgess Pub. Co., Minneapolis, Minnesota.
- Dubois, U., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. 1956. Colorimetric method for determination of sugar and related substances. *Anal. Chem.* 28: 350.
- Dunn, G. A. 1974. Model for starch breakdown in higher plants. *Phytochem.* 13: 1341.
- Dyer, T. A., and Novellie, L. 1966. Kaffircorn malting and brewing studies. *J. Sci. Fd. Agric.* 17: 449.
- El Tinay, A. H., Abdel, Gadir, A. M., and E. Hidai, M. 1979. Sorghum fermented Kisra bread. 1. Nutritive value of Kisra. *J. Sci. Food Agric.* 30: 859.
- Greenwood, C. T., and Milne, E. A. 1968a. Studies on starch degrading enzyme. VIII. A comparison of alpha-amylase from different sources: their properties and action patterns. *Die Stärke* 20: 139.
- Greenwood, C. T., and Milne, E. A. 1968b. Properties and action pattern of the alpha-amylase from barley, oats, rye and wheat. *Die Stärke.* 20: 101.
- Guardiola, J. L., and Sutcliffe, J. F. 1971. Content of protein hydrolysis in the cotyledons of germination per (Pisum sativum L.) seeds. *Ann. Botany.* 35: 791.
- Gunja, Smith, Z., Marshall, J. J., Smith, E. E., and Whelan, W. J. A. 1970. Glycogen debranching enzymes from cytophage. *FEBS Lett.* 12: 96.
- Jain, A. K., and Date, W. B. 1975. Relative amylase activity of some malted cereal grain. *Journal of Food Sci. and Tech.* 12: 151.

- Kneen, E., 1945. Sorghum amylase. *Cereal Chem.* 22: 112.
- Kruger, J. E. 1972a. Change in the amylase of hard red spring wheat during growth and maturation. *Cereal Chem.* 49: 379.
- Kruger, J. E. 1972b. Change in the amylases of hard red spring wheat during germination. *Cereal Chem.* 49: 391.
- Kruger, J. E., and Tkachuk, R. 1969. Wheat alpha-amylase 1. Isolation. *Cereal Chem.* 46: 219.
- Kulp, K. 1975. Carbohydrases. In *Enzyme in "food processing."* ed. by G. Reed, p. 54. Academic Press, N.Y.
- Lineback, D. R., and Ponpipon, S. 1977. Effect of germination of wheat, oats, and pearl millet on alpha-amylase activity and starch degradation. *Die Stärke.* 29: 52. 1977.
- Loyter, A., and Schramm, M. 1962. The glycogen-amylase complex as means of obtaining highly purified alpha-amylase. *Biochem. Biophys. Acta.* 65: 200.
- MacGregor, A. W. 1977. Isolation, purification, and electrophoretic properties of an alpha-amylase from malted barley. *J. Inst. Bres.* 83: 100.
- MacGregor, A. W., Thompson, R. G., and Meredith, W. O. S. 1974. Alpha-amylase from immature barley: purification and properties. *J. Inst. Brew.* 80: 181.
- Maeda, I., Kiribuchi, S., and Nakamura, M. 1978. Digestion of barley starch granules by the combined action of alpha- and beta-amylase purified from barley and barley malt. *Agric. Biol. Chem.* 42: 259.
- Marchylo, B. Kruger, J. E., and Irvine, G. N. 1976. Alpha-amylase from immature hard red spring wheat. 1. Purification and some chemical and physical properties. *Cereal Chem.* 53: 157.

- Miller, G. L. 1959. Protein determination for larger numbers of samples. *Anal. Chem.* 31: 964.
- Mitchell, E. B. 1972. Homogenous alpha-amylase from malted barley. *Phytochem.* 11: 1673.
- Montgomery, E. M., and Senti, F. R. 1958. Separation of amylose from amylopectin by an extraction-sedimentation procedure. *J. Polymer Sci.* 28: 1.
- Nelson, N. 1944. A photometric adaptation of the Somogi method for the determination of glucose. *J. Biol. Chem.* 153: 375.
- Paleg, L. G. 1965. Physiological effect of gibberellic acid. II. On starch hydrolysis enzymes of barley endosperm. *Plant Physiol.* 35: 902.
- Palmer, G. H. 1972. Morphology of starch granules in cereal grain and malts. *J. Inst. Brew.* 78: 326.
- Pharmacia. 1975. Beaded Sepharose 2B-4B-6B. Information Booklet. Pharmacia fine chemicals AB, Uppsala, Sweden.
- Robyt, J. F., and Whelan, W. J. 1968. The amylases. In: *Starch and its derivatives*, ed. by J. A. Radley, p. 430. Chapman and Hall, London.
- Sullins, L. W., and Rooney, R. D. 1977. In "Proceedings of a symposium on sorghum and millet for human food," ed. by D. A. V. Dendy, p. 91. Tropical Products Institute, London.
- Tkachuk, R., and Kruger, J. E. 1974. Wheat amylases. II. Physical characterization. *Cereal Chem.* 51: 508.
- Vogel, S., and Graham, M. 1978. Sorghum and Millet: Food Production and Uses. Intern. Dev. Research Center, 123e. Ottawa, Canada.
- Warchalewski, J. R., and Tkachuk, P. 1978. Durum wheat alpha-amylases: isolation and purification. *Cereal Chem.* 55: 146.

- Weber, K., and Osborn, M. 1969. The reliability of molecular weight determination by dodicyl sulfate-polacrylamid gel electrophoresis. J. Biol. Chem. 244: 4406.
- Whelan, W. J., 1964. Hydrolysis with β -amylase and preparation of the β -amylase limit dextrin of amylopectin, in: Methods Carbohyd. Chem., ed. by R. L. Whistler, p. 261. Academic Press, N.Y.

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Alpha-amylase was isolated from germinated pearl millet and purified using glycogen-amylase complex formation. Germination resulted in a 120 fold increase in specific activity of the enzyme over that of alpha-amylase from mature grain.

Alpha-amylolysis of millet starch and its fractions by purified alpha-amylase was studied using gel filtration. Raw millet starch is resistant to attack by alpha-amylase from germinated millet. Only limited degradation (26%) occurred after incubating starch with the enzyme for 24 hr. at 35°C. Millet amylose was readily hydrolyzed by purified millet alpha-amylase. After a 10 min. reaction period at 35°C, 36% of the molecule was degraded to molecular sizes of 40,000 to 10,000. Conversely, some portions of millet amylopectin were hydrolyzed slowly by alpha-amylase.

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