ACTIVITY OF α- AND β-AMYLASE AT LOW TEMPERATURES

by

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Thesis submitted to the Graduate Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Food Science and Technology

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November, 1974
Blacksburg, Virginia

ACKNOWLEDGMENTS

The author expresses sincere appreciation and gratitude to her major professor, Dr. Anthony Lopez, Department of Food Science and Technology, for suggestions, criticisms, and patience in the research and writing of this thesis.

Gratitude is expressed to the other members of the graduate committee, Drs. R. V. Lechowich and M. D. Pierson for assistance throughout the experiment, especially in finalizing the thesis.

The author is grateful to the Department of Food Science and Technology for both the opportunity to conduct this investigation and for financial support.

Appreciation is expressed to and for assistance in the statistical analysis of the research data.

Thanks go to and , for technical assistance.

The author is particularly indebted to her husband, , for his patience, understanding, encouragement, and assistance during her graduate studies.

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1.0 INTRODUCTION

Foods are composed of many complex compounds with widely varying properties. They are thus subject to a diversity of chemical reactions producing many changes. Enzymes are one of the complex compounds which occur naturally in most raw foods and other materials and can play a vital role in determining shelf life and overall quality of food products during processing, storage, and handling.

Enzymes cause various deleterious effects in many foods such as off-colors, off-flavors and odors, and the destruction of certain vitamins. On the other hand, the presence of naturally occurring enzymes can be used to advantage, such as the use of malted barley for starch conversions in brewing. It is apparent that both desirable and undesirable effects can result from enzyme activity depending on the conditions and food systems involved.

Many factors are involved in the rate of enzyme-catalyzed reactions. Some of these factors are enzyme and substrate concentrations, pH of the system, the presence and absence of certain chemicals and ions, and temperature. The catalytic activity of an enzyme is the result of its ability to lower the activation energy needed for the transformation of a substrate into other products.

The effects of temperature on enzyme activities have been of interest almost since enzymes were discovered over a century ago. Two major effects of temperature on enzyme activities have been considered. One has been on the enzyme-catalyzed reaction and the other

on the inactivation of the enzyme. The optimal temperature for most enzymatic reactions, with a few exceptions, is between 30°C (86°F) and 40°C (104°F). With an increase in temperature, the reaction rate increases, and for most enzymes, a rise of 10°C (18°F) will often double the reaction rate. This increase in activity with the rising of temperature will usually continue to the point of inactivation. The inactivation of enzyme activity was believed to be caused either by high or low temperatures. It is now generally realized that low temperatures and ice formation do not inactivate but only decrease enzyme activity. The rate of decrease is not the same for all enzyme-substrate systems.

Amylases are used as intentional additives in bread making and in the production of many other baked goods (Johnson, 1965). Recently, they have been used in the manufacture of sweet potato flakes from starchy and uncured roots (Hoover, 1966). The process consisted of treating the puree with a commercial amylolytic enzyme. It is thus obvious that these enzymes are of industrial and biological importance. A study of α- and β-amylase activity at low temperatures should contribute important information to food processing and storage operations.

The objectives of the research reported here were to determine:

(a) the activity of purified swine pancreatic α-amylase and of sweet potato β-amylase with soluble starch at temperatures ranging from 4°C (39.2°F) to -23°C (-9.4°F) over storage periods of 0 to 112 days;

(b) the effects of different levels of enzyme concentration on activity under the conditions specified in (a);

(c) cumulative maltose as an index of the activity of naturally occurring α- and β-amylase in sweet potato at 4°C (39.2°F) to -23°C (-9.4°F) over storage periods of 0 to 56 days; and

(d) the effects of low temperature on the stability of naturally occurring α- and β-amylase in sweet potato after the puree samples were stored at 4°C (39.2°F) to -23°C (-9.4°F) over storage periods of 0 to 56 days.

2.0 REVIEW OF LITERATURE

The amylases are hydrolytic enzymes which promote the decomposition of starch. In general, starches consist mainly of two chemically different substances, amylopectin and amylose. These two are the components with which we are essentially concerned in considering amylase action. Most natural starches contain 20-25% of amylose while the rest of the bulk is essentially amylopectin. Amylose consists of long unbranched molecules of roughly 300 glucose units average length (Meyer et al., 1940). Amylopectin consists of branched or laminated molecules with the average length of a terminal branch being from 12 to 18 units. A more recent review of the subject by Guthrie and Honeyman (1968) shows that amylose is a linear polysaccharide consisting of 1000 to 4000 D-glucose units depending on the source. Evidence suggested that units other than the α-(1$\rightarrow$4) linked D-glucopyranose units may be present. Amylopectin is a highly branched structure ranging as high as over one million D-glucose units for the size of the amylopectin molecule. Methylation and hydrolysis show that there is one non-reducing end group for every 20 to 25 D-glucose units. Evidence also suggested that some of the D-glucose units are joined to others through C_6, as well as C_1 and C_4. Thus these units constitute the branch points and the branched structure is completely random.

Ohlsson (1930) classified the amylases into two classes, the α- and β-types. The International Union of Biochemists Enzyme Commission

(Anonymous, 1961) assigned the identification number 3.2.1.1 and 3.2.1.2 to enzymes α- and β-amylase (trivial name), which carry the following systematic names: α-1,4-Glucan 4-glucanohydrolase and α-1,4-Glucan maltohydrolase, respectively.

The following major characteristics of α- and β-amylases can be listed.

α-Amylases:

(a) hydrolyze α-1,4-glucosidic linkages;

(b) produce end products which have the α-configuration of C_1 of the reducing glucose unit;

(c) possess an endo-attack mechanism;

(d) rapidly decrease the ability of amylose to stain blue with iodine;

(e) rapidly decrease the viscosity of starch solution; and

(f) possess the ability to by-pass α-1,6-glucosidic linkages (Robyt and Whelan, 1968).

β-Amylases:

(a) hydrolyze α-1,4-glucosidic linkages;

(b) produce end products which have the β-configuration at C_1 of the reducing glucose unit;

(c) possess an exo-attack mechanism;

(d) slowly decrease the ability of amylose to stain blue with iodine;

(e) slowly decrease the viscosity of starch solutions; and

(f) do not possess the ability to by-pass α-1,6-glucosidic linkages (Robyt and Whelan, 1968).

The β-amylases with which we are familiar at present are all of plant origin. Ungerminated grains such as wheat, barley, rice, and oats are the best sources of the β-amylase. They are also present in appreciable quantities in soybeans and in sweet potatoes (Giri, 1934; Balls et al., 1948; Caldwell and Adams, 1950; and Bernfeld, 1951). They are highly inactive on raw, native starch and bring about the hydrolysis of only the starch which has been rendered susceptible by mechanical injury, by treatment with acid, or by gelatinization (Geddes, 1946).

β-Amylase from sweet potato is now available commercially (Worthington Biochemical Corp.). It is the most widely used in research because it is relatively easy to prepare free from contamination with other amylases (French, 1960).

The source of α-amylases varies widely. They are prepared from animal tissue, plant materials, and bacteria. α-Amylase occurs along with β-amylase in germinated cereals and comprises the amylase of animal fluids and of certain bacteria and fungi (Geddes, 1946; Bernfeld, 1951). A more recent study by Ikemiya and Deobald (1966) demonstrated that freshly pressed sweet potato juice contains α-amylase enzyme. Its activity is readily apparent only at higher temperatures than are normally considered optimum for α-amylase activity. The α-amylase of malted barley, pancreatic α-amylase, and the α-amylase of Aspergillus oryzae are among those which have been most intensively studied and also most highly purified (Caldwell and Adams, 1950).

The α-amylases from different sources such as the pancreas, saliva, urine, blood, bacteria, molds, and malt, differ among themselves with respect to such properties as stability, pH optima, and other, but not to their specific actions on starch (Hopkins, 1946).

Human salivary α-amylase hydrolyzes the substrate in the same way as does crystalline swine pancreatic α-amylase. The enzyme hydrolyzes α-1,4-glucosidic bonds in polyglucosans (amylose, amylopectin, glycogen, and dextrins). The location in the molecule of the bond to be hydrolyzed is selected at random, but the terminal bonds are split much more slowly. The final products of action are maltose, limit dextrins of low degree of polymerization (3 to 7), and small amount of glucose. The α-1,6-glucosidic bonds are not hydrolyzed by this enzyme (Meyer et al., 1947; Fisher and Bernfeld, 1948).

Sweet potato β-amylase also hydrolyzes α-1,4-glucosidic bonds in polyglucosans (amylose, amylopectin, glycogen, and dextrins). In contrast to the action of α-amylase, the next to the last α-1,4-linkage from non-reducing end group of the substrate molecule is cleaved by β-amylase. Thus, one molecule of maltose after the other is detached from the substrate until the enzyme encounters an obstacle, that is, a branching point. Amylopectin yields about 60% maltose and 40% of a high molecular weight limit dextrin (Bernfeld, 1955). Amylose is completely converted into maltose when the reaction is carried out properly (Bernfeld and Gurther, 1948).

A review by Bernfeld (1951) reported that the degradation of amylose by α-amylase seems to depend on the enzyme concentration. The breakdown of amylose by α-amylase is somewhat disturbed by the

tendency of amylose in solution to form associated particles which are enzyme resistant. The smaller the enzyme concentration, the less amylose is combined in the enzyme-substrate complex which does not undergo association, and the more amylose becomes enzyme resistant by aging. The smaller the enzyme concentration, the lower the reducing value at which the rapid primary reaction slows down. Caldwell and Kung (1953) reported the influence of concentration of pancreatic α-amylase upon its stability in aqueous solution. When other conditions were held constant, pancreatic α-amylase exhibited more stability in aqueous solution as its concentration increased. They also mentioned that statements about amylase stability in aqueous solution should include its concentration as well as the other conditions. This was agreeable with Collier (1970) who emphasized that the conditions under any proposed test should be well defined in order to preclude misleading information.

Crystalline preparation of porcine pancreatic α-amylase, and of sweet potato β-amylase exhibit a decrease in specific activity upon dilution. This phenomenon can best be explained by a dissociation of the enzymes into enzymically inactive products. The enzyme concentrations at which specific activities drop to one half of their maximum values are 0.005 and 1 μg/ml of digest, respectively (Bernfeld et al., 1965).

Dixon and Webb (1964) advise of numerous pitfalls in working with pure enzymes. One of these concerns enzyme concentration. They stated that the usual quantity of an enzyme required in an activity test is of the order of 1 μg. Effects of contaminants are minimized

by this technique.

The stability and the activity of pancreatic α-amylase are exceedingly sensitive to its chemical environment. Little and Caldwell (1942) found that porcine pancreatic α-amylase was deactivated when ketene, phenylisocyanate, formaldehyde, and nitrous acid were reacted with the enzyme. They concluded that free amino groups were necessary for catalytic activity. Caldwell et al. (1945) also found that phenylmercuric chloride, iodoacetamide, and p-chloromercuribenzoate did not inactivate the enzyme, and thus sulfhydryl groups were not necessary for enzymic activity.

The incluence of certain cations and anions upon the activity of pancreatic α-amylase was reported. Roslyn and Caldwell (1948) observed that calcium ion was responsible for the activation of pancreatic α-amylase. Sherman et al. (1928) reported their work with starch solutions adjusted to pH values from pH 5.7 to pH 7.7 in 0.01 M phosphate. They found that pancreatic α-amylase required certain anions for its action and that chloride ions were outstanding in this respect. These findings were confirmed by Caldwell and Kung (1953). Pancreatic α-amylase in starch solution adjusted to pH 7.2 shows no measurable action unless chloride ions or certain other anions are also presented (Sherman et al., 1928). Calcium ions can not replace chloride ions in the activation of pancreatic α-amylase at pH 7.2. They also observed that beside activation, chloride ions also protect the amylase from inactivation in aqueous solution. Calcium ions also protect the enzyme from inactivation in aqueous

solution but they have no influence upon the activity as distinguished from the stability of the amylase. There is a fundamental difference between the influence exerted by chloride ions in activating pancreatic α-amylase and the influence exerted by calcium ions and chloride ions in protecting the amylase from inactivation on standing in dilute aqueous solution. It appears that chloride ions and, to a lesser extent, certain other anions, are necessary for the effective union of the amylase protein and its substrate.

β-Amylase is sensitive to sulfhydryl groups and is inhibited by heavy metal ions, p-mercuribenzoate, iodoacetamide and urea. Ascorbate inhibition has been attributed to cupric ion reduction and subsequent formation of an inactive cuprous enzyme complex (Rowe and Weill, 1962). Sweet potato β-amylase does not require chloride ion or any anions for effecting its activity. It is not activated by calcium ion either (Balls et al., 1948).

In a review by Geddes (1946), the optimum hydrogen ion activities of α- and β-amylases depend upon a number of factors such as purity of the preparation, enzyme and substrate concentration, nature of the electrolytes which are present, the temperature, and the length of time the reactions are carried out. Wheat α-amylase exerted its optimal activity at pH 4.9 to 5.3 when acting upon soluble potato starch in 0.02 to 0.06 M phosphate buffer for 30 min at 40°C (104°F). In studies of the influence of ten buffers of constant ionic strength on the activity of wheat β-amylase, as measured with 1% starch at 30°C (86°F), the optimal activities varied between pH 4.5 and 5.3, depending upon the buffer system used.

α- and β-amylases differ markedly in their stability to high hydrogen ion activities and to temperature, especially in crude aqueous extracts. β-Amylase is much more thermolabile than α-amylase, whereas α-amylase is more sensitive to high hydrogen ion activities.

Caldwell and Kung (1953) observed that at 2°C (35.6°F), there was no loss of amylase activity when solutions of three times crystallized pancreatic α-amylase, containing 0.044 mg of amylase per ml of buffer, were held at pH 7.19 or at pH 8.55 for 24 hr. Under similar conditions, the amylase lost 3 and 15% of its activity in 24 hr at pH 6.53 and at pH 5.18, respectively. At 25°C (77°F), solutions of pancreatic α-amylase lost their activity more rapidly when adjusted to pH 8.55 than when adjusted to pH 6.53. The loss in activity was most rapid and extensive when the solutions were adjusted to pH 5.18. Loss of activity was more rapid and more extensive at 40°C (104°F) than at 25°C (77°F) and the loss of activity was reduced when the solutions were adjusted to pH 6.53 than at pH 5.18. The pancreatic α-amylase seemed to be most stable at pH 6.6 and pH 7.2.

Bernfeld (1951) observed that swine pancreatic α-amylase is active between pH 3.8 and 9.4 with a distinct optimum at pH 6.9 which is the same as human salivary α-amylase. Balls et al. (1948) found that sweet potato β-amylase is most active between pH 4 and 5 in acetate buffer. The isoelectric point of sweet potato β-amylase, as determined by electrophoresis, is at pH 4.74 to 4.79 (England and Singer, 1950).

The literature on the influence of low temperatures was summarized by Hepburn (1915), who reported that a number of enzymes

survived prolonged exposure to temperatures varying from about 0°C (32°F) to as low as -191°C (-311.8°F, liquid air), either in tissues or in solution. Sizer (1943), in his review of the effects of temperature on enzyme kinetics also pointed out the fact that enzymes are not inactivated by storage at temperatures as low as -186°C (-302.8°F).

The preliminary experiments conducted by Diehl et al. (1939) indicated the persistence of enzymatic activity in blanched frozen peas which were stored for several months at -6.7°C (20°F). Other frozen vegetables (asparagus, lima beans, corn, spinach, and beans) packed without scalding and likewise stored for several months at -20.6°C (-5°F), -9.4°C (15°F), and -6.7°C (20°F) also showed persistence of enzymatic activity to a greater or lesser degree.

Tressler (1938) observed that the rate of enzyme action is greatly reduced by refrigeration, but at -17.8°C (0°F) enzyme activity is still sufficiently high to change the flavor of unblanched vegetables in a few weeks. However, if the temperature was lowered to -45.6°C (-50°F), the rate of enzyme action was so greatly reduced that most unblanched vegetables could be kept for six months or longer without the development of off-flavors.

Hartzler and Guerrant (1952) worked with broccoli, green beans, lima beans, spinach, and squash. After blanching for three time intervals between 0.5 and 4 min at 93°C (199.4°F), they observed that freezing and holding the vegetables in frozen stage at -18°C (-0.4°F) for 3, 6, and 9 months resulted in small decreases in enzyme activity.

Lund and Halvorson (1957) studied the effects of low temperature on enzymes and microorganisms. They found that freezing itself has

no deleterious effect on proteolytic enzymes, but gradual inactivation does occur during prolonged storage below freezing. In a model system, storage temperatures above -25°C (-13°F) are insufficiently low to stop enzymatic activity during normal storage of a year.

Even though freezing does not inhibit enzyme activity and enzyme action continues to occur in the frozen state, ice formation has a marked retarding effect (Kertesz, 1942). Lineweaver (1939) and Sizer and Josephson (1942) called attention to the fact that for the few cases investigated the velocity of enzyme reaction is faster in the supercooled state than in the frozen state at the same temperature. The velocity greatly decreases when the change of state occurs. This decrease must be due to increase in concentration of substrate and to other changed which take place in the physical and colloidal properties of a system when it passes from liquid to solid state.

Changes in enzyme activity on freezing are ascribed in part to changes in concentration of substrates and to other solutes in the medium following the removal of water as ice, and partly to a change in state of the enzyme itself (Kiermeier, 1952). Fennema (1971) reported that freezing sometimes increases reaction rates. This is because the rate-accelerating effect of solute concentration overbalances the rate-decreasing effect of lower temperatures and a net increase in reaction rate occurs. Maximum subfreezing rates are mostly from -1° to -15°C (30.2° to 5°F). Food quality can be affected by oxidation, insolubilization of proteins, and glycolysis. The greatest potential for damage is during freezing and thawing.

The combined effects of concentration of solutes and lowering

of temperature can greatly reduce enzyme specific activity (Tappel, 1966). One of the explanations offered in his review indicated that the greatly decreased rates are caused by limited diffusion of enzyme and substrate in the frozen aqueous system.

Cobet et al. (1961) reported that lipase, amylase, and phosphatase activity of grade A milk which had been kept for up to 30 days at -25°C (-13°F) was not affected. Pallavicini et al. (1970) also reported that the activity of o-diphenol oxidase, peroxidase, acid phosphatase, amylase, protease, and catalase in beans and peas were not influenced by freezing. They suggested that it might probably be due to the globular form of their protein molecules.

The degradation of starch in the bark and wood of the Korichnoe variety of apple trees was detected at temperatures as low as 1° to -20°C (33.8° to -4°F) (Ogolevets, 1966). Ptyalin, β-amylase, and invertase are active at -20°C (-4°F), but inactive at -40°C (-40°F). The enzyme activity was not irreversibly lost at -40°C (-40°F). The enzyme activity was not irreversibly lost at -40°C (-40°F), as transfer to higher temperature restored the activity.

Makoto and Motohiro (1955) reported the increase in the activity of α-amylase in rice koji during ripening at 15° to 17°C (59° to 62.6°F) and the decrease at -4° to 0°C (24.8° to 32°F).

Enzymolysis of a 1 to 2% solution of starch paste by α-amylase at 0°C (32°F) and -13°C (8.6°F) was studied (Veselov et al., 1970). At this temperature range, enzymolysis occurred intensively. High and low molecular weight dextrins, maltose, and glucose were detected in the product. The contact of the solution with the α-amylase

solution, both colled to 0°C (32°F), immediately reduced viscosity. Cryolysis of the starch paste solution at -13°C (8.6°F) produced decomposition of 12.9% of the paste at the most, whereas enzymolysis caused decomposition of over 89% of the starch paste.

In the manufacture of sweet potato flakes, the ratio of insoluble to soluble solids in the puree is very important from the standpoint of efficient production and product quality. When uncured and starchy roots are used, the resulting flakes are rather porous, with a low bulk density and do not retain their quality in storage as well as high-density flakes (Hoover, 1966). The presence of high β-amylase activity under certain conditions in some varieties results in high soluble solids in the cooked puree (Schwimmer, 1947; Balls et al., 1948).

Ikemiya and Deobald (1966) demonstrated the presence of α-amylase activity in raw sweet potato juice. High optimum activity temperature (70 to 75°C), heat stability at pH 6.0, and low activity at ordinary temperatures are among the unusual characteristics reported. Freshly harvested roots contain relatively small amounts of this enzyme which increases about six-fold after 9 month storage. This enzyme is distributed almost uniformly throughout the inner tissue of the root and is more soluble in water than in sweet potato juice.

Sweet potatoes apparently remain low in sugar content during the growing season (Hasselbring and Hawkins, 1915). The extensive conversion of starch into sugar appears to be inhibited by the activity of the vine. When the vines are destroyed, the transformation of carbohydrate into sugar starts even if the roots are left in the

ground. After harvest and storage at high temperature of 30°C (86°F), the conversion of starch to sugar is rapid at first, but soon becomes slower and approaches an equilibrium state. At low temperatures, 15.5° and 5°C (58.1° and 41°F), the rates of conversion are slower and the equilibirium is shifted to permit a greater concentration of sugar to accumulate. Evidently, hydrolysis of starch in the sweet potato results in the formation of reducing sugar, and sucrose is synthesized from the reducing sugar. This hydrolysis has also been demonstrated in the studies of Miyaki (1915), Gore (1923), and Culpepper and Magoon (1926). They have shown that the principal sugars resulting from starch hydrolysis are maltose and dextrin.

Deobald et al. (1969) showed that maltose formation in sweet potato puree was associated equally with the degree of heat treatment above starch gelatinization temperatures and with the amount of amylolytic activity in the purees. Cured roots having an excess of α-amylase required control measures to avoid over-conversion which would result in excessive amounts of maltose. Their study indicated two patterns of amylolysis in sweet potatoes. One pattern was associated with sucrose and glucose changes during storage. The other dealt with only maltose production during heat processing.

3.0 EXPERIMENTAL

3.1 Apparatus

3.11 pH meter

A Corning pH meter model 12, optional expanded scale, reading to one-hundredths, with calomel reference electrode, was used.

3.12 Analytical balance

Whenever possible, all weighings were performed on a Mettler Model B Gram-atic analytical balance with an accuracy of $\pm$ 0.02 mg.

A Sartorious balance model 2204 was used when bulky samples needed to be weighed.

3.13 Water bath

The water bath used in this study was a P.M. Thomson with a temperature controlled system, accurate to $\pm$ 1^{o}C.

3.14 Spectrophotometer

A Beckman DU-2 Ultraviolet Spectrophotometer with a wavelength range of 190-1000 mμ was used.

3.15 Sample storage containers

Polyethylene bottles, polyseal closures, having a capacity of 0.5 oz were used. These bottles were obtained from Preiser Scientific Inc., Charleston, W. Va. Polyethylene bottles were selected because of their durability, resistance to the high and low temperatures used in the experiment, and their chemically inert quality. Enzymes tend to adhere to glass and thus the use of glass containers was excluded.

3.16 Polyethylene bags

The bags were made of 1 mil polyethylene, double wall construction.

3.2 Materials

3.2.1 Soluble starch

The potato starch used in the study was certified A.C.S. grade in powder form (Fisher Scientific Co., Fairlawn, New Jersey).

