AN ABSTRACT OF THE THESIS OF

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Title: THE ISOLATION AND CHARACTERIZATION OF THE PECTIC ENZYMES OF THE MC FARLIN CRANBERRY

Abstract approved: Dr. H. Y. Yang

Cranberries are processed mainly for the production of jelly, sauce and juice. The content of pectic substances in the cranberry gives the fruit a desirable property for processing such products. Pectic enzymes which catalyze the hydrolysis of pectic substances may affect the consistency of these products.

The concern of this study was to isolate and characterize the pectic enzymes that may be found in the cranberry fruit. The methods utilized in the preparation of enzyme extracts were by (1) the preparation of acetone powder (2) the preparation of acetone powder in the presence of polyethylene glycol and (3) by extraction in the presence of polyvinylpyrrolidone. The following conclusions were made:

(1) Cranberry protein extracts were found to exhibit polygalacturonase activity.

(2) The cranberry polygalacturonase extract obtained by the

use of polyethylene glycol exhibited a 40.3 percent loss in viscosity over one percent pectate solution in citrate buffer at pH 5.0 and 30°C during the initial hour of the reaction. A 22.1 and 8.6 percent loss in viscosity was found when the polyvinylpyrrolidone extract and the acetone powder extract were used as the source of enzyme respectively.

(3) Cranberry polygalacturonase may be classified as an endo- type polygalacturonase which catalyzes a random hydrolysis of both low and high methoxyl pectic substances.

(4) Maximum activity of the cranberry polygalacturonase was found to be at pH 5.0.

(5) Sodium chloride concentration up to 0.6 M showed no significant effect on the polygalacturonase activity in a citrate buffer at pH 5.0.

(6) The cranberry polygalacturonase was inactivated when exposed to 100°C for 35 minutes at pH 5.0.

(7) Cranberry proteins were found to possess low pectin esterase activity. Optimum activity of cranberry pectin esterase occurred at pH 7.5 with sodium chloride concentration at 0.15M.

(8) Cranberry pectin esterase was inactivated when exposed to 100°C for five minutes.

The Isolation and Characterization of the Pectic Enzymes of the McFarlin Cranberry

by

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THE ISOLATION AND CHARACTERIZATION OF THE PECTIC ENZYMES OF THE MC FARLIN CRANBERRY

INTRODUCTION

The texture of fresh and processed fruits and vegetables is of considerable value to the food processor and the consumer. The presence of pectic substances in the plant cell plays an important role in maintaining rigidity to plant tissues and a certain consistency to fruit purees and juices. Pectic enzymes act on the polymer constituting the pectin molecule to produce smaller fragments, thus disrupting the rigidity of the tissue or the consistency of the juice or puree. These enzymes are called polygalacturonase, pectic esterase, and pectin transeliminase. They may be inherent in the plant tissue or may be produced by different types of microorganisms. The desirability of the cranberry fruit lies in its high content of pectin, which facilitates the production of cranberry sauce and jelly. Pectic enzymes from the cranberry could bring about a catalytic effect on the hydrolysis of the pectins present. As a result, the nature of the product will be affected.

In spite of the undesirable action of pectic enzymes on the texture of processed plant tissue, this same property is of beneficial use in the preparation of fruit juices. The pectic enzymes may be added to pureed fruits to facilitate the expression of a higher yield

of juice. This is accomplished by the ability of the enzymes to act on the middle lamella of the fruit cell to cause the disruption of the cell wall. Pectic enzymes are also used to clarify wines and fruit juices.

This study was initiated to determine and characterize the nature of the pectic enzymes of the McFarlin cranberries. The study involves extraction of the enzymes by different methods followed by determining their activity under different conditions.

LITERATURE REVIEW

The following discussion presents a review about pectic enzymes and their distribution in nature. A general classification of the enzymes, their properties, mode of action, methods of preparation, and determination will be included. The review will also include a brief presentation on the chemical constitution of the substrates, the pectic substances, as well as definitions of their various types.

Pectic Substances

Chemical Constitution

Pectic substances are polymers whose major units are made of galacturonic acids, linked by α-1, 4-glycosidic linkages. Approximately two thirds of the carboxylic acid groups on the polymer are esterified with methanol (Reed, 1966). Rhamnose, arabinose, galactose, and traces of other sugars are found to be accompanying the pectin molecule or linked as side groups to the main chain (McCready and Gee, 1960; Jansen et al., 1949). However, enzymic analysis using partially reduced pectins indicated that the main chain probably does not contain sugars other than anhydrogalacturonic acid (Solms and Deuel, 1955).

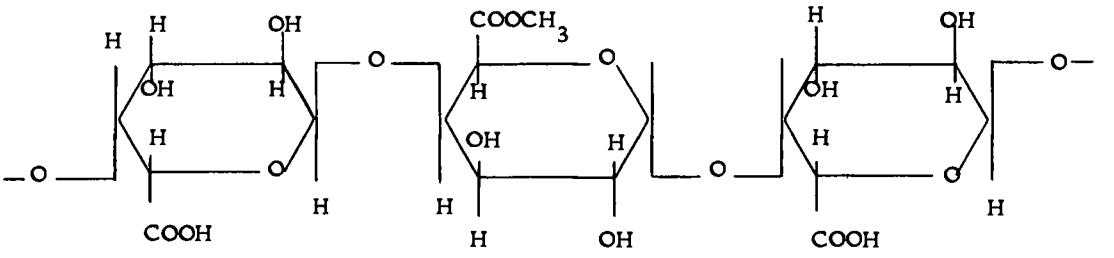

Figure 1. Schematic diagram of partially esterified polygalacturonic acid.

The degree of esterification represents the number of esterified carboxyl groups. When all carboxyl groups in pure polygalacturonic acids are all esterified, the methoxyl content is 16.32 percent and the degree of esterification is 100 percent (Doesburg, 1965).

Measurements of the molecular weights of pectic substances is made by osmotic pressure determinations, viscometric measurements and sedimentation velocity. The molecular weights from different sources range from 10,000 to 20,000 (Deuel and Stutz, 1958; Joslyn, 1962).

Both hydroxyl groups on carbon atoms 1 and 4 of the D-galacturonic acid molecule are in axial position making the pectin molecule a trans-α-1, 4-polysaccharide (Eliezen and Hyman, 1957), thereby hindering the free rotation at the glycosidic linkages and restricting flexibility. On the other hand, the secondary hydroxyl groups on carbon atoms 2 and 3, and the carboxyl group on carbon 5 are in equatorial position, making them easily accessible (Deuel

and Stutz, 1958). Historical and recent developments in extracting and characterizing the properties of pectic substances are reviewed by Kertesz (1952), Duel and Stutz (1958), Doesberg (1965), and Joslyn (1962).

Nomenclature

Due to past irregularity in the nomenclature of pectic substances, in 1944 the American Chemical Society adopted the following definitions (Baker et al., 1944).

Pectic Substances

Pectic substances is a group designation for those complex colloidal carbohydrate derivatives which occur in, or are prepared from, plants and contain a large proportion of anhydrogalacturonic acid units which are thought to exist in a chain-like combination. The carboxyl groups of polygalacturonic acids may be partly esterified by methyl groups and partly or completely neutralized by one or more bases.

Protopectin

The term protopectin is applied to the water-insoluble parent pectic substance which occurs in plants and which upon restricted hydrolysis, yields pectinic acids.

Pectinic Acids

The term pectinic acids is used for colloidal polygalacturonic acids containing more than a negligible proportion of methyl ester groups. Pectinic acids, under suitable conditions, are capable of forming gels (jellies) with sugar and acid or, if suitably low in methoxyl content, with certain metalic ions. The salts of pectinic acids are either normal or acid pectinates.

Pectin

The general term pectin (or pectins) designates those water soluble pectinic acids of varying methyl ester content and degree of neutralization which are capable of forming gels with sugar and acid under suitable conditions.

Pectic Acid

The term pectic acid is applied to pectic substances mostly composed of colloidal polygalacturonic acids and essentially free from methyl ester groups. The salts of pectic acid are either normal or acid pectates.

Classification of Pectic Enzymes

The importance of pectic enzymes in the food industry is of considerable value. In the limited observations on the formation of

pectic substances derived through the incorporation of various isotope-tagged compounds and groupings into pectins, Kertesz (1960) stated that we know little about the mechanism of pectin formation in plants and nothing about the enzymes responsible for their synthesis, especially those which partake in polymer formation. However, the information available concerning the enzymes which act on pectic substances is large. Demain and Phaff (1957), and Deuel and Stutz (1958) reviewed the literature of these enzymes. The two main groups of pectic enzymes have been described.

1. Depolymerizing enzymes which split the α-1,4-glycosidic bonds of pectic acid and pectinic acid. Enzymes responsible for hydrolyzing these bonds are referred to as polygalacturonases (PGs). These enzymes belong to the group called hydrolases because of their mechanism of action. Another enzyme causing depolymerization of pectic substances is called transeliminase. This enzyme is a depolymerase yet not a hydrolase. The mechanism of its action will be discussed under the heading Pectin Transeliminase.
2. The pectinesterases which act on pectins and pectinic acids by decreasing their degree of esterification and liberating equivalent amount of methanol and free carboxyl groups (Doesberg, 1965). The enzyme protopectinase is thought

to be responsible for the solubilization of the water insoluble pectic substance, the protopectin. However, there is no adequate indicative proof for the existence of protopectinase as a distinct enzyme. This will be discussed further under the heading Protopectinase.

Polygalacturonases (PGs)

Synonyms used in the literature include pectinase, pectolase, pectin glycosidase, and pectin depolymerase.

Mechanism of Action and Specificity

Polygalacturonases are regarded as hydrolases. A double replacement mechanism for the reaction is thought to be operative. According to this mechanism, the substrate is first attacked by a nucleophilic group of the enzyme , and subsequently the enzyme-substrate intermediate is attacked by the nucleophilic water molecules (Deuel and Stutz, 1958).

Many observations show that different polygalacturonases were found to act differently on pectic substances with different degrees of methylation and polymerization, and the extent of hydrolysis differ from one polygalacturonase to the other. Demain and Phaff (1957) based their classification of polygalacturonases on the substrate attacked, and the optimum pH values of the different

PG's. The latter criteria is not a popular one and is used only to a small extent. From the former criteria, it had been found that most of the pectic enzymes prefer either pectin or pectic acid as substrates (Demain and Phaff, 1957). The following schematic diagram suggests the possibilities of pectic substances break down by various types of polygalacturonases (Demain and Phaff, 1957).

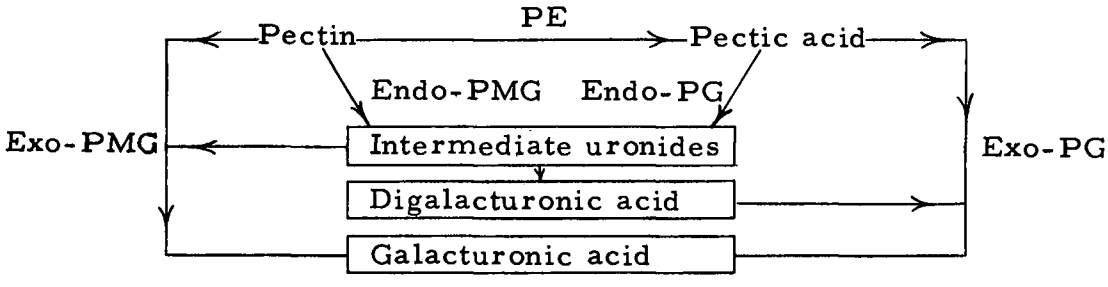

Figure 2. Mode of action of pectic enzymes

Random degradation of the pectin polymer is the most common type of breakdown encountered. However, a terminal mechanism has also been found during which galacturonic acid is liberated at the start of the reaction. A random type split is characterized by rapid lowering of viscosity and a slow increase in reducing power, while terminal attack causes very slow viscosity changes. The prefix "endo" has been applied to those enzymes carrying out random hydrolysis since the numerous bonds of the inner part of the chain are broken in preference to the few terminal linkages. Similarly "exo" refers to the enzymes causing terminal hydrolysis (Demain and Phaff, 1957). The following groups of polygalacturonases were

described by Demain and Phaff (1957):

Endopolymethylgalacturonase (Endo-PMG). This enzyme attacks pectin without previous deesterification, however, it is incapable of complete hydrolysis, not more than 26% of the total glycosidic bonds (Joslyn, 1962). As a result of this enzymatic hydrolysis, the hydrolysate probably contains uronides of 4 or 5 residues per molecule.

On the basis of optimum pH values of the enzyme, it was found that endo-PMG is of two different types, one that causes partial hydrolysis of pectins at pH 5.0 - 6.0 and called endo-PMG I, and the other causes the hydrolysis of pectin at pH 8.0 - 9.0 and referred to as endo-PMG II.

Endopolygalacturonase (Endo-PG). This enzyme carries out a random hydrolysis of pectic acid to the 70 percent level resulting in a mixture of digalacturonic acid and galacturonic acids (Demain and Phaff, 1957). Endo (1964 a, b, c) purified endopolygalacturonase from Coniothyrium diplodiella by ultra-centrifugation and free boundary electrophoresis. Three types of endo-polygalacturonases were identified (I, II, III). They differ by their degree of hydrolysis of pectic acids as well as their sedimentation coefficients, 2.68, 3.35, and 3.05 respectively. Optimum pH range was found to be between four and six. The action of endopolygalacturonase is very limited. The enzyme hydrolyzes pectic acid at least 5000 times as

fast as it does starch, maltose, sucrose, and carboxymethyl-cellulose (Lineweaver et al., 1949).

Exopolymethylgalacturonase (Exo-PMG). This enzyme hydrolyzes pectic substances only from one end of the chain molecule. They preferentially attack pectins with high degree of methylation (Deuel and Stutz, 1958). The breakdown of the pectin was found to go to completion. Matus (as cited by Demain and Phaff, 1957) in a study to differentiate between the two enzymes of fungal origin that hydrolyzed pectic acids and pectins from one end of the chain, found that as the degree of esterification was increased, the ratio of the enzymatic activity, in terms of liberated reducing groups to enzymatic activity in terms of viscosity also increased. The highly esterified compounds appeared to be attacked from the one end of the chain while random hydrolysis was the mechanism of pectic acid attack.

Exopolygalacturonase (Exo-PG). Compared to Exo-PMG, this enzyme attacks the pectic acid polymers and causes hydrolysis from the terminal end of the chain. Hydrolysis goes to completion by the liberation of galacturonic acid. Obtaining the enzyme from Aspergillus niger, Saito (1955) found evidence of the terminal attack by both paper chromatography studies as well as by comparing results of reducing group liberation and viscosity reduction. Endo (1964) extracting the enzyme from Coniothyrium diplodiella and purifying it

on DEAE cellulose and by column zone electrophoresis, found that it was most active in the pH range of 4.0 - 4.5 and that it was stable between the pH values of 3.0 and 6.0. Effect of temperature on exopolygalacturonase revealed that the enzyme was stable up to 55°C after which the activity tended to decrease. Endo (1964) also found that pectin was little affected by the same enzyme.

Preparation and Purification

Most of the polygalacturonases prepared for commercial use are obtained from microorganisms, mainly molds. Kertesz (1955) discussed the methods employed in the preparation and partial purification of these enzymes. However most of these preparations contained a mixture of polygalacturonases as well as pectinesterase. Pectinesterase can be inactivated from these preparations by allowing the crude enzyme extract to stand at pH 3.0 then adjusting the pH value to five. Ion exchange methods may also be used (Kertesz, 1951). Endo (1963) using column chromatography was able to fractionate different polygalacturonasesobtained from Coniothyrium diplodiella.

Stahmann (1963) reviewed several factors that make plant proteins unstable and difficult to deal with. The isolation of enzymes from plant tissue causes the dessembling of cellular components which brings about several chemical changes. Some plant tissues

contain a high concentration of tannins and that these polyphenols complex with proteins and cause their precipitation (Stahmann, 1963).

