News & Reviews

A MECHANISM FOR LOW TEMPERATURE INDUCED SUGAR ACCUMULATION IN STORED POTATO TUBERS: THE POTENTIAL ROLE OF THE ALTERNATIVE PATHWAY AND INVERTASE

Patricia M. Duplessis, Alejandro G. Marangoni, and Rickey Y. Yada[1]

Abstract

The accumulation of reducing sugars in stored potato tubers is of significant commercial importance because of its effect on processing quality. The process by which the accumulation of sugars occurs involves the interaction of many metabolic pathways and is yet to be fully described. Low temperature conditions result in an accumulation of ATP in potato tissue. Published evidence suggests that low temperature activation of the alternative pathway (cyanide resistant respiration) leads to decreased ATP levels and simultaneous increases in sucrose concentrations. This sucrose becomes the substrate for vacuolar acid invertase resulting in the accumulation of reducing sugars. Inhibition of the alternative pathway results in decreased sugar accumulation thereby minimizing the sucrose available to the acid invertase and the subsequent reducing sugar accumulation. Control of the alternative pathway on its own, or in combination with acid invertase activity, may provide insight into the phenomenon of low temperature sweetening in stored potato tubers.

Compendio

La acumulación de azúcares reductores en los tubérculos de papa almacenados tiene una importancia comercial significativa por su efecto en la calidad de procesamiento. El proceso por el cual ocurre la acumulación de azúcares involucra la interacción de muchas vías metabólicas y todavía falta describirlo en su totalidad. Las condiciones de baja temperatura dan como resultado la acumulación del ATP en el tejido de papa. Las evidencias publicadas sugieren que la activación de la vía alternativa (respiración resistente al cianuro) por bajas temperaturas disminuye los niveles de ATP y simultáneamente incrementa las concentraciones de sucrosa. Esta sucrosa

[1]Department of Food Science, University of Guelph, Guelph, ON , Canada N1G 2W1.
Accepted for publication September 10, 1996.
ADDITIONAL KEY WORDS: Cyanide resistant respiration, reducing sugars, sucrose, low temperature sweetening.

se convierte en el sustrato de la invertasa ácida vacuolar que origina la acumulación de azúcares reductores. La inhibición de la vía alternativa disminuye la acumulación de los azúcares y de esta manera minimiza la scurosa disponible para la invertasa ácida y la subsecuente acumulación de azúcares reductores. El control de la vía alternativa sola o en combinación con la actividad de la invertasa ácida puede proporcionar información sobre fenómeno de endulzamiento por baja temperatura en los tubérculos de papa almacenados.

Introduction

The potato is ranked fourth in production of all agricultural commodities in the world and produces more dry matter and protein per hectare than the major cereal crops (Horton, 1980). In countries with developed market economies, consumer preference drives the potato industry as evidenced by the expansion of the processing sector. Potato chips and french fries are two of the most popular processed potato products and consumer preference indirectly dictates the cultivars used to derive a satisfactory end product.

Presently, the primary problem associated with potato processing is the non-enzymatic browning of tissue that occurs under the high temperature conditions associated with frying when tissue reducing sugar levels are high (*i.e.*, Maillard reaction). The reducing sugars, glucose and fructose, combine with the α-amino groups of amino acids at the high temperatures used in frying operations and result in a product that is unacceptably dark. An ideal reducing sugar content is generally accepted to be 0.1% of the tuber fresh weight with 0.33% as the upper limit (Davies and Viola, 1992).

Higher reducing sugar content is generally associated with a product displaying a darker fried color and a more bitter flavor. Four main types of sugar accumulation or sweetening have been identified in potato tubers: 1)senescent sweetening resulting from the rapid accumulation of sugars after long term or high temperature storage; 2) sugar increases associated with rapid sprouting; 3) sweetening due to potato tubers being physiologically immature when placed under storage conditions; and 4) low temperature sweetening (LTS) caused by exposure to low temperatures (below 10 C) (Davies and Viola, 1992). The suitability of a cultivar for processing is dependent not only on its quality at harvest but also on its response to storage conditions (Burton and Wilson, 1978).

As food safety and environmental concerns rise, there is an increased need to develop storage regimes that do not involve the use of chemical sprout inhibitors. Chemical sprout inhibition permits the storage of potatoes at the intermediate temperatures (8-12 C) required for acceptable quality in processed products. Low temperature storage is an alternative that has been investigated. Advantages of low temperature storage include natural control of sprout growth, easier maintenance of the high humidity atmosphere

required to minimize evaporative losses and reductions in senescent sweetening and losses due to storage rot (Burton, 1969).

There are many benefits to low temperature storage but the associated accumulation of sugars in most cultivars used for processing overwhelms the benefits as it results in a potato tuber that is unsuitable for processing. Investigations into the causes of LTS have been ongoing since the 1930's and continue as researchers try to elucidate the mechanisms responsible for the phenomenon. With recent advances in potato breeding technology it should be possible to develop a cultivar which can be processed directly from low temperature storage but researchers must first determine which part of the starch-sugar conversion pathway to target. A cultivar with cold chipping ability would result in an improved processed product and would lead to increased industry efficiency. This review will concentrate on the phenomenon of LTS and attempt to explain a potential mechanism by which the process may occur.

The Potential Role of the Alternative Pathway and Invertase in Low Temperature Sweetening.

