AN ABSTRACT OF THE THESIS OF

MARTA LUISA DONDERO for the degree of MASTER OF SCIENCE

in Food Science and Technology presented on June 16, 1977

Title: PREPARATION OF A POTATO HYDROLYSATE WITH BACILLUS SUBTILIS α-AMYLASE

Abstract approved: M. W. Montgomery

Bacillus subtilis α-amylase was used to hydrolyze starch from peeled and whole potatoes (Solanum tuberosum). Effect of gelatinization temperature, pH, enzyme concentration, temperature of hydrolysis and presence of calcium ions were investigated. Optimum conditions for hydrolysis were when α-amylase levels were 0.3 percent of the starch concentration in the presence of 400 ppm calcium at pH 7.0 and 80°C for 2 hr. After centrifugation to remove residual material, the supernatants were adjusted to pH 5.0 to 5.5 with 30 percent sulfuric acid and heated at 100°C for 10 min to inactivate α-amylase. Treatment with four percent activated charcoal decolorized the clear hydrolysate before the product was spray dried.

The dried product was a light yellow powder, slightly sweet, relatively bland in taste and readily soluble in water. Dried potato hydrolysate made from peeled potatoes had a dextrose equivalent of 30 and contained 85 percent

carbohydrates, 8.4 percent protein and 5.5 percent ash; while the hydrolysate from whole potatoes had a dextrose equivalent of 26 and contained 86 percent carbohydrates, 8.4 percent protein and 6.0 percent ash. The composition of the carbohydrates of the two hydrolysates were similar except the hydrolysate from peeled contained a higher concentration of glucose and lower concentration of saccharides with a degree of polymerization greater than four.

At concentrations of greater than 40 percent, the potato hydrolysate made from whole potatoes did not show as high a viscosity as commercial corn syrup solids with a dextrose equivalent of 24; the potato hydrolysate adsorbed twice as much moisture as the commercial corn syrup solids at 75 percent relative humidity at 23°C for 30 days.

Substitution of sucrose with whole potato hydrolysate in chocolate milk revealed that substitution of two parts of sucrose by two parts of potato hydrolysate could be used with a slight loss of desirability. Fifty and one hundred percent addition of whole potato hydrolysate to a commercial dehydrated vegetable soup mix showed no decrease in the desirability of the product.

This work has shown that a useful potato hydrolysate containing significant amounts of protein can be prepared from either peeled or whole potatoes.

Preparation of a Potato Hydrolysate with *Bacillus subtilis* α-amylase

by

Marta Luisa Dondero

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

June 1978

APPROVED:

Associate Professor of Food Science and Technology
in charge of major

Head of Department of Food Science and Technology

Dean of Graduate School

Date thesis is presented June 16, 1977

Typed by Deanna L. Cramer for Marta Luisa Dondero

ACKNOWLEDGEMENTS

I wish to express my most sincere gratitude and appreciation to my advisor, Dr. Morris W. Montgomery for his guidance and valuable assistance during the course of this work especially in the preparation of this manuscript. The contribution of Dr. R. E. Wrolstad is also deeply appreciated.

Special thanks are extended to Mrs. Lois McGill for her generous cooperation in conducting the taste panel evaluations. Appreciation is expressed to Mr. D. L. Law for his help with the spray-drying of the samples.

The author also wishes to thank AID (Agency for International Development) which made my studies possible at the Department of Food Science and Technology, Oregon State University.

TABLE OF CONTENTS

Table of Contents -- continued

LIST OF FIGURES

LIST OF TABLES

To my husband, Manuel, for his patience, encouragement and understanding during my graduate study.

PREPARATION OF A POTATO HYDROLYSATE WITH *BACILLUS SUBTILIS* α-AMYLASE

INTRODUCTION

White potatoes (*Solanum tuberosum*) is one of the major crops in many countries of the world. Potatoes are either used for food as such or are transformed into processed products. Potato processing results in the production of substantial quantities of waste or by-products which are a considerable economic loss to the producer and processors of potatoes and is a potential pollutant to the environment.

Starch, the main component of potatoes, is the basic source of chemical energy and an important nutrient in the human diet. Recently, several methods have been developed for the conversion of corn starch into a wide variety of compositionally different corn sweeteners. The method first used for this conversion was acid hydrolysis of starch to dextrins, oligossacharides, maltose and glucose. Later, development of enzymic methods enabled the industry to produce syrup having specific composition depending on the mode of action of a particular enzyme. Enzymes also function under milder conditions, thus avoiding many of the undesirable side reactions. Used in low concentrations, removal of enzymes from the reaction system is generally not necessary. Recent advances with immobilized enzymes

further enhances the inherent advantages of enzyme catalysis systems.

The author is from Chile where sometimes a surplus of agricultural products such as potatoes exist. Therefore, this investigation is concerned with the development of an economical enzymic method for the preparation of a potato hydrolysate with a high nutritive and caloric content which could be used in a variety of food products.

REVIEW OF LITERATURE

Chemical Composition of the Potato Tuber

Literature on the chemistry of the potato is extensive, however, a clear picture of potato composition is difficult to obtain because of variations caused by area of growth, variety, maturity at harvest, storage conditions and other factors (Schwimmer and Burr, 1959). The following may be considered illustrative of Western Russet potato composition expressed in percentages on a dry basis (Treadway, 1967): starch 75 percent, nitrogenous compounds 10 percent, inorganic compounds 4.5 percent, sugars 2.5 percent, organic acids 2.5 percent, crude fiber 2.0 percent, pectic substances 1.0 percent, fatty substances 0.3 percent, and minor components 2.0 percent.

Characteristics of Potato Starch

Starch, comprising 65 to 80 percent of the dry weight of the potato tuber, is the food reserve carbohydrate polymer of plants. In the raw tuber, starch was shown to be present as microscopic granules, oval in shape, which were formed eccentrically around the hilum (Leach and Schoch, 1963). Greenwood and Thompson (1962) reported the granule size to be about 40 μm on the average. The two main components of starch, amylose and amylopectin were present in

a ratio of 1:3 (McCready and Hassid, 1947). Amylose is a flexible, linear chain molecule with an average degree of polymerization of 1,000 to 5,000 depending upon the source of tuber (Bottle and Gilbert, 1954). The glucose residues are joined by α-1,4 glycosidic linkages. Amylopectin is a branched chain glucose polymer in which the α-1,4 linked residues are branched by α-1,6 linkages every 20 glucosyl residues on the average (Schwimmer and Bevenue, 1956). Osman (1967) reported that the gelatinization temperature of potato starch was between 57 and 66°C.

Hydrolysis of Starch

A number of methods have been used to hydrolyze starch. All the methods involve the breakdown of starch molecules into simple sugars. Kirchoff (1811) is generally credited with being responsible for making a product known as starch sugar by cooking potato starch with acid.

The starch conversion process to produce a syrup sweetener was not used commercially in the United States until 1842. Today, corn is the main source of starch for the production of the various corn syrups and sugars.

Starch Conversion Products

Starch conversion products are classified by their dextrose equivalent or DE (Newton, 1970). DE is a measure of the reducing sugar content calculated as anhydrous

dextrose and expressed as a percentage of total dry substance. Maltodextrins are purified concentrated solutions (or dried products) of nutritive saccharides having a DE of less than 20; syrups have a DE range of 20 or higher; syrup solids are products obtained by the removal of most of the water from syrups with the production of powdered or granular material; dextrose is the term applied to the product obtained by the complete hydrolysis of starch to obtain D-glucose (Newton, 1970).

Methods of Hydrolysis

Hydrolysis of starch is accomplished by three different methods: acid conversion, acid-enzyme conversion and enzyme-enzyme conversion (Junk and Pancoast, 1973). Selection of the type of conversion method is dependent upon the type of syrup to be produced. The following discussion is taken from a review by Junk and Pancoast (1973).

Acid Conversion

When starch is hydrolyzed with acid as the catalyst a cleavage of the -C-O-C- linkages occur with the production of glucose and many of its polymers. If the process continues, the various polymers are hydrolyzed until essentially the only sugar which remains is glucose.

A starch slurry of about 35 to 40 percent dry substances is acidified with hydrochloric acid to pH 2.0 and

heated to 140-160°C. The process is terminated by adjusting the pH to 4.0 to 5.5 with sodium carbonate. The liquor is clarified by filtration and/or centrifugation to remove suspended materials and is concentrated by evaporation to an intermediate density of about 60 percent dried solids. The syrup is treated with powdered and/or granular carbon to effect further clarification and decolorization. This step may be followed by ion-exchange refining to remove soluble minerals, residual proteins, color and flavor and to lower the ash content. Final concentration is effected in large vacuum pans or continuous evaporators.

Enzymic Conversion

Use of enzymes to convert starch to sugars has several advantages over the acid conversion. Enzymes are more specific and function under milder conditions, thus avoiding many of the undesirable side reactions. Used in low concentrations, removal of enzymes from the reaction system is generally not necessary (McAllister *et al.*, 1975). Recent developments of immobilized enzymes which are adsorbed or chemically bounded to materials that are insoluble in the reaction medium further enhances the inherent advantages of enzyme catalysis systems (Zaborsky, 1973).

Starch Splitting Enzymes

Enzymes responsible for the breakdown of starch are widely distributed in nature. Among these are the amylases which act on starch, glycogen and derived polysaccharides to hydrolyse the α-1,4 linkages. Amylases may be divided into three groups. α-amylases (endoamylases) which hydrolyze at random the non-terminal α-1,4 glycosidic linkages. They cannot hydrolyze the α-1,6 branch points. β-amylases which hydrolyze units from the non-reducing end of the substrate (exoamylases) with the production of maltose as the only sugar; glucoamylases hydrolyze the α-1,4 and α-1,6 linkages of starch with the production of glucose.

α-amylases

α-amylases have been prepared from a variety of plants and animal sources. Schwimmer and Ball (1949) crystallized α-amylase from barley malt, Fischer and Stein (1954) from Aspergillus oryzae and Fischer and Stein (1961) from human saliva, Bacillus subtilis and porcine pancreas.

The mode of action, properties and degradation products differ somewhat depending on the source of the enzyme (Robyt and Whelan, 1968). α-amylase hydrolysates contain glucose, maltose and dextrins (Thoma et al., 1971).

The function of α-amylases is two fold (de Becze, 1965): 1) to liquefy the starch by cleaving at random the

large starch molecules and the long chains of dextrins as in the production of starchy syrup or other food ingredients; and 2) to accelerate the saccharification of starch by other enzymes as β-amylase and glucoamylase. These processes are more economical and result in better products than either acid conversion or acid-enzyme conversion.

Commercial α-amylase preparations of bacterial origin are considerably more heat stable. These amylases are preferred when amyloltic action is desired during the heating of raw starch beyond the gelatinization point, since with the beginning of gelatinization the granules become more susceptible to enzyme action (Leach and Schoch, 1961).

Bacillus subtilis α-amylase

α-amylase from Bacillus subtilis is particularly stable toward heat (Manning and Campbell, 1961) with a temperature optimum ranging between 60-70°C and an effective temperature range up to 90°C (Allen and Spradlin, 1974). The addition of calcium ions is generally recommended to achieve maximum protection of the enzyme against heat denaturation (Kulp, 1975). The optimum pH range is from 5.0 to 7.0 (Menzi et al., 1957). During the early stages of starch hydrolysis the action pattern of Bacillus subtilis α-amylase yields mainly dextrins with molecular weights greater than maltohexaose. Prolonged

hydrolysis with Bacillus subtilis α-amylase yields significant quantities of maltose, maltotriose and maltohexaose with smaller quantities of glucose, maltotetraose, maltopentaose and maltoheptaose (Robyt and French, 1963). The detailed mechanism has not been fully elucidated (Kulp, 1975).

Measurement of Amylase Activity

There are two assay methods for amylase activity which are based on physical changes in a starch molecule undergoing hydrolysis: 1) the reduction in iodine-staining ability of the substrate (Sandstedt et al., 1948); and 2) the decrease in the viscosity of the substrate (Marnett et al., 1948). There are also many methods for determining the number of reducing groups. A method using alkaline 3,5 dinitrosalicylate has been widely employed (Fischer and Stein, 1961). However, Robyt and Whelan (1965) found that alkaline 3,5 dinitrosalicylate did not yield equal reducing values for equimolar quantities of maltodextrins (Nelson, 1944).

Different α-amylase starch hydrolysates could have identical reducing powers but differ completely in carbohydrate composition and physical properties (Allen and Spradlin, 1974).

Thin-Layer Chromatographic Method for Identification of Oligosaccharides in Starch Hydrolysates

Identification of breakdown products resulting from the enzymic hydrolysis of starch requires a positive method such as thin-layer or paper chromatography. Paper chromatography is often a time-consuming method that requires up to 36 hr for development of the chromatograms (Whistler, 1955; Ough, 1964).

In recent years, thin-layer chromatography (TLC) has been the preferred method because it is rapid and sensitive. TLC with direct densitometry has proven to be a method equal in accuracy and superior in speed per sample to other chromatographic methods (Mansfield, 1973).

Different solvent systems have been used for determining the saccharide distribution in starch hydrolysates. Huber et al., (1966) used ethyl acetate-methanol-water (52:36:13 v/v) for the separation of monosaccharides to nonasaccharides. Later these authors (Huber et al., 1968) separated saccharides up to a degree of polymerization of thirty-five by using propanol-nitromethane-water (5:2:3 v/v) with four developments. Separation of glucose polymers with a degree of polymerization from one to nine were obtained with chloroform-acetic acid-water (10:79:11 v/v) and four developments (de Stefani and Ponte, 1968). Recently, Hansen (1975) proposed a method for the separation of oligosaccharides in starch hydrolysates with propan-2-ol-

acetone-1M lactic acid (4:4:2 v/v). To visualize the sugars a reagent consisting of aniline, diphenylamine and phosphoric acid was sprayed on the plates. The individual sugars appeared as blue spots on a white background (Hansen, 1975).

Application of Enzymes to Syrup Production

The use of enzymes has expanded the range of compositions of starch hydrolysates available. Malt diastase (α-amylase) in the preparation of malt syrup was the first application of enzymes to syrup production. Dale and Langlois (1940) proposed the use of fungal amylases. This led to products containing very high levels of glucose and maltose, with a wide range of glucose to maltose ratios (Bodnar et al., 1972). These syrups have practically no flavors other than that of the sugars and find large markets in the production of canned fruits, beverages, condiments and bakery goods. In contrast, syrups made by acid hydrolysis of starch could not exceed about 55 percent dextrose plus maltose without crystallization or development of unacceptable flavors; this resulted in lower product yields and a more costly purification (Barfoed, 1976).

Starch hydrolysate products of relatively low degree of hydrolysis have been made by treatment of starch with α-amylase (Morehouse et al., 1971). Syrups of this type are often converted to dry powders and find extensive use as

nutritious and soluble bulking agents (Murray and Luft, 1973; Murray, 1969; Murray and Ziemba, 1972).