3.2.2 Sodium hydroxide

Sodium hydroxide, certified A.C.S grade in pellet form (Fisher) was used to prepare solutions of 1 and 2 N concentrations. These were prepared by weighing 10 and 20 g of the pellets on an analytical balance, and the pellets dissolved in triple distilled, deionized water. The concentrated solutions were allowed to cool and diluted to 250 ml in a volumetric flask.

3.2.3 3,5-Dinitrosalicylic acid

3,5-dinitrosalicylic acid ("highest purity" Eastman) was used.

3.2.4 Sodium-potassium tartrate (Rochelle salt)

Sodium-potassium tartrate, Fisher certified A.C.S. grade in the crystal form was used.

3.2.5 Sodium phosphate monobasic

Certified A.C.S. sodium phosphate monobasic in crystal form (Fisher) was used to prepare a 0.2 M sodium phosphate monobasic solution. This solution was prepared by weighing 27.8 g of the chemical on an anlytical balance and diluting to 1000 ml in a 1000 ml volumetric flask with triple distilled, deionized water.

3.2.6 Sodium chloride

Sodium chloride, certified A.C.S. grade in crystal form (Fisher) was used.

3.2.7 Acetic acid, glacial

Acetic acid (99.7% w/w), reagent A.C.S. grade (Fisher) was used. A 0.2 M acetic acid solution was prepared by pipetting 11.55 ml acetic acid into approximately 250 ml triple distilled, deionized water in a 1000 ml volumetric flask. The solution was then diluted to 1000 ml.

3.2.8 Maltose

Maltose (malt sugar), reagent grade in powder form (Fisher) was used. A 3 μmole/ml solution was prepared by weighing 0.1081 g on an analytical balance and dissolved with a volume of distilled, deionized water. The concentrated solution was then diluted to 100 ml in a volumetric flask.

3.2.9 α-Amylase

Commercially prepared, purified, and standardized, swine pancreatic α-amylase (Worthington Biochemical Corporation, Freehold, N.J.) was used. This enzyme was available as a suspension in 0.5 saturated sodium chloride in 0.003 M calcium chloride. One ml of the suspension contains 49.85 mg of α-amylase. One enzyme unit, rated at 676 units/mg frees the soluble starch of 1 μmole of reducing groups (calculated as maltose) per minute at 25°C (77°F). The suspension has been found to be stable for six months when stored at 5°C (41°F).

3.2.10 β-Amylase

Commercially prepared, purified, and standardized sweet

potato β-amylase (Worthington Biochemical Corporation, Freehold, N.J.) was used. This enzyme was available as suspension of crystals in 0.6 saturated ammonium sulfate at pH 3.0. One ml of the suspension contains 60.6 mg of β-amylase. One enzyme unit, rated at 500 units per mg frees 1 μmole of β-maltose per minute at 25°C (77°F). The suspension has been found to be stable for six to 12 months when stored at 5°C (41°F).

3.2.11 Ethyl alcohol

Ethyl alcohol (95%), certified A.C.S., (Fisher) was used.

3.2.12 Water

Distilled, deionized water was used throughout the experiment, except that triple distilled, deionized water was used in the preparation of the enzyme solutions as well as the buffer solutions.

3.2.13 Sweet potato roots

Two varieties of sweet potatoes, Centennial and Julian, from the Eastern Shore of Virginia were used in the present study. They were freshly dug and treated approximately three days after harvest as follows:

(1) Cured-The roots of the Centennial variety were placed at 26.7°C (80°F) under 80% relative humidity for 10 days to "cure," and put in storage at 12.8°C (55°F) under 80% relative humidity for seven months. The roots were then removed from storage, made into purees, and treated further.

(2) Uncured-The roots of the Centennial and Julian varieties were made into purees ready for further treatment.

Sweet potato purees were prepared by washing and preheating the roots in a 54.5°C (130°F) water bath for 30 min. The preheated roots were then submerged in a 12% caustic soda solution heated to 100°C (212°F) for 5 min. The disintegrated peel was then mechanically loosened, removed with water, and all root defects were removed. The trimmed roots were then pulped through a 1/32 inch screen in a Rietz disintegrator.

3.3 Analytical Procedures

3.31 Preparation of reagents, substrate, and enzyme suspension

3.311 3,5-Dinitrosalicylic acid solution

One g of 3,5-dinitrosalicylic acid was weighed and moistened with distilled, deionized water. Twenty ml of 2 N sodium hydroxide solution was slowly added through a pipet and diluted with 50 ml of distilled, deionized water. After the solution cooled, 30 g of Rochelle salt was added and made up to 100 ml in a volumetric flask. The solution was filtered through a Pyrex C fritted Buchner funnel without vacuum. The filtrate was then stored in a stoppered brown bottle.

3.312 0.02 M Phosphate buffer, pH 6.0 and 6.9 with 0.0067 M sodium chloride

Fifty ml of 0.2 M sodium phosphate monobasic solution was pipetted into a 500 ml volumetric flask and 0.1960 g of sodium chloride was added. The volume was then made up to approximately 450 ml with water and the pH of the solution was adjusted to pH 6.0 by adding approximately 1.2 ml of 1 N sodium hydroxide. The volume of this buffer was then brought up to 500 ml and kept in a refrigerator at

4°C (39.2°F). The same procedure was used to prepare 0.02 M, pH 6.9 phosphate buffer with 0.0067 M sodium chloride by adding 5.3 ml of 1 N sodium chloride to the solution, bringing to 500 ml volume, and then also storing at 4°C.

3.313 0.016 M Acetate buffer, pH 4.8

Forty ml of 0.2 M acetic acid solution was pipetted into a 500 ml volumetric flask and diluted to approximately 450 ml with water. The pH of the solution was adjusted to pH 4.8 by adding approximately 4.2 ml of 1 N sodium hydroxide. The volume of the buffer was then brought up to 500 ml and kept in a refrigerator at 4°C (39.2°F).

3.314 Substrate preparation

3.3141 Substrate for purified swine pancreatic α-amylase

The substrate used was 1 and 2% soluble potato starch. These concentrations were prepared by suspending each portion of 1 and 2 g of soluble potato starch in 75 ml of 0.02 M phosphate buffer, pH 6.9 in a 100 ml volumetric flask. The suspensions were then heated in a boiling water bath until all the starch went into solution. They were allowed to cool to 25°C (77°F) and the volume brought to 100 ml with 0.02 M phosphate buffer, pH 6.9.

3.3142 Substrate for crude α-amylase extracted from sweet potatoes

A two percent substrate solution was prepared by weighing and suspending 2 g of soluble potato starch in 75 ml of 0.02 M phosphate buffer at pH 6.0. The suspension was then heated until the starch went into solution. The solution was cooled to 25°C (77°F) and the volume brought to 100 ml with 0.02 M phosphate buffer, pH 6.0.

3.3143 Substrate for commercially purified and crude β-amylase extracted from sweet potatoes

The substrate used was 1 and 2% soluble potato starch. Similar procedures described in Section 3.3141 were employed except 0.016 M acetate buffer, pH 4.8 was used as the solvent.

3.315 Enzyme preparation

3.3151 Commercially purified swine pancreatic α-amylase suspension

An enzyme suspension of 2941 μg/10 ml was prepared by diluting 0.059 ml of the original enzyme stock solution to a 10 ml volume using water. The suspension was then transferred into a polyethylene bottle and stored under refrigeration at 4°C (39.2°F). Subsequent aliquots were drawn from this stock suspension to prepare required enzyme concentrations.

3.3152 Commercially purified sweet potato β-amylase suspension

An enzyme suspension of 3333 μg/10 ml was prepared by pipetting 0.055 ml of the original enzyme stock solution into a 10 ml volumetric flask. Similar steps described in Section 3.3151 were followed.

3.3153 Crude maltose, α-, and β-amylase extracted from sweet potatoes

A representative 5 g of sweet potato puree was suspended in 50 ml of water and vigorously shaken by hand for 1 min. The suspension was then filtered through a Whatman No. 1 filter paper. An aliquot of the filtrate was used so that a desired dilution of the crude sweet potato maltose and α-, and β-amylase solution was obtained.

3.32 Assaying for enzyme activity

Enzyme assays were conducted under two sets of conditions.

The first set of conditions consisted of 3-minute storage time assays conducted at 25°C (77°F). These tests were performed to familiarize the operator with the procedures, to verify the feasibility of the system, to select enzyme and substrate concentrations, and to provide data for statistical evaluation of the accuracy and reproducibility of the experimental methods.

The second set of conditions involved enzyme assays of samples that had been in storage for varying lengths of time at different storage temperatures.

3.321 Enzyme activity determinations

3.3211 Determination of activity of commercially purified swine pancreatic α-amylase

The following procedures were described by Bernfeld (1951) and modified by Worthington Biochemical Corp. (1972):

a. One-half ml of 1 or 2% soluble starch solution in phosphate buffer, pH 6.9 was pipetted into a polyethylene bottle that was then immersed in a constant temperature water bath at 25°C (77°F).

b. One-half ml of the α-amylase suspension containing the desired concentration at 25°C (77°F) (Section 3.3151) was then added to the bottle.

c. The bottle containing enzyme-substrate mixture was incubated at 25°C (77°F) for 3 min.

d. One ml of the 3,5-dinitrosalicylic acid solution (Section 3.311) was added to the mixture.

e. Immediately, the bottle was immersed in boiling water for 5 min and then cooled in an ice bath.

f. Ten ml of water were added.

g. A representative aliquot of this unknown sample solution was transferred into a 1 cm square cuvette and percent transmittance of the solution determined at 540 mμ wavelength using a DU-2 Spectrophotometer. The reading was read against a solution described in step h.

h. A mixture of 1:1 ratio of water and 3,5-dinitrosalicylic acid solution was made up and was subjected to steps e through f. A representative aliquot of this solution was then used to set the percent transmittance of the spectrophotometer to 100.

i. Steps a through g were repeated with a blank sample which contained the soluble starch substrate without enzyme.

The percent transmittance reading of the unknown and the blank samples were computed into absorbancy readings. The value of the unknown reading minus the blank reading was read against the standard curve. The amount of maltose present in the unknown sample (μmole/3min) was then calculated. This value was then used to compute the "units of activity" using the formula:

$$\text{Units of activity} = \frac{\mu\text{mole maltose/min at } 25^{\circ}\text{C } (77^{\circ}\text{F})}{\text{mg enzyme}}$$

3.3212 Determination of activity of commercially purified sweet potato β-amylase

Similar procedures described in Section 3.3211 were employed except the soluble starch was suspended in acetate buffer, pH 4.8 (Bernfeld, 1955) and the enzyme suspension was purified sweet potato β-amylase. The activity was expressed into "units of activity" using the same formula described in Section 3.3211.

3.3213 Determination of naturally occurring maltose in sweet potatoes

A desired dilution of the extracted sweet potato solution was prepared (Section 3.3153). A 0.5 ml aliquot was taken and placed into a polyethylene bottle containing 0.5 ml water at 25°C (77°F). Procedure steps 3.3211 d to h were then employed and the amount of naturally occurring maltose in sweet potato was calculated using the formula:

$$\text{Naturally occurring maltose} = \mu\text{mole maltose} \times \text{dilution factor/g sample}$$

3.3214 Determination of activity of crude α-amylase extracted from sweet potatoes

Similar procedures described in Section 3.3211 were employed except the soluble starch substrate was dissolved in phosphate buffer, pH 6.0 (Ikemiya and Deobald, 1966). The crude α-amylase extracted from sweet potatoes as described in Section 3.3163 was used and the activity was expressed into "units of activity" at 25°C (77°F) using the formula:

$$\text{Units of activity} = \frac{\frac{\mu\text{mole M - NOM}}{\text{min}}}{\text{g Sample}} \times \text{DF}$$

where: M = Maltose

NOM = Naturally Occurring Maltose

DF = Dilution Factor

3.3215 Determination of activity of crude β-amylase extracted from sweet potatoes

Similar procedures described in Section 3.3212 were employed. The crude β-amylase extracted from sweet potatoes as described in

Section 3.3163 was used and the activity was expressed into "units of activity" at 25°C (77°F) using the formula given in Section 3.3214.

3.3216 Maltose standard curve calibration

The following procedures were used to establish a maltose standard curve necessary for the determination of α- and β-amylase activity in various consecutive experimentations:

a. A concentration series of 0, 0.3, 0.6, 0.9, 1.2, 1.5, 1.8, 2.1, 2.4, 2.7, and 3.0 μmole maltose/ml was prepared by pipetting 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 ml of maltose stock solution (3 μmole/ml) into each 0.5 oz polyethylene bottle. Water was added to each bottle to give a final volume of 1 ml of each concentration.

b. One ml of 3,5-dinitrosalicylic acid solution was pipetted into each of the bottles containing the maltose solutions.

c. The bottles containing the maltose and salicylic acid were immersed in boiling water for 5 min and immediately cooled in an ice bath.

d. Ten ml of water were added to each bottle.

e. Percent transmittance of each concentration was then determined with a Beckman DU-2 Spectrophotometer at 540 mμ. The treatment containing no maltose was used to set the percent transmittance of the spectrophotometer to 100.

f. The recorded percent transmittance was converted into absorbancy and plotted against the maltose concentrations on a graph paper.

3.33 Selection of substrate and enzyme concentrations

The enzyme assay procedures for purified swine pancreatic α-amylase and for purified sweet potato β-amylase described by Bernfeld (1951 and 1955) and modified by Worthington Biochemical Corp. (1972) were used for selecting enzyme and substrate concentrations. Further refinement of the procedures was accomplished by evaluating samples using different enzyme and substrate concentrations. Since substrate concentrations was not a variable in the study, the 2% substrate was used.

Enzyme concentrations were selected for the study that provided a steady rate of reaction and a good range of reading necessary for accurate determination of enzyme activity. Three enzyme concentrations of 0.5, 1.0, and 1.5 μg/sample were selected.

3.34 Evaluation of experimental method

The accuracy of the method was determined by conducting a statistically designed series of tests under assay procedures with results being statistically analyzed. On three consecutive days, a series of samples was run using the substrate concentration as suggested by the Worthington procedure and the 1 μg/sample enzyme concentration. Since all samples were prepared in triplicate, this required assay of nine samples plus nine blanks per day. To approximate the system of sample preparation to be used for samples prepared for subsequent storage, a batch system of substrate preparation was used. The batch was divided into three groups which were further divided into measured aliquots for triplicate samples and three measured aliquots for triplicate blanks.

These nine samples and nine blanks were properly labeled into groups (A, B or C) and replicates (I, II or III). The operator processed the samples according to the established procedures. Before the samples were read in a DU-2 Spectrophotometer, they were assigned at random three digit numbers. Original identification was recorded and removed from the samples. The samples were then rearranged at random and returned to the operator. This sample rearrangement was performed to eliminate possible operator bias in evaluating the reproducibility of the method. This same procedure was conducted daily over a three-day period. The results obtained were then statistically evaluated.

3.35 Sample preparation for storage

Sample preparation for storage included the treatment of starch substrate with different enzymes used in the study at different enzyme concentrations. The treated samples were frozen as described in 3.351 and put into storage. Volume and concentration of substrate as well as the different kinds of buffer solutions used for different enzyme treatments were similar to those described in the previous sections.

Sweet potato puree samples described in Section 3.2.13 were directly frozen and stored.

3.351 Freezing of samples

Samples were frozen using a mixture of dry ice and ethanol as a freezing medium that was found to be effective and had no adverse effects on enzyme activity (Mullenax, 1973). Freezing of samples was essential to minimize enzyme activity that would otherwise occur before samples would reach appropriate storage temperatures. The temperature

of this freezing medium was -55°C to -68°C (-67° to -90.4°F). This freezing technique was used to freeze samples treated with commercially purified enzymes and to freeze sweet potato puree samples.

3.3511 Freezing of samples treated with commercially purified swine pancreatic α-amylase and sweet potato β-amylase

The samples were immersed immediately in a mixture of dry ice and ethanol after addition of enzyme, but prior to placing into storage. The sample appeared solid in approximately 10 sec; however, to insure that the entire contents were solidly frozen, a twenty-second freezing time was provided. The frozen samples were then placed in storage.

3.3512 Freezing of sweet potato purees

Approximately 8 to 10 oz sweet potato puree samples (Section 3.2.13) were tightly packed in polyethylene bags and immediately immersed in a mixture of dry ice and ethanol for 20 min. After the treated samples appeared solid, they were put into storage at various temperatures.

3.36 Assaying for enzyme activity as well as cumulative maltose in sweet potatoes after storage

Samples were removed upon termination of each storage variable and the enzyme activity, with the exception of sweet potato puree samples, was immediately inactivated. The samples were then analyzed according to the previously described procedures.

3.361 Enzyme inactivation after storage

Of primary importance when samples were removed from storage was immediate inactivation of the enzymes. This was to insure that

subsequent activity measurement reflected activity during storage and not after samples were removed from storage.

When samples treated with commercially purified swine pancreatic α-amylase and sweet potato β-amylase were removed from storage for enzyme activity determination, their enzyme activities were terminated by immediately adding 1 ml of 3,5-dinitrosalicylic acid solution and then immersing samples in boiling water for 5 min.

Since the stability of the α- and β-amylase in sweet potatoes was the main objective of a part of this study, the enzyme activity was not inactivated after the samples were removed from storage. They were promptly prepared for water extractions of maltose, crude α- and β-amylase (see Section 3.3153).

3.37 Analysis of research data

Research data was accumulated and analyzed both graphically and statistically.

Statistical analysis was conducted by using a combined analysis of the Randomized Complete Block design, analysis of variance, Least Significant Difference Test, and Duncan's Multiple Range Test. The purpose of the analysis was to detect the level of significance of differences among variables in the study.

3.4 Enzyme Systems

Three systems were set up, as follows:

(a) Commercially purified α-amylase with soluble starch substrate in phosphate buffer pH 6.9.

(b) Commercially purified β-amylase with soluble starch substrate in acetate buffer pH 4.8.

(c) Sweet potato puree.

A diagram showing the various experimental design variables is presented on page 34.

3.41 Design variables

3.411 Source of purified enzymes

Commercially purified, swine pancreatic α-amylase and sweet potato β-amylase were selected for the present study. Three enzyme concentrations namely, 0.5, 1.0, and 1.5 μg/sample were included for both enzymes studied.

3.412 Source of crude α- and β-amylase

Since sweet potato is a rich source of α- and β-amylase, it was selected for this study.

3.413 Substrate

A 2% potato soluble starch dissolved in an appropriate buffer solution and hydrogen ion concentration was used throughout the present study.

3.414 Storage temperatures

Four storage temperatures namely 4°C (39.2°F), -13°C (8.6°F), -18°C (-0.4°F), and -23°C (-9.4°F) were employed throughout the present study.

The following guidelines were used in selecting the storage temperatures: (a) the 4°C temperature is within the range of temperatures used for the refrigeration of foods above freezing; (b) the -13°C represents a temperature just above what usually used for frozen food

storage; (c) the -18°C and the -23°C represent temperatures commonly used for commercial storage of frozen food.

3.415 Storage time

Storage time of starch substrates treated with commercially purified α- or β-amylase varied among the different storage temperatures studied. Treated samples held at 4°C were kept at that temperature for only 0, 15, 30, 45, 60, and 75 min before being analyzed for enzyme activity. Samples stored at -13°C were kept for 0, 1, 2, and 3 days while those stored at -18° and -23°C were kept for 0, 28, 56, 84, and 112 days.

Sweet potato puree samples were stored at 4°, -13°, -18°, and -23°C. Crude maltose, α-, and β-amylase were extracted after 0, 14, 28, 42, and 56 days storage at those temperatures.

The zero storage time samples were those which were treated with enzymes. They were immediately frozen by the rapid freezing technique and enzyme activity was immediately determined. A representative amount of sweet potato puree was frozen by the same method used for the systems with purified enzymes. Crude maltose and α- and β-amylase were immediately extracted. The zero storage time results were used as controls to compare with those samples which had been stored.

Triplicate samples were used throughout this experiment.

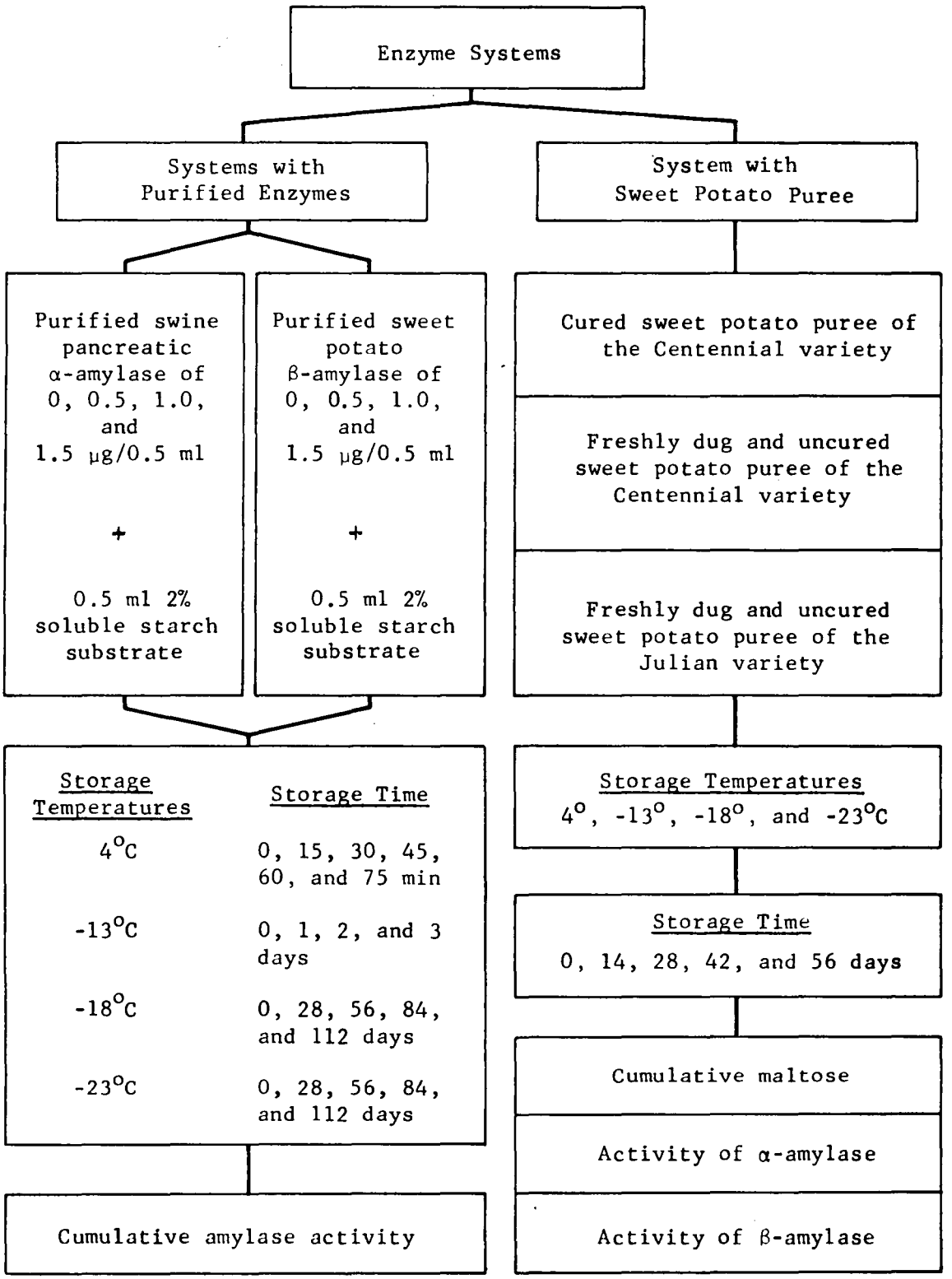

Diagram of Experimental Design

4.0 RESULTS AND DISCUSSION

4.1 Evaluation of Experimental Methods

The accuracy and reproducibility of experimental methods was evaluated statistically from data obtained from assays performed as described in Section 3.34.