Loomis and Battaile (1966) reviewed the various problems caused by the interaction of plant phenolic compounds and proteins. Phenols combine with proteins in two fundamentally different ways, reversibly by hydrogen bonding, and irreversibly by oxidation of quinones followed by covalent condensations of the quinones with reactive groups on the protein molecule. Loomis (1968) discussed the principles of enzyme isolation from phenol containing tissues by the use of phenol complexing agents namely polyvinylpyrrolidone and polyethylene glycol. Any means of binding tannins (flavolans) and the higher molecular weight polyphenols, is considered an important extraction aid in isolating enzymes from plants containing these substances (Badran and Jones, 1965).

Methods of Determination

PG activity was formerly measured by determining the amount of substrate left unchanged. This method involves the precipitation and determination of the remaining polymers of the pectic substance. The drawbacks of this procedure lie in the time consuming nature of the steps involved.

The two methods most widely used in determining the activity of polygalacturonases employ (1) the measurements of reducing

groups found and (2) the change in physical properties. Polygalacturonase catalyzes the hydrolysis of the α-1, 4-glycosidic linkages in pectic polyurosides. Every fissure of such a bondage produces a reducing group which was previously engaged in the formation of the polymer. The hypiodite method described by Willstätter and Scherdel is used for this determination (Jansen and MacDonnell, 1945).

Viscosity changes have been extensively used in the determination of pectin-polygalacturonase activity. After about one fourth of all glycosidic linkages have been hydrolyzed, the viscosity scarcely changes. The major viscosity changes occur very early in the reaction. For this reason, the measurements of viscosity changes is a very sensitive indication of even traces of pectin polygalacturonase action (Kertesz, 1951). A random type split of the α-1, 4-glycosidic bondage is characterized by a rapid lowering of viscosity and a slow increase in reducing power, while terminal attack causes very slow viscosity changes, although reducing group formation may take place at the very same ratio (Demain and Phaff, 1957).

Effect of External Factors on PG Activity

Effect of pH. pH values for the different polygalacturonase have been used by Demain and Phaff (1957) as a minor criterion to

differentiate them. The literature contains data which indicates optimum ranges from pH 3.0 to 5.0 (Kertesz, 1951). However a few extremes have been reported for polygalacturonases extracted from several types of bacteria which possess optimum activity at pH values between 7.0 and 8.0, while that of Penicillium chrysogenum is rapidly inactivated at such pH values (Kertesz, 1951).

Effect of Temperature. The temperature optimum of polygalacturonase is greatly dependent on experimental conditions, especially the length of heating time. When short periods of heating are used the temperature optimum appears to be much higher than when longer periods of exposure are applied (Kertesz, 1951). Tomatoes may contain an unusually heat resistant pectinolytic factor, with 20 percent of the original activity remaining after a heat treatment of one hour at 100°C in extracts saturated with salt (McCollach and Kertesz, 1948). Fungal pectinases in the form of dry powder are quite resistant to heat (Kertesz, 1951). Purified polygalacturonases of fungal sources were found to be somewhat more sensitive to temperature than corresponding crude preparations. Optimum temperatures are in the range of 45 to 55°C (Rahmann and Joslyn, 1953).

Activation, Inhibition and Inactivation. Because of the known activating effect of alkali chlorides on the deesterifying enzyme, it is difficult to say whether the observed activation of pectin

polygalacturonase action as reported by Pallmann (cited by Kertesz, 1951) was direct or caused by enhancement of the simultaneous deesterification which in turn facilitated pectin polygalacturonase action. Since there are several complicating factors not entirely eliminated in the above studies, as well as complications that may arise by the effect of the cations on the substrate, these reports should be regarded with reservation (Kertesz, 1951; Schubert, 1954).

Rahman and Joslyn (1953) studied the inhibitory effect of mercurous chloride, sodium floride, iodoacetic acid, and sulfur dioxide on polygalacturonase. No apparent inhibition was found. Alkali sulfonate was found to be an effective inhibitor for polygalacturonase in Pectinol M added to cherries at pH 3.0 to 4.0 (Yang et al., 1960; Steele and Yang, 1960). The inactivation effect of the detergent alkali sulfonate is much less effective on polygalacturonase than on pectin methyl esterase (Kertesz, 1951).

Hathaway and Seakins (1958) using viscometric measurements showed that tannins exert a powerful inactivation on the early stages of pectinolysis. Also, a pectinase inhibition was found in sweet potatoes and grape leaves by Uritani and Stahmann (1961), and by Bell and Etchells (1958) respectively. The inhibiting substance was stable to heat, nondialyzable and could not be completely precipitated with acetone or concentrated ammonium sulfate. Later work revealed that the inhibitor is a tannin-like substance (Porter et al., 1961).

Exposure of polygalacturonase to high acidity as well as low acidity decreases its activity. At pH 0.6 and 25°C a solution of polygalacturonase loses 90 percent of its activity in 20 minutes. When present in 0.5N NaOH, the enzyme was completely inactivated at 20°C in 40 minutes.(Kertesz, 1951).

Occurence of Polygalacturonase

Polygalacturonases, of the various types classified earlier, are found in fungi, yeasts, bacteria, and plants (Reed, 1966). The tomato and avacado polygalacturonases were found to have their activity qualitatively similar to that of fungal preparations. Plant polygalacturonases have been studied and found in cucumber vines (Bell et al., 1955). The enzyme was also found in beans, radishes, carrots, and cherries by Konovalova and by Ozawa (as cited by Reed, 1966).

Previous Work on Cranberry Polygalacturonase

Hobson (1962) extracted the proteins from cranberries with 2.25 grams of a 10:1 (w/w) mixture of sodium chloried and disodium salt of ethylene diamine tetra acetic acid. The substrate used for the polygalacturonase assay was Wichmann pectic acid (Newbold and Joslyn, 1952). The procedure used was the colorimetric method based on that of Willaman and Davidson (1924). No activity of the

enzyme was detected. Patterson et al. (1967) in studying the effect of bruising a cranberry, found that softening results from endogenous enzymatic degradation of pectic substances in the cell wall. No enzymatic activity was found in unbruised tissues. The method employed was the cup-plate method developed by Dingle et al. (1953).

Pectin Esterase

Action and Specifity

This enzyme catalyzes the hydrolysis of the methyl ester groups of pectinic acids and pectin liberating equivalent amounts of methanol (Demain and Phaff, 1957). PE is a highly specific enzyme which saponifies almost exclusively the methyl ester groups of pectic substances (Deuel and Stutz, 1958). Matus, as cited by Demain and Phaff (1957), in order to characterize the different polygalacturonases, used glycol esters of pectic acid to make sure that PE does not effect the structure of the substrate because of its inability to deesterify glycol esters. MacDonnell et al. (1950) found that methyl esters of polymanuronic acid were not attacked. The hydrolysis of methyl esters of pectic acids proceeds linearly along the chain molecule as successive methoxyl groups are split off (Schultz et al., 1945; Solms and Deuel, 1954).

Determination of Pectin Esterase Activity

The two methods that are used to determine the activity of pectin esterase make use of either the methanol liberated or the carboxyl group exposed upon the deesterification of the methylester on the pectin molecule. Determining the amount of methanol liberated is a laborious procedure. The methanol should be distilled and measured either colorimetrically or by gas liquid chromatography. For every methanol molecule liberated one carboxylic group will become available. The activity of pectin methyl esterase is conventionally determined by measuring the commensurate increase in acidity. This is usually accomplished by continuous titration with a standard alkali while maintaining the pH of the substrate-enzyme mixture at a constant value (Somogyi and Romani, 1964).

Preparation and Purification

Plant pectin esterases are strongly adsorbed on the water-insoluble cellular constituents, and therefore plant juices expressed directly or after freezing contain only a fraction of the enzyme present in the tissue (Kertesz, 1955). The enzyme can be easily separated from the water insoluble cellular tissues by the use of salt solutions, raising the tissue macerate pH above five, the addition of soluble pectin to the extracting medium or a combination of

these procedures (Kertesz, 1951; Kertesz, 1955; Jansen and Jang, 1960). During dialysis of the extracted enzymes, they may precipitate. However, they may be redissolved in a small quantity of salt solution (Kertesz, 1955). The precipitation during dialysis does not apply to all the pectin esterases obtained from different sources (McColloch et al., 1946).

Effects of External Factors on Activity

Effect of pH. The pH optimum for pectin esterase of higher plants is influenced by the concentration and kinds of cations present (Kertesz, 1955). In salt-free solutions, the pectin methyl esterase activity is nearly zero at pH 4.0 and it increases rapidly as the pH is raised to 8.0 (McColloch and Kertesz, 1947). The activity of pectin esterase at pH 5.7 was found to be much higher in the presence of 0.2 M cations. However, cations have no effect on the activity between pH 7.0 - 8.0, its chief usefulness is to counteract adverse pH conditions (Holden, 1946; Lineweaver and Ballou, 1945; McColloch and Kertesz, 1947). The cation activation of pectin esterase is to reverse the inhibition of the anionic carboxyl group. Thus cations appear to prevent the formation of enzyme-carboxyl complexes (Lineweaver and Ballou, 1945).

Effect of Temperature. The PE of higher plants is comparatively heat resistant. Above 60°C the enzyme is gradually inactivated.

At pH 4.0 the enzyme solution had to be heated to about 80°C for inactivation (Kertesz, 1939). The pectin esterase of molds is much more sensitive to heat than that of higher plants (McColloch and Kertesz, 1948). Mold pectin esterase can be completely inactivated at temperatures which do not noticeably affect the activity of the tomato enzyme. Atkins and Rouse (1953, 1954) discussed the time temperature relationships for the inactivation of pectin esterases in citrus juices. Kohn (1953), found that pectin esterase is more sensitive to heat than polygalacturonase in studying the pectic enzymes in the clarification of apple juice.

Activation, Inhibition, and Inactivation. The activation of PE by cations have been mentioned earlier. Moreover, salt activation of this enzyme falls into two classes with respect to the value of the cation component. With bivalent cations and pH 6.0, maximum activation is produced in the neighborhood of 0.03 M. At higher concentrations, under the same pH the activity is suppressed. Monovalent cations produced maximum activation at pH 6.0 in 0.1 M concentrations and do not suppress activity below 1.0 M concentration (Kertesz, 1951).

The presence of salt in the enzyme-substrate systems prevents the inhibition of the enzyme by the free carboxyl groups present on the pectinic acid substrate as well as those formed during the reaction by the formation of cation-carboxyl complexes (Kertesz,

1951; Lineweaver and Ballou, 1945). The pectin methyl esterase of higher plants is unusually resistant to the effect of chemical agents such as formaldehyde, iodine, iodoacetic acid, cyanide, and mercurial compounds. However, synthetic detergents inactivate the plant PE irreversibly, but not the fungal PE (Doesburg, 1965; Rahman and Joslyn, 1953).

Sucrose exhibits inhibitory effect on pectin methyl esterase at levels that delay gelation (above 13% sucrose)(Chang et al., 1965).

Pollard et al. (1958) in an investigation of pectin changes in cider fermentation found that tannins from the apple tissue inhibits the activity of pectin methyl esterase at varying degrees.

Occurance of Pectin Esterase

The enzyme occurs commonly in roots, stems, leaves, and fruits of many higher plants and is also produced by microorganisms. MacDonnell et al. (1960) in comparing the pectin esterases extracted from alfalfa, tomato, orange and fungi found that they differ quantitatively. Pectin methyl esterase was found in bananas (Hultin and Levine, 1965; Hultin et al., 1966), cherries, apples, pears (Reed, 1966). Pectin esterases were also found in Erwinia carotovora and Pseusomonas pumicola (Mills, 1949). Fungal pectin esterase was detected by Reid (1950). The PE found in Penicillium chrysogenium was an inducible enzyme, the formation of which was

stimulated by pectic substances (Phaff, 1947). Several observations indicated that the enzyme PE is closely associated with the insoluble fruit tissue and its extraction is facilitated by the presence of cations and or neutral pH or soluble pectins (Janssen and Jang, 1960; Polland and Kieser, 1951).

The concentration of pectin methyl esterase in plants was found to change at different stages of maturity (Reed, 1966).

Pectin Transeliminase (PTE)

Albersheim et al. (1960) showed that commercial pectic enzymes contained a fraction that attacks pectinic acid to yield products which possess a double bond between carbon four and carbon five of the galacturonic acid unit, and absorbs ultraviolet light with a maximum at 235 mμ. Albersheim and Killias (1962) describe the procedure for the purification of the enzyme. The enzyme acts only at the non-reducing end of uronide chains and cleaves unsaturated dimers. The following reaction shows the mechanism of PTE. (See Figure 3) Extracting the enzyme from Bacillus polymyxa, Nagel and Vaughn (1961) found that degradation of trigalacturonate yields an unsaturated dimer and a monogalacturonate. Also a degradation of a tetramer yields an unsaturated trimer and a monogalacturonate, while at a slower rate, a normal dimer and an altered dimer are produced (Anderson, 1963).

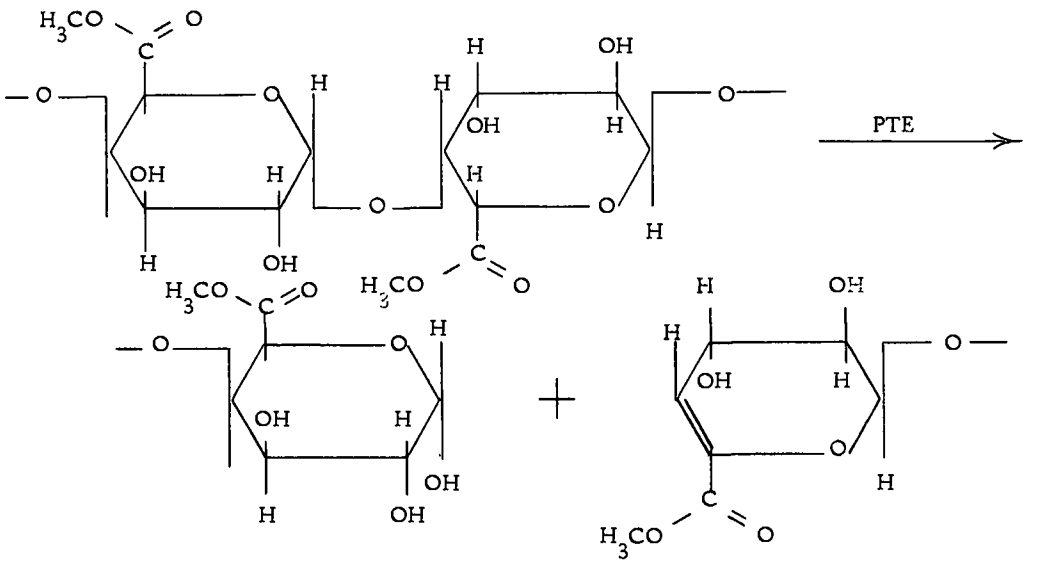

Figure 3. Mechanism of pectintranseliminase action

The optimum pH of the pectin transeliminase was shown to be between 5.1 and 5.3. Heating for a period of 20 minutes at 60°C resulted in a loss of 24 percent of the original activity (Albersheim et al., 1960).

Pectin transeliminase was detected in the following organisms: Clostridium multifermentans (MacMillan and Vaughn, 1964), Erwinia aroideae (Kertesz, 1955), Xanthomonas campestris (Starr and Nasuno, 1963) and in Pseudomonads (Preiss and Ashwell, 1963 a, b).

Protopectinase

The term protopectinase is applied to the enzyme which hydrolyzes or solvates protopectins to produce water soluble pectic

substances. Sloep (as cited by Doesberg, 1965) stated that the softening of fruits from Mespilus germanica was thought to be due to this enzyme. However, Roelfsen (1954) attributes this softening to enzymes other than a distinct protopectinase. Reed (1950) attributes the solubilization of protopectin to the combined action of pectin esterase and polygalacturonase that are bound to the cell tissue.