Dextrose is produced via the acid-enzyme or enzyme-enzyme conversion process. Pre-hydrolysis with acid or α-amylase to about 15 to 20 DE followed by saccharification to about 95 percent dextrose with glucoamylase are the steps used in the production of dextrose (Eichenberg, 1970).

A more recent application of enzyme technology has been the development of a new series of syrups with enhanced sweetening power. This is accomplished by treating glucose rich syrups with the enzyme, glucose isomerase, which isomerizes glucose to fructose (Mermelstein, 1975; Lloyd et al., 1972; Takasaki and Tanabe, 1971).

Functional Properties of Syrups

Hoover (1963) has illustrated the functional properties of corn syrup as related to the type of conversion and has prepared a list of functional uses of corn syrup in wide variety of foods.

Some of the most important properties are: solubility, humectancy and hygroscopicity, texture, bodying agent, cohesiveness, sweetness control, lowering of freezing point, osmotic pressure and preservative affects, control of dextrose and sucrose crystallization, foam stabilization, sheen production, etc. These properties change as the DE is raised or lowered. For a thorough explanation of the

functional properties of corn syrups the reader is referred to Junk and Pancoast (1973).

Enzymic Hydrolysis of Potato Starch

Raw potato starch is difficult to hydrolyze by α-amylase and no evidence of raw potato starch granule erosion or decrease of birefringence after 56 percent solubilization with selective granule digestion was shown by Leach and Schoch (1961). The surface of potato starch granules showed a much greater resistance to enzymic attack than that of wheat and corn. After the enzyme had penetrated into the granule the attack on the internal region was very rapid (Gallant et al., 1972). The hydrolysis occurred by a random attack, some of the granules were completely digested by amylases while others remained completely intact (Kulp, 1975).

Enzymic hydrolysis of potato starch by α-amylase at 0°C to -13°C was studied by Veselov et al. (1970). At these temperatures intensive hydrolysis occurred. Non-gelatinized potato starch was treated with bacterial α-amylase at 30°C at pH 4.7 and 6.5. The acid medium accelerated disruption of the starch granule structure and increased susceptibility to α-amylase (Dukhanina et al., 1970).

Degradation of potato starch by α-amylase in the presence of α-1,6 glycosidase yielded maltose as expected,

while glucose resulted from the action of immobilized glucoamylase on potato starch (Marshall and Whelan, 1971). Recent work in Czechoslovakia (Zelenka et al., 1972) also showed that α- and β-amylase could be used in the preparation of maltose syrup from potato starch. Products containing 97 to 99 percent glucose were obtained by the action of glucoamylase on potato starch. Addition of calcium chloride as activators of α-amylase did not improve the degree of potato starch hydrolysis (Pietrzak, 1973).

More papers have been published recently in Hungary, Russia, Denmark and Poland about the enzymic production of starch hydrolysates from potato starch (Ludving et al., 1970; Knudsen, 1969; Sroczynsky et al., 1972; Vecher et al., 1973).

Production of starch syrup from potato without prior isolation of the starch was studied by Heisler et al. (1951). Amylases produced more reducing sugars from isolated starch than from starch within intact potato tissue, under the same conditions. Using one percent fungal amylase, a sweet, amber colored syrup of pleasing taste was obtained from whole potatoes after carbon treatment and ion-exchange purification (Heisler et al., 1951). Later, Kask et al. (1969) obtained a high quality syrup by enzymic hydrolysis of potato pulp which had been previously washed with dilute alkali for extraction of the nitrogenous compounds.

MATERIALS AND METHODS

Raw Material

White potatoes (Solanum tuberosum) used in this study were U.S. No. 2 Oregon Russet potatoes obtained from a commercial supermarket during the summer of 1976. The potatoes were stored at 5°C until used in this study.

Amylase

Tenase, a food grade bacterial α-amylase (α-1,4-glucan-4-glucanohydrolase, E.C. 3.2.1.1) of Bacillus subtilis, was obtained from Marshall Division, Miles Laboratories. Tenase is an endo-amylase capable of randomly hydrolyzing the predominating α-1,4-glucosidic linkages of starch. Tenase was supplied as a brown non-viscous liquid (density of 1.15 to 1.25 g/ml).

Proximate Analysis

Analysis for moisture (A.O.A.C., 1975a), crude protein (A.O.A.C., 1975b), ether extract (A.O.A.C., 1975c), ash (A.O.A.C., 1975d), crude fiber (A.O.A.C., 1975e) and starch (A.O.A.C., 1965e) were carried out according to the "Official Methods of Analysis of the Association of Official Analytical Chemists."

Mineral Analysis

Determination of the mineral content of potato hydrolysates was performed in a Jarrel-Ash 82-500 Atomic Absorption/Flame Spectrometer. The determination was carried out by the Department of Agricultural Chemistry of Oregon State University.

Amino Acid Analysis

Amino acid content of the whole potatoes and of the spray dried product was determined according to the method of Spackman, Moore and Stein (1958) in an Automatic Amino Acid Analyzer, Beckman Model 120B, modified to use a single column system. The determination was carried out by the Biochemistry Department of Oregon State University.

Reducing Sugar Determination

The Nelson-Somogyi method (Nelson, 1944; Somogyi, 1945) based on the reduction of cuperic ion to cuperous ion by the presence of reducing sugars was used in this study. The cuperous ion concentration was determined spectrophotometrically with an arsenomolybdate solution. A standard curve was prepared (Figure 1) by plotting absorbance (at 520 nm) versus reducing sugar content (mg/ml) using glucose as standard (Baker's Analyzed Chemical Reagent).

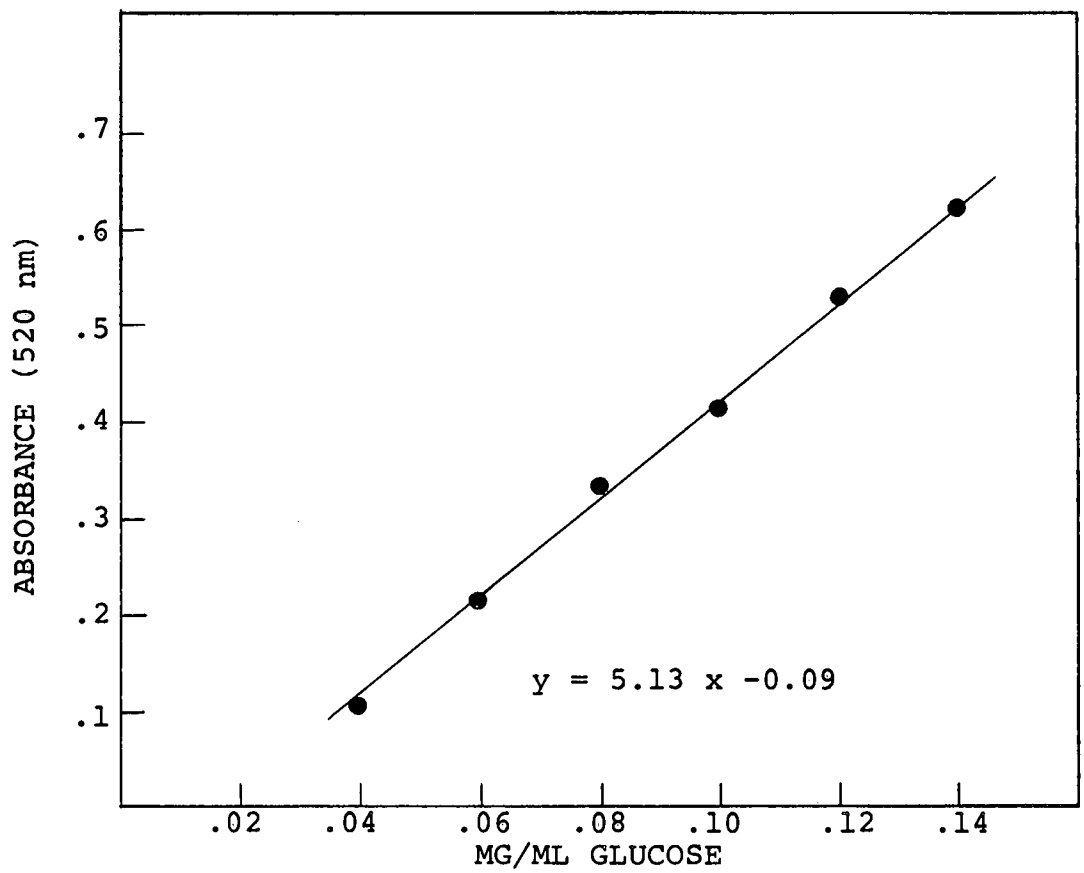

Figure 1. Nelson-Somogyi standard curve for glucose.

One ml aliquots of samples appropriately diluted to contain reducing sugars equivalent to 0.04 to 0.14 mg/ml of glucose were pipetted into 150 x 15 mm screw capped test tubes and mixed with 2 ml of Somogyi reagent (Somogyi, 1945). Following a reaction time of exactly 30 min in a boiling water bath, the tubes were placed in running cold tap water for 5 min. Two ml of Nelson reagent (Nelson, 1944) were pipetted into the solution, mixed and allowed to stand for 10 min. After dilution to 10 ml with distilled water, absorbance at 520 nm was measured in a Beckman Model DB Spectrophotometer against a reagent blank. Each sample was assayed in triplicate.

Dextrose equivalent (DE) was calculated using the following formula:

$$DE = \frac{(RS)}{(W)\,(\%\ DS)} \times 100 \times 100\%$$

where: RS = mg/ml of reducing sugar

W = weight (mg) of one ml aliquot of sample preparation

%DS = percent total dissolved solids

100 = converts % DS to DS

100% = convert DE to a percentage

The percent total dissolved solids was determined in a Bausch and Lomb ABBE-3L refractometer.

Hydrolysis of Potatoes

Optimum conditions for the hydrolysis of potato starch were determined with peeled potatoes. Once the optimum conditions of hydrolysis were determined, whole potatoes were used as raw material.

Preparation of Potatoes for Hydrolysis

Potatoes were cut quickly into 2 cm pieces, ground in an Osterizer Juicer model 361 and placed in Waring Blendor for 4 min. Four hundred g of ground potatoes were placed in a 6.5 x 3.5 inch screw capped glass jar equipped with a stirrer. The pH of the slurry was adjusted to 7.0 by addition of 50 percent sodium hydroxide. Four hundred ppm of calcium ions as calcium chloride and 0.1 percent of Tenase (based on the weight of starch, assuming 15 percent starch in the potato tissue) were added. The mixture was held in a constant temperature oil bath and thoroughly mixed with a mechanical stirrer. Temperature, pH and concentration of enzyme were varied in different experiments to determine the optimum conditions of hydrolysis.

Samples were removed at different hydrolysis periods and heat treated for 15 min at 100°C to inactivate the enzyme. After centrifugation at 1500 x G for 15 min at room temperature, reducing sugars in the supernatant were determined by the Nelson-Somogyi method.

For the preparation of large amounts of hydrolysate, inactivation of the α-amylase was accomplished by adjusting the sample to pH 5.0 to 5.5 with 30 percent sulfuric acid before heat treatment.

Concentration, Decoloration and Drying of Hydrolysate

The supernatant was concentrated under 50 mm pressure to about 30 percent solids in a Büchi Rotavapor. The concentrate was treated with activated charcoal (Darco S-51, I.C.I. United States, Inc.) at 80°C for 30 and 60 min with constant stirring. The mixture was filtered through Whatman No. 1 filter paper with a 5 mm layer of diatomaceous earth. Absorbance of the filtrate was measured at 440 nm in a Beckman DB recording spectrophotometer.

The filtrate was spray-dried in a Nichols/Niro centrifugal atomizer at the Oregon State University Seafoods Laboratory, Astoria. A cream white and light powder was obtained. This powder was stored in plastic bags at room temperature in a desiccator.

Viscosity and Hygroscopicity Determinations

The viscosity of the potato hydrolysate was determined using an Ostwald capillary viscometer at 23°C. Ten ml solutions containing different amounts of potato hydrolysate were used.

Viscosity was calculated in centipoise by the following equation:

$$\frac{\eta_1}{\eta_2} = \frac{s_1 t_1}{s_2 t_2}$$

where: η_1, s_1, t_1 represent viscosity, density and time respectively of efflux of the unknown

η_2, s_2, t_2 represent viscosity, density and time respectively of efflux of water used as reference.

The hygroscopicity of the potato hydrolysate was determined measuring the rate of moisture adsorption by exposing samples to 75 percent relative humidity at 23°C for one month (Morehouse et al., 1971).

Thin Layer Chromatography

TLC of saccharides from the potato hydrolysate was accomplished on pre-coated 20 x 20 cm x 0.25 mm thick silica gel 60 plates (E. Merck). Plates were conditioned in an oven at 110°C for 1 hr before 1 µl samples of 1 percent potato hydrolysate solutions were spotted on the plates together with 1 µl of 0.1 percent standard solutions. The plates were developed in propan-2-ol-acetone-1 M lactic acid (4:4:2 v/v/v) as the mobile phase (Hansen, 1976). After 3 hr, the plates were removed and dried in a stream of warm (60°C) air. To locate the sugar spots, the plates were sprayed with a reagent consisting of

aniline (1 ml), diphenyl amine (1 g), 85 percent phosphoric acid (10 ml) and ethanol (100 ml), before placing in an oven at 110°C for 1 hr. The individual sugars appeared as blue spots on a white background.

For quantification, the plates were scanned in a Schoeffel SD-3000 density computer. The densitometer was operated at 550 nm in the transmission mode with double beam system to provide background correction. The amount of each saccharide was determined by comparing the peak area with that of the corresponding saccharide standard.

Application of the Potato Hydrolysate

To evaluate the possible use of the potato hydrolysate as a sweetening agent and bodying agent, chocolate milk and vegetable soup were prepared.

Preparation of Chocolate Milk

A control chocolate milk drink similar to a commercially available chocolate milk was used as reference. The ingredients were in the following proportions (Tressler and Sultan, 1975): cocoa, 1.5 percent; sucrose, 6.0 percent; vanilla extract, 0.2 percent, salt, 0.2 percent, carrageenan, 0.04 percent, and homogenized milk, 92.06 percent.

One part of cocoa previously mixed with an equal weight of sucrose was added to 90 parts of homogenized milk and the mixture heated with stirring to 150°F.

Carrageenan was then added gradually and thoroughly stirred in. The remaining three parts of sucrose and other ingredients were added and the mixture heated at 145 to 150°F for 30 min. After rapid cooling, the product was refrigerated below 50°F until used. Samples were prepared the day before the panel test. In experiments with the potato hydrolysate, three samples of chocolate milk were prepared where the hydrolysate was substituted for the sucrose at levels of 33, 42 and 50 percent, respectively.