The results obtained over three consecutive days of similar assays revealed no significant difference between days or samples.

4.2 Enzyme Activity in the Systems with Purified Enzymes

4.21 α- and β-Amylase activity at 4°C (39.2°F)

A combined analysis of the Randomized Complete Block was used. It revealed a highly significant effect of enzyme concentration, holding time, and interaction between enzyme concentration and holding time on the activity of α- and β-amylase during storage at 4°C. Cumulative α- and β-amylase activity of all enzyme concentrations studied generally increased as the holding time was increased (Tables 1 and 2 and Figs. 1 and 2). The increase in activity at 15-minute intervals was statistically significant as determined by Duncan's Multiple Range Test (Tables 1 and 2). Activity was expressed as percentage increase over that of zero time storage samples. The increase in α-amylase activity after 75 min holding was 976.2% at the 0.5 µg concentration (Table 1). The increase was 1634.3 and 1458.1% over that of the zero storage time samples at the 1.0 and 1.5 µg

concentrations, respectively (Table 1). Similarly, percent increase of β-amylase activity over that of the zero storage time samples after 75 min holding was 912.6, 1202.8, and 1800.6% at the 0.5, 1.0, and 1.5 μg concentrations, respectively (Table 2). The high level of significance in the interaction between enzyme concentration and holding time implied that α- and β-amylase at different levels of concentration and holding times reacted differently. In general, as the enzyme concentration was increased there was a statistically significant increase in rate of activity as determined by the Least Significant Difference Test (Tables 3 and 4). It is likely that enzyme concentration could be a limiting factor affecting the rate of activity at a concentration as low as 0.5 μg. After 75 min holding periods, lower enzyme concentrations showed lower amounts of accumulated maltose (Tables 1 and 2). There was still an appreciable amount of α- and β-amylase activity at 4°C.

4.22 α- and β-Amylase activity at -13°C (8.6°F)

As found in the preceding Section (4.21), the statistical analysis revealed a highly significant effect of enzyme concentration, storage time, and interaction between enzyme concentration and storage time on the activity of α- and β-amylase during storage at -13°C.

Data in Tables 5 and 6 and Figs. 3 and 4 showed that the cumulative α- and β-amylase activity increased with storage time. The increase in activity at one-day intervals was statistically significant as determined by Duncan's Multiple Range Test (Tables 5 and 6). Activity was expressed as percentage increase over that of zero time storage samples. The increase in α-amylase activity after 3 days of

storage was 661.7, 1133.2, and 1722.7% at 0.5, 1.0, and 1.5 μg concentrations, respectively (Table 5). Similarly, the respective increase in β-amylase activity after 3 days of storage was 1044.7, 1114.5, and 1351.9% (Table 6). Statistical significance in the interaction between enzyme concentration and storage time implied that α- and β-amylase at different levels of concentrations and storage times reacted differently. The rate of α- and β-amylase activity increased as the concentration was increased. The differences were statistically significant (Tables 7 and 8). Enzyme concentration was probably the limiting factor affecting the rate of activity. Thus the cumulative activity was lower in the lower concentration treatments at the end of a three-day storage period. Evidently, α- and β-amylase activity at -13°C was still high.

4.23 α- and β-amylase activity at -18°C (-0.4°F)

Statistical calculations were performed by using the combined analysis of the Randomized Complete Block. A highly significant effect of enzyme concentration, storage time, and interaction between enzyme concentration and storage time on the activity of α- and β-amylase during storage at -18°C was still observed.

The cumulative α- and β-amylase activity increased as the storage time was increased (Tables 9 and 10 and Figs. 5 and 6). The increase in the activity at 28-day intervals was statistically significant at all concentration levels studied (Tables 9 and 10). Activity was expressed as percentage increase over that of zero time storage samples. The increase in α-amylase activity after 112 days of storage was 634.2, 678.9, and 783.9% at 0.5, 1.0, and 1.5 μg concentrations,

respectively (Table 9). Similarly, the respective increases for β-amylase activity were 684.4, 581.7, and 647.6% at 0.5, 1.0, and 1.5 μg concentrations (Table 10). The rate of activity was also different at different concentration levels. As the concentration was increased, the rate of activity increased. The differences in the rate of activity were statistically significant (Tables 11 and 12). At the end of a 112-day storage period, the cumulative activity was lower in the lower concentration treatments. Appreciable amount of α- and β-amylase activity was still going on at -18°C.

4.24 α- and β-Amylase activity at -23°C (-9.4°F)

A statistical analysis similar to that used in Section 4.23 showed a highly significant effect of enzyme concentration and storage time on the activity of α- and β-amylase during storage at -23°C. However, the interaction between enzyme concentration and storage time was not statistically significant.

The cumulative α- and β-amylase activity showed a trend to increase with storage time (Tables 13 and 14 and Figs 7 and 8). However, the increase in activity at 28-day intervals was not statistically significant at all levels studied with the exception at 0.5 μg concentration where the cumulative α-amylase activity at the end of 112 days of storage was significantly higher than that of the 0, 28, and 56 days of storage time (Table 13). A similar trend for β-amylase, though not statistically significant, was observed at 28-day intervals up to 56 days of storage (Table 14 and Fig. 8). After 84 and 112 days of storage, the increase in the cumulative activity for β-amylase was statistically different from that at 0, 28, and 56 days of storage

(Table 14). There was no statistical difference in the cumulative β-amylase activity between 84 and 112 days of storage.

Activity was expressed as percentage increase over that of zero time storage samples. The increase in α-amylase activity after 112 days of storage was 85.9, 42.1, and 39.0% at 0.5, 1.0, and 1.5 μg concentrations, respectively (Table 13). After 112 days of storage, β-amylase activity of samples treated with 0.5, 1.0, and 1.5 μg concentrations was 94.5, 124.6, and 120.1% more than the zero storage time activity (Table 14).

A trend in increase of rate of activity with increase in concentration was also observed. The cumulative activity was still lower in the lower concentration treatments. Evidently, measurable α- and β-amylase activity was still observed at -23°C but to a lesser degree when compared to the results with the higher storage temperatures.

4.25 Magnitude of activity under various low storage temperatures in systems with purified enzymes

Results indicate that α- and β-amylase in the systems with purified enzymes were still active at temperature as low as -23°C. However, rates of activity were lower as storage temperatures decreased. Comparison of data in Tables 1, 5, 9, and 13 show that α-amylase activity decreased at lower storage temperatures. After 75 min, cumulative activities of α-amylase at 4°C were 0.8825, 1.7395, and 2.4773 μmole maltose at 0.5, 1.0, and 1.5 μg concentrations, respectively (Table 1). At similar respective concentrations, α-amylase cumulative activities at -13°C after one day were 0.4539,

0.7702, and 1.1489 μmole maltose (Table 5). After 28 days, the cumulative activities at -18°C were 0.2173, 0.3579, and 0.5799 μmole maltose (Table 9).

Statistical analysis of the difference in cumulative activity of α-amylase at -18°C and -23°C clearly demonstrated that enzyme activity decreased with decreasing temperatures (Table 15). The decrease in activity was statistically significant at all concentration levels studied (Table 15). At 1.5 μg concentration cumulative activity after 112 days of storage at -18°C was 1.3816 μmole maltose while at -23°C it was 0.2173 μmole maltose (Table 15).

The β-amylase activity also decreased at lower storage temperatures. At 0.5, 1.0, and 1.5 μg concentrations, the cumulative activities of β-amylase at 4°C after 75 min holding were respectively 0.9792, 1.9373, and 2.9593 μmole maltose (Table 2); at -13°C after one day of storage they were 0.6028, 1.0799, and 1.4352 μmole maltose (Table 6); and at -18°C after 28 days of storage they were 0.3477, 0.5466, and 0.5995 μmole maltose (Table 10).

Statistical analysis of the difference in β-amylase cumulative activity at -18° and -23°C indicated a decrease in activity significant at all concentration levels (Table 16). When activities at 1.5 μg concentration after 112 days were compared, it was observed that the activity at -18°C was 1.1887 μmole maltose while at -23°C it was 0.3499 μmole (Table 16).

4.3 Enzyme Activity in the System with Sweet Potato Puree

4.31 Cumulative maltose in cured sweet potatoes of the Centennial variety

When puree samples of cured sweet potatoes of the Centennial variety were stored at 4°C for a period of 14, 28, 42, and 56 days, they showed an increase in the amount of cumulative maltose over that of the zero storage time sample (Table 17 and Fig. 9). The increase reached its peak at 28 days of storage and declined thereafter (Table 17 and Fig. 9). The differences in the cumulative maltose at 14-day intervals were statistically significant as determined by Duncan's Multiple Range Test (Table 17). The highest increase was 30.8% over that of the zero storage time at 28 days of storage (Table 17). The rate of increase declined after 28 days of storage and was only 6.0% over that of the zero storage time after 56 days of storage (Table 17). This decline in activity could be due to spoilage resulting in loss of enzyme activity. Spoilage could also result in a change in the chemical environment. Such a change has been reported to greatly affect stability and activity of amylases. Giri (1934) reported that when sweet potato samples were allowed to ferment, the enzyme activity was generally reduced.

When the puree samples were stored at -13°C, -18°C, and -23°C for a period of 14, 28, 42, and 56 days, a fluctuation in the amount of cumulative maltose was observed (Table 17 and Fig. 9). However, there was no statistically significant difference in the change of cumulative maltose at the end of 56 days of storage. The fluctuation in the amount of cumulative maltose over the entire storage period

could be due to an error in analytical technique as well as an error in the sub-sampling of the puree samples for cold storage.

Data in Table 18 indicate that cumulative maltose content at -13°C, -18°, and -23°C was significantly different from that at 4°C. Cumulative maltose content at -18°C was significantly different from that at -13°C only after 42 and 56 days of storage. Similarly, significant difference in the content of cumulative maltose between -23° and -13°C was observed only at 56 days of storage (Table 18). There was no significant difference between storage at -18° and -23°C (Table 18). Apparently, α- and β-amylase activity in cured sweet potato puree samples of the Centennial variety were inhibited by storage temperatures of -13°, -18°, and -23°C.

4.32 Cumulative maltose in uncured sweet potatoes

4.321 Centennial variety

Puree samples of uncured sweet potatoes of the Centennial variety when stored at 4°C for a period of 14 and 28 days also revealed an increase in the amount of cumulative maltose over that of the zero storage time sample (Table 19 and Fig. 10). This increase at 14-day intervals was statistically significant and the peak was at 28 days of storage. On a percentage basis, the increase was 29.5 and 57.2% over that of the zero storage time at 14 and 28 days, respectively (Table 19). After 28 days of storage, the amount of cumulative maltose decreased. At the end of 56 days, cumulative maltose was 38.5% less than that of the zero storage time (Table 19 and Fig. 10). This decrease in cumulative maltose could have been caused by microbiological activity at 4°C.

When the puree samples were stored at -13°, -18°, and -23°C for a period of 14, 28, 42, and 56 days, a slight increase in the amount of cumulative maltose over that of the zero storage time sample was observed (Table 19 and Fig. 10).

The cumulative amount of maltose formed at -13°, -18°, and -23°C was significantly different from that at 4°C (Table 20). At -18° and -23°C, the cumulative maltose was significantly different from that at -13°C only after 28 and 42 days of storage, respectively (Table 20). There was no significant difference between storages at -18° and -23°C (Table 20). Evidently, α- and β-amylase activity in uncured sweet potato puree of the Centennial variety was inhibited by storage temperatures of -13°, -18°, and -23°C.

4.322 Julian variety

Puree samples of uncured sweet potatoes of the Julian variety when stored at 4°C for 14, 28, and 42 days also had more cumulative maltose over that of the zero storage sample (Table 21 and Fig. 11). The differences at 14-day intervals were statistically significant and the highest increase was 69.9% over that of the zero storage time sample after 28 days of storage (Table 21 and Fig. 11). However, after 28 days of storage, the rate of increase declined (Table 21 and Fig. 11). The effects of long storage and spoilage on puree samples may have contributed to this decline.

A consistent but slight increase in the amount of cumulative maltose over that of the zero storage time sample was observed in puree samples stored at -13°, -18°, and -23°C for a period of 14, 28, 42, and 56 days (Table 21). However, as observed in the Centennial

variety, this increase was less than that of samples stored at 4°C.

Cumulative maltose at -13°, -18°, and -23°C was significantly different from that at 4°C as shown by the data in Table 22. In general, there was no significant difference in the cumulative maltose among samples stored at -13°, -18°, and -23°C. Apparently, α- and β-amylase activity in the Julian variety of uncured sweet potato puree was inhibited to a great extent during storage at temperatures of -13°, -18°, and -23°C.

4.4 Stability of α- and β-Amylase in the System with Sweet Potato Puree after Various Storage Conditions

4.41 Crude α- and β-amylase extracted from cured sweet potatoes of the Centennial variety

Crude α- and β-amylase were extracted from cured sweet potato puree samples of the Centennial variety. They were still active after the puree samples were stored at 4°C for 14 and 28 days (Tables 23 and 24 and Figs 12 and 13). When compared to the zero storage time sample, the activity was almost the same with no statistical differences detected. After 42 and 56 days of storage, the activity decreased approximately 9 fold and was statistically significant from that of 0, 14, and 28 day storage samples (Tables 23 and 24 and Figs 12 and 13). The decrease in α-amylase activity was 92.7 and 97.1% under that of the zero storage time samples at 42 and 56 days of storage, respectively (Table 23). Also, the β-amylase activity decline was approximately 96.0% under that of the zero storage time sample after 42 days (Table 24). This decline in activity was in agreement with cumulative

maltose values (Table 17 and Fig. 9). The aging effect and spoilage of the puree samples could contribute to this decline in α- and β-amylase stability after 42 and 56 days of storage at 4°C.

After puree samples were stored at -13°, -18°, and -23°C for 14, 28, 42, and 56 days, the extractable crude α- and β-amylase were still active (Tables 23 and 24 and Figs. 12 and 13). Evidently, the activity of both enzymes was somewhat inhibited by various low temperature treatments but their stability were not affected. Once the temperature was brought back to 25°C, the activity was restored.

Comparison of the activity of the α- and β-amylase extracted from the samples stored at 4°, -13°, -18°, and -23°C for 0, 14, 28, 42, and 56 days showed that at 42 and 56 days of storage, the activity of the enzymes from samples stored at 4°C was significantly reduced (Tables 25 and 26). Some significant differences in the stability of the enzymes were observed among samples stored at -13°, -18°, and -23°C (Tables 25 and 26). In general, the stability of α- and β-amylase was not affected by temperatures at -13°, -18°, and -23°C (Tables 25 and 26).

4.42 Crude α- and β-amylase extracted from uncured sweet potatoes

4.421 Centennial variety

Crude α- and β-amylase extracted from uncured sweet potato puree samples of the Centennial variety were still active after the puree samples were stored at 4°C for 14 days (Tables 27 and 28 and Figs. 14 and 15). When compared to the zero storage time samples, α-amylase activity was not statistically different. However, a 10% decrease in the activity of β-amylase was observed and was statistically

significant (Table 28). After 28, 42, and 56 days of storage, the activity of both enzymes drastically decreased and were statistically different from those of 0 and 14 day storage samples (Tables 27 and 28 and Figs. 14 and 15).

At 4°C the decreases in α-amylase activity were 59.3, 94.5, and 97.7% under those of the zero storage time samples at 28, 42, and 56 days of storage, respectively (Table 27). Similarly, the respective decreases in β-amylase activity were 63.4, 97.1, and 97.6% under those of the zero storage time samples after 28, 42, and 56 days of storage (Table 28). The decline in α-amylase activity, except after 28 days of storage, agreed with cumulative maltose values (Table 19 and Fig. 10). The different trend observed at 28-days of storage (Figs. 10 and 14) could be explained by the fact that only 59.3% of the enzyme was inactivated in comparison to the zero storage time sample (Table 27). The rest of the α-amylase was presumably still active and could have contributed to the increase in the amount of cumulative maltose shown in Table 19 and Fig. 10. Moreover, the cumulative maltose was an end product of the joint action of α- and β-amylase present in the sweet potato puree. The increase in cumulative maltose was thus more likely to be observed.

The decrease in β-amylase activity after 14 and 28 days of storage did not agree with the cumulative maltose values in Table 19 and Fig. 10. However, since only 10% and 63.4% of the enzyme lost its activity after 14 and 28 days of storage, respectively, the same explanation given above could apply.

The stability at 4°C of uncured sweet potato puree of the

Centennial variety was compared to that of cured puree of the same variety. In uncured puree samples (Tables 27 and 28 and Figs. 14 and 15) the stability dropped after 14 days while that of the cured samples (Tables 23 and 24 and Figs. 12 and 13) stayed at the same level for up to 28 days, and dropped thereafter. Visual observation indicated that spoilage of the samples from uncured sweet potatoes occurred more readily than in samples from cured roots. Higher microbial contamination of the samples prepared from the uncured sweet potatoes may have been the reason.

Crude α- and β-amylase extracted from uncured puree samples were still active after storage of samples at -13°, -18°, and -23°C for 14, 28, 42, and 56 days (Tables 27 and 28 and Figs. 14 and 15). Evidently, the activity of both enzymes was again somewhat inhibited by the various low temperature treatments below -13°C but their stability were not affected.

The activity of α- and β-amylase from samples stored at 4°, -13°, -18°, and -23°C after 14, 28, 42, and 56 days was compared. It was found that the activity of the enzymes extracted after 28 days and longer storage at 4°C was less than that of samples stored at -13°, -18°, and -23°C (Tables 29 and 30). There was no significant difference in the stability of the enzymes extracted from samples stored at -13°, -18°, and -23°C (Tables 29 and 30).

4.422 Julian variety

α- and β-Amylase activity in uncured sweet potato puree samples of the Julian variety was determined after 14, 28, 42, and 56 days of storage at 4°C. The crude enzymes extracted from samples

stored for 14 days were still active (Tables 31 and 32 and Figs. 16 and 17). A drastic decrease in the stability of the enzymes was observed after 28, 42, and 56 days of storage. The decrease for both enzymes was approximately 86.0, 93.0, and 98.0% under that of the zero storage time samples at 28, 42, and 56 days of storage, respectively (Tables 31 and 32 and Figs 16 and 17). The decrease in activity of both enzymes at 28 days of storage did not agree with the data in Table 21 and Fig. 11, which showed an increase in cumulative maltose after 28 days. The same explanation discussed in Section 4.421 could apply.

After the puree samples were stored at -13°, -18°, and -23°C for 14, 28, 42, and 56 days, the extractable crude α- and β-amylase were still active (Tables 31 and 32 and Figs. 16 and 17). Apparently, the enzyme activity was somewhat inhibited by the various low temperature treatments below -13°C but its stability was not affected.

The stability of both crude enzymes extracted from samples stored at 4°C was significantly reduced after 28 days of storage when compared to those extracted from samples under -13°, -18°, and -23°C treatments (Tables 33 and 34). There was no significant difference in the stability of the enzyme extracted from puree samples stored at -13°, -18°, and -23°C (Tables 33 and 34).

4.5 Comparison of Amylase Activity in the System with Sweet Potato Puree and the Systems with Purified Enzymes

As storage temperatures were lowered, it was anticipated that enzyme activity would decrease. Such observation was made in the systems with purified enzymes (Tables 1, 2, 5, 6, 9, 10, 13, and 14

Figs. 1, 2, 3, 4, 5, 6, 7, and 8). In the system with sweet potato puree a decrease in enzyme activity was also observed when storage temperature was lowered from 4° to -13°C (Tables 17, 19, and 21 and Figs. 9, 10, and 11). However, enzyme activity did not decrease below that observed at -13°C when storage temperature was -18° or -23°C. That indicates that in the system with sweet potato puree α- and β-amylase activity was inhibited at -13°C and below (Tables 17, 19, and 21; Figs. 9, 10, and 11).

Evidently, natural α- and β-amylase in the sweet potato puree act differently from purified enzymes in a model system. α- and β-Amylase present in the sweet potato system were more sensitive to low temperature treatments and the activity was retarded. On the contrary, α- and β-amylase in the systems with purified enzymes were more resistant to low temperature treatments and a measurable amount of activity was still observed at -23°C.

In accordance with the Q_{10} theory, reaction rates are lower when temperatures decrease. Results of this study indicate that the systems containing purified enzymes may follow that theory. The data obtained with the sweet potato puree system, on the other hand, points out that this system follows the Q_{10} theory less closely. The higher chemical complexity of the sweet potato puree system may be a factor contributing to the different behaviour of the two systems.

4.6 Implications to Food Storage and Quality

It is well known that several factors of food quality are subjected to deterioration by enzymatic activity. Attributes such as

flavor, odor, color, and others are among those directly affected. This applies especially to foods that are preserved, for the most part, by reliance upon low temperature.

Data from this study indicate that α- and β-amylase in the systems with purified enzymes showed activity that was considered significant from the standpoint of food quality at all levels of enzyme concentration and at all storage temperatures that were included in the study. However, the sweet potato puree system showed lower enzyme activity at the same storage temperature. From these observations, several implications concerning storage and handling of food can be drawn.

It is likely that enzyme concentration is a critical factor in enzyme reactions at low temperatures. It was apparent from this study that the highest concentration of either α- or β-amylase used in the study exhibited the greatest activity at all temperatures studied. This observation may have a bearing upon systems that depend on controlled enzymatic activity for flavor development, as in foods that are aged or matured by naturally present enzymes. Enzyme inactivation before the product is put into storage seems well justified when practicable.

Since amylase activity in the sweet potato puree was inhibited at -13°C, it is reasonable to expect other starchy food items to show similar trends under corresponding storage conditions. Dependence upon temperatures below -13°C to control amylase activity in similar food items may be effective.

5.0 SUMMARY AND CONCLUSIONS

α- and β-Amylase activity was determined at 4°, -13°, -18°, and -23°C (39.2°, 8.6°, -0.4°, and -9.4°F) in three different systems. The systems used were: (a) purified swine pancreatic α-amylase with starch; (b) purified sweet potato β-amylase with starch; and (c) sweet potato puree made from fresh sweet potatoes. In the systems containing purified enzymes, the variables were enzyme concentration, storage temperature, and storage time. In the sweet potato puree system the variables were sweet potato variety, curing, and storage temperature and time.