The Role and Importance of Pectic Enzymes on the Texture of Fruits and Vegetables

Pectic substances play an essential role in the desirable consistency of processed fruits and vegetables. The content of these substances and their properties which include solubilization and jellying is largely dependent on the action of pectic enzymes (McColloch and Kertesz, 1949). McCready and McComb (1954) investigated and characterized the changes that pectic substances undergo during ripening and found that the degree of methylation in peaches and pears drops from 86 percent in the unripe fruit to 40 percent after ripening. No polygalacturonase was detected in unripe fruits, but in ripe pears and avocadoes PG exhibited a 0.001 and 0.035 millimole of bonds split/hr/gram of fruit respectively. This suggests that pectic enzymes come in contact with the pectic

substances during ripening and hydrolyzes them, thus becoming less effective in maintaining a firm structure in the fruits and become less important in contributing to the consistency of processed foods.

Studies on the influence of ripening on the quality and quantity of pectins have been demonstrated in apples (Woodmansee et al., 1959), clingstone and freestone peaches (Postmayr et al., 1956), tomatoes (Kertesz, 1938), and Bartlett pears (Dome et al., 1956). These studies revealed that a significant increase in soluble solids during ripening occurred and that it was thought to be partly due to the conversion of insoluble protopectin to soluble pectins. There is little doubt that the transformation of protopectin to soluble pectin is enzymatically catalyzed (Reed, 1966). However, the fact that the enzyme protopectinase, which is defined as the enzyme responsible for the solubilization of protopectin, have not been isolated, Joslyn (1962) suggested that protopectinase and polygalacturonase are identical and that the solubilization of pectic substances is brought about by polygalacturonase.

In studying the effect of pectin methyl esterase in snap beans, Van Buren et al. (1962) found that the enzyme converts pectins to pectic acids which in the presence of cations forms pectates and consequently an increased firmness of the tissue was observed. At moderate blanching temperatures, the activity of the pectin esterase increases, resulting in an increased content in pectate which serve

as an intercellular cement.

The softening of canned apricots was studied by Luh and Dastur (1966), who explained the loss in firmness to the conversion of protopectin to water soluble pectin due to either enzymatic degradation or chemical hydrolysis.

The softening of cucumbers have been studies by Etchells et al. (1955) and found that a number of fungi (Mucor, Fusanium, Aspergillus) were growing on the cucumber flower and suspected them to be a primary source of the enzyme.

Steele and Yang (1960) found that "Cats Claws" disease of Bing and Lambert cherries contained a large concentration of polygalacturonase, which caused rapid softening of brined cherries when 10 percent by count of the infected cherries were brined with firm Bing cherries.

Other than the deteriorating effect of pectic enzymes, Schilt (as cited by Doesberg, 1965) reviewed the use of pectic enzymes for industrial, analytical, and pharmaceutical purposes. Pectin methyl esterase has been used in the production of low methoxyl pectins (Hill et al., 1949). Polygalacturonases are applied in fruit juice manufacturing. The enzyme when added to the milled pulp, facilitates pressing the juice from the fruits.

Polygalacturonases are widely used in the clarification of fruit juices and wines. Endo (1965 a, b, c, d, e) studied the classification

of apple juice by the joint action of polygalacturonase and pectin esterase. The mechanism of clarification was thought to be by solubilizing the insoluble pectin bound to the suspended particles, followed by a decrease in viscosity of soluble pectin, which results in the flocculation of suspended particles. For the clarification and pressing of fruit juices, it is desirable to produce preparations of pectinolytic enzymes which are balanced in their content of pectin esterases and polygalacturonases (Doesberg, 1965).

MATERIALS AND METHODS

This study was undertaken primarily to investigate the pectic enzymes in cranberries, namely polygalacturonase, pectin esterase, protopectinase, and transeliminase.

Phenolic compounds, namely tannins, are abundant in the nature cranberry and upon maceration of the berries for enzymes extraction, these compounds may react with the proteins reversibly by hydrogen bonding or irreversibly by oxidation thus affecting the activity of the enzymes. The methods used to avoid this phenomenon were by precipitating the proteins from the berries with acetone, combination of acetone and polyethylene glycol (Badran and Jones, 1965) and by extraction in the presence of polyvinyl pyrrolidone (Loomis, 1968).

The above extracted proteins were suspended in water, precipitated with $(NH_4)_2SO_4$, dialyzed against distilled water and then used to test the activity of the pectic enzymes.

Source of the Fruit

The McFarlin cranberries, Vaccinium macrocarpon, used for this study were grown in Markham, Washington. The ripe harvested berries were placed in polyethylene bags, sealed in tin containers, frozen and stored at a temperature of -20°C.

Extraction and Preparation of Cranberry Pectins

Preparation of Alcohol Insoluble Solids (AIS)

Frozen cranberries were blended in a Waring blender for 30 seconds with sufficient (95%) ethanol making the final concentration to be 70% alcohol (w/v). To inactivate the enzymes, the slurry was heated to 80.0°C (177°F) for 30 minutes. Hereafter the slurry was blended in a Waring blender for one minute and kept at 80°C for 30 minutes. After immediate cooling to room temperature the slurry was filtered through a nylon cloth, washed several times with 70 percent alcohol and the solids air dried. The air dried solids were suspended in ethanol to make the final concentration 60 percent alcohol (w/v). The suspension was allowed to stand overnight for 20 hours. The suspended slurry was stirred, then filtered on a Bücchner funnel, resuspended in acetone to make the final concentration 50 percent (w/v) and allowed to stand overnight. Thereafter, the suspension was filtered by suction through a Bücchner funnel, washed with acetone, and air dried.

Isolation of the Water Soluble Pectins

Twenty grams of the AIS were dispersed in 800 mls of water and stirred for 20 hours. Toluene was added at a one percent level as a bacterio-static agent. The suspension was then filtered through

a nylon cloth and the filtrate centrifuged at 1475 x G for ten minutes. This procedure was repeated six times until a negative anthrone test (Helbert and Brown, 1957) was evident. The supernatants were mixed and the water soluble pectins were precipitated by slow addition of 95 percent alcohol acidified with one percent hydrochloric acid until a final concentration of 60 percent alcohol was reached. The mixture was allowed to stand for 20 hours after which it was centrifuged. The precipitated pectin was washed twice with 70 percent alcohol and recentrifuged. The precipitated fraction was washed twice with acetone and air dried followed by drying at 60°C in vacuum oven over 29 inch gauge for 24 hours. The dried water soluble pectins were ground in a Wiley hammermill (screen 60). The powder was used as substrates in the viscometric studies to determine the polygalacturonase activity of the cranberry.

Isolation of Sodium Hexametaphosphate (Calgon) Soluble Pectins

The residue left after isolating the water soluble pectins was suspended in 800 mls of water containing 0.4 percent of hexametaphosphate. The suspension, with toluene added at the one percent level as a bacteriostatic agent, was stirred for 20 hours after which it was filtered through a nylon cloth followed by centrifugation at 1475 x G for ten minutes. This extraction procedure was repeated twice. Ethyl alcohol was added slowly to the supernatants to

accomplish the precipitation of the pectins until a final concentration of 60 percent alcohol was reached. The ethanol used was acidified with one percent concentrated HCl. The suspended material was allowed to stand overnight before centrifuging at 1475 x G for ten minutes. The precipitate was washed twice with 70 percent alcohol followed by washing with acetone and then air dried. The air dried hexametaphosphate (calgon) soluble pectins were dried at 60°C in a vacuum oven under 29 inches of vacuum, thereafter the dried material was ground in a Wiley hammermill (screen 60). This ground material was used as a substrate to determine the viscometric changes due to the cranberry polygalacturonase.

Methoxyl Content and Equivalent Weight of Pectic Substances

Equivalent Weight of Pectic Substances

The method employed for this determination was similar to that described by Owens et al. (1952). To an accurately weighed sample (approximately 0.500 grams) of the pectic substance, five mls of ethanol were added to enhance the solubilization of the pectin. One hundred mls of carbon dioxide free water were added and the solutions titrated slowly with 0.0497N NaOH to pH 7.5 using a Corning pH meter (model 7). Eq. wt. $= \frac{1000 \text{ x wt. of sample (grams)}}{\text{N x mls of NaOH}}$. The equivalent weight can be defined as the number of grams of

pure polygalacturonic acids which corresponds with an equivalent of free carboxyl groups.

Methoxyl Content of Pectic Substances

The method employed was that of Owens et al. (1952). To the neutralized solution used for equivalent weight determination, 25 mls of 0.2496N NaOH were added. The solution was allowed to stir for 30 minutes before an equivalent amount of standard hydrochloric acid (0.254N) was added from a buret. The solution was finally slowly titrated with 0.0497N NaOH to pH 7.5 end point. Percent of Methoxyl Content = $\frac{\text{N x mls of NaOH x 3.1}}{\text{wt. of Sample (grams)}}$.

Preparation of the Insoluble Residue of the Alcohol Insoluble Solids

After extracting the hexametaphosphate soluble pectins, the residue was suspended in distilled water, filtered through a nylon cloth to remove the hexametaphosphate. This procedure was repeated four times. The washed residue was then suspended in acetone, air dried followed by drying at 60°C in a vacuum oven under 29 inches of vacuum. The dried material was ground in a Wiley hammermill (screen 60) and stored in a screw cap jar to be used as substrate in studying the action of cranberry proteins.

Extraction and Preparation of Cranberry Proteins

Preparation of Acetone Powder

With all equipment pre-cooled at -20°C, a 100 grams of washed frozen cranberries were converted to pulp in a Waring blender at full speed for three minutes in the presence of acetone (-20°C), one to five w/v. The slurry was filtered by suction through Whatman No. 1 filter paper. The cake was then washed several times with 200 ml portions of cold acetone after which it was allowed to dry in a dessicator over concentrated H_2SO_4 under vacuum for 20 hours at room temperature. The acetone powder was stored in a tight flask at -20°C. The yield was 4.33 grams per 100 grams of frozen tissue.

Preparation of Acetone Powder in Presence of Polyethylene Glycol (PEG)

The method employed was similar to that of Badran and Jones (1965) modified as follows: 100 grams of frozen cranberries were added to 100 mls of two percent polyethylene glycol. Cold acetone (-20°C) was also added in the ratio of one to five (suspension:acetone). The mixture was blended in a Waring blender for three minutes at full speed. The suspension was filtered by suction through Whatman No. 1 filter paper. The cake was then washed with several volumes of cold acetone then dried over concentrated H_2SO_4 under vacuum at

room temperature for 20 hours. The powder was stored at -20°C.

Preparation of Cranberry Proteins in Presence of Polyvinylpyrrolidone (PVP)

The method employed was the modified procedure developed by Loomis (1968). One hundred grams of frozen cranberries were ground with a mortar and pestle using liquid nitrogen to arrest the biochemical reactions during the process and facilitate grinding. The liquid nitrogen frozen powder was suspended in citrate buffer pH 5.0 containing 100 grams of polyvinylpyrrolidone. The buffer was prepared by mixing 0.52 grams of citric acid and 8.68 grams of sodium citrate then diluting to 1000 mls. This suspension was stirred with a magnetic stirrer for 30 minutes then filtered through a nylon cloth. The residue was reextracted with 300 mls of buffer. The combined filtrates were centrifuged in a Surval centrifuge at 27,000 x G to remove the particulate matter. From the clear supernatant, the cranberry proteins were precipitated with ammonium sulfate (75 percent saturation).

Precipitation and Dialysis of Cranberry Proteins

Four grams of the acetone powder prepared were suspended in cold 800 mls of 0.15M NaCl solution and stirred gently for 30 minutes. The suspension was squeezed through a nylon cloth

followed by filtration through Whatman No. 1 filter paper. This procedure was repeated by resuspending the residue in 200 mls of 0.15M NaCl and the filtrates combined.

The solubilized proteins were precipitated by the slow addition of ammonium sulfate until a 75 percent saturation was reached. The suspension was allowed to stand for 18 hours. Having a density lower than that of the 75 percent saturated ammonium sulfate solution, the salted out proteins were skimmed off the top and dissolved in 40 mls of cold distilled water. The protein solution was dialyzed against distilled water in a cellophane tubing for 20 hours. All steps were carried out at 4°C.

The protein dialysate was used as the source of enzyme to determine the pectinolytic properties of cranberry proteins.

Nitrogen Determinations of Cranberry Protein Extracts

The micro-Kjeldahl method used for nitrogen determination was similar to that of the AOAC (1960). Two mls of the protein dialysate were pipetted into a 2.1 x 20 cm test tube. Two grams of K_2SO_4, 40 mgms of HgO, and two mls of concentrated H_2SO_4 were added. The mixture was digested for one hour after the solution became clear and then allowed to cool. Two mls of H_2O were added to dissolve the solids. The contents were transferred into a micro-Kjeldahl distillation apparatus. Eight mls of 50 percent NaOH

were added. The distillates were trapped in five mls of four percent boric acid solution containing two drops of bromocresol green and methyl red indicator, then titrated with a standardized HCl solution.

Viscometric Determinations to Characterize Cranberry Polygalacturonase

The procedure employed in this study was similar to that of Bell et al. (1955). A 1.2 percent solution of the pectic substance in a sodium hydroxide-citric acid buffer (pH 5) was prepared. The buffer was made by mixing two grams of NaOH and five grams of citric acid in 1500 mls of distilled water and adjusting the pH to five using a Corning pH meter (Model 7). Ten mls of this solution were placed in an Oswald viscometer and placed in a water bath maintained at 30 °C. Two mls of cranberry dialysates were added to the ten mls pectic substance solution making a final concentration of one percent. Flow time readings were made initially, and at different intervals for a period of 20 hours. The percent loss in viscosity was calculated as follows:

$$\frac{A-B}{A-W} \times 100 = \text{percent loss in viscosity}$$

A - Initial time of drainage
B - Time of drainage at end of period
W - Flow time of water

Bell et al. (1955) established a table to convert the percent loss in

viscosity to pectinolytic units. With 100 units of activity equaling 50 percent loss in viscosity of a one percent pectate-pectinolytic enzyme solution at 30°C and pH 5.0 for 20 hours. When the percent loss in viscosity values are plotted against the log of the concentration or of the time, the data are nearly linear except below the ten percent level. The relative viscosity was calculated as follows: $\frac{n_1}{n_2} = \frac{d_1 t_1}{d_2 t_2}$ with n_1, d_1, t_1 corresponding to viscosity, density, and flow time of the sample and n_2, d_2, t_2 represent the values for distilled water.

Determination of pH Optimum for the Polygalacturonase Activity

Sodium hydroxide-citric acid buffers having different values of pH were prepared from the previously discussed buffer preparation by adjusting the values with dilute solutions of sodium hydroxide and citric acid as necessary. Solutions of the pectic substances were made using the buffers with different pH values and viscometric changes were determined at 30°C using the protein dialysate preparation described previously as an enzyme source.

Effect of Cranberry PG on Different Pectic Substances

Four different types of pectic substances were used as substrate on which the activity of cranberry polygalacturonase was

determined. Two of the substrates were of citrus origin obtained from Sunkist Growers Inc., Sodium polypectate No. 6024 and Citrus Pectin No. 3442. The remaining two were the water soluble and the hexametaphosphate soluble pectins extracted from the cranberries. Solutions of 1.2 percent of the four different substrates were prepared in sodium hydroxide : citric acid buffer pH 5.0. Using two mls of the cranberry protein dialysate as the enzyme source and ten mls of the substrate, viscosity determinations were made initially and at intervals over a period of 20 hours. The substrate-enzyme mixtures were maintained at 30°C.

Effect of Salt Concentration on PG Activity

Using 0.2, 0.4, and 0.6M NaCl pH 5 buffers, a 1.2 percent Sunkist polypectate solution was prepared. With these solutions as substrates and two mls of the protein dialysate as enzyme, viscometric changes were made to determine the effect of NaCl on the pectinolytic activity of cranberry proteins initially and after 20 hours at 30°C.

Effect of Heat on Polygalacturonase Activity

Aliquotes of the protein dialysate adjusted to pH 5.0 with citrate buffer were placed in test tubes and heated at 100°C for the following periods of time: 5, 10, 15, 25, 35, and 45 minutes. Immediately

after the lapse of each period, one tube was removed and immersed in crushed ice. These heat treated samples were used to study the effect of heat on the polygalacturonase activity in cranberry proteins. The viscometric procedure as described earlier was used to determine the effect of heat on cranberry polygalacturonase.

