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A STUDY OF EFFECTS OF SPROUT INHIBITORS ISOPROPYL N-(3-CHLOROPHENYL) CARBAMATE AND MALEIC HYDRAZIDE ON ATP AND PPi DEPENDENT PHOSPHOFRUCTOKINASES AND SUGARS DURING STORAGE OF SELECTED POTATO CULTIVARS

presented by

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By

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ABSTRACT

A STUDY OF EFFECTS OF SPROUT INHIBITORS ISOPROPYL N-(3-CHLOROPHENYL) CARBAMATE AND MALEIC HYDRAZIDE ON ATP AND PPi DEPENDENT PHOSPHOFRUCTOKINASES AND SUGARS DURING STORAGE OF SELECTED POTATO CULTIVARS

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This work was aimed at understanding possible effects of the sprout inhibitor isopropyl (N-(3-Chlorophenyl)) carbamate (CIPC) and maleic hydrazide (MH) on activities of ATP and pyrophosphate (PPi) Fructose-6-phosphate phosphotransferases (ATP-PFK and PPi-PFK) since these may be the key enzymes regulating entry of hexoses into the glycolytic pathway. A loss or reduction in activities of these enzymes may lead to accumulation of sugars in stored potatoes.

In the first phase of this study activity of PPi-PFK and ATP-PFK and corresponding changes in sucrose, glucose and fructose were measured in CIPC and MH treated Atlantic potatoes stored at 3.3 and 10°C with 90% RH for six months. Storage at 3.3°C resulted in a high accumulation of sugars compared to 10°C. However, the activities of enzymes were not different, at either of the two temperatures or in sprout inhibitor treated samples, suggesting little effect of CIPC, MH or temperature on these enzymes. It is likely that the metabolic reactions governing changes in sugars in stored potatoes are under fine enzymatic control involving immediate changes in these enzymes' activity. PPi-PFK was more predominant in potatoes than ATP-PFK.

In the second phase of the study, control and CIPC treated samples of Atlantic, Saginaw Gold and Onaway potatoes were analyzed for the afore mentioned enzymes and glucose after storage at 3.3 and 10^{o}C with 90% RH for five weeks. The activity of PPi-PFK was more predominant than ATP-PFK in all varieties. A statistical analysis of the data showed significant differences in PPi-PFK ($p=0.001$) and ATP-PFK ($p=0.008$) activity among the varieties. The glucose content was not significantly correlated with either PPi-PFK ($p=0.051$; $r=-0.253$) or, ATP-PFK ($p=1.00$; $r=-0.043$). No significant effects of CIPC or the storage temperatures were observed on activities of these enzymes. However, the activity of PPi-PFK in Onaway (which accumulated more glucose) was lower than in Atlantic and Saginaw Gold.

DEDICATED TO

My late Grandfather who was the most kind man I've known, my parents for their love and support and my former Professor, Dr. Ted Hedrick for his faith in me and his support in achieving my academic goals.

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Minoo and my children, Roomi and Nihal. They endured this journey with joy. My daughter Roomi spent many hours typing this manuscript and my son Nihal provided hope to me. My wife Minoo helped me on many weekends with sugar analysis and enzyme extractions.

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INTRODUCTION

Most potatoes used by the processing industry are stored for several months. Their suitability for processing into products depends on sugar content and sprouting. Sprouting can be minimized by holding potatoes at low temperatures but such temperatures also cause starch-sugar interconversion and processing of potatoes into dark colored products as a result of reaction between reducing sugars and amino acids in potatoes.

Commercially, potatoes are stored up to 6-7 months at 10-12^{o}C with the aid of a sprout inhibitor isopropyl N-(3-chlorophenyl) carbamate (CIPC). Another sprout inhibitor maleic hydrazide (MH) is applied as a short term sprout control measure. While these two sprout inhibitors influence many aspects of tuber metabolism and effectively inhibit sprout growth, their effect on carbohydrate metabolism in stored potatoes has not been fully investigated. The basic objective of this work was to gain insight into mechanisms which might govern the effects of these sprout inhibitors on glycolytic enzymes ATP and Pyrophosphate (PPi) Fructose-6-phosphate phosphotransferases (ATP-PFK and PPi-PFK) which regulate entry of hexoses into the glycolytic pathway.

A two phase study was conducted; 1) the influence of CIPC and MH on ATP-PFK and PPi-PFK and sugars in Atlantic (a chipping cultivar) potatoes stored at 3.3 and 10^{o}C for six months, 2) the activity of these two enzymes and glucose in Atlantic, Saginaw Gold and Onaway potatoes stored at the afore mentioned temperatures for five weeks.

LITERATURE REVIEW

POTATO (*Solanum tuberosum*) AS A MAJOR FOOD CROP

The potato is one of the world's major food crops and is widely used as a staple food. Since potato tubers are almost 80% water and are living organisms, they are affected by the surrounding temperature and humidity and are susceptible to attack by a variety of pathogens. Processed products utilize approximately 50% of the total U.S. potato production and about 61% of the per capita potato consumption is in processed form (Talburt, 1987).

Processing of potatoes requires special care during production and storage. While moisture loss, sprouting, senescent sweetening and decay of potatoes are associated with a high storage temperature, low temperatures (which could prevent sprouting and extend the storage life of potatoes) foster development of sucrose and reducing sugars - glucose and fructose (Burton and Wilson, 1978). Potatoes with high reducing sugars process into dark colored products (chips, french fries and dehydrated products), because of the reaction between these sugars and amino acids in the potato cells (Schwimmer et al., 1957; Shallenberger et al., 1959; Wunsch and Schaller, 1972).

SUGARS IN POTATOES

Starch and sugars are maintained in equilibrium in potatoes during different stages of growth. Sucrose is high in immature tubers and decreases gradually while the starch increases as the tubers attain physiological maturity (Iritani and Weller, 1977). Varieties differ in their capacity to form sugars and within a given variety, sugar content of tubers at the same stage of development may be different (Agle and Woodbury, 1968; Morrell and ApRees, 1986). In selected potato cultivars (Fig 1), sucrose was found to range from 4 - 11 mg/g in immature tubers and 1 - 3.5 mg/g of fresh weight in mature tubers (Sowokinos and Preston, 1988). Reducing sugars, which are relatively high in immature tubers, decrease with maturity and should reach a minimum value by the time of harvest (Ronsen and Frogner, 1968; Mazza et al., 1983; Weaver and Timm, 1983).

STARCH - SUGAR TRANSFORMATION IN STORED POTATOES

Starch to sugar transformation in potatoes is a varietal characteristic influenced by: 1) stress during growth (e.g moisture, heat stress, nutrient imbalance etc.), which interupts normal tuber development, 2) relative maturity at the harvest, and 3) storage environment (Iritani and Weller, 1980). During storage, temperature, enzyme systems, the amyloplast membrane surrounding the starch granules and inorganic ions are believed to be important in starch - sugar transformation. Their role is briefly discussed here.

A) Role of Temperature

Temperature is perhaps the most important factor in the post harvest life of fresh fruits and vegetables. It effects biological reactions, including respiration, which operate properly within a narrow range of temperatures. Within this range, the Arrhenious plot (which represents effect of temperature on enzymic/chemical reaction) is relevant (Kader,

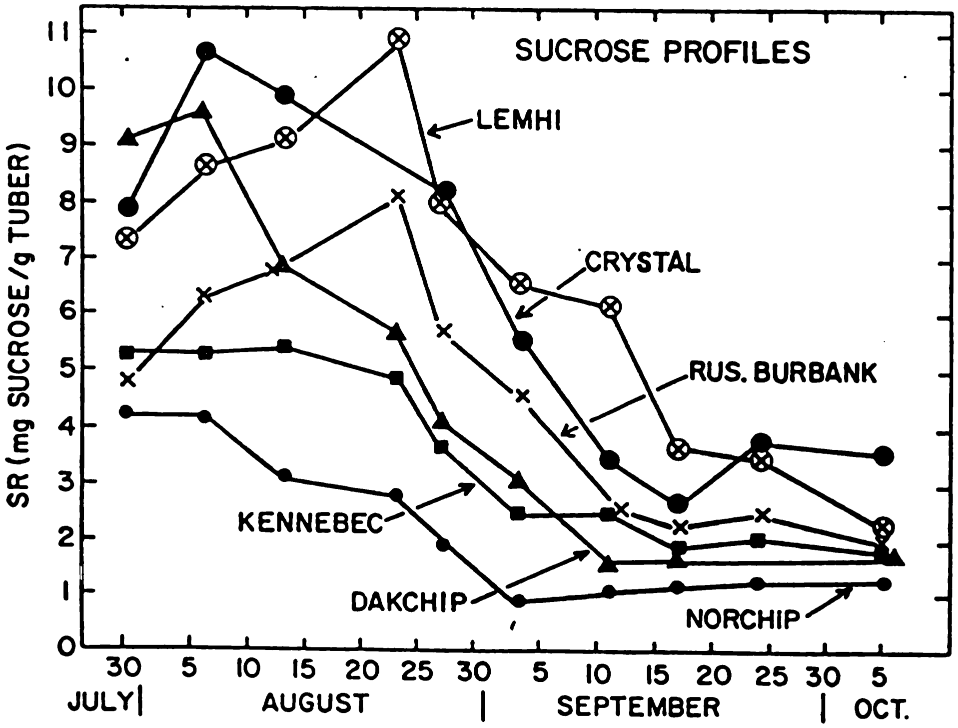


Figure 1. Sucrose content in different potato varieties during growth (Sowokinos and Preston, 1988).

1987; Phan, 1987).

For most biological reactions the velocity of reaction (Q_{10}) is doubled when the temperature rises by 10°C. The Q_{10} concept can be applied to respiration almost ideally. Respiration slows down at lower temperatures and this is the basis for low temperature storage.

Many plant species when exposed to temperatures in the range of 0 to 12°C fail to maintain normal physiological functions and exhibit visual signs of metabolic disorder (discoloration, softening, etc.). This effect of low temperature on chill sensitive plants is thought to be due to changes in the physical properties of cellular membranes and in the kinetics of membrane bound enzymes (Raison, 1974). In chill sensitive plants at a particular temperature, a phase change (physical structure of membrane changes from more fluid to a solid gel) occurs, leading to increased permeability of the membranes, decreased activity of membrane bound enzymes and accumulation of certain metabolites.

The membrane basically is a fluid entity in which the tertiary structure of the protein is interdependent on the membrane lipids. Exposure to low temperatures affects fluidity of the lipids and produces a change in the protein conformation. Many membrane proteins are enzymically active so conformational changes would affect the conformation of active sties and alter the kinetics of reaction catalyzed by the membrane bound enzymes (Raison, 1974).

In potato tubers which accumulated reducing sugars at low temperatures, studies using spin-labeled lipid probes showed decreased lipid fluidity, indicating physical change in membrane (Shekhar and Iritani, 1979). Potato tubers, are not considered chill sensitive in the classical sense because the membranes of mitochondria from potato tubers exhibit no break in the slope of an Arrehenius plot over the range 0 to 25^{o}C (Raison et al., 1971). However, the metabolic transformation of starch to sugar which occurs in potatoes does seem to exhibit sensitivity to low temperatures.

In mature potato tubers stored at 0^{o}C for 2 wks, starch was reported to decrease from 67 to 61% (dry wt. basis), whereas sucrose increased from 1.07 to 6.65%, glucose from 0.62 to 0.79% and fructose from 0.17 to 1.50% (Arreguin-Lozano and bonner, 1949). The decrease in starch content in this study was almost proportional to increases in sugars. Samotus and Schwimmer (1962a), found that during the post harvest storage of immature and mature potato tubers at 0^{o}C for 6 wks, fructose accumulation was maximum in immature tubers, but mature tubers had twice as much sucrose as fructose. Ewing et al., (1981), reported that the two reducing sugars respond more or less in parallel manner to low temperatures.

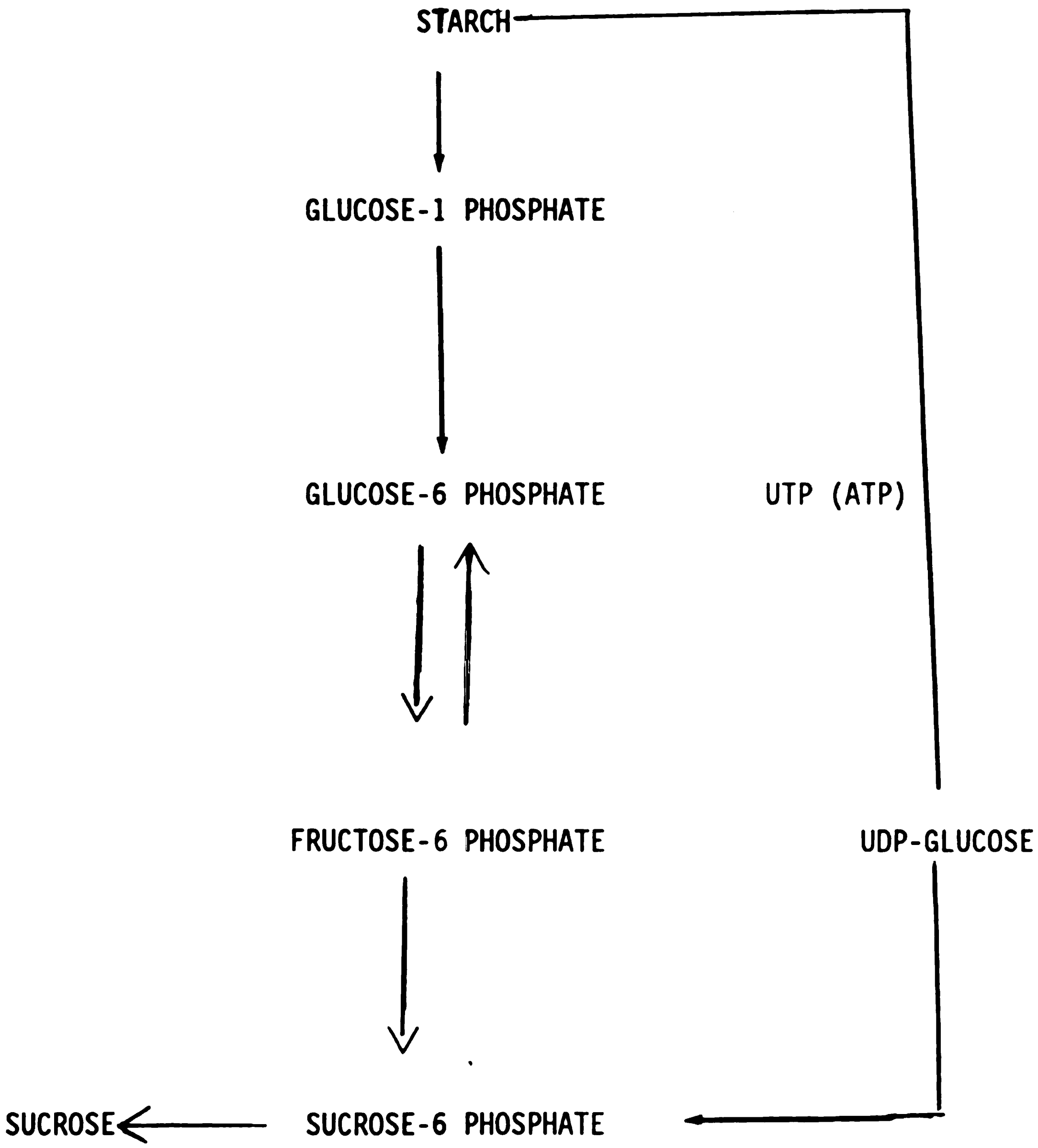
Isherwood (1973) followed the interconversion of starch to sugars and changes in respiration in stored potatoes transferred from 10 to 2^{o}C and back to 10^{o}C. In potatoes transferred from 10 to 2^{o}C, the levels of sucrose rose immediately whereas glucose and fructose did not begin to rise until after two days and showed only a small change up to 8 days. The respiration rate, which was below the initial value for the first 5-8 days, rose to a maximum at 14 days before returning to the initial value after 28 days. In tubers transferred from 2-10^{o}C the sugars declined steadily, while the respiration rate reached a maximum after 10 days and then slowly declined to a value slightly above the initial.

The results of this study suggested that during the formation of sucrose at low temperature approximately 1 mol of ATP (as UTP) was required to form 1 mol of sucrose (Fig 2). During conversion of sugars to starch at 10^oC, the sucrose is first hydrolyzed to hexoses which are converted to starch (Fig 3). The calculated ratio of moles of ATP needed per mol of anhydrohexoses formed was 2 in this conversion.

Thus, 3 mol of ATP was shown to be required for each cycle of breakdown and synthesis of starch. These calculations were based on the basal respiration rate of the tubers and the knowledge that a certain proportion of respiration is coupled to phosphorylation. The fact that the ATP equivalents were so close to prediction values suggests that only a small amount of high energy phosphate compounds were expended in the actual transport of intermediates across the membranes in the cell. Isherwood (1973), concluded that the increased respiration which accompanied chilling was related quantitively to the conversion of starch to sucrose. He also showed that sucrose was produced before the reducing sugars. However, in a later study, Isherwood (1976), compared changes in hexose phosphates and other phosphate esters with sugar changes during low temperature storage. The phosphate esters increased within the first two days, but sucrose and reducing sugars did not accumulate until more than 10 days of storage. A lag period of approximately 7 days has been shown before sugars start to build up in potatoes (Fig 4, 5 and 6), at low temperatures (Hyde and Morrison, 1964; Workman et al., 1979; Coffin et al., 1987).

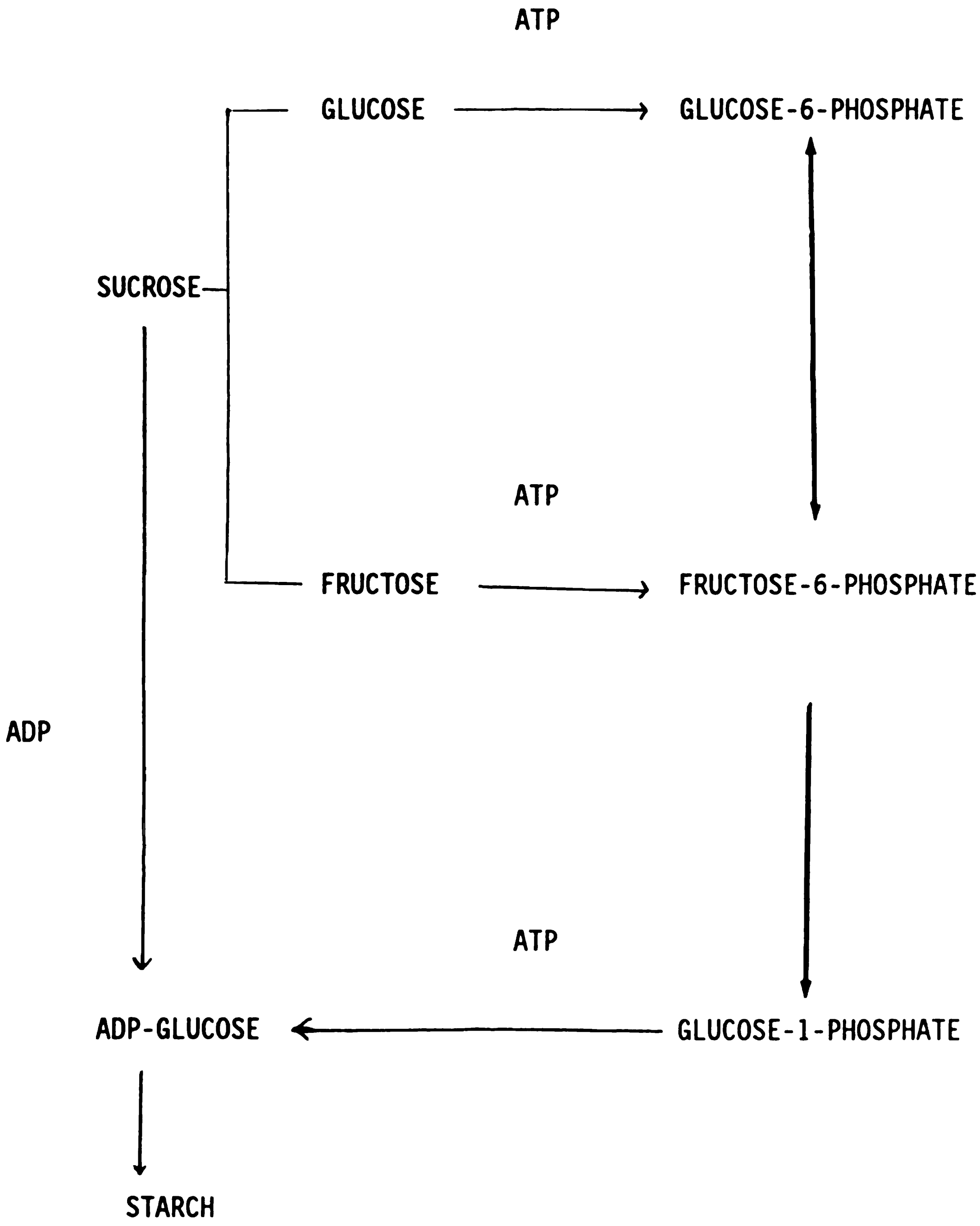
Respiration in potato tubers is also a variety characteristic. Physiological maturity, sprout growth and injury or previous stress to the tubers, influence respiration. Immature and injured tubers respire much more intensively than mature sound tubers. The respiration rate of several potato cultivars was reported to decrease as the storage

Figure 2. A scheme for the conversion of starch to sucrose in potato tubers during low temperature storage.



(ISHERWOOD, 1973)

Figure 3. A scheme for the conversion of sugars to starch at high temperature.



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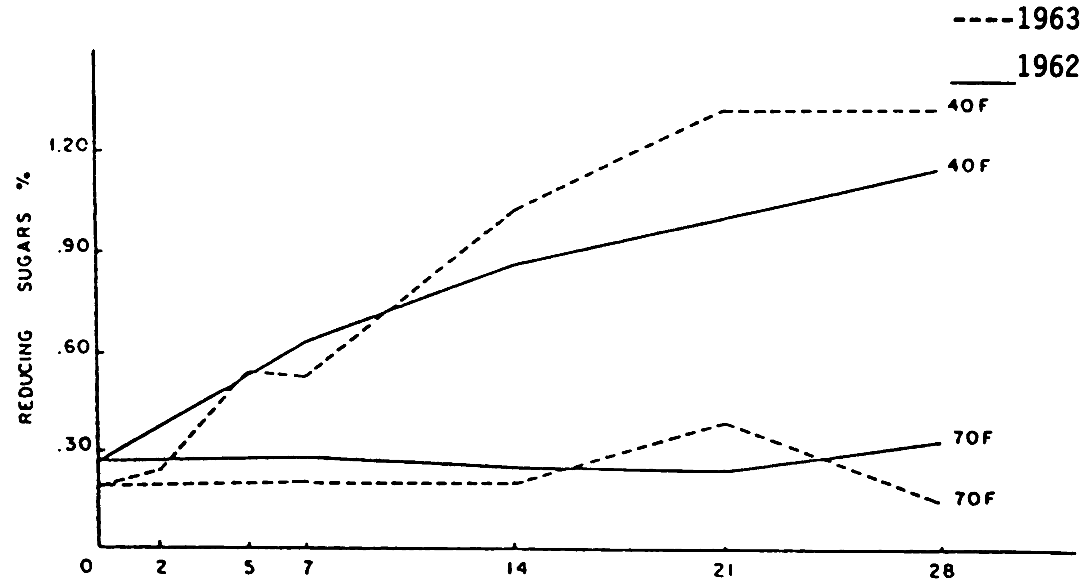


Figure 4. Effect of storage at 4.4 and 10^{o}C on reducing sugars in potatoes (Hyde & Morrison, 1964).