Results can be summarized as follows.

1. In the systems with purified enzymes, cumulative α- and β-amylase activity at all storage temperatures studied increased as the storage time and as the enzyme concentration increased.

2. There was appreciable α- and β-amylase activity in the systems with purified enzymes at all storage temperatures studied. Lower activity was usually observed at lower temperatures. Both enzymes were still active at -23°C.

3. In the system with sweet potato puree, cumulative maltose at 4°C increased as the storage time was increased up to 28 days. After 42 and 56 days of storage there was a decrease in cumulative maltose. That implies that α- and β-amylase were active at 4°C for up to 28 days of storage and that the activity decreased thereafter.

4. Cumulative maltose in the sweet potato puree system stored at -13° to -23°C for 56 days remained quite constant at the level present in the zero storage time samples. This implied that the activity of α- and β-amylase was inhibited at those low temperatures.

5. Varietal difference and curing did not affect trends in enzyme activity observed in 3 and 4 above.

6. α- and β-amylase activity in cured sweet potatoes stored at 4°C was stable for 28 days, while the uncured samples showed stable activity for 14 days. After those periods of stability, enzyme activity decreased considerably.

7. Generally, α- and β-amylase in the sweet potato puree samples were stable for 56 days at -13°, -18°, and -23°C.

8. When stored at -13°, -18°, and -23°C, α- and β-amylase in the sweet potato puree system acted differently than in the systems with purified enzymes. In the sweet potato puree system, the enzymatic activity was inhibited at -13°C while in the systems with purified enzymes activity persisted at -23°C.

9. Overall results indicate that in starchy foods in general, amylase activity at -13°C would be essentially negligible if they behave like sweet potatoes.

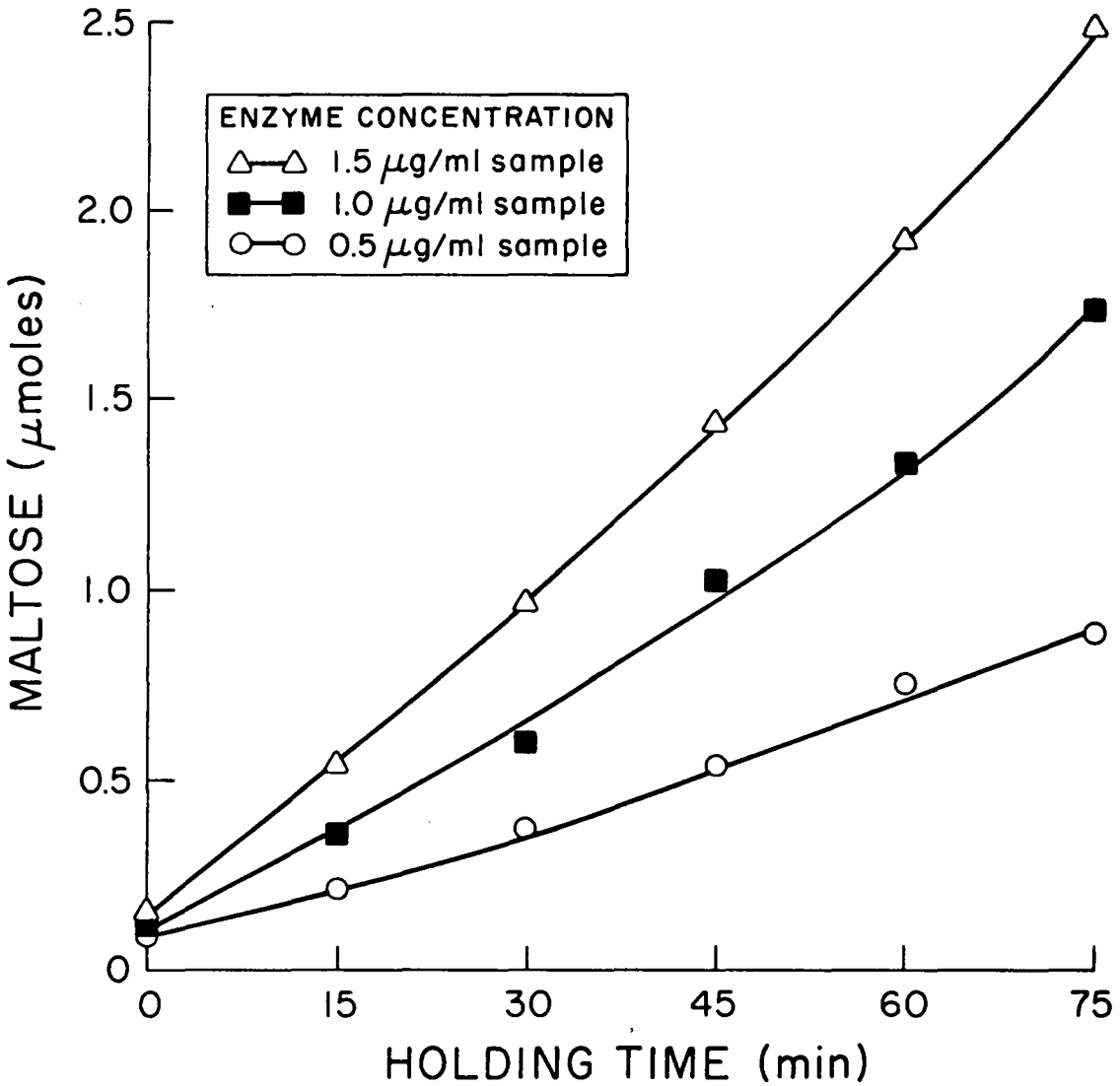

Fig. 1. Effect of holding time at 4°C (39.2°F) on cumulative activity of different concentrations of purified swine pancreatic α-amylase with 2% soluble starch substrate.

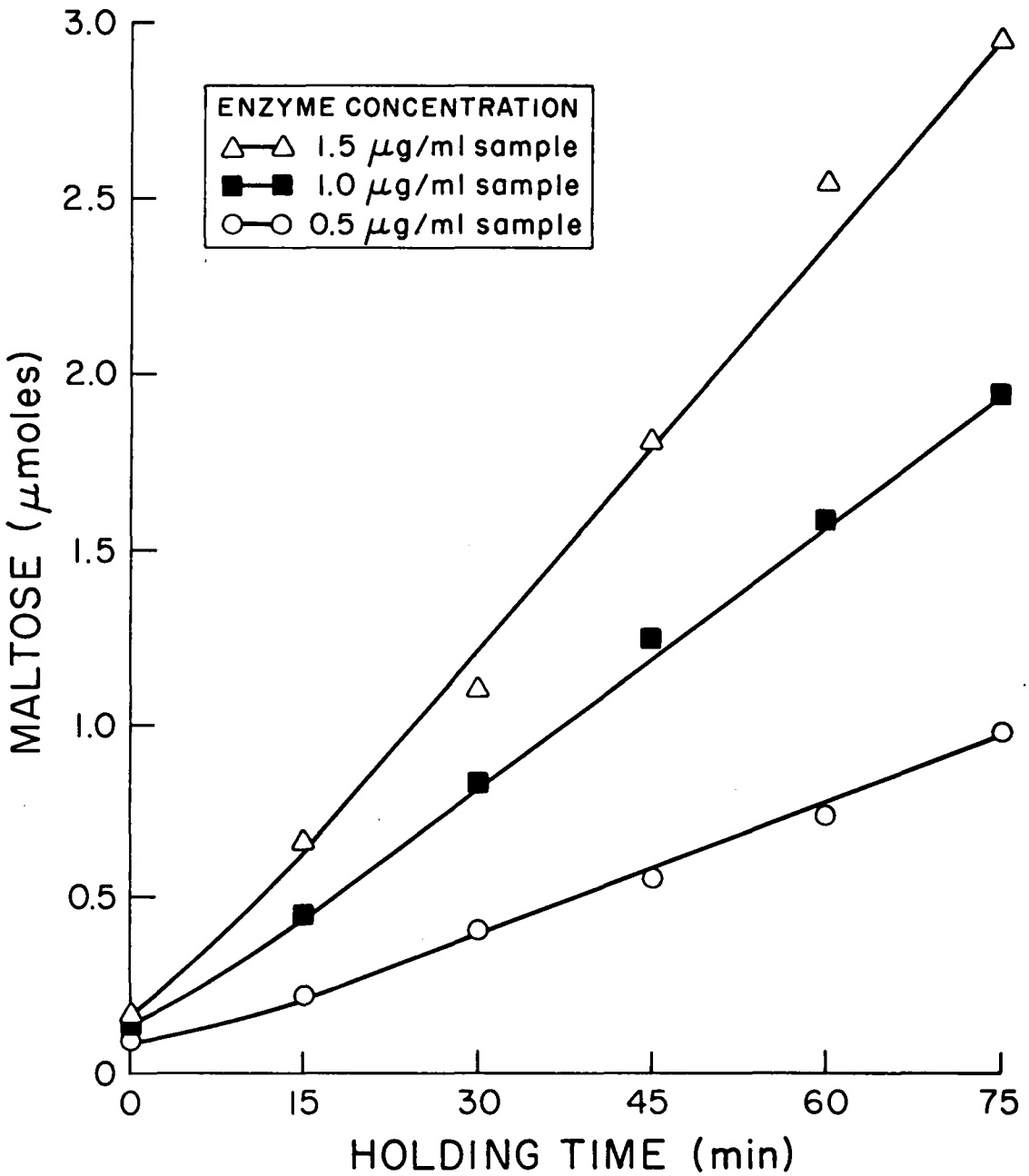

Fig. 2. Effect of holding time at 4°C (39.2°F) on cumulative activity of different concentrations of purified sweet potato ß-amylase with 2% soluble starch substrate.

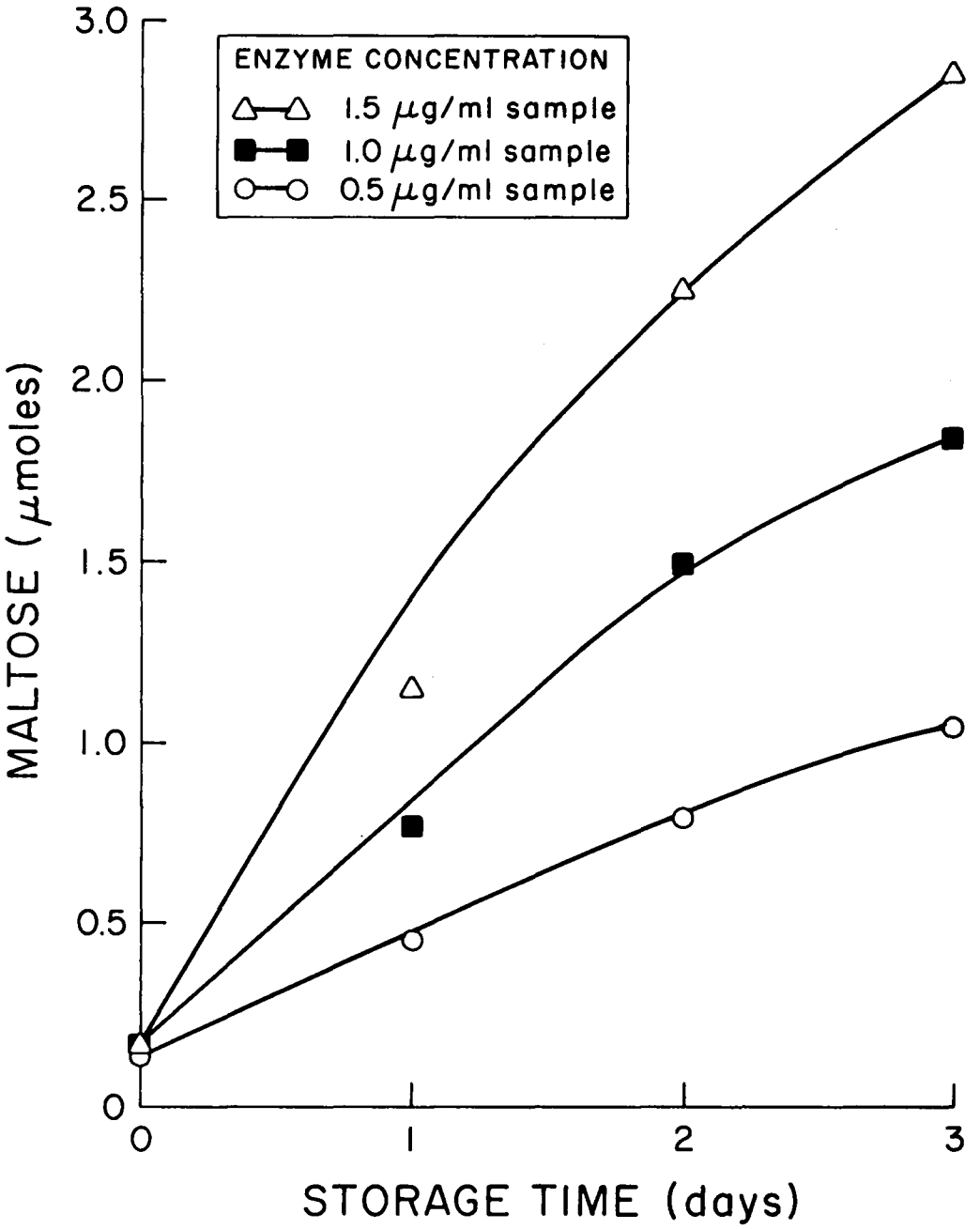

Fig. 3. Effect of storage time at -13°C (8.6°F) on cumulative activity of different concentrations of purified swine pancreatic α-amylase with 2% soluble starch substrate.

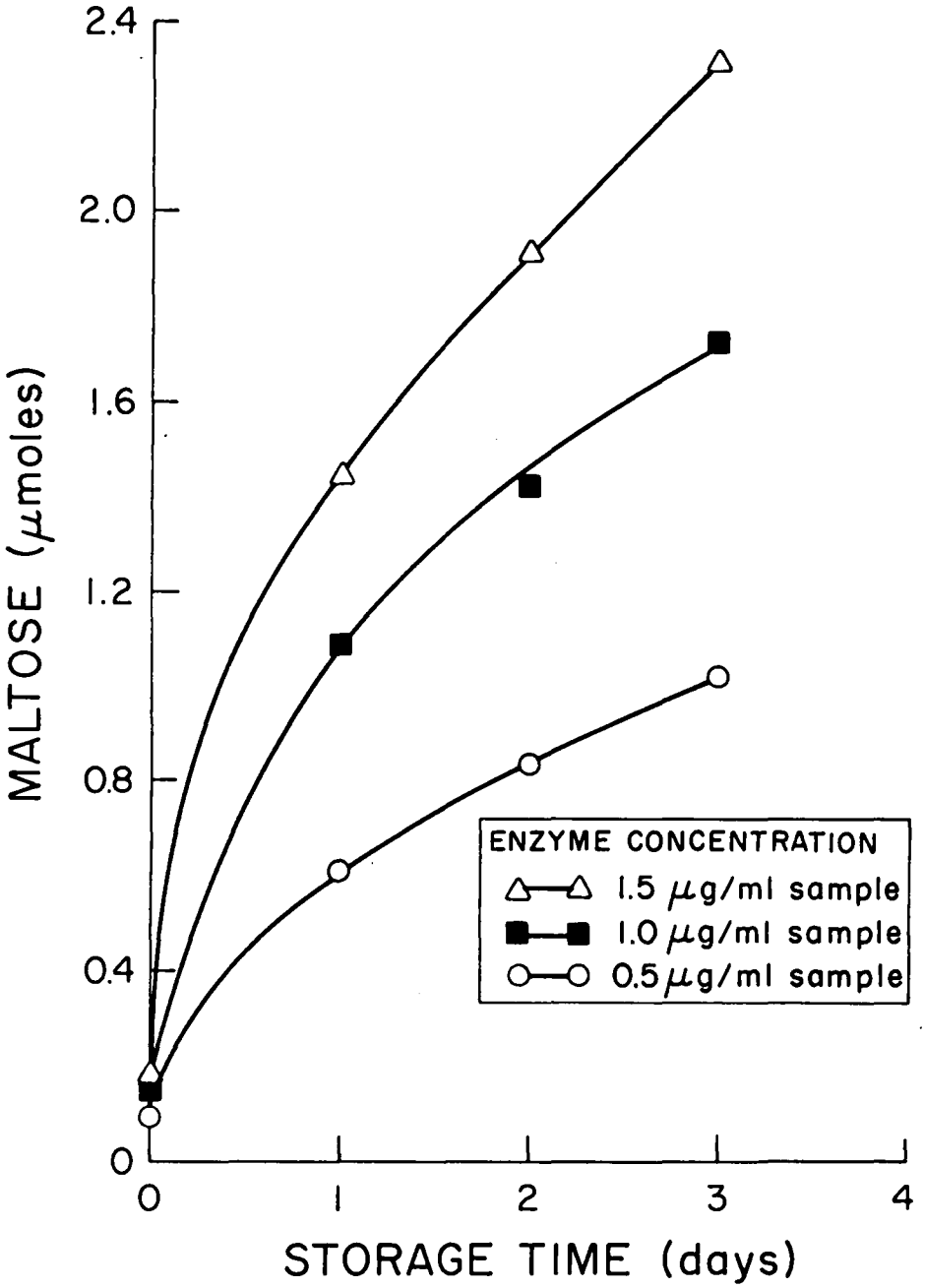

Fig. 4. Effect of storage time at -13°C (8.6°F) on cumulative activity of different concentrations of purified sweet potato β-amylase with 2% soluble starch substrate.

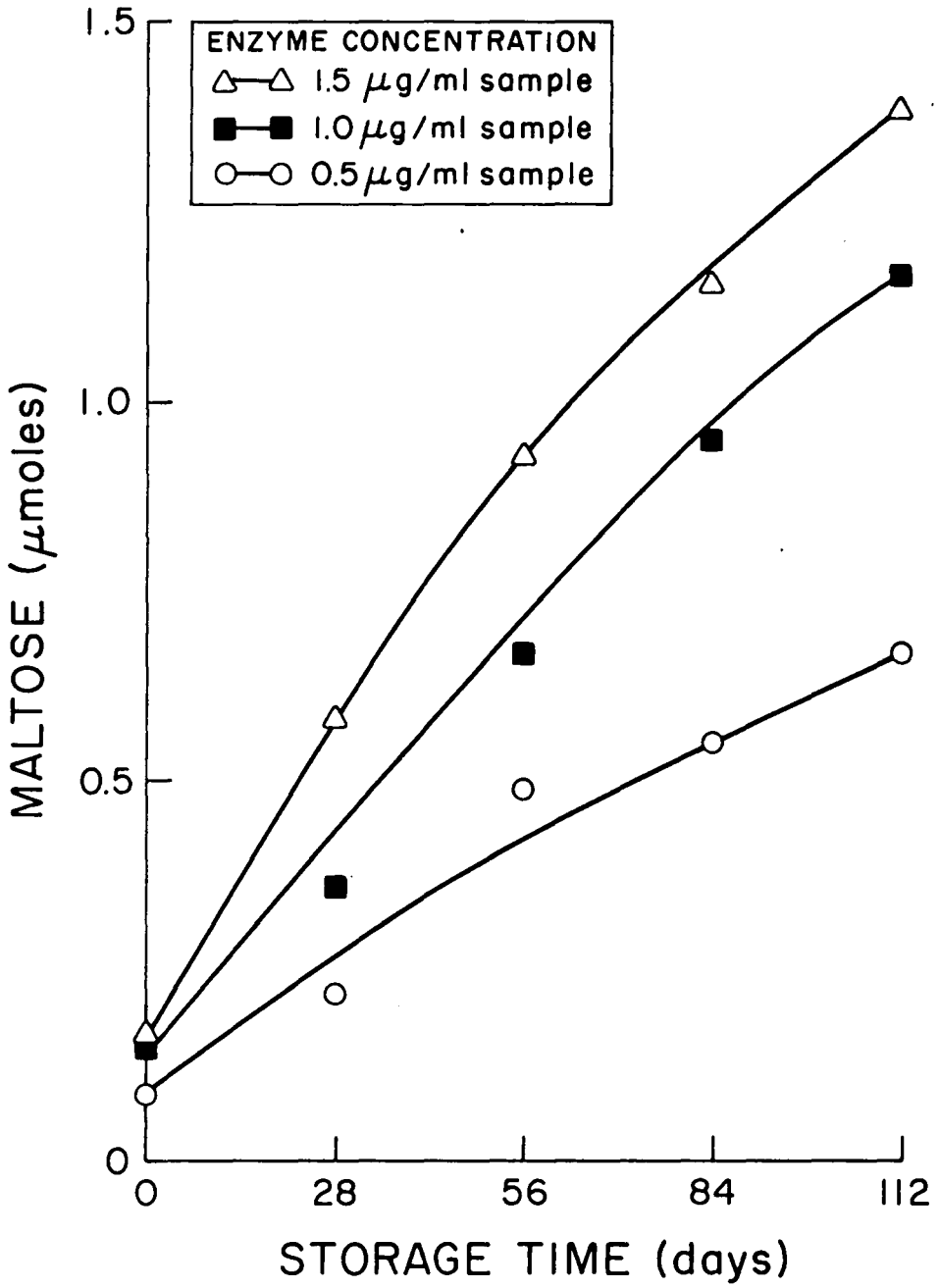

Fig. 5. Effect of storage time at -18°C (-0.4°F) on cumulative activity of different concentrations of purified swine pancreatic α-amylase with 2% soluble starch substrate.

