CHARACTERIZATION OF THE SOLUBLE CARBOHYDRATE COMPONENTS IN TUBERS OF THE JERUSALEM ARTICHOKE (HELIANTHUS TUBEROSUS L.)

BY

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A THESIS SUBMITTED TO

THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE

FOOD SCIENCE DEPARTMENT

UNIVERSITY OF MANITOBA

WINNIPEG, MANITOBA

OCTOBER, 1988

ISBN 0-315-47898-5

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TABLE OF CONTENTS

PAGE

CHAPTER 4

CHAPTER 5

ABSTRACT

The Jerusalem artichoke (Helianthus tuberosus L.) is a tuber producing crop indigenous to North America. High carbohydrate yields are possible with this crop. The storage carbohydrate of the Jerusalem artichoke is inulin. Inulin is a linear polymer of fructose. The number of fructofuranose residues in the chain varies greatly, it is for this reason that inulin has been arbitrarily defined as having a degree of polymerization (DP) of 20-30.

Several methods were utilized to extract the available carbohydrate from the tuber. This was done to maximize carbohydrate recovery from the tubers. It was found that carbohydrate levels approaching or even apparently exceeding the theoretical maximum amount of carbohydrate present in the tuber could be extracted.

Commercial preparations of inulin were characterized using differential scanning calorimetry (DSC). It was found that each concentration of inulin had its own characteristic endotherm.

Commercial standards were fractionated by gel filtration chromatography. It was found to be possible to separate mixtures of seven or more standards of different molecular weights using gel filtration chromatography. The tuber extracts prepared earlier were subjected to this technique and it was found that the raw extracts could be fractionated into 7-10 distinct peaks as a result of gel filtration chromatography. Gel filtration was

found to be useful in the characterization of the carbohydrate extracts by effecting fractionation into more homogeneous subsamples and also allowing estimation of their molecular size and therefore DP.

ACKNOWLEDGEMENTS

For my Mother and Father

I would like to express my deepest appreciation and gratitude to Dr. E. D. Murray, Head, Department of Food Science, University of Manitoba. Without his patience, understanding and unfailing support this project would not have been completed.

I would like to thank Dr. N. A. M. Eskin, Department of Foods and Nutrition, Faculty of Human Ecology, University of Manitoba and Dr. G. Mazza, Canada Department of Agriculture Research Station, Morden, Manitoba for their careful review of this thesis.

Special thanks go to Export Packers Company Limited--Science and Technology Centre for their support during this period.

LIST OF FIGURES

PAGE

LIST OF TABLES

1. INTRODUCTION.

The Jerusalem artichoke (Helianthus tuberosus L.) is a tuber crop native to North America. The unusual and interesting characteristics of the artichoke give the possibility of many diverse uses for this Manitoba-grown crop. The artichoke can be used and is marketed as a table vegetable although the crop is not well-known in North America. The storage carbohydrate of the artichoke is a fructofuranoside known as inulin. Since inulin is apparently not digested directly by humans and therefore has a low caloric value it can be used in specialty food products such as low calorie flour and pasta. The yields of this crop are favorable when compared to those of corn and sugar beets and consequently the tuber might be an economical source material for the production of fuels such as ethanol. The sugar residues in the inulin molecule are in the form of fructose and as a result the tuber might represent a superior source material for the production of high fructose syrup, a sweetener preferred by the beverage industry because of its greater sweetness and lower caloric value relative to sucrose. Considering the many potential uses of this crop, only some of which have been mentioned here, it is surprizing that the crop is not more fully utilized or more well-known in North America.

In order to more fully utilize this Manitoba-grown crop, it is necessary to have a good understanding of the crop and its characteristics. It is for this reason that the goal of this study was to characterize the soluble carbohydrate components of the Jerusalem artichoke so that, with more known about this

fraction, utilization of the crop might be enhanced.

2. REVIEW OF LITERATURE.

2.1 The Jerusalem Artichoke - What's in a Name

Jerusalem artichoke, the common name for Helianthus tuberosus, has resulted in confusion about the origins of the name as well as confusion with other plants. There are several theories about how the Jerusalem artichoke received its name. One theory is based on information published in an herbal in 1618 in which it was reported that the tubers were cultivated in Ter Neusen, Holland (Heiser,1976). It is possible that Ter Neusen was converted to Jerusalem in transit between Holland and England. The second theory was proposed in the early 19th century in which it was suggested that Jerusalem was derived from a corruption of the Italian name Girasole articiocco. Ironically, soup made from Jerusalem artichokes is called Palestine Soup. The "artichoke" part of the name is easy to explain because both Champlain and Lescarbot compared its taste to that of the globe artichoke (Cynara scolymus).

The globe artichoke is a distant relative of the Jerusalem artichoke; both are members of the sunflower family. The globe artichoke is much more familiar to the North American public than is the Jerusalem artichoke. The similarity of the two names has perhaps been a detriment because it has resulted in confusion between the two plants. For example: in a newspaper article about Jerusalem artichokes published in the Winnipeg Free Press (Speirs,1984) the title referred to artichoke "hearts". It went on to state that "... there is more to the potentially

profitable plant than its edible heart, considered a culinary delicacy". This statement indicates clearly that Jerusalem artichokes were mistaken for globe artichokes. As early as 1629 Parkinson had complained about the suitability of the name "Jerusalem artichoke" and suggested that they be called Potatoes of Canada (Heiser,1976). The name Jerusalem artichoke has persisted for over 350 years; however, a name change may be in order if the identity of the crop is to be firmly established. Several names have been suggested, sunchoke and sunroot being the most popular. In the body of this text the crop will be referred to as Jerusalem artichokes or more often simply as artichoke tubers.

2.2 Agronomic Aspects

The Jerusalem artichoke is a plant native to North America. It grows well in the agricultural conditions that prevail in the prairie provinces of Canada. The Jerusalem artichoke has also been cultivated in other areas of the world, namely the U.S.A., France, Japan, the Netherlands as well as the U.S.S.R. (Fleming and GrootWassink,1979).

The Jerusalem artichoke is related to the sunflower plant but unlike the sunflower plant it is cultivated for its tubers and not for its seeds. The flowers of the Jerusalem artichoke do produce seeds, however propagation generally takes place through planting of the tuber.

The Jerusalem artichoke plant generally grows from 1.5 to 2 meters in height (Fleming and GrootWassink,1979). The height of

the plant as well as the degree of branching vary considerably with the variety. The tuber shape is usually knobby but can also be smooth depending upon the variety grown. Ideally, the tubers should be smooth in order to facilitate harvesting and washing. The color of the tubers can be either white, yellow, red or purple (Heiser,1976).

The Jerusalem artichoke is of interest because high tuber yields can be achieved with a minimum of management. When the crop is grown under non-stress conditions the cultivar that is chosen very often has a greater effect on the yield than do management practises. Even the addition of fertilizer and irrigation do not greatly affect the yield (Dorrell and Chubey,1977).

The Jerusalem artichoke tuber matures in roughly 130 days (Stauffer et al.,1975). Tuber weight increases substantially during the late stages of maturation. This increase in tuber yield is due partly to increases in tuber numbers but mainly to dramatic increases in tuber size (Bacon and Loxley,1952). The observed increase in tuber size was accompanied by an increase in the percentage of total reducing sugars on a fresh weight basis.

The harvest of Jerusalem artichoke tubers is similar to that of potatoes and can be carried out using slightly modified potato equipment. Harvest of the tubers generally takes place in the late fall but the crop may also be overwintered in the soil and then harvested in the spring. The harvest date is one of the few factors that must be closely monitored in the management of this crop. For example, Dorrell and Chubey (1977) have found that a delay in the date of harvest from mid September to late October

generally resulted in increased amounts of total reducing sugars but that the fructose concentration present in the tubers decreased. This was accompanied by an increase in the proportion of glucose (both fructose and glucose concentrations are expressed as a percentage of the total reducing sugars). These changes in the relative proportions of fructose and glucose would seem to suggest that shifts in the carbohydrate composition of the tuber occurs during maturation.

The Jerusalem artichoke appears to be reasonably resistant to disease as well as insect infestation during growth. However, severe white mold and soft rot problems do exist during storage (Stauffer et al.,1975). The thin epidermal layer of the tuber is extremely sensitive to damage. The entire crop can be dessimated by white mold and soft rot in a matter of weeks. It is therefore essential that this storage problem be resolved if long term storage of the tubers is to be feasible. The use of mold inhibitors, low temperature storage and dehydration prior to storage have been suggested as means to reduce storage problems encountered with Jerusalem artichoke tubers.

The major use for Jerusalem artichoke tubers would probably be as a source of fructans, either in the form of free fructose or short oligofructans. It would therefore be of interest to compare the yield potential of artichoke tubers with those of other crops utilized for their sugar contents, those of interest being corn and sugar beets.

It can be seen from Table 1 that the carbohydrate yield fluctuates considerably for all three crops. This variation is

likely due, at least in part, to variations in the growing conditions that prevailed. In Canada the carbohydrate yield for artichoke tubers varies from 1,790 to 15,240 kg/ha. A generally accepted yield for this crop is 6,460 kg/ha (Chubey and Dorrell, 1974; Charleton,1984). Corn generally yields between 3,630 and 4,150 kg/ha and sugar beets yield 4,920 to 6,610 kg of carbohydrate per hectare. Overall, the carbohydrate yield of artichoke tubers compares favorably with the yields of either corn or sugar beets.

Table 1: Carbohydrate Yield Data for Jerusalem Artichoke, Corn and Sugar Beet.

Country	Carbohydrate Yield kg/ha
Jerusalem Artichoke Tubers[a]	
Canada	1790-15,240
USA	3840-7170
Netherlands	3590-6050,7260
France	2440-7620
Germany	1210-3220
USSR	1600-2400,9530[c]
Corn Kernels[b]	
Canada	3630-4150
USA	3370
Sugar Beets	
Canada	4920-6610
USSR	3030

[a] adapted using standard compositions of 20% dry matter and 80% carbohydrate.
[b] adapted using standard compositions of 82% dry matter and 75% carbohydrate.
[c] sunflower-artichoke hybrid.

Source: Fleming and GrootWassink,1979.

2.3 Potential Uses of Tubers

The high yield potential of artichoke tubers makes it desirable to review the possible uses of this crop. The most obvious use is that as a food vegetable. The artichoke tuber has never received widespread acceptance as a food crop. Greater potential for this food probably exists when in a more processed form such as flour. Another possible use would be as a starting material for the production of fuel grade ethanol. In light of recent studies on the effect of fructooligosaccharides on human health another potential use for the artichoke tuber might be as a source material for the production of fructooligosaccharides (Hidaka et al.,1986). The last and apparently most promising potential for the artichoke tuber lies in its use for the production of high fructose syrup.

As previously stated the first and most obvious use of this crop would be for the fresh food market. While there are many ways to prepare this vegetable, the public has not embraced the artichoke as a food vegetable. Consumers are simply not aware of what artichokes are or what they can be used for. This is an area where education of the public could be used to create demand for a "new" food crop which of course would have a beneficial effect on Canadian agriculture.

Perhaps a more viable food use for this crop would be in a more processed form, for example, as Jerusalem artichoke flour. The fact that inulin is not digested directly by humans indicates that the crop may be suitable for use in the production of calorie-reduced foods. This characteristic would be of

considerable attraction for North America's weight-concious society. Jerusalem artichoke flour has a caloric value of 8.4 KJ/g (Hoehn,1982) as compared with caloric values of 15.2 KJ/g and 15.0 KJ/g for wheat and rye flours (Chan,1983). This type of flour could be used as a low calorie filler in products such as pasta. The flavor of Jerusalem artichoke flour must be bland if its use is to gain widespread acceptance. However, there is a flavor problem associated with the flour. Its flavor has been described as "earthy" by Chan (1983) thus limiting its use in this capacity for the time being.

Another potential use for the artichoke tuber is as a source material for the production of fuel grade ethanol. The high yield of fermentable carbohydrate possible with the artichoke tuber makes potential ethanol yields of 56 hL/ha possible (Williams and Ziobro,1982). In the future increasing government regulation pertaining to permissible levels of environmental pollutants will create a demand for fuel grade ethanol. For example, Colorado motorists will likely be the first Americans required to use gasohol (90% gasoline/10% ethanol) in winter in an attempt to reduce airborne carbon monoxide emissions (Anonymous,1987).

A new possible use for the artichoke tuber might be as a source material for the production of fructooligosaccharides. A recent study has examined the effects of a new sweetener, Neosugar, a commercially available fructooligosaccharide, on intestinal flora and human health (Hidaka et al.,1986). Neosugar is a mixture of short chain fructans (GF_2,GF_3 and GF_4). When this product was incorporated into various foods and ingested by

humans several significant occurrences were observed. The Neosugar was selectively utilized by a group of organisms known as bifidobacteria and this led to an increase in their number in the gut. The change in the makeup of the intestinal flora resulted in relief of constipation or loose stools. Other beneficial effects that were also observed were improvement in serum lipids, total cholesterol, triglycerides, blood glucose and blood pressure (Hidaka et al.,1986). These findings warrant continued investigation and may result in an innovative use for the artichoke tuber.

The last and possibly the most promising use for artichoke tubers is in the production of high fructose syrup. The beverage industry is currently the biggest user of high fructose syrups but other sectors of the food industry such as the confectionery, bakery and dairy industries are also beginning to look to fructose as a sweetener. This trend toward the increased use of fructose may have some repercussions on the sugar market. It has been predicted that the use of sucrose will decline dramatically over the next 20 years (Anonymous,1985), mainly because competition from fructose and other sweeteners will strain the already complex system of price supports and import quotas in effect in the U.S.A..

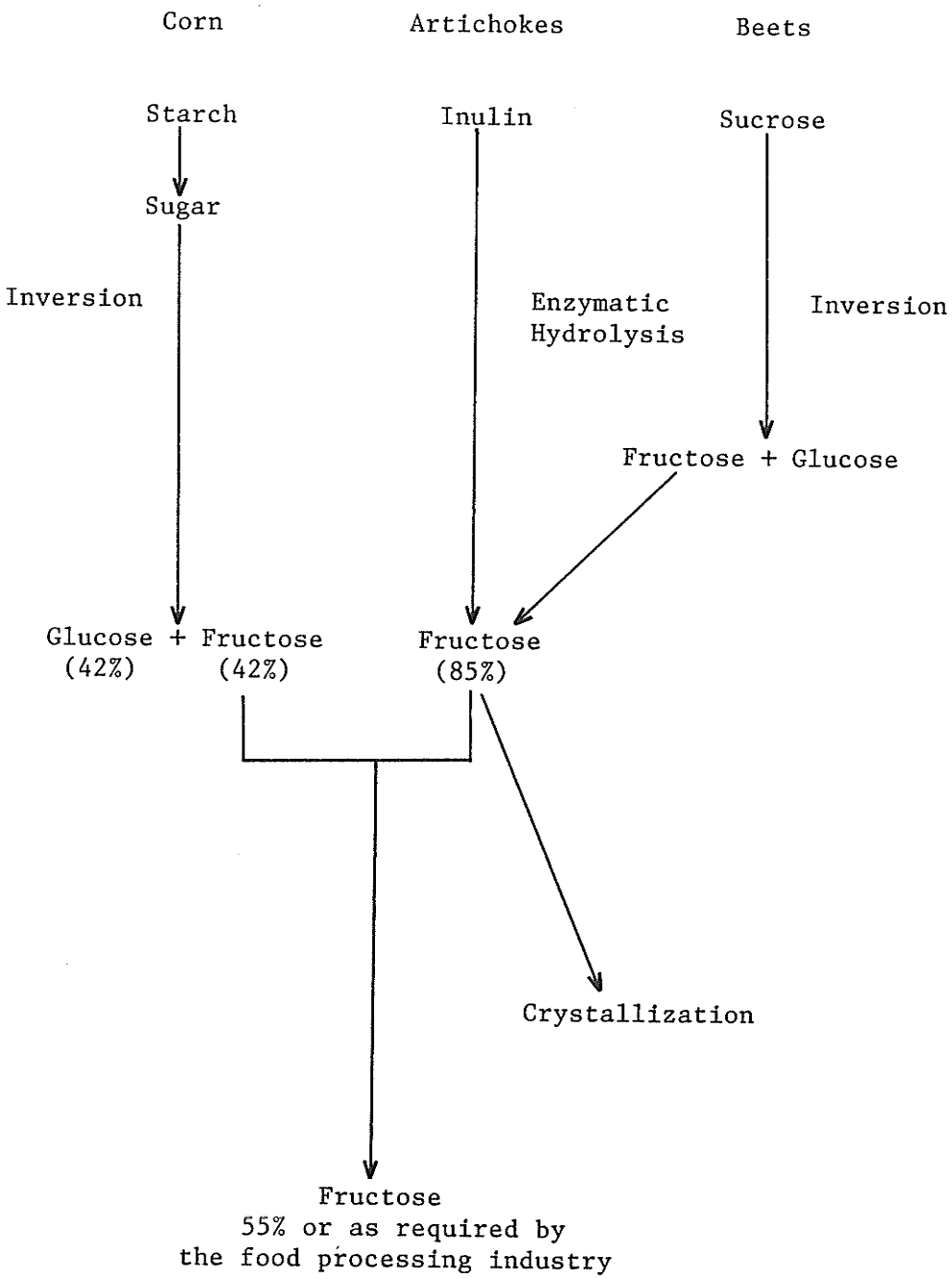
Fructose is the sweetest naturally occurring sugar, with a sweetness of 1.5 to 1.7 times that of sucrose. This makes the use of fructose as a sweetener very attractive because the desired level of sweetness in a food can be achieved using less fructose than sucrose. Also, this means that a food item

sweetened with fructose would have about one third fewer calories than one sweetened with sucrose.

High fructose syrups are generally made from corn starch by a highly technical process involving a multi-step enzymatic hydrolysis of corn starch. This hydrolysis results in the production of glucose which is then isomerized to fructose. Isomerization of glucose to fructose can, at best, result in a 50% fructose-50% glucose syrup providing that the reaction is allowed to go to equilibrium. Time considerations usually limit this to a 42% fructose/58% glucose syrup which must then be fractionated using chromatographic techniques to produce a 90% fructose syrup. This 90% fructose stream is then used to enrich a portion of the original 42% fructose stream (Luenser,1983).