Action of Cranberry Proteins on the Insoluble Residue of the Cranberry AIS

The method used was described by Gizis (1964). Three grams of the cranberry insoluble residue powder were suspended in 150 mls of citric acid - NaOH buffer pH 5.0. To half the suspension, four mls of cranberry protein dialysate were added while four mls of buffer were added to the other. The samples were placed in a shaker-incubator at an adjusted temperature of 30°C. Aliquotes were removed, filtered through Whatman No. 1 filter paper at initial and different time intervals to determine the concentration of soluble pectates present. The method employed was similar to that of Dubois et al. (1956). One ml of the filtrate was diluted to 25 mls with distilled water in a volumetric flask. One ml of the diluted sample was analyzed for the soluble carbohydrates present.

Determination of Pectin Esterase in Cranberries

The assay method used was described by Kertesz (1955). Fifty mls of one percent pectin solution made 0.15M NaCl, then adjusted to

pH 7.5, were placed in a 100 ml beaker attached to a constant temperature water bath. Five mls of enzymic solution was added to the beaker and the pH immediately readjusted to pH 7.5 and the time noted. The solution was maintained at pH 7.5 by titration with a 0.05M NaOH.

The activity was expressed in micro equivalents of ester hydrolyzed per gram of acetone powder per hour.

Preparation of Cranberry PE

A 0.25 grams of acetone powder whose method of preparation was described in an earlier section were dispersed in ten mls of 0.15M NaCl and stirred continuously for fifteen minutes. The dispersion was centrifuged at 25,000 x G for 15 minutes and the supernatant was used to determine the activity of pectinesterase.

Determination of the Optimum pH

To determine the pH optimum for cranberry pectin esterase, the assay was carried by measuring the number of carboxyl groups liberated as a result of the deesterification of the methyl ester on the pectin molecule by pectin esterase. The pH values 5.0, 6.0, 7.0, 7.5, and 8.0 in the enzyme-substrate system were maintained by continuous titration with a standardized NaOH solution. A one

percent Sunkist Pectin (3442) was used for substrate and maintained at 30°C.

Effect of Salt Concentration on PE Activity

NaCl was added to 50 mls of one percent Sunkist pectin (3442) solutions making a final concentration of 0.15 and 0.30M. One sample contained no NaCl. These were used as a substrate to determine the effect of salt on PE activity at pH 7.5 and 30°C.

Transeliminase Activity of Cranberry Proteins

The procedure followed was described by Albersheim et al. (1960). The activity was followed by measuring the changes in light absorption on a Beckman DB spectrophotometer at 235 mμ of a 0.5 percent pectin solution (Sunkist 3442) at a pH of 5.2 in the presence of the enzyme. The dialysate of the ammonium sulfate precipitate was used as the enzyme source. The buffer was a 0.1M citrate-phosphate prepared by mixing equivalent amounts of Na_2HPO_4 and citric acid then adjusting the pH to a value of 5.2. A unit of pectin transeliminase was defined as the amount of enzyme in 0.1 mls of protein dialysate which will cause the absorption of light at 235 mμ by 2.0 ml of a 0.5 percent citrus pectin solution contained in a one cm cuvette to be increased by one optical density unit in one minute.

Fractionation of Cranberry Proteins on DEAE Cellulose Column Chromatography

Twenty-five grams of DEAE cellulose obtained from Eastman Organic Chemicals were dispersed in 500 mls of 1N NaOH and stirred for 30 minutes. The suspension was filtered through a Büchner funnel and washed with 1N NaOH until the filtrates were colorless. Sufficient 1 N NaOH was added to make the suspension strongly acidic. This was then filtered and washed with distilled water until all the acid was removed.

The DEAE cellulose was then resuspended in 1N NaOH solution, filtered, and washed free of alkali with water before being adjusted to the pH of the selected buffer (Peterson, 1962). The cellulose was then placed on a Büchner funnel and washed several times with the starting buffer pH 5.0 prepared by diluting five grams of citric acid and two grams of NaOH and making the volume to 1500 mls. The washed DEAE cellulose was resuspended in the same buffer solution, stirred then allowed to settle thus making possible the removal of the fine particles by siphoning. This procedure was repeated several times until the supernatant solution was almost clear. The suspension was placed in a two liter bottle with a nozzle on the lower rim. Then the suspension was diluted with the starting buffer making a final concentration of about 60 mls per gram of cellulose and continually stirred on a magnetic stirrer. The

suspension was then allowed to drip into a glass chromatographic column (2 x 50 cm) at the bottom of which a compressed glasswool plug was placed topped by a stainless steel screen (80 mesh) then filled with the starting buffer. The cellulose was allowed to settle by gravity. The column was equilibrated overnight with the starting buffer at 4°C, then mounted on a fraction collector equipped with ten ml volumetric siphon. Eighteen mls of protein dialysate (Method of preparation was discussed earlier) were carefully added to the column and a sodium chloride gradient elution was started. The elution gradient was prepared by placing 50 mls of 0.01, 0.05, 0.10, 0.30, and 1.0M NaCl citric acid, NaOH buffer solution pH 5.0 in the chambers of a Buchler Varigrad in an increasing order. The rate of elution was 60 mls per hour. The gradient was then followed by the addition of 200 mls of 1.0M NaCl to the last chamber. Absorbancy of the fractions was measured on a Beckman DB spectrophotometer at 280 mμ. The fractions were then tested for the polygalacturonase activity by the viscometric method. Sodium chloride concentration in the fractions was determined by the Chromate Indicator method (Rich, 1963). The column used was 2 x 27 cm.

RESULTS AND DISCUSSION

This research was designed to determine the nature and activity of the pectinolytic enzymes as extracted from the McFarlin cranberry. Since the method of extracting the enzymes affect their activity, three methods of extraction were investigated. The extracted enzyme preparations were further fractionated on a DEAE cellulose column chromatography and the polygalacturonase activity of each fraction was determined. Viscosity changes caused by the cranberry polygalacturonase were determined on citrus pectin, citrus polypectate as well as on both water soluble pectins and hexametaphosphate soluble pectins extracted from the cranberries.

Analysis of Substrates

The four types of pectic substances, their methoxyl content, and equivalent weight values used for substrates in this study are shown in Table 1.

Table 1 shows that a difference exists between citrus and cranberry pectins, both in methoxyl content and equivalent weight values. There is considerable variation in the methods used to extract pectic substances from fruits. The procedure used and the stage of maturity of the fruit have a large effect on the physical and chemical properties of the pectin molecules.

Table 1. Methoxyl content and equivalent weight values of citrus and cranberry pectic substances

Substrate	Methoxyl Content %	Equivalent Weight
Sunkist Pectin 3442	8.74	1529.9
Sodium Polypectate 6024	1.22	8383.8
Cranberry Water Soluble Pectins	5.86	2109.1
Cranberry Calgon Soluble Pectins	4.50	296.7

Extraction, Precipitation and Dialysis of Cranberry Proteins

Three different methods for extracting the cranberry proteins were used. The first method was by the preparation of cranberry acetone powder at -20°C. The second procedure was similar to that reported by Badran and Jones (1965) in which an acetone powder of the cranberries was prepared in the presence of polyethylene glycol at -20°C. The third method employed was similar to that of Loomis and Battaile (1966) in which polyvinylpyrrolydone was utilized. The protein in the latter method was extracted in citrate buffer pH 5.0. In the first two methods, the protein was solubilized in water from the prepared powder. The three extracted protein fractions were precipitated by ammonium sulfate at 75 percent saturation then dialyzed against distilled water for 20 hours. The dialysate was

used to study the polygalacturonase activity.

Determination of Cranberry Polygalacturonase Activity

Effect of Extraction Procedure on PG Activity

Following the viscometric changes of a one percent pectic substance in a sodium hydroxide-citric acid buffer at pH 5.0 and 30°C proved to be a sensitive method in determining the activity of polygalacturonase in cranberry proteins. The determination of the number of reducing groups formed as a result of polygalacturonase activity was found to be very slow and was abandoned for the former viscometric method. The viscometric method is particularly sensitive to detect endo-polygalacturonases activities which attack the pectin molecule at random. In an enzyme-substrate system, pectin solutions show a large decrease in viscosity and a small increase in reducing groups during the initial stage of degradation (Demain and Phaff, 1957; Deuel and Stutz, 1958).

Table 2 shows the loss in viscosity of a 1.0 percent sodium polypectate in NaOH-citric acid buffer (pH 5.0) at 30°C for a period of 20 hours using the three different types of protein extracts as enzyme sources. The same table also shows that the proteins extracted by acetone in conjunction with polyethylene glycol has a higher activity than the proteins extracted either by acetone alone

or by the method which employed polyvinyl pyrrolidone. Non-enzymatic hydrolysis of the substrate was evident in the blank samples where no protein was added to the system.

The amount of nitrogen per two milliliters of dialysate obtained from the method in which PVP was employed was higher than the dialysate obtained when acetone-polyethylene glycol was used in the preparation. The lowest value was obtained from the acetone powder dialysed extract.

The initial rates presented in Table 2, when conditions of the substrate-enzyme systems are more uniform and better known, gave a better concept of the different activities for the three different enzyme extracts. The initial rate corresponds to the change occurring in the first hour of the reaction. The activity of the acetone-polyethylene extracted proteins show approximately 4.7 and 1.8 fold increase over the acetone powder and polyvinyl pyrrolidone extracted proteins respectively.

Figure 4 shows the course of action of the three different enzyme extracts on one percent sodium polypectate solution at pH 5.0 and 30°C. After a loss of 40-60 percent of the viscosity, the rate of the reaction tended to decrease slowly until no significant change in the rate of viscosity change was observed.

Table 2. Action of cranberry enzyme extracts at pH 5.0 and 30°C. One percent sodium polypectate was used as substrate (means of five determinations).

Method for Enzyme Extraction	% loss in viscosity			Initial rate % loss/hour	mg N_2/2 ml Dialysate
	Enzyme Added	Blank	Difference		
Acetone Powder	69.0	7.4	61.6	8.6	0.154
Acetone - PEG	94.5	7.4	87.1	40.3	0.191
PVP	88.0	7.9	81.1	22.1	0.214

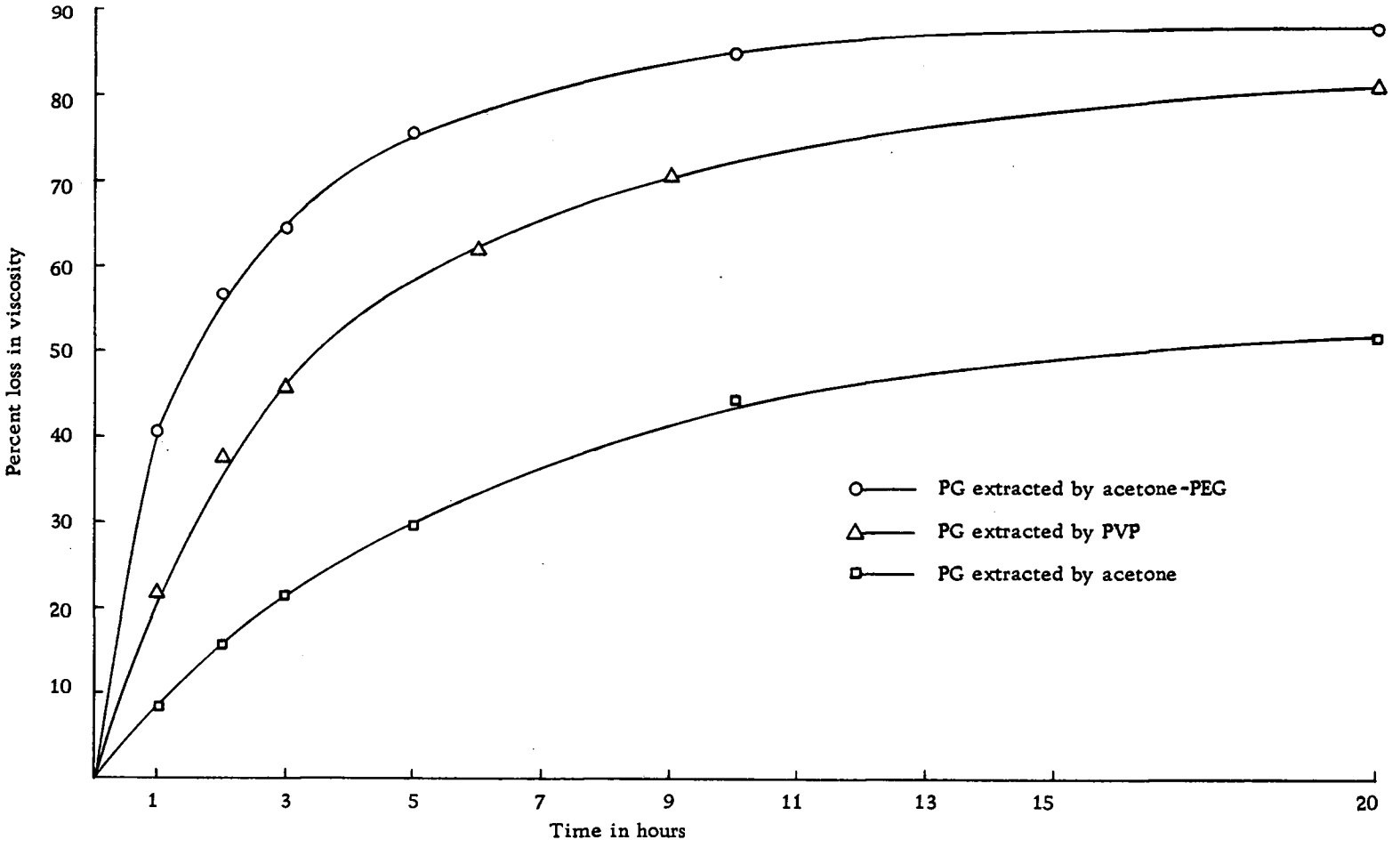

Figure 4. Percent loss in viscosity of one percent sodium polypectate solution at pH 5.0 and 30°C, using cranberry polygalacturonase prepared by three different methods.

pH Optimum for the Cranberry Polygalacturonase Activity

Studies to find the pH optimum were determined using 0.25 percent sodium polypectate solution at 30°C and maintaining constant pH values of 3, 4, 4.5, 5, 5.5, 6, and 7. Two milliliters of one to two dilution of the protein dialysate prepared by acetone and polyethylene glycol were used for an enzyme source.

Figure 5 shows the percent loss in viscosity of the substrate due to the pectinolytic action of cranberry proteins over a period of one hour at the different pH values. The highest activity was observed at pH 5.0. Moreover, at pH values below five, the activity decreased slowly while at pH values above 5.0, the activity of the enzyme dropped sharply with almost complete loss in activity at pH 7.0.

The use of a one percent solution of the substrate to determine pH optimum was abandoned because of the jellying effect at pH values below four. Figure 6 shows that pectate molecules were subjected to hydrolysis in the absence of enzyme preparation and this effect increased as the pH value decreased.