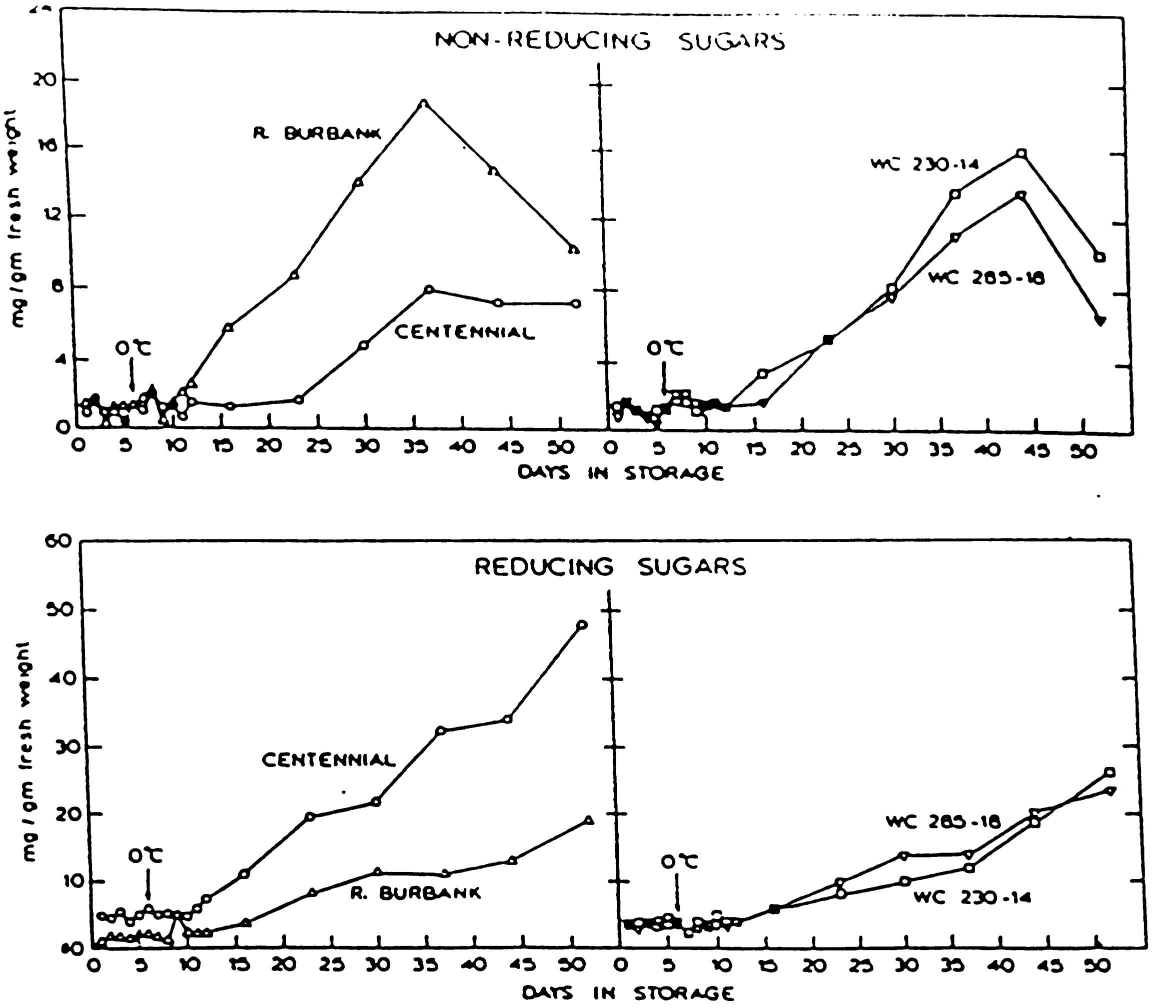
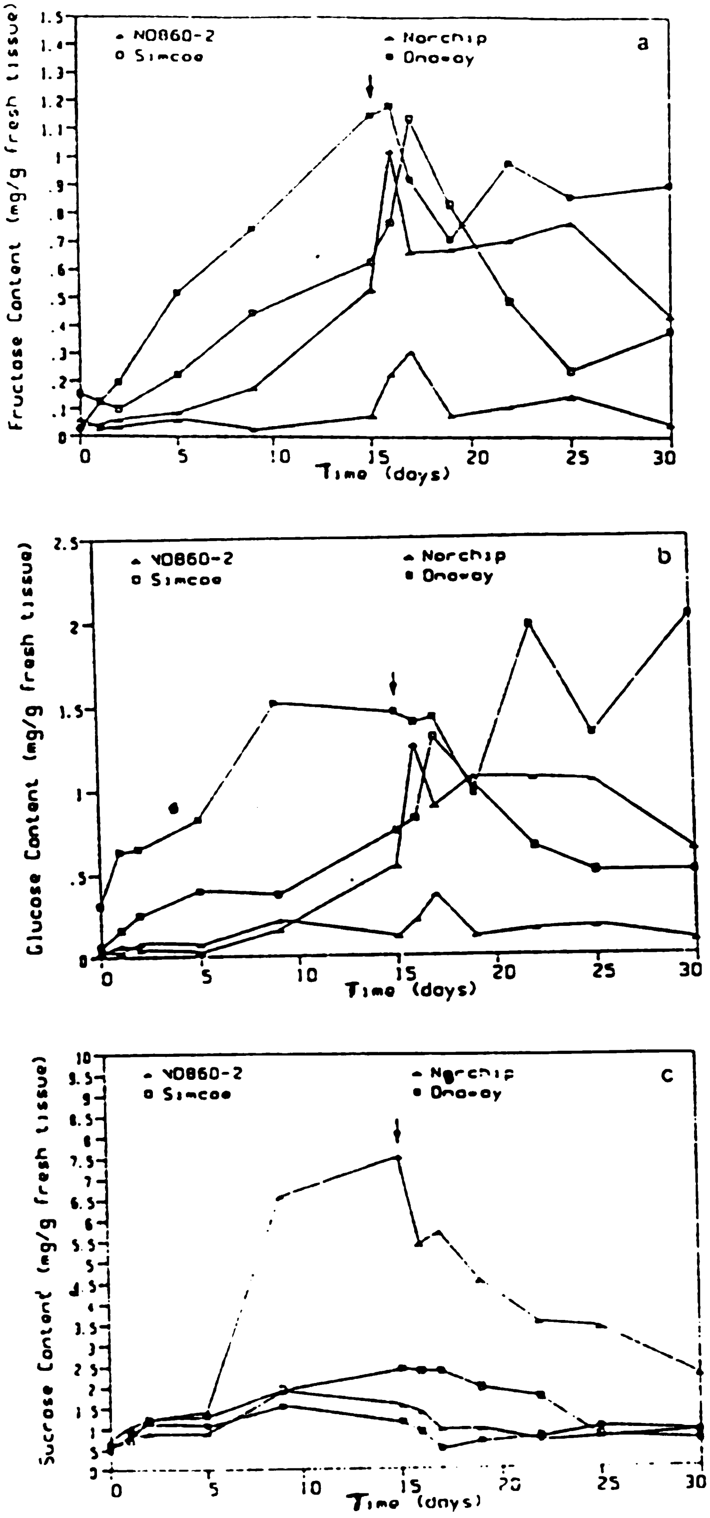


Figure 5. Sugar development in potatoes during storage at 0°C (Workman et a., 1979).



Arrows indicate time of transfer to 20°C.

Figure 6. Sugar content in potatoes stored at 5°C (Coffin et. al., 1987).

temperature decreased from 20 to 0°C, but the respiratory change was unrelated to increase in sugar content (Workman et al., 1979). Similar results were obtained by Dwelle and Stallknecht (1978). The respiration in stored tubers is reported to be minimum at about 7°C (Fig 7).

Maximum Limit of Sugars for Chipping Potatoes

Chipping potential of various cultivars at harvest and during storage has been investigated (Sowokinos, 1978; Nelson and Sowokinos, 1983; Mazza et al., 1983; Santerre et al., 1986). Recently, Sowokinos and Preston (1988), suggested monitoring chemical maturity of potatoes to be processed into chips. The chemical maturity reached by the cv. Norchip (a chipping potato) at 10°C in stress-free storage is reflected by a sucrose level of less than or equal to 1.0 mg/g of tuber, and a glucose level of less than or equal to 0.035% (or 0.35 mg/g tuber) on a fresh wt. basis. Sowokinos and Preston (1988), suggested that processing cultivars should experience a safe value close to that observed for Norchip. Non-processing cultivars may have sucrose values close to the limit indicated for sucrose but their glucose concentration is normally several fold higher. These safe limits for sucrose and glucose assume that potatoes are: 1) healthy and sprout free; 2) stored at approximately 10°C to insure minimum sugar development and 3) the varieties have genetic potential to reach acceptable sugar levels at the time of harvest and are able to maintain these levels in storage.

B) Role of Amyloplast Membrane

Permeability changes in the amyloplast membrane surrounding the starch granules may have a role in starch breakdown and sugar synthesis

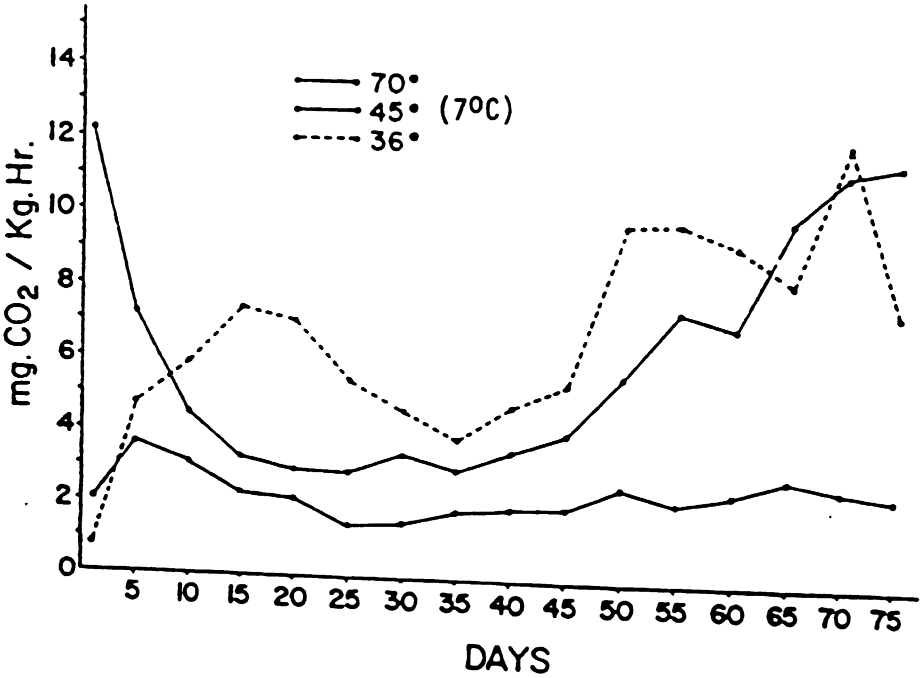


Figure 7. Respiration rates of Russet Burbank potatoes at different storage temperatures (Boe et. el., 1974).

in potato tubers at low temperatures. Ohad et al. (1971), followed the changes in morphology of cells from developing and mature tubers stored at 4 and 25^{o}C. The electron micrographs showed disintegration of the plastid (amyloplast) layer surrounding the starch at the lower temperature. Isherwood (1976) and Hazel and Sterling (1978), reported that the amyloplast membrane was intact in cold stored potatoes. In senescent tubers however, this membrane showed signs of disintegration and fragility.

Electron micrographs of translucent or glassy-end tubers having high levels of free sugar also revealed that the amyloplast membrane was intact and continuous around starch granules (Sowokinos et al., 1985). Analysis of starch grains from tubers stored at 10 and 2^{o}C have shown that a large part of the K, Na, Cl, citrate and glucose -6-phosphate were inside the amyloplast membrane but the sugar at 2^{o}C storage was outside, therefore, sweetening may involve transport of metabolites through the membrane (Isherwood, 1976). Workman et al., (1976), also reported increased membrane permeability in low temperature stored potatoes. Although a membrane may appear intact, this does not preclude alterations in its fine structure, composition and permeability. Van Es and Hartmans (1987), suggested that in the amyloplast membrane starch is hydrolyzed to glucose, which in turn, is partly converted to fructose. Glucose and fructose are enzymatically coupled forming the disaccharide sucrose which can be translocated and converted back into glucose and fructose by the cold activated enzyme invertase (Fig 8).

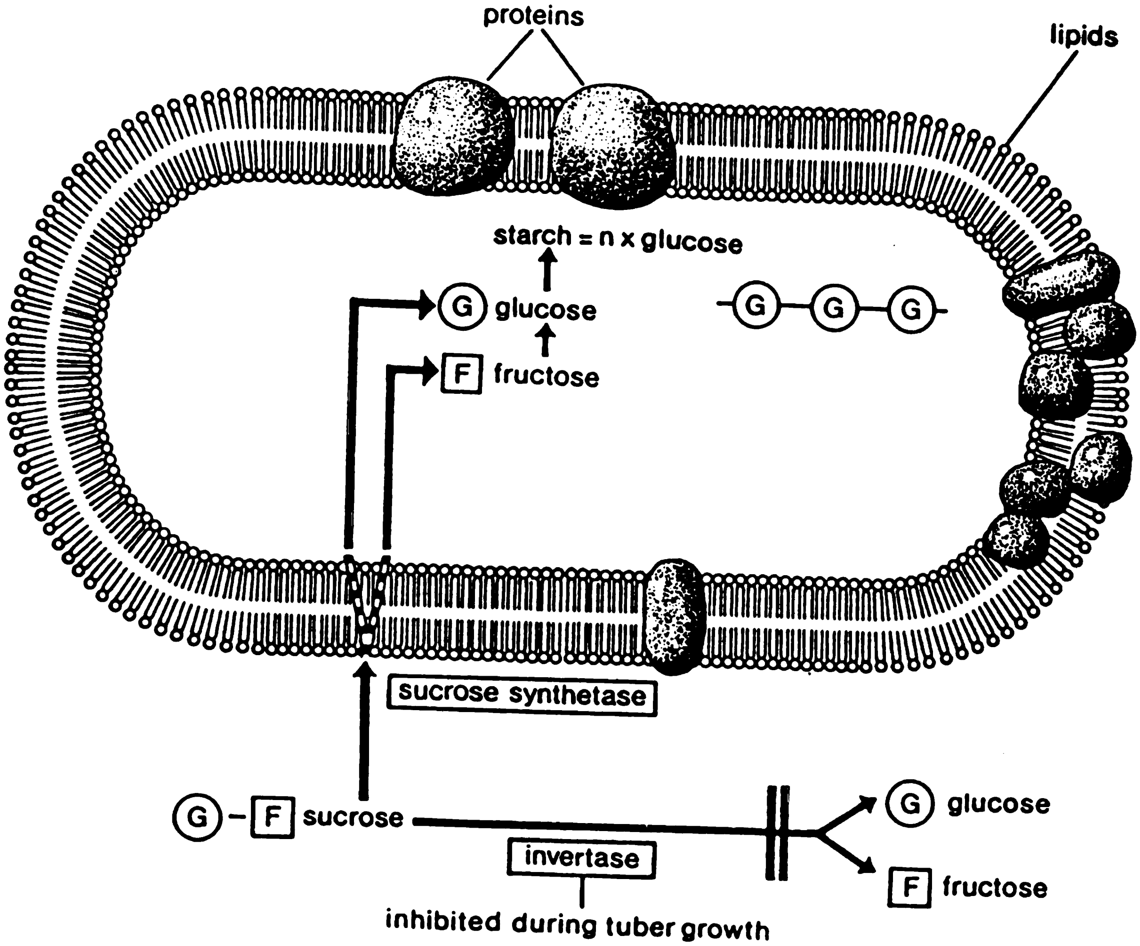


Figure 8. Possible role of amyloplast membrane in cold induced sugar formation (Van Es and Hartmans, 1987).

C) Role of Enzymes

Invertase (EC 3.2.1.26)

In potato tubers an invertase system consisting of the enzyme and an endogenous invertase inhibitor has been reported (Schwimmer et al., 1961; Pressey and Shaw, 1966). Freshly harvested mature tubers have been shown to contain low levels of invertase and high levels of invertase inhibitor (Pressey, 1969). In sprouting tubers however, the invertase activity is reported to be about five times higher than in unsprouted tubers (Schwimmer et al., 1961).

Evidence of invertase with two pH maxima (acid invertase optimum: pH 3.8 to 5.5 and alkaline invertase having optimum pH: 7.0 to 8.5) with separate roles in regulation of sucrose metabolism in growing carrot roots has been provided (Ricardo and ApRees, 1979; Lyne and ApRees, 1971). It was suggested that the acid invertase, possibly located at the tonoplast, directs sucrose to the cells in which the demand for sucrose was high and alkaline invertase, located in the cytoplasm, was active in the cells that stored starch. However, in growing potato tubers neither of the invertases were detected in sufficient activity to suggest their role in metabolizing sucrose delivered to the developing tubers (Morrell and ApRees, 1986).

Tishel and Mazelis (1966), observed that at low temperature (2^{o}C) the increase in sucrose content matched the increase in invertase activity, followed by an increase in reducing sugars. However at 10^{o}C no such increase in invertase activity of sugars was obtained.

Ewing and McAdoo (1971), showed that the invertase activity is under regulation of an inhibitor which forms an undissociable complex with the enzyme. Thus, when potatoes are held at low temperatures, the increased invertase activity brings about a reduction in inhibitor activity. At high temperatures, high inhibitor and low invertase activities have been reported (Pressey and Shaw, 1966; Anderson and Ewing, 1978). This relationship between temperature, levels of sugars and the invertase system holds only when the potatoes are stored continuously, either at high, or low temperatures. Storage of potatoes at alternating temperatures has shown relatively low sugar increases at 4°C and high increases at 18°C (Pressey and Shaw, 1966). While temperature-induced invertase activity may be responsible in part for accumulation of reducing sugars in potatoes at low temperatures, other factors such as utilization of hexoses cannot be ignored.

Sucrose Synth(et)ases (EC 2.4.1.13)

Sucrose synthase catalyzes a reversibile transglycosylation reaction:

$$\text{Sucrose + UDP} \rightleftharpoons \text{UDP Glucose + D-fructose}$$

This reaction is a major mechanism by which translocated sucrose can be directly utilized to provide tissues with sugar nucleotide intermediates, primarily UDP-glucose via which starch is synthesized in the reserve tissues. Slabnic et al., (1968), reported the important role of this enzyme in the formation of sugar nucleotides UDP-glucose and ADP-glucose. In growing potato tubers, the activity of this enzyme was shown to be well in excess of rates of sucrose breakdown but decreased sharply after harvest (Pressey, 1970; Morrell and ApRees, 1986). However, no significant difference in activity of this enzyme was found in potatoes stored either at low or high temperatures (Pressey, 1970; Sowokinos, 1973; Pollock and ApRees, 1975), indicating that the enzyme has little to do with sugar accumulation at low temperatures.

In contrast, the activity of sucrose phosphate synthetase (EC 2.4.1.14), which had much lower activity than sucrose synthase at the time of harvest has been shown to match the accumulation of sugars at low temperatures (Pressey, 1970; Pollock and ApRees, 1975). The reaction catalyzed by this enzyme is irreversible so it is likely that this enzyme has some role in sucrose formation in cold stored tubers.

Phosphorylase (EC 2.4.1.1)

Phosphorylase initiates degradation of glycogen in animal tissues, but its role in plants is not very well established. Gerbrandy and Verleur (1971), suggested that this enzyme is mainly concerned with digestion of starch. In potato tubers, phosphorylase activity was shown to be unrelated to sugar accumulation at low temperatures (Ioannou et al., 1973; Chism and Hard, 1975; Kennedy and Isherwood, 1975).

In _in vitro_ experiments, crystalline muscle phosphorylase was reported to be only weakly inhibited by chlorogenic acid, but crude potato phosphorylase was inhibited 5-6 times more by this compound while sucrose and other sugars were shown to inhibit adsorption of phosphorylase on starch. (DeFekete, 1966; Blank and Sondheimer, 1969). Although chlorogenic acid, polyphenoloxidase and phosphorylase are present in potato tubers there is no evidence to suggest that this system has a regulatory role in intact potatoes. Sowokinos (1976), suggested that the non-photosynthetic starch biosynthesis in potato tubers may be associated with the activity of ADP-glucose pyrophosphorylase.

Phosphofructokinase (PFK) (EC 2.7.1.11)

Since PFK catalyzes the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate in the first step unique to glycolysis, this enzyme may regulate the entry of hexose phosphates into glycolysis (Uyeda, 1979; ApRees, 1980; Turner and Turner, 1980). Possibly cold lability of PFK in potatoes stored at low temperatures restricts glycolysis and cause hexose phosphates to accumulate. The increased availability of hexose phosphates may lead to an enhanced synthesis of sucrose (Pollock and ApRees, 1975).

It has been reported that when potato tubers are transferred from 25 to 2°C, carbohydrate metabolism is not only reduced in rate but is also redirected. A particularly marked aspect of this redirection is a reduction in proportion of hexose 6-phosphates that enter the respiratory pathway and a diversion of these compounds to sucrose. Such a diversion is precisely what would be expected if the enzymes that control glycolysis are sensitive to cold (Dixon and ApRees, 1980b).

In an *in vitro* study, Pollock and ApRees (1975) found that lowering the temperature to that which leads to sweetening reduced the activity of PFK more than some other enzymes of carbohydrate metabolism. Because PFK is an oligomeric enzyme, lowering the temperature could weaken the hydrophobic bond (which contributes to the stability of this enzyme at ordinary temperature) in the oligomeric complex. This could lead to dissociation and consequent loss of activity (Uyeda, 1979). This was demonstrated in PFK extracted from rabbit muscle, chicken liver and

Bacillus licheniformis where lowering the temperature caused dissociation of enzyme into inactive sub units (Block and Frieden, 1974; Kono and Uyeda, 1973; Marschke and Bernlohr, 1973). Spontaneous dissociation and loss of activity of purified PFK extracted from potato tubers has also been reported (Dixon et al., (1981). However, in *in vivo*, the behavior of this enzyme may be affected by the cytoplasmic solutes which could afford some protection from the effects of cold.

Pyrophosphate-dependent Phosphofructokinase (EC 2.7.1.90)

The inorganic pyryphosphate dependent phosphofructokinase (PPi-PFK) uses PPi instead of ATP as a phosphoryl donor. This pyrophosphate: fructose-phosphate phosphotransferase is Mg^{2+} dependent and catalyzes the reversible reaction:

$$\text{PPi} + \text{Fructose-6-P} \rightleftarrows \text{Pi} + \text{Fructose-1, 6-P}_2$$

This enzyme has been found in some bacteria and in plants (O'Brien et al., 1975; Carnal and Black, 1983; ApRees et al., 1985).

The root and stem homogenates of germinating corn seedlings have been shown to have more PPi -PFK than ATP-PFK activity, whereas expanding leaves have more of ATP-PFK (Smyth et al., 1985). The activity of plant PPi-PFK is reported to be dependent on the presence of fructose 2,6-diphosphate (Sabularse, and Anderson, 1981).

The role of allosterically regulated PPi-PFK from plants is not clear, though the following suggest a glycolytic function (Carnal and Black, 1983; Schaftingen, 1987):

a) The activity of PPi-PFK predominates over that of ATP-PFK in several plants which rely on glycolysis during the night for CO_2 fixation.

b) Plant PPi-PFK is stimulated in the glycolytic rather than in the gluconeogenic direction.

c) PPi has been detected in plants at concentrations of 5 to 40 nmols/g fresh weight. However, it is not known in which sub cellular compartment this inorganic ion is located.

Storage tissues such as potatoes have been shown to contain 4 times as much PPi-PFK activity as ATP-PFK (Scaftingen et al., 1982). A molecular comparison of PPi-PFK and ATP-PFK from potato tubers showed that the two enzymes are composed of distinctly unrelated polypeptides and a direct conversion between the two is unlikely (Kruger and Hammond, 1988).

It is possible that PPi-PFK (or ATP-PFK) has a role in mediating the entry of hexoses into the glycolytic pathway (Fig 9), and a loss of enzymes may be responsible for accumulation of reducing sugars in the tissues, since they are not converted via glycolysis. A study of these two enzymes therefore, may provide some evidence of the pathway controlling starch to sugar transformation at low temperatures.

Other Enzymes

Some other enzymes likely to have a role in carbohydrate metabolism at low temperatures have also been studied. Arreguin-Lozano and Bonner (1949), reported that amylase was not a factor in starch breakdown and sugar formation at low temperatures. They also observed negligible

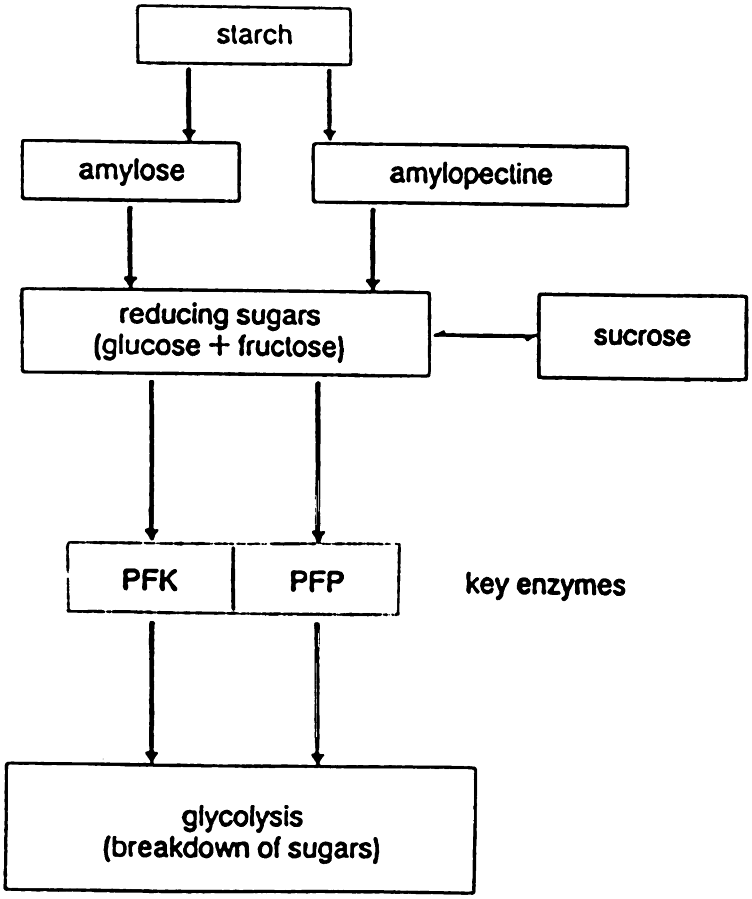


Figure 9. Possible role of ATP-PFK and PPi-PFK in sugar accumulation at low temperature (Van Es and Hartmans, 1987).