The beauty of the artichoke tuber is that the storage carbohydrate is in the form of inulin, which is a polymer of fructose. When this carbohydrate is hydrolyzed the simple sugar produced will already be in the form of fructose and as a result no isomerization would be required. Also, fructose syrup production from inulin is not affected by isomerization equilibrium limitations. Hydrolysis of inulin with an average DP of 20 (19 fructose/1 glucose) would result in a 95% fructose syrup. This would eliminate the need for the chromatographic fractionation step. This type of syrup could be used to enrich the 42% fructose made from corn starch or fructose from other sources such as invert sugar made from beets. In this way, a new crop (artichokes) could benefit a struggling crop (sugar beets) as shown in Figure 1.

Figure 1: Possible scheme for the production of high fructose products using two prairie grown crops



The use of artichoke and beet tubers for the production of high fructose syrup could lead to some processing simplifications over corn and should lead to greater cost efficiencies. This advantage, when combined with the higher yield potential of artichoke tubers over corn would make the artichoke tuber a very economical source of fructose provided that the process limitations of fructose production from artichoke tubers can be worked out.

2.4 The Nature of Inulin and Related Polyfructans in Jerusalem Artichoke Tubers

Inulin is a carbohydrate that is widely distributed throughout the plant kingdom. It is generally found as a storage carbohydrate in the Compositae and Graminae families, some well known examples of these being chicory, dahlia and the Jerusalem artichoke. Inulin is a polymer of fructose and is the most widely studied of the polyfructans. It was first isolated from Jerusalem artichokes by Rose in 1804. Thomson was credited with coining the word inulin in 1811 (McDonald,1946).

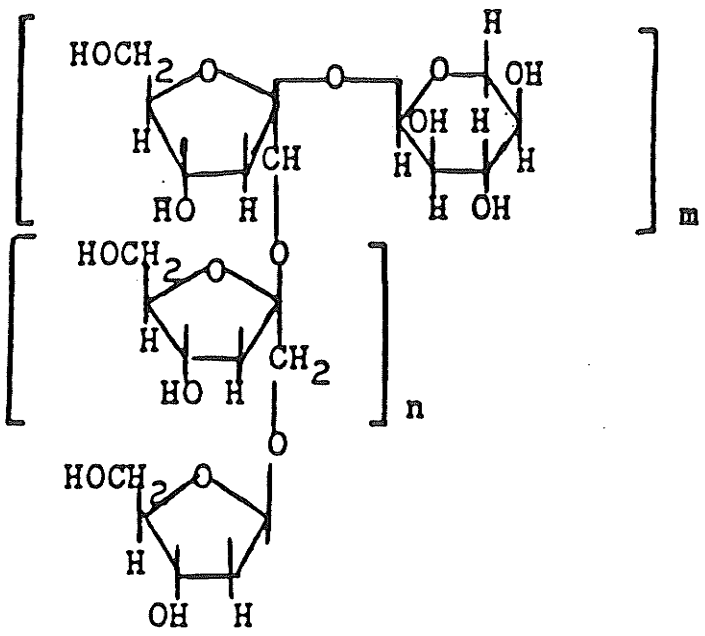
2.4.1 Structure of Inulin and Related Fructosans

Inulin is a polymer of fructose. It is made up of a beta-D-fructofuranose chain which is terminated by an alpha-D-glucopyranose residue. The fructofuranose units are joined by beta(2->1) linkages and the terminal glucopyranose unit is attached to the fructofuranose chain by a beta2->alpha1 bond

(Figure 2). The number of fructofuranose (fructosyl) residues in the chain varies greatly; it is for this reason that inulin has been arbitrarily defined as having a degree of polymerization (DP) of 20-30 (Edelman and Jefford,1964).

It has long been known that inulin is extremely polydisperse meaning that a homologous series of polyfructans exist together when in solution. Fructans ranging in size from DP 2-50 were reported in artichoke tubers in addition to the monosaccharides fructose and glucose (Edelman and Jefford,1964). The general formula for inulin and its related polyfructans consists of a chain of fructose residues bound to a terminal glucose residue $F-[F]_n-G$. The polymer is linked in a linear fashion, consequently the average chain length can be ascertained by determining the fructose to glucose ratio. A high fructose to glucose ratio would indicate that the average chain length was very long. Also, the higher this ratio the greater the percentage of fructose in the polymer.

Figure 2: Inulin Molecule



m: terminal sucrosyl unit

n: fructofuranose unit

Source: McKay, 1982

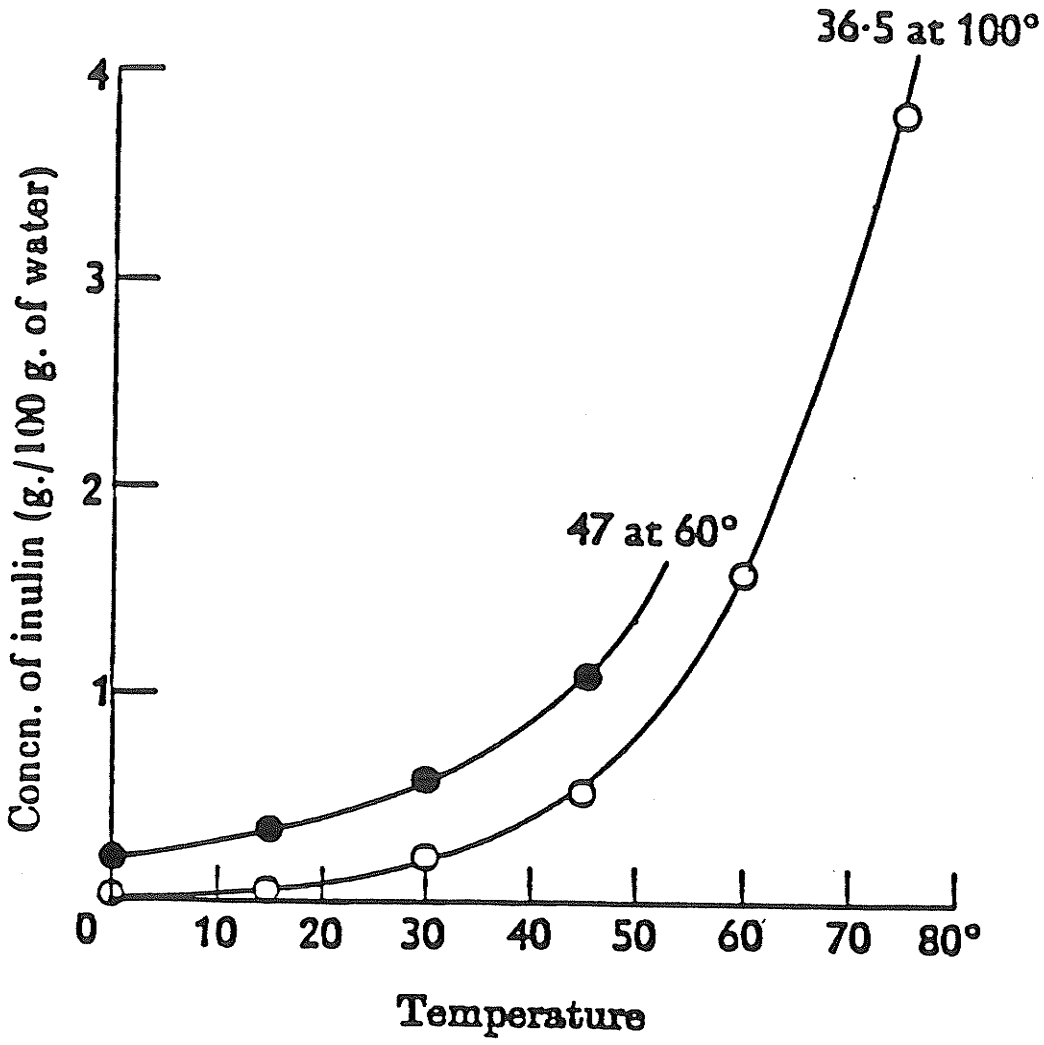
2.4.2 Solubility Behavior of Inulin

Inulin exhibits some very interesting physical properties particularly with respect to its solubility behavior. This carbohydrate is sparingly soluble in water. Less than 0.2 g of water-recrystallized inulin will dissolve in 100 g of water at 30°C (Phelps,1965). As the temperature of the solution is increased a dramatic increase in solubility occurs. At 100°C, 36.5 g of inulin will dissolve in 100 g of water (Figure 3). This represents a 200-fold increase in solubility. The huge increases in solubility between 60° and 100°C are thought to indicate that some kind of structural change takes place in the molecule.

Inulin has a tendency to form metastable solutions. If the temperature of a saturated inulin solution is decreased, dissolution should occur. If crystallization is induced the excess solute should precipitate out of solution fairly rapidly. In the case of inulin solutions, precipitation of excess solute can take as long as several weeks until the true equilibrium of the solution is reached.

Several theories about the unusual physico-chemical properties of inulin solutions have been advanced. It has been suggested that since inulin is very susceptible to hydrolysis, even by carbon dioxide due to a slight acidic effect, that simply dissolving it may result in some limited hydrolysis (Phelps,1965). However, if this type of hydrolysis does occur in significant amounts it should result in increased solubility at moderate temperatures since lower DP fructans are more soluble than higher DP fructans at the same temperature. It is also

Figure 3: Solubility of inulin in water at various temperatures



○ Sample A: water-recrystallized inulin
● Sample B: ethanol-recrystallized inulin

Source: Phelps, 1965

possible that temperature can have a significant hydrolytic effect on inulin (Marchessault et al.,1980). This may explain the huge increases in solubility between 60° and 100°C. As the temperature of the solution is increased, long fructan polymers become more soluble. Phelps (1965) determined that at boiling water temperatures the proportion of low DP fructans increases markedly compared with solutions at 60°C. Heat will not only increase the solubility but will also cause hydrolysis of these polyfructans, thereby generating the much more soluble low DP fructans and dramatically increasing the solubility of the fructans present (Phelps,1965). This type of superdynamic system may explain the unusual solubility curve of inulin.

It has also been suggested that inulin is polymorphic. Yanovsky (1933) made several interesting observations about inulin isolated from chicory and dahlia. The first was that water-recrystallized inulin was less soluble than the ethanol-recrystallized form while exhibiting very similar solubility behavior. It was also observed that chicory inulin was much more soluble than inulin separated from dahlia. It was postulated that this unusual behavior occurred because inulin existed in two different forms but no strong supporting data were presented and no further publications supporting this hypothesis have been found.

2.4.3 Conformational and Configurational Properties of Inulin

Many workers have attempted to determine the shape and structure of the inulin molecule. It must be noted however that no one method can conclusively establish the configuration and conformation of a molecule. Inulin is thought to be a linear molecule (Akazawa,1965). Its unusual physical properties led researchers to formulate theories about its shape and structure. Phelps (1965) suggested two steric models of inulin. The first in which the polyfructan chain assumed a zig-zag shape in solution. The second model depicted inulin as a helical molecule with four fructose residues per turn. The first model was rejected in favor of the second based on the fact that the theoretical axial ratio of the inulin helix was more in line with the calculated value than was that of the zig-zag ribbon model.

Conformational analysis of inulin was carried out by Marchessault (1980) using X-ray and electron diffraction data. Here too, it was concluded that inulin was probably helical. It must be pointed out that this work was done on inulin crystallized from aqueous solution and that the structure of crystalline inulin may differ dramatically from that when in solution or when in the tuber.

The first major configurational study of inulin was conducted by Middleton (1977). Middleton agreed with the Phelps suggestion that the structure of inulin was probably helical but that the proposed value of four fructose residues per turn of the helix was probably unrealistic since steric repulsion between different parts of the polyfructan chain would occur at every

turn of the helix. An alternate value of 3.9 fructose residues/turn of the helix was suggested by Middleton.

2.5 Seasonal Changes in the Carbohydrate Composition of Jerusalem Artichoke Tubers

It would seem that the ultimate long term and stable potential for the use of artichoke tubers lies in the production of high fructose syrup. To ensure a year-round supply of the tubers it is necessary to understand the changes that occur within the tuber so that the tubers can be stored successfully for any given application.

Artichoke tubers normally contain a homologous series of fructans ranging from sucrose to inulin. The carbohydrate composition of artichoke tubers changes in a fairly systematic way during the stages of growth, dormancy and germination. Generally during maturation the fructose concentration is variable until early October and then begins to decrease until late October. Dorrell and Chubey (1977) found that the fructose concentration (expressed as a percentage of total reducing sugars) declined from 82.4% in late September to 71.8% in late November. Bacon and Loxley (1952) observed that the level of ketoses (assumed to be fructose) decreased from 95% in August to 85% in March on artichoke tubers which were stored in the ground until just prior to the analysis. This decrease in fructose was accompanied by an increase in glucose (Dorrell and Chubey,1977). Meanwhile the total reducing sugars present increased until late

October and then leveled off until the spring (Bacon and Loxley,1952).

During storage dramatic changes occur in the carbohydrate composition of the tuber especially with respect to the degree of polymerization. Generally polysaccharides tend to be converted to lower DP carbohydrates during storage. The observed downward shift in DP is accompanied by a corresponding change in the fructose to glucose ratio. Bacon and Loxley (1952) found that this increase in the proportion of low molecular weight fructans was quite substantial. Paper partition chromatography was used by Bacon and Edelman (1951) to quantitate the different carbohydrates present. In August low molecular weight fructans represented only 9% of the sugars present while in April this figure had risen to 38% for the Yorkshire variety. The same trend was observed for the Sussex variety; in September only 8% of the sugars were low molecular weight fructans while in April they represented 48%. Both varietal examples would seem to indicate that a great deal of hydrolytic activity occurs in the tubers at this time.

During storage the quantity of high molecular weight fructans decreases and the quantity of low molecular weight fructans increases. It has been previously stated that this type of fructan is terminated by a glucose residue. The shift toward low molecular weight fructans of course results in a decrease in the fructose to glucose ratio. The fructose concentration is consistently lower after storage than at harvest. Dorrell and Chubey (1977) found that at harvest tubers contained 78.2% fructose (expressed as a percentage of the total reducing

sugars). After storage this level had decreased to 68.0%. It has been pointed out that decreasing fructose levels were accompanied by increasing glucose levels. It has been suggested by Bacon and Edelman (1951) that during storage a redistribution of fructose occurs. During storage the average DP decreases (Rutherford and Weston,1968). This change is accompanied by a decrease in the fructose to glucose ratio. It is still not known whether metabolism of fructose occurs during storage resulting in an apparent increase in glucose or whether isomerization of fructose to glucose occurs (Dorrell and Chubey,1977).

2.6 Factors Affecting Storage of Artichoke Tubers

During growth artichoke tubers are remarkably disease and insect resistant (Stauffer et al.,1975). However, some disease problems do exist. Pseudomonas infections have caused severely reduced plant stands in Manitoba and Minnesota (Kiehn and Chubey,1982) and Sclerotinia sclerotiorum (Lib.) has been found to have detrimental effects on artichoke tubers both in the field and in storage (Kiehn and Chubey,1982).

One of the problems associated with this crop is that of minimizing tuber deterioration during storage. In order to achieve successful storage of artichoke tubers several factors must be taken into consideration. First it is desirable to minimize tuber losses due to microbial spoilage. These losses can be devastating, destroying as much as 50% of the tubers in as little as eight weeks (Hoehn and Murray,1982). Second, storage conditions must be such that breakdown of high DP fructans is

minimized. This will ensure a high fructose to glucose ratio. And third, the storage conditions that are chosen must minimize losses of total carbohydrate due to respiration during dormancy.

The artichoke tuber has a thin epiderm (Stauffer et al.,1975; Kiehn and Chubey,1982). This thin skin makes the tuber very susceptible to physical damage during harvest and predisposes it to moisture loss plus breakdown by disease organisms during storage (Kiehn and Chubey,1982). As a result, conditions such as white mold and soft rot are potential problems in artichoke tubers (Stauffer et al.,1975).

It is necessary to determine proper storage practises for the artichoke tuber so that physical deterioration of the tuber as well as undesirable changes in the quality and quantity of the carbohydrate are minimized. Predictably, conflicting accounts about what factors constitute ideal storage conditions can be found in the literature. McGlumphy et al. (1933) reported that storage temperatures of 0°-1.7°C and relative humidities of 82-92% provided the most satisfactory storage conditions for artichoke tubers. During storage, conversion of fructose polymers to short chain oligomers occurs. Edelman and Jefford (1964,1968) reported that these changes were much more pronounced at 2°C than at 20°C. Naturally, one would wish to prevent fructosan breakdown as much as possible in order to maintain a high fructose to glucose ratio. At temperatures of 20°C tubers would be much more susceptible to microbial breakdown (Edelman and Jefford,1968). These workers also found that losses of carbohydrate at low storage temperatures were insignificant when

compared with carbohydrate losses at 20°C. On the other hand Fleming and GrootWassink (1979) found that carbohydrate losses were not related to the storage temperature. These workers reported that samples stored at temperatures ranging from -1.5° to 14°C all lost 4-5% of total reducing sugar content (dry basis) after a storage period of three months. At the higher storage temperature of 25°C, microbial deterioration made the tubers unsuitable for food uses in as little as one month. However, fructose still represented 80-88% of the total reducing sugar content (Fleming and GrootWassink,1979) indicating that the carbohydrate had a high average DP or that little depolymerization had taken place.

Generally, cold storage conditions with controlled humidity will inhibit microbial growth and thereby reduce tuber deterioration. In addition it is also desirable to maintain a high carbohydrate content as well as a high fructose to glucose ratio. Workers have not yet agreed on the best conditions and methods for doing so. It appears to be quite difficult to maintain tuber integrity, carbohydrate content as well as a high fructose to glucose ratio in cold storage conditions. This may be the reason that some workers have investigated other methods of storage. It has been found that tubers can remain in the ground during winter and then be retrieved in the spring in satisfactory condition. The fructose level expressed as percentage of total reducing sugars decreased somewhat from 77.8% to 73.8% from November to April (Bacon and Edelman,1951). However if tubers are overwintered in the soil problems of tuber access may arise during harvesting. It is thought that dehydration of artichoke

tubers prior to storage may be an effective technique for overcoming storage problems associated with this crop while reducing bulk storage volume at the same time (Mazza,1984). Hoehn and Murray (1982) found several advantages associated with dehydration prior to storage. There was no spoilage of the tubers, insignificant breakdown of inulin occurred over a storage period of several years as a result of dehydration and that subsequent storage of the tubers at ambient storage conditions would therefore be inexpensive. The major disadvantage of this technique was the cost of dehydration, estimated in 1982 to be $16.60/Tonne of fresh tubers. Other methods of storage that have been investigated include dessication (McGlumphy et al.,1933; Dykins et al.,1933; Hoehn and Murray,1982) and frozen storage (Fleming and GrootWassink,1979).