Effect of Salt Concentration on Polygalacturonase Activity

Kertesz (1952) reported that salt may have some influence on some polygalacturonase preparations. The effect of sodium chloride

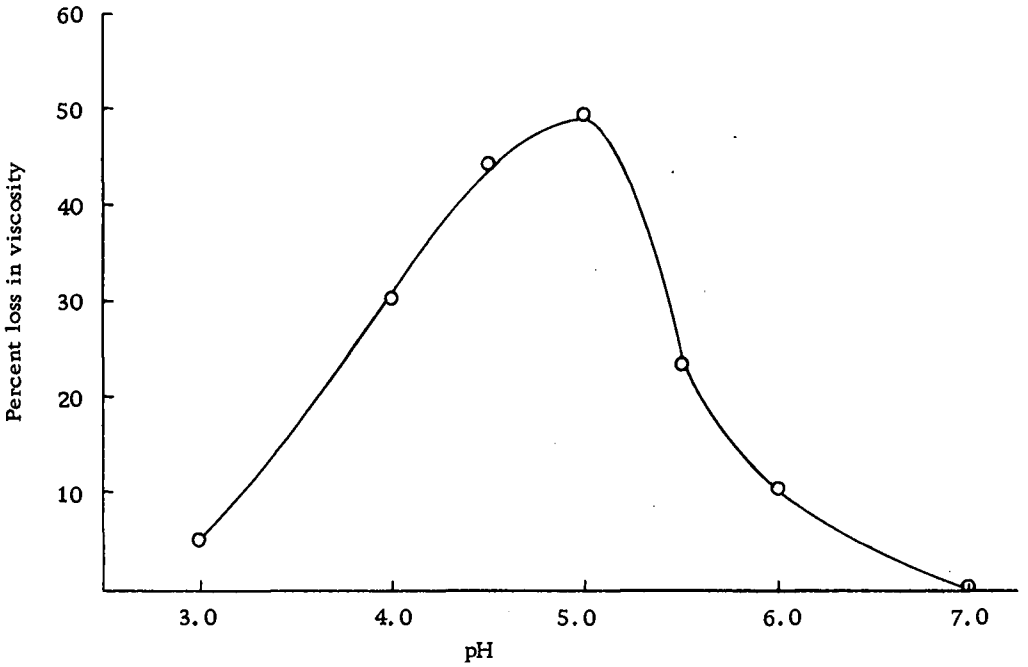

Figure 5. Effect of pH on cranberry polygalacturonase activity on sodium polypectate at 30°C.

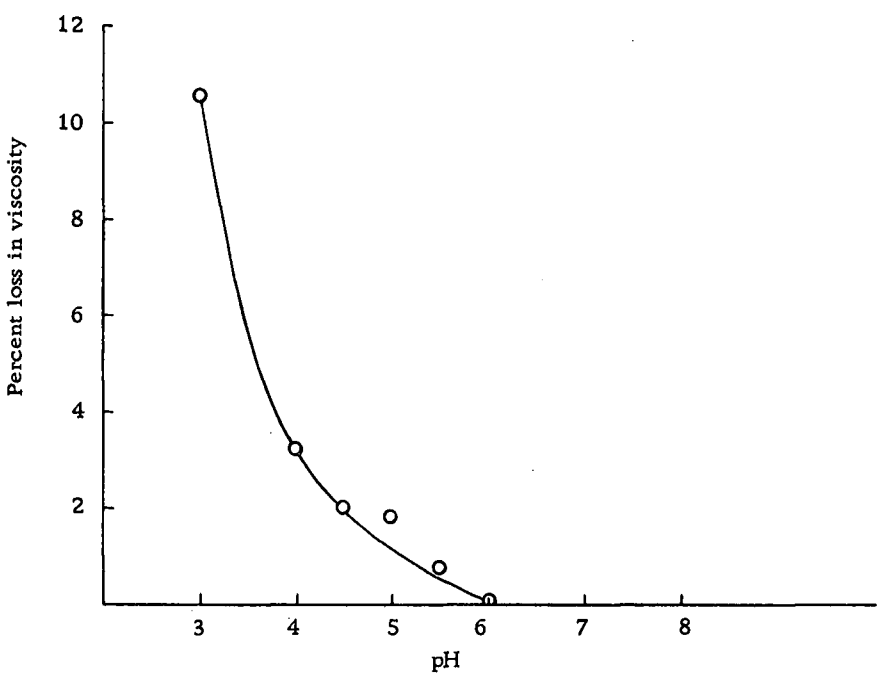

Figure 6. Percent loss in viscosity of 0.25 percent sodium polypectate solution at different pH and 30°C.

on the cranberry polygalacturonase was determined by using 0.2, 0.4, and 0.6M of the salt in the enzyme-substrate system. Table 3 shows the percent loss in viscosity of one percent sodium pectate solutions at pH 5.0 and 30°C using different salt concentrations for a period of 20 hours. The data on Table 3 indicate that sodium chloride influence on the cranberry pectinolytic activity was very small. Initial rate values corresponding to the change in viscosity during the first hour of the reaction were found to be insignificant. The sodium ion concentration in the buffer used was 0.033M.

Table 3. Effect of NaCl Conc. on the Pectinolytic Activity of Cranberry Proteins (means of five determinations)

NaCl Added (Moles/liter)	% loss in Viscosity over a 20 hr period	Initial Rate	Standard Deviation
0.00M	87.1	40.3	0.22
0.20M	85.8	39.7	0.98
0.40M	85.7	39.2	1.04
0.60M	85.7	39.5	1.46

Polygalacturonase Activity of Cranberry Proteins on Different Pectic Substances

The activity of cranberry proteins extracted by the method employing acetone and polyethylene glycol was determined using the following four different substrates, sodium polypectate (Sunkist 6042), citrus pectin (Sunkist 3442), cranberry water soluble pectin

and cranberry sodium hexametaphosphate (calgon) soluble pectins.

Figures 7, 8, 9, and 10 show the course of action of cranberry polygalacturonic enzyme on the four different substrates by determining the change in viscosity of a one percent substrate concentration over a period of 20 hours, at pH 5.0 and 30°C. Two milliliters of cranberry protein dialysate corresponding to 0.19 milligrams nitrogen was used as the enzyme source. A citric acid - NaOH buffer solution pH 5.0 was used in the preparation of the enzyme-substrate system. The system contained toluene at the one percent level as a bacteriostatic agent.

Table 4 shows that the percent loss in viscosity over a period of 20 hours using cranberry protein dialysate as enzyme source and one percent solutions of the four different substrates at pH 5.0 and 30°C.

The catalytic effect of cranberry proteins exhibited a decrease in viscosity on the four different substrates used. The activity of the polygalacturonase was slightly higher on citrus sodium polypectate than citrus pectin. This may be due to the lower degree of methoxyl content in the former substrate.

Also, a slightly higher activity of the enzyme was exhibited on the cranberry calgon soluble pectins over that of the water soluble pectins. The calgon soluble pectins possess a lower methoxyl content. A significant difference in the catalytic effect of cranberry

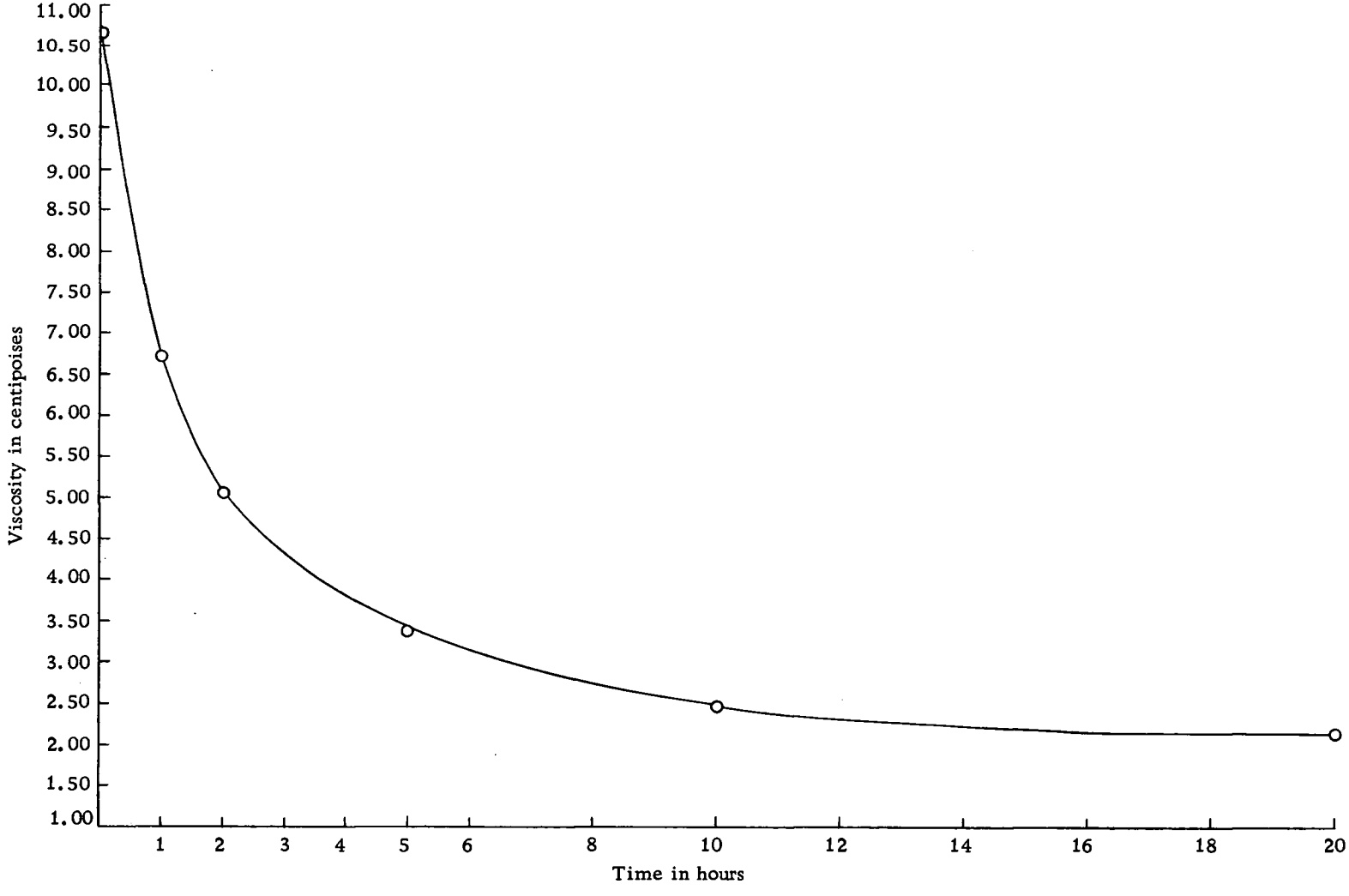

Figure 7. Change in viscosity of sodium polypectate (Sunkist 6042) solution due to the action of cranberry polygalacturonase at pH 5.0 and 30°C.

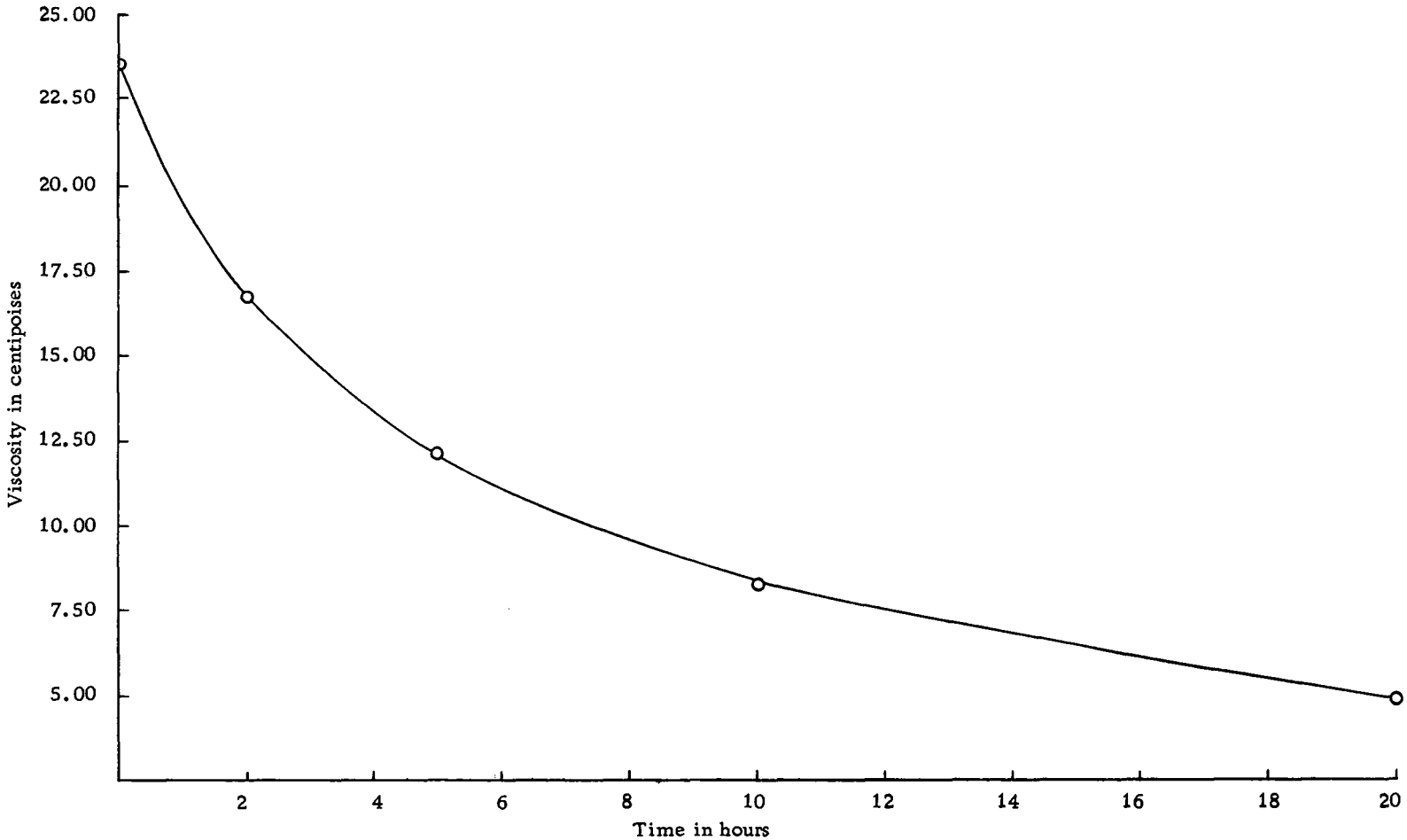

Figure 8. Change in viscosity of citrus pectin (Sunkist 3442) due to the action of cranberry polygalacturonase at pH 5.0 and 30°C.

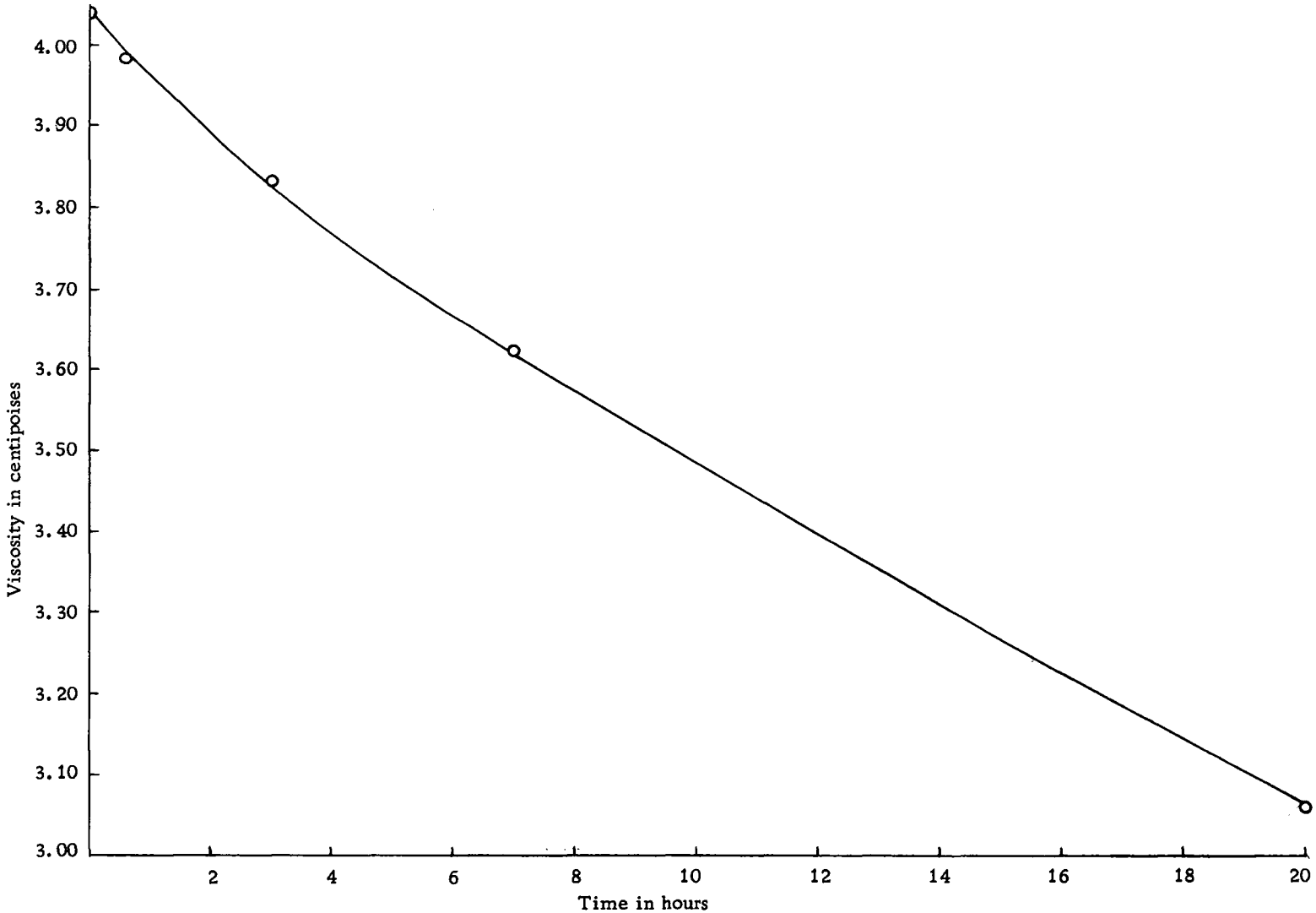

Figure 9. Change in viscosity of cranberry water soluble pectin due to the action of cranberry polygalacturonase at pH 5.0 and 30°C.