Vegetable Soup

Lipton Spring Vegetable Soup was used as dry soup mix. Levels of 50, 100 and 150 percent (on dry weight basis) of potato hydrolysate were added to the soup mixture. Samples were prepared according to instructions on the package on the same day of the panel test and kept warm in a double boiler.

Flavor Panel Evaluation

Three samples together with a known reference were served at the same time in paper cups coded with three digit numbers. Forty judges were used for each taste panel. The samples were evaluated for appearance, consistency, flavor and overall desirability using a 9 point hedonic scale ranking from 9 "extremely desirable" to 1 "extremely undesirable" (Figure 2).

Department of Food Science and Technology
OREGON STATE UNIVERSITY

PRODUCT: _____ NAME: _____

DATE: _____

Please write the sample number in the space following the statement which best describes your opinion of the sample. Be sure and score samples on all four quality factors.

	Appearance	Consistency	Flavor	Overall Desirability
9 - Extremely desirable				
8 - Very desirable				
7 - Moderately desirable				
6 - Slightly desirable				
5 - Neither				
4 - Slightly undesirable				
3 - Moderately undesirable				
2 - Very undesirable				
1 - Extremely undesirable				

WHICH SAMPLE DID YOU PREFER? _____

WHY?

Figure 2. Preference panel ballot.

RESULTS AND DISCUSSION

Composition of the Potatoes

Proximate composition of peeled and whole potatoes are presented in Table 1. Starch was 73 to 77 percent of the dry weight of the potato tuber and calorically is the most important nutritional component. Crude fiber content was

Table 1. Proximate composition of Oregon Russet potatoes.[a]

	Peeled potatoes (percent)	Whole potatoes (percent)
Moisture	82.50	81.50
Total solids	17.50	18.50
Protein	1.95	2.25
Ash	0.90	1.00
Ether extract	0.10	0.15
Carbohydrates		
starch	13.60	13.50
crude fiber	1.00	1.75

[a]Mean of duplicate samples.

higher in whole potatoes and has been reported to be largely cell wall components including lignin and suberin, a constituent of the peel (Schwimmer and Burr, 1959). The amount of fat present in the potato tuber was 0.1 to 0.15 percent. Cadwell et al. (1945) found carotene in the

potato tuber which was responsible for the yellow flesh coloration. Levels of protein in potatoes reported in Table 1 agree with those reported in the literature (Schwimmer and Burr, 1959). Potato protein has a good amino acid balance for human nutrition being rich in lysine and tryptophan (Schuphan, 1970). Seventy percent of potato protein has been reported to be a globulin known as tuberin (Schwimmer and Burr, 1959). The ash forms some 5.0 to 5.5 percent of the dry matter of the tuber and is slightly higher in unpeeled potatoes.

Hydrolysis of Potatoes

Effect of Gelatinization Temperature on Potato Hydrolysis

To determine the effect of gelatinization temperature on potato hydrolysis by B. subtilis α-amylase the potato slurry was adjusted to pH 7.0, 0.1 percent of Tenase and 400 ppm calcium as calcium chloride were added. The slurry was maintained at temperatures from 60 to 100°C for 30 min to allow gelatinization of the starch. After this treatment 0.1 percent more enzyme was added and the samples were placed in a 70°C oil bath.

The results (Figure 3 and Table 2) reveal that after 30 min of hydrolysis, reducing sugars were lowest at 60°C and highest at 80°C. As expected heat inactivation of α-amylase was observed at 100°C. Higher susceptibility to

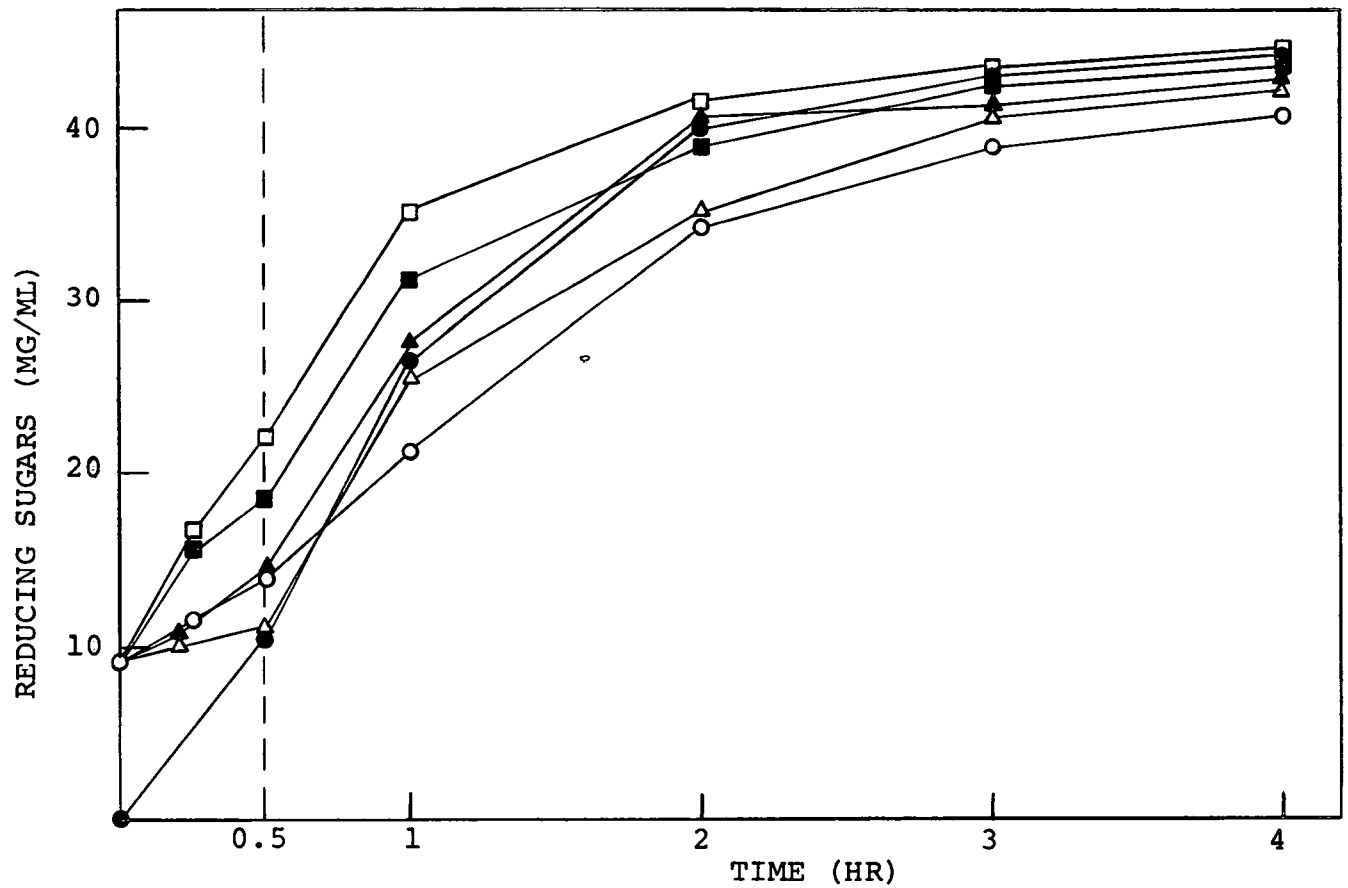

Figure 3. Effect of gelatinization temperature on hydrolysis of potato slurry with B. subtilis α-amylase. Potato starch at 70°C, ●—● ; potato homogenate at 60°C, ○—○ , at 70°C ■—■ ; at 80°C □—□ ; at 90°C ▲—▲ , at 100°C △—△ .

enzymic hydrolysis for the gelatinized starch than for the granular form was reported by several researchers (Leach and Schoch, 1961; Walker and Hope, 1963; Kulp, 1972). Leach (1965) reported a gelatinization temperature for potato

Table 2. Effect of gelatinization temperature on hydrolysis of potato slurry with B. subtilis α-amylase.

Time hr	Potato starch 70°C	60°C	70°C	80°C	90°C	100°C
		(mg reducing sugars/ml)				
0	0.0	9.3	9.3	9.3	9.3	9.3
0.25		11.5	15.7	16.5	10.9	11.0
0.50	10.4	13.9	18.7	22.0	14.6	11.0
1.00	26.8	21.3	31.1	35.3	27.7	26.0
2.00	40.0	34.3	39.2	41.5	40.6	35.1
3.00	43.5	36.9	42.6	43.0	41.6	41.8
4.00	44.0	40.9	43.6	44.5	43.2	43.4

starch ranged between 56-66°C. The starch granules exhibited exceptionally high swelling indicating weak internal bonding. Leach and Schoch (1961) also found no correlation between granule size (surface area) and extent of solubilization.

Purified potato starch was hydrolyzed at a faster rate than starch in peeled potato homogenate (Figure 3). This observation agrees with those of Heisler et al. (1951).

Effect of Temperature on Potato Hydrolysis

To determine the optimum temperature for the hydrolysis of potato starch, the slurry was prepared as stated in the previous section. Gelatinization was at 80°C for 30 min and temperatures between 50° to 90°C were used for the 4 hr hydrolysis period. Results presented in Figure 4 and Table 3 indicate that 80°C was the optimum temperature for the action of Tenase. Higher temperatures as 85°C and 90°C decreased the activity of Tenase.

Table 3. Effect of temperature on hydrolysis of potato slurry with B. subtilis α-amylase.

Time of hydrolysis (hr)	50°C	60°C	70°C	80°C	85°C	90°C
	(mg of reducing sugars/ml)					
0.5	22.0	22.1	22.1	22.0	22.1	22.0
1.0	30.8	31.1	31.1	35.0	31.9	28.1
1.5	35.6	37.4	38.0	39.2	34.9	31.1
2.0	37.4	38.0	39.2	44.5	36.9	32.1
3.0	40.4	41.4	42.6	49.0	40.2	35.0
4.0	41.7	42.5	43.6	50.2	42.7	40.2

The difference in heat stabilities of various α-amylases were studied by Miller et al. (1953). The following α-amylase activities were retained at 80°C: fungal α-amylase 1 percent, cereal α-amylase 25 percent and a bacterial α-amylase 92 percent. The decrease in activity was probably due entirely to thermal denaturation (Robyt and Whelan,

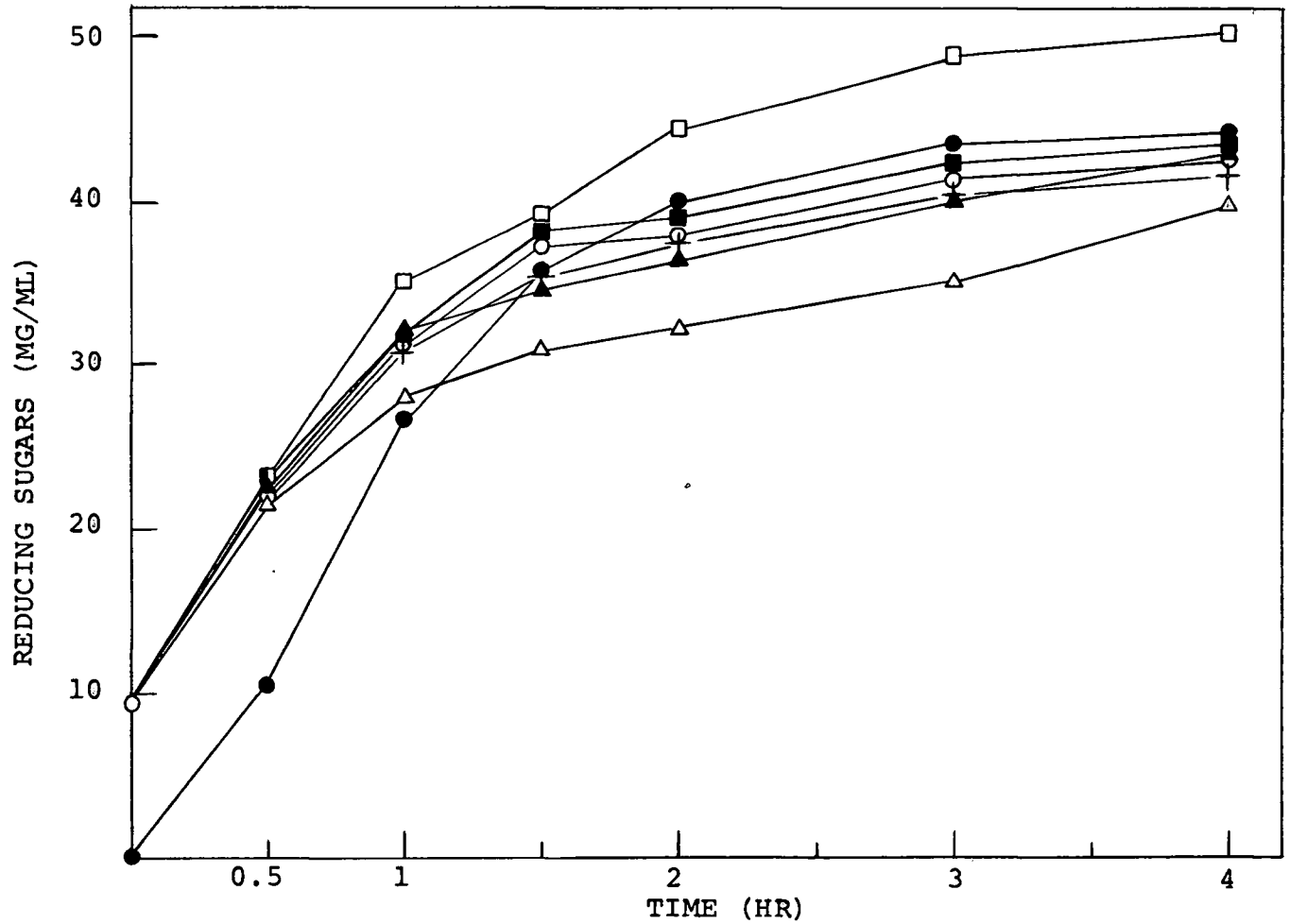

Figure 4. Effect of temperature on hydrolysis of potato slurry with B. subtilis α-amylase. Potato starch at 70°C, ●—● ; potato homogenate at 50°C, +—+ ; at 60°C, ○—○ ; at 70°C, ■—■ ; at 80°C, □—□ ; at 85°C, ▲—▲ , at 90°C, △—△.

1968). Another observation from Figure 4 was that a slow increase in the reducing sugar formation after 1.5 hr of hydrolysis. One explanation for this given by Whitaker (1972) was that the rate of hydrolysis decreased with a decreasing degree of polymerization.

Effect of pH on Potato Hydrolysis

The effect of pH on potato hydrolysis with B. subtilis α-amylase is shown in Figure 5. These data reveal that optimum pH for the hydrolysis occurred at pH 7.0.