2.7 Carbohydrate Metabolism in Artichoke Tubers

Artichoke tubers contain a variety of carbohydrates ranging from sugars such as fructose, glucose and sucrose to fructan chains containing as many as 50 fructose residues (Edelman and Jefford,1968). A number of changes are assumed to occur in the carbohydrate composition of artichoke tubers throughout the life cycle of the tuber. These changes involve the quantity of carbohydrate as well as factors such as chain length. The metabolic pathway present in the artichoke tuber must accomodate these seasonal changes and permit rapid changes in the chain length of these carbohydrates. The changes that occur in the fructans are attributable to the endogenous enzyme systems

which are in operation in the artichoke tuber. Two main enzyme systems as well as at least one minor enzyme system are known to regulate fructan metabolism within the artichoke tuber (Edelman and Jefford, 1968). These systems are: the synthetic system involving the transferases; the hydrolytic system involving the hydrolases; and finally the invertase system.

2.7.1 Sucrose-Sucrose 1^F-Fructosyltransferase (SST)

The SST enzyme is present in tubers during growth and high levels of this enzyme will induce polymerization since SST catalyses the formation of 1^F-fructosylsucrose. This trisaccharide is synthesized from two sucrose molecules in the following manner (Edelman and Jefford,1968).

$$G\text{-}F + G\text{-}F \longrightarrow G\text{-}F\text{-}F + F$$

sucrose 1^F-fructosylsucrose

The enzyme is highly specific for sucrose. Its action is essentially irreversible and it does not catalyse the formation of fructans above the trisaccharide level. The role of SST in fructan synthesis can be seen in Figure 4. The SST enzyme disappears rapidly when the tubers have stopped growing.

The carbohydrate 1^F-fructosylsucrose is the only trisaccharide found in artichoke tuber extracts. This trisaccharide is an important intermediate in the synthesis of fructosans; 1^F-fructosylsucrose is the preferred fructose donor for the synthesis of longer polymers.

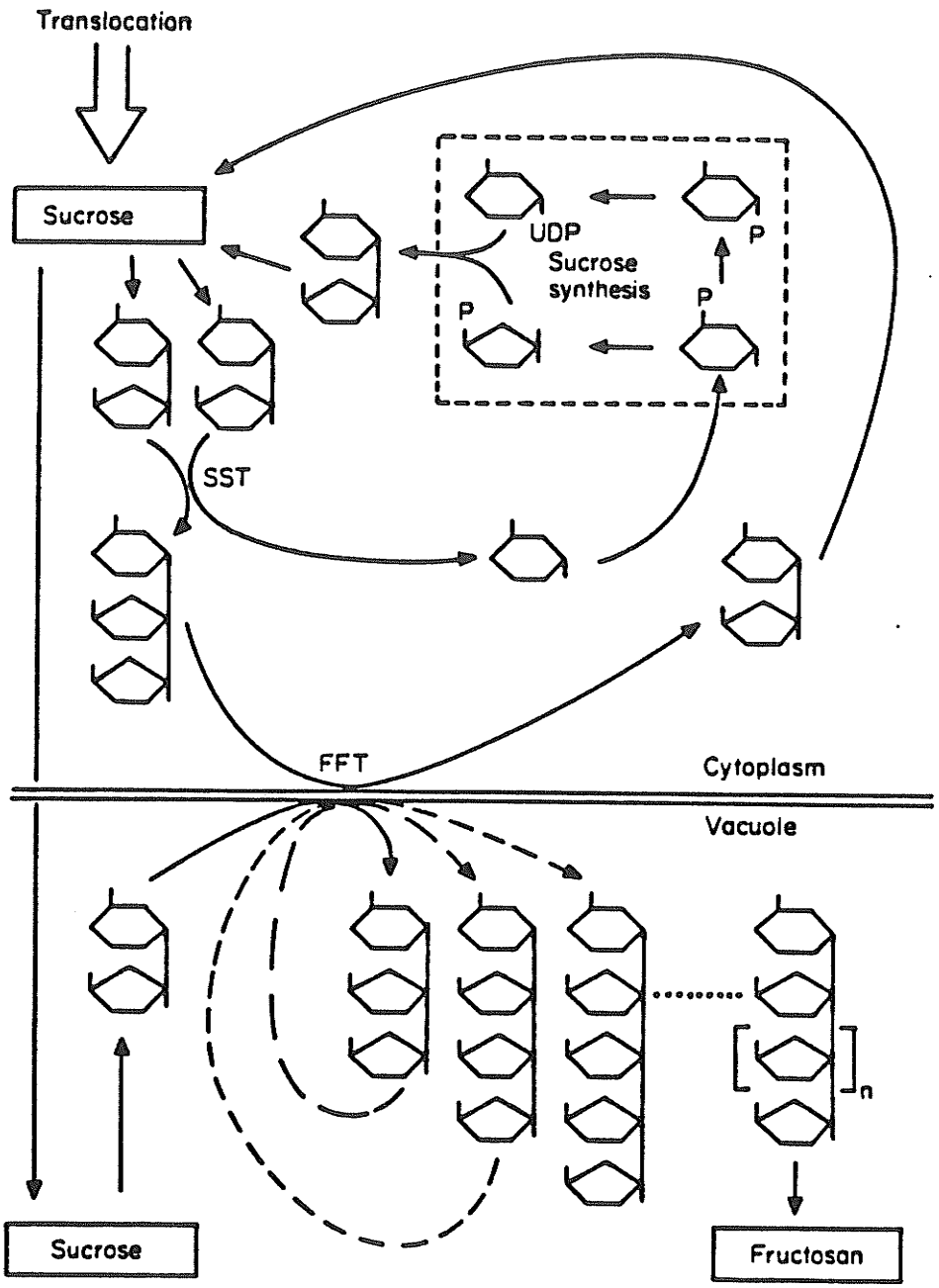


Figure 4: Scheme for polymerization of sucrose to fructose in cells of developing artichoke tuber.
Source: Edelman and Jefford, 1968

2.7.2 Beta(2-->1) Fructan 1^F-Fructosyltransferase (FFT)

The FFT enzyme catalyses the transfer of fructose from donor to acceptor molecules thereby permitting the formation of long chain fructans, 1^F-fructosylsucrose is the primary donor for this reaction.

$$G\text{-}F\text{-}F_n + G\text{-}F\text{-}F_m \longleftrightarrow G\text{-}F\text{-}F_{n-1} + G\text{-}F\text{-}F_{m+1}$$

The FFT enzyme is highly specific, as a result only straight chain fructans are formed. It demonstrates no hydrolytic activity. The function of this enzyme in fructan synthesis is illustrated in Figure 4. The FFT activity is present during all phases of growth but decreases during storage.

2.7.3 The Role of Sucrose in Fructan Metabolism

Artichoke tubers have the ability to synthesize sucrose during growth, dormancy and sprouting. Sucrose can be synthesized from either glucose or fructose or a combination of both sugars. It is thought that the synthesis of sucrose follows the usual pathway involving uridine diphosphate glucose (UDPG) as shown in Figures 4 and 5 (Edelman and Jefford,1968).

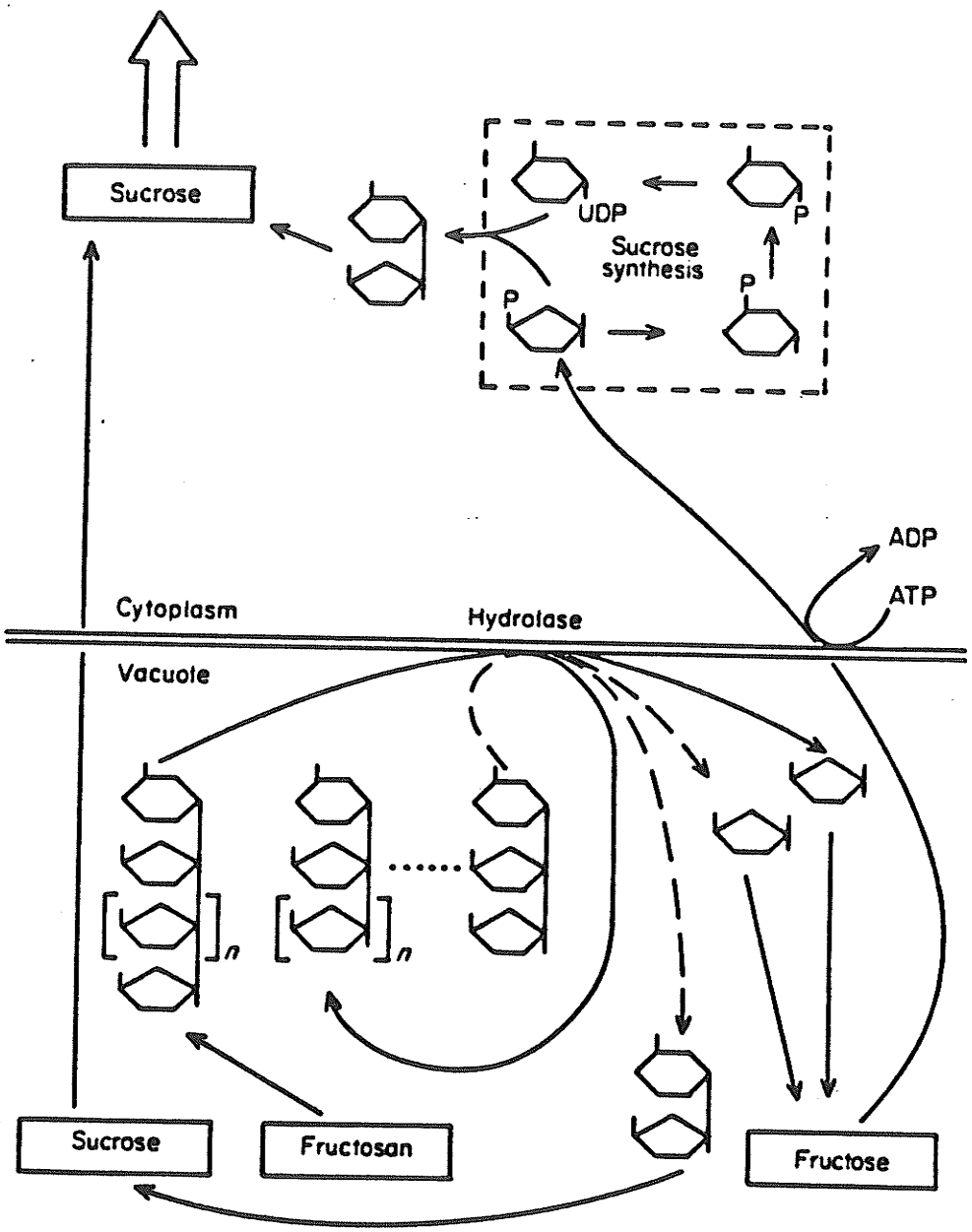


Figure 5: Scheme for depolymerization of fructosans to sucrose in cells of sprouting artichoke tuber.
Source: Edelman and Jefford, 1968

Sucrose seems to have an important role in the regulation of fructan synthesis as well as fructan breakdown. It is the "currency" of fructan metabolism and serves as a substrate for the synthesis of 1^F-fructosylsucrose which in turn is the primary donor in the synthesis of higher fructans. Sucrose also affects the rate at which other metabolic processes occur. For example, the sucrose level can affect the length and number of fructosyl chains by monopolizing FFT. The FFT enzyme is responsible for the synthesis of long chain fructans. Sucrose cannot act as a fructan donor but may accept fructose in this reaction.

$$G\text{-}F\text{-}F + G^{*}\text{-}F^{*} \longleftrightarrow G\text{-}F + G^{*}\text{-}F^{*}\text{-}F$$

1^F-fructosylsucrose sucrose

This has been called the "clutch" mechanism by Edelman and Jefford (1968) and essentially results in the inhibition of fructan synthesis because even though the enzyme is actively turning over fructose molecules, the cycle is a futile one, resulting in no fructan synthesis. Sucrose also has an inhibitory effect on the hydrolytic enzymes responsible for fructan breakdown. It can be seen that sucrose is an important control point in the synthesis and breakdown of carbohydrates within the artichoke tuber.

2.7.4 Beta(2-->1) Fructan 1^F-Fructanohydrolase

Two hydrolases (also known as inulases) have been identified in artichoke tubers by Edelman and Jefford (1964). These have been identified as hydrolase A and hydrolase B. Little distinguishes the two hydrolases except their relative rates of reaction on inulin and its related fructans. These enzymes catalyse the following reaction.

$$G\text{-}F\text{-}F_n \longrightarrow G\text{-}F\text{-}F_{n-1} + F$$

The action of these hydrolases is highly specific and as a result they break only the beta(2-->1) bond between the terminal fructose and its adjacent fructose molecule. No transfructosylation occurs. Fructan polymers are eventually hydrolysed to a mixture of fructose and sucrose but the enzyme is incapable of hydrolysing sucrose to fructose and glucose. The entire scheme for the depolymerization of fructosans can be seen in Figure 5. It is interesting to note that sucrose inhibits hydrolase activity. Depolymerization is enhanced when the tubers are stored at low temperatures (2°C) because the activity of these hydrolases increases under those conditions.

2.7.5 Invertase

Small amounts of invertases are present in artichoke tubers, mainly when depolymerization is occurring. Invertases catalyse the synthesis of sucrose and trisaccharide but they can also hydrolyze them. Invertases cannot therefore be responsible

for large scale production of polyfructans. Although they do exercise a certain control over sucrose, a key control point in fructan synthesis, it has been thought that these invertases represent a relatively unimportant aspect of fructan metabolism.

3. MATERIALS AND METHODS

3.1 Artichoke Tubers

Artichoke tubers (Helianthus tuberosus L.), varieties Columbia and NC10-39 were supplied courtesy of Dr. G. Mazza of the Canada Department of Agriculture Research Station in Morden, Manitoba. The tubers were harvested in the fall of 1983 and 1984. The tubers were stored in plastic bags at 4°C.

3.2 Chemicals

Inulin (purified from chicory) was purchased from BDH. Fructose was obtained from the Fisher Scientific Company in New Jersey. Bio-Gel P-2 and Bio-Gel P-6 were obtained from Bio-Rad. Maltotriose, maltotetraose, maltopentaose and maltohexaose were purchased from Sigma. All Other Chemicals used were of analytical grade.

3.3 Proximate Analysis of Artichoke Tubers

Proximate analyses were carried out on two varieties of 1984 artichoke tubers, Columbia and NC10-39. All analyses for moisture, nitrogen, crude fat and ash were performed according to A.O.A.C. (1975) specifications. The total carbohydrate content of the tubers was determined by difference. All analyses were carried out in triplicate.

3.4 Methods for the Determination of Carbohydrates

3.4.1 Total Soluble Carbohydrates

Total soluble carbohydrates were determined in artichoke extracts using the method of Dubois et al.,1956. Fructose was used as the standard sugar in the preparation of the calibration curve.

3.4.2 Free Reducing Sugars

Free reducing sugars were determined in artichoke extracts using the Folin and Wu method (A.O.A.C.,1975). Parallel determinations were made on water as well as on a fructose standard working solution (0.2 mg/mL).

3.4.3 Total Hydrolyzed Reducing Sugars

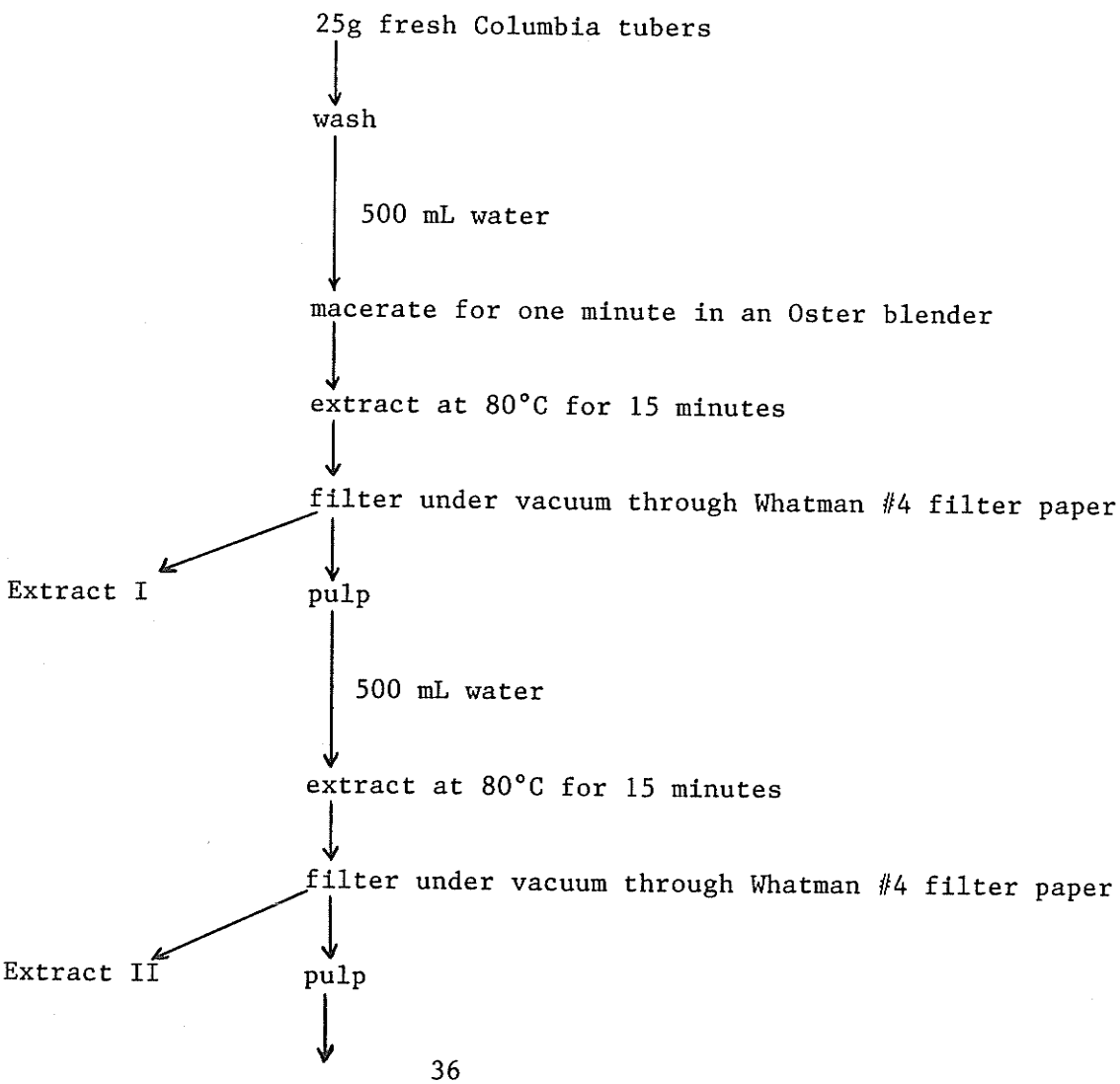
The total hydrolyzed reducing sugars were determined in artichoke extracts using a modification of the Folin and Wu method. The pH of the extracts was decreased to 1.5 using 4N HCl. The samples were incubated at 80°C for one hour in a waterbath in order to inactivate endogenous enzymes. The samples were cooled and then the pH was increased to 5.5 using 4N NaOH. Appropriate dilutions were made and the total hydrolyzed reducing sugars were determined as outlined in section 3.3.2.