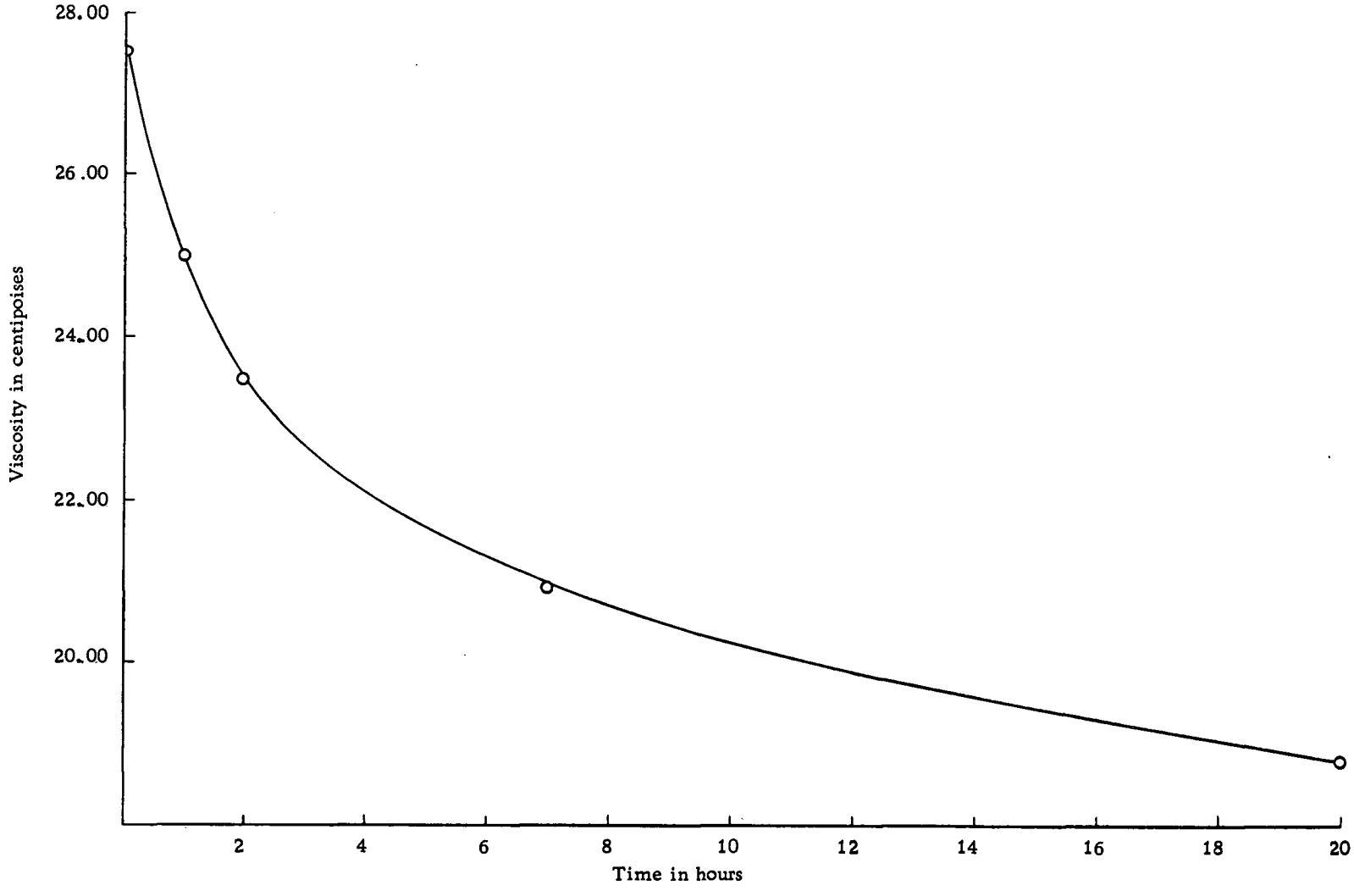

Figure 10. Change in viscosity of cranberry calgon soluble pectin due to the action of cranberry polygalacturonase at pH 5.0 and 30°C.

proteins was evident between cranberry and citrus pectins. The pectinolytic enzyme showed a higher activity rate when the latter was used for substrate. This may be ascribed to the difference in the chemical structure of both substrates. Three of the four blank samples, where no protein was added, exhibited approximately similar losses in viscosity. However, citrus pectin blank sample showed a lower loss than the other three substrates. This may be attributed to the high methoxyl content of the citrus pectin which may interfere by decreasing the rate of acid hydrolysis (Gizis, 1964).

Table 4. Percent drop in viscosity of one percent substrate solutions after 20 hrs. at 30°C

Substrate	Percent Loss in Viscosity (Means of Five Determinations)			
	Protein Dialysate Added	Blank	Difference	Standard Deviation
Sodium Polypectate (Sunkist 6042)	94.5	7.4	87.1	0.22
Citrus Pectin (Sunkist 3442)	86.1	3.7	82.1	0.42
Cranberry Water Soluble Pectins	38.2	8.0	30.2	0.17
Cranberry Calgon Soluble Pectins	42.0	8.2	33.8	0.17

Effect of Cranberry Protein Concentration on Polygalacturonase Activity

This study was conducted to find the relationship between the enzyme concentration and the course of activity of its pectinolytic property. The different protein concentrations used in this study corresponded to 19.1, 9.55, 4.77, and 2.38 x 10^{-2} milligrams of nitrogen. One percent sodium polypectate solution was used as substrate at pH 5.0 and 30°C. Figure 11 shows the course of action of the different protein concentrations over a period of 20 hours. Figure 12 shows the relationship between protein concentration against the initial rates exhibited by the four different concentrations used. The initial rate corresponds to the change occurring during the first hour of the reaction. When the rate of activity of the enzyme results in a viscosity loss over 15 percent per hour, a linear relationship existed.

Heat Inactivation of Cranberry Polygalacturonase

The effect of heat on the stability of cranberry pectinolytic enzyme was studied by exposing the protein dialysate extracted by acetone-polyethylene glycol for different time intervals at 100°C. The percent loss in viscosity of a one percent sodium polypectate solutions in citric acid-NaOH buffer pH 5 and 30°C was determined using the heated protein samples as the source of enzyme. The blank samples contained two milliliters of buffer instead of protein

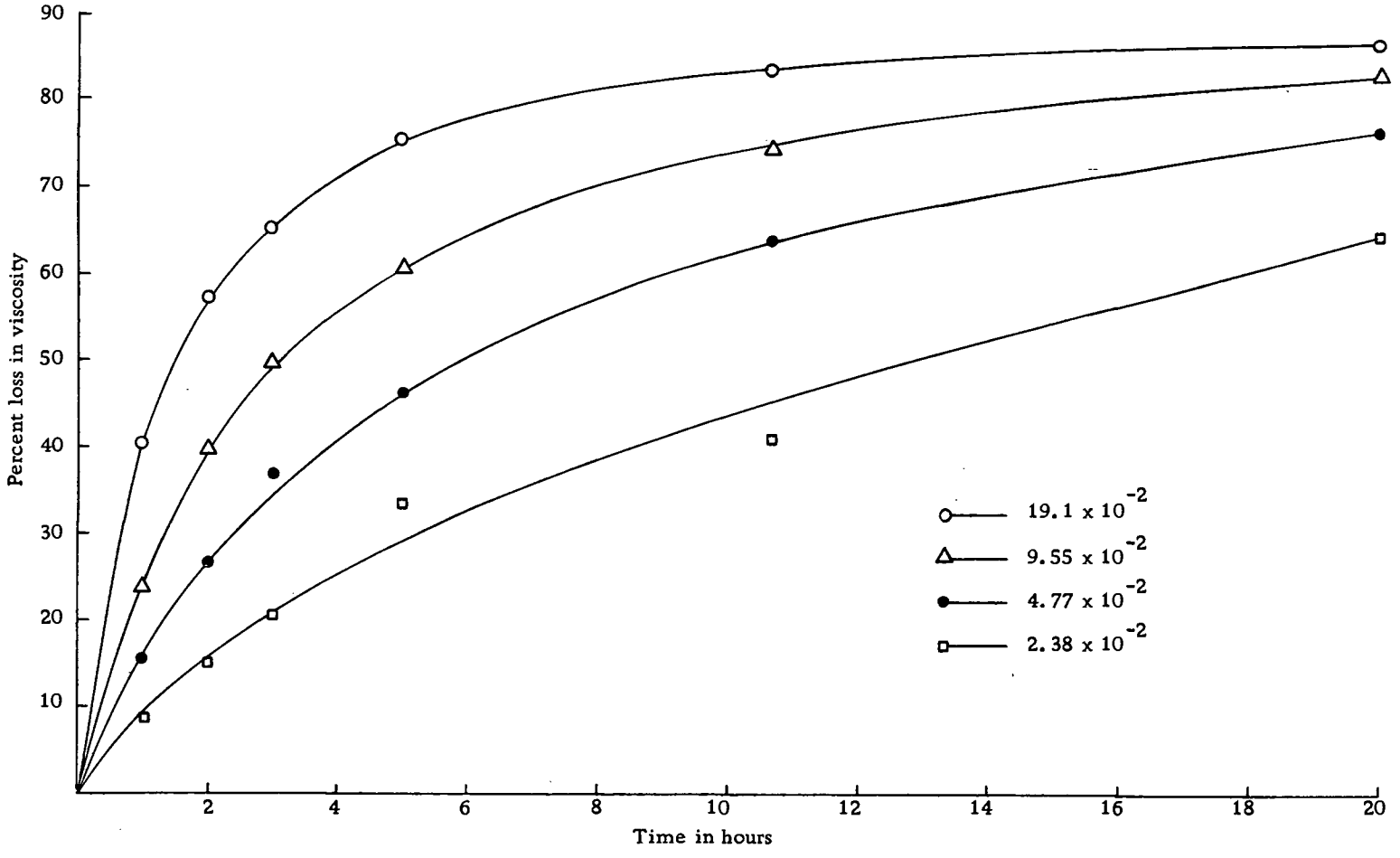

Figure 11. The relation between cranberry protein concentration and its polygalacturonase activity on one percent citrus polypectate solution at pH 5.0 and 30°C.

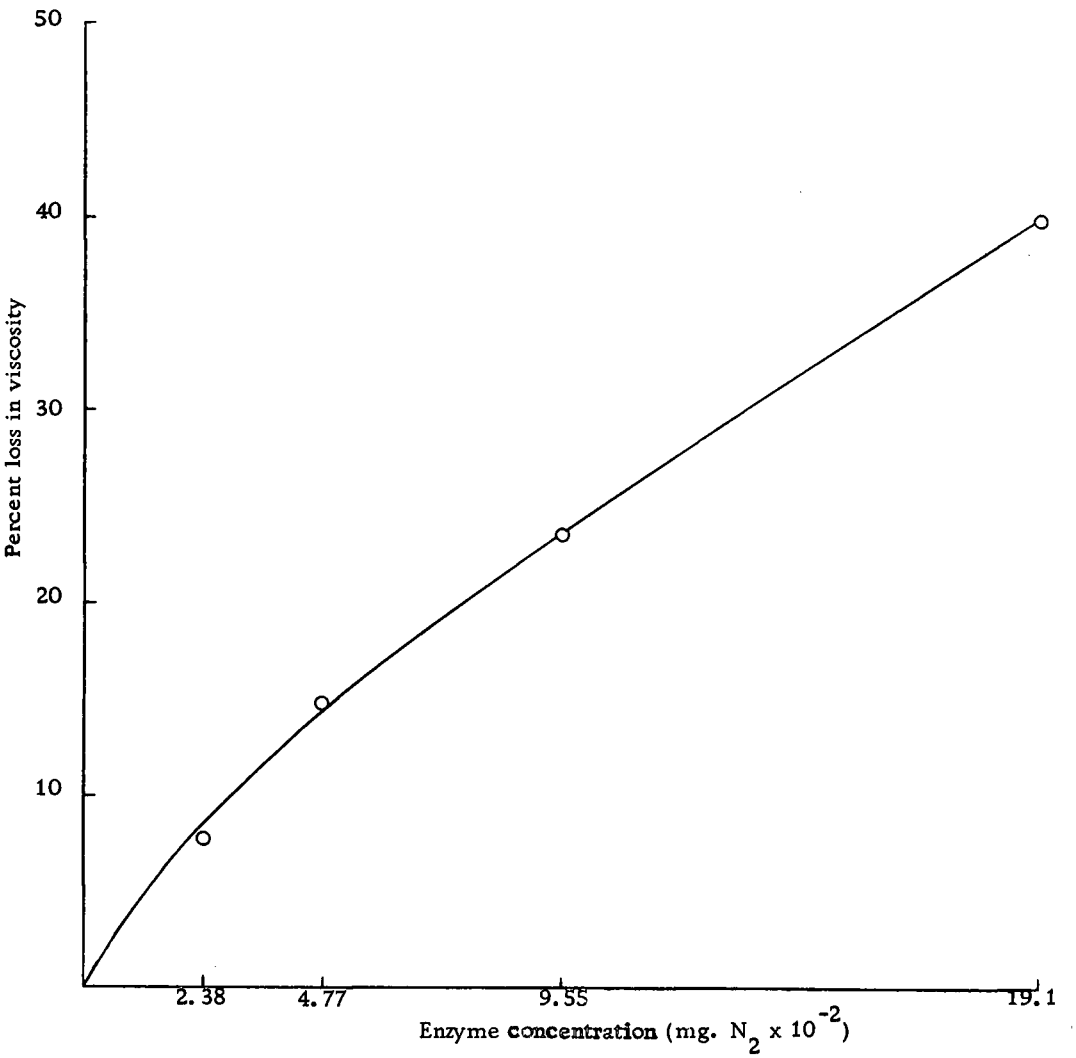

Figure 12. The relation between different enzyme concentrations of cranberry polygalacturonase and their initial rate of activity on one percent polypectate solution at pH 5.0 and 30°C.

solution. Table 5 shows the percent loss in viscosity of the sodium polypectate solution over a period of 44 hours.