The maximum activities of α-amylase have been shown to be in the acid region between 4.5 and 7.0. Significant differences in the shapes of the curves and in the position of the activity optima have been shown to depend on the enzyme source (Kulp, 1975). Optimum pH for mammalian α-amylase from human saliva and porcine pancreas was relatively narrow, extending from 6.0 to 7.0 (Fischer et al., 1960). The optimum value for B. subtilis α-amylase on the other hand was relatively broad with the activity not changing significantly between pH 5.0 and 7.0 (Menzi et al., 1957). However, in the trials presented in Figure 5, almost no liquefaction was observed at pH 5.0 and a very thick slurry with low reducing sugar content was obtained. The possible presence of other non-starch constituents of the potatoes might have some influence in this behavior. Heisler et al. (1951) reported that α-amylases were more effective in

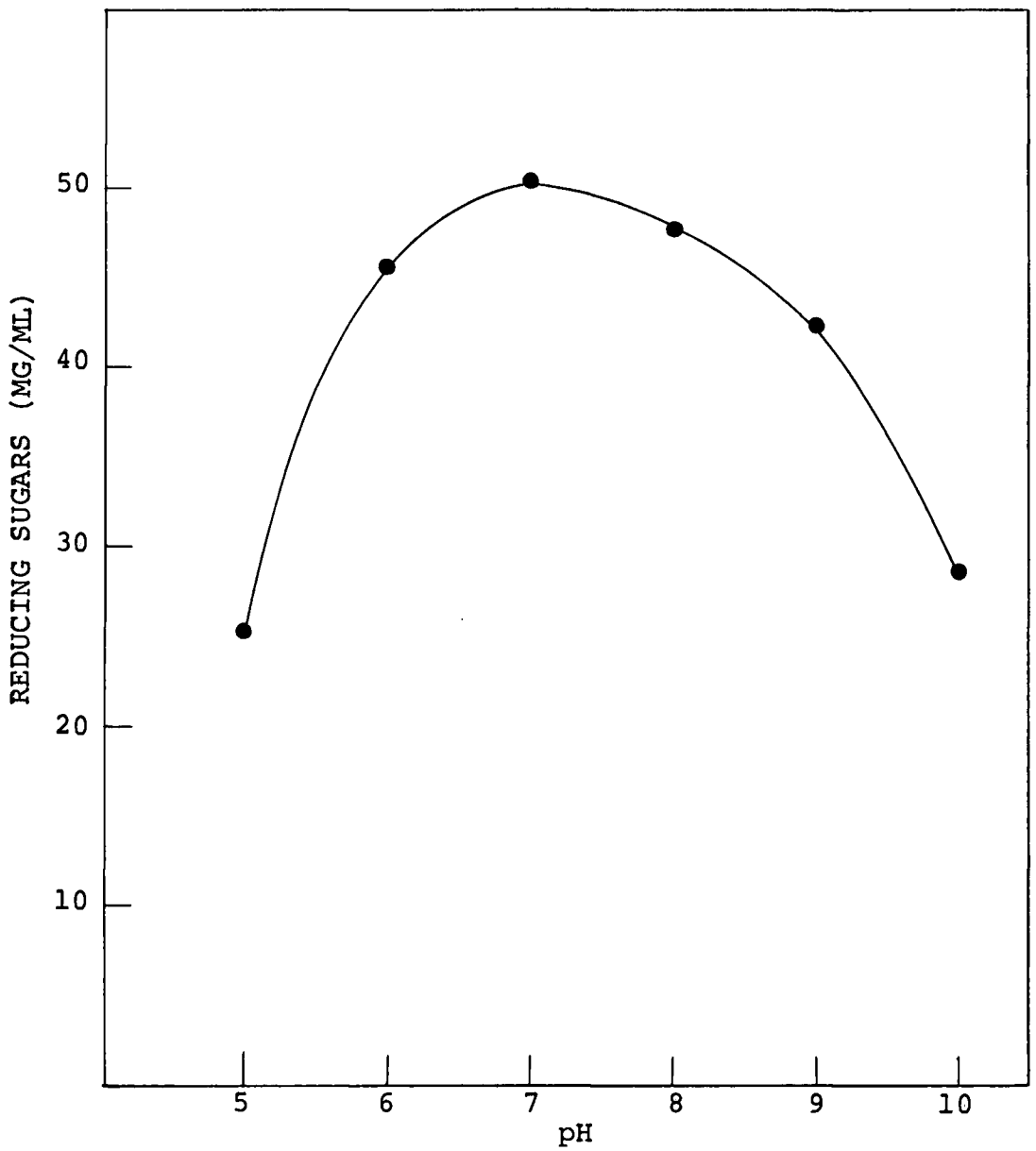

Figure 5. Effect of pH on hydrolysis of potato slurry with B. subtilis α-amylase.

converting pure starch to sugars, than starch in potato tissue under the same conditions.

Effect of Enzyme Concentration on Potato Hydrolysis

Concentrations from 0.1 to 0.7 percent of B. subtilis α-amylase (based on 15 percent starch content) were added to the potato slurry at 80°C. Results are presented in Figure 6 and Table 4. No significant differences were observed by using 0.3, 0.4, 0.5, or 0.7 percent of α-amylase

Table 4. Effect of enzyme concentration on hydrolysis of potato slurry.

Time of hydrolysis (hr)	0.1%	0.2%	0.3%	0.4%	0.5%	0.7%
	(mg of reducing sugars/ml)					
0.5	28.2	36.8	37.7	43.4	47.8	49.0
1.0	35.8	43.5	49.0	51.6	51.6	52.7
1.2	40.5	46.7	54.4	55.3	56.0	57.8
2.0	43.8	49.0	57.2	57.3	57.6	57.8

after 2 hr of hydrolysis. This behavior might be due to the low activity of the enzyme on the products with intermediate degree of polymerization, consequently a decrease in the rates of hydrolysis was observed (Whitaker, 1972).

Robyt and French (1963, 1967), Thoma and Spradlin (1970), did not detect any degradation of maltose, maltotriose, or maltopentaose when they used ten times the amount of B. subtilis α-amylase as employed in the breakdown of

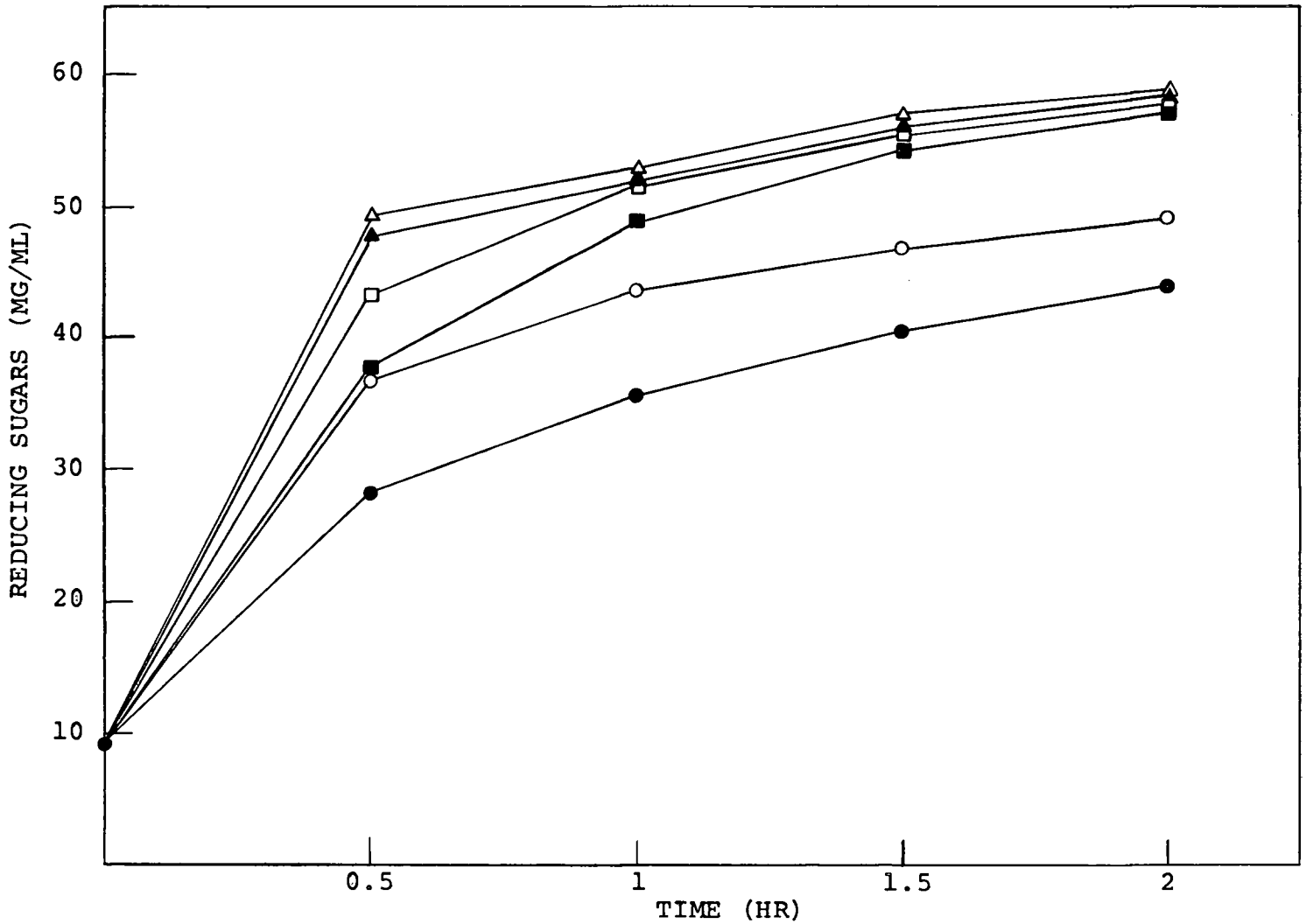

Figure 6. Effect of enzyme concentration on hydrolysis of potato slurry with B. subtilis α-amylase. Concentration of 0.1%, ●—●; 0.2%, ○—○; 0.3%, ■—■; 0.4%, □—□; 0.5%, ▲—▲; 0.7%, △—△.

amylose and amylopectin. These workers observed that maltohexaose was slowly converted to glucose and maltopentaose.

Concentration of 0.3 percent α-amylase was chosen for the subsequent studies on potato hydrolysis. Tenase is an inexpensive enzyme with a price of 0.60 cents per pound so that the process does not appear to be expensive. Generally 0.1 to 0.2 percent α-amylase on a dry starch basis is used to liquefy starch slurry (Petersen, 1975). Vance et al. (1972) have provided an enzyme process for starch liquefaction by using 0.25 percent α-amylase added in three steps to get a product with a DE from 9 to 30.

Effect of the Presence of Calcium on Potato Hydrolysis

Results presented in Figure 7 show a sharp decrease in the formation of reducing sugars in the absence of calcium ions.

All α-amylases have been found to be calcium requiring enzymes containing at least one atom of calcium per molecule (Fischer and Stein, 1960; Vallee et al., 1959). In the presence of calcium, the α-amylases were more resistant to denaturation at extremes of pH, temperature or treatment with urea. Stein et al. (1964) showed that when calcium was completely removed from B. subtilis α-amylase by EDTA or by electrodialysis activity lost could be restored on addition of calcium. Hsui et al. (1964) reported that B. subtilis

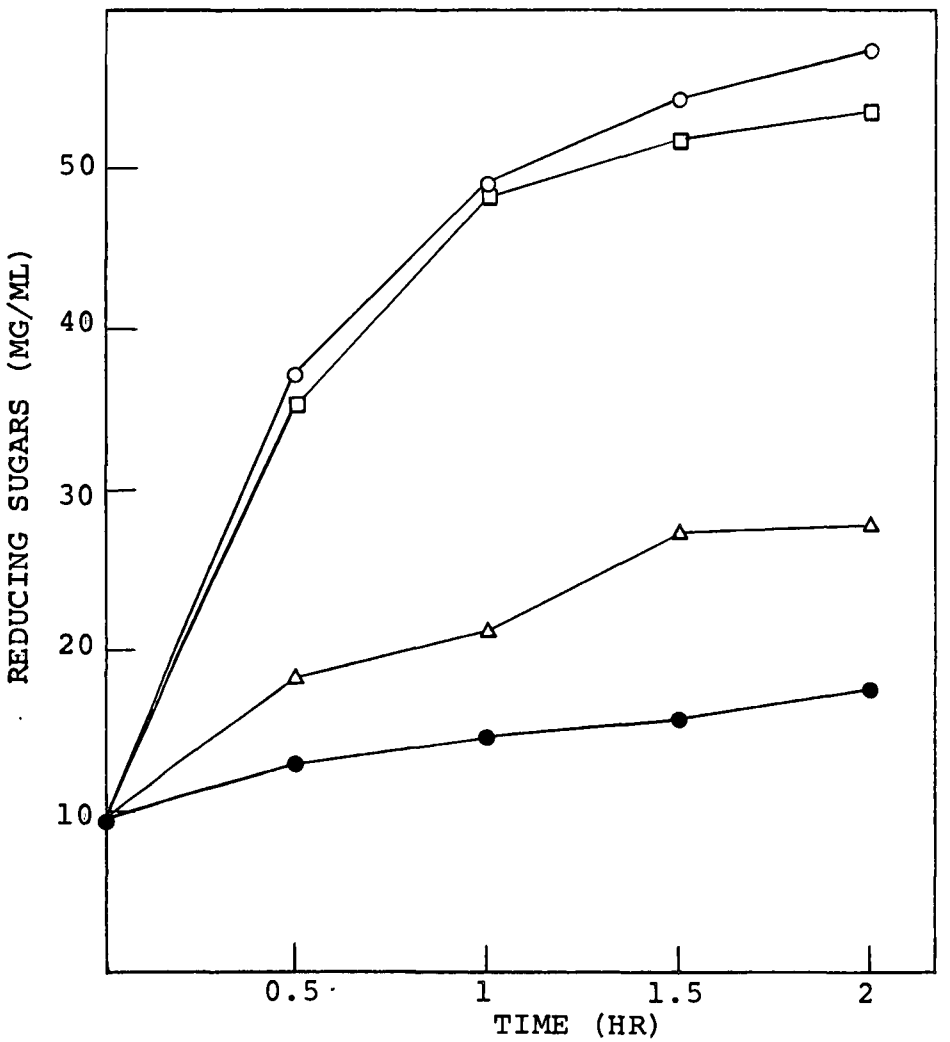

Figure 7. Effect of the presence of calcium ions on hydrolysis of potato slurry with B. subtilis α-amylase. Hydrolysis without enzyme, ●—● ; with calcium chloride, ○—○ ; without calcium chloride, △—△ ; with whole potatoes □—□ .