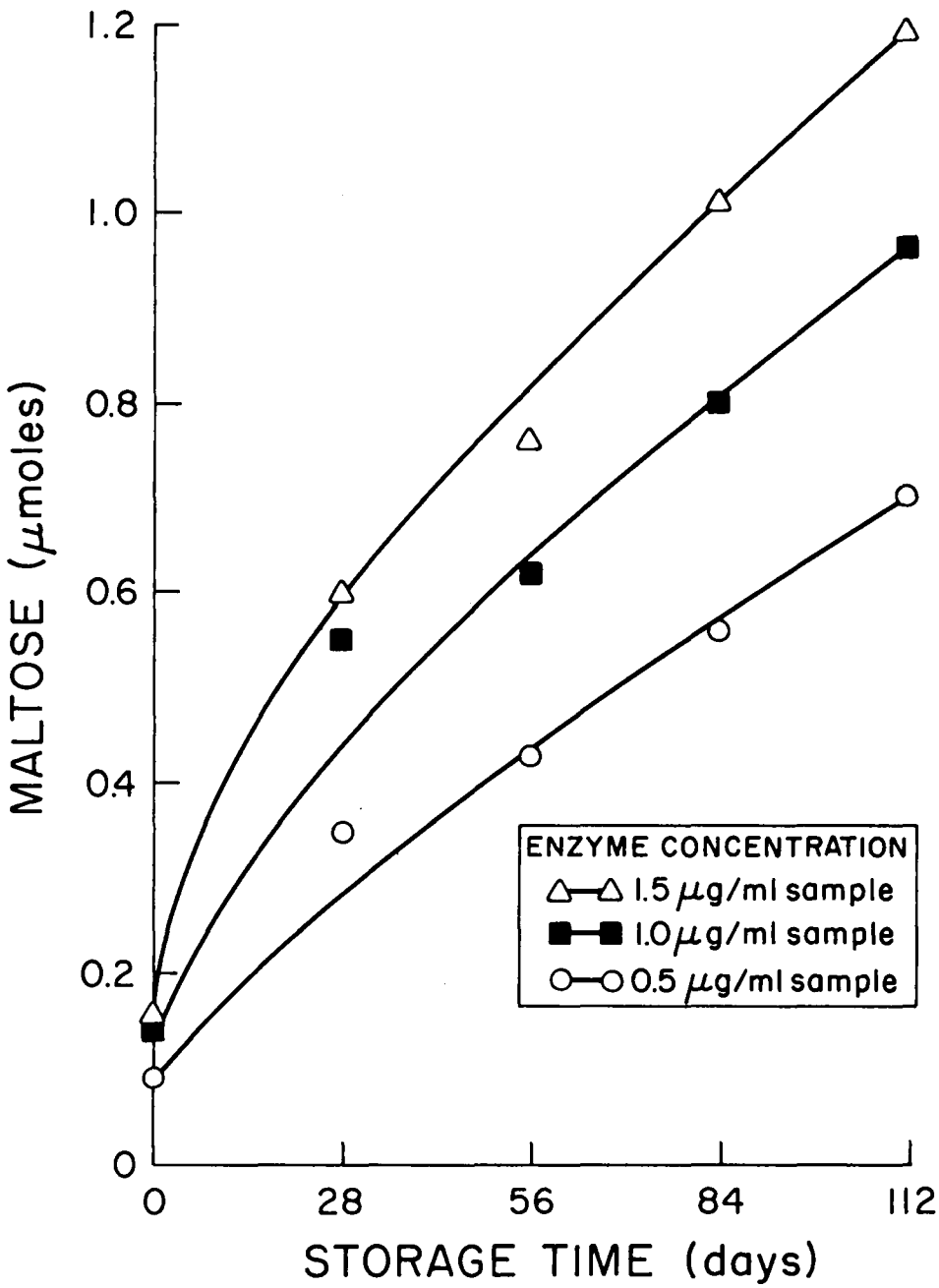

Fig. 6. Effect of storage time at -18°C (-0.4°F) on cumulative activity of different concentrations of purified sweet potato β-amylase with 2% soluble starch substrate.

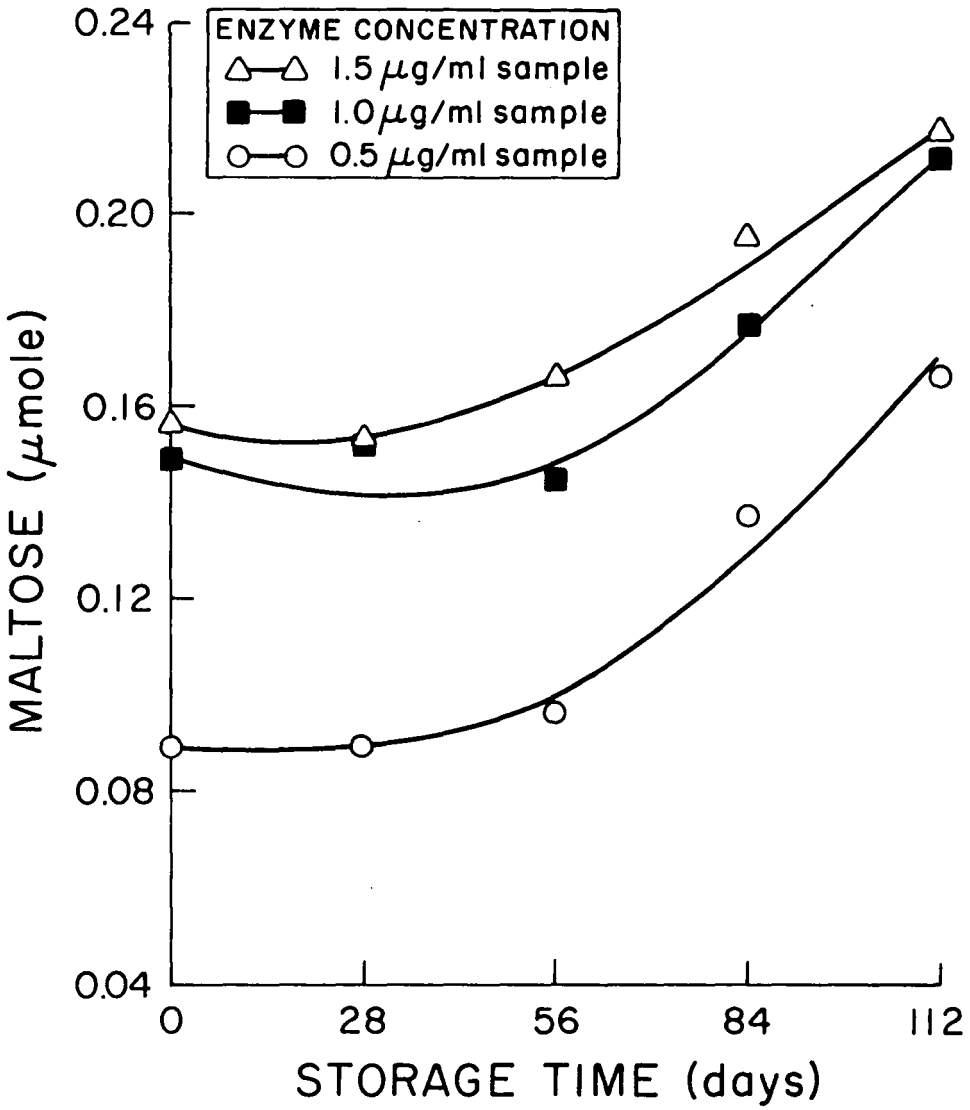

Fig. 7. Effect of storage time at -23°C (-9.4°F) on cumulative activity of different concentrations of purified swine pancreatic α-amylase with 2% soluble starch substrate.

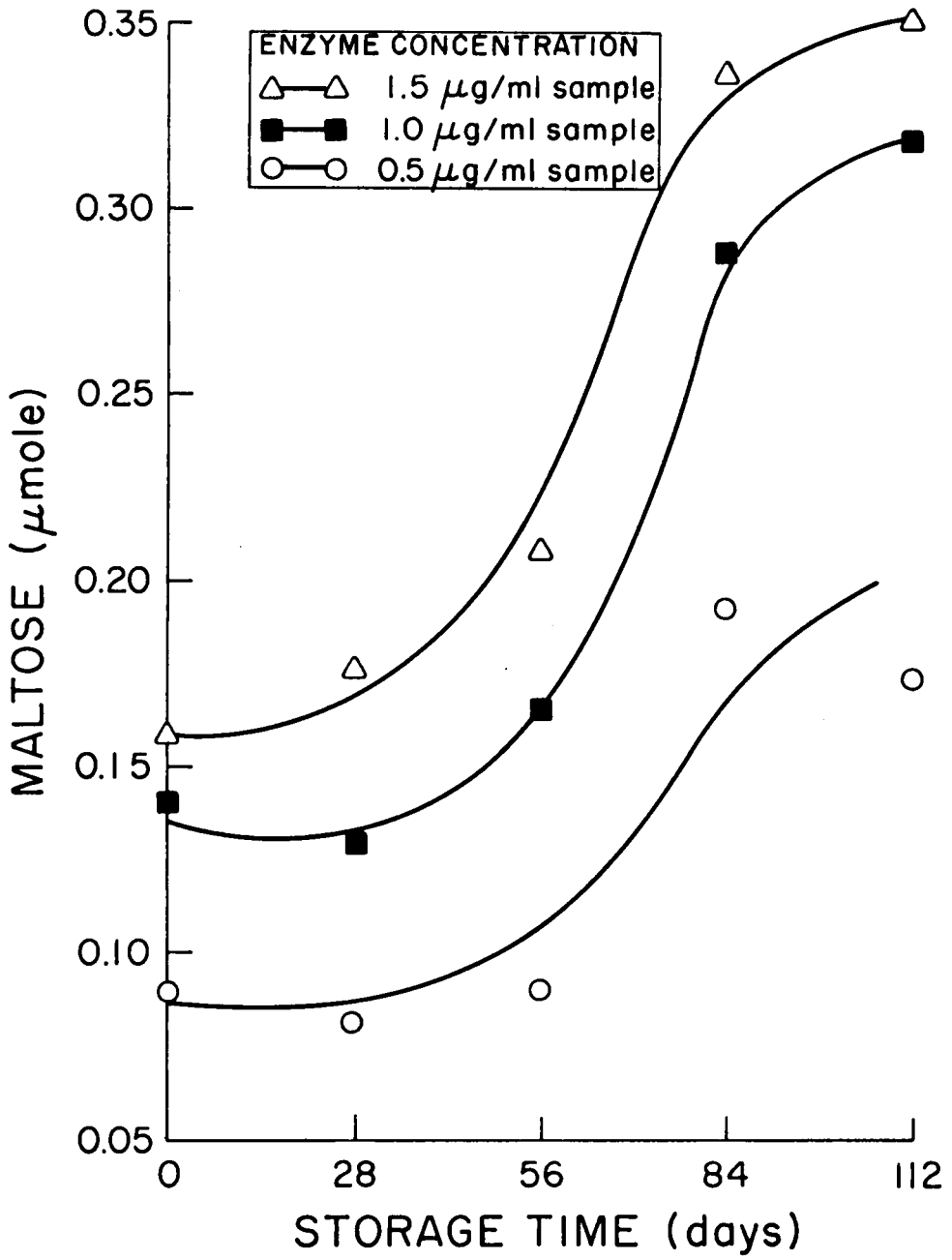

Fig. 8. Effect of storage time at -23°C (-9.4°F) on cumulative activity of different concentrations of purified sweet potato β-amylase with 2% soluble starch substrate.

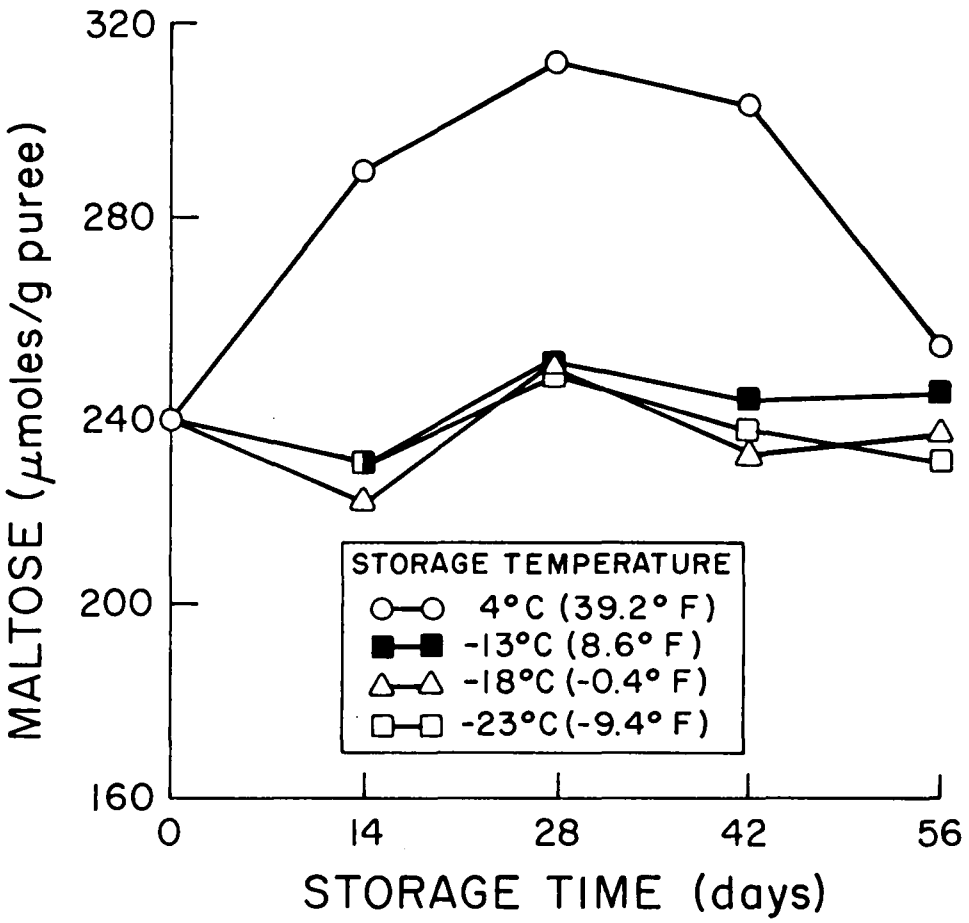

Fig. 9. Effect of storage time at different temperatures on cumulative maltose in cured sweet potatoes of the Centennial variety.

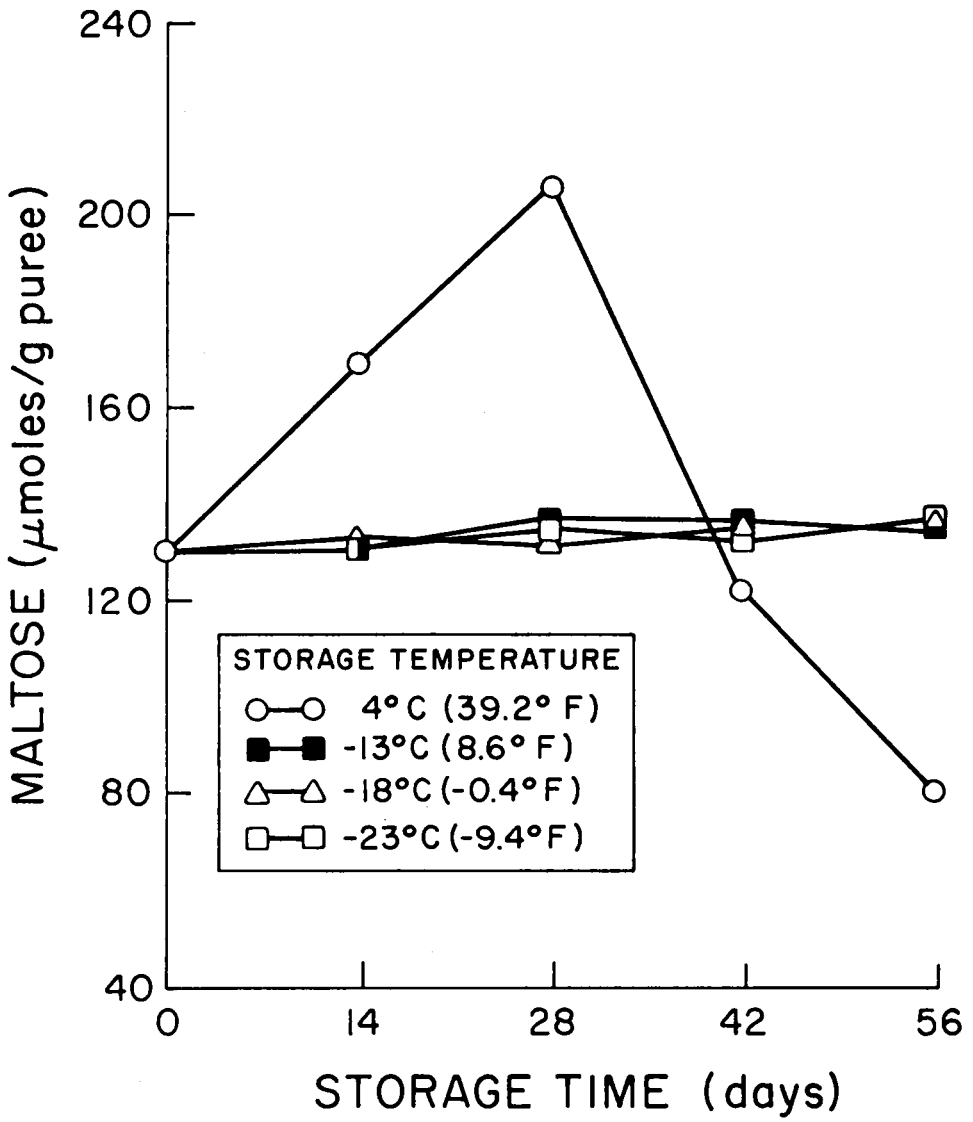

Fig. 10. Effect of storage time at different temperatures on cumulative maltose in uncured sweet potatoes of the Centennial variety.

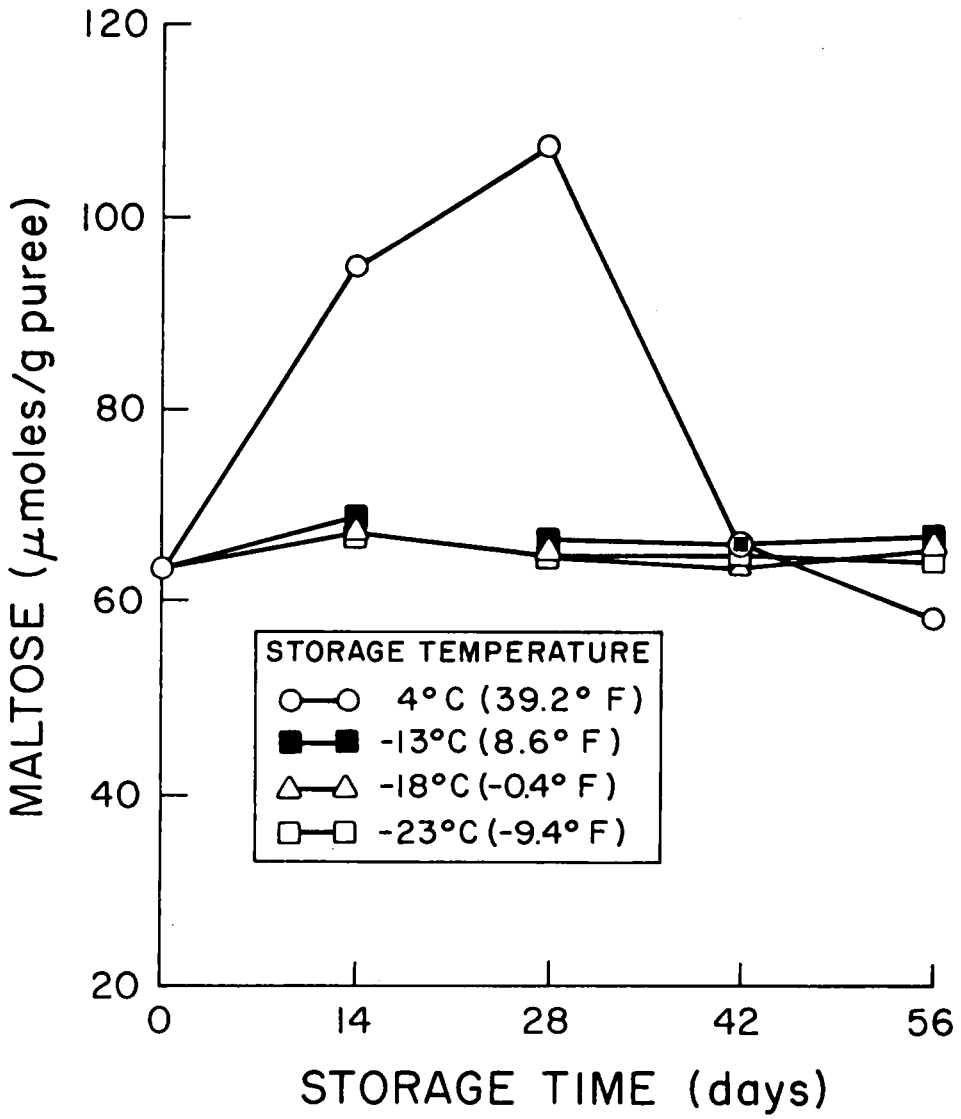

Fig. 11. Effect of storage time at different temperatures on cumulative maltose in uncured sweet potatoes of the Julian variety.

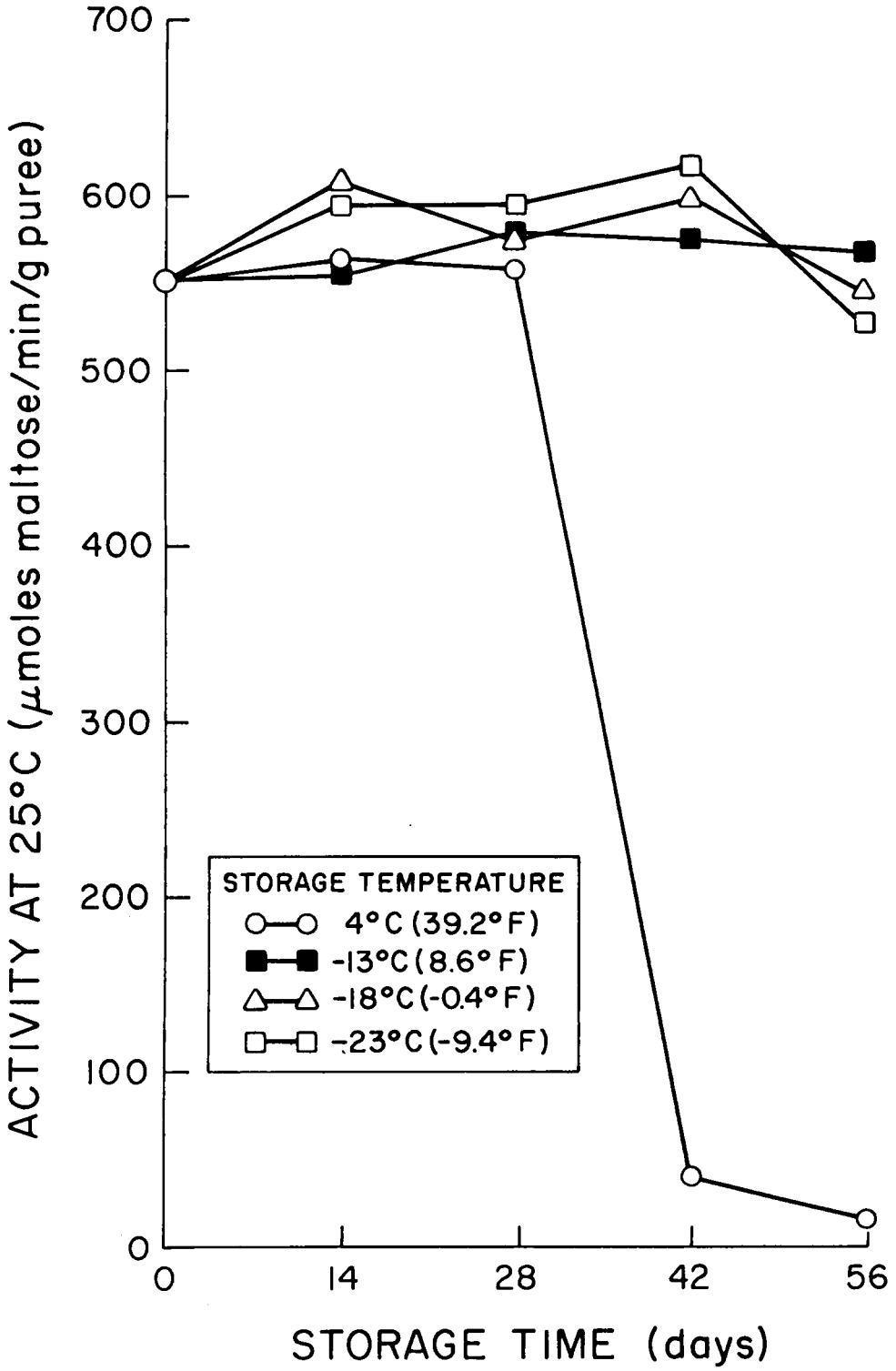

Fig. 12. Effect of storage time at different temperatures on activity of α-amylase in cured sweet potatoes of the Centennial variety.