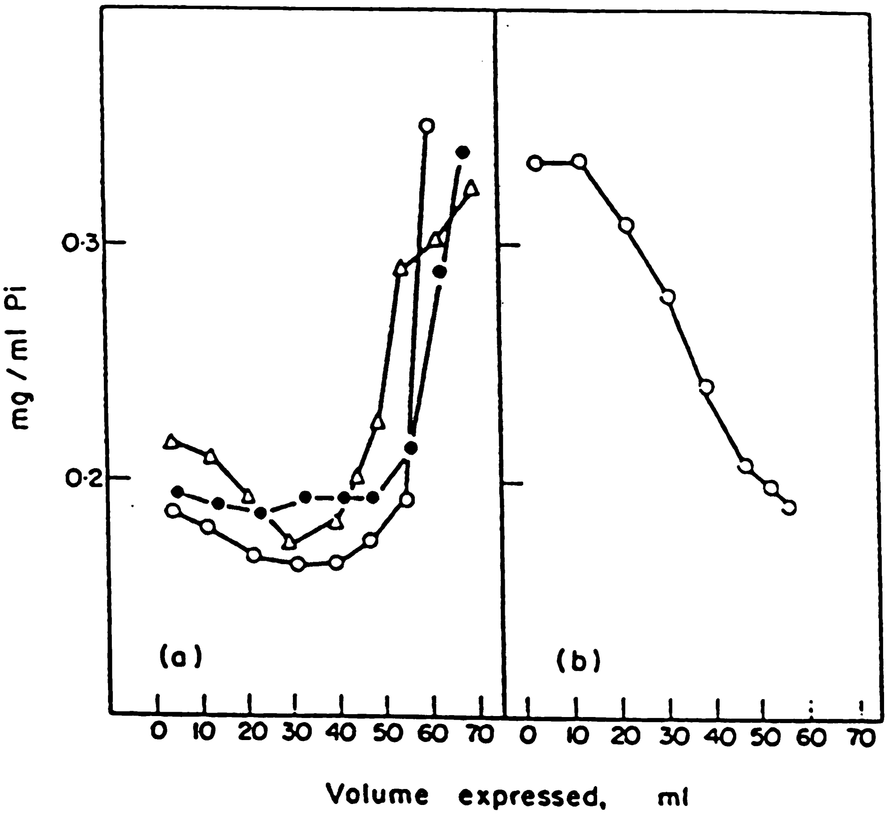
differences in activities of phosphatases (which attack hexose phosphates to yield corresponding hexoses) from tubers stored at 9 and 25°C.

No detectable effects on the activity of glucose-6-phosphate dehydrogenase (which can shift glycolytic intermediates to pentose pathway), glyceraldehyde phosphate dehydrogenase (which catalyzes conversion of glyceraldehyde 3-phosphate to 1,3- diphosphoglycerate in the glycolytic pathway) and aldolase (which catalyzes breakdown of hexoses to trioses and would determine hexose concentration) was observed in potatoes stored at either 2 or 10°C for 2 weeks (Pollock and ApRees, 1975). Pyruvate kinase is suggested to play a role in determining the level of intermediates of glycolytic and oxidative pentose pathway and indirectly influence PFK (Dixon and ApRees, 1980b).

D) Role of Inorganic Ions

Inorganic phosphorus is an activator of phosphorylase so an increase in its concentration is believed to play a role in starch to sugar conversion at low temperatures. This ion is also an inhibor of ADPG pyrophosphorylase a key enzyme in starch synthesis (Sowokinos and Preiss, 1982). Increased inorganic phosphorus content has been reported in potato tubers and in their expressed sap (Fig 10) from potatoes stored at low temperature (Samotus and Schwimmer, 1962b; Isherwood and Kennedy, 1975; Shekhar and Iritani, 1978).

Disruption/modification of membranes may lead to leakage of inorganic ions from the cytoplasm and activate enzymatic degradation of starch. Calciumn, magnesium and potassium were found to have no correlation with reducing sugar development but a highly significant



(a) Pi —○— 10°: —●— storage for 4 days at 2° after transfer from 10°: —△— 7 days at 2° after transfer from 10°. (b) Pi —○— after freezing and thawing.

Figure 10. Composition of the sap expressed from the tubers stored at 10 and 2°C (Isherwood and Kennedy, 1975).

positive correlation was obtained between inorganic Pi and reducing sugar accumulation at low temperature (Shekhar and Iritani, 1978).

SPROUT INHBITION IN POTATOES

A major portion of potatoes used by the processing industry are stored at $12^{o}C$ for several months. Potatoes held at these temperatures often yield chips and other processed products of better texture, color and flavor than those from lower storage temperature, however, these tubers sprout rapidly.

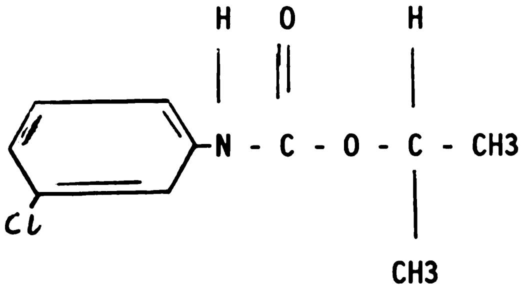
Sprouting in potatoes is a normal physiological phenomenon following dormancy, and causes potatoes to soften, lose weight, nutrients and processing quality. Sprouting can be prevented by:

A) Storage at low temperatures of about $3\text{-}4^{o}C$ with high humidity for 5-8 months after harvest. These temperatures however, cause an accumulation of sugars in potatoes.

B) Irradiation can prevent sprouting of potato tubers through prevention of cell division. Irradiation of food products however, is still a safety concern. Also, irradiation of potatoes is reported to cause a number of side effects such as black spot, after cooking darking, storage rot etc. (Buitelar, 1987).

C) Chemical Sprout Inhibitors

Among the chemical compounds, isopropyl N- (3- chlorphenyl) carbamate (CIPC, chlorpropham) and maleic hydrazide are most widely used. CIPC like other carbamate herbicides derive its basic structure from carbamic acid (NH_2 COOH), and has this molecule as a basic part of its structure (Ashton and Craft, 1981):



[Structure of CIPC]

CIPC is synthesized commercially by the reaction of 3- chlorphenyl isocynate with isopropyl alcohol, or 3- chloraniline with isopropyl chlorformate. CIPC is very effective as a sprout inhibitor for stored potatoes (Marth and Schultz, 1952; Heinze, et al., 1955). This compound is applied as an aerosol by means of special equipment, which vaporizes the liquid compound and distributes it through the potato storage bin's internal ventilation system. It can also be applied as an emulsion formation on unsprouted tubers out of storage. The minimum concentration of this compound in the peel layer of potatoes for complete inhibition of sprouts is empirically estimated as 20 ppm (Corsini et al., 1979). CIPC must reach all eyes (buds) to be effective (Kim et al., 1972).

Moorland (1967), classified into three broad categories the primarybiochemical responses induced by herbicides:

a) respiration and mitochondrial electron transport,

b) photosynthesis and Hill reaction,

c) nucleic acid and protein synthesis.

The effect of CIPC on respiration and potato constituents has been discussed below to gain an understanding of its mechanism of action.

Respiration

Respiration can be considered to procede through tree steps: a) glycolysis (conversion of sugars to pyruvic acid), b) tricarboxylic acid cycle (metabolism of pyruvic acid to CO_2 through a series of organic acids), and c) oxidative phosphorylation and transport (transfer of electrons from organic acid to ATP). Glycolysis occurs in cytoplasm whreas the TCA cycle, oxidative phosphorylation, and electron transport take place in mitochondria.

Craft and Audia (1959), reported that the oxygen uptake in potato tubers dipped in 0.5% suspension of CIPC was not significantly different from untreated tubers. However, Boe et al., (1974), showed that when CIPC was applied as a commercial formulation the respiration rate of CIPC treated tubers was less than untreated tubers after about 80 days in storage at 9^oC (Fig 11).

Carbohydrates

Zaehringer et al., (1966), reported that CIPC treated potatoes stored at 7.2^oC for upto 12 months had no effect on sugar accumulation and on chip color. Cash et al. (1988), also found little effect of CIPC on glucose and on chip color of Atlantic potatoes stored at 11^oC in a commercial storage facility. However, in their study slight variation in fructose and sucrose content was found. Post harvest application of 0.5% CIPC, as a dip was shown to reduce starch degradation when potato tubers were kept in an evaporative cooling chamber but starch

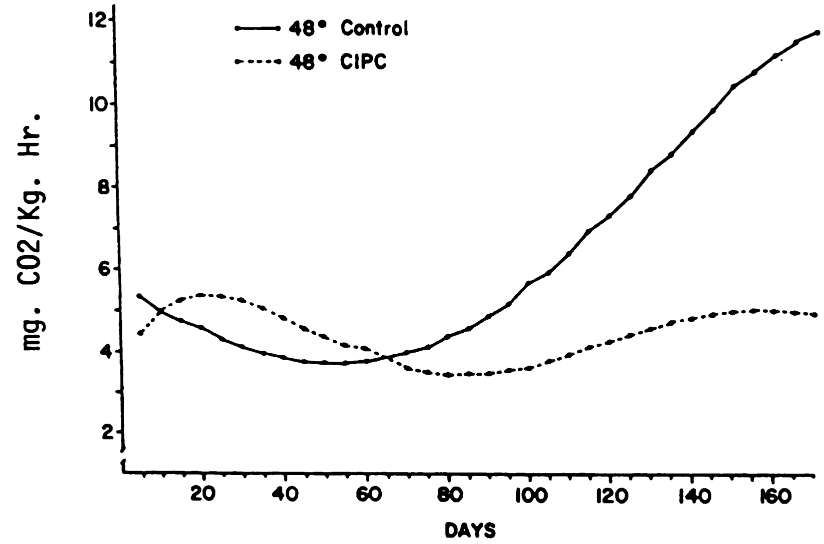


Figure 11. Respiration rate of CIPC treated tubers (Boe et. al., 1974).

degradation was faster at refrigerated storage conditions (Khurana, et al., 1985).

Proteins and Enzymes

While looking at the sprout inhibiting mechanism of CIPC its effect on protein synthesis and on enzymes have been studied. Nowak (1977a and 1977b), reported that CIPC prevented changes in soluble protein fraction in stored tubers. It also inhibited protein synthesis and ribonuclease activity in the eyes of the tubers, but had no effect on this enzyme in the parenchyma cell. Earlier, Vanwinkle (1975), had reported that CIPC did not act through blocking of new messenger RNA. Chung (1985), reported little effect of CIPC on ATP-PFK, PPi-PFK, mitochondrial respiratory activity and reducing sugars during storge of Nooksack, a non-chipping potato cultivar, at 20-30^{o}C for 18 days. Ravamel and Tissut (1984), reported that treatment of dormant tubers with 1% chlorpropham powder inhibited cellular division in the buds for several months but the mitochondria extracted from treated tubers were similar to those of untreated tubers (Fig 12).

Phenolic and Ascorbic Acid

The phenolic content was found to be higher in CIPC treated tubers than in controls and CIPC treated tubers stored at 5^{o}C were significantly lower in ascorbic acid than the controls (Ponnampalam and Mondy, 1986).

Glycoalkaloids

Glycoalkaloids, a potent cholinesterase inhibitor occur in potato

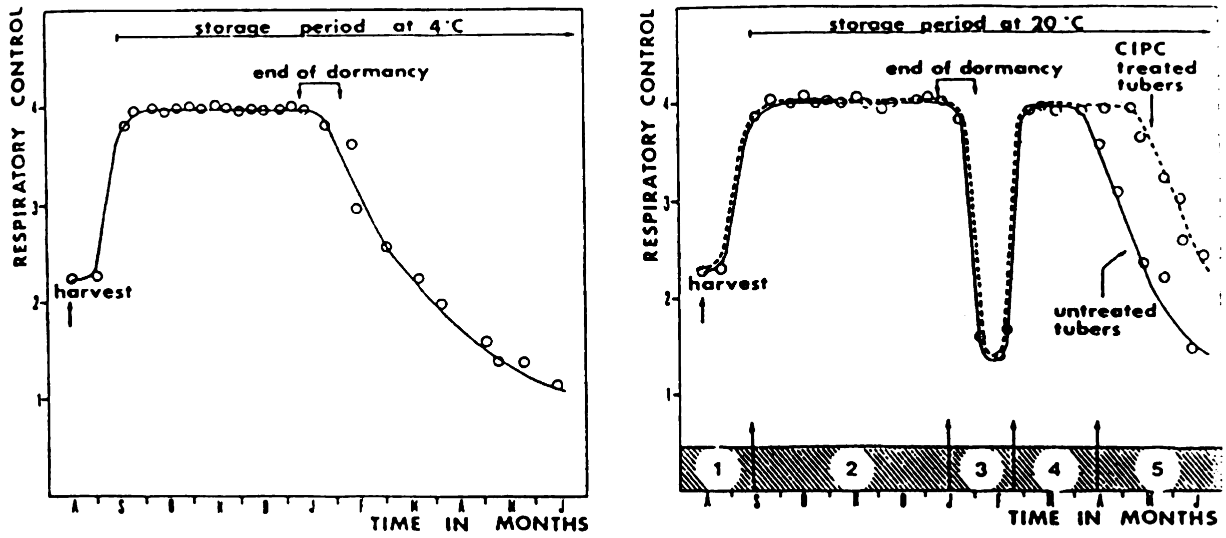


Figure 12. Mitochondrial changes in CIPC treated, stored tubers.(Ravanel and Tissut, 1984).

tubers in various amounts depending on variety, stage of development, and environmental conditions. Glycoalkaloids increase markedly during tuber sprouting (Gull and Isenberg, 1960). An average glycoalkaloid concentration of 2 to 10 mg/100g fresh wt. in potatoes has been reported, and a concentration in excess of 20 mg/100g fresh wt. constitutes a potential health risk (Wu and Salunkhe, 1977; Van Es and Hartman, 1987). CIPC was reported to have an inhibitory effect on wound induced glycoalkaloid formation in potato tubers. But, it had no effect on existing glycoalkaloids and light induced glycoalkaloid formation (Wu and Salunkhe, 1977). However, Mondy and Ponnampalam (1985), reproted that CIPC not only supressed sprouting but also reduced the rate of glycoalkaloid synthesis and could be particularly advantageous in hot climates.

Maleic Hydrazide (MH)

MH is defined chemically as 1,2-dihydro 3,6 pyridazinedione. It is a stable, non-volatile powder which is sparingly soluble in water but will react readily with basic compounds to form more soluble compounds.

The exact mechanism by which MH inhibits sprouting is not known, however, it is believed that MH may act as an antiauxin, interferring with the synthesis and activity of the plant hormones, indoleacetic acid or giberellic acid. It is also shown to inhibit a number of enzymes, e.g dehydrogenase, phosphatase and, phenolase.

When MH was applied to the foliage at the time of blossom abscission, good sprout control on harvested tubers with no reduction in yield was obtained (Sawyer and Dallyn, 1958). Application of MH was also reported to yield better shaped, marketable potatoes (Weis, et al.

1980) and to lower glycoalkaloid content (Mondy, et al., 1978). In MH treated potatoes enzymatic darkening was found to be greater, and the crude lipid and phospholipid content lower. The fatty acid composition of the phospholipid fraction of MH-treated tubers showed a significant increase in saturated fatty acids and a decrease in unsaturated fatty acits. Since lipid content of the tuber is an important determinant of cellular integrity, the altered lipid composition due to MH treatment may be an important factor contributing to tubers increased susceptibility to bruising and discoloration (Mueller & Mondy, 1977).

LIST OF REFERENCES

Agle, W.M., and Woodbury, GW. 1968. Specific gravity-dry matter relationship and reducing sugar changes affected by potato variety, production area and storage. Am. Potato J. 45:119-131.

Anderson, R.S. and Ewing, E.E. 1978. Partial purification of potato tuber invertase and its proteinaceous inhibitor. Phytochemistry. 17:1077-1081.

ApRees, T. 1980. Integration of pathways of synthesis and degradation of hexose phosphates. In Biochemistry of Plants. Vol. III. Ed. Preiss. J. Academic Press. New York. pp. 1-39.

ApRees, T., Green, J.H. and Wilson, P.M. 1985. Pyrophosphate: fructose 6-phosphate 1-phosphotransferase and glycolysis in non-photosynthetic tissue of higher plants. Biochem. J. 227:199-204.

Arreguin-Lozano, B. and Bonner, J. 1949. Experiment on sucrose formation by potato tubers as influenced by temperature. Plant Physiology. 24:720-738.

Ashton, F.M. and Crafts, A.S. 1981. Mode of action of herbicides. 2nd edition. John Wiley & Sons, New York., pp. 180-181.

Blank, GE. and Sondheimer, E. 1969. The influence of polyphenols on potato phosphorylase. Phytochemistry. 8:823-826.

Block, P.E. and Frieden, C. 1974. pH induced cold lability of rabbit skeletal muscle phosphofructokinase. Biochemistry 13:4191-4196.

Boe, A.A., Woodbury, GW. and Lee, T.S. 1974. Respiration studies on Russet Burbank Potato Tubers: Effects of storage temperature and chemical treatments. Am. Potato J. 51:355-360.

Buitelar, N. 1987. Sprout inhibition in ware potato storage. In Storage of Potatoes. Ed. Rastovaski, A. & Vans Es. A. et al. Pudoc, Wageningen. pp. 331-341.

Burton, W.G and Wilson, A.R. 1978. The sugar content and sprout growth of tubers of potato cultivar record, grown in different localities, when stored at 10, 2 and 20^{o}C. Potato Res. 21:145-162.

Carnal, N.W. and Black, C.C. 1983. PFK activities in photosynthetic organisms. Plant Physiology. 71:150-155.

Cash, J.N., Sinha, N.K. and Chase, R.W. 1988. The influence of CIPC on potato carbohydrates during commercial production and storage. Michigan Potato Research Report. Vol. 20. Michigan State University, Agricultural Research Station, East Lansing, Michigan.

Chism, GW. and Hard, N.F. 1975. Influence of temperatures on kinetic properties of phosphorylase from two varieties of potato tubers. J. Food Sci. 40:94-96.

Chung, C. 1985. Effect of temperature and chlorpropham on phosphofructokinases, mitochondrial respiration and reducing sugars in dormant Nooksack potato tubers. Ph.D. Thesis. University of Missouri, Columbia.

Coffin, R.H., Yada, R.Y., Parkin, K.L., Grodzinski, B. and Stanley, D.W. 1987. Effect of low temperature storage on sugar concentrations and chip color of certain processing cultivars and selections. J. Food Sci. 52:639-645.

Corsini, D., Stallknecht, G and Sparks, W. 1979. Changes in chlorpropham residues in stored potatoes. Am. Potato J. 56:41-50.

Craft, C.C. and Audia, W.V. 1959. Some effects of Isopropyl N-(3-Chloropheny carbamate on respiration, water uptake and ion leakage of potato tissue. Am. Potato J. 36:386-393.

DeFekete, M.A.R. 1966. Interaction of amyloplast with enzymes. I phosphorylase adsorption on amyloplasts in viciafaba. Biochem. Biophys. 116:368-374.

Dixon, W.L. and ApRees, T. 1980a. Identification of the regulatory steps in glycolysis in potato tubers. Phytochemistry. 19:1297-1301.

Dixon, W.L. and ApRees, T. 1980b. Carbohydrate metabolism during cold induced sweetening of potato tubers. Phytochemistry. 19:1653-1656.

Dixon, W.L., Franks, F. and ApRees, T. 1981. Cold lability of phosphofructokinases from potato tubers. Phytochemistry. 20:969-972.

Dwelle, R.B. and Stallknecht, G 1978. Respiration and sugar content of potato tubers as influenced by storage temperature.. Am. Potato J. 55:561-571.

Ewing, E.E., Senesac, A.H. and Sieczka, J.B. 1981. Effects of the periods of chilling and warming on potato sugar content and chipping quality. Am. Potato J. 58:239-246.

Gerbrandy, S.J. and Verleur, J.D. 1971. Phosphorylase isoenzymes: location and occurance in different plant organs in relation to starch metabolism. Phytochemistry. 10:261-.

Gull, D.P. and Isenberg, F.M. 1960. Chlorophyll and solanine content and distribution in four varieties of potato tubers. Proc. Amer. Soc. Hort. Sci. 75:545.

Hazel, Y. and Sterling, C. 1978. Integrity of amyloplast membrane in stored potato tubers. Z. Pflanzenphysiol. 90:373-378.

Heinz, P.H., Marth, P.C. and Craft, C.C. 1955. Further tests with 3-chloro-isopropyl-N-phenyl carbamate as asprout inhibitor for potato tubers. Am. Potato J. 32:357-361.

Hyde, R.B. and Morrison, J.W. 1964. The effect of storage temperature on reducing sugars, pH and phosphorylase enzyme activity in potato tubers. Am. Potato J. 41:163-168.

Ioannou, J., Chism, G and Hard, N.F. 1973. Molecular species of phosphorylase in post harvest potato tubers. J. Food Sci. 38:1022-23.

Iritani, W.M. and Weller, L.D. 1977. Changes in sucrose and reducing sugar content of Kennebec and Russet Burbank tubers during growth and post harvest holding temperatures. Am. Potato J. 54:395-404.

Iritani, W.M. and Weller, L.D. 1980. Sugar development in potatoes. Washington State University Cooperative Extension Bulletin 0717:3-16.

Isherwood, F.A. 1973. Starch-sugar interconversion in Solanum tuberosum. Phytochemistry. 12:2579-2591.

Isherwood, F.A. and Kennedy, M.GH. 1975. The composition of expressed sap from cold stored potatoes. Phytochemistry. 14:83-84.

Isherwood, F.A. 1976. Mechanism of starch-sugar interconversion in Solanum tuberosum. Phytochemistry. 15:33-41.

Kader, A.A. 1987. Respiration and gas exchange of vegetables. In post harvest physiology of vegetables. Ed. Weichman, J. Mercel Dekhar Inc., NY. pp:25-33.

Kennedy, M.GH. and Isherwood, F.A. 1975. Activity of phosphorylase in Solanum tuberosum during low temperature storage. Phytochemistry. 14:667-670.

Khurana, D.S., Randhawa, K.S. and Bajaj, K.L. 1985. Carbohydrate content of potato tubers treated with CIPC. J. Sci. Food, Agric. 36:959-962.

Kim, M.S.L., Ewing, E.E. and Sieczka, J.B. 1972. Effects of chlorpropham (CIPC) on sprouting of individual potato eyes and on plant emergence. Am. Potato J. 49:420-430.

Kruger, N.J. and Hammond, J.B.W. 1988. Molecular comparison of pyrophosphate and ATP dependent fructose 6-P 1-phosphotransferases from potato tuber. Plant Physiol. 86:645-648.

Kono, N. and Uyeda, K. 1977. Chicken liver phosphofructokinase II cold inactivation. J. Bio. Chem. 248:8603-8609.

Lyne, R.L. and ApRees, T. 1971. Invertase and sugar content during differentiation of roots of pisumstivum. Phytochem. 10:2593-2599.

Lyons, J.M. 1973. Chilling injury in plants. Annual Review. Plant Physiology. 24:445-466.

Marth, P.C. and Schultz, E.S. 1952. A new sprout inhibitor for potato tubers. Am. Potato J. 29:268-.

Marschke, C.K. and Bernlohr, R.W. 1973. Purification and characterization of PFK of Vacillus licheniformis. Arch. of Biochem. & Biophys. 156:1-16.

Mazza, G, Hung, J. and Dench, M.J. 1983. Processing/nutritional quality changes in potato tubers during growth and long term storage. Can. Inst. Food Sci. Technol. J. 16:39-44.

Mondy, N.I., Tymiak, A. and Chandra, S. 1978. Inhibition of glycoalkaloid formation in potato tubers by sprout inhibitor maleic hydrazide. J. Food Sci. 43:1033-1035.

Mondy, N. and Ponnampalam, R. 1985. Effects of sprout inhibitor isopropyl-N-(3-chlorophenyl)- carbamate on total glycoalkaloid content of potatoes. J. Food Sci. 50:258-261.

Moorland, D.E. 1967. Mechanism of action of herbicides. Ann. Rev. Plant Physiology. 18:365-386.

Morrell, S. and ApRees, T. 1986. Sugar metabolism in developing tubers of Solanum tuberosum. Phytochemistry. 25:1579-1585.

Mueller, T.O. and Mondy, N.I. 1977. Effect of sprout inhibition on lipid composition of potatoes. J. Food Sci. 42:618-621.

Nelson, D.C. and Sowokinos, J.R. 1983. Yield and relationship among tuber size, sucrose and chip color in six potato cultivars on various harvest dates. Am. Potato J. 60-949-958.

Nowak, J. 1977a. Biochemical changes in stored potato tubers with different rest periods. I. Influence of storage temperature and isopropyl phenyl carbamates (IPC) and CIPC on protein changes. Z. Pflanzenphysiol. 81:113-124.

Nowak, J. 1977b. II. Influence of the storage temperature and isopropyl carbamate on enzyme activities. Z. Pflanzenphysiol. 81:125.

O'Brien, W.E., Bowien, S. and Wood, H.G 1975. Isolation and characterization of a pyrophosphate dependent phosphofructokinase from propionibacterium shermanii. The J. of Biological Chemistry. 250:8690-8695.

Ohad, I., Friedburg, I. Neeman, Z. and Schramm, M. 1971. Biogenesis and degradation of starch. The fate of amyloplast membranes during maturation and storage of potato tubers. Plant Physiol. 47:465-.

Phan, C.T. 1987. Temperture effects on metabolism. In post harvest physiology of vegetables. Ed. Weichman, J. Mercel Dekhar Inc., NY. pp:173-180.

Pollock, C.J. and ApRees, T. 1975. Activities of enzymes of sugar metabolism in cold-stored tubers of Solanum tuberosum. Phytochemistry. 14:613-617.

Pressey, R. and Shaw, R. 1966. Effect of temperature on invertase, invertase inhibitor and sugars in potato tubers. Plant Physiol. 41:1657-1661.

Pressey, R. 1969. Role of invertase in the accumulation of sugars in cold stored potatoes. Am. Potato J. 46:291-297.

Pressey, R. 1970. Changes in sucrose synthetase and sucrose phosphate synthetase activities during storage of potatoes. Am. Potato J. 47:245-251.

Ponnampalam, R. and Mondy, N.I. 1986. Effect of sprout inhibitor isopropyl-N-3 chloropheynl carbamate on phenolic and ascorbic acid content of potatoes. J. Agric. Food Chem. 34:262-263.

Raison, J.K., Lyons, J.M., Mehlhorn, R.J. and Keith, A.D. 1971. Temperature induced phase change in mitochondria membranes detected by spin-labeling J. Biol. Chem. 246:4036-4040.

Raison, J.K. 1974. A biochemical explanation of low temperature stress in tropical and sub-tropical plants. In mechanism of regulation of plant growth. Ed. Bieleski, R.L., Ferguson, A.R. and Cresswell, M.M. The Roayl Soc. of New Zealand. pp:487-497.