α-amylase required four atoms of calcium per molecule of protein. Calcium was speculated to impart to the B. subtilis α-amylase molecule the same structural rigidity conferred by -S-S- linkages. Lloyd and Lodge (1971) proposed the use of 0.52 percent of calcium as acetate, formate, propionate or lactate to enhance the storage stability of α-amylase. Calcium ions were not reported to be involved in the hydrolysis step but stabilized the compact architecture of the protein molecule to maintain an enzymatically active configuration (Robyt and Whelan, 1968). Allen and Spradlin (1974) reported that B. subtilis α-amylase should be stabilized with 150 to 400 ppm calcium ions when operating at temperatures above the gelatinization temperature of starch.

Results of hydrolysis without added α-amylase is also shown in Figure 7. A slight increase in reducing sugars was obtained. This increase was probably due to the presence of natural α- and β-amylases in potatoes (Schwimmer, 1953). Hydrolysis of whole potatoes gave results similar to those of peeled potatoes. However, the syrups from whole potatoes were darker in color than those obtained from peeled potatoes. Higher polyphenol oxidase activity and higher levels of phenolic compounds have been found in the outer portion of the potato tuber (eyes and peel) (Amberger and Schaller, 1973). These factors could account for the darker color of syrup from the whole potatoes.

Inactivation of B. subtilis α-amylase

Inactivation of B. subtilis α-amylase can be achieved thermally by raising the temperature to 100°C and holding for 10 to 20 min or by lowering the pH with hydrochloric or sulfuric acid to pH 4.0 (Miles Laboratories Inc., 1976). The pH adjustment was the preferred method since the hydrolysate darkened at pH 7.0 and high temperature. Inactivation of α-amylase in peeled potato hydrolysate by adjusting the pH to 4.3 with 6N hydrochloric acid resulted in an acid flavor in the product. With whole potato hydrolysate a combination of adjusting the pH to 5.0 to 5.5 with 30 percent sulfuric acid and heating to 100°C for 10 min gave the least browning of the syrup.

Schachtel et al. (1959) studied the influence of pH on browning of glucose syrup. At acid pH the browning was due to the formation of hydroxymethyl furfural. With increasing pH, browning due to hydroxymethyl furfural was replaced by the Maillard reaction. Between pH 4.0 to 5.0 minimum color formation was observed. Framkevicz and Short (1970) have described a process where α-amylase was inactivated by fluorosilicate compounds with a high degree of control over starch liquification in a starch slurry. These compounds are nontoxic and may be used in food applications.

Purification and Drying of Potato Hydrolysate

Activated charcoal was used to decolorize the potato hydrolysate. Results presented in Table 5 indicate that after 30 min at 80°C the decolorizing action of active charcoal was almost complete. Color of the potato hydrolysate decreased as the concentration of activated charcoal was increased to four percent (Figure 8 and Table 5). Above this level only a slight change was observed.

Table 5. Decoloration of potato hydrolysate with activated charcoal.

Percent active charcoal[a]	Peeled potatoes		Whole potatoes
	30 min (absorbance 440 nm)	60 min	30 min (absorbance 440 nm)
0.00	1.046	1.046	1.523
0.25	0.824	0.796	1.431
0.50	0.757	0.757	1.398
1.00	0.638	0.629	1.155
2.00	0.469	0.444	0.824
4.00	0.268	0.268	0.523
6.00	0.260	0.180	0.398

[a]Based on dissolved solids.

Two concentrations (2 and 4 percent) of activated charcoal were selected for trials with spray dried products. No differences in color were observed in the final product made from peeled potatoes after spray-drying. In the case

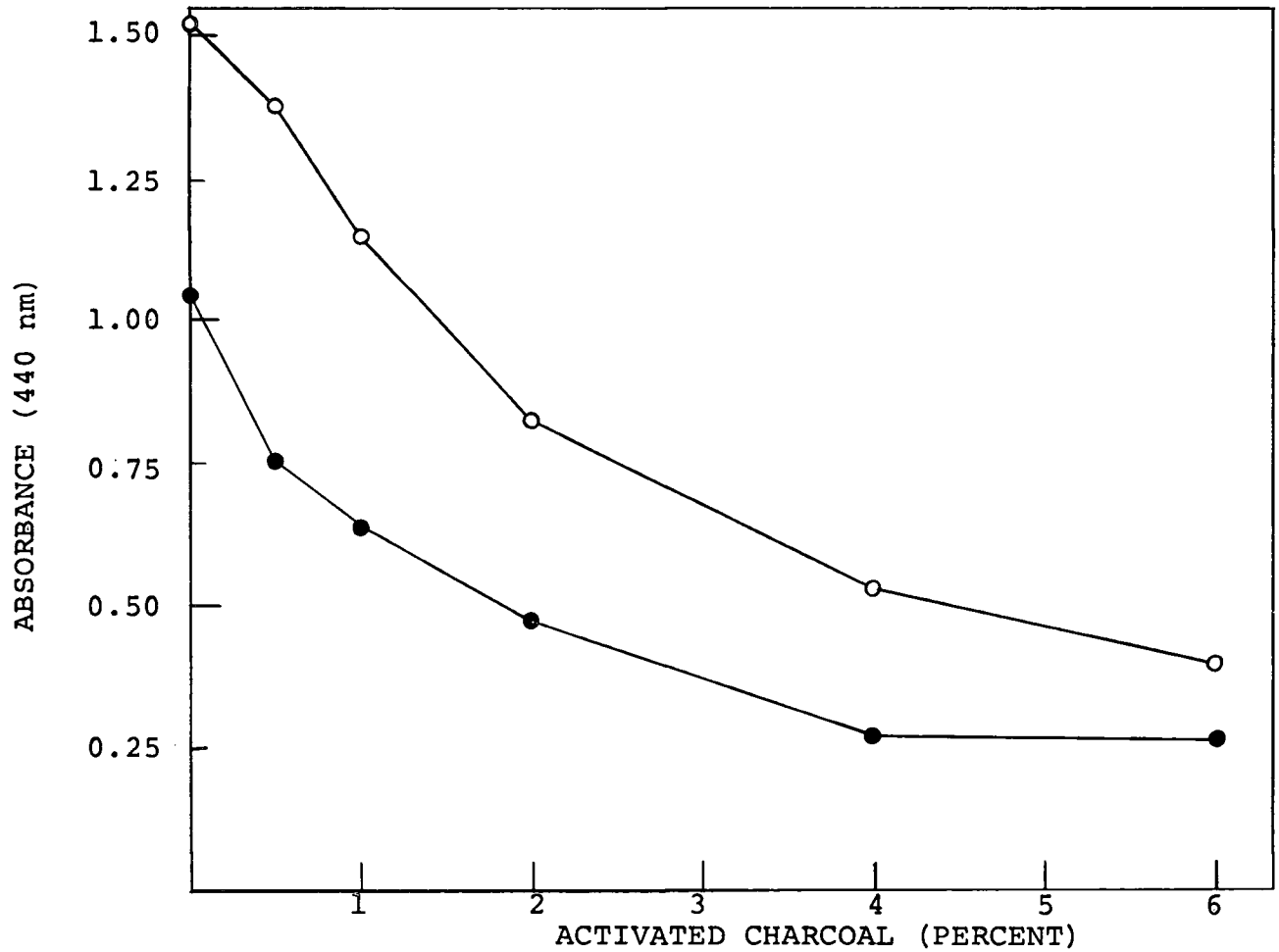

Figure 8. Decoloration of potato hydrolysate with activated charcoal. Syrup from peeled potatoes, ●—● ; syrup from whole potatoes, ○—○ .

of the hydrolysate from whole potatoes a darker color was always observed and four percent activated charcoal was used.

The decoloration process removes certain dissolved substances such as proteins, color bodies, odors and partially removes soluble minerals and other impurities due to the adsorptive properties of the activated charcoal (Hyndshaw, 1975). A method of purifying and decolorizing sugars liquors such as starch hydrolysates has been reported by Corson and Johnson (1970). In this process the liquors are passed through a series of filters whereby the downstream filter contains the freshest carbon filter cake. Hyndshaw (1975) reviewed a complete discussion about the use of activated charcoal for refining of sugar syrups with reference to: structure of activated charcoal, mechanisms of adsorption, evaluation of activated charcoal on basis of adsorption isotherms and methods of activated carbon treatment of sugar syrups.

To produce corn syrup solids and maltodextrins the syrups are dried in spray or vacuum drum dryers to markedly lower the moisture content to less than 3.5 percent (Corn Refiners Assoc., 1976). Spray-drying proved to be the best method to obtain syrup solids. Cagley (1972) reported that by using a stainless steel spray drying system a variety of medium and low conversion syrups could be dried and physical properties such as product viscosity, body and texture

controlled. The dryer can be run two to five days continuously before cleaning and production has been increased 60 percent.

After decoloration, the potato hydrolysate was concentrated to 25 to 30 percent solid material for more efficient spray-drying. Drying was at 4 kg/cm^2 and 80°C. At higher temperatures of drying the color of the hydrolysate turned a dark brown.

Proposed Flow Sheet for Potato Hydrolysis by *B. subtilis* α-amylase

The scheme shown in Figures 9 and 10 represents the different steps in the hydrolysis of peeled and whole potatoes by *B. subtilis* α-amylase. With peeled potatoes the residue left after hydrolysis represents about 2.5 percent on a wet basis (14 percent on a dry basis) while the residue from whole potatoes represents 3.1 percent (17 percent on a dry basis). The residue has a high fiber content particularly that from whole potatoes.

Liquefaction was nearly complete; only 3.5 percent of the initial starch content in the mixture was not hydrolyzed and remained in the residue. This was possibly due to incomplete disintegration of the cells which did not liberate the starch for enzyme action. The dried potato hydrolysate would represent 12 to 15 percent yield on a wet basis.

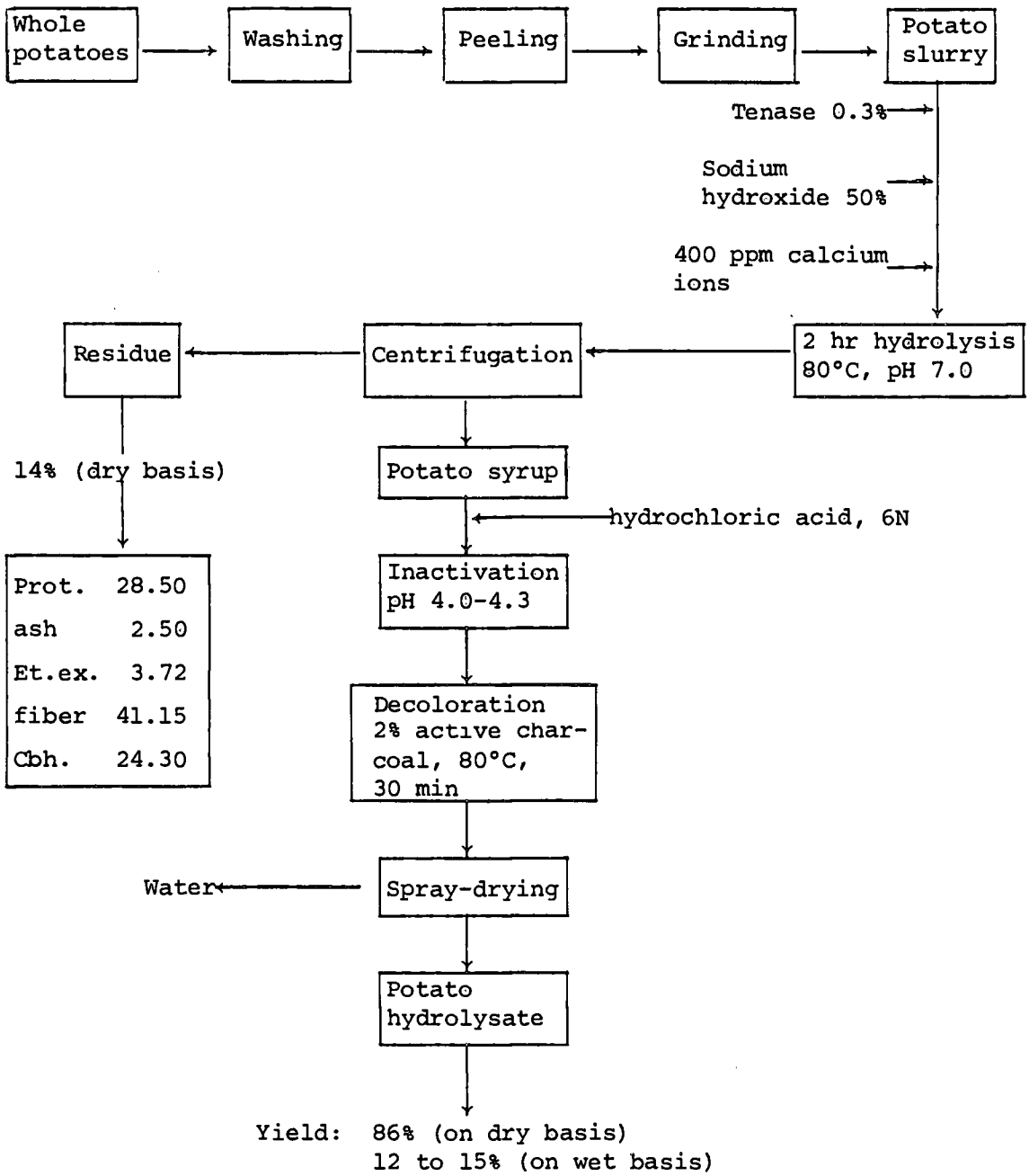

Figure 9. Flow sheet for peeled potato hydrolysate by B. subtilis α-amylase.