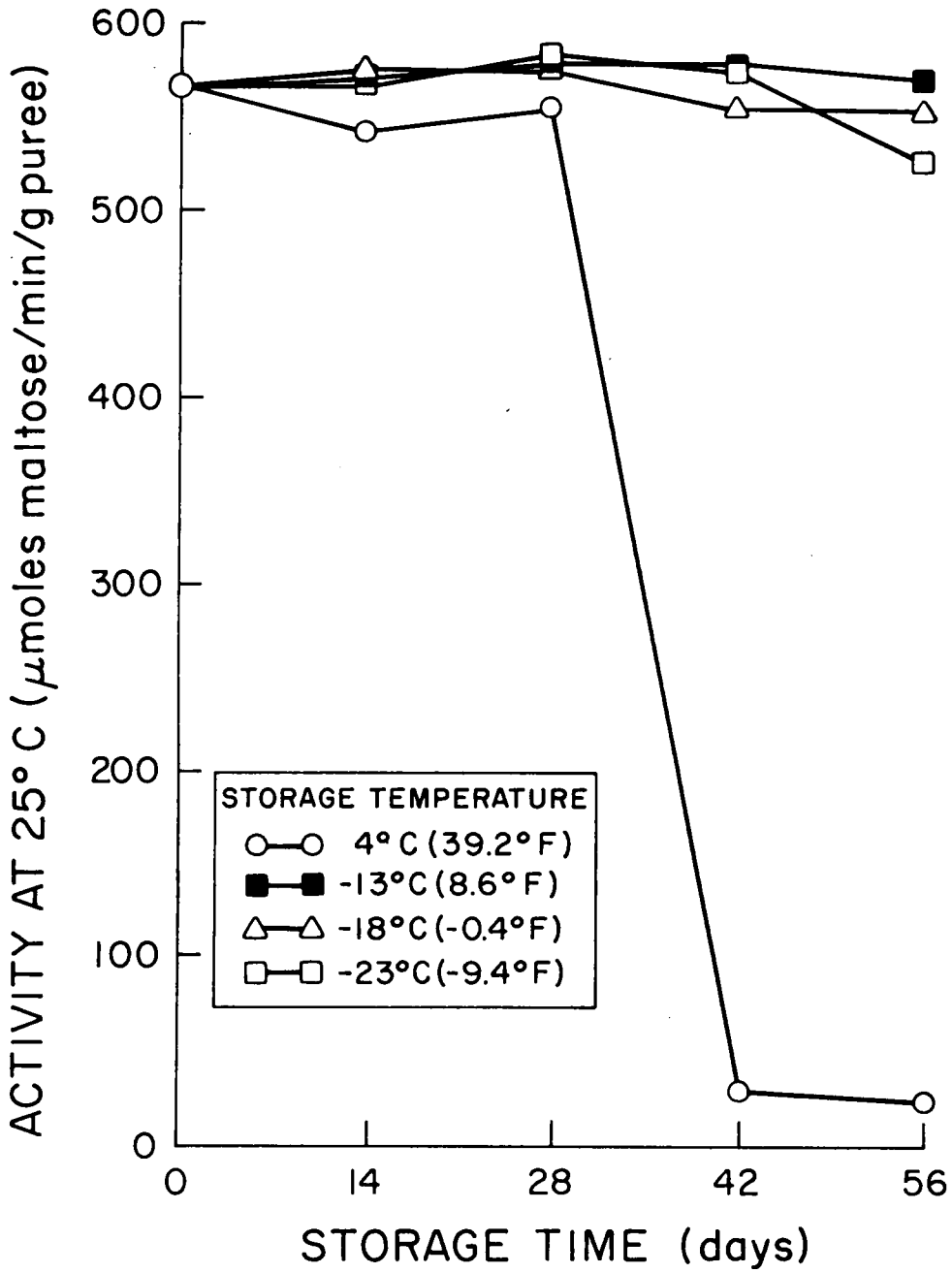

Fig. 13. Effect of storage time at different temperatures on activity of ß-amylase in cured sweet potatoes of the Centennial variety.

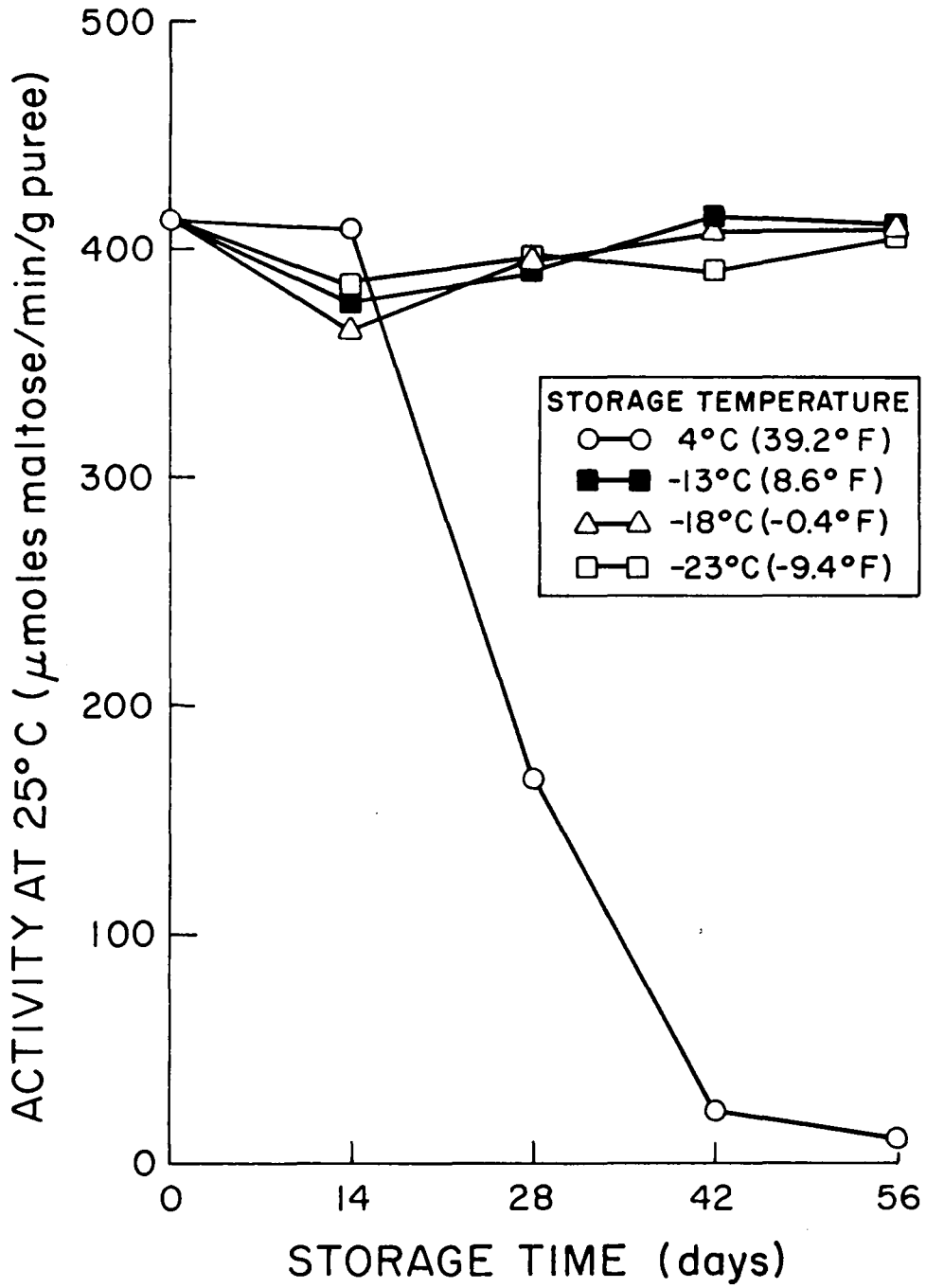

Fig. 14. Effect of storage time at different temperatures on activity of α-amylase in uncured sweet potatoes of the Centennial variety.

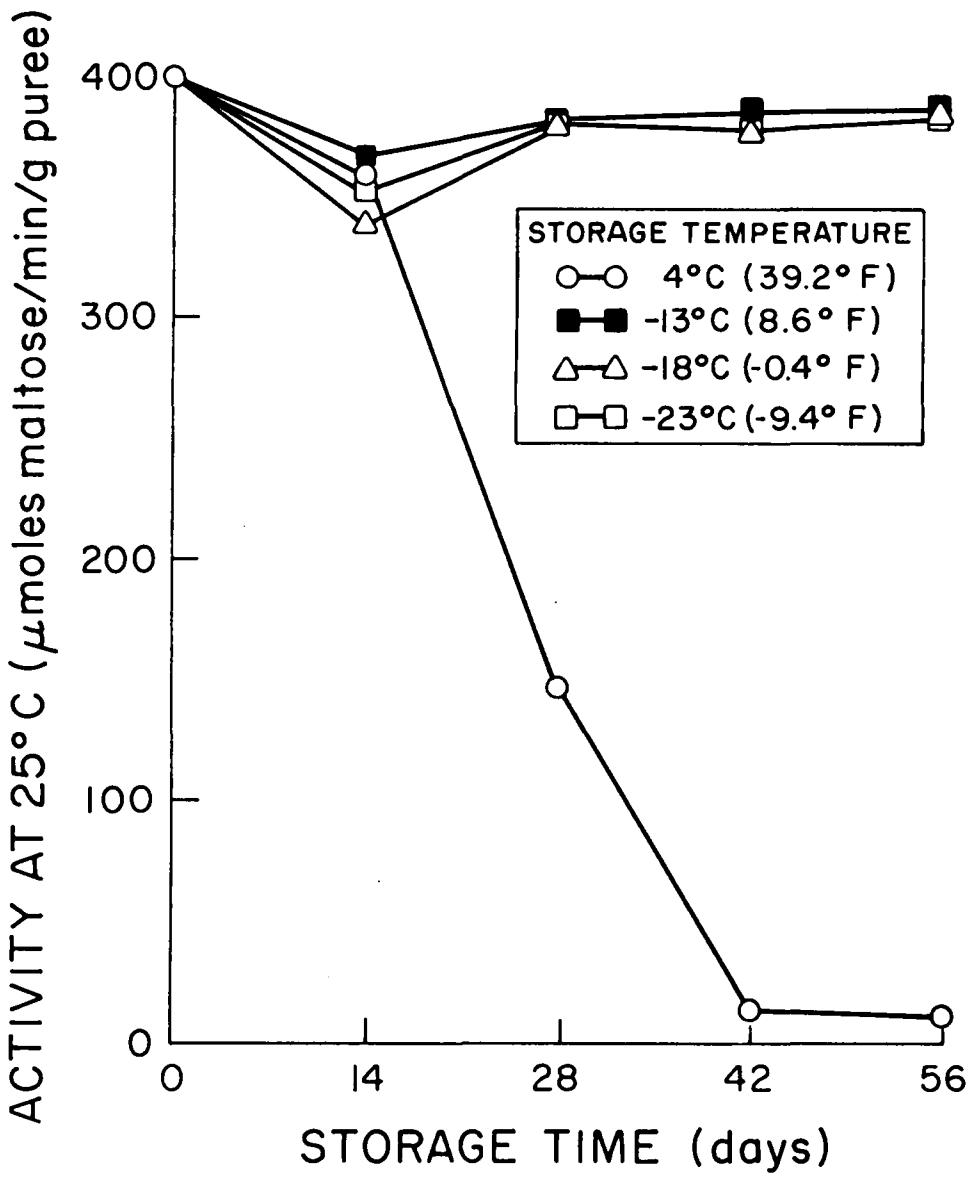

Fig. 15. Effect of storage time at different temperatures on activity of ß-amylase in uncured sweet potatoes of the Centennial variety.

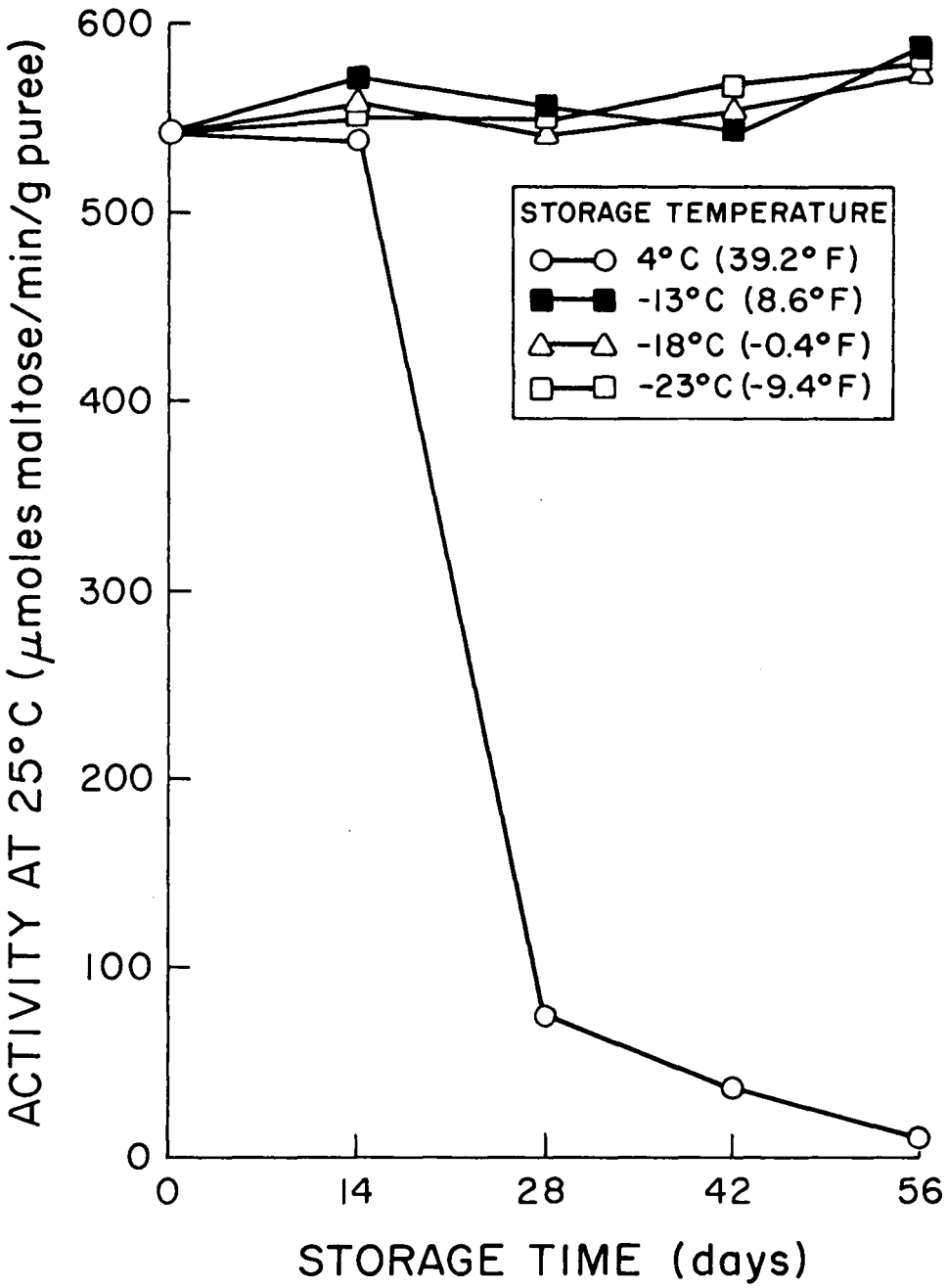

Fig. 16. Effect of storage time at different temperatures on activity of α-amylase in uncured sweet potatoes of the Julian variety.

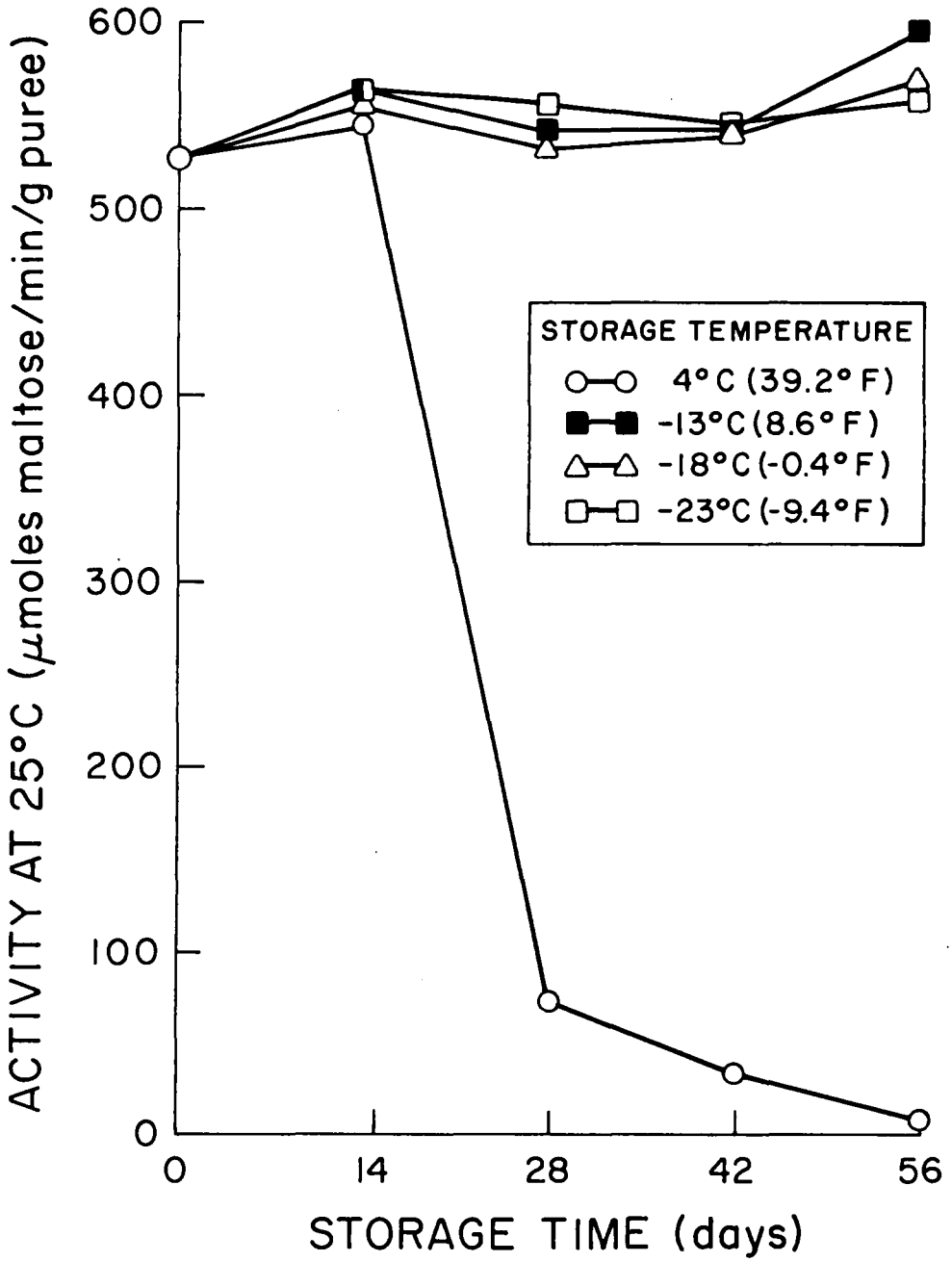

Fig. 17. Effect of storage time at different temperatures on activity of β-amylase in uncured sweet potatoes of the Julian variety.

Table 1. Effect of holding time at 4°C (39.2°F) on cumulative activity of different concentrations of purified swine pancreatic α-amylase with 2% soluble starch substrate.

Holding time (min)	Cumulative activity (μmoles maltose)[a]					
	Enzyme concentration (μg/ml)					
	0.5		1.0		1.5	
	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)
0	0.0820 a		0.1003 a		0.1590 a	
15	0.2110 b	+157.3	0.3551 b	+254.0	0.5470 b	+244.0
30	0.3766 c	+359.3	0.6021 c	+500.3	0.9728 c	+511.8
45	0.5326 d	+549.5	1.0257 d	+922.6	1.4492 d	+811.5
60	0.7546 e	+820.2	1.3326 e	+1228.6	1.9191 e	+1107.0
75	0.8825 f	+976.2	1.7395 f	+1634.3	2.4773 f	+1458.1

[a] Data are average of three replicates.

[b] Means within columns followed by a common letter are not significantly different at 5% level as determined by Duncan's Multiple Range Test.

[c] Data are based on cumulative activity and are expressed as percent increase over the zero storage time samples.

Table 2. Effect of holding time at 4°C (39.2°F) on cumulative activity of different concentrations of purified sweet potato β-amylase with 2% soluble starch substrate.

Holding time (min)	Cumulative activity (μmoles maltose)[a]					
	Enzyme concentration (μg/ml)					
	0.5		1.0		1.5	
	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)
0	0.0967 a		0.1487 a		0.1557 a	
15	0.2230 b	+130.6	0.4553 b	+206.2	0.6683 b	+329.2
30	0.4102 c	+324.2	0.8404 c	+465.2	1.1019 c	+607.7
45	0.5565 d	+475.5	1.2459 d	+737.9	1.8066 d	+1060.3
60	0.7395 e	+864.7	1.5889 e	+968.5	2.5506 e	+1538.2
75	0.9792 f	+912.6	1.9373 f	+1202.8	2.9593 f	+1800.6

[a] Data are average of three replicates.

[b] Means within columns followed by a common letter are not significantly different at 5% level as determined by Duncan's Multiple Range Test.

[c] Data are based on cumulative activity and are expressed as percent increase over the zero storage time samples.

Table 3. Comparative effect of enzyme concentration at 4°C (39.2°F) on cumulative activity of purified swine pancreatic α-amylase with 2% soluble starch substrate after equal holding times.

Enzyme concentration	Cumulative activity (μmoles maltose)[a]					
	Holding time (min)					
	0	15	30	45	60	75
0.5 μg/ml	0.0820	0.2110	0.3766	0.5326	0.7546	0.8825
1.0 μg/ml	0.1003	0.3551	0.6021	1.0257	1.3326	1.7395
Mean difference[b]	0.0183	0.1441**	0.2255**	0.4931**	0.5780**	0.8570**
0.5 μg/ml	0.0820	0.2110	0.3766	0.5326	0.7546	0.8825
1.5 μg/ml	0.1590	0.5470	0.9728	1.4492	1.9191	2.4773
Mean difference[b]	0.0770**	0.3360**	0.5962**	0.9166**	1.1645**	1.5948**
1.0 μg/ml	0.1003	0.3551	0.6021	1.0257	1.3326	1.7395
1.5 μg/ml	0.1590	0.5470	0.9728	1.4492	1.9191	2.4773
Mean difference[b]	0.0587*	0.1919**	0.3707**	0.4235**	0.5865**	0.7378**

[a] Data are average of three replicates.