Table 5. Effect of heat on cranberry polygalacturonase

Period of exposure to heat (min.)	Means of Five Determinations		
	Percent loss in viscosity	Loss due to catalytic action	Standard deviation
0	94.7	80.6	----
5	58.5	43.3	0.36
10	39.36	24.3	0.65
15	30.3	15.2	0.72
25	18.78	3.7	1.32
35	15.9	0.8	0.0

Figure 13 shows the thermal death rate curve of cranberry pectinolytic enzymes. At pH 5.0, the activity of the polygalacturonase was inactivated after 35 minutes of exposure to 100°C. McColloch and Kertesz (1948) indicated that tomatoes contain an unusually heat resistant pectinolytic factor with 20 percent of the original activity remaining after a heat treatment of one hour at 100°C. Moreover, Gizis (1964) found that strawberry pectinolytic enzymes were completely inactivated when exposed to 100°C for 35 minutes.

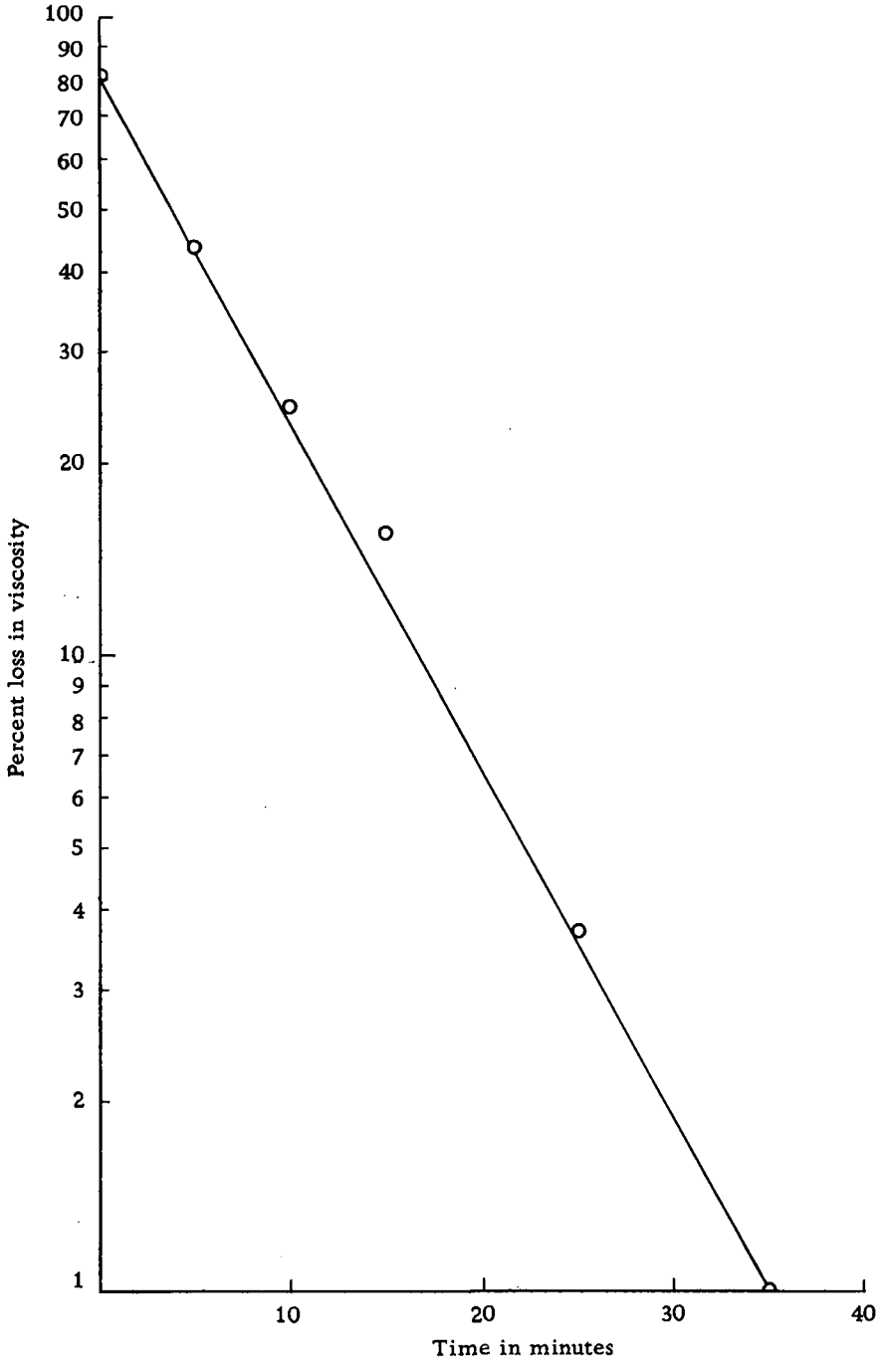

Figure 13. Thermal death rate curve for the cranberry PG activity at pH 5.0 on sodium polypectate.

Chromatographic Separation of Cranberry Proteins on DEAE Cellulose Column

A DEAE cellulose column was used to fractionate cranberry proteins. These proteins were extracted with acetone-polyethylene glycol, precipitated by ammonium sulfate (75 percent saturation), dialyzed against distilled water followed by dialysis against citric acid:sodium hydroxide buffer pH 5.0 for equilibration. Eighteen mls of the dialysate were placed on the DEAE cellulose column and eluted with citric acid-NaOH buffer pH 5.0 containing a sodium chloride gradient. Ten milliliter fractions were collected with a fraction cutter at a rate of 60 mls per hour. The fractions collected were assayed for polygalacturonase activity by determining the percent loss in viscosity of a one percent sodium polypectate solution at pH 5 and 30°C. Table 6 shows the activity of polygalacturonase of the different fractions collected from the DEAE cellulose column.

Figure 14 gives the relationship between the fraction numbers, their extinction at 280 mμ and their effect on one percent polypectate solution.

Figure 15 indicates the sodium chloride concentration in different fractions obtained from the DEAE cellulose column. The results on Table 6 show that the cranberry pectinolytic enzymes were concentrated between eluate eight and fourteen with highest activity present in eluate number nine. Peaks two and three on

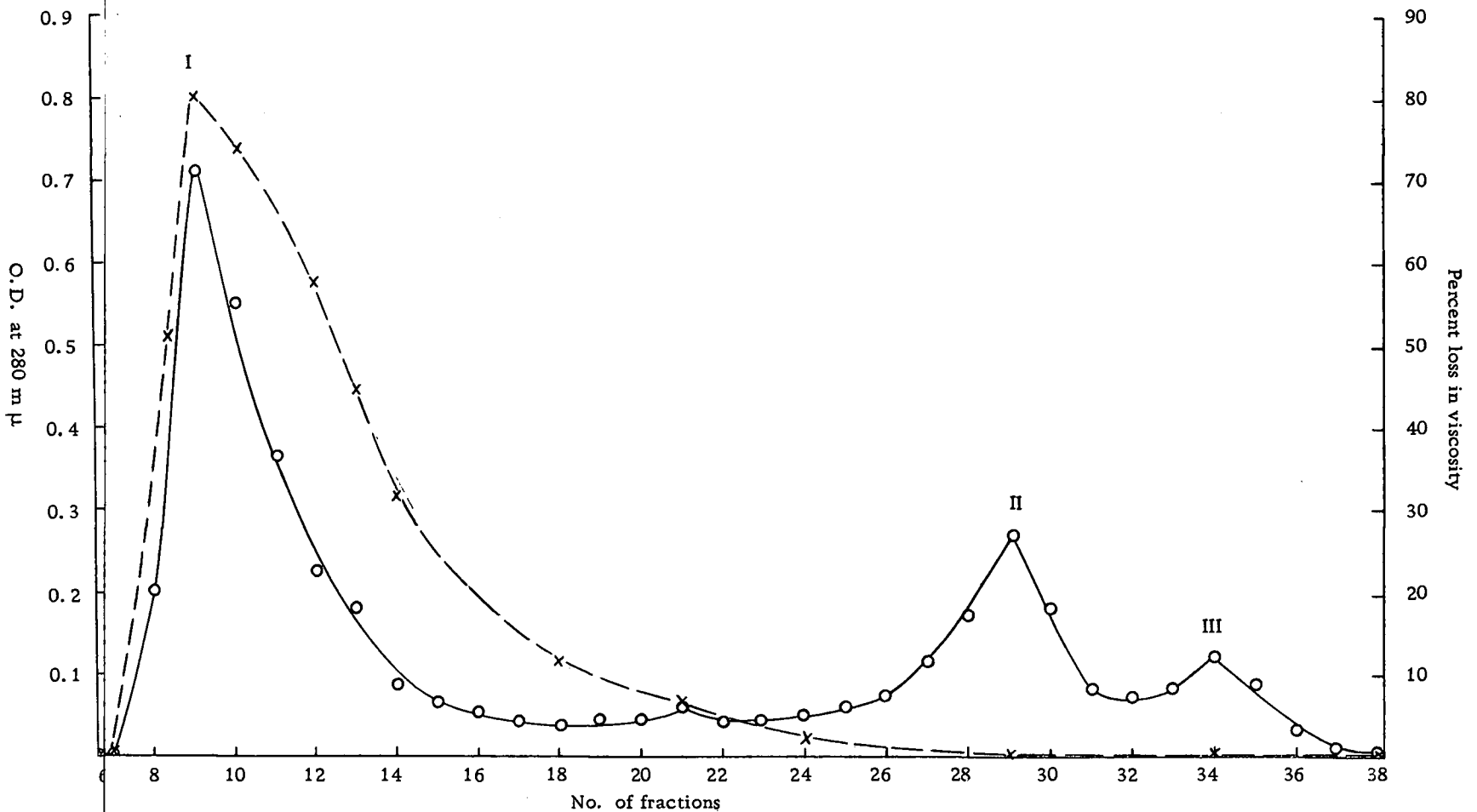

Figure 14. Chromatography of cranberry protein dialysate on DEAE cellulose column and the polygalacturonase activity of the fractions at pH 5.0 and 30°C.

x——— % loss in viscosity

o——— O.D. at 280 mμ

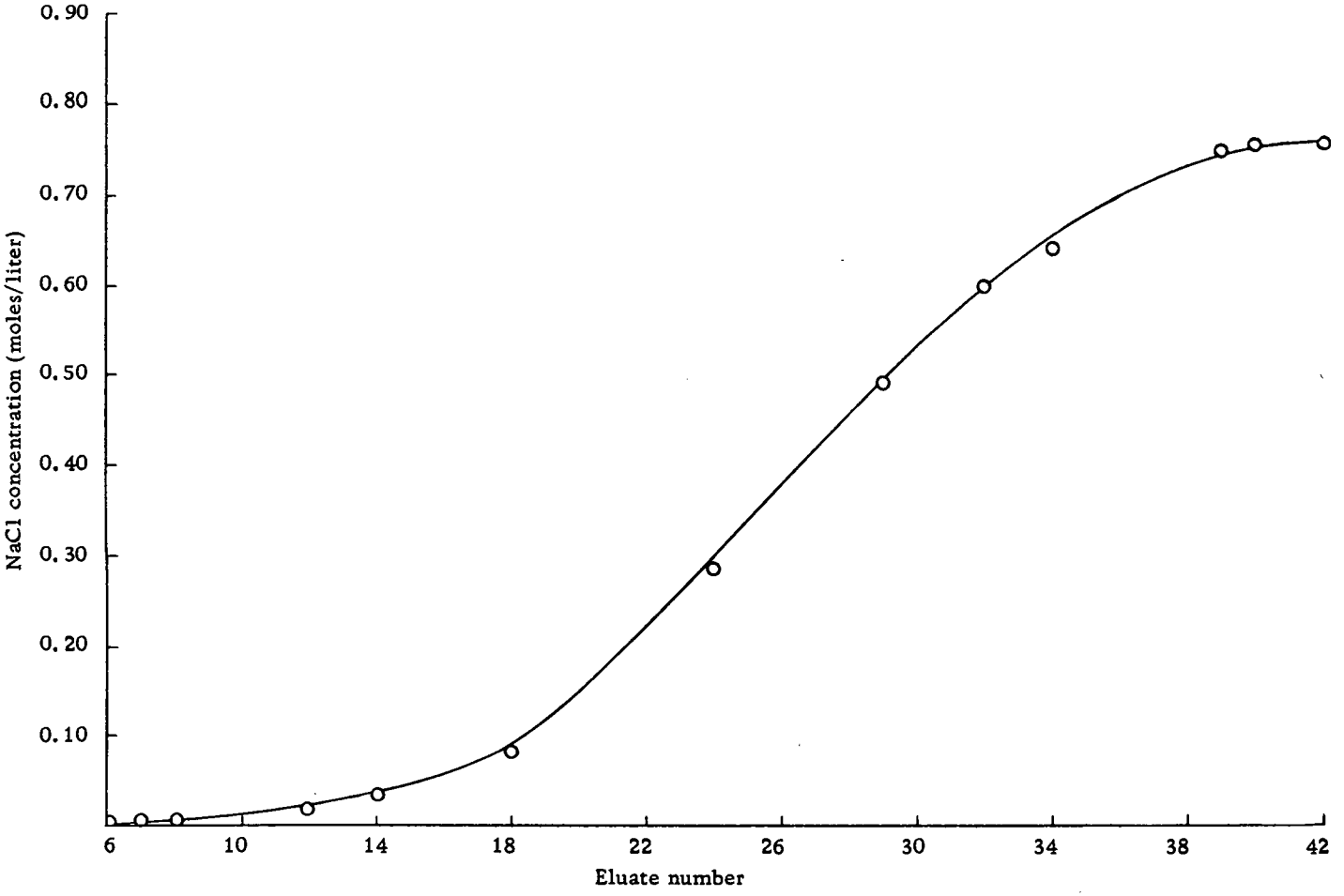

Figure 15. Sodium chloride concentration in eluates from DEAE cellulose column.

Figure 12, did not show any pectinolytic activity. The DEAE cellulose column enabled us to separate the proteins which possess a polygalacturonase activity from proteins which exhibited no activity.

Table 6. The pectinolytic activity of DEAE cellulose column chromatography fractions in one percent sodium polypectate at pH 5.0 and 30°C

Fraction	Percent loss in viscosity due to pectinolytic activity	O. D. at 280 mμ
1-7	----	-----
8	51.8	0.235
9	80.5	0.71
10	75.0	0.58
11	65.0	0.35
12	57.5	0.225
13	45.0	0.18
14	32.5	0.09
18	11.5	0.035
21	7.9	0.045
24	2.4	0.05
29	----	0.27
34	----	0.110
40	----	-----

Effect of Cranberry Proteins on the Insoluble Residue of Cranberries and Alcohol Insoluble Solids

After preparing the cranberry insoluble residue as described in the Methods and Materials section, the action of the cranberry

proteins on this residue was determined. This was carried by suspending three grams of the insoluble residue in 150 mls of citric acid:NaOH buffer pH 5.0. To half of the suspension four mls of cranberry protein dialysate, as prepared by acetone-polyethylene glycol method, were added while four mls of buffer were added to the other portion. After placing the samples in a shaker-incubator at 30°C, aliquots were removed and total soluble carbohydrate concentration was determined by the phenol-sulfuric acid method of Dubois et al. (1956). Table 7 shows the results obtained.

Table 7. Effect of cranberry proteins on the insoluble residue of the AIS

Time of Incubation	Soluble sugars expressed as galacturonic acid in µg/ml		
	Sample	Blank	Difference
5 hours	1370	1047	323
19 hours	1573	1110	466
29 hours	1653	1160	493
43 hours	1657	1160	497

The insoluble residue of the Alcohol Insoluble Solids is mainly composed of protopectin and cellulytic matter. When protopectins were hydrolyzed by two percent NaOH and soluble carbohydrates determined on the protopectin free residue, no change in total sugar was found. Thus, the increase in total soluble carbohydrates in the former experiment (Table 7) may be attributed to the action of cranberry proteins.

Pectin Transeliminase in Cranberry Proteins

The activity of pectin transeliminase was followed by determining the change in light absorption at 235 mμ of a 0.5 percent pectin solution in 0.1M citrate-phosphate buffer pH 5.2, after the addition of the protein dialysate prepared from the acetone-polyethylene glycol protein preparations. No activity of transeliminase was found.