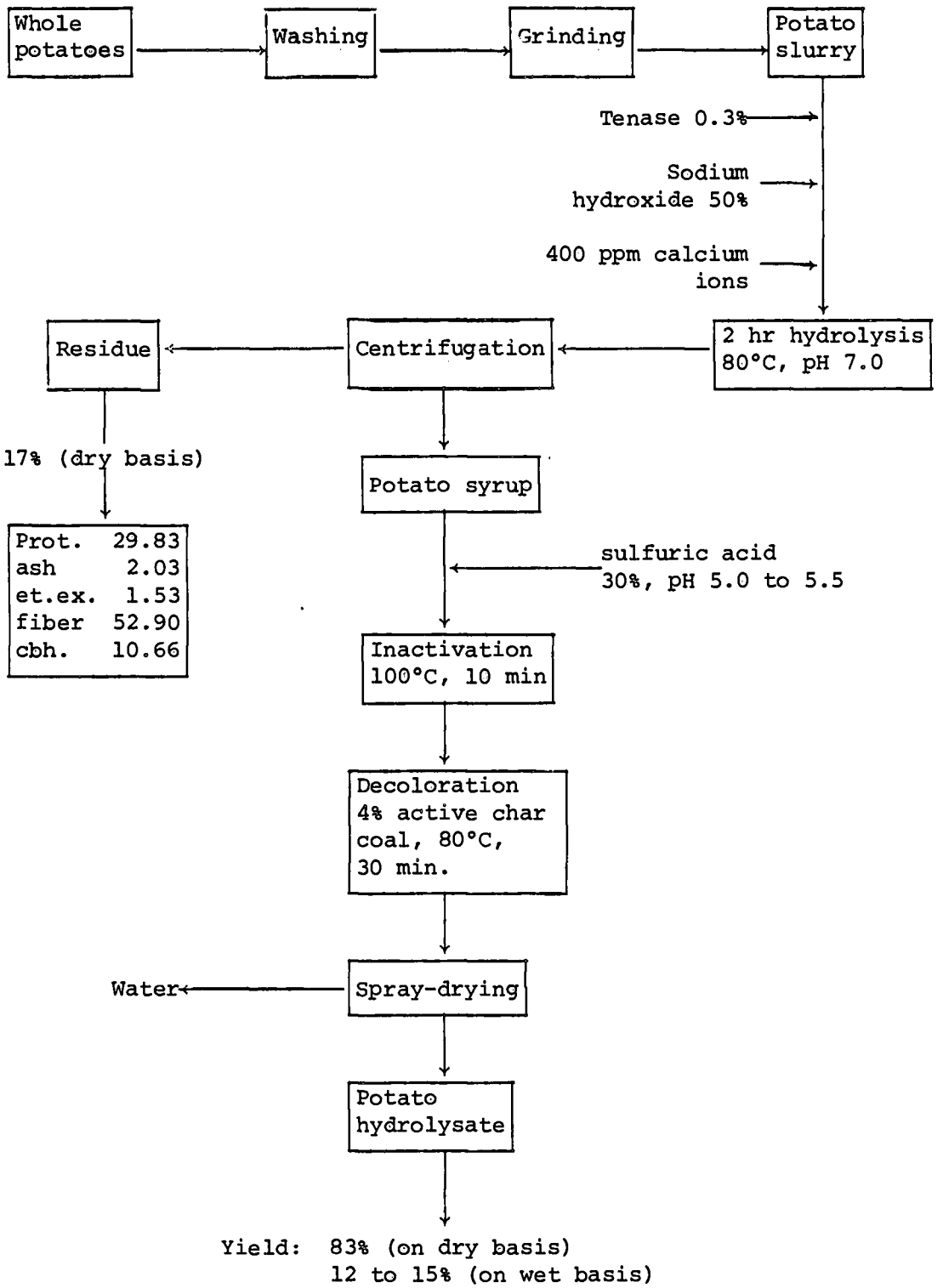

Figure 10. Flow sheet for whole potato hydrolysate by B. subtilis α-amylase.

Composition of the Potato Hydrolysate

Proximate Composition

Potato hydrolysate obtained after spray-drying the syrup from peeled and whole potatoes were submitted to proximate analysis. The results are presented in Table 6. Protein content of the hydrolysate was higher than expected,

Table 6. Composition of potato hydrolysate.

	From peeled potatoes	From whole potatoes
Moisture	2.50%	1.40%
Protein (Nx 6.25)	8.45%	8.46%
Ash	5.50%	5.96%
Ether extract	0.07%	0.05%
Carbohydrate	84.80%	86.00%
DE	30	26
Calcium	457 ppm	495 ppm
Sodium	455 ppm	495 ppm
Iron	36 ppm	35 ppm
Zinc	18 ppm	35 ppm
Copper	5.7 ppm	2.1 ppm
Potassium	2.13%	2.59%
Magnesium	0.13%	0.15%
Sulfate	0.47%	1.30%
Chloride	2.17%	0.68%
Phosphorus	0.13%	0.12%

indicating considerable retention of the potato protein in the hydrolysate. High ash values were due to the salts formed by pH adjustment. DE of the peeled potato

hydrolysate was slightly higher (30) than the DE of whole potato hydrolysate (26). Both hydrolysates presented similar chemical composition.

Samples of ash from the hydrolysate were submitted to mineral analysis by atomic absorption spectrophotometry. High calcium was due to the 400 ppm of calcium chloride added to the potato slurry to stabilize the α-amylase. The hydrolysate from peeled potatoes showed higher chlorine value than the whole potato hydrolysate. This is probably due to the hydrochloric acid added to inactivate the α-amylase. Higher sulfate values in the whole potato hydrolysate were due to the use of sulfuric acid to inactivate the α-amylase. To reduce ash content in potato hydrolysates ion-exchange resins could be used for refining the syrup but a considerable increase in cost would result.

Amino Acid Content of Potato Hydrolysate

To determine the nutritional value of the protein in the potato hydrolysate, amino acid composition of the hydrolysate was determined. Samples of whole potatoes and the hydrolysate were submitted to acid hydrolysis followed by amino acid analysis. The spectrum of amino acids was shown by peaks on a semilog chart and the percent of amino acid in both samples were calculated mathematically.

Results shown in Table 7 reveal that aspartic acid and glutamic acid were the amino acids present in highest

amounts in whole potatoes and the hydrolysate. Tyrosine, proline and phenyl alanine were not retained in the hydrolysate. The recovery of the other amino acids was low. Tryptophan was not included because it is destroyed during acid hydrolysis of the protein.

Table 7. Amino acid content in whole potatoes and its hydrolysate.[a]

Aminoacid	Whole potatoes	Potato hydrolysate	Recovery (percent)
Lysine	3.52	1.62	46.31
Histidine	1.25	0.59	47.20
Arginine	4.23	2.03	47.99
Aspartic acid	17.92	22.44	125.22
Threonine	2.78	0.97	34.89
Serine	3.23	1.23	38.08
Glutamic acid	23.13	21.17	91.53
Proline	3.30	--	--
Glycine	2.52	0.31	12.30
Alanine	3.10	1.27	40.97
Cysteine	--	--	--
Valine	4.94	2.12	42.91
Methionine	1.96	0.28	14.29
Isoleucine	3.13	1.16	37.06
Leucine	4.36	0.76	17.43
Tyrosine	3.43	--	--
Phenyl alanine	4.89	--	--

[a]Values given as percent of amino acid per 100 g of potato protein.

The amino acids lost during the preparation of the hydrolysate could remain in the residue, which contained

about 29 percent protein on a dry basis or be adsorbed on the charcoal in the decoloration step.

Neuberger and Sanger (1942) have reported that about two-thirds of the total nitrogen in potatoes was present as free amino acids. Asparagine and glutamine were the free amino acids present in the highest amounts (Talley and Porter, 1970; Milder and Bakena, 1956). Glutamine and asparagine together with arginine were the forms in which adsorbed nitrogen was accumulated in the tuber before being further metabolized (Talley and Porter, 1970; Milder and Bakena, 1956).

Since the α-amylase preparation did not contain protease activity (Miles Laboratories Inc., 1976), the proteins were not hydrolyzed during preparation of the hydrolysate. The presence of amino acids in the hydrolysate increases the nutritional value of the potato hydrolysate.

Carbohydrate Composition of the Potato Hydrolysate

TLC was used to separate and transmission densitometry was used to quantify the saccharides.

First attempts were with ethyl acetate-methanol-water (52-36-13 v/v) (Huber et al., 1966). Presence of seven saccharides was indicated but separation was poor. Better resolution was obtained with propan-2-ol-acetone-1M lactic acid (4-4-2 v/v) as solvent system.

A photograph of a TLC plate is presented in Figure 11, where saccharides of whole potato hydrolysate were separated together with standards. The main problem with this technique was spraying the TLC plate with the developing reagent. Careful and uniform spraying was required to produce reproducible results. To help overcome this problem, standards were included on each plate and the samples were run twelve times.

A densitometer scan of a TLC chromatogram of the whole potato hydrolysate is shown in Figure 12. Separation between saccharides with degree of polymerization five and six was not complete.

Table 8 shows the carbohydrate composition of the potato hydrolysate. For comparison the composition of an acid-enzyme converted corn syrup with a DE range from 26 to 33 is also given. The main difference between the two potato hydrolysates was that the glucose (DP1) content was lower in the hydrolysate made from whole potatoes. Maltotriose (DP3) and maltotetraose (DP4) were similar.

Junk and Pancoast (1973) have pointed out that syrups which have relatively the same DE value can differ completely in the carbohydrate distribution pattern depending on the method of conversion. α-amylase has a specific preference to accumulate higher levels of DP6, DP7, and DP3 (Robyt and French, 1963; Greenwood and Milne, 1968; Thoma and Spradlin, 1970).

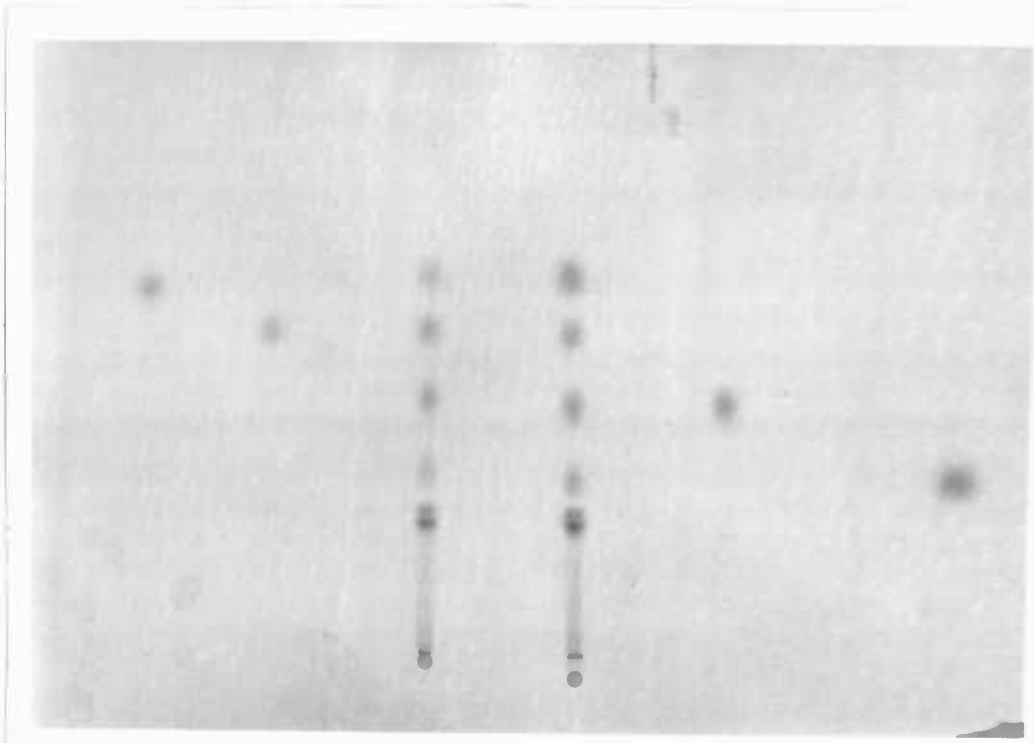

Figure 11. TLC of potato hydrolysates. From left to right, glucose, maltose, whole potato hydrolysate, maltotriose and maltotetraose.

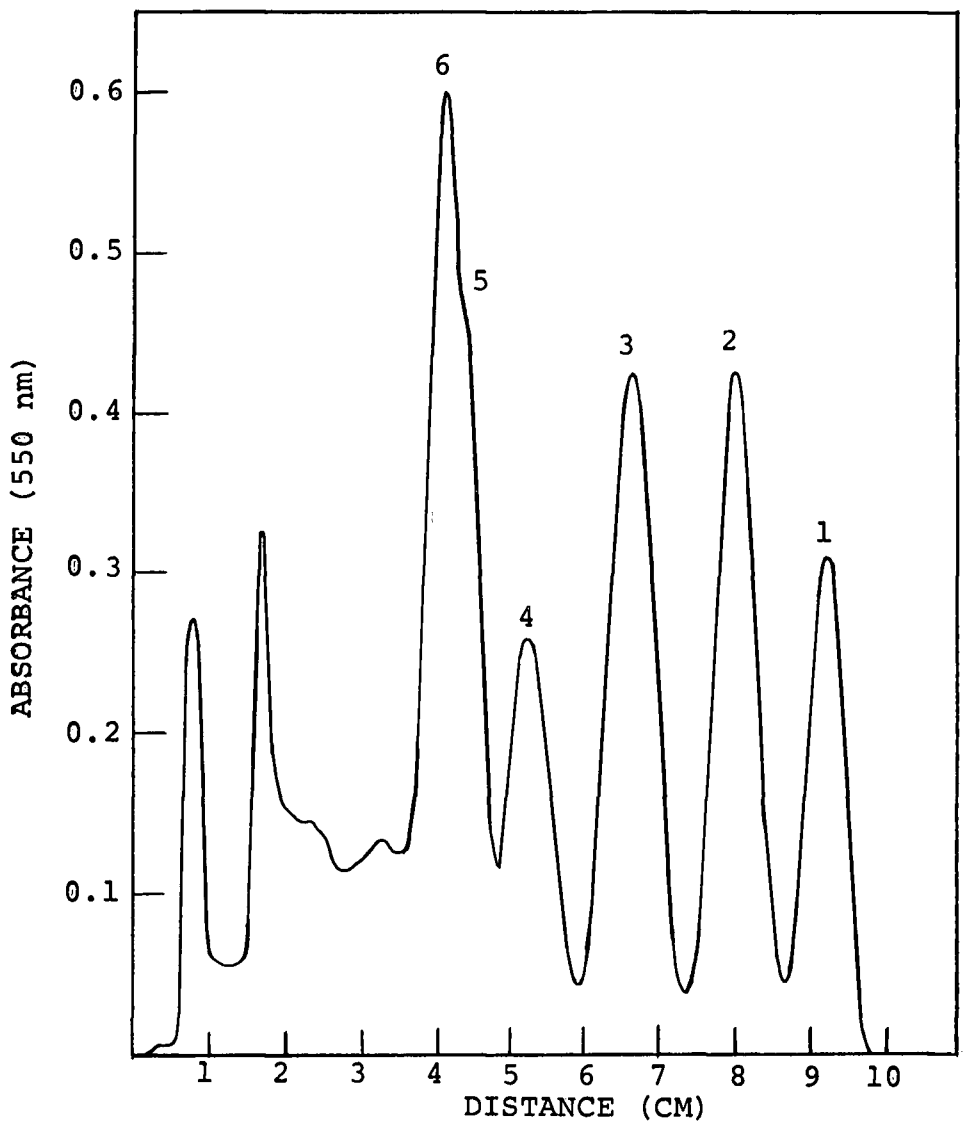

Figure 12. Densitometer scan of thin-layer chromotogram of whole potato hydrolysate. Peak 1 to 6: monosaccharide to hexosaccharide.