[b] Tabulated L.S.D. values of enzyme concentrations x storage time required for significance at 5% and 1% levels were 0.0523 and 0.0702, respectively.

Asterisks indicate that means are statistically different at 5% (*) or 1% (**) level.

Table 4. Comparative effect of enzyme concentration at 4°C (39.2°F) on cumulative activity of purified sweet potato β-amylase with 2% soluble starch substrate after equal holding times.

Enzyme concentration	Cumulative activity (μmoles maltose)[a]					
	Holding time (min)					
	0	15	30	45	60	75
0.5 μg/ml	0.0967	0.2230	0.4102	0.5565	0.7395	0.9792
1.0 μg/ml	0.1487	0.4553	0.8404	1.2459	1.5889	1.9373
Mean difference[b]	0.0522*	0.2323**	0.4302**	0.6894**	0.8494**	0.9581**
0.5 μg/ml	0.0967	0.2230	0.4102	0.5565	0.7395	0.9792
1.5 μg/ml	0.1557	0.6683	1.1019	1.8066	2.5506	2.9593
Mean difference[b]	0.0592**	0.4453**	0.6917**	1.2501**	1.8111**	1.9801**
1.0 μg/ml	0.1487	0.4553	0.8404	1.2459	1.5889	1.9373
1.5 μg/ml	0.1557	0.6683	1.1019	1.8066	2.5506	2.9593
Mean difference[b]	0.0070	0.2130**	0.2615**	0.5607**	0.9617**	1.0220**

[a] Data are average of three replicates.

[b] Tabulated L.S.D. values of enzyme concentrations x storage time required for significance at 5% and 1% levels were 0.0438 and 0.0588, respectively.

Asterisks indicate that means are statistically different at 5% (*) or 1% (**) level.

Table 5. Effect of storage time at -13°C (8.6°F) on cumulative activity of different concentrations of purified swine pancreatic α-amylase with 2% soluble starch substrate.

Storage time (days)	Cumulative activity (μmoles maltose)[a]					
	Enzyme concentration (μg/ml)					
	0.5		1.0		1.5	
	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)
0	0.1377 a		0.1487 a		0.1563 a	
1	0.4539 b	+229.6	0.7702 b	+418.0	1.1489 b	+635.1
2	0.7894 c	+473.3	1.4915 c	+903.0	2.2515 c	+1340.5
3	1.0489 d	+661.7	1.8338 d	+1133.2	2.8487 d	+1722.6

[a] Data are average of three replicates.

[b] Means within columns followed by a common letter are not significantly different at 5% level as determined by Duncan's Multiple Range Test.

[c] Data are based on cumulative activity and are expressed as percent increase over the zero storage time samples.

Table 6. Effect of storage time at -13°C (8.6°F) on cumulative activity of different concentrations of purified sweet potato β-amylase with 2% soluble starch substrate.

Storage time (days)	Cumulative activity (μmoles maltose)[a]					
	Enzyme concentration (μg/ml)					
	0.5		1.0		1.5	
	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)
0	0.0893 a		0.1413 a		0.1590 a	
1	0.6028 b	+575.0	1.0799 b	+664.3	1.4352 b	+802.6
2	0.8307 c	+830.2	1.4244 c	+908.1	1.9128 c	+1103.0
3	1.0222 d	+1044.7	1.7161 d	+1114.5	2.3085 d	+1351.9

[a] Data are average of three replicates.

[b] Means within columns followed by a common letter are not significantly different at 5% level as determined by Duncan's Multiple Range Test.

[c] Data are based on cumulative activity and are expressed as percent increase over the zero storage time samples.

Table 7. Comparative effect of enzyme concentration at -13°C (8.6°F) on cumulative activity of purified swine pancreatic α-amylase with 2% soluble starch substrate after equal storage times.

Enzyme concentration	Cumulative activity (μmoles maltose)[a]			
	Storage time (days)			
	0	1	2	3
0.5 μg/ml	0.1377	0.4539	0.7894	1.0489
1.0 μg/ml	0.1487	0.7702	1.4915	1.8338
Mean difference[b]	0.0110	0.3163**	0.7021**	0.7849**
0.5 μg/ml	0.1377	0.4539	0.7894	1.0489
1.5 μg/ml	0.1563	1.1489	2.2515	2.8487
Mean difference[b]	0.0186	0.6950**	1.4621**	0.7998**
1.0 μg/ml	0.1487	0.7702	1.4915	1.8338
1.5 μg/ml	0.1563	1.1489	2.2515	2.8487
Mean difference[b]	0.0076	0.3787**	0.7600**	1.0149**

[a] Data are average of three replicates.

[b] Tabulated L.S.D. values of enzyme concentrations x storage time required for significance at 5% and 1% levels were 0.0237 and 0.0322, respectively.

Asterisks indicate that means are statistically different at 5% (*) or 1% (**) level.

Table 8. Comparative effect of enzyme concentration at -13°C (8.6°F) on cumulative activity of purified sweet potato β-amylase with 2% soluble starch substrate after equal storage times.

Enzyme concentration	Cumulative activity (μ moles maltose)[a]			
	Storage time (days)			
	0	1	2	3
0.5 μg/ml	0.0893	0.6028	0.8307	1.0222
1.0 μg/ml	0.1413	1.0799	1.4244	1.7161
Mean difference[b]	0.0520*	0.4771**	0.5937**	0.6939**
0.5 μg/ml	0.0893	0.6028	0.8307	1.0222
1.5 μg/ml	0.1590	1.4352	1.9128	2.3085
Mean difference[b]	0.0697**	0.8324**	1.0821**	1.2863**
1.0 μg/ml	0.1413	1.0799	1.4244	1.7161
1.5 μg/ml	0.1590	1.4352	1.9128	2.3085
Mean difference[b]	0.0177	0.3553**	0.4884**	0.5924**

[a] Data are average of three replicates.

[b] Tabulated L.S.D. values of enzyme concentrations x storage time required for significance at 5% and 1% levels were 0.0476 and 0.0647, respectively.

Asterisks indicate that means are statistically different at 5% (*) or 1% (**) level.

Table 9. Effect of storage time at -18°C (-0.4°F) on cumulative activity of different concentrations of purified swine pancreatic α-amylase with 2% soluble starch substrate.

Storage time (days)	Cumulative activity (μmoles maltose)[a]					
	Enzyme concentration (μg/ml)					
	0.5		1.0		1.5	
	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)
0	0.0893 a		0.1487 a		0.1563 a	
28	0.2173 b	+143.3	0.3579 b	+140.7	0.5799 b	+271.0
56	0.4877 c	+446.1	0.6659 c	+347.8	0.9333 c	+497.1
84	0.5518 d	+517.9	0.9456 d	+535.9	1.1560 d	+639.6
112	0.6556 e	+634.2	1.1582 e	+678.9	1.3816 e	+783.9

[a] Data are average of three replicates.

[b] Means within columns followed by a common letter are not significantly different at 5% level as determined by Duncan's Multiple Range Test.

[c] Data are based on cumulative activity and are expressed as percent increase over the zero storage time samples.

Table 10. Effect of storage time at -18°C (-0.4°F) on cumulative activity of different concentrations of purified sweet potato β-amylase with 2% soluble starch substrate.

Storage time (days)	Cumulative activity (μmoles maltose)[a]					
	Enzyme concentration (μg/ml)					
	0.5		1.0		1.5	
	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)
0	0.0893 a		0.1413 a		0.1590 a	
28	0.3477 b	+289.4	0.5463 b	+286.6	0.5995 b	+277.0
56	0.4312 c	+382.9	0.6229 c	+340.8	0.7643 c	+380.7
84	0.5551 d	+521.6	0.7976 d	+464.5	1.0132 d	+537.2
112	0.7005 e	+684.4	0.9633 e	+581.7	1.1887 e	+647.6

[a] Data are average of three replicates.

[b] Means within columns followed by a common letter are not significantly different at 5% level as determined by Duncan's Multiple Range Test.

[c] Data are based on cumulative activity and are expressed as percent increase over the zero storage time samples.

Table 11. Comparative effect of enzyme concentration at -18°C (-0.4°F) on cumulative activity of purified swine pancreatic α-amylase with 2% soluble starch substrate after equal storage times.

Enzyme concentration	Cumulative activity (μmoles maltose)[a]				
	Storage time (days)				
	0	28	56	84	112
0.5 μg/ml	0.0893	0.2173	0.4877	0.5518	0.6556
1.0 μg/ml	0.1487	0.3579	0.6659	0.9456	1.1582
Mean difference[b]	0.0594*	0.1406**	0.1782**	0.3938**	0.5026**
0.5 μg/ml	0.0893	0.2173	0.4877	0.5518	0.6556
1.5 μg/ml	0.1563	0.5799	0.9333	1.1560	1.3816
Mean difference[b]	0.0670**	0.3626**	0.4456**	0.6042**	0.7260**
1.0 μg/ml	0.1487	0.3579	0.6659	0.9456	1.1582
1.5 μg/ml	0.1563	0.5799	0.9333	1.1560	1.3816
Mean difference[b]	0.0076	0.2220**	0.2674**	0.2104**	0.2234**

[a] Data are average of three replicates.

[b] Tabulated L.S.D. values of enzyme concentrations x storage time required for significance at 5% and 1% levels were 0.0472 and 0.0635, respectively.

Asterisks indicate that means are statistically different at 5% (*) or 1% (**) level.

Table 12. Comparative effect of enzyme concentration at -18°C (-0.4°F) on cumulative activity of purified sweet potato β-amylase with 2% soluble starch substrate after equal storage times.

Enzyme concentration	Cumulative activity (μmoles maltose)[a]				
	Storage time (days)				
	0	28	56	84	112
0.5 μg/ml	0.0893	0.3477	0.4312	0.5551	0.7005
1.0 μg/ml	0.1413	0.5463	0.6229	0.7976	0.9633
Mean difference[b]	0.0520*	0.1986**	0.1917**	0.2425**	0.2628**
0.5 μg/ml	0.0893	0.3477	0.4312	0.5551	0.7005
1.5 μg/ml	0.1590	0.5995	0.7643	1.0132	1.1887
Mean difference[b]	0.0697**	0.2518**	0.3331**	0.4581**	0.4882**
1.0 μg/ml	0.1413	0.5463	0.6229	0.7976	0.9633
1.5 μg/ml	0.1590	0.5995	0.7643	1.0132	1.1887
Mean difference[b]	0.0177	0.0532*	0.1414**	0.2156**	0.2254**

[a] Data are average of three replicates.

[b] Tabulated L.S.D. values of enzyme concentrations x storage time required for significance at 5% and 1% levels were 0.0500 and 0.0674, respectively.

Asterisks indicate that means are statistically different at 5% (*) or 1% (**) level.

Table 13. Effect of storage time at -23°C (-9.4°F) on cumulative activity of different concentrations of purified swine pancreatic α-amylase with 2% soluble starch substrate.

Storage time (days)	Cumulative activity (μmole maltose)[a]					
	Enzyme concentration (μg/ml)					
	0.5		1.0		1.5	
	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)
0	0.0893 a		0.1487 a		0.1563 a	
28	0.0893 a	0.0	0.1520 a	+2.2	0.1523 a	-2.6
56	0.0967 a	+8.2	0.1447 a	-2.7	0.1660 a	+6.2
84	0.1377 ab	+54.2	0.1770 a	+19.0	0.1950 a	+24.8
112	0.1660 b	+85.9	0.2113 a	+42.1	0.2173 a	+39.0

[a] Data are average of three replicates.

[b] Means within columns followed by a common letter are not significantly different at 5% level as determined by Duncan's Multiple Range Test.

[c] Data are based on cumulative activity and are expressed as percent change as compared to the zero storage time samples.

Table 14. Effect of storage time at -23°C (-9.4°F) on cumulative activity of different concentrations of purified sweet potato β-amylase with 2% soluble starch substrate.

Storage time (days)	Cumulative activity (μmole maltose)[a]					
	Enzyme concentration (μg/ml)					
	0.5		1.0		1.5	
	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)
0	0.0893 a		0.1413 a		0.1590 a	
28	0.0820 a	-8.2	0.1303 a	-7.8	0.1760 a	+10.7
56	0.0893 a	0.0	0.1663 a	+17.7	0.2080 a	+30.8
84	0.1917 b	+114.7	0.2880 b	+103.8	0.3364 b	+111.6
112	0.1737 b	+94.5	0.3173 b	+124.6	0.3499 b	+120.1

[a] Data are average of three replicates.

[b] Means within columns followed by a common letter are not significantly different at 5% level as determined by Duncan's Multiple Range Test.

[c] Data are based on cumulative activity and are expressed as percent change as compared to the zero storage time samples.

Table 15. Comparative effect of storage temperature on cumulative activity of different concentrations of purified swine pancreatic α-amylase with 2% soluble starch substrate after equal storage times.

	Cumulative activity (μmoles maltose)[a]				
	Storage time (days)				
	0	28	56	84	112
0.5 μg/ml					
at -18°C (-0.4°F)	0.0893	0.2173	0.4877	0.5518	0.6556
at -23°C (-9.4°F)	0.0893	0.0893	0.0967	0.1377	0.1660
Mean difference[b]	0.0	0.1280**	0.3910**	0.4141**	0.4896**
1.0 μg/ml					
at -18°C (-0.4°F)	0.1487	0.3579	0.6659	0.9456	1.1582
at -23°C (-9.4°F)	0.1487	0.1520	0.1447	0.1770	0.2113
Mean difference[b]	0.0	0.2059**	0.5212**	0.7686**	0.9469**
1.5 μg/ml					
at -18°C (-0.4°F)	0.1563	0.5799	0.9333	1.1560	1.3816
at -23°C (-9.4°F)	0.1563	0.1523	0.1660	0.1950	0.2173
Mean difference[b]	0.0	0.4276**	0.7673**	0.9610**	1.1643**

[a] Data are average of three replicates.

[b] Tabulated L.S.D. values of storage temperatures x storage time required for significance at 5% and 1% levels were 0.0541 and 0.0721, respectively.

Asterisks indicate that means are statistically different at 5% (*) or 1% (**) level.

Table 16. Comparative effect of storage temperature on cumulative activity of different concentrations of purified sweet potato β-amylase with 2% soluble starch substrate after equal storage times.

	Cumulative activity (μmoles maltose)[a]				
	Storage time (days)				
	0	28	56	84	112
0.5 μg/ml					
at -18°C (-0.4°F)	0.0893	0.3477	0.4312	0.5551	0.7005
at -23°C (-9.4°F)	0.0893	0.0820	0.0893	0.1917	0.1737
Mean difference[b]	0.0	0.2657**	0.3419**	0.3634**	0.5268**
1.0 μg/ml					
at -18°C (-0.4°F)	0.1413	0.5463	0.6229	0.7976	0.9633
at -23°C (-9.4°F)	0.1413	0.1303	0.1663	0.2880	0.3173
Mean difference[b]	0.0	0.4160**	0.4566**	0.5096**	0.6460**
1.5 μg/ml					
at -18°C (-0.4°F)	0.1590	0.5995	0.7643	1.0132	1.1887
at -23°C (-9.4°F)	0.1590	0.1760	0.2080	0.3364	0.3499
Mean difference[b]	0.0	0.4235**	0.5563**	0.6768**	0.8388**

[a] Data are average of three replicates.

[b] Tabulated L.S.D. values of storage temperatures x storage time required for significance at 5% and 1% levels were 0.0541 and 0.0721, respectively.

Asterisks indicate that means are statistically different at 5% (*) or 1% (**) level.

Table 17. Effect of storage time at different temperatures on cumulative maltose in cured sweet potatoes of the Centennial variety.

Storage time (days)	Cumulative maltose (μmoles maltose/g puree)[a]							
	Storage temperature							
	4°C (39.2°F)		-13°C (8.6°F)		-18°C (-0.4°F)		-23°C (-9.4°F)	
	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)
0	238.30 a		238.30 a		238.30 a		238.30 ab	
14	289.70 b	+21.6	229.80 b	-3.6	221.65 b	+7.0	229.80 a	-3.6
28	311.70 c	+30.8	249.63 c	+4.8	249.63 c	+4.8	246.80 b	+3.6
42	302.77 d	+27.1	242.55 ac	+1.8	231.33 a	-2.9	235.47 a	-1.2
56	252.47 e	+6.0	243.97 ac	+2.4	235.47 a	-1.2	229.80 a	-3.6

[a] Data are average of three replicates.

[b] Means within columns followed by a common letter are not significantly different at 5% level as determined by Duncan's Multiple Range Test.

[c] Data are based on cumulative maltose and are expressed as percent change as compared to the zero storage time samples.

Table 18. Comparative effect of storage temperature on cumulative maltose in cured sweet potatoes of the Centennial variety after equal storage times.

Storage temperature	Cumulative maltose (μmoles maltose/g puree)[a]				
	Storage time (days)				
	0	14	28	42	56
4°C (39.2°F)	238.30	289.70	311.70	302.77	252.47
-13°C (8.6°F)	238.30	229.80	249.63	242.55	243.97
Mean difference[b]	0.0	59.90**	62.07**	60.22**	8.50*
4°C (39.2°F)	238.30	289.70	311.70	302.77	252.47
-18°C (-0.4°F)	238.30	221.65	249.63	231.33	235.47
Mean difference[b]	0.0	68.05**	62.07**	71.44**	17.00**
4°C (39.2°F)	238.30	289.70	311.70	302.77	252.47
-23°C (-9.4°F)	238.30	229.80	246.80	235.47	229.80
Mean difference[b]	0.0	59.90**	64.90**	67.30**	22.67**

Table continued

Table 18 (Continued)

Storage temperature	Cumulative maltose (μmoles maltose/g puree)[a]				
	Storage time (days)				
	0	14	28	42	56
-13°C (8.6°F)	238.30	229.80	249.63	242.55	243.97
-18°C (-0.4°F)	238.30	221.65	249.63	231.33	235.47
Mean difference[b]	0.0	8.15	0.0	11.22*	8.50*
-13°C (8.6°F)	238.30	229.80	249.63	242.55	243.97
-23°C (-9.4°F)	238.30	229.80	246.80	235.47	229.80
Mean difference[b]	0.0	0.0	2.83	7.08	13.17**
-18°C (-0.4°F)	238.30	221.65	249.63	231.33	235.47
-23°C (-9.4°F)	238.30	229.80	246.80	235.47	229.80
Mean difference[b]	0.0	8.15	2.83	4.14	5.67

[a] Data are average of three replicates.

[b] Tabulated L.S.D. values of storage temperatures x storage time required for significance at 5% and 1% levels were 8.5054 and 11.3798, respectively.

Asterisks indicate that means are statistically different at 5% (*) or 1% (**) level.

Table 19. Effect of storage time at different temperatures on cumulative maltose in uncured sweet potatoes of the Centennial variety.

Storage time (days)	Cumulative maltose (μmoles maltose/g puree)[a]							
	Storage temperature							
	4°C (39.2°F)		-13°C (8.6°F)		-18°C (-0.4°F)		-23°C (-9.4°F)	
	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)
0	131.25 a		131.25 a		131.25 a		131.25 a	
14	170.00 b	+29.5	131.63 a	+0.3	133.10 ab	+1.4	132.39 ac	+0.9
28	206.29 c	+57.2	137.21 b	+4.5	132.39 ac	+0.9	135.32 bc	+3.1
42	122.91 d	-6.4	137.56 b	+4.8	136.08 bc	+3.7	133.10 ab	+1.4
56	80.78 e	-38.5	136.43 b	+4.0	136.45 b	+4.0	136.81 b	+4.2

[a] Data are average of three replicates.

[b] Means within columns followed by a common letter are not significantly different at 5% level as determined by Duncan's Multiple Range Test.

[c] Data are based on cumulative maltose and are expressed as percent change as compared to the zero storage time samples.

Table 20. Comparative effect of storage temperature on cumulative maltose in uncured sweet potatoes of the Centennial variety after equal storage times.

Storage temperature	Cumulative maltose (μmoles maltose/g puree)[a]				
	Storage time (days)				
	0	14	28	42	56
4°C (39.2°F)	131.25	170.00	206.29	122.91	80.78
-13°C (8.6°F)	131.25	131.63	137.21	137.56	136.43
Mean difference[b]	0.0	38.37**	69.08**	14.65**	55.65**
4°C (39.2°F)	131.25	170.00	206.29	122.91	80.78
-18°C (-0.4°F)	131.25	133.10	132.39	136.08	136.45
Mean difference[b]	0.0	36.90**	73.90**	13.17**	55.67**
4°C (39.2°F)	131.25	170.00	206.29	122.91	80.78
-23°C (-9.4°F)	131.25	132.39	135.32	133.10	136.81
Mean difference[b]	0.0	37.61**	70.97**	10.19**	56.03**

Table continued

Table 20 (Continued)

Storage temperature	Cumulative maltose (μmoles maltose/g puree)[a]				
	Storage time (days)				
	0	14	28	42	56
-13°C (8.6°F)	131.25	131.63	137.21	137.56	136.43
-18°C (-0.4°F)	131.25	133.10	132.39	136.08	136.45
Mean difference[b]	0.0	1.47	4.82*	1.48	0.02
-13°C (8.6°F)	131.25	131.63	137.21	137.56	136.43
-23°C (-9.4°F)	131.25	132.39	135.32	133.10	136.81
Mean difference[b]	0.0	0.76	1.89	4.46*	0.38
-18°C (-0.4°F)	131.25	133.10	132.39	136.08	136.45
-23°C (-9.4°F)	131.25	132.39	135.32	133.10	136.81
Mean difference[b]	0.0	0.71	3.33	2.98	0.36

[a] Data are average of three replicates.

[b] Tabulated L.S.D. values of storage temperatures x storage time required for significance at 5% and 1% levels were 3.7160 and 4.9720, respectively.

Asterisks indicate that means are statistically different at 5% (*) or 1% (**) level.

Table 21. Effect of storage time at different temperatures on cumulative maltose in uncured sweet potatoes of the Julian variety.

Storage time (days)	Cumulative maltose (μmoles maltose/g puree)[a]							
	Storage temperature							
	4°C (39.2°F)		-13°C (8.6°F)		-18°C (-0.4°F)		-23°C (-9.4°F)	
	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)
0	63.25 a		63.25 a		63.25 a		63.25 a	
14	94.92 b	+50.1	68.79 b	+8.8	67.30 b	+6.4	67.19 b	+6.2
28	107.43 c	+69.9	66.37 ce	+4.9	64.89 ac	+2.6	65.08 a	+2.9
42	65.99 d	+4.3	65.26 de	+3.2	63.43 a	+0.3	64.89 a	+2.6
56	58.24 e	-7.9	66.55 e	+5.2	65.80 bc	+4.0	64.34 a	+1.7

[a] Data are average of three replicates.