Ravamel, P. and Tissut, M. 1984. Mitochondrial changes during storage of untreated and CIPC treated potatoes. Pesticide Biochem. and Physiol. 22:1-7.

Ricardo, C.P.P. and ApRees, T. 1979. Invertase activity during the development of carrot roots. Phytochemistry. 9:239-247.

Ronsen, K. and Frogner, S. 1969. The influence of storage and conditioning on the content of reducing sugars in potatoes grown in Norway. Eur. Potato J. 12:122-133.

Sabularse, D.C. and Anderson, R.L. 1981. D-fructose 2,6 bisphosphate: A naturally occuring activator for inorganic pyrophosphate. D-fructose-6-phosphate 1-phosphotransferase in plants. Biochem. Biophys. Res. Communications. 103:848-855.

Samotus, B. and Schwimmer, S. 1962a. Predominance of fructose accumulation in cold stored immature potato tubers. J. Food Sci. 27:1-4.

Samotus, B. and Schwimmer, S. 1962b. Effect of maturity and storage on distribution of phosphorous among starch and other components of potato tubers. Plant Physiol. 37:519-522.

Santerre, C.R., Cash, J.N. and Chase, R.W. 1986. Influence of cultivar, harvest date and soil nitrogen on sucrose, Sp-Gr. and storage stability of potatoes grown in Michigan. Am. Potato J. 63:99-109.

Sawyer, R.L. and Dallyn, S.L. 1958. Timing maleic hydrazide sprays to stage of plant development. Am. Potato J. 35:620-625.

Schaftingen, V.E., Lederer, B., Bartons, R. and Hers, H.G 1982. A kinetic study of pyrophosphate: F 6-p phosphotransferases from potato tubers. Eur. J. Biochem. 129:191-195.

Schaftingen, V.E. 1987. Fructose 2,6-bisphosphate. In Adv. Enzymology. 59:315-395.

Schwimmer, S., Hendel, C. Herrington, W. and Olson, R. 1957. Interrelation among measurement sof browning of processed potatoes and sugar components. Am. Potato J. 34:119-132.

Schwimmer, S. 1958. Influenc eof polyphenols and potato components on potato phosphorylase. J. Biochem. 232:715-721.

Schwimmer, S., Makower, R.U. and Rorem, E.S. 1961. Invertase and invertase inhibitor in potato. Plant Physiol. 36:313-316.

Shallenberger, R.S., Smith, O. and Treadway, R.H. 1959. Role of sugars in the browning reaction. J. Agr. Food Chem. 7:272-277.

Shekher, V.C. and Iritani, W.M. 1978. Starch to sugar interconversion in Solanum tuberosum L. I. influence of inorganic ions. Am. Potato J. 55:345-350.

Smyth, D.A., Wu, Min-Xian and Black, C.C. 1984. Phosphofructokinase and fructose 2,6-bisphosphatase activities in developing corn seedlings (Zea Mays L.). Plant Science Letters. 33:61-70.

Sowokinos, J.R. 1973. Maturation of Solanum tuberosum. Comparative sucrose and sucrose synthetase levels between several good and poor processing varieties. Am. Potato. J. 50:234-247.

Sowokinos, J.R. 1976. Pyrophosphorylase in Solanum tuberosum. Plant Physiology. 57:63-68.

Sowokinos, J.R. 1978. Relation of harvest sucrose to processing maturity and storage life of potatoes. Am. Potato J. 50:333-334.

Sowokinos, J.R. 1981. Pyrophosphorylases in Solanum tuberosum. II. Catalytic properties and regulation of ADP glucose and UDP glucose pyrophosphorylase in potatoes. Plant Physiology. 69:1459-1466.

Sowokinos, J.R. and Preiss, J. 1982. Pyrophosphorylases in Solanum tuberosum III. Purification, physical and catalytic properties of ADP-glucose pyrophosphorylase in potatoes. Plant Physiol. 68:924-929.

Sowokinos, J.R., Lulai, E.C. and Knoper, J.A. 1985. Translucent tissue defects in Solanum tuberosum L. I. Alteration in amyloplast membrane integrity, enzyme activities, sugars, and starch content. Plant Physiology. 78:489-494.

Sowokinos, J.R. and Preston, D.A. 1988. Maintenance of potato processing quality by chemical maturity monitoring Station Bulletin 586-1988 (Item No. Ad-SB-3441) Minesota Agricultural Experiment Station.

Talburt, W.F. 1987. History of potato processing In Potato Processing 4th Edition. Ed. Talburt, W.F. and Smith, O. AVI, NY. pp:1-9.

Tishel, M. and Mazelis, M. 1966. The accumulation of sugars in potato tubers at low temperature and some associated enzymatic activities. Phytochemistry. 5:895-902.

Turner, J.P. and turner, D.H. 1980. The regulation of glycolysis and the pentose phosphate pathway. In the Biochemistry of plants. Vol. 2. Academic Press, NY. pp:279-316.

Uyeda, K. 1979. Phosphofructokinase. Adv. Enzymol. 48:193.

Van Es, A. and Hartmans, K.J. 1987. Starch and sugars during tuberization, storage and sprouting In Potato Storage. Ed. Rastovaski, A. and Van Es. A. et al. Pudoc, Wageningen. pp:79-113.

Van Winkle, D. 1975. An investigation into the mode of action of isopropyl meta-chlorocarbanilate (chloropham) in potato tissue. Ph.,D. Thesis, Cornell University, NY.

Weaver, M.L. and timm, H. 1983. Significance of the fructose/glucose ratio in screening potto breeding lines with processing potential. Am. Potato J. 60:329-338.

Weis, GG, Schoenemann, J.A. and Groskopp, M.D. 1980. Influence of time of application of maleic hydrazide on the yield and quality of Russet Burbank potatoes. Am. Potato J. 57:197-204.

Workman, M., Kerschner, E. and Harrison, M. 1976. The effect of storage factors on membrane permeability and sugar content of potatoes and decay by Erwinia carotovora and Fusarium roseum. Am. Potato J. 53:191-204.

Workman, M. Cameron, A. and Twomey, J. 1979. Influence of chilling on potato tuber respiration, sugar. O-dihydroxyphenolic content and membrane permeability. Am. Potato J. 56:277-288.

Wu, M.T. and Salunkhe, D.K. 1977. Inhibition of wound induced glycoalkaloid formation in potato tubers (Solanum tuberosum L.) by isopropyl-N-(3-chlorophenyl)-carbamate. J. Food Sci. 42:622.

Wunsch, A. and Schaller, K. 1972. Interactin of sugars and amino acids in development of color in chips. Potato Res. 15:12-23.

Zaehringer, M.V., Cunningham, H.H. and Sparks, W.C. 1966. Sugar content and color of Russet Burbank potatoes as related to storage temperature and sprout inhibitors. Am. Potato J. 43:305-314.

CHAPTER 1

INFLUENCE OF ISOPROPYL N-(3-CHLOROPHENYL) CARBAMATE (CIPC) AND MALEIC HYDRAZIDE (MH) ON ATP AND PPi-DEPENDENT PHOSPHOFRUCTOKINASES (PFK) AND SUGARS IN ATLANTIC POTATOES STORED AT 3.3 AND 10°C

ABSRACT

Specific activities of ATP and pyrophosphate (PPi) fructose-6-phosphate phosphotransferases (ATP-PFK and PPi-PFK) and corresponding changes in sucrose, glucose and fructose were measured in CIPC and MH treated Atlantic potatoes stored at 3.3 and 10°C for six months. The basic objective was to gain an insight into the possible pathway(s) controlling changes in sugars in CIPC and MH treated, stored potatoes. Storage at 3.3°C resulted in a high accumulation of sugars compared to 10°C. However, the activities of enzymes were not different at the two temperatures, suggesting little effect of CIPC, MH or temperature on these enzymes. The specific activity of PPi-PFK was found to be greater than ATP-PFK.

INTRODUCTION

Atlantic, a medium to late season maturity cultivar with high specific gravity and yield, is reported to be an excellent chipping potato (Webb, et al., 1978). This variety is the most popular variety grown in Michigan for processing into chips. Since, a portion of this cultivar is stored for several months, sprout control and low sugar levels during storage are important to insure good quality potatoes to the chipping industry.

It is well known that holding potatoes at low temperature delays sprouting, but these conditions contribute to sucrose and reducing sugar accumulations, which cause dark colored chips when tubers are processed. The conventional means of minimizing sugar levels is to recondition the potatoes at room temperature for 1-3 weeks after removal from cold storage.

Potatoes may be held for up to 6-7 months at 10-12^{O}C with the aid of a sprout inhibitor such as CIPC which is applied as an aerosol through the potato storage air system. MH is applied to the potato foliage 2-3 wks before top kill for sprout control on a short term storage.

CIPC may influence many aspects of tuber metabolism. It has been reported to inhibit the movement of nitrogenous substances from the parenchyma to the external part of the tuber and prevent changes in the soluble proteins. It has also been shown to inhibit protein synthesis and the activity of enzymes associated with protein synthesis in the eyes of tubers but had no effect on enzyme activity in the parenchyma cells (Nowak 1977a, 1977b). Ravanel and Tissut (1984), reported CIPC treated tubers maintained moisture and dry matter content for a long period. They also reported a mild inhibition of electron transfer when the mitochodria from potato tubers were saturated with CIPC. These changes in treated tubers reflect CIPC's effect on cell metabolism and growth (sprouting) process.

CIPC's effects have also been reported on glycoalkaloid, ascorbic acid and phenolic contents of potatoes (Wu & Salunkhe, 1977; Mondy & Ponnampalam, 1985; Ponnampalam & Mondy, 1986). Zaehringer et al., (1966), reported little effect of CIPC or MH on sugar accumulation in potato tubers stored at 7.2°C, but Vanvliet and Schriemer (1963), found an increase in sugar content in CIPC treated tubers stored at 10-15°C. These conflicting reports indicate a need for studies to understand CIPC's effect on sugar changes in stored potatoes.

Use of MH has been shown to produce more uniform shaped potatoes (Weis et al., 1980). It is reported to lower glycoalkaloid content (Mondy et al., 1978), but its use is also observed to promote enzymatic darkening in the tubers (Mueller & Mondy, 1977). Few reports are available on MH effects on carbohydrate metabolism in stored tubers.

Pollock & ApRees (1975) and Dixon et al., (1981) have suggested lability of ATP-PFK as being responsible for accumulation of sugars at low temperatures. Some recent reports (Carnal & Black, 1983; Schaftingen et al., 1982; Morrel & ApRees, 1986), have pointed out pyrophosphate (PPi) dependent PFK as being important in glycolysis in plant tissues. Reduction or loss of activity of these enzymes due to CIPC or MH can cause sugar accumulation in stored potatoes.

Since, CIPC and MH are being routinely used as sprout inhibitors, the basic aim of this study was to gain an understanding of their effect on sugars and on the rate-limiting glycolytic enzymes: ATP-PFK and PPi-PFK (which regulate entry of hexoses into glycolytic pathway), in Atlantic potatoes stored at 3.3 and 10°C for six months.

MATERIAL & METHODS

POTATOES

Atlantic potatoes, grown on a sandy loam soil with optimum production management inputs at the Montcalm Research Farm of Michigan State University were harvested at maturity. Maleic hydrazide 1.5 k was applied as a spray 3 weeks prior to top kill. MH treated and untreated samples containing approximately 30 lb. potatoes each were placed in mesh bags. All potatoes were held at room temperature for three weeks to allow for wound healing CIPC was applied as an aerosol by a commercial applicator. Subsequently, all samples (the controls, CIPC, MH and CIPC + MH treated) were stored at 3.3 and 10^0 with 90% RH for six months.

Extraction and Assay for ATP-PFK and PPi-PFK

A) Extraction

Crude extracts for enzyme assays were prepared according to Chung (1985). Ten g of potato obtained from center cuts of 3-4 uniform sized potato tubers were mixed in a prechilled waring blender for 1.5 min., with 30 ml of extraction medium containing 50 mM Tris-HCl (pH 8.0), 20 mM potassium acetate, 2 nM dithiothreitol, 4 mM magnesium chloride and 2 mM disodium pyrophosphate. The homogenate was filtered through cheese cloth and centrifuged at 20,000 g in a refrigerated centrifuge (Sorvall RC 2-B, Dupont Instruments, Newtown, Conn.) for 10 min.

B) Assay

The assay of enzymes on freshly prepared extracts was according to Schaftingen et al., (1982) with modifications based on Siga Product Note No. F-2258, Appendix 1 (Siga Chemical Co., St. Louis, MO 63178). ATP-PFK and PPi-PFK activities were determined by following the rate of change in absorbance at 340 nm due to oxidation of NADH for six min., immediately after addition of ATP, in case of ATP-PFK, and disodium-Pyrophosphate for PPi-PFK. The reaction rates were corrected for endogenous NADH oxidation. A Lamda Perkin Elmer Spectrophotometer (Perkin Elmer Corporation, IL) with enzyme kinetics software package, equilibrated to 30^{o}C was used. Background correction was made with water as blank.

Protein Analysis

Protein measurements on crude enzyme extracts prepared from potatoes were made according to a dye binding technique (Coomassie Brilliant Blue G-25), using bovine serum albumin as a standard (Bradford, 1976). The binding of the dye to protein causes a shift in the absorption maximum of dye from 465 to 495 nm, which was measured. The dye was prepared by dissolving 100 mg of Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol. To this, 100 ml 85% (w/v) phosphoric acid were added and the resulting solution diluted to one liter with distilled water then filtered through a milipore filter (0.47 mm filter paper). A standard bovine serum albumin (BSA) solution was prepared by dissolving 100 mg dehydrated, moisture free BSA in 100 ml 0.15 M Nacl. For protein measurements, amounts containing 10-100 ug of BSA (0.01-0.10 ml standard solution) were used. The volume of standard protein solution was adjusted to 0.1 ml with the enzyme extraction buffer, 5 ml dye were added and the contents mixed well before measuring absorbance after 5 min. at 595 nm against a blank made up of 5 ml dye and 0.1 ml extraction buffer. The standard curve for bovine serum albumin, which is shown in Appendix 2, was used to calculate the protein content in enzyme extracts.

Sugar Analysis

A) Standard Preparation

A standard containing sucrose, glucose and fructose was prepared for high pressure liquid chromatography (HPLC) analysis as described by Wilson et al., (1981), by dissolving 250 mg of each sugar in 25 ml deionized distilled water in a volumetric flask. A 10 ml aliquot from this solution was mixed with 10 ml HPLC grade methanol, followed by centrifugation at 27,000 g for 10 min. in a refrigerated centrifuge. After centrifugation, the supernatant was clarified using milipore filter (0.45 um filter paper). A standard curve was prepared by injecting 5, 10, 15, 18, 20, 40 and 50 ul of the standard solution and quantification of each sugar was based on a standard curve (Appendices 3, 4, and 5).

B) Sample Preparation

Samples for sugar analysis by HPLC were also prepared according to Wilson et al., (1981). A 200 g random sample of fresh potato tissue obtained from center cuts of 4-5 uniformed sized tubers was extracted using an Acme juicerator (Model #6001 Acme juicer Mfg Co., Sierra Madre, CA). A 10 ml aliquot of the potato extract was prepared for analysis by

HPLC in the same manner as the 10 ml standard solution. Fresh samples of 20-50 ul (depending on concentration of sugars in the samples) were injected into HPLC for analysis. All measurements were made in duplicate.

C) HPLC Equipment

A waters model M-45 solvent delivery system, a U6K injector, and a model R-401 RI detector (Waters Associates, Inc., Milford, MA 01757), along with Beckman 10 inch recorder were used.

D) Column

For separation of sguars, an Alltech 600CH silica based amino bonded phase column with a direct connect refillable guard column with pellicular NH_2 packing was used.

E) Mobile Phase

The mobile phase was acetonitrile-water (80+20).

F) Operating Conditions

Flow rate: 1.6 ml/min., attenuation: 4X, and chart speed: 0.2 inch per min.

CIPC Residual Analysis

The CIPC residual analysis in the potato peels was adapted from Gard & Ferguson (1963) and Ferguson and Gard (1969). A macerated sample was hydrolyzed in alkali and the hydrolysis product 3-chloranaline, was steam distilled and collected in HCl. The analysis was completed by diazotizing the analine with sodium nitrite followed by coupling with N-(1-naphthyl) ethyldiamine. The absorbance of the color complex formed was measured spectrophotometriclaly using Spectronic 21D (Milton Roy, Rochester, NY).

A) Standard Curve for CIPC Residual Analysis

A standard curve was made with 3-chloroanaline (P-1271, Eastman Kodak Co.), according to Gard and Ferguson (1963). In this procedure, 0.1 g of 3-chloroanaline was dissolved in 1 M HCl and made to one liter with 1 M HCl in a volumetric flask. Ten ml of this solution was further diluted to 1000 ml with 1 M HCl, so that 1 ml of final dilution was equivalent to 0.001 mg of 3-chloroanaline or 0.0017 mg of CIPC. A standard curve was made by measuring 0.0, 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0 ml of this standard solution in a series of 50 ml volumetric flasks. The volume of each flask was made to 40 ml with 1 M HCl to which 1 ml freshly prepared 2% sodium nitrite was added. The contents of each flask were mixed well and held for 20 min. to allow for diazotization. Subsequently, 1 ml freshly prepared 10% sulfamic acid was added to each flask and the contents allowed to stand for 15 min. for complete destruction of nitrite. Finally, 5 ml of 2% N-1-naphthylethylenediamine dihydrochloride was added to each flask and the contents made to 50 ml with 1 M HCl. After 90 min. the absorbance of the color complex formed was measured at 540 nm with water as blank. The standard curve for CIPC residual analyusis is shown in Appendix 6.

B) Sample Preparation for CIPC Residual Analysis

Potato peels from 4-5 uniform sized, unwashed tubers (with loose dirt brushed off) were obtained by peeling potatoes to approximately 2 mm thickness using a kitchen knife (Corsini et al., 1979). The peels from all the potatoes were mixed and a homogenous sample of approximately 25 g was used for hydrolysis and distillation.

a) Alkaline Hydrolysis

The peels from potatoes were homogenized in a waring blender with 100 ml distilled water. The homogenized slurry was transferred to a 2000 ml round bottom distillation flask to which 50 ml 30% sodium hydroxide (prepared from reagent grade pellets) was added through a dispensing funnel.

The hydrolysis and distillation apparatus (Appendix 7) was according to Gard and Ferguson (1963), with some modification to prevent overflow during 3-4 hours required form complete hydrolysis. Following hydrolysis, distillation was started without dismantling the apparatus and 20-25 ml distillates were collected in a beaker containing 10 ml of 3 M HCl. The distillate collected was filtered through Whatman No. 42 filter paper and the clear filterate was spectrophotometrically analyzed for CIPC residue in the same manner as the standard.

Chip Color Measurement

Potato chips were prepared from potatoes stored at 3.3 and 10^{o}C according to Gould and Plimpton (1985). Five or six uniform sized tubers selected at random were washed and sliced into chips with a manual slicer (The Eagle Tool & Machine Co., Inc., Springfield, OH) to approximately 0.125 to 0.175 cm thickness. The tuber slices were collected and washed in a container with cold water. Approximately 25-

30 slices were collected, washed one more time in cold water then fried in corn oil at 188°C for 130 sec., in a thermostatistically controlled fryer (Model F-49, Wells Mfg Co.,, Verdi, NV). The chip color was evaluated using Agtron E-10 colorimeter (Filper Magnuson, Reno, NV).

Calculations

1) Enzyme (PPi-PFK & ATP-PFK) activity:

$$\text{Unit/mg protein} = \frac{\text{Delta A 340 nm/min.} \times 3}{6.22 \times 10^{-3} \times 2 \times (\text{mg enzyme as protein/ml reaction mixture})}$$

Delta A 340 nm = change in absorbance/min.

3 = volume of reaction mix in the cuvette

6.22×10^{-3} = extinction coefficient of NADH (2 accounts for 2 mole of NADH oxidized per mole of fructose, 1,6 bisphosphate formed)

2) $$\text{Sugar (mg/g)} = \frac{\text{Factor} \times \text{peak height} \times 1000 \times 20\text{ ml} \times \text{wt. of extract (g)}}{\text{volume injected} \times 10\text{ ml} \times \text{wt. of sample (g)}}$$

Factor was arrived at from the standard curve of each sugar and gives mg of each sugar/peak height.

3) CIPC Residual analysis:

$$\text{ppm of CIPC} = \frac{\text{mg of 3-chloroanaline} \times 1.67 \times 1000}{\text{wt. of the sample (g)}}$$

Statistical Analysis

Statistical analysis was done with MSTAT microcomputer program, version 4.0 (Michigan State University and Agriculture University of Norway).

RESULTS AND DISCUSSION

Sugar changes at 3.3°C

The effect of holding the potato tubers at 3.3°C on sucrose, glucose and fructose in control, CIPC, MH and CIPC+MH, treated Atlantic tubers was determined by HPLC (Fig 1, 2 & 3). A representitave chromatogram of sugar analysis in potatoes is shown in Appendices 8 and 9. Fructose peak emerged first followed by glucose and sucrose and the time taken for three sugars to elute was about 10 min. There was an increase in the three sugars during the first 2-3 months, followed by a decline at 4 months. Fructose did show a slight increase in MH and CIPC treated samples at 6 month. The increase in glucose and fructose was more pronounced than sucrose in all the samples.

Statistical analysis showed a significant difference in changes in sugars over the storage period ($P < 0.05$), but sugar accumulations in treated and control samples at 3.3°C were not significantly different (Appendix 11). The six months' average data (Table 1) showed a lower content of sucrose but higher glucose and fructose respectively in CIPC (1.71 mg/g, 3.63 mg/g and 2.98 mg/g fresh weight), CIPC+MH (1.80 mg/g, 3.93 mg/g and 3.23 mg/g) and MH (1.92 mg/g, 3.41 mg/g and 2.85 mg/g) treated tubers compared to control samples (2.11 mg/g, 3.28 mg/g and 2.79 mg/g). This is in contrast to Khurana et al., (1985), who reported more total sugars in control samples than in CIPC treated tubers stored at 2-3°C. However, Zaehringer et al., (1966), reported no effect of CIPC or MH on sugar accumulation in Russet Burbank potatoes stored at

7.2°C. This is expected because the increase in sugars starts rapidly below 4°C (Burton, 1965). Earlier, Amir et al., (1977) reported a rapid rise in ATP and a fall in respiration below the initial value and no detectable increase in sugars immediately after exposure to cold temperatures. The increase in sugar formation was accompanied by a decrease in ATP content and dinitrophenol (an uncoupler of oxidative phosphorylation), blocked sugar formation. The rise in ATP immediately following exposure to cold could be in response to tubers increased energy requirements.

In the present study, maximum sucrose, glucose and fructose accumulations on fresh weight basis were 2.95 mg/g, 5.37 mg/g and 4.70 mg/g respectively in the control and 2.01 mg/g, 5.96 mg/g and 3.57 mg/g in CIPC treated Atlantic tubers after 2-3 months at 3°C. However, the extent to which sugars can accumulate during storage is a variety characteristic. Coffin et al. (1987) reported low levels of sugars (sucrose - 1.30 mg/g, glucose - 0.56 mg/g and fructose - 0.46 mg/g) in ND-860-2, compared to Kennebec, which accumulated more reducing sugars (1.83 mg/g glucose and fructose each) than sucrose (1.48 mg/g) when stored at 4°C for 3 months. Workman et al., (1979) reported total reducing sugars as high as 48 and 19 mg/g fresh weight in Centennial and Russet Burbank tubers stored at 0°C for 50 days.

In the present study, all treatments held at 3.3°C showed a rise in sugar content indicating that sprout inhibitors had little effect on the accumulation of sugars at low temperatures.

Sugar changes at 10°C

Control and MH treated tubers began sprouting after 2 months of storage at 10°C (although sprouting in MH treated tubers was slightly less severe), while CIPC and CIPC+MH treated tubers were sprout free even after six months of storage. Acceptable color chips could be made with these tubers as compared to tubers stored at 3.3°C (Table 1). Increase in all three sugars was less at 10°C than at 3.3°C (Fig 4, 5 & 6). This is in agreement with Khurana et al. (1965) who reported a decrease in starch breakdown and reducing sugar accumulation in CIPC treated potatoes stored at room temperature. Craft and Audia (1959) using potato chip color as an index of sugar accumulation showed that CIPC treated tubers held at 12.8°C for 10 wks responded esentially the same as untreated tubers.

The results of this study suggest that treatment with CIPC and storage at 10°C causes little sugar accumulation and the treated tubers maintain their chipping quality for a longer period provided proper humidity is maintained during storage. This is in contrast to an earlier held view that suppressing sprouting with chemical sprout inhibitors could cause a greater sugar accumulation since sugar formed is not utilized in the growth of sprouts (Vanvliet and Schriemer, 1963 and Burton, 1965).

There was no advantage to using both MH and CIPC in terms of relative reduction in sugar levels or improvement of chip color.

Effect on Enzymes ATP-PFK and PPi-PFK

In the present study, the approach was to determine if storage temperature and sprout inhibitors affect ATP-PFK or PPi-PFK and whether these enzymes influence in sugar changes in stored potatoes.

At the beginning of the experiment, tests were made with pure PPi-

PFK from potato tuber (Siga product No. P 2258) and ATP-PFK from rabbit liver (Siga product No. P 8134) to ascertain the recovery of these enzymes' activity under the extraction and assay procedure followed for the potato samples. In case of added PPi-PFK and ATP-PFK, only 80% and 28% activities, respectively were recoverable. Pollock and ApRees (1975) reported "for PFK (EC.2.7.1.11) at least 70% of the added enzyme was lost regardless of whatever precautions we took." The fact that properties of enzyme from plant and mammalian sources may not be the same could also cause lower recovery of ATP-PFK.