Table 8. Carbohydrate composition of the products obtained from hydrolysis of potatoes by B. subtilis α-amylase.[a]

	Whole potato hydrolysate (percent)	Peeled potato hydrolysate (percent)	Corn syrup[c] (acid enzyme conversion) (percent)
DE	26	30	26 - 30
DP1[b]	11.3 ± 0.30	16.7 ± 0.47	8.0 - 9.0
DP2	15.4 ± 0.34	14.3 ± 0.27	10.0 - 13.0
DP3	15.3 ± 0.26	14.4 ± 0.20	10.0 12.0
DP4	9.9 ± 0.28	9.9 ± 0.35	6.0 - 9.0
higher	47.9 ± 0.43	44.6 ± 0.57	60.0 - 63.0

[a]All values are means of 12 determinations ± standard error.
[b]DP is degree of polymerization.
[c]Junk and Pancoast (1973).

Functional Properties of the Whole Potato Hydrolysate

The potato hydrolysate produced was a light yellow-powdered solid, slightly sweet, relatively bland in taste and readily soluble in water.

Figure 13 shows how the viscosity of the hydrolysate compares with a commercial corn syrup solid of 24 DE. The higher hydrolysate concentrations demonstrated higher viscosities. Small differences were observed at low levels of concentration but at 60 percent solids, a higher increase in viscosity of the commercial corn syrup solids was observed. The relatively high percentage of polysaccharides

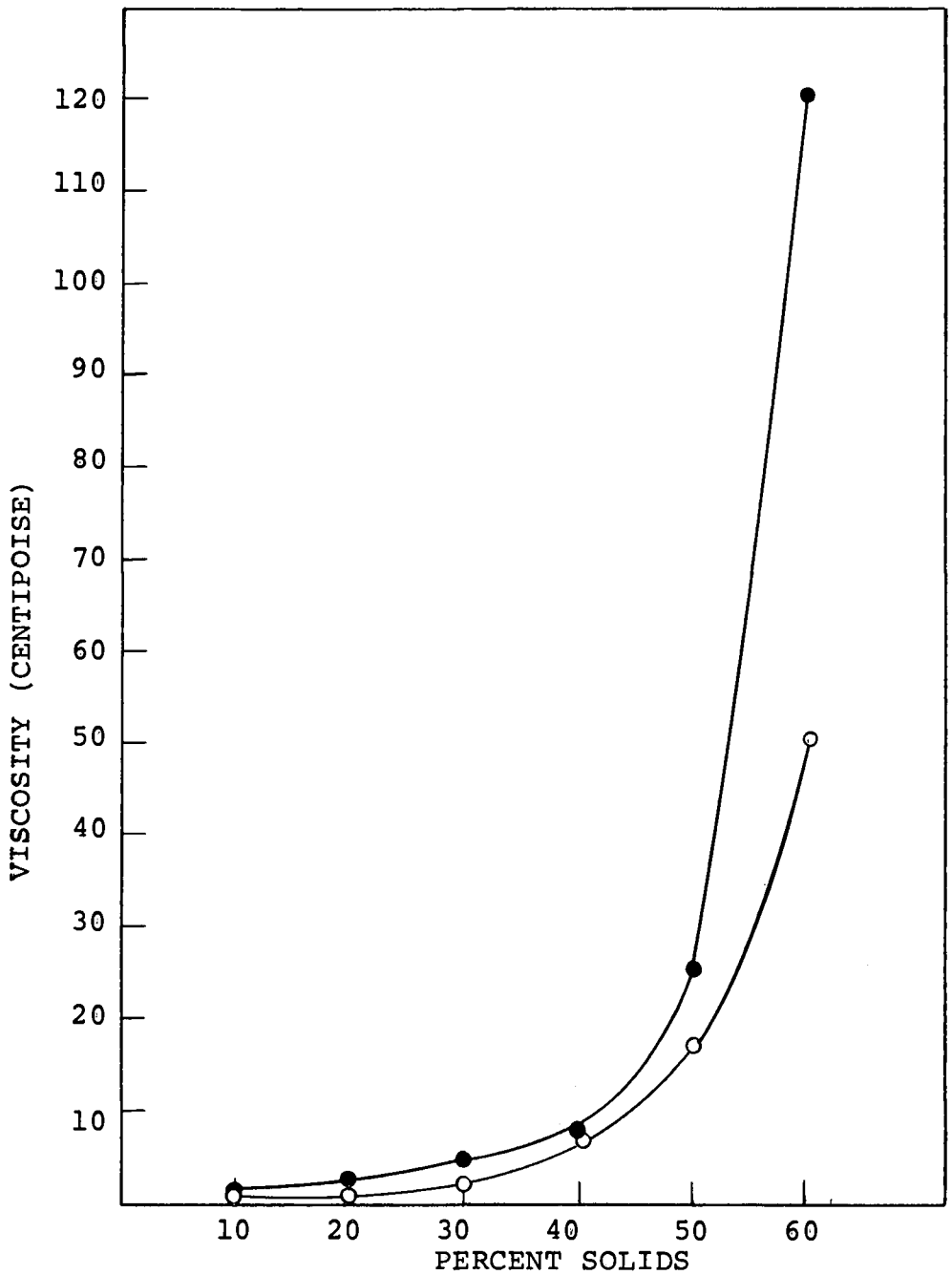

Figure 13. Viscosity of whole potato hydrolysate at 23°C. Potato hydrolysate from whole potatoes, ○—○ ; corn syrup solids Star-Dri 24R, A.E. Staley Manufacturing Co., ●—● .

present in the hydrolysate were responsible for the high viscosity, cohesiveness and bodying effects.

Figure 14 shows the rate of moisture adsorption by exposure of samples of the whole potato hydrolysate and the 24 DE commercial corn syrup solid to 75 percent relative humidity for one month. The results show that the potato hydrolysate absorbed more moisture than the corn syrup solids. Thus, the potato product was much more hygroscopic than the corn syrup solids which might give some adhesion problems in some dried food mixes.

Syrup solids are one of the most essential ingredients in the manufacture of high quality ice cream products. They can raise the total solids without contributing excessive sweetness (Drusendahl, 1951). The potato hydrolysate product could be used to adjust sweetness and solids content, to influence body density and to contribute to the nutritive value of foods in which it was used.

Sensory Evaluations

Chocolate Milk

The sensory panel evaluations of the chocolate milk were carried out by substituting equal parts of sucrose with the dried whole potato hydrolysate. Judgements for appearance, consistency, flavor and overall desirability are presented in Table 9.

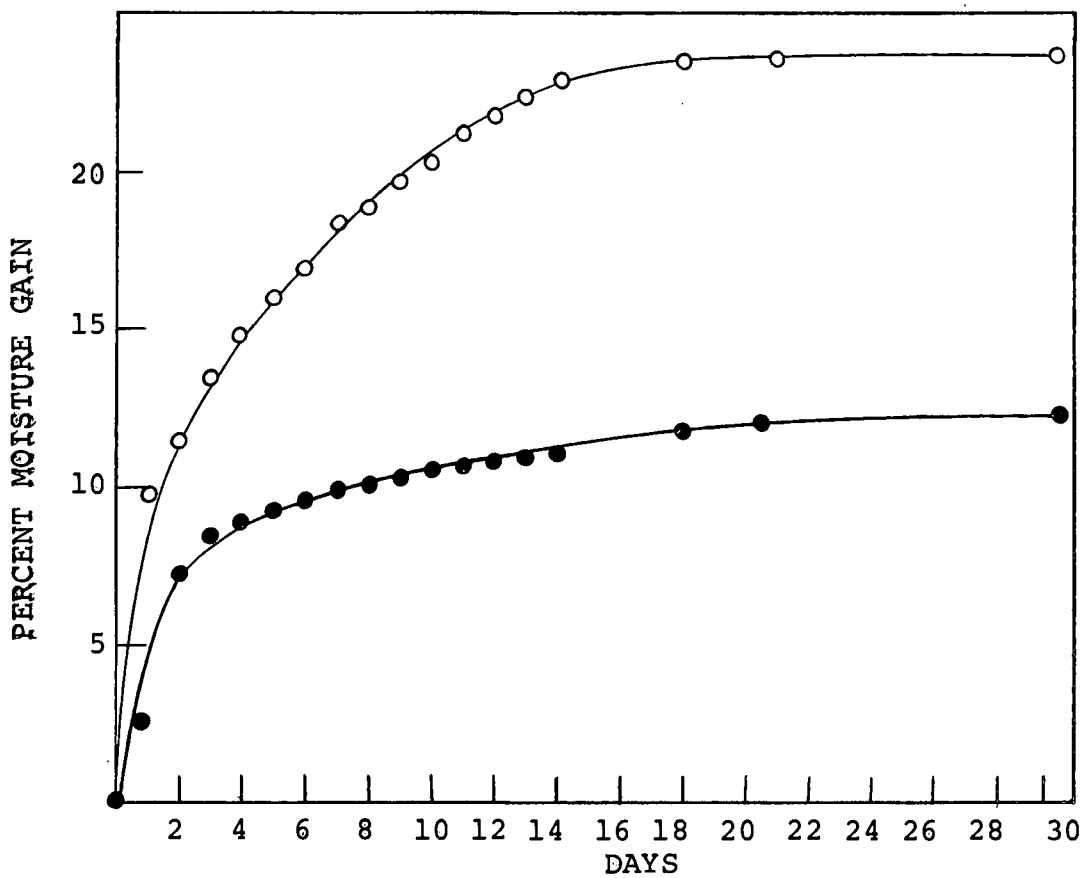

Figure 14. Moisture adsorption by whole potato hydrolysate at 23°C and 75% relative humidity. Potato hydrolysate from whole potatoes, ○——○ ; corn syrup solids Star-Dri 24R, A. E. Staley Manufacturing Co., ●——● .

Table 9. Mean panel scores for the chocolate milk with increasing levels of whole potato hydrolysate.[a]

Sample Potato sucrose/hydrol.	Scores[b,c] Appearance	Consistency	Flavor	Overall desirability
6 /0	7.40[d]	7.18[d]	7.18[d]	7.10[d]
3 /3	7.43[d]	7.68[e]	4.78[g]	5.03[g]
3.5/2.5	7.55[d]	6.88[d]	5.68[f]	5.68[f]
4 /2	7.53[d]	7.18[d]	6.53[e]	6.38[e]
LSD (0.05)	0.31	0.42	0.61	0.59

[a] n = 40 judges.

[b] Range of scores: 9 "extremely desirable" to 1 "extremely undesirable."

[c] In a column, mean scores with the same superscript are not significantly different ($P < 0.05$).

No significant differences ($P < 0.05$) were noted in the appearance of the samples. The sample with 3.0 parts of potato hydrolysate had a significantly more desirable consistency than the control and the other two samples containing lower levels of whole potato hydrolysate. The flavor and overall desirability evaluations showed significant differences between all the samples. With increasing levels of potato hydrolysate, lower scores were obtained. However, with 2.0 parts of potato hydrolysate scores were in the "slightly desirable" range.

From the results of the panel evaluation, the substitution of 2.0 parts of sucrose by 2.0 parts of potato

hydrolysate could be used with a slight loss of desirability of the chocolate milk.

Dehydrated Vegetable Soup Mix

Levels of 50, 100 and 150 percent (on dry weight basis) of whole potato hydrolysate were added to a commercial dehydrated vegetable soup mix. The potato hydrolysate increased the total solids of the soup. The scores of the panel test are presented in Table 10.

Table 10. Mean panel scores for the dehydrated vegetable soup mix.[a]

Sample % Potato hydrolysate	Scores[b,c] Appearance	Consistency	Flavor	Overall desirability
0	6.75[d]	6.98[d]	6.33[df]	6.43[de]
50	7.33[e]	7.08[d]	6.95[e]	6.88[d]
100	7.25[e]	7.08[d]	6.88[ed]	6.88[d]
150	7.33[e]	6.80[d]	5.73[f]	5.88[e]
LSD (0.05)	0.41	0.36	0.61	0.57

[a] n = 40 judges.

[b] Nine point hedonic scale: 9 "extremely desirable", 1 "extremely undesirable".

[c] In a column mean scores with the same superscript are not significantly different ($P < 0.05$).

Significantly more desirable differences ($P < 0.05$) were noted in the appearance of all three samples containing potato hydrolysate. Consistency scores of all the samples were not significantly different. The flavor

scores were significantly better than the control in the samples with 50 percent potato hydrolysate. Samples containing 100 and 150 percent potato hydrolysate were scored not significantly different from the control. With overall desirability, the 150 percent addition was scored significantly less desirable than the 50 and 100 percent samples. However, none of the three samples containing potato hydrolysate were significantly different from the control.

From these results, the addition of 50 and 100 percent of potato hydrolysate to vegetable soup mix could result in an economical means to increase the solids and caloric content and weight without decreasing the desirability of the product.

SUMMARY AND CONCLUSIONS

Hydrolysates from peeled and whole potatoes were prepared with B. subtilis α-amylase. Optimum conditions of hydrolysis were obtained by using α-amylase at 0.3% of the starch content in the presence of 400 ppm of calcium ions at pH 7.0 and 80°C for 2 hr. Under these conditions, 96 percent of the starch was hydrolyzed into smaller saccharides. The product obtained from subsequent centrifugation, inactivation at 100°C for 10 min, decolorization with four percent activated charcoal and spray-drying possessed acceptable properties for use in food products.

The dried potato hydrolysate was readily soluble in water at room temperature and contained about 8.5 percent protein. This protein would increase the nutritive value of foods in which the product was used.

Although the potato hydrolysate prepared in this work appears to be inexpensive to prepare and to have uses in certain food applications, considerably more research is required to investigate further applications of this product to the manufacture of food products.

BIBLIOGRAPHY

Allen, W. G. and Spradlin, J. E. 1974. Amylases and their properties. Brewers Digest 49(7):48.

Amberger, A. S. and Schaller, K. L. 1973. Compounds of value in different potato varieties with regard to manufacturing quality. I. The topographic distribution of phenolic compound in the tuber. Chemie Mikrobiologie technologie der Lebensmitted 2(2):39.

A.O.A.C. 1975a. Official Methods of Analysis. 12th ed. p. 123, 564, 7.003, 31.005. Assoc. of Official Analytical Chemists, Washington, D.C.

_____. 1975b. Official Methods of Analysis. 12th ed. p. 130, 7.016. Assoc. of Official Analytical Chemists, Washington, D.C.

_____. 1975c. Official Methods of Analysis. 12th ed. p. 135, 7.046. Assoc. of Official Analytical Chemists, Washington, D.C.

_____. 1975d. Official Methods of Analysis. 12th ed. p. 566, 31.013. Assoc. of Official Analytical Chemists, Washington, D.C.

_____. 1975e. Official Methods of Analysis. 12th ed. p. 138, 7.057. Assoc. of Official Analytical Chemists, Washington, D.C.

_____. 1975f. Official Methods of Analysis. 12th ed. p. 334, 22.045. Assoc. of Official Analytical Chemists, Washington, D.C.

Barfoed, H. C. 1976. Enzymes in starch processing. Cer. Food World 21(11):588.

Bodnar, D. A., Hinman, C. W. and Nelson, W. J. 1972. Method of making a starch conversion syrup. U.S. Patent 3,644,126.