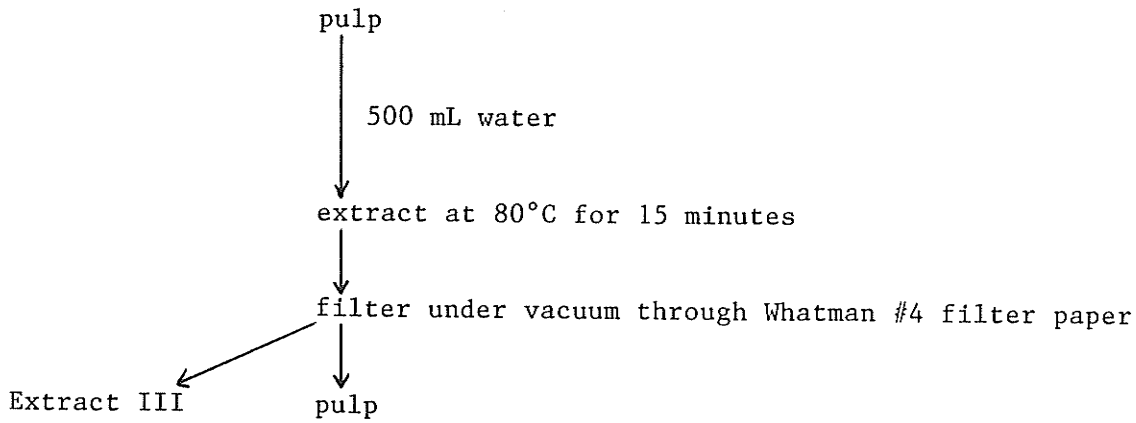
3.5 Extraction Procedures

Artichoke tubers (Helianthus tuberosus L.), varieties Columbia and NC10-39 obtained from the Canada Department of Agriculture Research Station in Morden, Manitoba were used in the preparation of carbohydrate extracts.

3.5.1 Extraction of 1983 Artichoke Tubers (var. Columbia)

The overall extraction procedure is tabulated below:

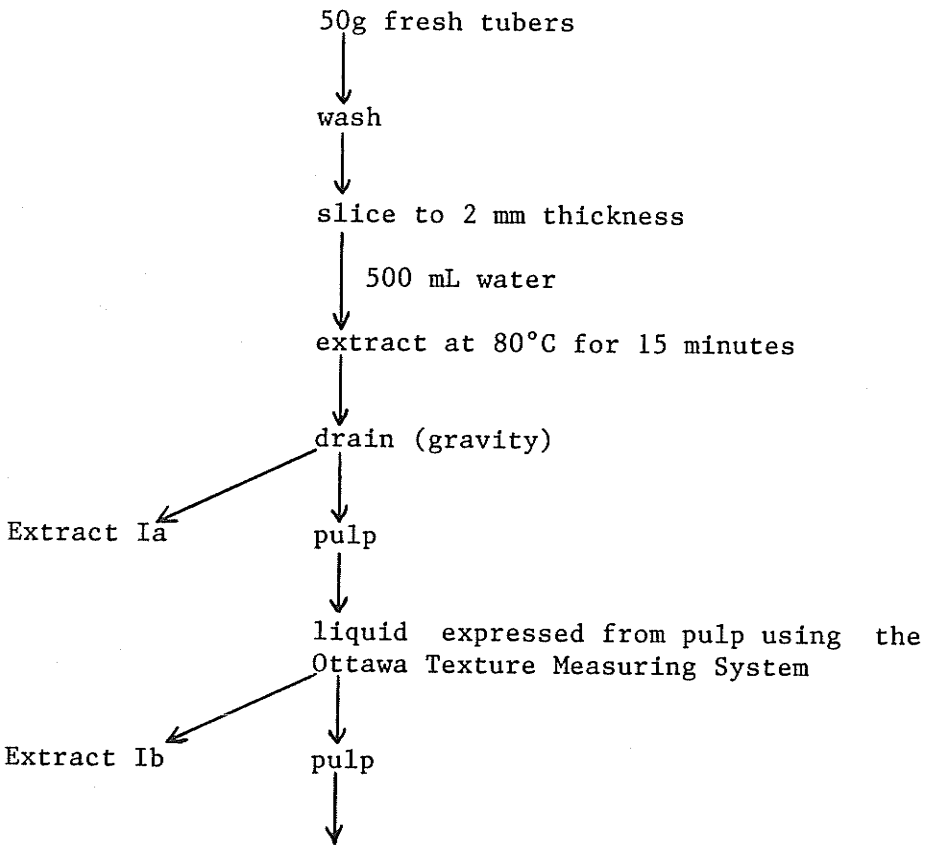


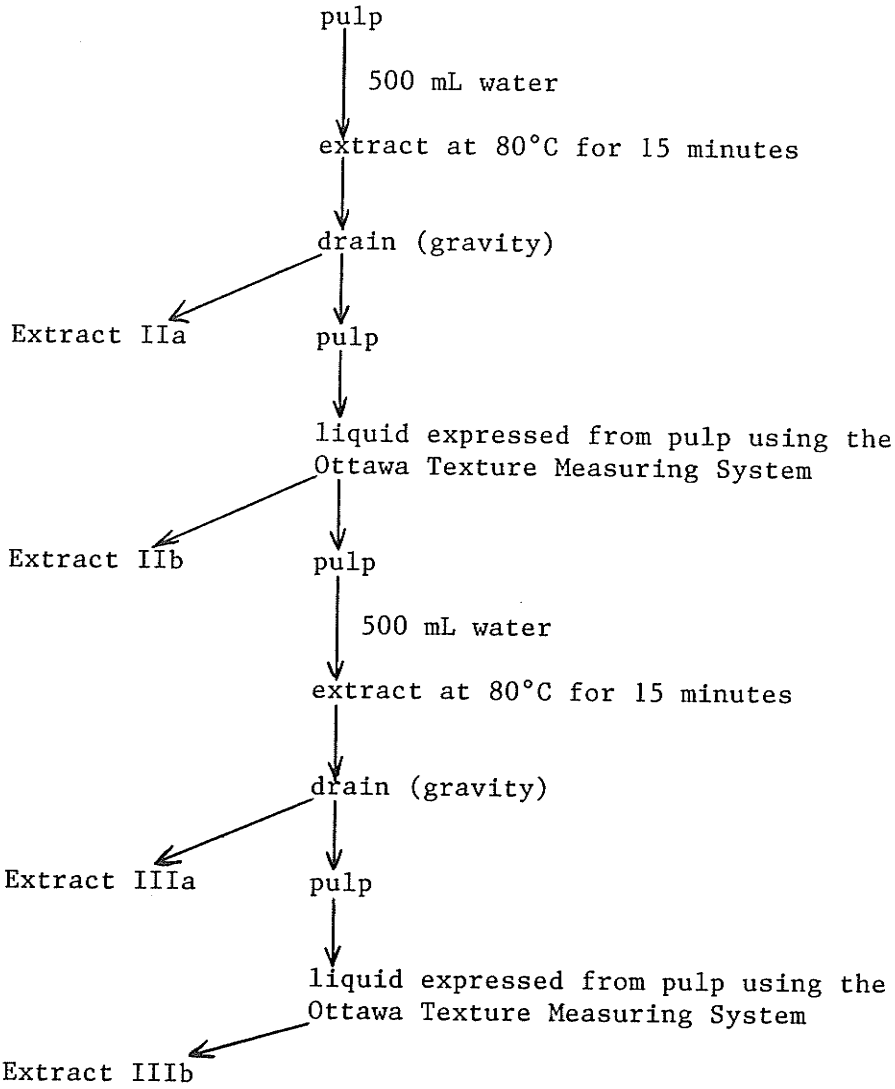


Three replicates were carried out in quadruplicate (12 determinations per value).

3.5.2 Extraction of 1984 Artichoke Tubers (var. Columbia)

The following procedure was used for the preparation of extracts from 1984 Columbia tubers.



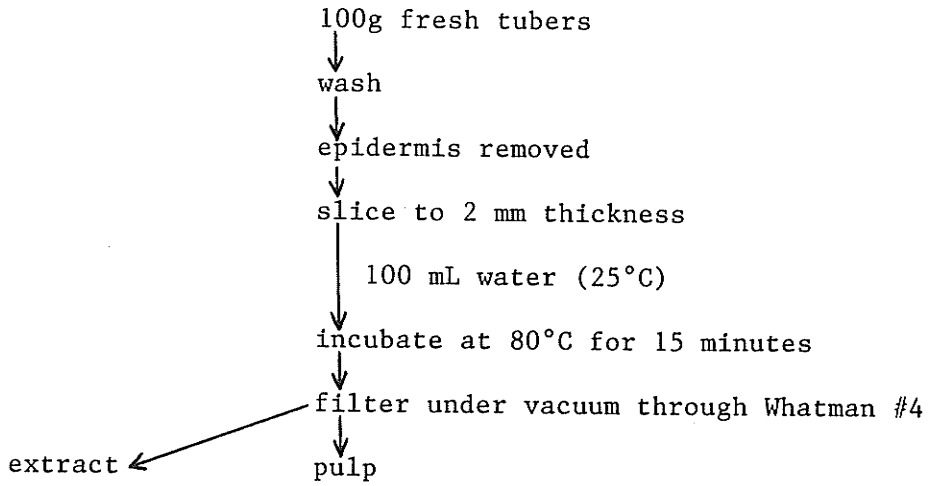


Two replicates were carried out in duplicate (four determinations per value).

3.5.3 Extraction of 1983 Artichoke Tubers (var. Columbia) for Chemical and Chromatographic Analyses

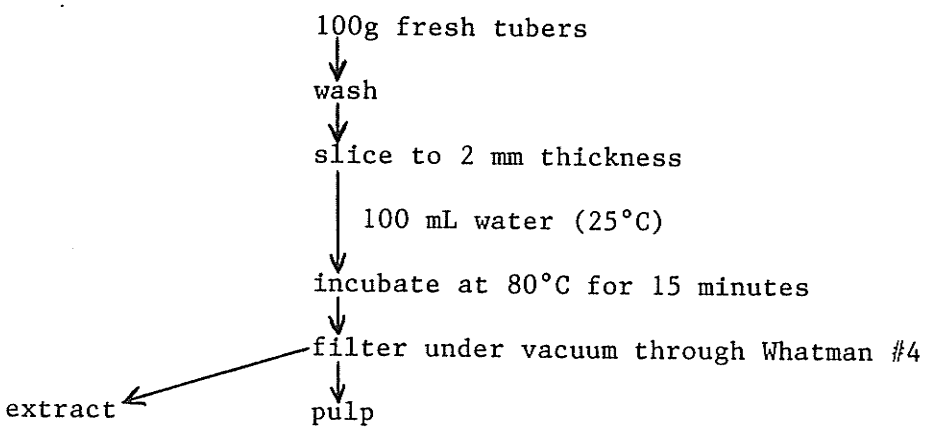
A total of six different extraction methods were employed. They are as follows:

Extraction A



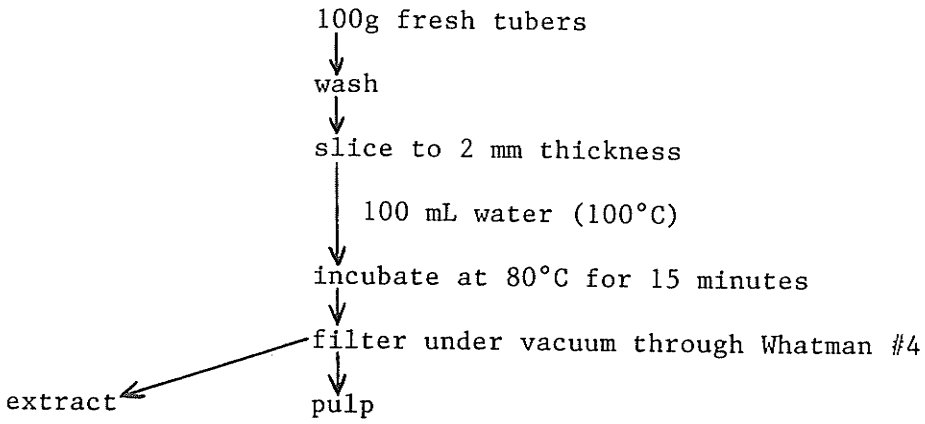
Both pulp and extract were lyopholized prior to analysis.

Extraction B



Both pulp and extract were lyophilized prior to analysis.

Extraction C



Both pulp and extract were lyophilized prior to analysis.

The remaining three extraction methods were identical to the above three methods except that the samples were macerated instead of sliced. The samples were macerated in an Oster blender for one minute. All extractions were carried out in triplicate.

3.6 Chromatographic Analysis

3.6.1 Preparation of Extracts for Chromatographic Fractionation

Fifty grams of fresh artichoke tubers were washed and blotted dry with paper towels. The sample was macerated for one minute with 100 mL of distilled water in an Osterizer blender. The sample was incubated at 80°C for 15 minutes in a waterbath. The slurry was then filtered under vacuum through a Buchner funnel using Whatman #4 filter paper. The filtrate was diluted 1:10 with distilled water prior to fractionation.

3.6.2 Column Characteristics

A jacketed 2.5 x 75 cm column from Pharmacia (Uppsala, Sweden) was used. Bio-Gel P-2 was used as the stationary phase. It was purchased from Bio-Rad Laboratories (Richmond, California). A Heto circulating waterbath (Birkerod, Denmark) was used to elevate the temperature of the column to 60°C. An LKB peristaltic pump was used to pump degassed water (80°C) containing 0.02% sodium azide through the column at a rate of 25 mL/h.

3.6.3 Fractionation of Artichoke Extracts by Gel Filtration Chromatography

A 2 mL aliquot of extract prepared as per section 3.6.1 was applied to the column. Fractions of 4 mL each were collected overnight using an LKB 2112 Redirac fraction collector. In the case of the 1983 extracts (Section 3.5.3) the extracts were lyophilized and then rehydrated to a concentration of 0.4% w/v just prior to fractionation. Two mL aliquots were applied to the column. In all cases, one replicate of each extraction procedure was submitted for chromatographic analysis and it was run on two columns simultaneously.

3.6.4 Fractionation of Carbohydrate Standards by Gel Filtration Chromatography

Commercially prepared fructose, sucrose, maltotriose,

maltotetraose, maltopentaose, maltohexaose and inulin standards were fractionated by gel filtration chromatography. Each commercial standard was hydrated to concentrations of 0.1%, 0.05% and 0.025% w/v. Two mL aliquots of each concentration were fractionated on Bio-Gel P-2. In the case of the pooled samples 5 mg of each standard were weighed out. These were dissolved together in 20 mL of distilled water. Two mL aliquots were fractionated on Bio-Gel P-2. Fractionations were carried out on two columns simultaneously.

3.6.5 Chemical Analysis of Fractionated Extracts

All chromatographically collected fractions were analyzed for total carbohydrate using the phenol-sulfuric acid method (Dubois et al.,1956). All chromatographic work was carried out in duplicate.

3.7 Thermal Analysis of Commercial Inulin Samples

Differential scanning calorimetry was carried out using a Dupont Model 990 Thermal Analyzer. A heating rate of 10°C/min was employed. Lyophilized inulin samples previously fractionated by gel filtration chromatography were combined with varying ratios of water and 5-10 mg of the slurry was weighed into a DSC pan and was sealed. The samples were scanned from 10°C to 110°C. Sand was used as a reference.

4. Results and Discussion

4.1. Analysis of Tuber Composition

Proximate analysis of two varieties of 1984 tubers was carried out and the results are presented in Table 2. The vacuum oven method and lyophilization were used to determine the water content of the tubers. Water was the major component of the tubers representing 77.96% of the tuber weight for Columbia and 78.19% of the tuber weight for the NC10-39 variety. The protein, crude fat and ash were relatively minor components representing 2.66, 0.06 and 0.96% respectively for Columbia variety and 2.49, 0.06 and 1.10% respectively for the NC10-39 variety. The major component of interest in this study is the carbohydrate component. The carbohydrate content was determined by difference and was 18.36% for the Columbia variety and 18.16% for the NC10-39 variety. The type of carbohydrate present in artichoke tubers can be divided naturally into two groups. The first being the water soluble carbohydrates comprising fructose, sucrose, fructan polymers of various lengths (DP) and inulin. The second main category of carbohydrate present in the tuber is the water insoluble fraction comprising cellulose, hemicellulose and possibly lignin. Fleming and GrootWassink (1979) report that cellulose and hemicellulose constitute 13% of the dry weight of Jerusalem artichoke tubers. Very little work has been carried out on this fraction. It has not been well characterized. This study will focus on the characterization of the water soluble carbohydrates in Jerusalem artichoke tubers.

Table 2 Composition of Artichoke Tubers

	Columbia Fresh Weight (%)	NC10-39 Fresh Weight (%)
Water	77.96 ± 0.71	78.19 ± 0.44
Carbohydrate[1]	18.36 ± 0.79	18.16 ± 0.60
Protein[2]	2.66 ± 0.05	2.49 ± 0.06
Lipid	0.06 ± 0.002	0.06 ± 0.002
Ash	0.96 ± 0.03	1.10 ± 0.10

[1]Carbohydrate content was determined by difference.

[2]Protein content was determined by multiplying the percent nitrogen value by 6.25.