The ATP-PFK activity observed in this study ranged from 3-39 nmoles/min/mg protein (Fig 7), which compares with values of 1-30 nmoles/min/mg protein, reported by Carnal and Black (1983) in several plant species. Kruger et al. (1988) reported a value of 12.9 nmoles/min/mg protein. Carnal and Black (1983) also indicated that the extractable ATP-PFK activities are the lowest displayed by enzymes catalyzing reaction in the glycolytic sequence. The activity of PPi-PFK in many plant species was found to be 4 to 70 fold higher than ATP-PFK.

The specific activity of PPi-PFK measured in this study ranged from 11 to 127 nmoles/min/mg protein (Fig 8, 9 & 10). The total protein content in the crude extracts for enzyme assay are given in Appendix 10. It is evident that PPi-PFK is the predominant enzyme in the potato samples analyzed. Neither temperature nor sprout inhibitors effected these enzymes. Nowak (1977b) also reported little effect of CIPC on enzymes in the parenchyma cells. Chung (1985) found little evidence of CIPC effects on ATP-PFK, PPi-PFK, mitochondrial respiratory activities or reducing sugar content during a short term storage of potatoes. Dixon et al. (1981), however, in an <u>in vitro</u> study using differential scanning calorimetry found exothermic dissociation of purified ATP-PFK obtained from potato tubers at 286K. They argued that as this temperature is close to that at which tubers start accumulating sugars, inactivation of ATP-PFK at low temperatures could possibly be the cause for sugar increases. In the present study, activities of enzymes at 10 and 3.3°C were not different. However, the activities of the enzymes declined over the storage period. It is likely that the metabolic reactions leading to sugar changes are under a fine control with a quick change in enzyme activity. Also, in intact tissues cytoplasmic fluid may offer some protection to these enzymes so that alteration in enzyme activity due to low temperature or sprout inhibitors in crude extracts are not seen.

CIPC Residual Analysis

The residual CIPC analysis was conducted to determine what effect, if any, CIPC concentration has on sugars and enzymes in potatoes. After commercial application of CIPC, the initial CIPC content was determined to be 55.20 ppm, which decreased to 9.5 ppm at 3.3°C and 2.0 ppm at 10°C respectively after six months (Fig 11). Corsini et al. (1979) reported an average CIPC content of 51 ppm after commercial aerosol application. Cash et al. (1988) observed no sprouting in potatoes having 1.8 ppm residual CIPC after six months storage at 11°C. Hajslova and Davidek (1986) reported gradual decrease in CIPC content to 2.10 mg/kg during storage.

Although the residual level of CIPC is in agreement with published values we do not find a relationship between CIPC concentration in the tubers and its effect on either enzymes studied or sugar content. In fact the treated tubers were not significantly different from controls at 3.3^{o}C (Appendix 11).

CONCLUSION

In this study, the influence of sprout inhibitors CIPC and MH on sucrose, glucose, fructose and enzymes PPi-PFK and ATP-PFK in Atlantic potatoes stored for six months at 3.3 and 10°C was investigated. CIPC, MH or a combination of these two sprout inhibitors (CIPC+MH) had little effect on the increase in sugar accumulation at 3.3°C. The control and MH treated tubers began sprouting after two months, although sprouting in the MH treated tubers was not as severe as in the controls. The CIPC and CIPC + MH treated potatoes were sprout free and maintained low sugar levels throughout the study and processed into acceptable color chips.

Both the enzymes PPi-PFK and ATP-PFK were detected in Atlantic potatoes. PPi-PFK's activity was more predominant than ATP-PFK however, neither temperature nor sprout inhibitors caused any significant alteration in activities of these enzymes. The variations in enzymes and sugars in potatoes stored at 3.3°C were significant ($P<0.05$) over the storage period. But there were no significant correlations between enzyme activities and sugars. It is likely that the metabolic reactions governing changes in sugars are under a fine enzymatic control involving immediate change in enzyme activity.

The residual CIPC level declined more in tubers stored at 10°C than in 3.3°C. However, relationships between sugar accumulation, enzyme activities or residual CIPC level were not discernable.

REFERENCES

Amir, J., Kahn, V. and Unterman, M. 1977. Respiration, ATP level, and sugar accumulation in potato tubers during storage $4^{0}C$. Phytochemistry. 16:1495-1498.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding Analytical Biochemistry. 72:248-254.

Burton, W.G 1965. The sugar balance in some British potato varieties during storage. I. Preliminary observations. Eur. Potat J. 8:80-91.

Carnal, N.W. and Black, C.C. 1983. PFK activities in photosynthetic organisms. Plant Physiology. 71:150-155.

Cash, J.N., Sinha, N.K. and Chase, R.W. 1988. The influence of CIPC on potato carbohydrates during commercial production and storage. Michigan Potato Research Report. Vo. 20. Michigan State University, Agricultural Research Station, East Lansing, Michigan.

Chung, C. 1985. Effect of temperature and chlorpropham on phosphofructokinases, mitochondrial respiration and reducing sugars in dormant Nooksack potato tubers. Ph.D. thesis. University of Missouri, Columbia.

Coffin, R.H., Yada, R.Y., Parkin, K.L., Grodzinski, B. and Stanley, D.W. 1987. Effect of low temperature storage on sugar concentrations and chip color of certain processing cultivars and selections. J. Food Sci. 52:639-645.

Corsini, D., Stallknecht, G and Sparks, W. 1979. Changes in chlorpropham residues in storage potatoes. Am. Potato J. 56:43-50.

Craft, C.C. and Audia, W.V. 1959. Some effects of isopropyl N-(3-Chlorphenyl) carbamate on respiration, water uptake and ion leakage of potato tissue. Am. Potato J. 36:386-393.

Dixon, W.L., Franks, F. and ApRees, T. 1981. Cold lability of phosphofructokinases from potato tubers. Phytochemistry. 20:969-972.

Ferguson, C.E. and Gard, L.N. 1969. IPC and CIPC residue analysis. J. Agr. Food Chem. 17:1062-1065.

Gard, L.N. and Ferguson, C.E. 1963. Determination of micro amounts of isopropyl N-(3-chlorophenyl) carbamate (CIPC) in milk and urine excreted from dairy cows. J. Agri. and Food Chem. 11:234-237.

Gould, W.A. and Plimpton, S. 1985. Quality evaluation of potato cultivars for processing Ohio Agricultural Research and Development Center, Wooster, Ohio, Research Bulletin No. 1172:8-10.

Hajslova, J. and Davidek, J. 1986. Sprout inhibitors IPC and CIPC in treated potatoes. Nahrung,. 30:75-79.

Khurana, D.S., Randhawa, K.S. and Bajaj, K.L. 1985. Carbohydrate content of potato tubers treated with CIPC. J. Sci. Food, Agric. 36:959-62.

Kruger, N.J., Hammond, J.B.W. and Burrell, M.M. 1988. Molecular characterization of four forms of phosphofructokinase purified from potato tuber. Archives of Biochem. Biophysics. 267:690-700.

Mondy, N.I., Tymiak, A. and Chandra, S. 1978. Inhibition of glycoalkaloid formation in potato tubers by sprout inhibitor maleic hydrazide. J. Food Sci. 43:1033-1035.

Mondy, N.I. and Ponnampalam, R. 1985. Effects of sprout inhibitor isopropyl-N-(3-Chlorophenyl) carbamate on total glycoalkaloid content of potatoes. J. Food Sci. 50:258-261.

Morrell, S. and ApRees, T. 1986. Sugar metabolism in developing tuberosum. Phytochemistry. 25:1579-1585.

Mueller, T.O. and Mondy, N.I. 1977. Effect of sprout inhibition on lipid composition of potatoes. J. Food Sci. 42:618-621.

Nowak, J. 1977a. Biochemical changes in stored potato tubers with different rest periods. I. Influence of storage temperature and isopropylphenyl carbamates (IPC) and CIPC on protein changes. Z Pflanzen Physiol. 81:113-124.

Nowak, J. 1977b. II. Influence of the storage temperature and isopropyl phenyl carbamates on enzyme activities. Z. Pflanzen Physiol. 81:125-.

Pollock, C.J. and ApRees, T. 1975. Activities of enzyems of sugar metabolism in cold-stored tubers of Solanum tuberosum. Phytochemistry. 14:613-617.

Ponnampalam, R. and Mondy, N.I. 1986. Effect of sprout inhibitor isopropyl-N-(3-Chlorophenyl) carbamate on phenolic and ascorbic acid content of potatoes. J. Agric. Food Chem. 34:262-263.

Ravmel, P. and Tissut, M. 1984. Mitochondrial changes during storage of untreated and CIPC treated potatoes. Pesticide Biochem. and Physiol. 22:1-7.

Schaftingen, V.E., Lederer, B., Bartons, R. and Hers, H.G 1982. A kinetic study of pyrophosphate: F 6-P phosphotransferases from potato tubers. Eur. J. Biochem. 192-195.

Van Vliet, W.F. and Schreimer, W.H. 1963. High temperature storage of potatoes with the aid of sprout inhibitors. Eur. Potato J. 6:201-217.

Webb, R.E., Wilson, D.R., Shumaker, J.R., Graves, B., Henninger, M.R., Watts, J., Frank, J.A. and Murphy, H.J. 1978. Atlantic a new potato variety with high solids, processing quality and resistance to pests. Am. Potato J. 55:141-145.

Weis, GG, Schoenemann, J.A. and Groskopp, M.D. 1980. Influence of time of application of maleic hydrazide on the yield and quality of Russet Burbank potatoes. Am. Potato J. 57:197-204.

Wilson, A.M., Work, T.M., Bushway, A.A. and Bushway, R.J. 1981. HPLC determination of fructose, glucose and sucrose in potatoes. J. Food Sci. 46:300-301.

Workman, M., Cameron, A. and Twomey, J. 1979. Influence of chilling on potato tuber respiration, sugar, O-dihydrophenolic content and membrane permeability. Am. Potato J. 56:277-288.

Wu, M.T. and Salunkhe, D.K. 1977. Inhibition of wound induced glycoalkaloid formation in potato tubers by isopropyl-N-(3-Chlorophenyl) carbamate. J. Food Sci. 42:622.

Zaehringer, M.V., Cunningham, H.H. and Sparks, W.C. 1966. Sugar content and color of Russet Burbank potatoes as related to storage temperature and sprout inhibitors. Am. Potato J. 43:305-314.

Table 1. Average sugar content and chip color of Atlantic potatoes stored at 3.3 and 10^{o}C for six months.

Sample	Temperature (^{o}C)	Sugar content (mg/g fresh wt.) Sucrose	Glucose	Fructose	Chip** Color
Control	3.3	2.11±0.80	3.28±1.21	2.79±1.26	34
	10*	0.59±0.16	0.30±0.03	0.23±0.01	69
MH	3.3	1.92±1.07	3.41±0.90	2.85±1.00	32
	10*	0.51±.06	0.40±0.15	0.22±0.10	66
CIPC	3.3	1.71±0.21	3.63±1.01	2.98±0.87	32
	10	0.50±0.11	0.24±0.13	0.21±0.10	69
CIPC+MH	3.3	1.80±0.32	3.93±1.05	3.23±0.69	31
	10	0.47±0.16	0.25±0.08	0.19±0.08	62

* Data for two months only, as sprouting started in these samples.

** Agtron chip color: > 60 = excellent; 56-60 = acceptable.

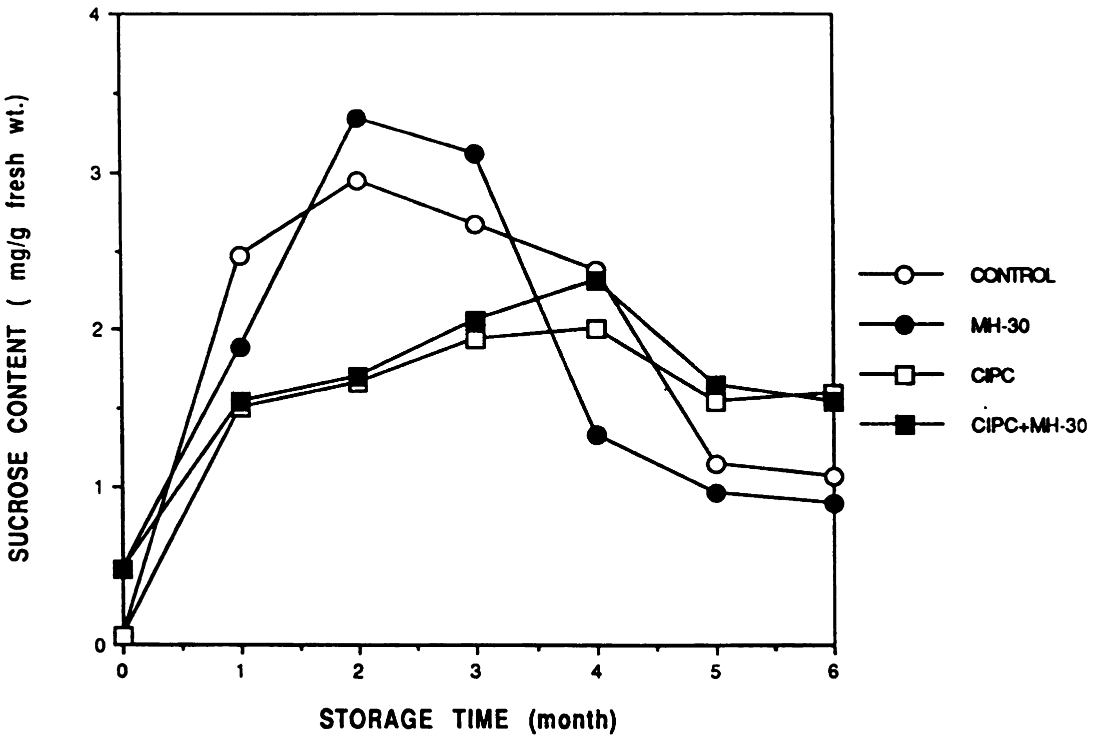


Figure 1. Sucrose content of Atlantic potatoes at 3.3^{o}C.

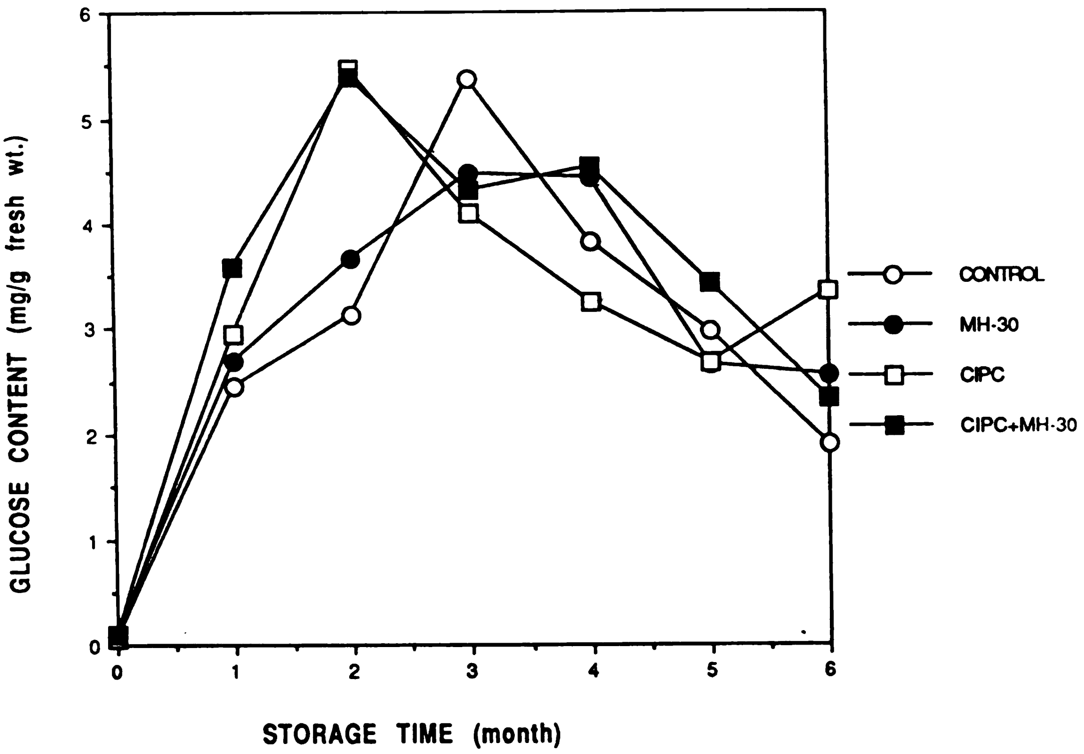


Figure 2. Glucose content of Atlantic potatoes at 3.3°C.

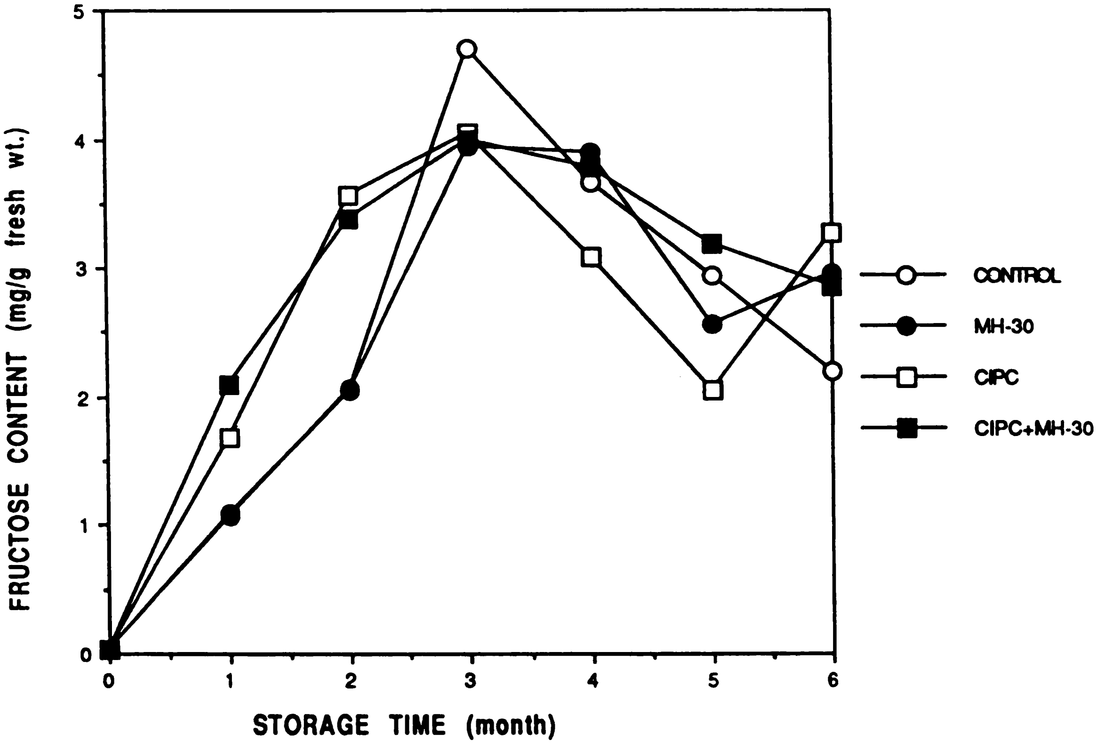


Figure 3. Fructose content of Atlantic potatoes at 3.3°C.

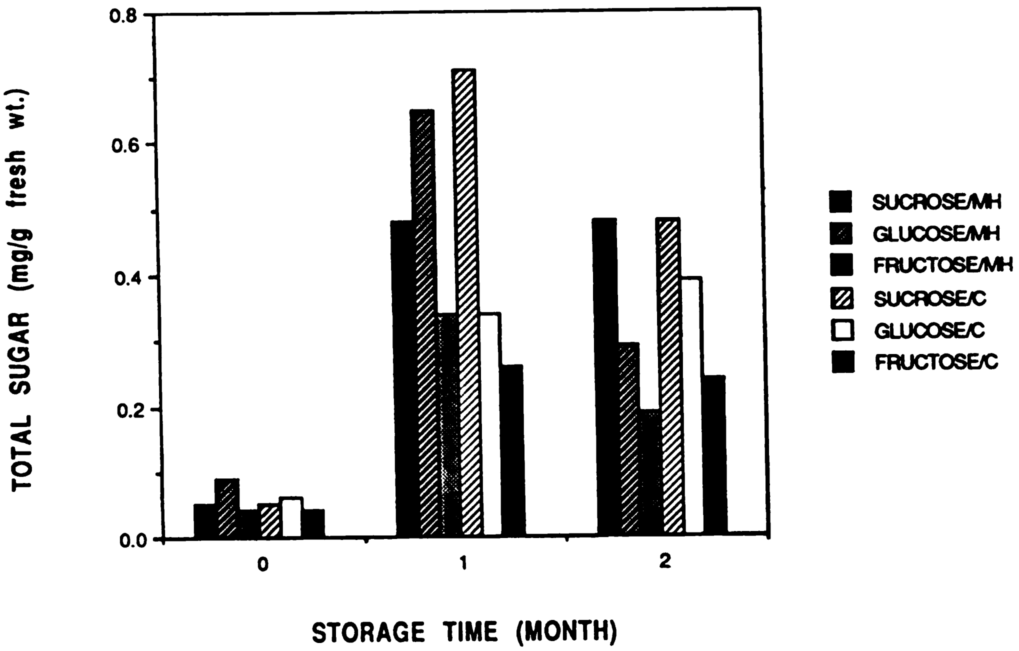


Figure 4. Sugar content in control & MH treated Atlantic potatoes stored at 10°C.

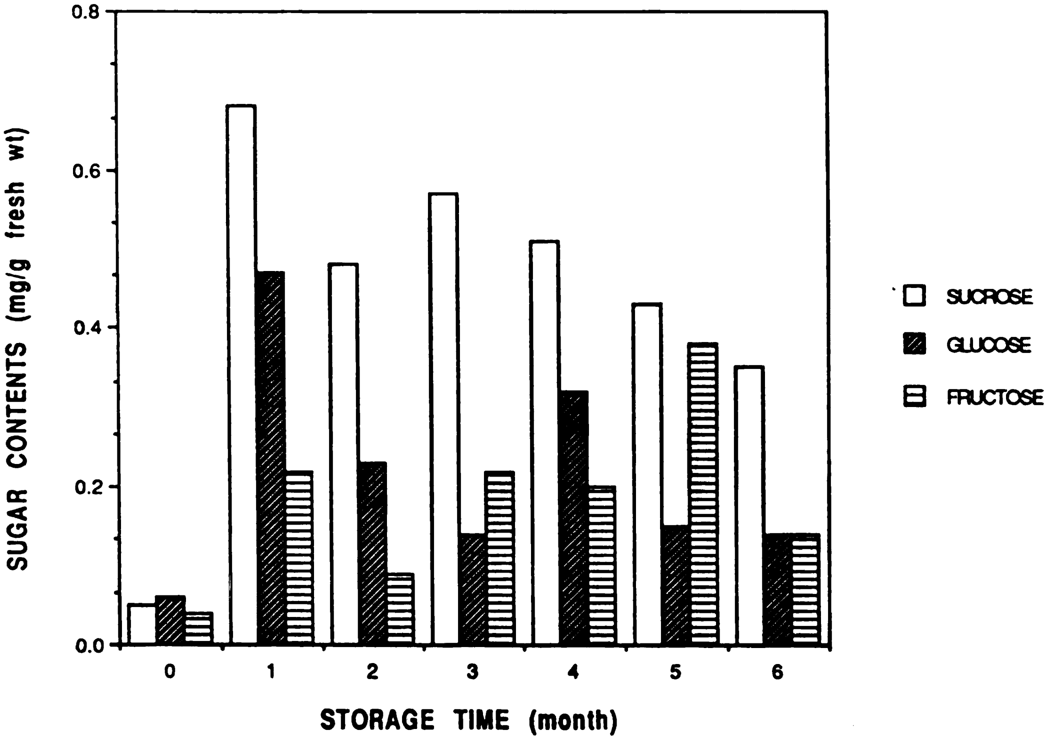


Figure 5. Sugar contant in CIPC treated Atlantic potatoes stored at 10°C.

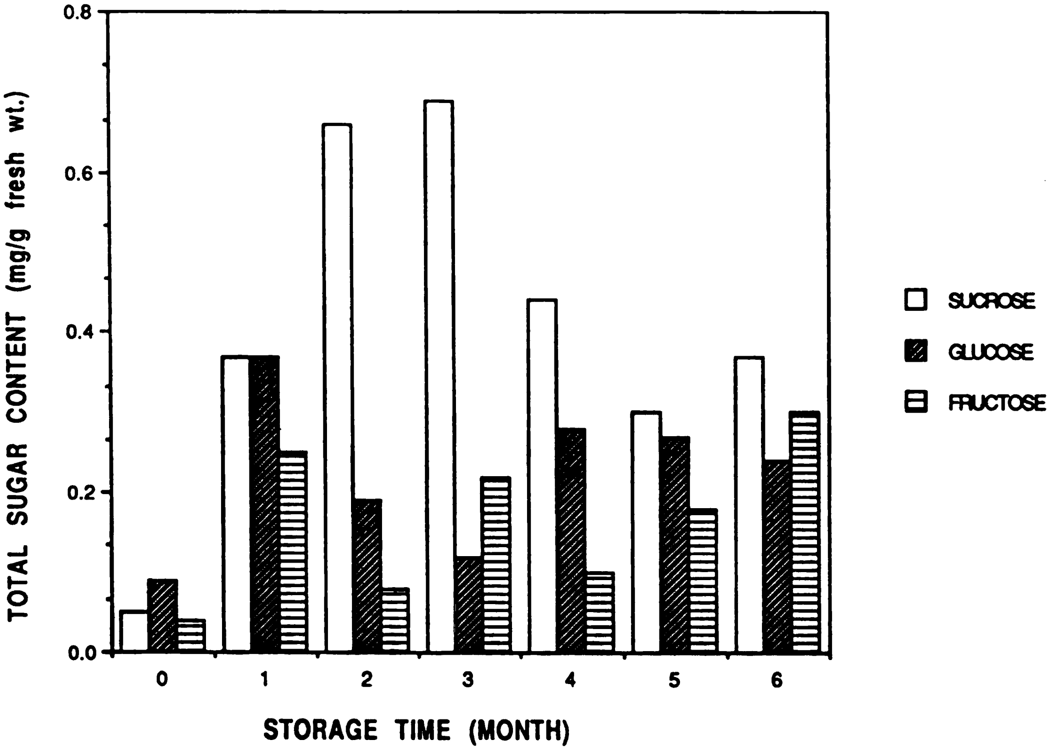


Figure 6. Sugar content in CIPC + MH treated Atlantic potatoes at 10°C.