Botle, R. T. and Gilbert, G. A. 1954. A magnification procedure for the estimation of the reducing end groups of the components of starch. Chem. and Ind. 39:1201.

Burton, W. G. 1966. The Potato. H. Veenman and Zonen N.V. Holland.

Cadwell, J. S., Brunstetter, B. C., Culpepper, C. W. and Ezell, B. D. 1945. Causes and control of discoloration in dehydration of white potatoes. Canner 100(13):25.

Cagley, P. M. 1972. Spray dryer increases production, produces wider variety of products, increases production 60 percent, operates continuously 2-5 days without cleaning. Food Processing 33(3):35.

Corn Refiners Assoc. 1965. Corn Syrup and Sugars. 3rd ed. Corn Refiners Assoc., Washington, D.C.

Dale, J. K. and Langlois, D. P. 1940. Starch conversion syrup. U.S. Patent 2,201,609.

de Becze, G. I. 1965. Enzymes, industrial. In Encyclopedia of Chemical Technology. Vol. 8, p. 173. John Wiley and Sons, Inc.

Denault, L. J. and Underkofler, L. A. 1963. Conversion of starch by microbial enzymes for production of syrups and sugars. Cereal Chem. 40:619.

de Stefani, A. I. and Ponte, J. G. 1968. Separation of sugars by thin layer chromatography. J. Chromatogr. 34:116.

Drusendahl, L. G. 1951. The use of corn syrup and corn syrup solids in ice cream. Ice Cream Trade J. 3:44.

_____. 1964. Guide for proper selection of your corn sweeteners. Ice Cream Trade Review 6:30.

Dukhanina, L. A., Sukhorukova, T. I., Lukyanov, A. B. 1970. Effect of alpha-amylase on non-gelatinized potato starch. Prikladnaya Biokkimiya i Mikrobiologiya 6:254.

Eichenberger, W. R. 1970. Dextrose manufacture. In Syrup Proc. Products of the Wet Corn Milling Industry in Food. Corn Refiners Assoc., Washington, D.C.

Fischer, E. H. and Stein, E. A. 1960. Alpha-amylases. In The Enzymes. Vol. 4, p. 313. Academic Press, N.Y.

_____. 1961. Alpha-amylase from human saliva. Biochem. Preparations 8:27.

Framkevicz, J. A. and Short, S. M. 1970. Fluorsilicate inactivation of alpha-amylases. U.S. Patent 3,513,072.

Gallant, D. L., Mercier, C. E. and Guilbot, A. S. 1972. Electron microscopy of starch granules modified by bacterial alpha-amylase. Cereal Chem. 49:354.

Greenwood, C. T. and Thompson, J. E. 1962. Physiochemical studies on starches. Part XXIV. The fractionation and characterization of starches of various plant origins. J. Chem. Soc. (1):222.

Greenwood, C. T. and Milne, E. A. 1968. Starch degrading and synthesizing enzymes. In, Advances in Carbohydrate Chemistry, Vol. 23, p. 281. Academic Press, N.Y.

Hansen, S. A. 1975. Thin layer chromatographic methods for identification of oligosaccharides in starch hydrolysates. J. Chromatogr. 105, 388.

Heisler, E. G., Treadway, R. H., Osborne, M. F. and Clennan, M. L. 1952. Enzymic hydrolysis of potatoes. Am. Pot. J. 29:37.

Hendel, C. E., Bwor, H. K. and Boogs, M. M. 1951. Factors affecting storage stability of potato granules. U.S. Dept. Agr., Bur. Agr. and Ind. Chem. Mimeo Circ. Ser. AIC-303.

Hoover, W. J. 1963. The technological development and increased usage of glucose syrup. Paper presented Amsterdarm Sept. 11. Corn Refiners Assoc., Washington, D.C.

_____. 1964. Corn sweetners with full spectrum of functional properties. Food Process. Catalog 1964-65 edition.

Huber, C. N., Scobell, H. D. and Fischer, E. E. 1968. Thin layer chromatography of the maltooligo and megalosaccharides with mixed support and multiple irrigations. Anal. Chem. 40:207.

Huber, C. N., Scobell, H. D. and Tai, H. I. 1966. Determination of saccharide distribution of corn syrup by direct densitometry of thin layer chromatograms. Cereal Chem. 43:342.

Hyndshaw, A. Y. 1975. Selecting a powdered activated carbon. Sugar y Azucar 70(5):44.

Junk, W. R. and Pancoast, H. M. 1973. Handbook of Sugars. p. 95. AVI Publishing Co., Westport, Conn.

Kask, K. A., Kestner, A. I. and Kipper, H. Y. 1969. Production of starch syrup from potatoes. Tr. Tallin. Politekn. Inst. Ser. A. 273:59.

Kipper, H.Y., Sildruk, A. G., Kask, K. A. and Kostner, A. J. 1969. Production of syrup from potatoes. Tr. Tallin. Politekn. Inst. Ser. A. 273:69.

Kirchhoff, G. S. 1811. Observations, experiences et notices interessantes faites et comuniquees a l'academie. Acad. Imperiale des Sci. de St. Petersborg. Memoires 4:27.

Knudsen, F. E. and Karkalas, J. A. 1969. Enzymic production of starch hydrolysates. Staerke 21(11):284.

Kulp, K. 1972. Physiochemical properties of starches of wheats and flours. Cereal Chem. 49:697.

_____. 1975. Carbohydrases. In Enzymes in Food Processing, p. 53. Academic Press, N. Y.

Leach, H. W. and Schoch, T. J. 1961. Structure of the starch granule. II. Action of various amylases on granular starches. Cereal Chem. 40:618.

Leach, H. W. 1965. Gelatinization of starch. In Starch, Chemistry and Technology. Vol. 1, p. 289. Academic Press, N. Y.

Lloyd, N. E. and Lodge, R. E. 1971. Stabilization of alpha-amylase preparations by incorporating high levels of calcium. U.S. Patent 3,524,798.

Lloyd, N. E., Lewis, L. T., Logan, R. M. and Patel, D. N. 1972. Process for isomerizing glucose to fructose. U.S. Patent 3,694,134.

Ludving, L. S. and Szatmazi, E. A. 1970. Enzymic production of starch syrup and investigation of the nature of sugars in the syrup. Szeszipar 18(5/6):129.

MacAllister, R. V., Warddrup, E. K., and Schmyder, B. J. 1975. Modified starches, corn syrup containing glucose and fructose and crystalline destrose. In Enzymes in Food Processing, p. 331. Academic Press, N.Y.

Manning, G. B. and Campbell, L. L. 1961. Thermostable alpha-amylase of Bacillus stearothermophilus. J. Biol. Chem. 236:2952.

Mansfield, C. T. 1973. Use of thin-layer chromatography in determination of carbohydrates. In Quantitative Thin Layer Chromatography," p. 79. John Wiley and Sons, N. Y.

Marnet, L. F., Selman, R. W., and Summer, R. J. 1948. A modified amylograph method for the rapid determination of flour amylase activity. Cereal Chem. 25:127.

McCready, R. M. and Hassid, W. Z. 1947. Separation and quantitative estimation of amylose and amylopectin in potato starch. J. Am. Chem. Soc. 65:1154.

McFarren, E. F., Brand, K. L. and Ruthowski, I. P. 1951. Quantitative determination of sugars on filter paper chromatograms. Anal. Chem. 23:1146.

Menzi, R. A., Stein, E. A. and Fischer, E. H. 1957. Propietes de deux alpha-amylases de Bacillus subtilis sur les enzymes amylolytiques. Helv. Chim. Acta 40:534.

Mermelstein, N. A. 1975. Immobilized enzymes produce high fructose corn syrup. Food Techn. 29(6):20.

Milder, E. G. and Bakema, K. I. 1956. Effect of the nitrogen, phosphorous, potassium and magnesium nutrition of potato plants on the content of free amino acid and on the amino acid composition of the protein of soil. Pl. Soil 7:135.

Miles Laboratories, Inc. 1976. Tenase. Technical information.

Miller, B. S., Johnson, J. A. and Palmer, D. L. 1953. A comparison of cereal, fungal and bacterial alpha-amylase as supplement for breadmaking. Food Techn. 7:38.

Morehouse, A. L., Guillenwater, D. L. and Murray, D. G. 1971. Preparation of low DE hydrolysates. Paper presented at 56th annual meeting of Am. Assn. of Cereal Chem.

Murray, D. G. 1969. New starch hydrolysates. Improve product formulation. Food. Eng. 41(6):87.

_____. 1969. Makes low DE cereal solids more versatile. Food Eng. 41(5):78.

Murray, D. G. and Ziemba, J. V. 1972. New starch hydrolysates spur product development. Food Eng. 44(3):88.

Murray, D. G. and Luft, L. R. 1973. Low DE corn starch hydrolysates. Food Techn. 27:32.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153:375.

Newton, J. M. 1970. Corn syrups. In Syrup Proc. Products of the Wet Corn Milling Industry in Food. Corn Refiners Assoc., Washington, D. C.

Neuberger, A. F. and Sanger, F. Y. 1942. Nitrogen of the potato. Biochem. J. 36:662.

Osman, E. M. 1967. Starch in the food industry. In Starch Chemistry and Technology. Vol. 2, p. 163. Academic Press, N. Y.

Ough, D. L. 1964. Chromatographic determination of saccharides in starch hydrolysates. In Methods in Carbohydrate Chemistry. p. 91. Academic Press, N.Y.

Pietrzak, R. and Niedzwiecka, J. 1973. Effectiveness of new types of amylolytic preparations for hydrolysis of potato mash. Prozemysl Fermentacyjny i Rolni 17:15.

Ramakrishnan, C. V. 1974. Use of enzymes in starch industry. J. Scient. Ind. Res. 33:143.

Robyt, J. F. and French, D. J. 1963. Action pattern and specificity of an amylase from B. subtilis. Arch. Biochem. and Biophys. 100:451.

Robyt, J. F. and Whelan, W. J. 1965. Anomalous reduction of alkaline 3,5, dinitrosalicylate by oligosaccharides and its bearing on amylase studies. Biochem. J. (95):10p-11p.

Robyt, J. F. and French, D. J. 1967. Multiple attack hypothesis of alpha-amylase action: Action of porcine pancreatic, human salivary, and Aspergillus oryzae alpha-amylases. Arch. Biochem. and Biophys. 122:8.

Robyt, J. F. and Whelan, W. J. 1968. The alpha-amylases. In Starch and Its Derivatives. p. 430. Chapman and Hall, London.

Sandstedt, R. M., Kneen, E. and Blish, H. J. 1939. A standardized Wohgemuth procedure for alpha-amylase activity. Cereal Chem. 16:712.

Schachtel, R. M., Braverman, J. B. S. and Groag, N. 1959. Non-enzymatic browning in commercial glucose syrup. Bull. Res. Council Israel 7:37.

Schwimmer, S. A. and Balls, A. K. 1949. Isolation and properties of crystalline alpha-amylase from germinated barley. J. Biol. Chem. 179:1063.

Schwimmer, S. A. 1953. Enzyme system of the white potato. J. Agr. Food Chem. 1(7):1063.

Schwimmer, S. A. and Bevenue, A. L. 1956. A reagent for the differentiation of 1,4 and 1,6 linked glucosaccharides. Science 123:543.

Schwimmer, S. A. and Burr, H. K. 1959. Structure and chemical composition of the potato tuber. In Potato Processing. p. 12, AVI Publishing Co., Westport, Conn.

Schuphan, W. E. 1970. Control of plant proteins, the influence of genetics and ecology of food plants. In Protein as Human Foods. p. 245. AVI Publishing Co., Westport, Conn.

Somogyi, M. F. 1945. A new reagent for the determination of sugars. J. Biol. Chem. 160:61.

Sroczynski, A. and Pierzgalski, T. 1972. Physiocochemical properties of enzymic starch hydrolysates. Roczniki Technologii i Chemii Zywnosci 22(2):173.

Stein, E. A. and Fischer, E. H. 1964. Alpha-amylases as calcium-metalloenzymes. I. Preparation of calcium free apoamylases by chelation and electrodialysis. Biochemistry 3:56.

Sweely, C. C., Bentley, R., Makita, M. and Wells, W. M. 1963. Gas liquid chromatography of trimethylsilyl derivatives of sugars and related substances. J. Am. Chem. Soc. 85:2497.

Takasaki, Y. and Tanabe, O. 1971. Enzyme method for converting glucose into glucose syrup to fructose. U.S. Patent 3,616,221.

Talley, E. A. and Porter, W. L. 1970. Chemical composition of potatoes. VIII. Effect of variety, location and year of growth on the content of nitrogen compound. Am. Pot. J. 46(1):231.

Thoma, J. A. and Spradlin, J. E. 1970. The action pattern of amylases. Brewer Digest 45(1):58.

Thomas, J. A., Spradlin, J. E. and Dygert, S. A. 1971. Plant and animal amylases. In The Enzymes. Vol. 5, p. 115. Academic Press, N. Y.

Touchtone, J. C. 1973. Quantitative thin-layer chromatography. John Wiley and Sons, N. Y.

Treadway, R. H. 1967. Manufacture of potato starch. In Starch Chemistry and Technology. Vol. 2, p. 87. Academic Press, N. Y.

Tressler, D. K. and Sultan, W. J. 1975. Food Processing Formulary. Vol. 2. Cereals, Baked Boods, Dairy and Egg Products. p. 271. AVI Publishing Company, Inc. Westport, Conn.

Vallee, B. L., Stein, E. A. and Summerwell, W. N. 1959. Metal content of alpha-amylases of various origins. J. Biol. Chem. 234:2901.

Vance, R. V., Rock, A. O. and Carr, P. W. 1972. Enzyme liquefaction using calcium salts, U.S. Patent 3,654,081.

Veselov, A. I., Uvarow, I. P. and Uznadke, E. I. 1970. Enzymolysis of potato starch by alpha-amylases at 0°C to -13°C. Prikladnaya Biokhimiya Mikrobiologiya 6(2):201.

Vecher, A. S. and Levitskaya, M. V. 1973. Change in the carbohydrate complex of potato pulp during its enzymic hydrolysis. Vestsi Akad. Navuk Belarus. SSR, Ser. Biyal. Nayuk (3):108.

Walker, G. I. and Hope, P. M. 1963. The action of some alpha-amylases on starch granules. Biochem. J. 86: 452.

Whistler, R. H. and Hickson, J. L. 1955. Determination of some components in a syrup by quantitative paper chromatography. Anal. Chem. 27:1544.

Zaborsky, O. 1973. Inmobilized enzymes. CRC Press, Cleveland, Ohio.

Zelenka, S., Malcher, J. A. and Medal, K. 1972. Enzymic hydrolysis of starch and its application in Czechoslovakian syrup production. Shornick Vysoke Skoly Chemicko-Technologicke Proze. E. 35:367.