4.2 Extraction of Water-Soluble Carbohydrate from Artichoke Tubers

A 100 gram sample of artichoke tubers contains about 18% of total carbohydrate (Table 2). Of this amount about 13% is water insoluble carbohydrate such as cellulose (Fleming and GrootWassink, 1979). Therefore about 16 grams of the carbohydrate present in a 100 gram sample of artichoke tubers may be estimated to be water soluble.

Water soluble carbohydrates were extracted from 1983

Columbia variety tubers. The tubers were washed and water was added to extract the carbohydrate. The ratio of tuber to water for each extraction was constant at 1:20. Each sample of tuber was extracted with water 3 times. The tubers were macerated in an Oster blender for one minute to facilitate extraction of the carbohydrate. The slurry was held at 80°C for 15 minutes before vacuum filtration. The results are presented in Table 3. It can be seen that 97% of the total amount of soluble carbohydrate extracted came out in the first extract. Much lower yields of carbohydrate were obtained in the second and third extracts. From a commercial point of view it would not be worthwhile to carry out any more than one extraction since the cost of extracting the remaining available carbohydrate would probably be greater than the value of the extra carbohydrate extracted.

Table 3 Efficiency of Carbohydrate Extraction from 1983 Columbia Tubers

Extract	Grams of Soluble Carbohydrate per 100 Grams of Tubers using Two Analytical Methods	
	Phenol Sulfuric Acid Method	Folin-Wu Method
I	19.46 ± 0.58	14.96 ± 0.06
II	0.42 ± 0.10	0.36 ± 0.07
III	0.03 ± 0.03	0.04 ± 0.02
Total	19.91 ± 0.71	15.36 ± 0.15

In a second extraction experiment 1984 Columbia tubers were washed and then sliced to 2mm thickness. Water was added in a ratio of 1:10 (w/v). The mixture was held in a waterbath at 80°C for 15 minutes. The fluid was drained from the slices and the liquid was then expressed from the pulp using the Ottawa Texture Measuring System. The pulp was extracted in this manner three times. The results of this extraction study on carbohydrate recovery from 1984 Columbia variety tubers are shown in Table 4. There are dramatic differences in carbohydrate recovery between Tables 3 and 4. For Table 4, only 50-56% of the total extracted carbohydrate came out in the first extract. Whereas for Table 3, 97% of the soluble carbohydrates extracted came out in the first extract. In addition to the observed differences in the carbohydrate extraction profiles between Tables 3 and 4, the total amount of soluble carbohydrate extracted also varied considerably. It can be seen in Table 3 that a total of 19.91 grams of soluble carbohydrate was extracted per 100 grams of tubers (using the phenol-sulfuric acid method) whereas for Table 4 a total of only 15.35 grams of carbohydrate/100 grams of tubers was extracted.

Table 4 Efficiency of Carbohydrate Extraction from 1984 Columbia Tubers

Extract	Grams of Soluble Carbohydrate per 100 Grams of Tubers using Two Analytical methods	
	Phenol Sulfuric Acid Method	Folin-Wu Method
Ia	6.13 ± 0.64	6.60 ± 0.56
Ib	1.53 ± 0.19	1.34 ± 0.19
IIa	4.43 ± 0.58	3.14 ± 0.35
IIb	0.90 ± 0.08	0.85 ± 0.08
IIIa	2.00 ± 0.07	1.94 ± 0.27
IIIb	0.36 ± 0.04	0.34 ± 0.05
Total	15.35 ± 1.60	14.21 ± 1.50

There were several important differences between the two extraction methods which probably account for some of the differences that were observed. First, for Table 3, the extract was separated from the tuber pulp by filtration under vacuum whereas for Table 4 the extract was separated from the pulp by gravity filtration. Vacuum filtration may have been a more effective method of drawing moisture out of the sample than was gravity filtration thereby increasing the recovery of soluble carbohydrate. Second, in Table 4 the tuber pulp was expressed using the OTMS prior to subsequent extractions whereas for Table 3 the pulp was not expressed. The concentration of the

carbohydrate in the expressed liquid (Table 4) was found to be very high, yet the overall recovery of carbohydrate was lower than that in Table 3. Third, for Table 3, the tuber to water ratio used in the extraction was 1:20; for Table 4 this ratio was 1:10. The solubility curve shown in Figure 3 of the literature review indicates that inulin is soluble in water at about the 4% level at 80°C (the temperature at which the extractions were done). Although this indicates that solubility restrictions should not have been a limiting factor in these extractions, it appears however that the larger water to tuber ratio may have been a factor which could explain the more efficient carbohydrate extraction seen in Table 3. Finally, for Table 3 the samples were macerated, and for Table 4, they were sliced. The greater surface area of the macerated sample may have made solubilization of the carbohydrate easier thereby accounting for the greater amount of carbohydrate extracted in Table 3 as well as for the high carbohydrate recovery seen in the first extraction in Table 3.

It can be seen that the Folin-Wu test consistently gives lower results than the phenol-sulfuric acid test. A modification of the Folin-Wu test was used to determine the total hydrolyzed reducing sugar content of the extracts. The pH of the extracts was decreased to 1.5 using 4N HCl. The samples were then incubated at 80°C for one hour in a waterbath in order to allow the carbohydrate to be hydrolyzed. It would appear that these conditions might be insufficient for complete hydrolysis of the carbohydrate. It was also observed that the phenol-sulfuric acid test sometimes indicated carbohydrate levels above the

theoretical maximum present in the tubers. It would appear that the more rigorous phenol-sulfuric acid test (which utilizes concentrated sulfuric acid to hydrolyze the carbohydrate) is giving false high results. These factors must be taken into consideration at all times, not just when mass balance studies are being done.

It is apparent that the amount of carbohydrate that was extracted from the tubers approached or appeared to exceed the theoretical amount of water-soluble carbohydrate present in the sample. This would indicate that highly effective extraction of available carbohydrate from artichoke tubers is possible.

4.3 Characterization of Commercial Standards on Bio-Gel P-2 Columns

A total of seven commercial carbohydrates were characterized on acrylamide cross-linked adsorbant gels. These carbohydrate standards were: fructose, sucrose, maltotriose, maltotetraose, maltopentaose, maltohexaose and inulin. When each of these carbohydrates was characterized individually by gel filtration using Bio-Gel P-2 the pattern of elution was consistent i.e. the same carbohydrate always eluted in the same place. When this series of carbohydrates was combined and run as a pooled sample it was possible to clearly separate each of the carbohydrate standards on the Bio-Gel P-2 using water as the eluent (Figure 6). Because the individual elution patterns were known it was possible to conclude that the carbohydrates, when combined, were eluted in decreasing order of molecular size. The

technique of gel filtration would therefore be useful not only to characterize different raw materials but to fractionate molecules of this type and also to estimate their molecular weights.

When known amounts of the carbohydrate standards were applied to Bio-Gel P-2 and the quantity of eluted carbohydrate in each fraction assayed, interesting patterns of carbohydrate recovery were observed. These are presented in Table 5. Interestingly, five of the carbohydrates that were examined showed recovery rates of greater than 100%, i.e. once again appearing to be in excess of the theoretical maximum; only fructose and inulin had recovery rates less than 100%. This observation was thought to have occurred because throughout all column work, estimations of carbohydrate recovery were based on calculations made from the fructose standard curve. When individual standard curves were constructed and used to calculate individual peak recoveries, all were less than 100% (Table 5). This illustrates the importance of working with the correct standards in a correct manner. This is especially applicable when working with unknown quantities.

FIGURE 6 FRACTIONATION OF CARBOHYDRATE STANDARDS ON BIO-GEL P-2

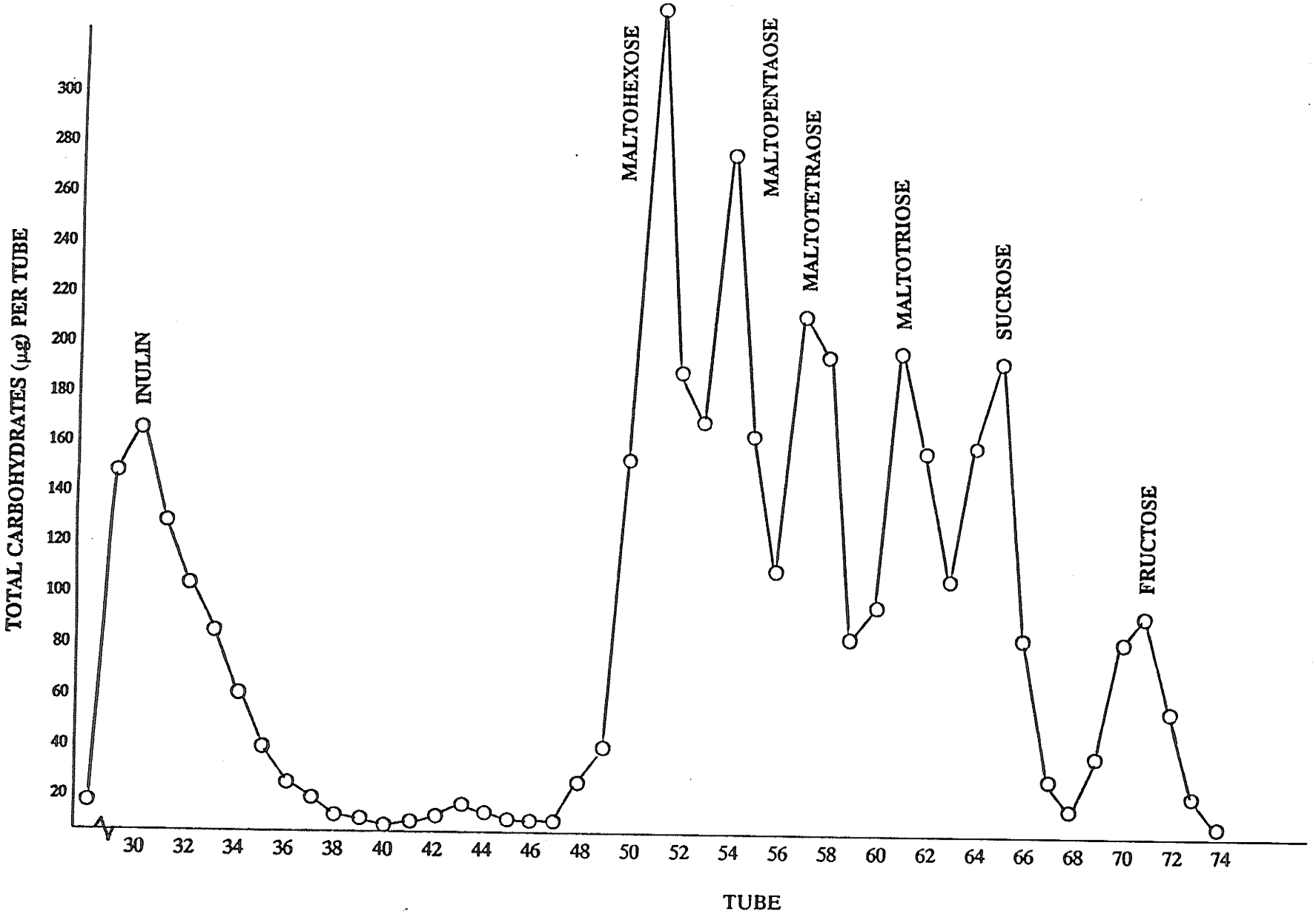


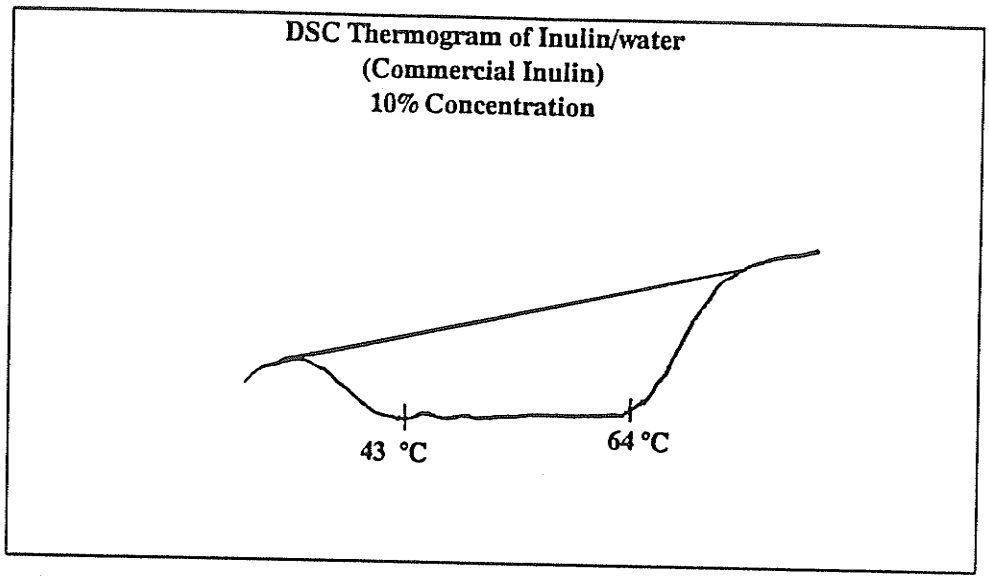
Table 5 Column Recoveries on Bio-Gel P-2

Carbohydrate	Fructose Standard Used to Determine Each Carbohydrate Recovery (%)	Respective Standards Used to Determine Each Carbohydrate Recovery (%)
Fructose	57	57
Sucrose	111	58
Maltotriose	146	75
Maltotetraose	137	75
Maltopentaose	113	57
Maltohexaose	114	53
Inulin	52	

4.4 Thermal Characterization of Commercial Inulin

Commercial inulin purified from chicory (BDH) was characterized by differential scanning calorimetry. Samples containing from 10-50% inulin in water were examined. It was found that each concentration had its own characteristic endotherm. These can be seen in Figures 7, 8 and 9.

FIGURE 7 DSC THERMOGRAMS OF VARIOUS CONCENTRATIONS OF COMMERCIAL INULIN IN WATER.



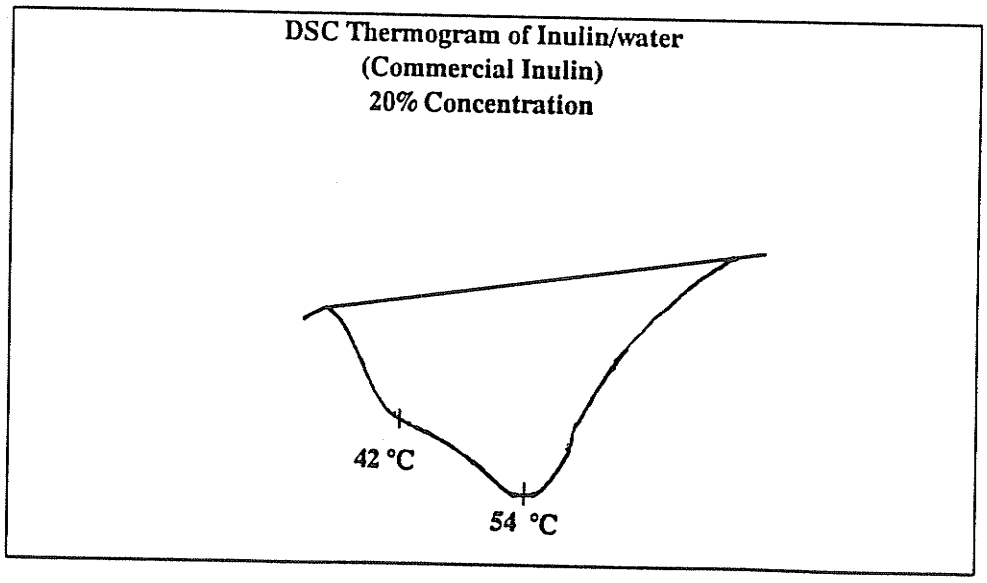
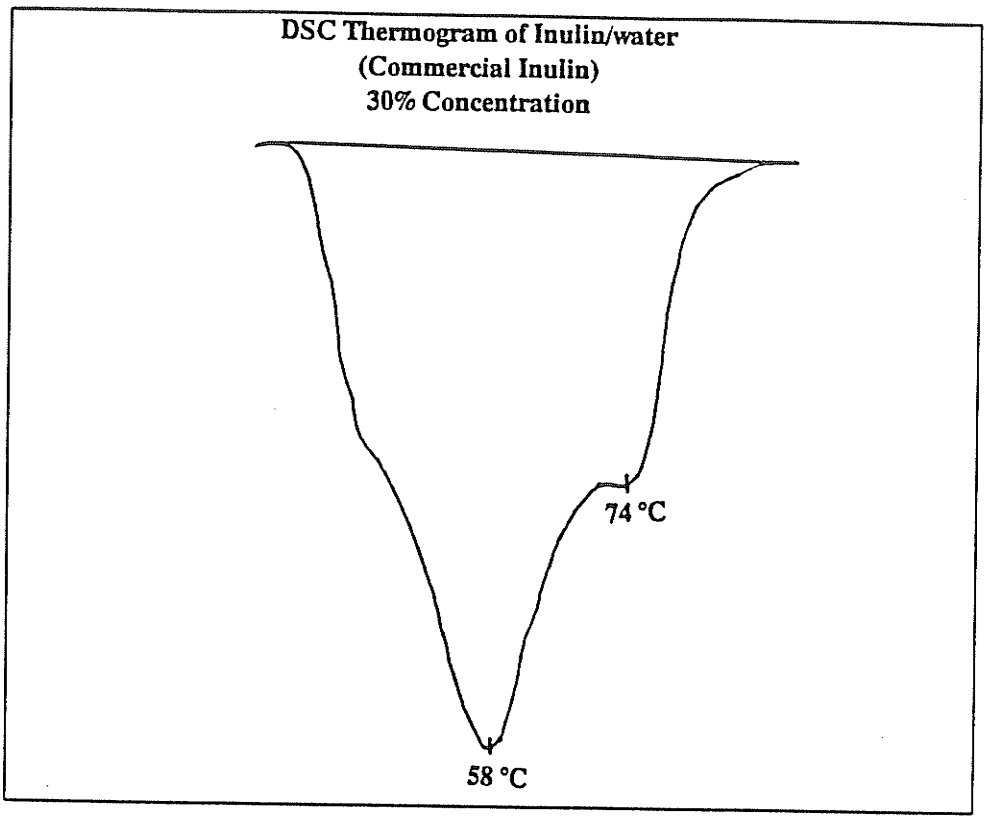


FIGURE 8 DSC THERMOGRAMS OF VARIOUS CONCENTRATIONS OF COMMERCIAL INULIN IN WATER.