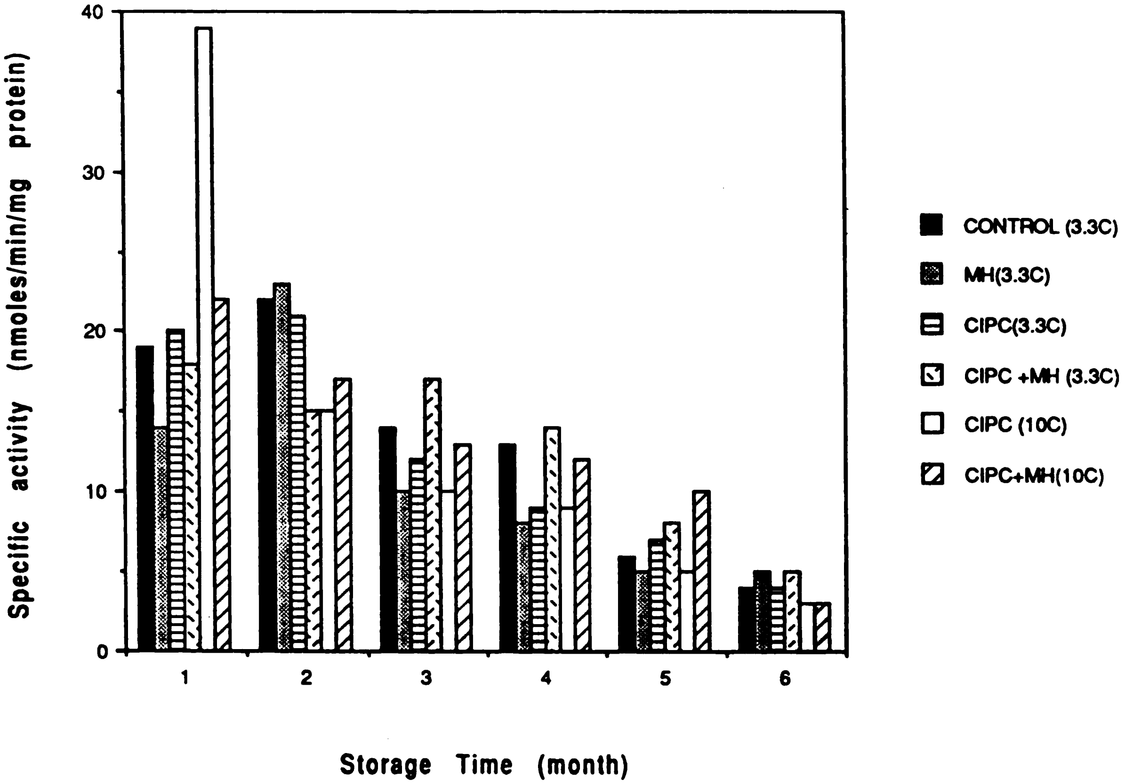


Figure 7. Specific activity of ATP-PFK in Atlantic potatoes.

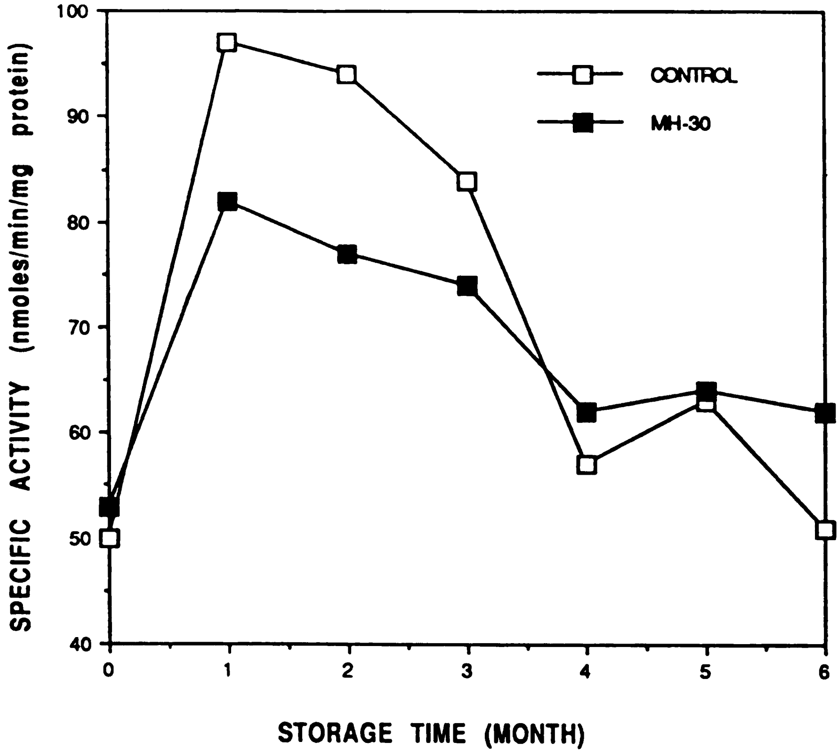


Figure 8. Activity of PPi-PFK in control & MH treated Atlantic potatoes at 3.3^{o}C.

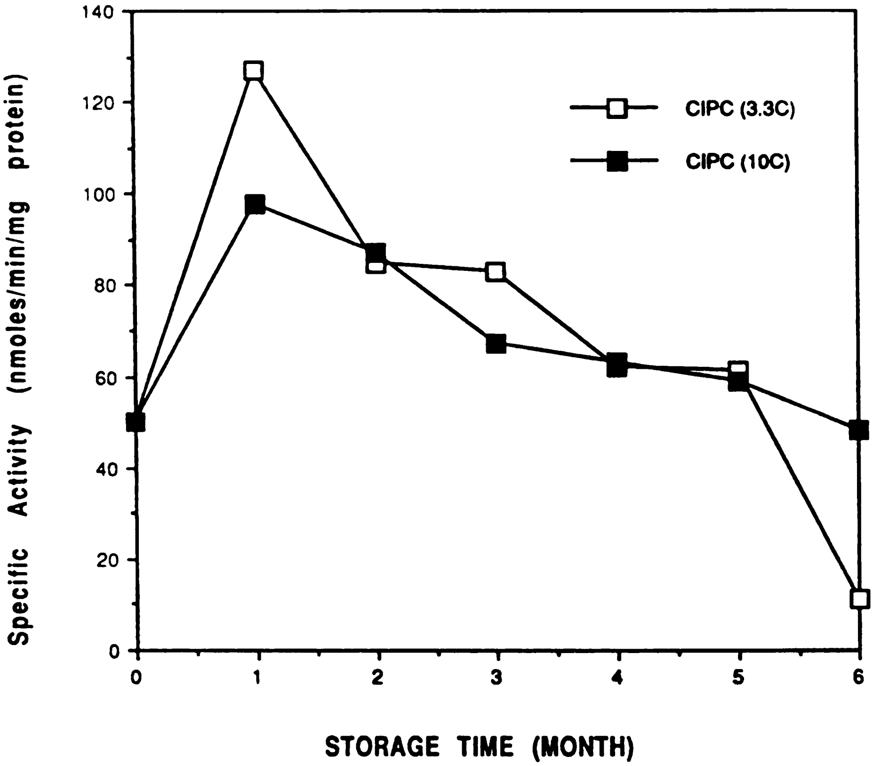


Figure 9. Activity of PPi-PFK in CIPC treated Atlantic potatoes.

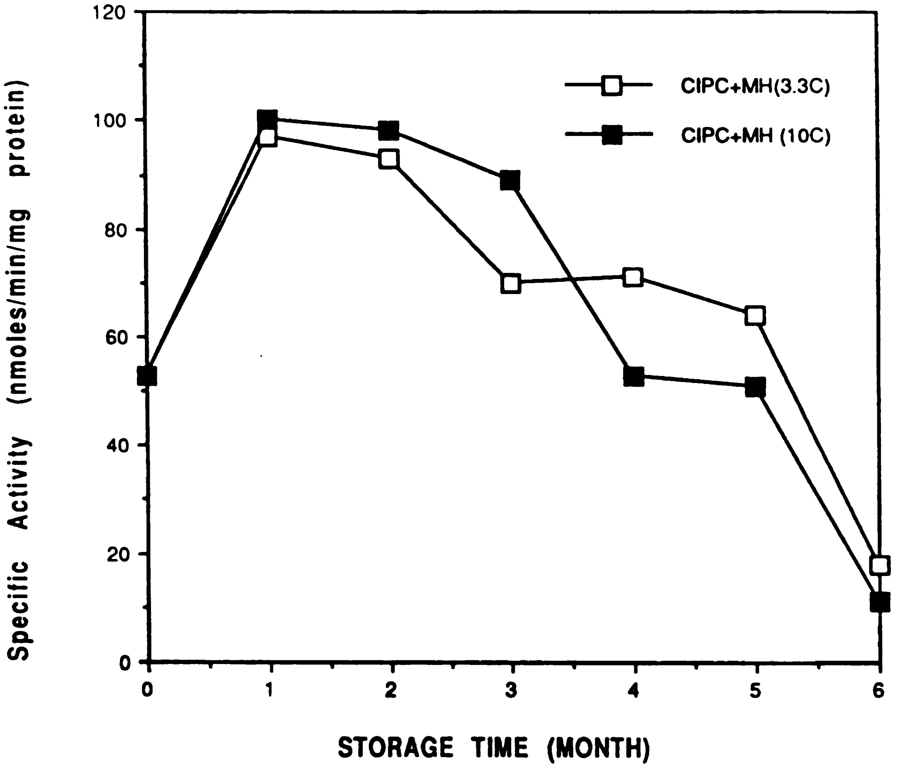


Figure 10. Activity of PPi-PFK in CIPC + MH treated Atlantic potatoes.

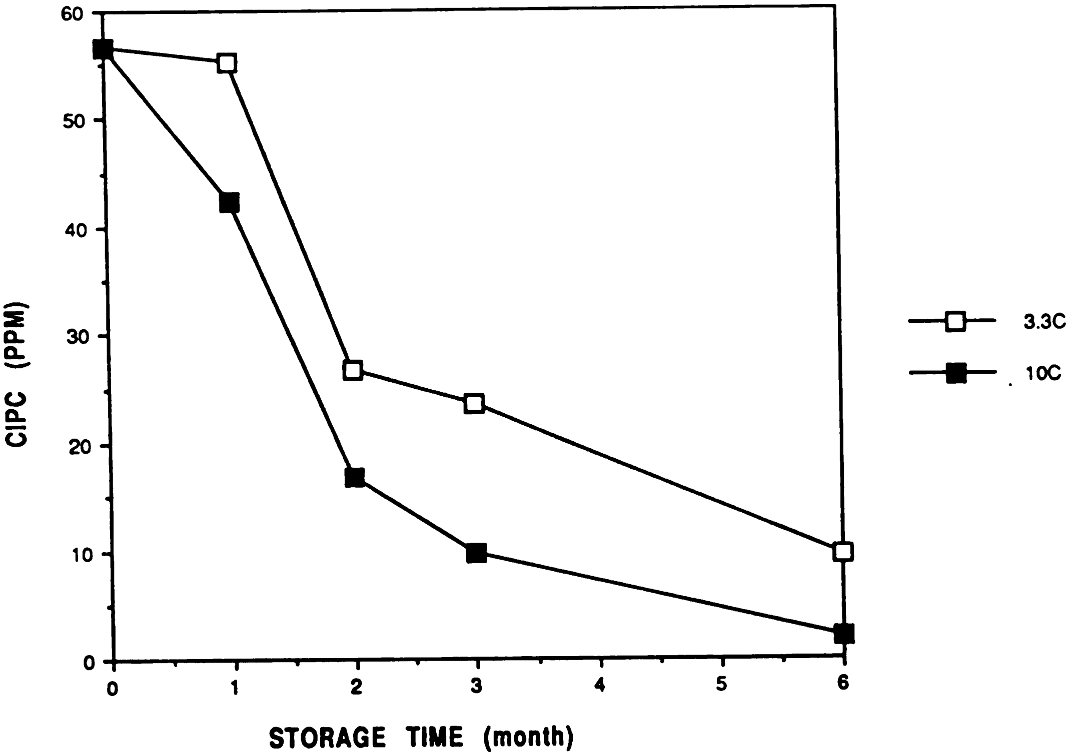
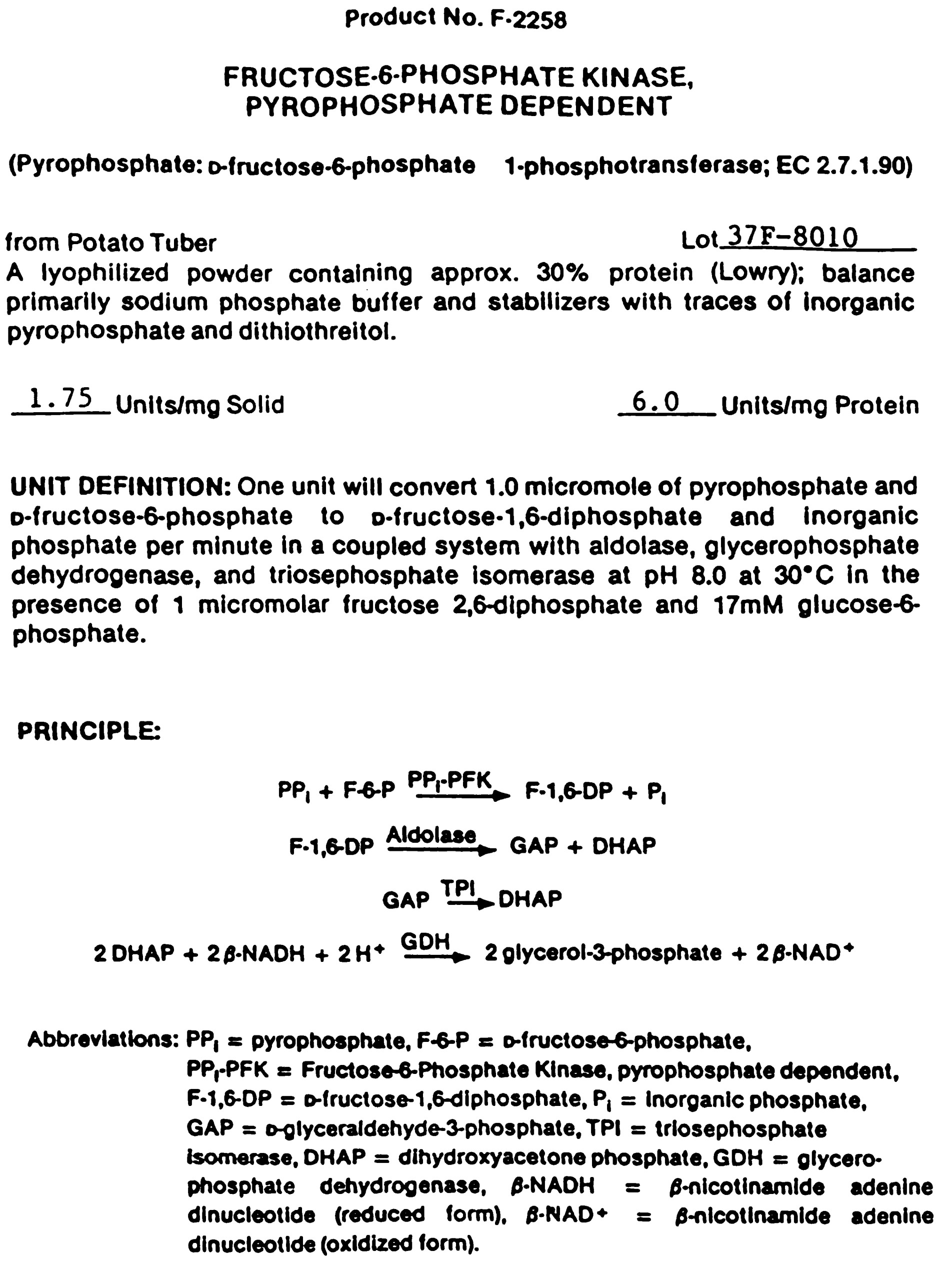


Figure 11. CIPC residual analysis in potato peels.

Appendix 1. Procedure for enzyme assay.



Product No. F-2258

FRUCTOSE-6-PHOSPHATE KINASE, PYROPHOSPHATE DEPENDENT

(Pyrophosphate: D-fructose-6-phosphate 1-phosphotransferase; EC 2.7.1.90)

from Potato Tuber Lot 37F-8010

A lyophilized powder containing approx. 30% protein (Lowry); balance primarily sodium phosphate buffer and stabilizers with traces of inorganic pyrophosphate and dithiothreitol.

1.75 Units/mg Solid

6.0 Units/mg Protein

UNIT DEFINITION: One unit will convert 1.0 micromole of pyrophosphate and D-fructose-6-phosphate to D-fructose-1,6-diphosphate and inorganic phosphate per minute in a coupled system with aldolase, glycerophosphate dehydrogenase, and triosephosphate isomerase at pH 8.0 at 30°C in the presence of 1 micromolar fructose 2,6-diphosphate and 17mM glucose-6-phosphate.

PRINCIPLE:

$$\mathrm{PP_i + F\text{-}6\text{-}P \xrightarrow{PP_i\text{-}PFK} F\text{-}1,6\text{-}DP + P_i}$$

$$\mathrm{F\text{-}1,6\text{-}DP \xrightarrow{Aldolase} GAP + DHAP}$$

$$\mathrm{GAP \xrightarrow{TPI} DHAP}$$

$$\mathrm{2\,DHAP + 2\beta\text{-}NADH + 2H^+ \xrightarrow{GDH} 2\,glycerol\text{-}3\text{-}phosphate + 2\beta\text{-}NAD^+}$$

Abbreviations: PP_i = pyrophosphate, F-6-P = D-fructose-6-phosphate, PP_i-PFK = Fructose-6-Phosphate Kinase, pyrophosphate dependent, F-1,6-DP = D-fructose-1,6-diphosphate, P_i = inorganic phosphate, GAP = D-glyceraldehyde-3-phosphate, TPI = triosephosphate isomerase, DHAP = dihydroxyacetone phosphate, GDH = glycerophosphate dehydrogenase, β-NADH = β-nicotinamide adenine dinucleotide (reduced form), β-NAD+ = β-nicotinamide adenine dinucleotide (oxidized form).

Appendix 1 Cont. Procedure for enzyme assay.

Enzyme Activity Assay

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Pipet the following reagents into a quartz or silica cuvet (1 cm light path):

2.00 ml - Reagent A	(75 mM Tris, Prod. No. T-1503, -7.5 mM Magnesium Chloride, Prod. No. M-0250, adjusted to pH 8.0 at 30°C with HCl.)
0.10 ml - Reagent B	(4.3 mM β-NADH, Stock No. 340-110. Reconstitute one vial with 3.0 ml of Reagent A.)
0.10 ml - Reagent C	(Aldolase, Prod. No. A-7145, diluted to 10 units/ml with deionized water. NOTE: ONLY THIS ALDOLASE PRODUCT NUMBER IS SUITABLE.)
0.10 ml - Reagent D	(GDH/TPI Mixed Enzymes, Product. No. G-6755, diluted to 10 GDH units/ml with deionized water.)
0.10 ml - Reagent E	(75 mM D-Fructose-6-Phosphate, Potassium Salt, Prod. No. F-1502, in deionized water.)
0.30 ml - Reagent F	(175 mM D-Glucose-6-Phosphate, Monosodium Salt, Prod. No. G-7879, in deionized water.)
0.10 ml - Reagent G	(30 μM Fructose 2,6-Diphosphate, Prod. No. F-7006, in Reagent A.)
0.10 ml - Reagent H	(PP_i-PFK Enzyme Solution, 0.25 units/ml in deionized water.)

Mix by inversion, equilibrate to 30°C using a suitable thermostated spectrophotometer with air or a cuvet containing H_2O as a reference. Add:

0.10 ml - Reagent J	(75 mM Pyrophosphate, Disodium, Prod. No. P-8135, titrated to pH 8.0 at 30°C.)

Immediately mix and record the ΔA_{340nm} per minute. Correct the ΔA_{340nm} for any blank rate obtained before adding the pyrophosphate.

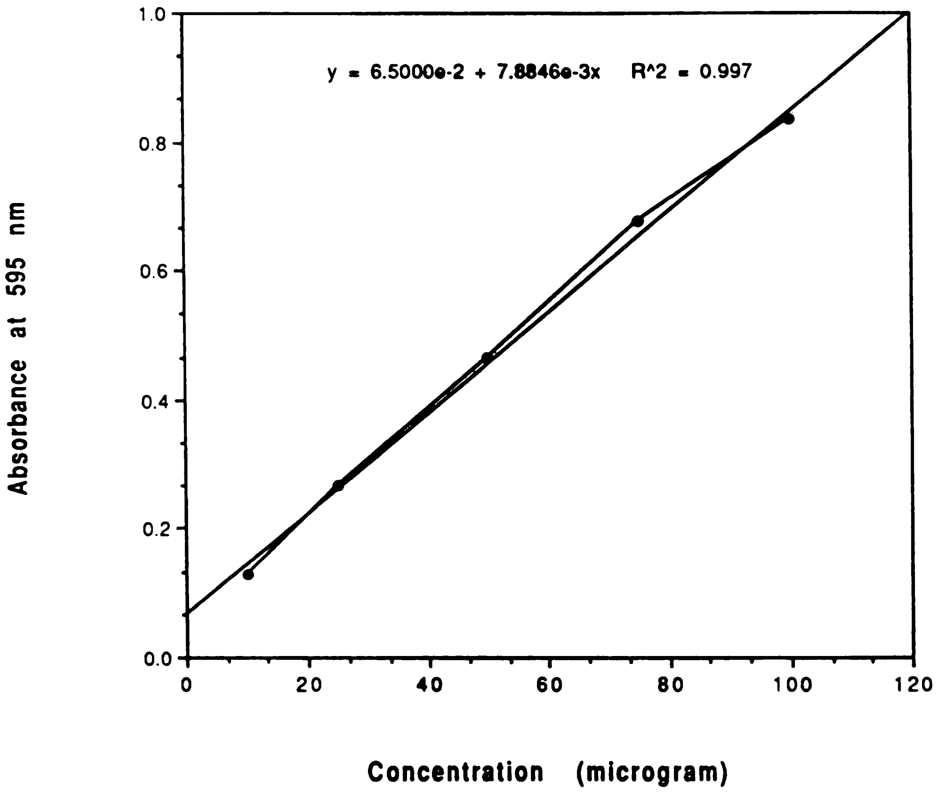
CALCULATION:

$$\text{Units/Cuvet} = \frac{\Delta A_{340\,nm}/\text{minute} \times 3}{6.22 \times 2}$$

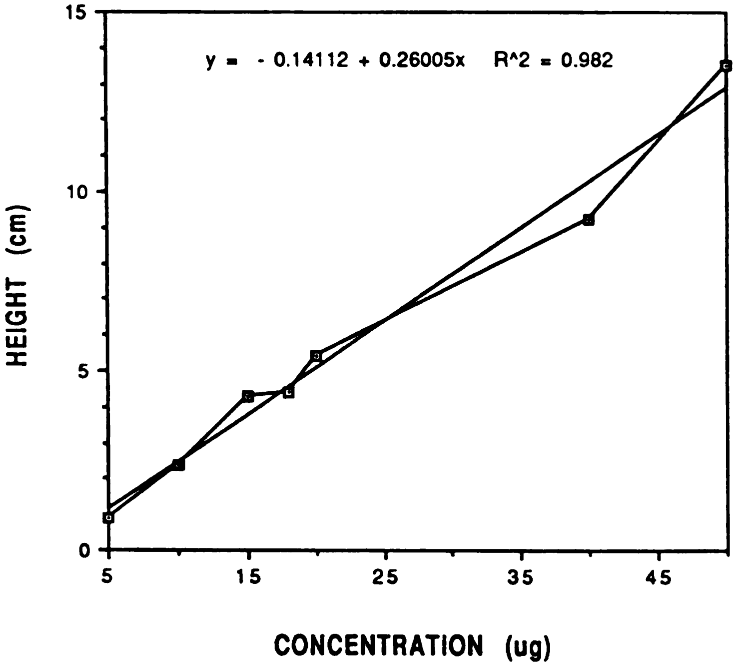
3 = Volume of reaction mix in cuvet in ml

6.22 = Extinction coefficient of β-NADH (2 accounts for the 2 NADH oxidized per 1 PP_i consumed)

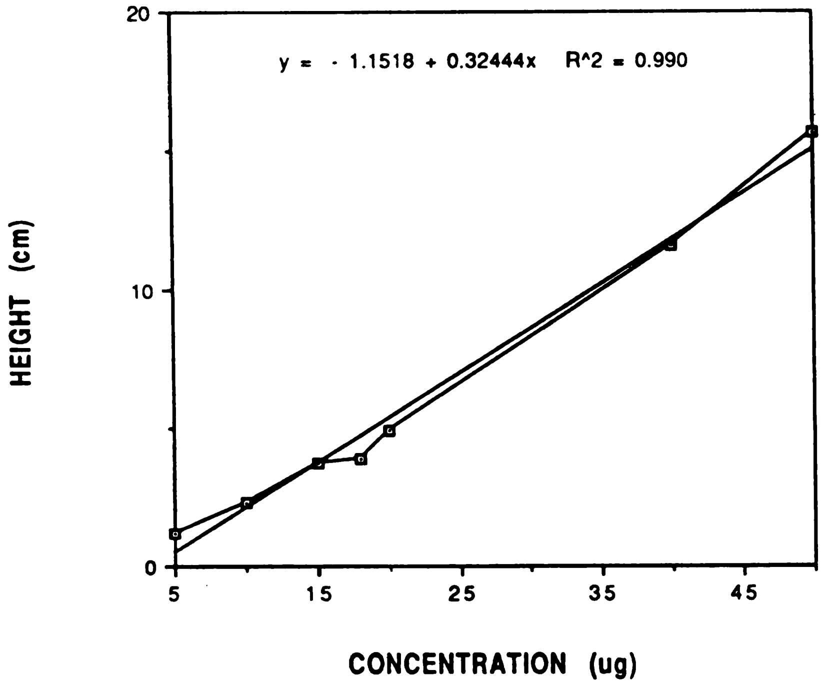
Appendix 2. Standard curve for protein.