[b] Means within columns followed by a common letter are not significantly different at 5% level as determined by Duncan's Multiple Range Test.

[c] Data are based on cumulative maltose and are expressed as percent change as compared to the zero storage time samples.

Table 22. Comparative effect of storage temperature on cumulative maltose in uncured sweet potatoes of the Julian variety after equal storage times.

Storage temperature	Cumulative maltose (μmoles maltose/g puree)[a]				
	Storage time (days)				
	0	14	28	42	56
4°C (39.2°F)	63.25	94.92	107.43	65.99	58.24
-13°C (8.6°F)	63.25	68.79	66.37	65.26	66.55
Mean difference[b]	0.0	26.13**	41.06**	0.73	8.31**
4°C (39.2°F)	63.25	94.92	107.43	65.99	58.24
-18°C (-0.4°F)	63.25	67.30	64.89	63.43	65.80
Mean difference[b]	0.0	27.62**	42.54**	2.56*	7.56**
4°C (39.2°F)	63.25	94.92	107.43	65.99	58.24
-23°C (-9.4°F)	63.25	67.19	65.08	64.89	64.34
Mean difference[b]	0.0	27.73**	42.35**	1.10	6.10**

Table continued

Table 22 (Continued)

Storage temperature	Cumulative maltose (μmoles maltose/g puree)[a]				
	Storage time (days)				
	0	14	28	42	56
-13°C (8.6°F)	63.25	68.79	66.37	65.26	66.55
-18°C (-0.4°F)	63.25	67.30	64.89	63.43	65.80
Mean difference[b]	0.0	1.49	1.48	1.83	0.75
-13°C (8.6°F)	63.25	68.79	66.37	65.26	66.55
-23°C (-9.4°F)	63.25	67.19	65.08	64.89	64.34
Mean difference[b]	0.0	1.60	1.29	0.37	2.21*
-18°C (-0.4°F)	63.25	67.30	64.89	63.43	65.80
-23°C (-9.4°F)	63.25	67.19	65.08	64.89	64.34
Mean difference[b]	0.0	0.11	0.19	1.46	1.46

[a] Data are average of three replicates.

[b] Tabulated L.S.D. values of storage temperatures x storage time required for significance at 5% and 1% levels were 1.9460 and 2.6037, respectively.

Asterisks indicate that means are statistically different at 5% (*) or 1% (**) level.

Table 23. Effect of storage time at different temperatures on activity of α-amylase in cured sweet potatoes of the Centennial variety.

Storage time (days)	Units of activity (μmoles maltose/min/g puree at 25°C)[a]							
	Storage temperature							
	4°C (39.2°F)		-13°C (8.6°F)		-18°C (-0.4°F)		-23°C (-9.4°F)	
	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)
0	550.27 a		550.27 a		550.27 b		550.27 b	
14	561.04 a	+2.0	555.84 a	+1.0	608.71 a	+10.6	593.38 a	+7.8
28	559.30 a	+1.6	578.50 a	+5.1	575.02 d	+4.5	594.32 a	+8.0
42	40.11 b	-92.7	574.94 a	+4.5	597.29 ad	+8.5	616.38 a	+12.0
56	15.77 c	-97.1	566.91 a	+3.0	545.14 b	+0.9	527.58 b	-4.1

[a] Data are average of three replicates.

[b] Means within columns followed by a common letter are not significantly different at 5% level as determined by Duncan's Multiple Range Test.

[c] Data are based on unit of activity and are expressed as percent change as compared to the zero storage time samples.

Table 24. Effect of storage time at different temperatures on activity of β-amylase in cured sweet potatoes of the Centennial variety.

Storage time (days)	Units of activity (μmoles maltose/min/g puree at 25°C)[a]							
	Storage temperature							
	4°C (39.2°F)		-13°C (8.6°F)		-18°C (-0.4°F)		-23°C (-9.4°F)	
	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)
0	569.81 a		569.81 a		569.81 a		569.81 a	
14	534.14 d	-4.7	570.68 a	+0.2	573.47 a	+0.6	569.18 a	-0.1
28	555.49 ad	-2.5	576.37 a	+1.2	576.20 a	+1.1	581.88 a	+2.1
42	28.25 b	-95.0	576.13 a	+1.1	555.43 a	-2.5	575.48 a	+1.0
56	21.74 b	-96.2	567.69 a	-0.4	553.58 a	-2.9	525.68 b	-7.8

[a] Data are average of three replicates.

[b] Means within columns followed by a common letter are not significantly different at 5% level as determined by Duncan's Multiple Range Test.

[c] Data are based on unit of activity and are expressed as percent change as compared to the zero storage time samples.

Table 25. Comparative effect of storage temperature on activity of α-amylase in cured sweet potatoes of the Centennial variety after equal storage times.

Storage temperature	Units of activity (μmoles maltose/min/g puree at 25°C)[a]				
	Storage time (days)				
	0	14	28	42	56
4°C (39.2°F)	550.27	561.04	559.30	40.11	15.77
-13°C (8.6°F)	550.27	555.87	578.50	574.94	566.91
Mean difference[b]	0.0	5.20	19.20	534.83**	551.14**
4°C (39.2°F)	550.27	561.04	559.30	40.11	15.77
-18°C (-0.4°F)	550.27	608.71	575.02	597.29	545.14
Mean difference[b]	0.0	47.67**	15.72	557.18**	529.37**
4°C (39.2°F)	550.27	561.04	559.30	40.11	15.77
-23°C (-9.4°F)	550.27	593.38	594.32	616.38	527.58
Mean difference[b]	0.0	32.34**	35.02**	576.27**	511.81**

Table continued

Table 25 (Continued)

Storage temperature	Units of activity (μmoles maltose/min/g puree at 25°C)[a]				
	Storage time (days)				
	0	14	28	42	56
-13°C (8.6°F)	550.27	555.84	578.50	574.94	566.91
-18°C (-0.4°F)	550.27	608.71	575.02	597.29	545.14
Mean difference[b]	0.0	52.87**	3.48	22.35	21.77
-13°C (8.6°F)	550.27	555.84	578.50	574.94	566.91
-23°C (-9.4°F)	550.27	593.38	594.32	616.38	527.58
Mean difference[b]	0.0	37.54**	15.82	41.44**	39.33**
-18°C (-0.4°F)	550.27	608.71	575.02	597.29	545.14
-23°C (-9.4°F)	550.27	593.38	594.32	616.38	527.58
Mean difference[b]	0.0	15.33	19.30	19.09	17.56

[a] Data are average of three replicates.

[b] Tabulated L.S.D. values of storage temperatures x storage time required for significance at 5% and 1% levels were 24.0008 and 32.1119, respectively.

Asterisks indicate that means are statistically different at 5% (*) or 1% (**) level.

Table 26. Comparative effect of storage temperature on activity of β-amylase in cured sweet potatoes of the Centennial variety after equal storage times.

Storage temperature	Units of activity (μmoles maltose/min/g puree at 25°C)[a]				
	Storage time (days)				
	0	14	28	42	56
4°C (39.2°F)	569.81	543.14	555.49	28.25	21.74
-13°C (8.6°F)	569.81	570.68	576.37	576.13	567.69
Mean difference[b]	0.0	27.54*	20.88	547.88**	545.95**
4°C (39.2°F)	569.81	543.14	555.49	28.25	21.74
-18°C (-0.4°F)	569.81	573.47	576.20	555.43	553.58
Mean difference[b]	0.0	30.33**	20.71	527.18**	531.84**
4°C (39.2°F)	569.81	543.14	555.49	28.25	21.74
-23°C (-9.4°F)	569.81	569.18	581.88	575.48	525.68
Mean difference[b]	0.0	26.04*	26.39*	547.23**	503.94**

Table continued

Table 26 (Continued)

Storage temperature	Units of activity (μmoles maltose/min/g puree at 25°C)[a]				
	Storage time (days)				
	0	14	28	42	56
-13°C (8.6°F)	569.81	570.68	576.37	576.13	567.69
-18°C (-0.4°F)	569.81	573.47	576.20	555.43	553.58
Mean difference[b]	0.0	2.79	0.17	20.70	14.11
-13°C (8.6°F)	569.81	570.68	576.37	576.13	567.69
-23°C (-9.4°F)	569.81	569.18	581.88	575.48	525.68
Mean difference[b]	0.0	1.50	5.51	0.65	42.01**
-18°C (-0.4°F)	569.81	573.47	576.20	555.43	553.58
-23°C (-9.4°F)	569.81	569.18	581.88	575.48	525.68
Mean difference[b]	0.0	4.29	5.68	20.05	27.90*

[a] Data are average of three replicates.

[b] Tabulated L.S.D. values of storage temperatures x storage time required for significance at 5% and 1% levels were 21.1449 and 28.2909, respectively.

Asterisks indicate that means are statistically different at 5% (*) or 1% (**) level.

Table 27. Effect of storage time at different temperatures on activity of α-amylase in uncured sweet potatoes of the Centennial variety.

Storage time (days)	Units of activity (μmoles maltose/min/g puree at 25°C)[a]							
	Storage temperature							
	4°C (39.2°F)		-13°C (8.6°F)		-18°C (-0.4°F)		-23°C (-9.4°F)	
	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)
0	411.66 a		411.66 a		411.66 a		411.66 a	
14	409.04 a	-0.6	375.99 b	-8.7	362.97 b	-11.8	381.61 b	-7.3
28	167.76 d	-59.3	390.22 ab	-5.2	395.67 a	-3.9	395.96 ab	-3.8
42	22.74 b	-94.5	410.81 a	-0.3	405.01 a	-1.6	389.15 ab	-5.5
56	9.57 b	-97.7	408.27 a	-0.8	408.26 a	-0.8	406.88 ab	-1.2

[a] Data are average of three replicates.

[b] Means within columns followed by a common letter are not significantly different at 5% level as determined by Duncan's Multiple Range Test.

[c] Data are based on unit of activity and are expressed as percent change as compared to the zero storage time samples.

Table 28. Effect of storage time at different temperatures on activity of β-amylase in uncured sweet potatoes of the Centennial variety.

Storage time (days)	Units of activity (μmoles maltose/min/g puree at 25°C)[a]							
	Storage temperature							
	4°C (39.2°F)		-13°C (8.6°F)		-18°C (-0.4°F)		-23°C (-9.4°F)	
	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)
0	399.13 a		399.13 a		399.13 a		399.13 a	
14	359.41 b	-9.9	365.89 b	-8.3	339.08 b	-15.1	352.37 bc	-11.7
28	146.29 c	-63.4	380.58 ab	-4.7	378.48 ac	-5.2	379.95 ac	-4.8
42	11.72 d	-97.1	384.26 ab	-3.7	376.55 c	-5.7	370.07 c	-7.3
56	9.55 d	-97.6	385.10 ab	-3.5	381.30 ac	-4.5	382.53 ac	-4.2

[a] Data are average of three replicates.

[b] Means within columns followed by a common letter are not significantly different at 5% level as determined by Duncan's Multiple Range Test.

[c] Data are based on unit of activity and are expressed as percent change as compared to the zero storage time samples.

Table 29. Comparative effect of storage temperature on activity of α-amylase in uncured sweet potatoes of the Centennial variety after equal storage times.

Storage temperature	Units of activity (μmoles maltose/min/g puree at 25°C)[a]				
	Storage time (days)				
	0	14	28	42	56
4°C (39.2°F)	411.66	409.04	167.76	22.74	9.57
-13°C (8.6°F)	411.66	375.99	390.22	410.81	408.27
Mean difference[b]	0.0	33.05*	222.46**	388.07**	398.70**
4°C (39.2°F)	411.66	409.04	167.76	22.74	9.57
-18°C (-0.4°F)	411.66	362.97	395.67	405.01	408.26
Mean difference[b]	0.0	46.07**	227.91**	382.27**	398.69**
4°C (39.2°F)	411.66	409.04	167.76	22.74	9.57
-23°C (-9.4°F)	411.66	381.61	395.96	389.15	406.88
Mean difference[b]	0.0	27.43*	228.20**	366.41**	397.31**

Table continued

Table 29 (Continued)

Storage temperature	Units of activity (μmoles maltose/min/g puree at 25°C)[a]				
	Storage time (days)				
	0	14	28	42	56
-13°C (8.6°F)	411.66	375.99	390.22	410.81	408.27
-18°C (-0.4°F)	411.66	362.97	395.67	405.01	408.26
Mean difference[b]	0.0	13.02	5.45	5.80	0.01
-13°C (8.6°F)	411.66	375.99	390.22	410.81	408.27
-23°C (-9.4°F)	411.66	381.61	395.96	389.15	406.88
Mean difference[b]	0.0	5.62	5.74	21.66	1.39
-18°C (-0.4°F)	411.66	362.97	395.67	405.01	408.26
-23°C (-9.4°F)	411.66	381.61	395.96	389.15	406.88
Mean difference[b]	0.0	18.64	0.29	15.86	1.38

[a] Data are average of three replicates.

[b] Tabulated L.S.D. values of storage temperatures x storage time required for significance at 5% and 1% levels were 25.3043 and 33.8560, respectively.

Asterisks indicate that means are statistically different at 5% (*) or 1% (**) level.

Table 30. Comparative effect of storage temperature on activity of β-amylase in uncred sweet potatoes of the Centennial variety after equal storage times.

Storage temperature	Units of activity (μmoles maltose/min/g puree at 25°C)[a]				
	Storage time (days)				
	0	14	28	42	56
4°C (39.2°F)	399.13	359.41	146.29	11.72	9.55
-13°C (8.6°F)	399.13	365.89	380.58	384.26	385.10
Mean difference[b]	0.0	6.48	234.29**	372.54**	375.55**
4°C (39.2°F)	399.13	359.41	146.29	11.72	9.55
-18°C (-0.4°F)	399.13	339.08	378.48	376.55	381.30
Mean difference[b]	0.0	20.33	232.19**	364.83**	371.75**
4°C (39.2°F)	399.13	359.41	146.29	11.72	9.55
-23°C (-9.4°F)	399.13	352.37	379.95	370.07	382.53
Mean difference[b]	0.0	7.04	233.66**	358.35**	372.98**

Table continued

Table 30 (Continued)

Storage temperature	Units of activity (μmoles maltose/min/g puree at 25°C)[a]				
	Storage time (days)				
	0	14	28	42	56
-13°C (8.6°F)	399.13	365.89	380.58	384.26	385.10
-18°C (-0.4°F)	399.13	339.08	378.48	376.55	381.30
Mean difference[b]	0.0	26.81**	2.10	7.71	3.80
-13°C (8.6°F)	399.13	365.89	380.58	384.26	385.10
-23°C (-9.4°F)	399.13	352.37	379.95	370.07	382.53
Mean difference[b]	0.0	13.52	0.63	14.19	2.57
-18°C (-0.4°F)	399.13	339.08	378.48	376.55	381.30
-23°C (-9.4°F)	399.13	352.37	379.95	370.07	382.53
Mean difference[b]	0.0	13.29	1.47	6.48	1.23

[a] Data are average of three replicates.

[b] Tabulated L.S.D. values of storage temperatures x storage time required for significance at 5% and 1% levels were 20.3604 and 27.2412, respectively.

Asterisks indicate that means are statistically different at 5% (*) or 1% (**) level.

Table 31. Effect of storage time at different temperatures on activity of α-amylase in uncured sweet potatoes of the Julian variety.

Storage time (days)	Units of activity (μ moles maltose/min/g puree at 25°C)[a]							
	Storage temperature							
	4°C (39.2°F)		-13°C (8.6°F)		-18°C (-0.4°F)		-23°C (-9.4°F)	
	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)
0	541.80 a		541.80 b		541.80 b		541.80 b	
14	539.19 a	-0.5	571.23 ac	+5.4	560.31 ab	+3.4	554.91 bc	+2.4
28	75.77 d	-86.0	556.91 bc	+2.8	542.98 b	+0.2	552.94 bc	+2.1
42	36.08 c	-93.3	545.95 b	+0.8	556.71 ab	+2.8	569.09 ac	+5.0
56	10.35 b	-98.1	589.08 a	+8.7	576.41 a	+6.4	580.91 a	+7.2

[a] Data are average of three replicates.

[b] Means within columns followed by a common letter are not significantly different at 5% level as determined by Duncan's Multiple Range Test.

[c] Data are based on unit of activity and are expressed as percent change as compared to the zero storage time samples.

Table 32. Effect of storage time at different temperatures on activity of β-amylase in uncured sweet potatoes of the Julian variety.

Storage time (days)	Units of activity (μmoles maltose/min/g puree at 25°C)[a]							
	Storage temperature							
	4°C (39.2°F)		-13°C (8.6°F)		-18°C (-0.4°F)		-23°C (-9.4°F)	
	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)	Mean[b]	Change[c] (%)
0	527.70 a		527.70 a		527.70 a		527.70 a	
14	544.65 a	+3.2	564.77 b	+7.0	556.53 bc	+5.5	565.30 b	+7.1
28	73.64 b	-86.1	542.88 ab	+2.9	533.52 ac	+1.1	556.15 b	+5.4
42	34.50 c	-93.5	542.15 ab	+2.7	541.42 ac	+2.6	545.04 ab	+3.3
56	9.25 c	-98.3	595.39 d	+12.8	569.24 b	+7.9	559.64 b	+6.1

[a] Data are average of three replicates.

[b] Means within columns followed by a common letter are not significantly different at 5% level as determined by Duncan's Multiple Range Test.

[c] Data are based on unit of activity and are expressed as percent change as compared to the zero storage time samples.

Table 33. Comparative effect of storage temperature on activity of α-amylase in uncured sweet potatoes of the Julian variety after equal storage times.

Storage temperature	Units of activity (μmoles maltose/min/g puree at 25°C)[a]				
	Storage time (days)				
	0	14	28	42	56
4°C (39.2°F)	541.80	539.19	75.77	36.08	10.35
-13°C (8.6°F)	541.80	571.23	556.91	545.95	589.08
Mean difference[b]	0.0	32.04**	481.14**	509.87**	578.73**
4°C (39.2°F)	541.80	539.19	75.77	36.08	10.35
-18°C (-0.4°F)	541.80	560.31	542.98	556.71	576.41
Mean difference[b]	0.0	21.12	467.21**	520.63**	566.06**
4°C (39.2°F)	541.80	539.19	75.77	36.08	10.35
-23°C (-9.4°F)	541.80	554.91	552.94	569.09	580.91
Mean difference[b]	0.0	15.72	477.17**	533.01**	570.56**

Table continued

Table 33 (Continued)

Storage temperature	Units of activity (μmoles maltose/min/g puree at 25°C)[a]				
	Storage time (days)				
	0	14	28	42	56
-13°C (8.6°F)	541.80	571.23	556.91	545.95	589.08
-18°C (-0.4°F)	541.80	560.31	542.98	556.71	576.41
Mean difference[b]	0.0	10.92	13.93	10.76	12.67
-13°C (8.6°F)	541.80	571.23	556.91	545.95	589.08
-23°C (-9.4°F)	541.80	554.91	552.94	569.09	580.91
Mean difference[b]	0.0	16.32	3.97	23.14	8.17
-18°C (-0.4°F)	541.80	560.31	542.98	556.71	576.41
-23°C (-9.4°F)	541.80	554.91	552.94	569.09	580.91
Mean difference[b]	0.0	5.40	9.96	12.38	4.50

[a] Data are average of three replicates.

[b] Tabulated L.S.D. values of storage temperatures x storage time required for significance at 5% and 1% levels were 23.2395 and 31.0933, respectively.

Asterisks indicate that means are statistically different at 5% (*) or 1% (**) level.

Table 34. Comparative effect of storage temperature on activity of β-amylase in uncured sweet potatoes of the Julian variety after equal storage times.

Storage temperature	Units of activity (μmoles maltose/min/g puree at 25°C)[a]				
	Storage time (days)				
	0	14	28	42	56
4°C (39.2°F)	527.70	544.65	73.64	34.50	9.25
-13°C (8.6°F)	527.70	564.77	542.88	542.15	595.39
Mean difference[b]	0.0	20.12	469.24**	507.65**	586.14**
4°C (39.2°F)	527.70	544.65	73.64	34.50	9.25
-18°C (-0.4°F)	527.70	556.53	533.52	541.42	569.24
Mean difference[b]	0.0	11.88	459.88**	506.92**	559.99**
4°C (39.2°F)	527.70	544.65	73.64	34.50	9.25
-23°C (-9.4°F)	527.70	565.30	556.15	545.04	559.64
Mean difference[b]	0.0	20.65	482.51**	510.54**	550.39**

Table continued

Table 34 (Continued)

Storage temperature	Units of activity (μ moles maltose/min/g puree at 25°C)[a]				
	Storage time (days)				
	0	14	28	42	56
-13°C (8.6°F)	527.70	564.77	542.88	542.15	595.39
-18°C (-0.4°F)	527.70	556.53	533.52	541.42	569.24
Mean difference[b]	0.0	8.24	9.36	0.73	26.15*
-13°C (8.6°F)	527.70	564.77	542.88	542.15	595.39
-23°C (-9.4°F)	527.70	565.30	556.15	545.04	559.64
Mean difference[b]	0.0	0.53	13.27	2.89	35.75**
-18°C (-0.4°F)	527.70	556.53	533.52	541.42	569.24
-23°C (-9.4°F)	527.70	565.30	556.15	545.04	559.64
Mean difference[b]	0.0	8.77	22.63	3.62	9.60

[a] Data are average of three replicates.

[b] Tabulated L.S.D. values of storage temperatures x storage time required for significance at 5% and 1% levels were 24.9707 and 33.4095, respectively.

Asterisks indicate that means are statistically different at 5% (*) or 1% (**) level.

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ACTIVITY OF α- AND β-AMYLASE AT LOW TEMPERATURES

by

Supanit Hiranpradit

(ABSTRACT)

α- and β-Amylase activity were determined at 4°, -13°, -18°, and -23°C (39.2°, 8.6°, -0.4°, and -9.4°F) in systems with purified enzymes and in a system with sweet potato puree for different periods of time. In the systems with purified enzymes, commercially purified swine pancreatic α-amylase and sweet potato β-amylase at 0, 0.5, 1.0, and 1.5 μg/0.5 ml concentrations were used to react with 0.5 ml of a 2% potato soluble starch substrate for 112 days. In the system with sweet potato puree, samples prepared from cured and uncured roots were frozen and stored for 56 days. The cumulative enzyme activity was determined after different storage times. The stability of the enzymes as affected by low temperatures was determined in the sweet potato puree.

In the systems with purified enzymes, cumulative α- and β-amylase activity at all storage temperatures studied increased as the storage time and enzyme concentration were increased. Both enzymes were still active at -23°C.

In the system with sweet potato puree, α- and β-amylase were active at 4°C for up to 28 days, but were inhibited at -13°, -18°, and -23°C.

In the cured sweet potato puree samples of the Centennial variety, α- and β-amylase were stable for up to 28 days at 4°C. Enzyme stability in samples from uncured roots of two varieties of sweet potatoes was

not affected for up to 14 days of storage at 4°C, but decreased considerably thereafter.

α- and β-Amylase stability in all sweet potato puree samples stored for 56 days at -13°, -18°, and -23°C was not affected.