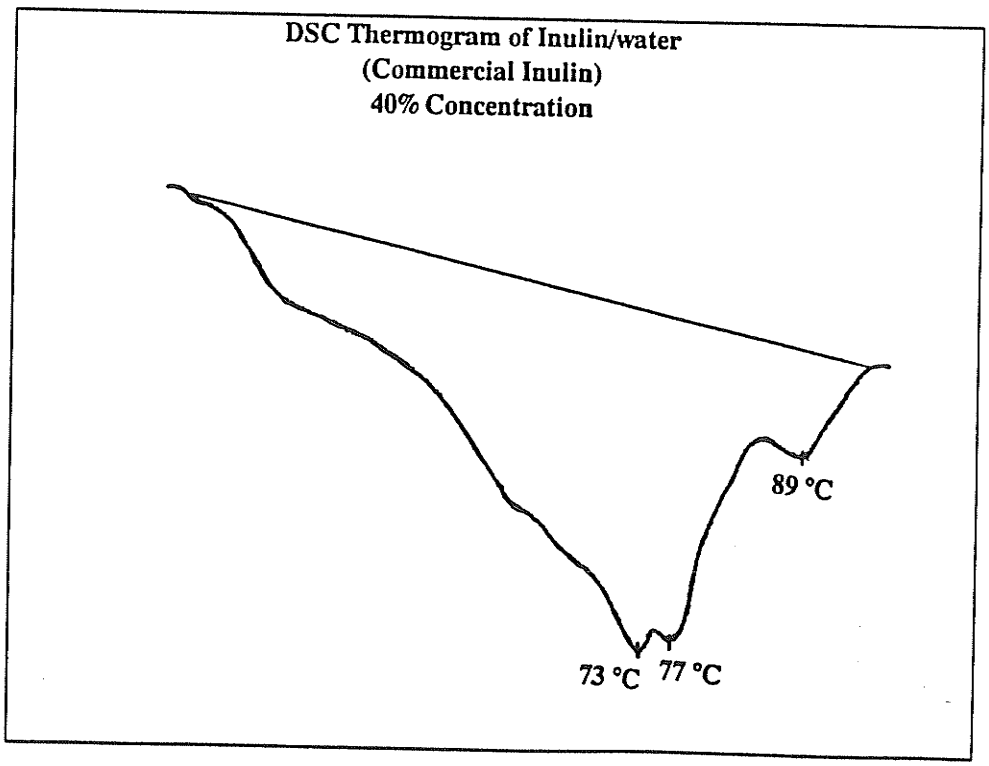
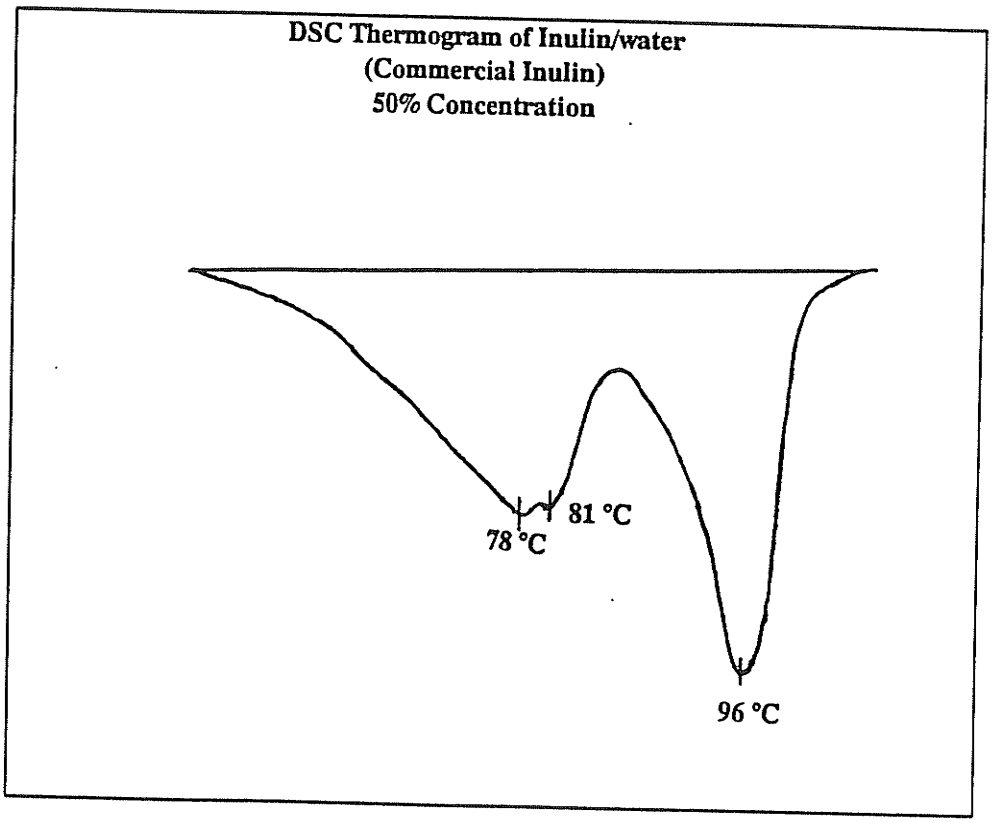


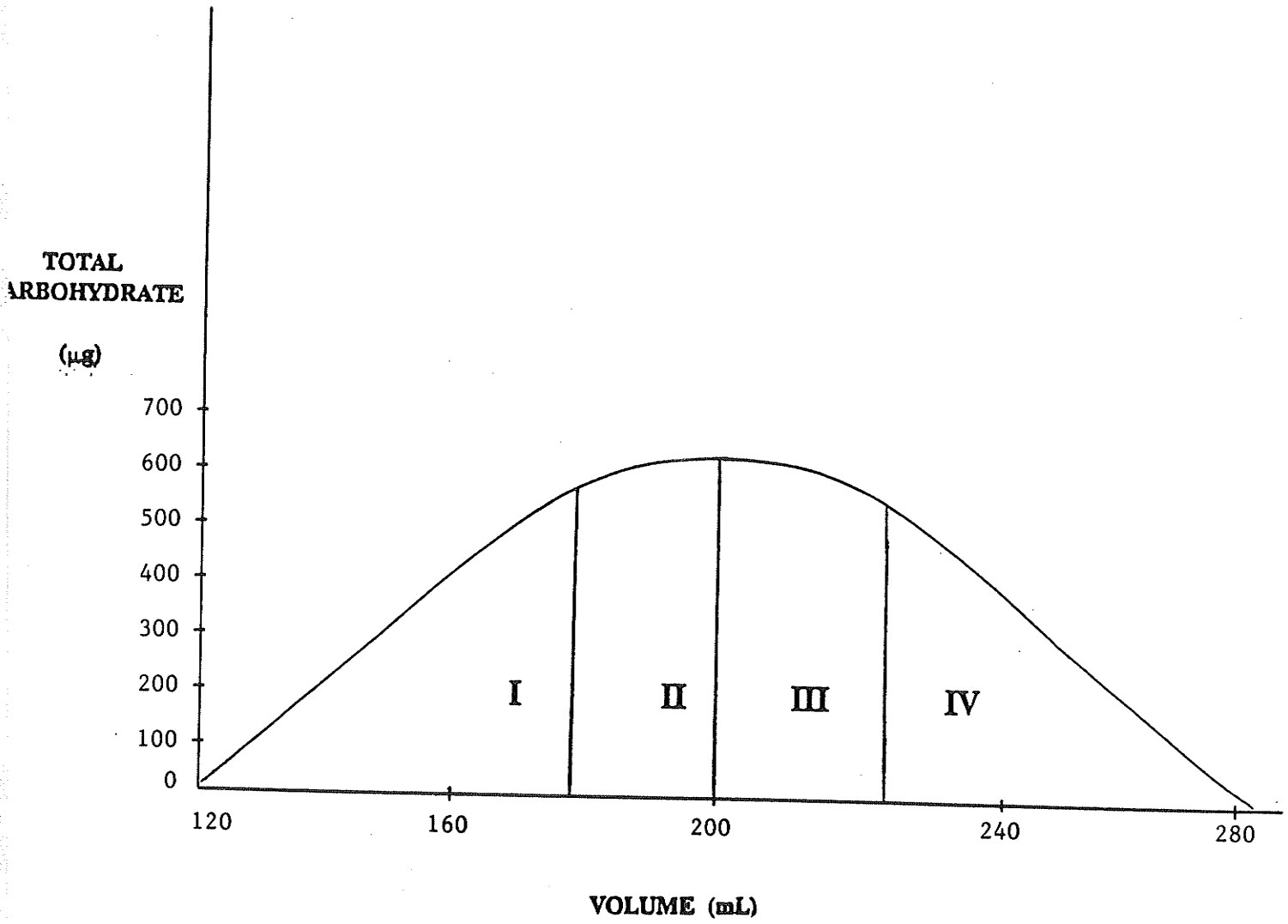
FIGURE 9 DSC THERMOGRAMS OF VARIOUS CONCENTRATIONS OF COMMERCIAL INULIN IN WATER.



It can be seen that the greater the concentration of inulin, the higher the transition temperature. This phenomenon has been well documented on thermal studies of starch (Donovan, 1979). It appears that when water is a limiting factor, thermally resistant portions of a polymer will become even more resistent. It can also be seen from Figures 7, 8 and 9 that at higher concentrations of inulin more than one endotherm occurred. This phenomenon has also been observed in starch (Donovan, 1977; Donovan, 1980). Starch is a well known heterogeneous polymer. It is thought that more than one mechanism is responsible for the thermal behavior of starch. Multiple endotherms may therefore indicate that more than one mechanism of action is being observed with inulin.

In the next phase of work on commercial inulin a preparation of inulin was fractionated on Bio-Gel P-6 (Figure 10). Since Bio-Gel P-6 fractionates molecules on the basis of size, the larger molecules are eluted at the head of the peak (Fraction I) and the smaller molecules are eluted at the tail (Fraction IV). The peak was divided into four fractions of approximately equivalent carbohydrate contents and these four fractions from a number of runs were collected and lyophilized in preparation for thermal analysis. When these fractions were examined by differential scanning calorimetry it was observed that each fraction had its own characteristic endotherm (Figures 11 and 12).

FIGURE 10 FRACTIONATION OF COMMERCIAL INULIN ON BIO-GEL P-6



It was found that the transition temperature generally decreased as the size of the polyfructan decreased with the exception of Fraction I. This would seem to indicate that less heat is required to disorder small molecules and more heat is required to disorder large molecules i.e. larger molecules would appear to have a self-stabilizing ability.

It was also found that thermograms of the smaller polyfructans were much sharper and well defined than those fractions containing the larger polymers. This may indicate that fractions containing the lower molecular weight fructans were more homogeneous than those containing the larger polyfructans.

Starch has been very well characterized by DSC and for this reason it is tempting to utilize the knowledge gained from the extensive research that has been done on starch to explain the thermal behavior of inulin. However, the work done on starch may not be comparable with that done on inulin because of the large structural differences between these two polysaccharides. Starch is a large and heterogeneous polymer with highly branched as well as linear portions. Inulin, on the other hand, is a relatively small polysaccharide which is polydisperse and is not thought to have any branching. As a result, the thermal behavior of starch may be the result of completely different mechanisms than those of inulin.

It can be said for any molecule that the observed endotherm represents absorption of heat by the sample which effects a change in that sample (either chemical or physical). With inulin, the exact nature of that change is unknown because

inulin has not been well characterized by DSC. There are several possible explanations to describe what is happening to inulin as a result of the heating process.

First, the observed thermograms may represent dissolution endotherms i.e. the application of heat causes the sample to dissolve. This is a distinct possibility considering the relatively low solubility of inulin at room temperature and the great increase in solubility that occurs at temperatures above 60 °C (Figure 3). Second, these endotherms may represent melting transitions as those seen in work done on starch. According to the Flory-Huggins theory, melting temperature is a function of water content (Donovan, 1979). It was found that when the reciprocal melting points as determined from the endotherms in Figures 7, 8 and 9 were plotted against the volume fraction of water in the sample that a straight line was obtained (Figure 13).

This straight line relationship would appear to indicate that these endotherms represent melting transitions. Recently however, questions have arisen on the validity of applying the Flory-Huggins theory to predict polymer behavior (Biliaderis,1986). The Flory-Huggins theory as previously mentioned indicates a relationship between polymer melting temperature and concentration of that polymer - under equilibrium conditions. Since many of the changes that occur in polymer/solvent systems as a result of the heating process are irreversible such a system cannot be said to be in equilibrium.

FIGURE 11 DSC THERMOGRAMS OF FRACTIONATED COMMERCIAL INULIN (30% w/w)

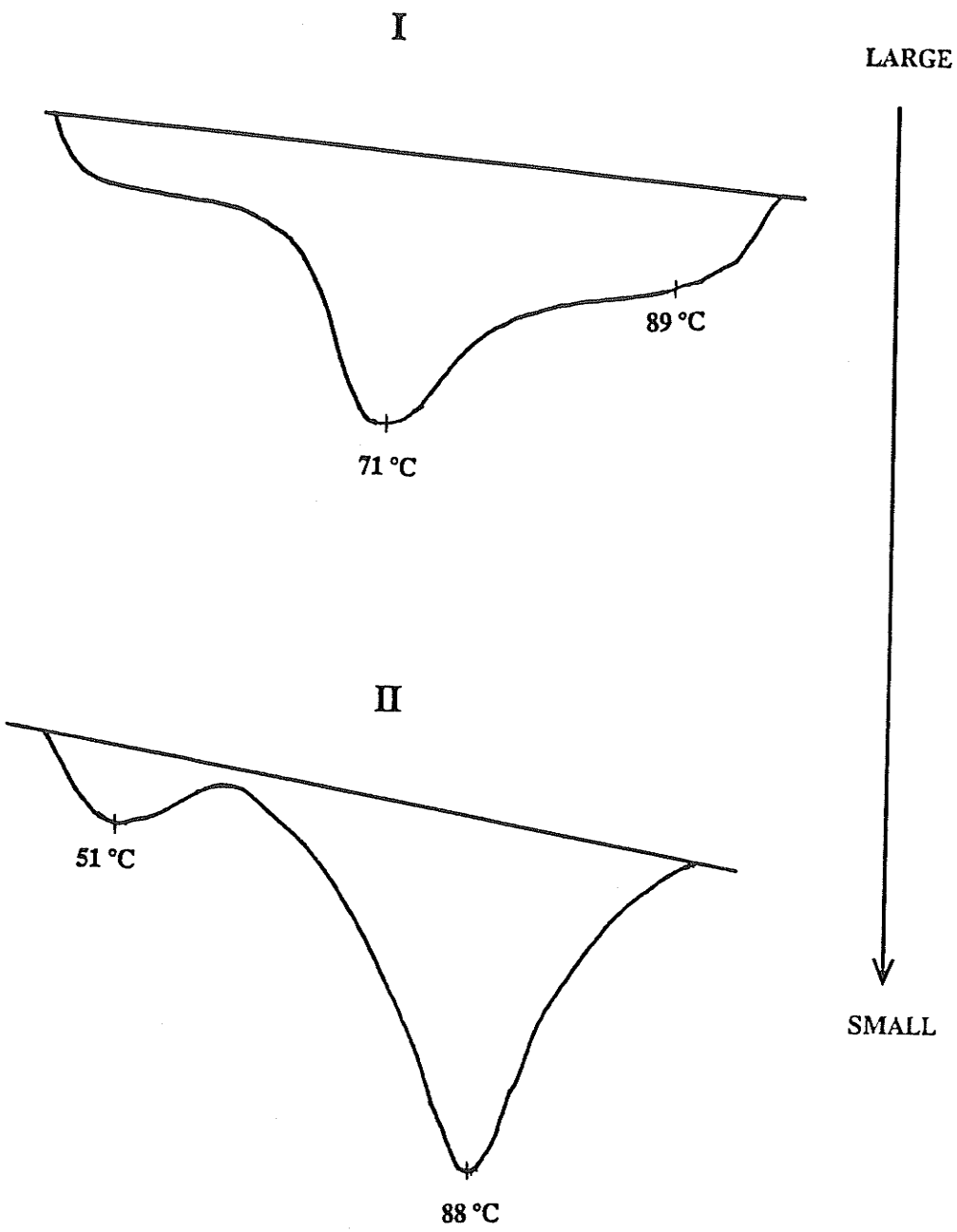


FIGURE 12 DSC THERMOGRAMS OF FRACTIONATED COMMERCIAL INULIN (30% w/w)