Pectin Esterase in the Cranberrry

Due to the fact that pectin esterase is strongly attached to the cellular constituents of the plant, the enzyme was extracted with 0.15M NaCl solution from the cranberry acetone powder. The activity of pectin esterase was determined by measuring the increase in carboxyl groups on the pectin molecule.

pH Optimum of Pectin Esterase

Studies to determine the pH optimum for the activity of cranberry pectin esterase were made by continuous titration of the liberated carboxyl groups produced. Six pH values were used at which the enzyme-substrate system was maintained in order to measure the activity. Figure 16 shows the relationship of the enzyme activity at the different pH values used.

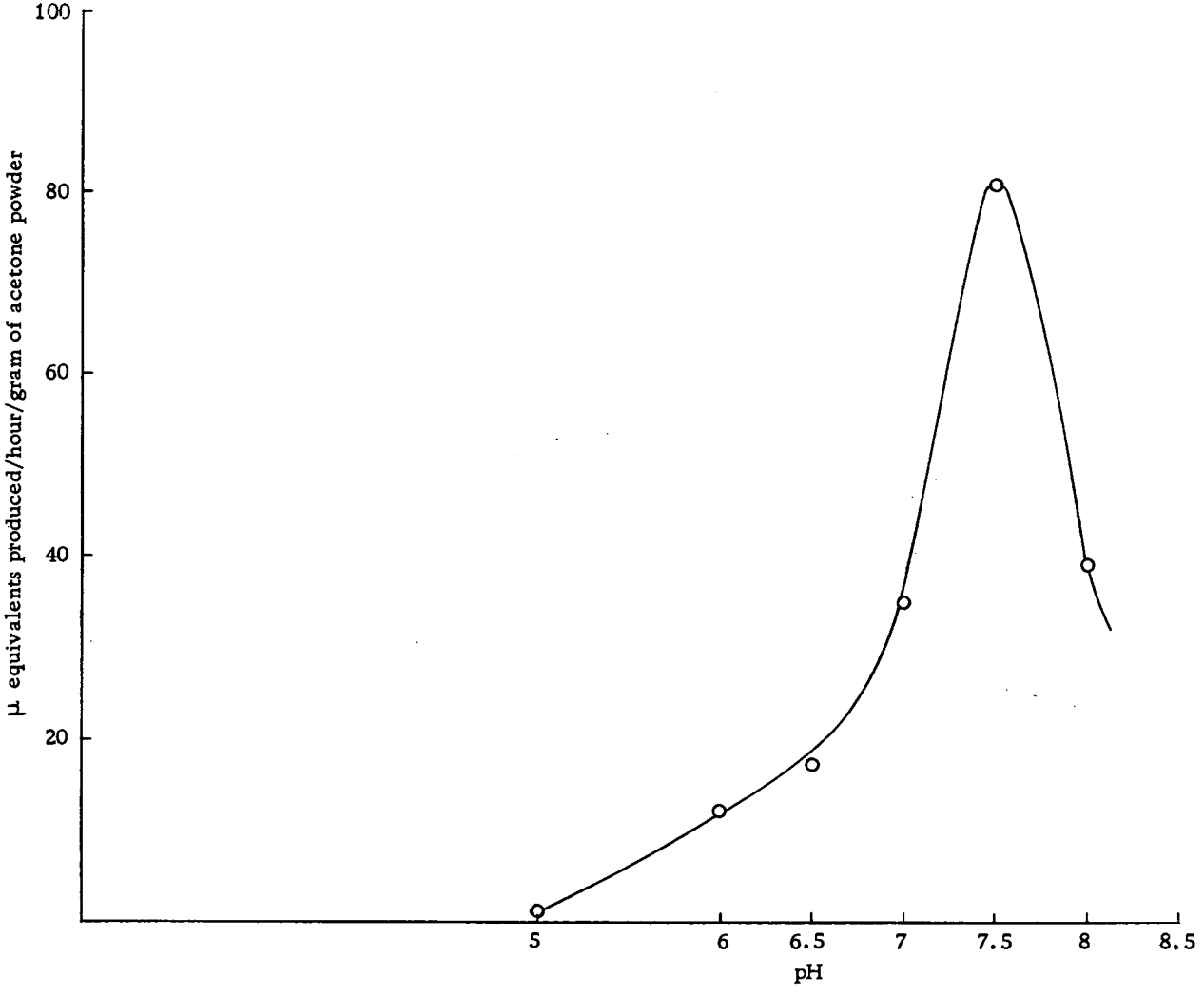

Figure 16. Activity of PE of cranberries at different pH values, using citrus pectin as substrate.

Table 8 gives the means of four determinations of the pectin esterase activity at the different pH values used, at 30°C. The substrate used was a one percent solution of Sunkist pectin (3442) containing 0.15M NaCl.

Table 8. Effect of pH on the activity of cranberry pectin esterase

pH	μ Equivalents/ gram acetone powder/ hour	Standard deviation	Per 100 grams of cranberries
5	0.00	0.00	0.000
6.0	12.0	0.30	52.2
6.5	17.0	0.22	73.6
7.0	35.0	0.41	151.6
7.5	81.0	0.41	350.7
8.0	39.0	0.41	168.9

The data on Table 8 shows that the highest activity of cranberry pectin esterase was found at pH 7.5. No activity was evident at pH 5.0. Alkaline hydrolysis of the methyl ester at pH values above 8.0 making the results unreliable.

Pectin Esterase Activity

When using the protein dialysate of acetone powder after precipitation with 75 percent saturation of ammonium sulfate as an enzyme source, the activity of the enzyme was found to be very slow

and insignificant. This was abandoned for the acetone powder extracted with 0.15M NaCl solution as a source of the enzyme. Figure 17 shows the course of action of cranberry proteins on one percent citrus pectin solution (3442) at pH 7.5 and 30°C. The amount of enzyme used was equivalent to one gram of acetone powder. According to Kertesz (1959), demethylation of the pectin does not affect the viscosity of their solutions except in presence of calcium ions. Under such conditions, the gelation properties of the pectin are affected.

Influence of Salt on Pectin Esterase Activity

The activity of cranberry pectin esterase was determined by using three 50-ml aliquots of one percent citrus pectin solution containing no sodium chloride, 0.15M NaCl and 0.30M NaCl at pH 7.5 and 30°C. Table 9 shows the effect of NaCl on the activity of the enzyme.

Table 9. Effect of NaCl on cranberry pectin esterase activity at pH 7.5

M NaCl	Activity units of enzyme per gram of acetone powder	Activity units/100 grams of fresh tissue
0.00	4.0	17.2
0.15	81.0	350.7
0.30	76.0	329.1

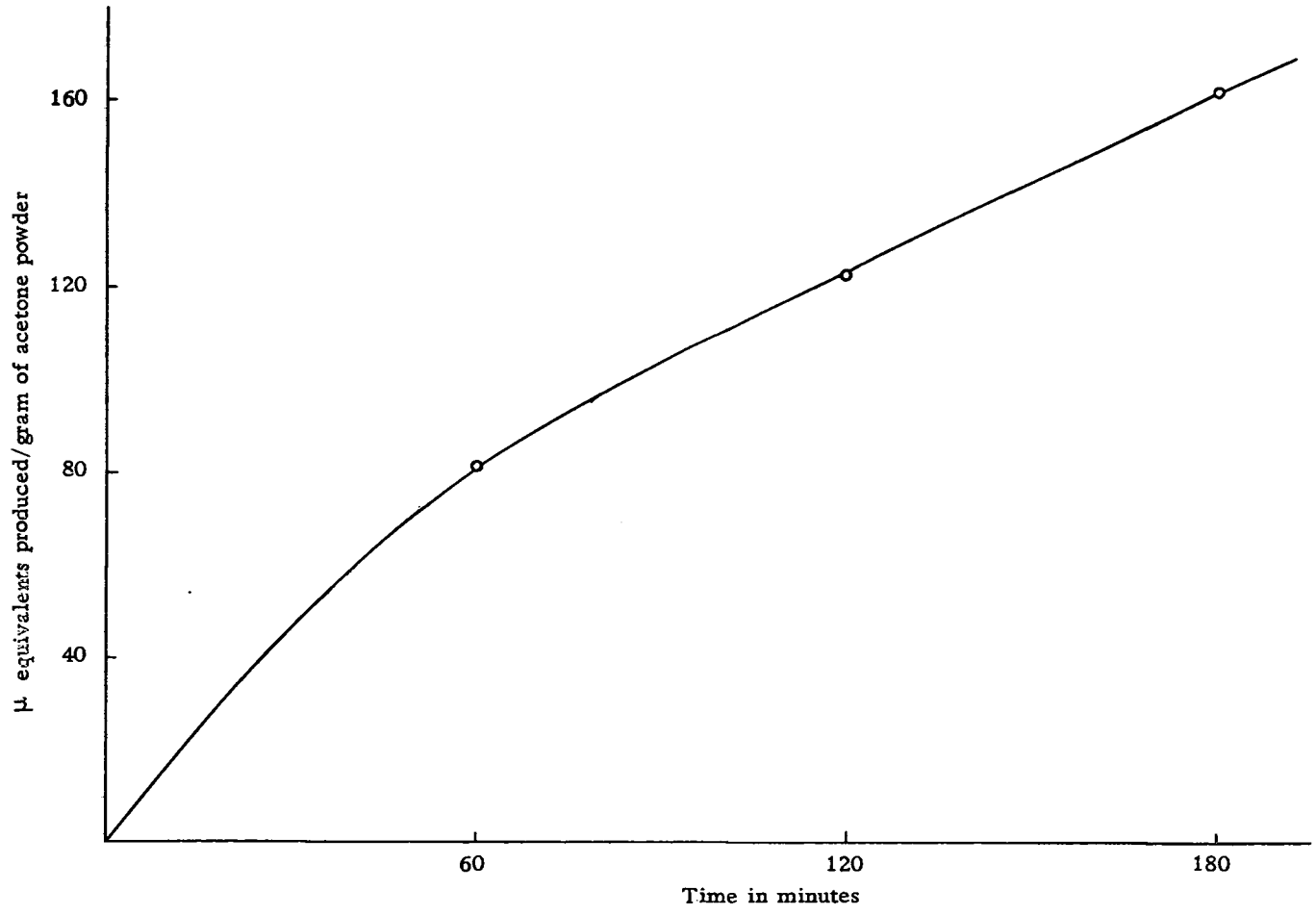

Figure 17. Cranberry PE activity on one percent citrus pectin solution (0.15M NaCl) at pH 7.5 and 30°C.

A unit of pectin esterase corresponds to microequivalents of ester hydrolyzed per hour. It is evident from the results in Table 9 that the NaCl concentration increased the activity of the enzyme at 0.15M above which only slight decrease in activity occurred. When no salt was added to the system the activity of the enzyme was comparatively low. Lineweaver and Ballou (1945) proposed a hypothesis regarding the effect of cations on pectin esterase activity. It was assumed that cations prevent the inhibition of pectin esterase by the pectin carboxyl groups by forming cation-carboxyl complex.

Heat Inactivation of Pectin Esterase

The effect of heat on cranberry pectin esterase activity was determined by exposing the enzyme extract to 100°C for one, two, three, and five minutes. The activity of the heat treated enzyme preparation was determined by following the amount of carboxyl groups exposed as a result of the catalytic hydrolysis of the methyl ester on a one percent citrus pectin (3442) solution at 30°C and maintaining the pH at 7.5. NaCl concentration in the substrate corresponded to 0.15M. Table 10 shows the results obtained and are the means of three determinations.

Table 10. Influence of heat on cranberry pectin esterase

Time of exposure to 100°C (Minutes)	μ equivalents/ gram of acetone powder/ hour	Standard deviation
0	81.00	0.41
1	37.10	2.67
2	25.17	1.88
3	17.23	0.40
5	00.00	0.00

Table 10 shows that cranberry pectin esterase was inactivated when exposed to 100°C for five minutes.

DISCUSSION

The data shown in Tables 2 and 4 and Figures 7, 8, 9, and 10, show that a loss in viscosity was exhibited on solutions of pectic substances due to the presence of cranberry proteins. Thus cranberry proteins possess the pectinolytic factor polygalacturonase which hydrolyzes the glycosidic linkages of a pectin polymer.

The method of enzyme extraction from the cranberry proved to be essential in obtaining an enzyme extract which exhibits a high rate of activity. The use of phenol complexing agents prevents the phenol-protein interaction which could cause a decrease in the activity of the enzyme. Table 2 and Figure 4 show that when the phenol complexing agents are used in extracting the proteins from the cranberries, a higher activity of the polygalacturonase was evident. Moreover, when different types of phenol complexing agents are used, the protein extracts prepared possess different enzyme activity. Results in Table 2 show that when the protein extracted by the use of acetone in conjunction with polyethylene glycol a higher activity was observed over the acetone or the polyvinylpyrrolidone protein extracts. This may be attributed to the stronger affinity of the polyethylene glycol in the presence of acetone to the phenolic compounds.

Table 4 and Figures 7, 8, 9, and 10 indicate that the activity

of the polygalacturonase on the different pectic substances used for substrates was rapid during the initial period of the reaction. This rapid loss in viscosity suggests a random split of the pectin polymer. The polygalacturonase which possess this criterion in their course of action, are referred to as endoenzyme. A slight pectinolytic preference for the citrus sodium polypectate over citrus pectin was evident. The latter possess a higher metholyl content, see Table 1.

The action of cranberry polygalacturonase was also demonstrated on cranberry water soluble pectin and the calgon soluble pectin. The difference in the activity of polygalacturonase on both was slight. Moreover, the enzyme showed a considerably higher activity on citrus pectins than on cranberry pectins. This may be attributed to the difference in structure between the pectins of both sources.

The foregoing discussion indicates that the cranberry polygalacturonase possesses the ability to randomly hydrolyze pectic substances with high and low methoxyl content.

Optimum pH for the cranberry polygalacturonase lies in the range of 5.0. The enzyme was inactive at neutral pH values as shown in Figure 5. However, although enzyme activity decreases with a decrease in pH, slight activity was observed at pH 3.0. The influence of temperature on the cranberry polygalacturonase is shown in Table 5. The enzyme was found to be inactivated after 35 minutes

at 100°C. It may be said that the enzyme is highly resistant to heat.

The action of cranberry proteins on the insoluble residue of the cranberry alcohol insoluble solids as shown in Table 7, shows that the protein exhibited a solubilization effect on the insoluble parent material protopectin. Usually, the concentration of protopectin in fruits decreases with maturation. This may be attributed to its contact with pectic enzymes which may hydrolyze it into the soluble form.

Pectin esterase activity of the cranberries was found to be low as shown on Table 8 and Figure 17. Optimum pH activity was found at a value of 7.5, with no apparent activity at pH 5.0. Heating enzyme preparations for five minutes at 100°C caused complete inactivation. NaCl at a concentration of 0.15M gave an optimum pectin esterase activity. In the absence of the salt, the activity was negligible.

SUMMARY AND CONCLUSION

The work done in this study concerns the isolation and characterization of the pectic enzymes in cranberries. The following conclusions could be drawn:

1. Polygalacturonase was found in cranberry proteins. The enzyme could be classified as an endoenzyme that has the ability to catalyze the hydrolysis of pectins with both high and low methoxyl content.
2. The use of a phenol binding agent in the enzyme preparation was effective in obtaining a polygalacturonase extract which possessed a relatively high activity.
3. The polygalacturonase showed a higher activity on citrus pectins as compared to cranberry pectins.
4. pH value of 5.0 was found to give optimum activity for cranberry polygalacturonase.
5. The enzyme polygalacturonase was inactivated after 35 minutes of exposure to 100°C at pH 5.0.
6. Sodium chloride up to a concentration of 0.6M gave no significant affect on cranberry polygalacturonase activity in a citric acid - sodium hydroxide buffer pH 5.0.
7. Cranberry proteins were found to possess pectin esterase which exhibited a low activity when compared to strawberry and tomato

pectin esterase.

8. Optimum pH value for pectin esterase was 7.5.
9. Pectin esterase was inactivated when exposed for five minutes at 100°C.
10. NaCl in the pectin esterase-substrate system gave optimum activity at 0.15M concentration.

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