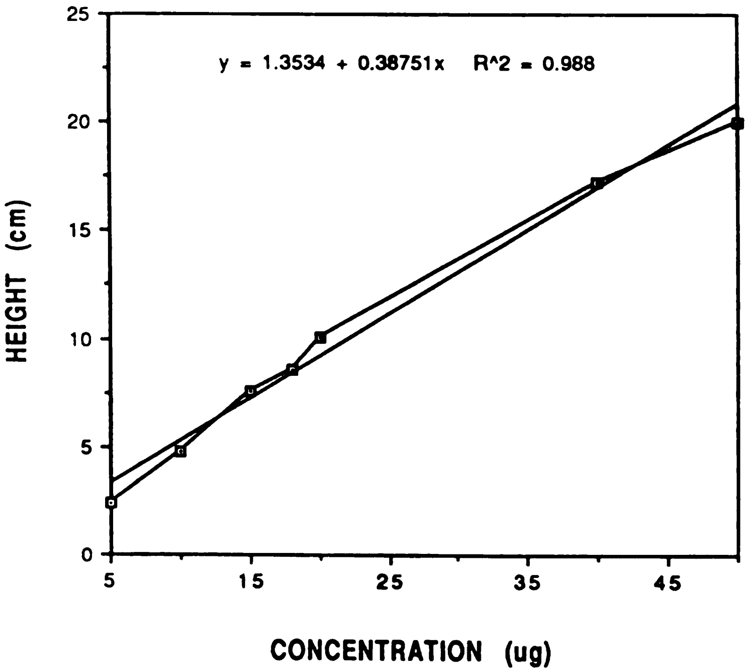
Appendix 3. Standard curve for sucrose.



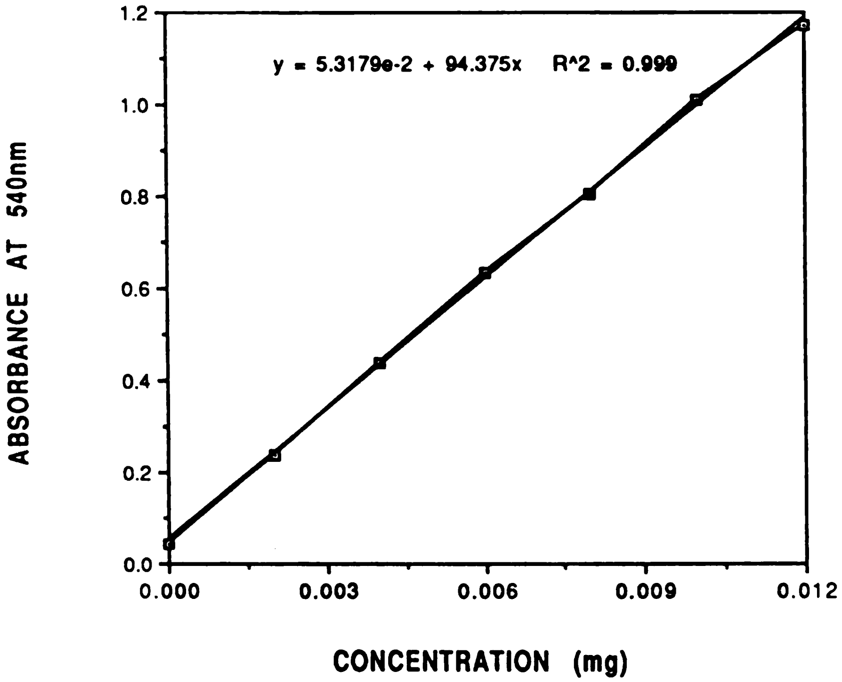
Appendix 4. Standard curve for glucose.



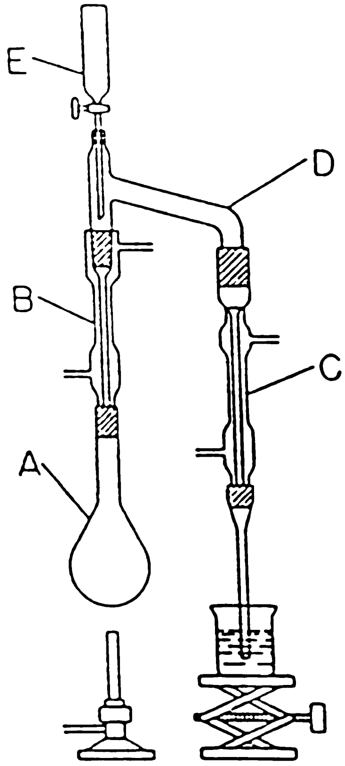
Appendix 5. Standard curve for fructose.



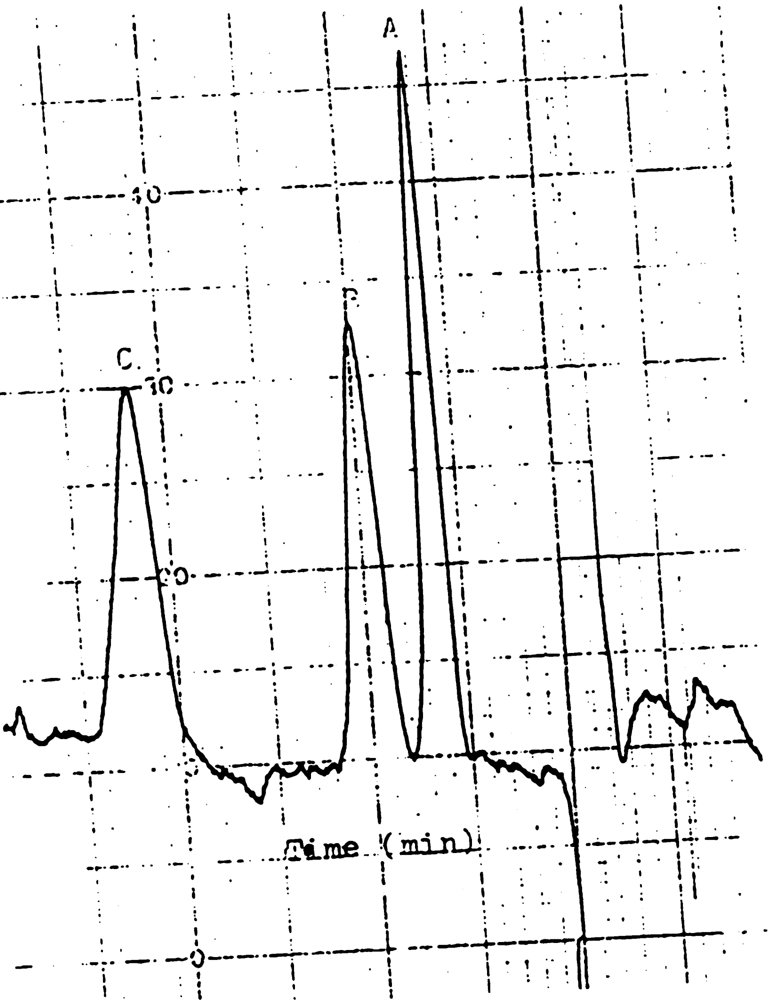
Appendix 6. Standard curve for chloroanaline.



Appendix 7. Hydrolysis and distillation apparatus for CIPC residual analysis.



Appendix 8. Chromatogram of fructose, glucose and sucrose in standard sugar solution.



A = Fructose
B = Glucose
C = Sucrose

Appendix 9. Chromatogram of fructose, glucose and sucrose in Atlantic potatoes stored at 3.3°C.

A = Fructose
B = Glucose
C = Sucrose

Appendix 10. Average protein content in the crude enzyme extracts of Atlantic potatoes stored at 3.3 and $10^{o}C$ for six months.

Treatment	Temperature (^{o}C)	Protein (mg/ml)
Control	3.3	0.96±0.11
Control	10*	0.79±0.01
MH	3.3	0.96±0.11
MH	10*	0.93±0.06
CIPC	3.3	0.85±0.11
CIPC	10	0.85±0.09
CIPC+MH	3.3	0.81±0.12
CIPC+MH	10	0.83±0.11

* Data for two months as the tubers began sprouting.

Appendix 11. Analysis of variance showing the effect of treatment and time of storage at 3.3°C on PPi-PFK and ATP-PFK activities and sugars in Atlantic potatoes.

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-Value	Prob.
Variable 3					
PPi-PFK					
Total	23	13685.96			
Treatment	3	99.46	33.153	0.15	
Time	5	10330.71	2066.142	9.52	.000
Error	15	3255.79	217.053		
Variable 4					
ATP-PFK					
Total	23	879.96			
Treatment	3	17.46	5.819	0.89	
Time	5	764.21	152.842	23.32	.000
Error	15	98.29	6.553		
Variable 5					
Sucrose					
Total	23	10.24			
Treatment	3	0.55	0.183	0.61	
Time	5	5.16	1.032	3.42	.029
Error	15	4.53	0.302		
Variable 6					
Glucose					
Total	23	23.56			
Treatment	3	1.46	0.487	1.05	.399
Time	5	15.14	3.029	6.53	.002
Error	15	6.96	0.464		
Variable 7					
Fructose					
Total	23	21.65			
Treatment	3	0.84	0.281	1.04	.401
Time	5	16.78	3.356	12.48	.000
Error	15	4.03	0.269		

CHAPTER 2

ACTIVITIES OF PPi-PFK AND ATP-PFK IN THREE SELECTED CULTIVARS DURING A SHORT TERM STORAGE AT 3.3 AND 10^{0}C

ABSRACT

This work was aimed at understanding possible effects of the sprout inhibitor CIPC on enzymes ATP-PFK and PPi-PFK, key enzymes regulating entry of hexoses into the glycolytic pathway. Samples of Atlantic, Saginaw Gold and Onaway potatoes from Michigan State University's Montcalm Research Farm were treated with CIPC as a dip after a three week period of wound healing The CIPC treated and untreated tubers were analyzed for the afore mentioned enzymes and glucose after stoage at 3.3 and 10^{o}C were 90% RH for five weeks.

The activity of PPi-PFK was more predominant than ATP-PFK in all varieties. A statistical analysis of the data showed significant differences in PPi-PFK ($p=0.001$) and ATP-PFK ($p=0.008$) activity among the varieties. The glucose content was not significantly correlated with either PPi-PFK ($p=0.051$; $r=-0.253$) or ATP-PFK ($p=1.00$; $r=-0.043$). No significant effects of CIPC or the storage temperatures were observed on activities of these enzymes. However, the activity of PPi-PFK in Onaway (which accumulated more glucose) was lower than in Atlantic and Saginaw Gold.

INTRODUCTION

As with most living organisms, carbohydrates are the principal energy source in stored potato tubers, and the main pathway of carbohydrate degradation is glycolysis where glucose is converted to pyruvate with the concommitant product of ATP. In the previous study (chapter 1), an attempt was made to determine if sprout inhibitors CIPC and MH effect sugar metabolism in stored Atlantic potatoes via their effect on enzymes-ATP-PFK and PPi-PFK, which regulate the entry of hexose mono phosphates into glycolysis (Dixon & ApRees, 1980; Morrell & ApRees, 1986). At the conclusion of the previous study, it was realized that since sugar formation is a varietal characteristic and even within a variety, sugar content varies (Agle & Woodbury, 1968; Nelson and Sowokinos, 1983), a study of these enzymes in other varieties would provide more information about whether the primary site of action of CIPC is the single metabolic reaction controlled by these enzymes.

In the present study, activities of ATP-PFK & PPi-PFK, along with changes in glucose were monitored in Atlantic, Saginaw Gold & Onaway potatoes harvested in 1989. Saginaw Gold is a yellow flesh cultivar with mid-season maturity which produces acceptable chip color. Onaway on the other hand, is an early maturity, nonchipping, table use potato.

The aim of this study was to compare the differences in activiteis of the above two enzymes in different cultivars and to more fully understand their role in reducing sugar accumulation in stored potatoes.

MATERIALS AND METHODS

POTATOES

Atlantic, Onaway and Saginaw Gold potatoes were grown on a sandy loam soil with the same production management inputs at the Michigan State University's Montcalm Research Farm and harvested at maturity. Approximately 30 lbs of each variety were placed in mesh bags and held at 16°C for three weeks for suberizatin before CIPC was applied as a dip. Subsquently, the control and the CIPC treated samples were stored at 3.3 and 10°C with 90% humidity.

SUGAR ANALYSIS

A) Sample Preparation and Analysis

The preparation of sample for glucose analysis was according to Sowokinos and Preston (1988). A 200 g sample of fresh potato tissue obtained from center cuts of 4-5 randomly selected, uniform sized tubers was extracted using an Acme juicerator (Model #6001 Acme juicer Mfg, Co., Sierra Madre, CA). Following extraction, three 100 ml volumes of cold distilled water were passed through the juicerator, allowing one to two minutes between each wash. The potato extract was made to 430 ml with cold distilled water and the diluted potato extract was held at refrigerator temperature for one hour to allow for mutarotation of all glucose to the D-glucose. A 10 ml portion of the extract was placed in a vial and used for analysis. An automatic sampling device measured 0.5 ml potato extract in the glucose analyzer (YSI-2000, glucose and Llactate analyzer, Yellow Springs Instrument Co., Inc., Yellow Springs, OH). All samples were analyzed in duplicate. The glucose analyzer uses combination of mono and dibasic sodium phosphate buffer (0.47 M, pH 8.0) and calibrates automatically with a standard glucose solution at the beginning of analysis and after every five samples.

Extraction and Assay of Enzymes PPi-PFK and ATP-PFK

The extraction and assay of these two enzymes were the same as described in Chapter 1.

Calculation

Glucose content (mg/g fresh weight) =

$$\frac{\text{YSI glucose reading in g/liter} \times 430\ \text{ml}}{200\ \text{g}}$$

RESULTS AND DISCUSSION

Effect of CIPC and temperature on:

Glucose

Changes in glucose were determined because this sugar can provide a fairly precise estimate of the chipping quality of tubers (Mazza, 1983; Shallenberger et al., 1959). An increase in glucose in the control as well as CIPC treated tubers from all three varieties was observed at 3.3°C (Figs. 1, 2 and 3). At 10°C, glucose contents of Atlantic and Saginaw Gold potatoes were almost similar; Onaway had high glucose content at harvest and remained higher than maximum values (0.35 mg/g fresh wt.) recommended for chip making (Sowokinos and Preston, 1988). This variety showed maximum glucose accumulation at the end of five week period. Chase et al., (1988) reported unacceptable agtron chip color score in chips made from this variety upon harvest.

Enzyme PPi-PFK

Figs. 4, 5 and 6 show the activity of PPi-PFK in Atlantic, Saginaw Gold and Onaway potatoes sstored at 3.3 and 10°C for five weeks. There is a slight decline in activity of this enzyme from the third week onwards at both the temperatures. PPi-PFK activity in Onaway was similar to the other two varieties in the first two weeks but showed sharp decreases later on. Statistical analysis of the data showed significant differences ($p=.001$) in PPi-PFK activity among varieties and over time. The glucose content is not significantly correlated

$(p=0.051)$ with PPi-PFK activity $(r=-0.253)$ (Appendices 1 and 2). However, the negative correlation indicates an inverse relationship between this enzyme and glucose accumulation during storage, i.e. a high PPi-PFK activity is associated with low glucose content and vice-versa. This is important as it supports the view that this enzyme may have a role in mediating the entry of hexoses into glycolytic pathway. A loss or reduction in its activity may cause accumulation of sugars as they do not get converted via glycolysis. Earlier, Dixon and ApRees (1980), proposed that lowering the temperature could restrict glycolysis to a greater extent than other reactions that utilize hexose phosphates.

Table 1, gives the average specific activities of PPi-PFK, ATP-PFK and glucose content over five weeks storage at 3.3 and $10^{O}C$. Onaway, a non chipping cultivar relatively low PPi-PFK activity and high glucose content when compared with Atlantic and Saginaw Gold.

ATP-PFK

The activity of ATP-PFK in Atlantic, Saginaw Gold and Onaway is shown in Figs. 7, 8 and 9, respectively. As has been observed in the previous study, ATP-PFK activity is 8-10 fold lower than PPi-PFK activity measured in the tubers. Carnal and Black (1983) reported PPi-PFK activity greater than ATP-PFK in many plant tissues. Schaftingen et al., (1982) reported 4 times as PPi-PFK than ATP-PFK in potato homogenates.

Statistical analysis of the data shows no significant correlation $(p=1.0)$ between glucose content and the activity of this enzyme $(r=-0.043)$. However, there is a significant difference in the activity of this enzyme among varieties ($p=.008$) and over temperature of storage ($p=.05$) (Appendices 2 and 3).

CONCLUSION

In this study, glucose and the enzymes PPi-PFK and ATP-PFK were monitored in Atlantic, Saginaw Gold and Onaway potatoes stored for five weeks at 3.3 and 10^{o}C. A gradual increase in glucose in all the varieties was observed at 3.3^{o} and as in the previous study, PPi-PFK was the predominant enzyme in all the varieties. A statistically significant difference in PPi-PFK and ATP-PFK activities among the varieties was obtained, however, the glucose content was not signficantly correlated with either PPi-PFK ($p=0.051$); $r=-0.253$) or ATP-PFK ($p=1.0$; $r=-0.043$).

REFERENCES

Agle, W.M. and Woodbury, GW. 1968. Specific gravity-dry matter relationship and reducing sugar changes affected by potato variety, production area and storage. Am. Potato J. 45:119-131.

Carnal, N.W. and Black, C.C. 1983. PFK activities in photosynthetic organisms. Plant Physiology. 71:150-155.

Chase, R.W., Silva, GH., Douches, D., Kitchen, R.B., Leep, R.H. and Hammerschmidt, R. 1988. Potato variety evaluations. Michigan Potato Reearch Report. 20:5-36. Michigan State University Agricultural Experiment Station.

Dixon, W.L. and ApRees, T. 1980. Carbohydrate metabolism during cold-induced sweetening of potato tubers. Phytochemistry. 19:1653-1656.

Mazza, G 1983. A comparison of methods for sugars in potatoes. Can. Inst. Food Tech. 16:234.

Morrell, S. and ApRees, T. 1986. Sugar metabolism in developing tubers of Solanum tuberosum. Phytochemistry. 25:1579-1585.

Nelson, D.C. and Sowokinos, J.R. 1983. Yield and relationship among tuber size, sucrose and chip color in six potato cultivars on various harvest dates. Am. Potato J. 60:949-958.

Schaftingen, V.E., Lederer, B., Bartrons, R. and Hers, H.G 1982. A kinetic study of pyrophosphate:F 6-P phosphofructotransferases from potato tubers. Eur. J. Biochem. 129:191-195.

Shallenberger, R.S., Smith, O. and Treadway, R.H. 1959. Role of sugars in the browning reaction. J. Agr. Food Chem. 7:274-277.

Sowokinos, J.R. and Preston, D.A. 1988. Maintenance of potato processing quality by chemical maturiety monitoring Station Bulletin 686-1988 (Item No. AD-SB-3441), Minnesota Agricultural Experiment Station.

Table 1. Activity of PPi-PFK, ATP-PFK and Glucose Content in Three Selected Potato Varieties During Storage at 3.3 and 10^{o}C.

Variety	Storage Temp. (^{o}C)	Glucose (mg/g)	PPi-PFK (nmoles/min/mg protein)	ATP-PFK (nmoles/min/mg protein)	Protein Content in the Crude Enzyme Extract mg/ml
I. Atlantic					
1 Control	3.3	2.17±0.60	91.35±19.15	9.28±5.31	0.98±0.06
2 Control	10	0.24±0.06	85.41±34.80	8.00±4.40	1.04±0.09
3 CIPC	3.3	2.13±0.61	87.40±27.56	8.57±4.64	1.01±0.22
4 CIPC	10	0.20±0.04	84.33±22.72	5.67±3.84	1.00±0.07
II. Saginaw Gold					
1 Control	3.3	2.00±0.57	95.95±17.35	8.62±4.80	0.97±0.42
2 Control	10	0.31±0.04	95.07±13.83	9.39±4.57	1.01±0.08
3 CIPC	3.3	2.58±0.53	90.10±13.02	10.71±10.03	0.96±0.08
4 CIPC	10	0.29±0.62	97.47±15.76	8.84±3.67	1.02±0.14
III. Onaway					
1 Control	3.3	2.76±0.50	71.27±64.22	10.00±6.49	0.63±0.07
2 Control	10	1.36±0.15	64.65±45.78	8.17±4.31	0.67±0.07
3 CIPC	3.3	3.03±0.44	71.57±63.17	9.81±4.62	0.54±0.09
4 CIPC	10	1.48±0.03	60.81±35.48	9.34±5.60	0.68±0.17

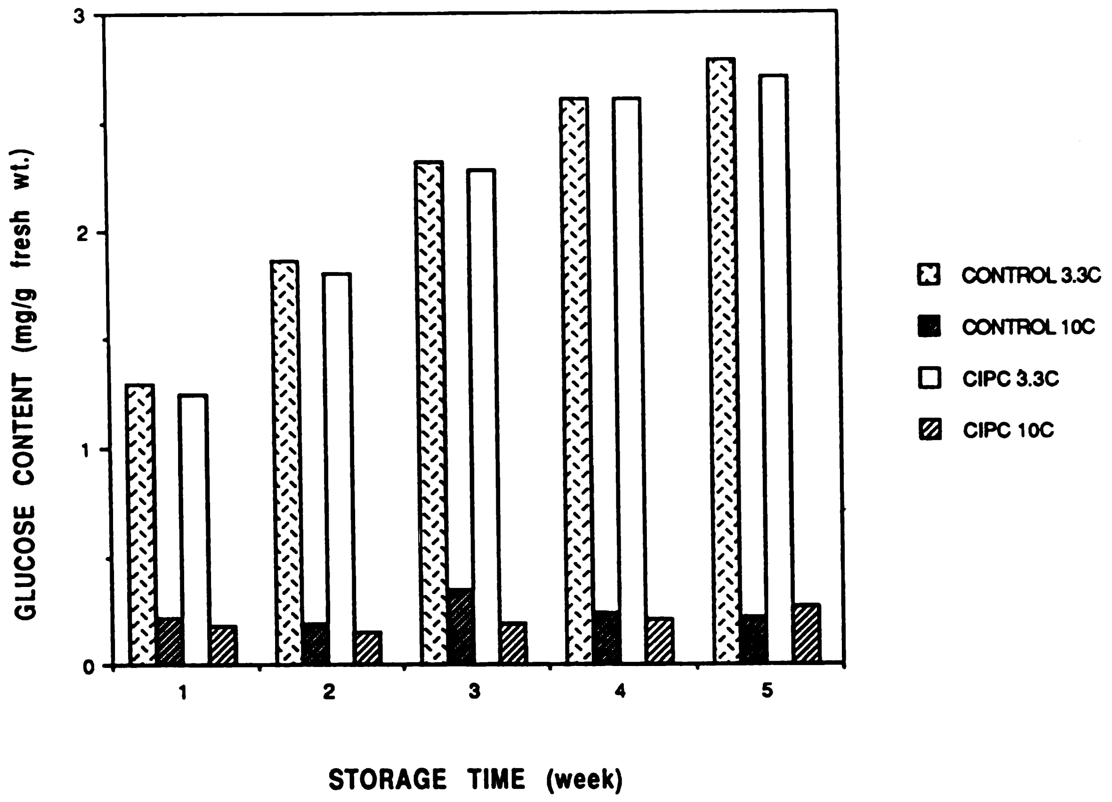


Figure 1. Glucose content in Atlantic potatoes.