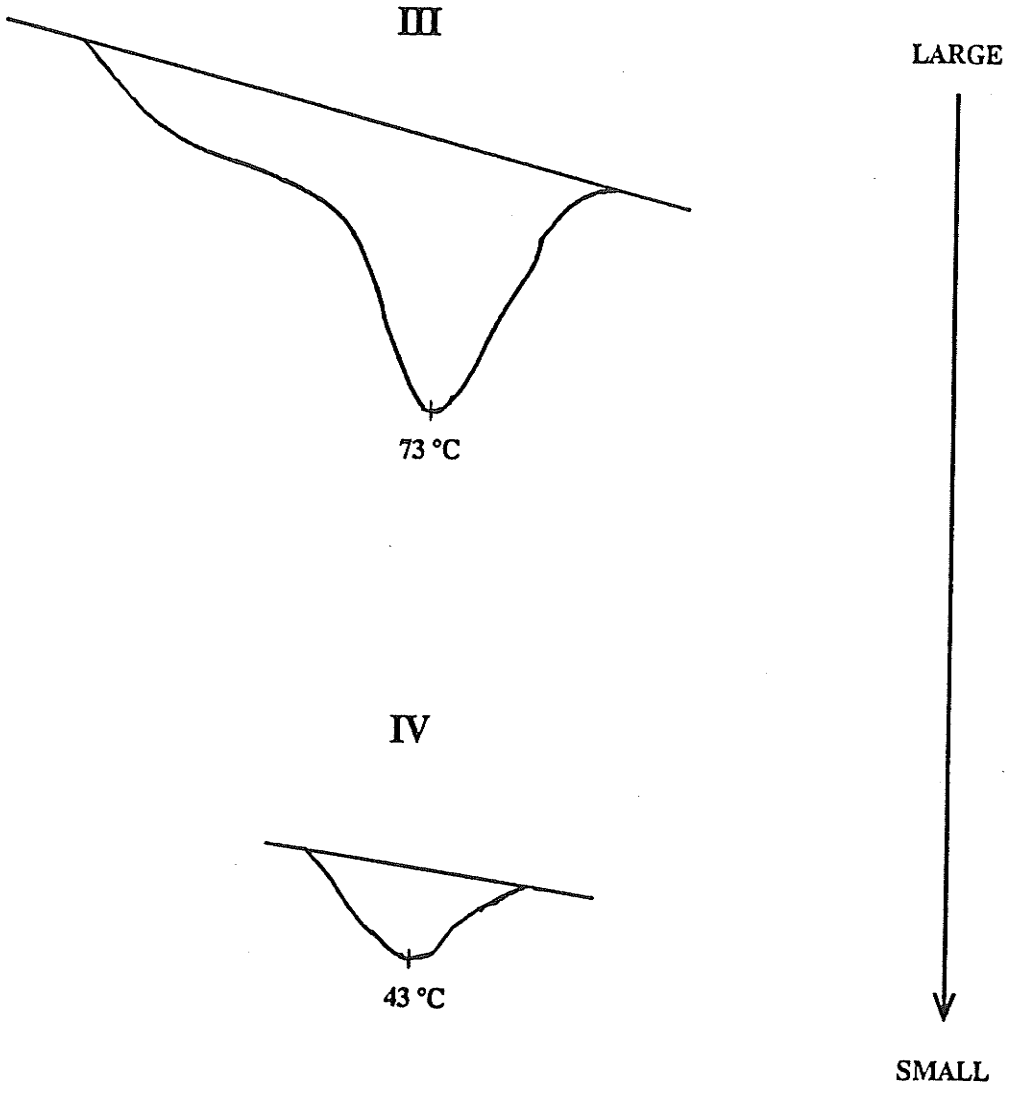
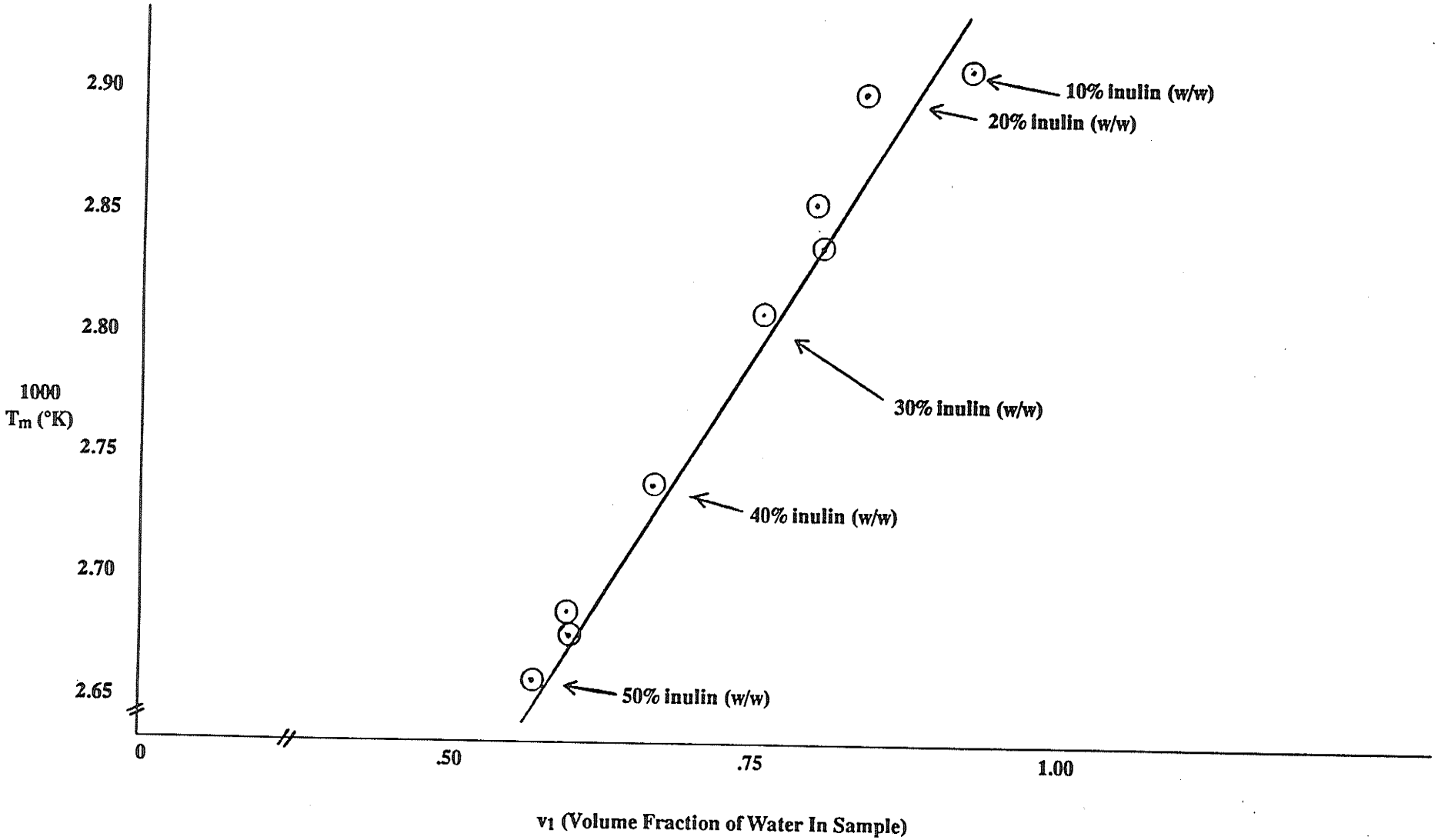


Figure 13 The reciprocal melting point of commercial inulin in the presence of water verses the volume fraction of water in the sample



Third, the endotherms in Figures 7, 8, 9, 11 and 12 may represent conformational or configurational changes in inulin not associated with phase change transitions. For example, inulin is easily degraded on heating in water (Marchessault et al., 1980). Middleton (1977) states that inulin is essentially a compact rather than an extended molecule. It is therefore possible that the observed endotherms are the result of denaturation such as simple cleavage or unwinding of the inulin molecule.

Finally, it is possible that more than one mechanism is being observed i.e. the resulting endotherm reflects a net absorption of energy which is the result of several changes occurring within the sample simultaneously. A central problem remains, that being to differentiate between dissolution, melting, denaturation and complex endotherms.

4.5 Maturation Study of 1984 Columbia and NC10-39 Varieties of Artichoke Tubers

Two varieties of artichoke tubers, Columbia and NC10-39, were received from Morden on a weekly basis over a period of 54 days in the latter part of the growth cycle of the plant (from August 16, 1984 to October 9, 1984). The tubers were washed, dried and then macerated for one minute in an Oster blender with water in order to extract the carbohydrate. The samples were held at 80°C for 15 minutes prior to vacuum filtration in order to inactivate endogenous enzymes present in the extracts. The extracts were diluted 1:10 with distilled water. Extracts were

prepared from both varieties of tubers and were characterized by gel filtration. This study was undertaken in order to examine any changes or trends in the DP profile that might occur during maturation.

Extracts from both Columbia and NC10-39 varieties of artichoke tubers were chromatographed on Bio-Gel P-2. The quantity of eluted carbohydrate in each test tube was assayed. The pattern of carbohydrate fractionation for Columbia and NC10-39 varieties for week one of the study can be seen in Figures 14 and 15 respectively. The profiles for both varieties were remarkably consistent with those of the commercial standards (Figure 6). Since the elution pattern of the commercial carbohydrate standards was known and was consistent with those of the varietal extracts, the degrees of polymerization of the eluted peaks were deduced by their place of elution. Several of the peaks in Figures 14 and 15 were eluted earlier than the peaks of the carbohydrate standards, fructose through maltohexaose (Figure 6). These peaks were thought to be larger in molecular size than maltohexaose and were therefore designated as DP 7, DP 8, DP 9 and DP $\geqslant$ 10 (listed in inverse order of elution).

FIGURE 14 FRACTIONATION OF ARTICHOKE EXTRACTS BY GEL FILTRATION ON BIO-GEL P-2 (COLUMBIA). WEEK ONE OF MATURATION STUDY.

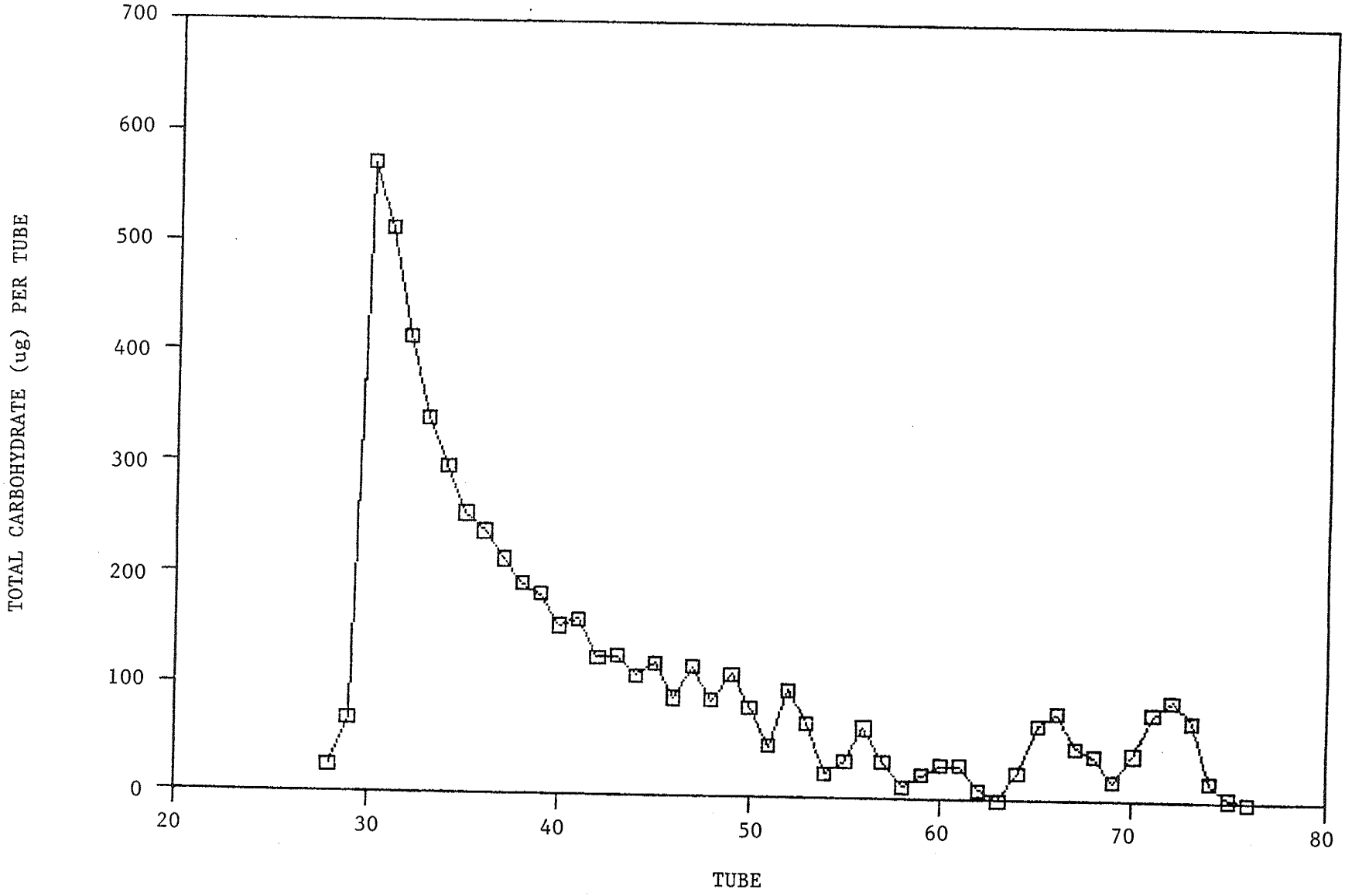
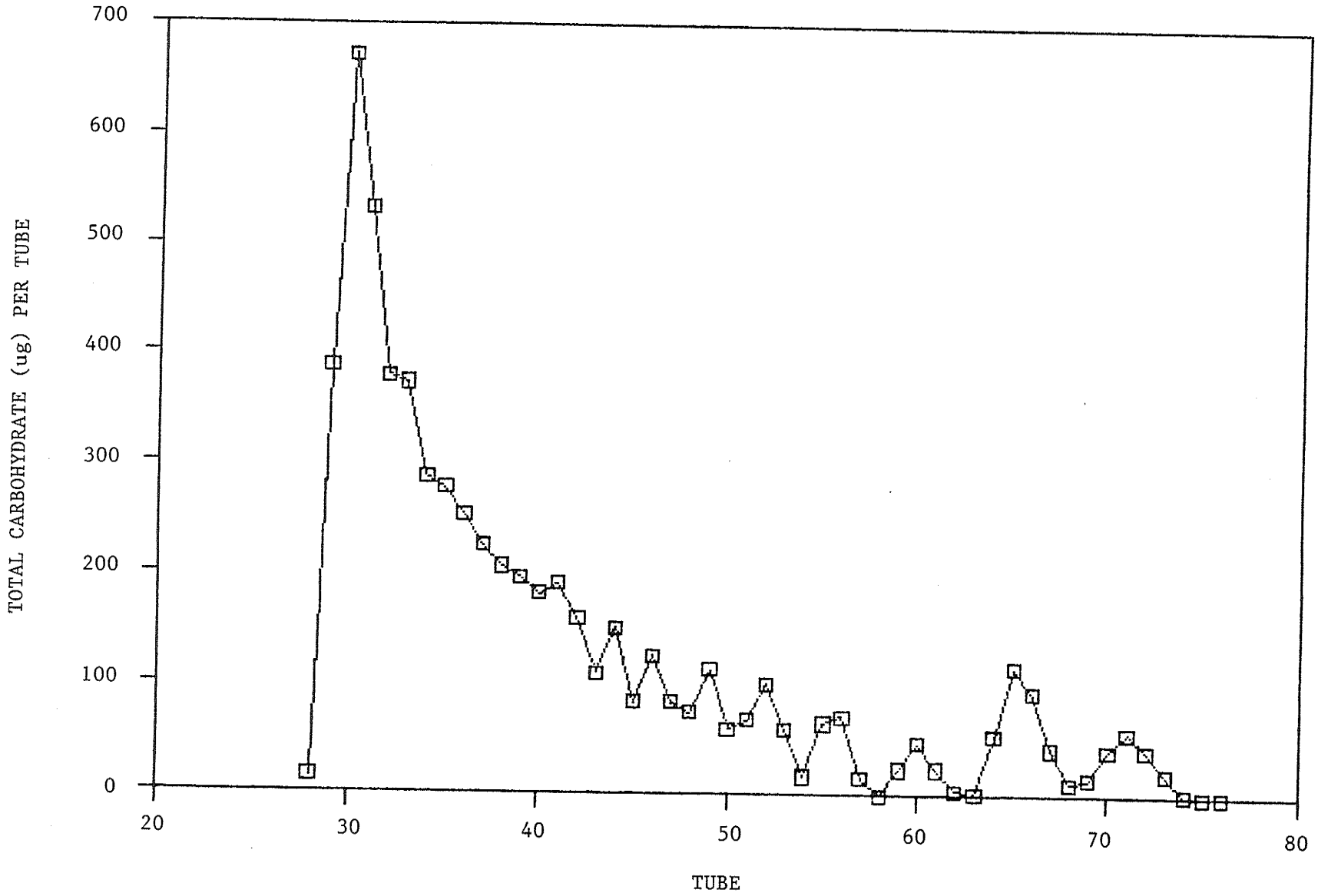


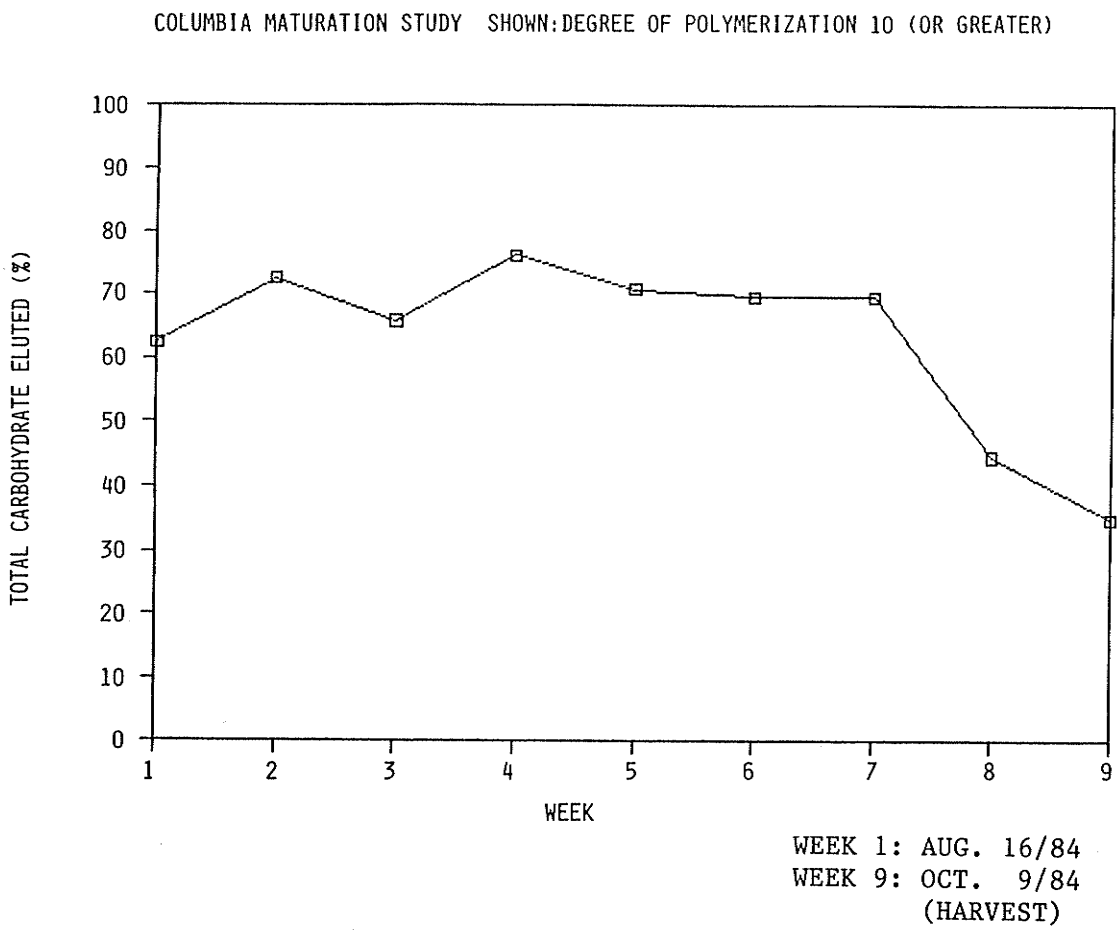
FIGURE 15 FRACTIONATION OF ARTICHOKE EXTRACTS BY GEL FILTRATION ON BIO-GEL P-2 (NC10-39). WEEK ONE OF MATURATION STUDY.



The changes in DP pattern over time in Columbia and NC10-39 extracts can be seen in Figures 16 and 17 respectively. Interestingly, no major differences between varieties were observed over time. Both varieties showed considerable variation in their DP profiles from week to week. An interesting situation was observed during week five with the NC10-39 variety (Figure 17). For this week the fraction containing fructans of DP 10 or greater decreased dramatically while fractions representing DP values 1-7 increased. This was perhaps due to endogenous tuber enzyme activity during preparation of the extract which resulted in breakdown of the high DP fructans with concomitant increases in the low DP fructans. Edelman and Bacon (1951) and Bacon and Edelman (1951) have demonstrated that enzyme hydrolysis does occur during extraction. This indicates that in order to study the range of fructans in maturing tubers or to produce high DP fractions, enzymes must be inactivated prior to or during extraction. This would minimize hydrolysis so that a more accurate picture of the carbohydrate profile can be obtained.

Generally speaking, although the results showed considerable variation in DP profile from week to week several trends were observed. First, that as the harvest date drew near that the fraction containing DP 1 decreased substantially. Rutherford and Weston (1968) found that at harvest fructose and glucose represented less than 1% of the sugars present in artichoke tubers. Second, it was interesting to note that by the end of the study that the sum of the low DP fructans (1-10) had increased while the high DP fraction (>10) had decreased. Since inulin is a storage carbohydrate, logically one would expect the

FIGURE 16 CHANGES WITH MATURITY IN DEGREE OF POLYMERIZATION IN ARTICHOKE TUBERS (COLUMBIA)



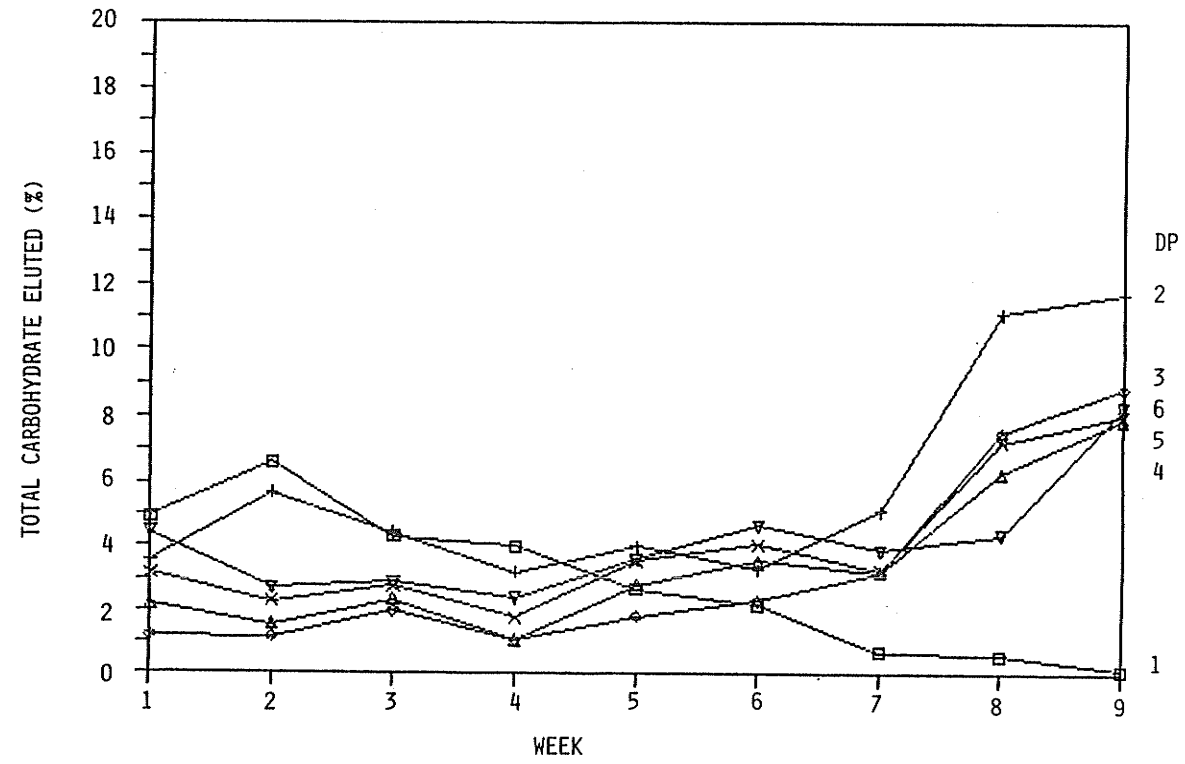
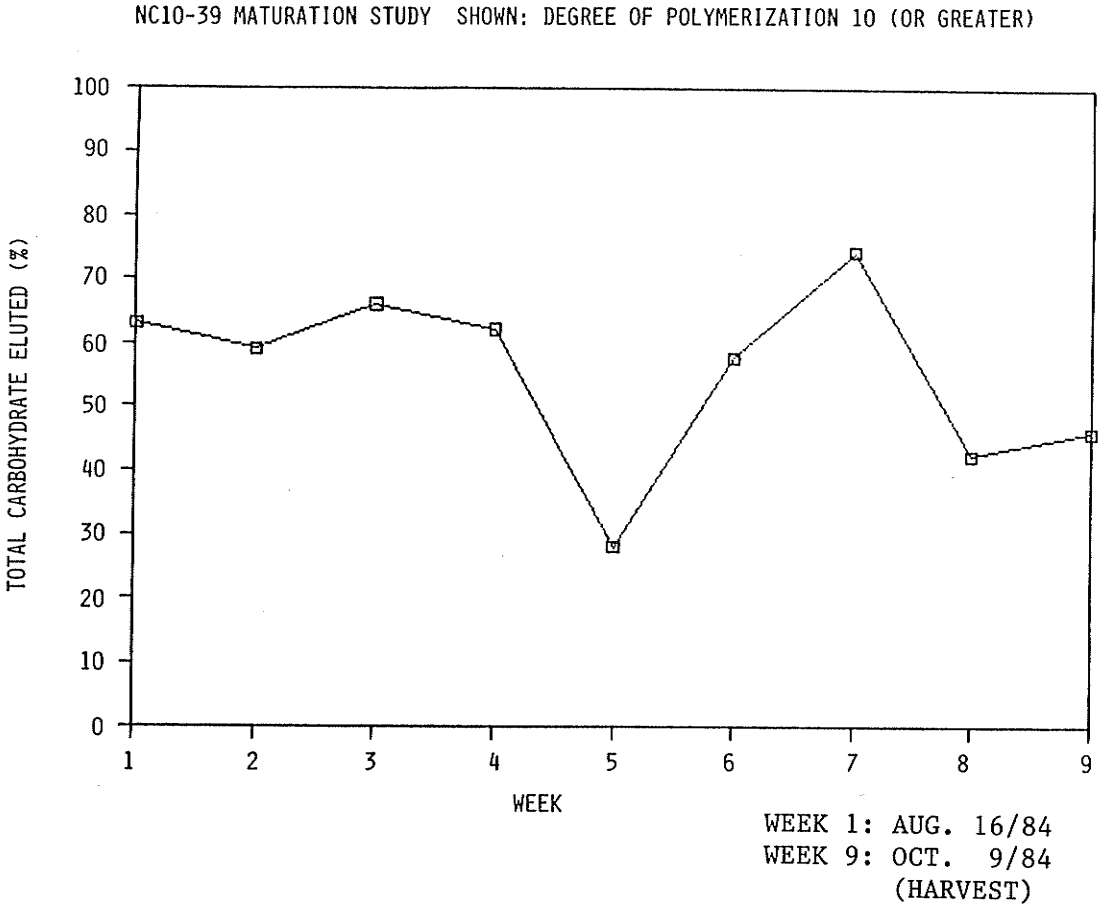
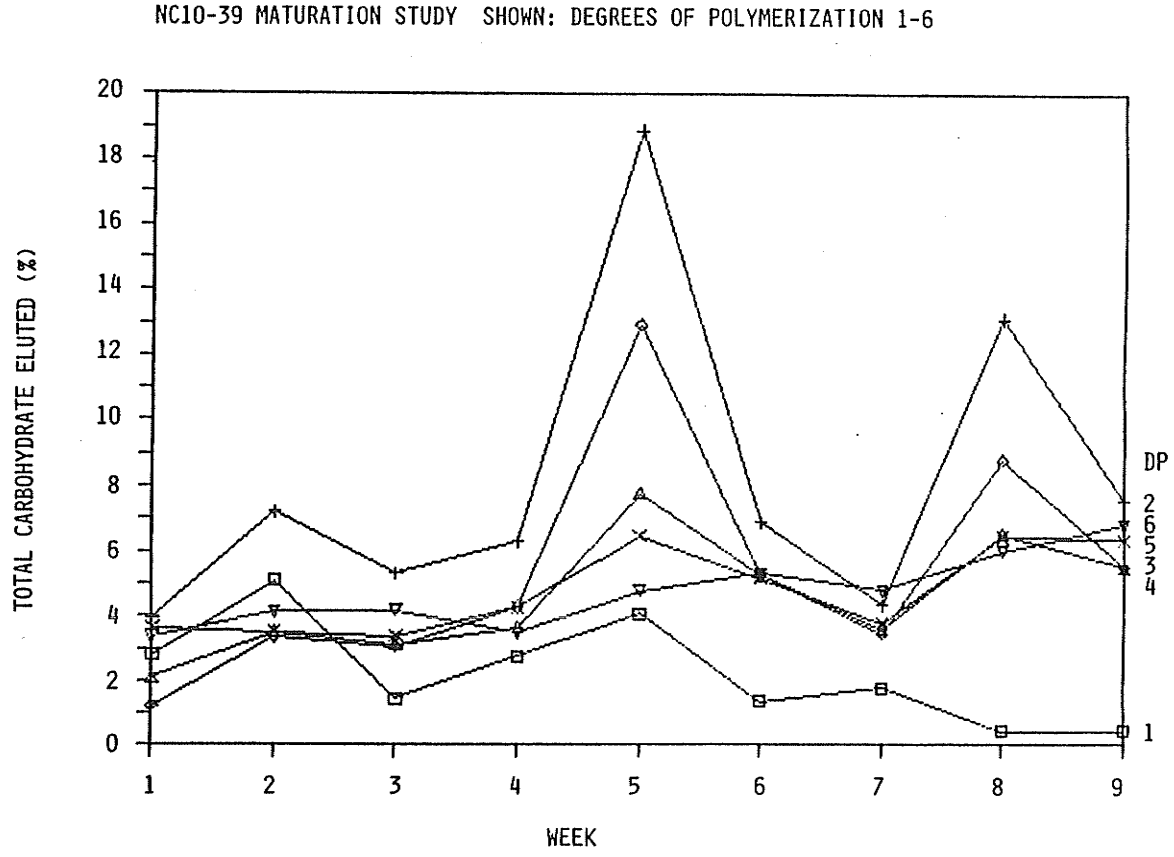


FIGURE 17 CHANGES WITH MATURITY IN DEGREE OF POLYMERIZATION IN ARTICHOKE TUBERS (NC10-39)





level of the high DP fructans to be at their highest at this time. Praznik and Beck (1987) have found significant quantities of very high DP fructans in their artichoke extracts. The fact that more high DP fructans were not detected in this maturation study could have been the result of endogenous enzyme activity within the prepared extracts. It is possible that incubation at 80°C was not rigorous enough to inactivate all of the endogenous enzymes present in the extracts. It is also possible as Praznik and Beck (1987) suggest that other parameters such as tuber size may affect the carbohydrate composition of the artichoke extracts.

5. CONCLUSIONS AND RECOMMENDATIONS.

Throughout the course of this work characterization of fairly raw extracts was carried out. It is concluded that the samples were handled in a manner such that changes between extraction and analysis occurred. It is believed that some of the results were observed because changes within the sample being characterized had occurred somewhat randomly, and also because of analytical anomalies. It is recommended that extraction techniques which minimize or at least more closely control changes to the material of interest be used. A second recommendation would be that characterization studies be carried out on material that is as pure as possible so that endogenous compounds such as enzymes do not interfere with the studies at hand. It was found that when more highly purified samples were analyzed, as in the case of the thermal characterization of commercial inulin, that much more consistent data were obtained.

Future studies that might be undertaken include optimization of the tuber-to-water ratio for the best extraction of inulin. Also of interest would be an investigation of the effect of the heat used during the extraction process on inulin, the carbohydrate of interest.

BIBLIOGRAPHY.

Akazawa,T. 1965. Reserve Polysaccharides. In: Plant Biochemistry. Bonner,J. and Varner,J.E. (eds.). Academic Press, New York and London.

Anonymous. 1985. Study predicts sugar's demise. Dairy Field 168(3):12.

Anonymous. 1987. Colorado to restrict gasoline use in winter. Canadian and Engineering News. June 29. Page 8.

Bacon, J.S.D. and Edelman, J. 1951. The carbohydrates of the Jerusalem artichoke and other Compositae. Biochem. J. 48:114.

Bacon, J.S.D. and Loxley, R. 1952. Seasonal changes in the carbohydrates of the Jerusalem artichoke tuber. Biochem. J. 51:208.

Chan, C.M. 1983. Flavor Components of Jerusalem artichoke tubers (Helianthus tuberosus L.). M.Sc. Thesis, University of Manitoba, Winnipeg, Canada.

Charleton, J.R. and Associates. 1984. Fructose from artichoke tubers - an assessment. Food and Consumer Industries Products Branch, Department of Regional and Industrial Development. Ottawa, Canada.

Chubey, B.B. and Dorrell, D.G. 1974. Jerusalem artichoke, a potential fructose crop for the prairies. Can. Inst. Food Sci. Technol. J. 7(2):98.

Chubey, B.B. and Dorrell, D.G. 1983. The effect of fall and spring harvesting on the sugar content of Jerusalem artichoke tubers. Can. J. Plant Sci. 63:1111.

Dykins, F.A. and Englis, D.F. 1933. Production of a palatable artichoke syrup. II. Hydrolysis of the polysaccharide material. Ind. Eng. Chem. 25:1165.

Dorrell, D.G. and Chubey, B.B. 1977. Irrigation, fertilizer, harvest dates and storage effects on the reducing sugar and fructose concentrations of Jerusalem artichoke tubers. Can. J. Plant Sci. 57:591.

Edelman, J. and Jefford, T.G. 1964. The metabolism of fructose polymers in plants. Biochem. J. 93:148.

Edelman, J. and Jefford, T.G. 1968. The mechanism of fructosan metabolism in higher plants as exemplified in Helianthus tuberosus . New Phytol. 67:517.

Fleming, S.E. and GrootWassink, J.W.D. 1979. Preparation of high fructose syrup from the tubers of the Jerusalem artichoke (Helianthus tuberosus L.). CRC Critical Reviews in Food Science and Nutrition. p.1.

Heiser, C.B.Jr. 1976. Sweet potato - Jerusalem artichoke. In: The Sunflower. Heiser, C.B.Jr. University of Oklahoma Press, U.S.A..

Hidaka,H., Eida, T., Takizawa, T., Tokunaga, T. and Tashiro, Y. 1986. Effects of fructooligosaccharides on intestinal flora and human health. Bifidobacteria Microflora 5(1):37.

Hoehn, E. 1982. Food potential of Jerusalem artichoke. Manitoba Agronomists Annual Conference (1982). University of Manitoba. Winnipeg, Canada.

Hoehn, E. and Murray, E.D. 1982. Review of Jerusalem artichoke tuber research program. Department of Food Science, University of Manitoba. Winnipeg, Canada.

Kiehn, F.A. and Chubey, B.B. 1982. Agronomics of Jerusalem artichoke. Agriculture Canada. Research Station: Morden, Manitoba.

Luenser, S.J. 1983. Microbial enzymes for industrial sweetener production. In: Developments in Industrial Microbiology. 24:79.

Marchessault, R.H., Bleha, T., Deslandes, Y. and Revol, J.-F. 1980. Conformation and crystalline structure of (2-->1)-Beta-D-fructofuranan (inulin). Can. J. Chem. 58:2415.

McDonald, E.J. 1946. The polyfructans and difructose anhydrides. Adv. Carbo. Chem. 2:254.

McGlumphy, J.H., Eichinger, J.W.Jr., Hixon,R.M. and Buchanan, J.H. 1933. Commercial production of levulose. I - General considerations. Ind. Eng. Chem. 23:1202.

McKay, C.J. 1982. The enzymatic hydrolysis of inulin and related fructosans. M.Sc. Thesis, University of Manitoba, Winnipeg, Canada.

Middleton, E. 1977. The molecular configuration of inulin: implications for ultrafiltration theory and glomerular permeability. J. Memb. Biol. 34:93.

Phelps, C.F. 1965. The physical properties of inulin solutions. Biochem. J. 95:41.

Praznik, W. and Beck, R.H.F. 1987. Inulin composition during growth of tubers of Helianthus tuberosus. Agric. Biol. Chem. 51(6):1593.

Spiers, D. 1984. Artichokes capture hearts of local businessmen. Winnipeg Free Press. October 13th. Winnipeg, Canada.

Rutherford, P.P. and Weston, E.W. 1968. Carbohydrate changes during cold storage of some inulin-containing roots and tubers. Phytochemistry 7:175.

Stauffer, M.D., Chubey, B.B. and Dorrell, D.G. 1975. Jerusalem artichoke, formulating the potential of a new crop. Can. Agricultural 7:34.

Williams, L.A. and Ziobro, G. 1982. Processing and fermentation of Jerusalem artichoke for ethanol production. Biotech. Letters 4:45.

Yanovsky, E. and Kingsbury, R.M. 1933. Solubility of inulin. J. Amer. Chem. Soc. 55:3658.