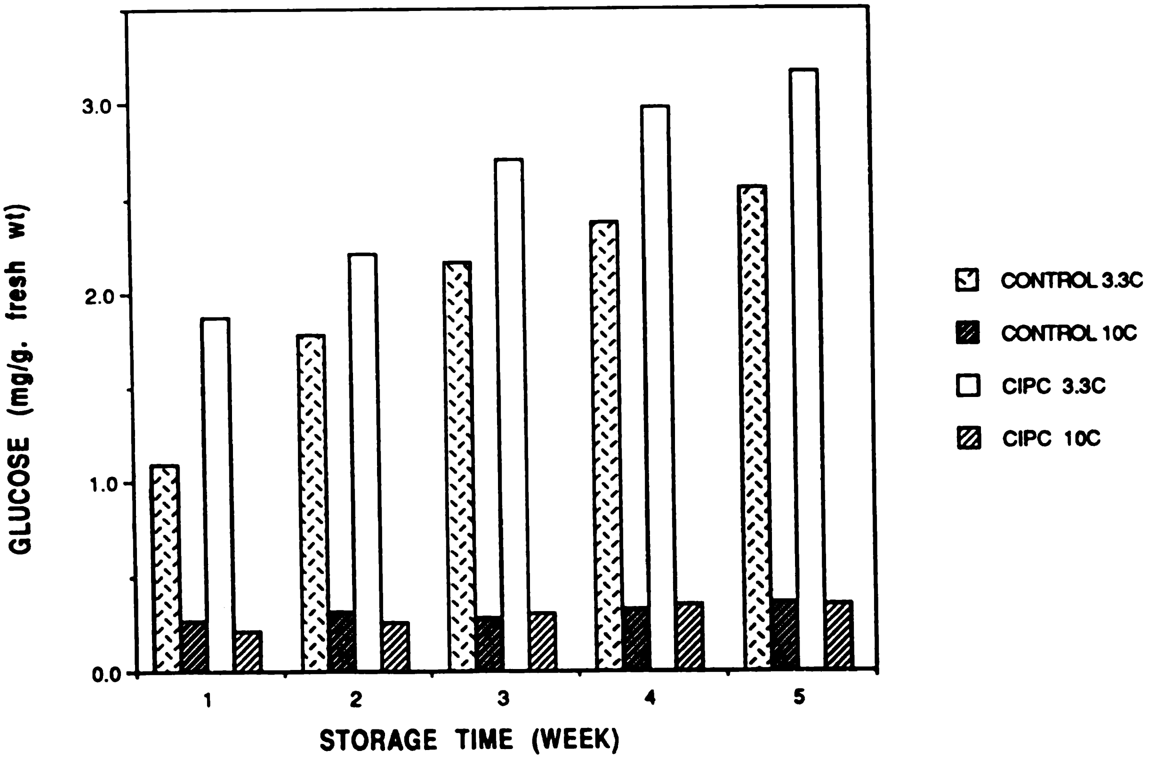


Figure 2. Glucose content in Saginaw Gold potatoes.

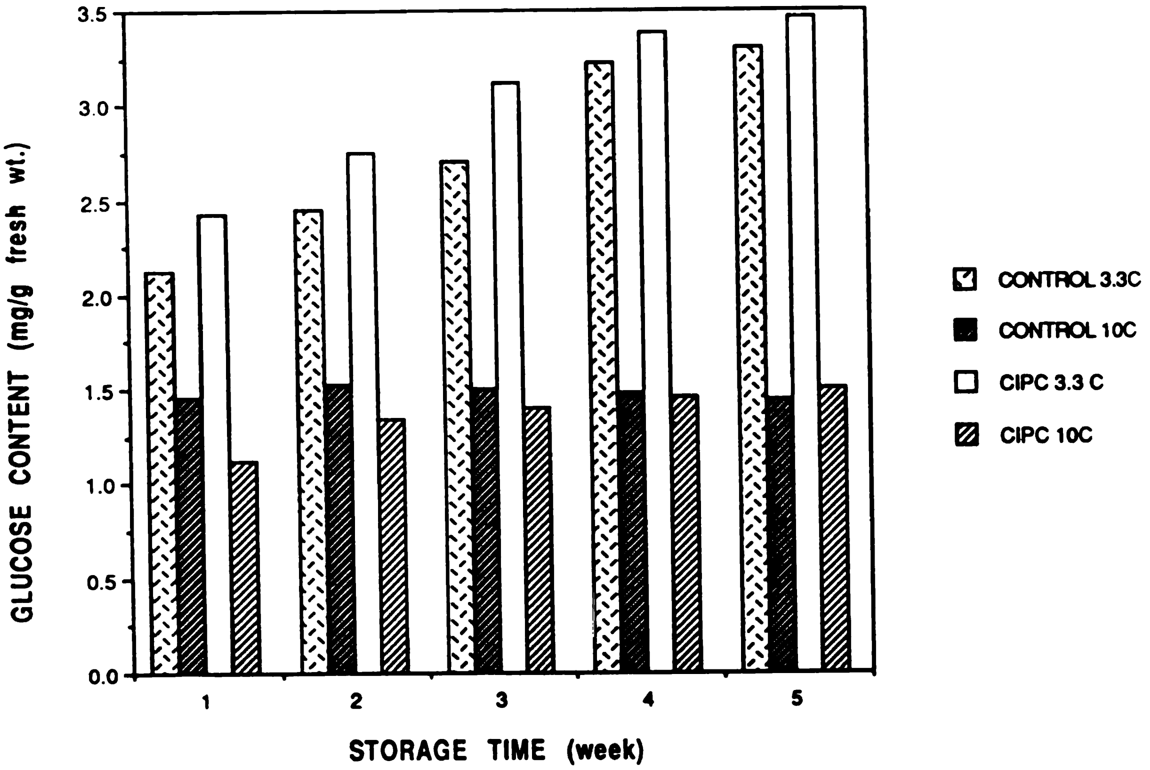


Figure 3. Glucose content in Onaway potatoes.

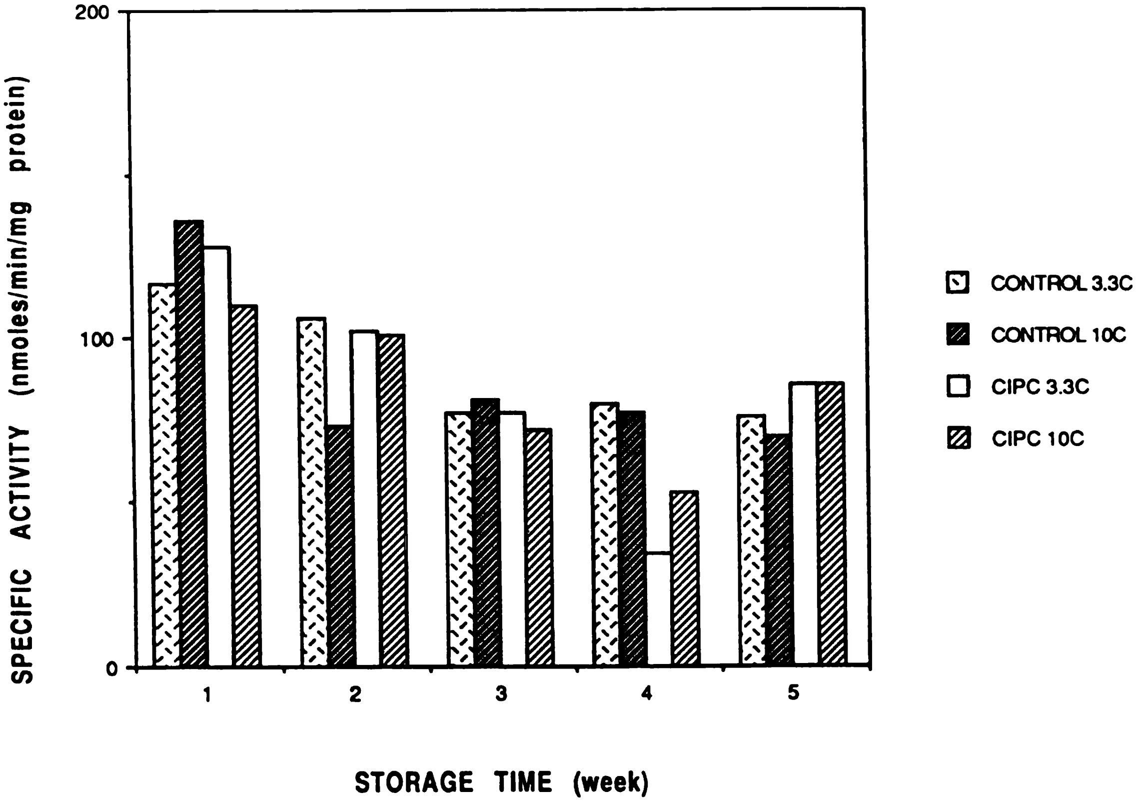


Figure 4. Activity of PPi-PFK in Atlantic potatoes.

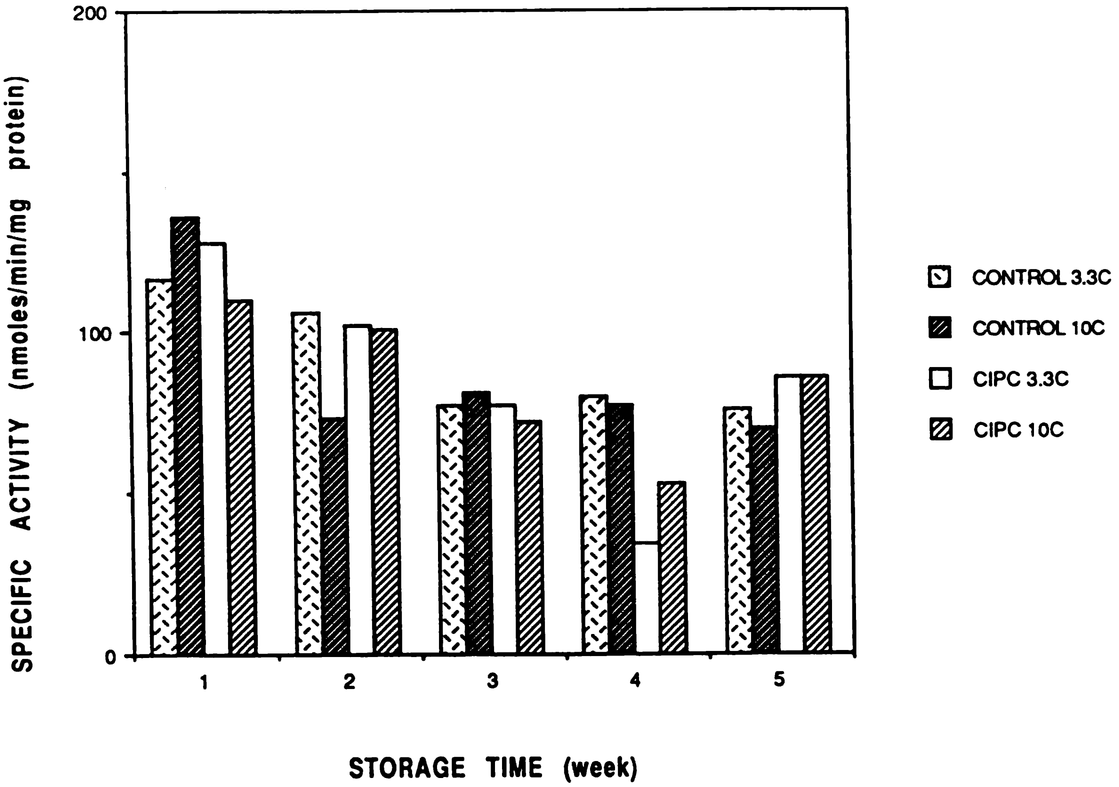


Figure 4. Activity of PPi-PFK in Atlantic potatoes.

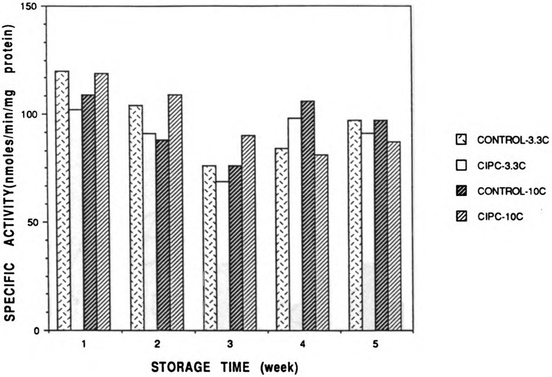


Figure 5. Activity of PPi-PFK in Saginaw Gold potatoes.

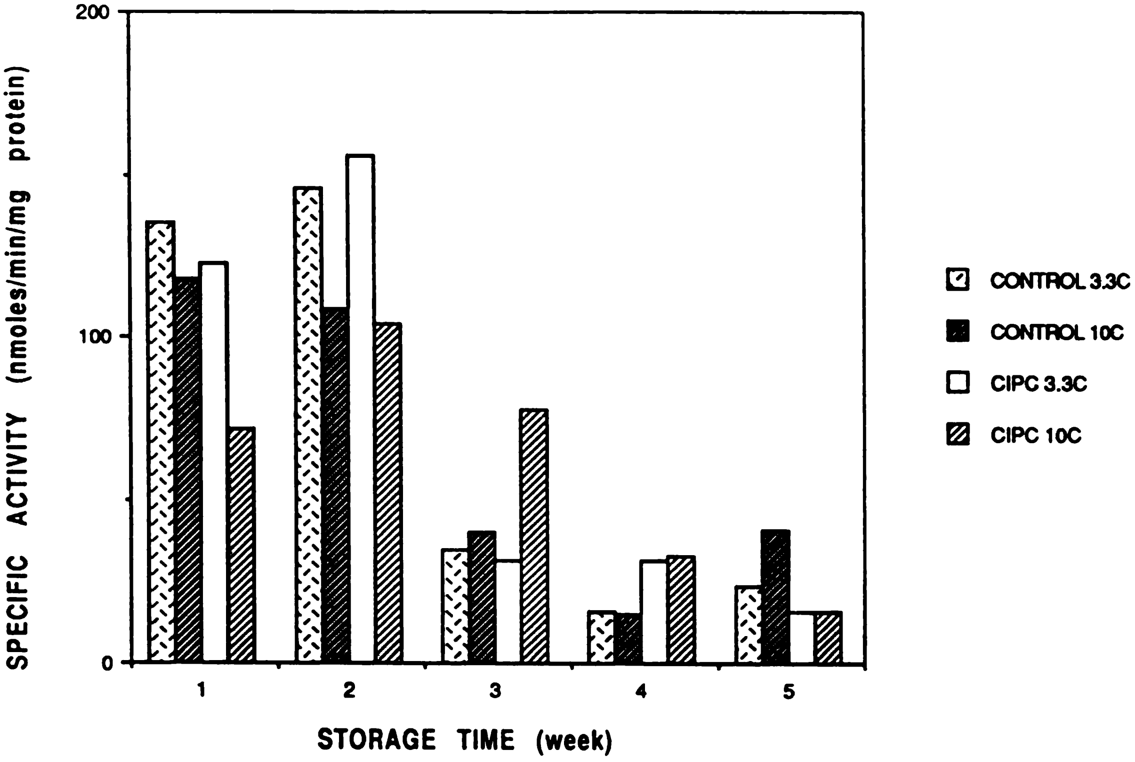


Figure 6. Activity of PPi-PFK in Onaway potatoes.

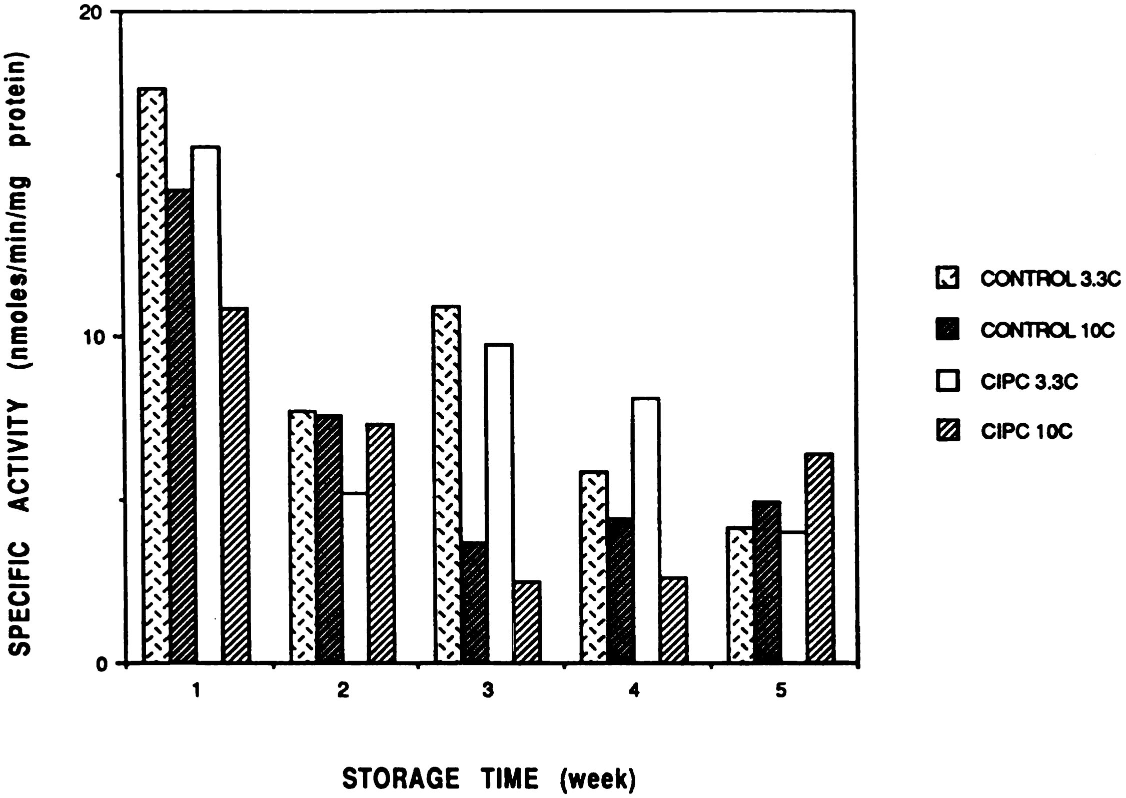


Figure 7. Activity of ATP-PFK in Atlantic potatoes.

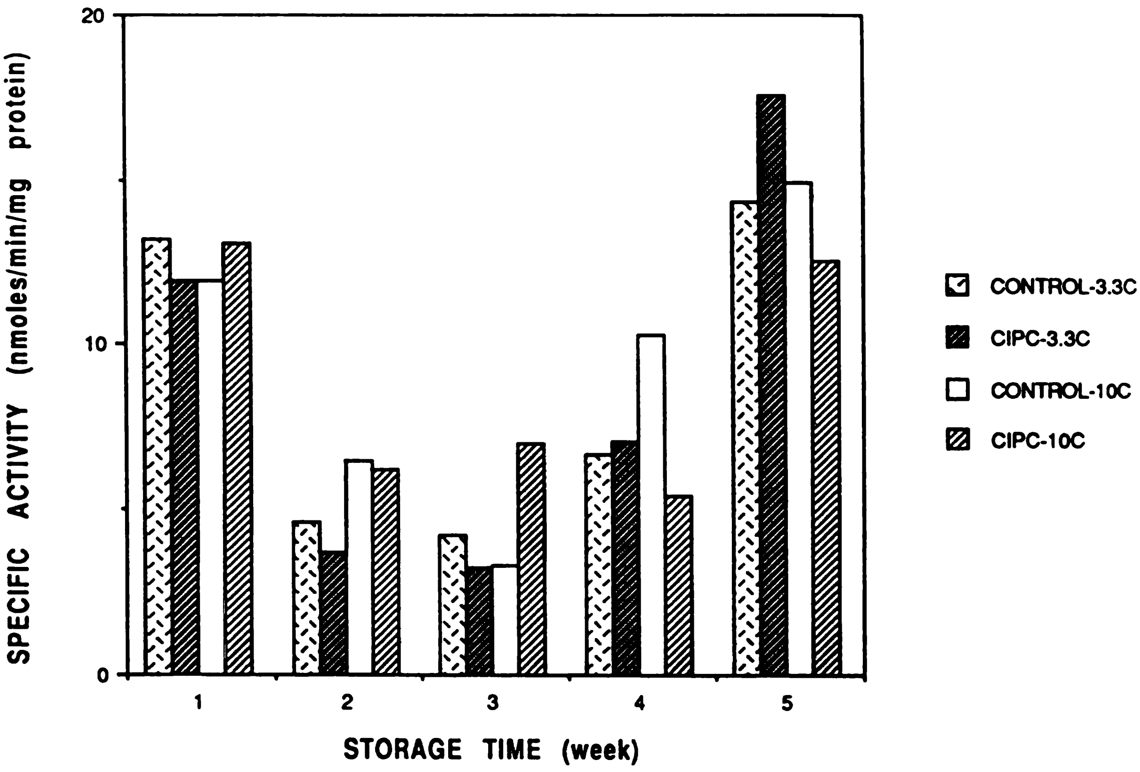


Figure 8. Activity of ATP-PFK in Saginaw Gold potatoes.

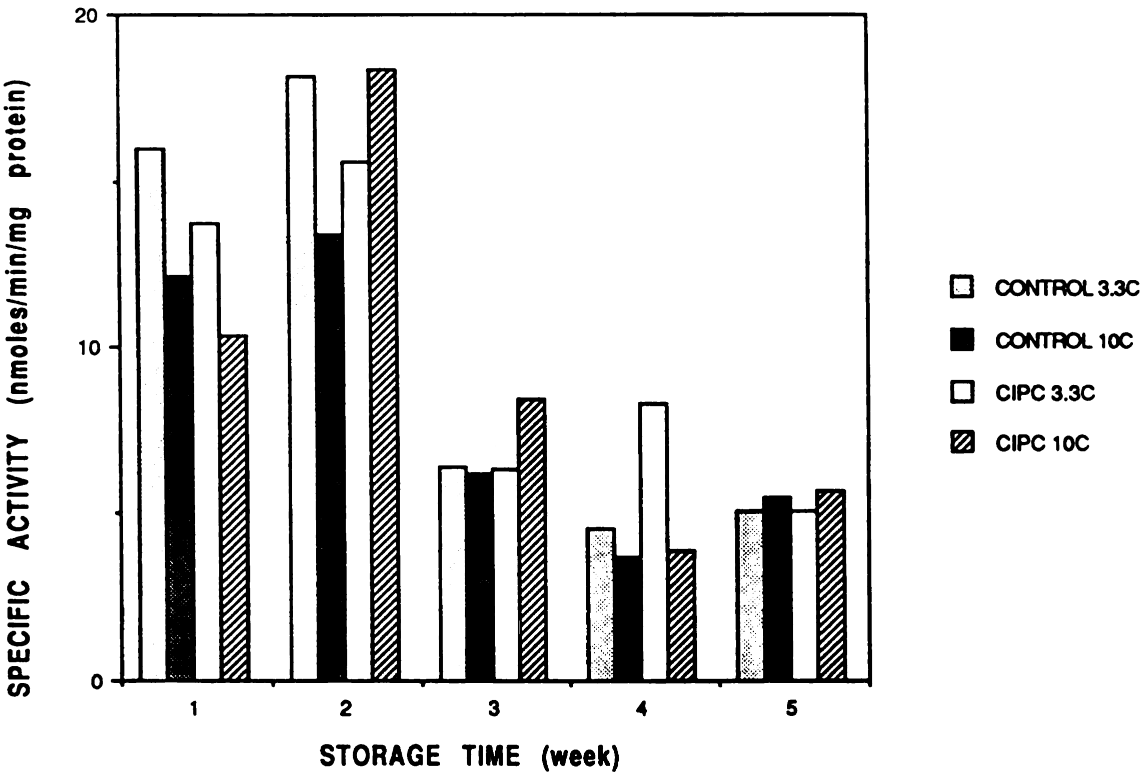


Figure 9. Activity of ATP-PFK on Onaway potatoes.

Appendix 1. Analysis of variance showing effect of variety, time and temperature on PPi-PFK activity in potatoes.

```
29.   User supplied K values


     A N A L Y S I S    O F    V A R I A N C E    T A B L E

                      Degrees of      Sum of                         F
Code    Source          Freedom      Squares     Mean Square      Value        Prob
-----------------------------------------------------------------------------------
   1   Variety                2      8161.90        4080.950      17.90        .001
   2   Time                   4     33632.93        8408.233      36.88        .000
   3   Variety x Time         8     17393.77        2174.221       9.54        .002
   4   Temperature            1       236.02         236.017       1.04        .338
   5   Variety x Temperature  2       266.63         133.317       0.58
   6                          4      1593.07         398.267       1.75        .232
   7                          8      3532.03         441.504       1.94        .184
   8                          1        33.75          33.750       0.15
   9                          2        10.30           5.150       0.02
  10                          4       999.33         249.833       1.10        .420
  11                          8      1148.37         143.546       0.63
  12                          1        16.02          16.017       0.07
  13                          2       100.23          50.117       0.22
  14                          4       626.40         156.600       0.69
 -15    Error                 8      1824.10         228.013

  Coefficient of Variation=     18.20%
```

Appendix 2. Correlation between enzymes and glucose content in potato varieties stored at 3.3°C and 10°C.

```
Function: CORR
Data case no.  1  to  60
Without selection


 ppi-pfk
Variable   5 Average =       82.95 Variance =      1179.23
 glucose
Variable   7 Average =        1.55 Variance =         1.17

Number =  60
Covariance =        -9.39 Correlation =   -0.253
Intercept  =      2.21          Slope =  -0.008
Standard Error of the Slope =   0.004
Standard Error of the Estimate =   1.055
Student's T value      1.991 Probability =    .051

 pfk'
Variable   6 Average =        8.48 Variance =        19.53
 glucose
Variable   7 Average =        1.55 Variance =         1.17

Number =  60
Covariance =        -0.20 Correlation =   -0.043
Intercept  =      1.63          Slope =  -0.010
Standard Error of the Slope =   0.032
Standard Error of the Estimate =   1.089
Student's T value      0.325 Probability = 1.0
```

Appendix 3. Analysis of variance describing effect of variety, time and temperature on ATP-PFK activity.

```
User supplied K values(15)


   A N A L Y S I S     O F     V A R I A N C E     T A B L E

                         Degrees of      Sum of                        F
Code    Source             Freedom      Squares    Mean Square    Value       Prob
-------------------------------------------------------------------------------------
   1 Variety                   2          26.67          13.335    9.32       .008
   2 Time                      4         470.77         117.692   82.30       .000
   3 Variety x Time            8         438.37          54.797   38.32       .000
   4 Temperature               1           7.59           7.590    5.31       .050
   5 Variety x Temperature     2          39.53          19.767   13.82       .002
   6                           4          37.95           9.487    6.63       .011
   7                           8          36.63           4.578    3.20       .060
   8                           1           4.44           4.439    3.10       .116
   9                           2           8.36           4.182    2.92       .111
  10                           4           8.78           2.195    1.54       .280
  11                           8          28.84           3.605    2.52       .106
  12                           1           2.23           2.235    1.56       .246
  13                           2           2.51           1.257    0.88
  14                           4          28.09           7.022    4.91       .026
 -15 Error                     8          11.44           1.430

 Coefficient of Variation=    14.11%
```