The production of free sugars in potato tubers is certainly not controlled by a single factor, but by the interaction of several pathways of carbohydrate metabolism including starch synthesis, glycolysis, mitochondrial respiration and gluconeogenesis (Sowokinos, 1994). Starch is the energy and carbon source for the production of sugars induced by stress (Isherwood, 1973). The mechanism(s) responsible for the stress-induced sweetening of potato tissue at the molecular level is still unexplained but the levels of cellular regulation include: 1) hormones; 2) membrane structure and function; 3) compartmentalization and concentration of key ions, substrates, enzymes and other effectors; and 4) enzyme synthesis and/or activity (Sowokinos, 1990).

In addition to the changes in sugar accumulation patterns observed at storage temperatures below 5 C, respiration changes have also been observed (Sherman and Ewing, 1982). Respiration decreases as storage temperature decreases but at storage temperatures below 5 C respiration is stimulated. There is a brief respiratory burst followed by a subsequent decrease in respiration rate to a new steady state (Amir *et al.*, 1977; Isherwood, 1973). The initial respiratory burst has been attributed to the combination of cytochrome-mediated and cyanide-resistant respiration (Sherman and Ewing, 1982).

Purvis and Shewfelt (1993) stated that unstressed tissue possesses biosynthetic defense and repair mechanisms which prevent membrane degradation. Membrane degradation will lead to physiological deterioration (Marangoni *et al.*, 1996b). Metabolic disorders due to stress can result from either: 1) compromised defense or repair mechanisms; 2) an increase in degradative reactions that exceed the capacity of defense or repair mechanisms; or 3) a combination of both processes (Purvis and Shewfelt, 1993). The production of free radicals in response to stress is a well documented phenomenon (Purvis and Shewfelt, 1993; Kumar

and Knowles, 1993). Increased production of active oxygen species is often a result of the disruption of metabolism involving electron transport systems. Under stress conditions, the cell is unable to inactivate the free radicals produced and deterioration ensues. This deterioration results in altered cellular composition which could lead to the deregulation of metabolic processes and sub-cellular decompartmentation. Alterations of the cellular environment can change the normal cycles of the cell and result in altered behavior as observed by Marangoni *et al.* (1996a). Oscillations in sugar concentrations can be identified as the potato tuber attempts to reach a new steady state in its stressed environment.

Purvis and Shewfelt (1993) discussed the possible involvement of the alternative oxidase as a mediator of resistance to stress. Plant tissues with the ability to reduce the content of active oxygen species are often more resistant to stresses, including low temperature exposure. The alternative pathway was discovered in potato tubers as a cyanide stimulated respiration (Hanes and Barker, 1931) and is therefore also known as cyanide resistant respiration. The alternative pathway consists of an alternative terminal oxidase that accepts electrons from the ubiquinone pool (Fig. 1), reduces O_2 to H_2O, but does not conserve energy as in cytochrome mediated respiration (Laties, 1982; Lance, 1981). The alternative path branches at or near coenzyme Q and is nonphosphorylating (Laties, 1982). The pathway became known as the alternative pathway, suggesting that two ways to reach oxygen were possible for the electrons produced by the oxidation of Krebs cycle substrates. The alternative oxidase is localized in the mitochondria (Fig. 1) (Lance, 1981).

It has been suggested that the alternative pathway functions only during periods of high cellular energy charge or when there is an imbalance between the supply of carbohydrates and the requirement for carbohydrates for structural growth, energy production, storage, and osmoregulation (Lambers, 1982; Elthon *et al.*, 1986). Evidence also indicates that changes in the physical characteristics of cellular membranes (*i.e.* mitochondrial membranes) may activate the alternative pathway (Solomos and Laties, 1975). The flow of electrons through the alternative pathway could enhance oxygen utilization (*i.e.* respi-

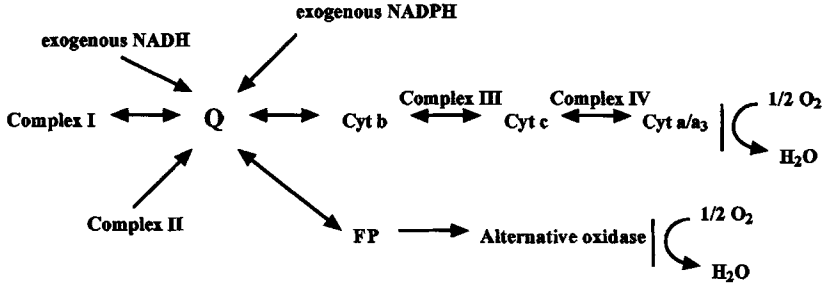

FIG. 1. Pathways of electron transport in plant mitochondria. Q is a mobile pool of ubiquinone which transfers electrons to complex III (the cytochrome b-c complex). FP is the flavoprotein intermediate of the alternative pathway. Adapted from Bryce and Hill, 1993.

ration) when energy demands of the tissues are low and respiratory substrates are high. Thus the alternative pathway could reduce the potential for the generation of oxygen-derived free radicals when the availability of respiratory electrons to the dehydrogenase complexes exceeds the capacity of the cytochrome oxidase (Purvis and Shewfelt, 1993). Strong evidence for the enhanced formation of free radicals in tubers under low temperature conditions were presented by Wismer (1995) in a comparison of the LTS sensitive Norchip and the LTS tolerant ND860-2.

Amir *et al.* (1977) conducted a kinetic study of the relationship between respiration rate, sugar content and ATP levels in cold stored potato tubers (Fig. 2). Results showed an immediate decrease in respiration rate upon storage at 4 C (Fig. 2a). The respiratory minimum is concomitant with an ATP maximum which is followed by a respiratory burst and a rapid decline in ATP content (Fig. 2a, 2c). This evidence suggests the presence of an active alternative pathway in cold stressed tubers. The alternative pathway could utilize the excess ATP and minimize free radical production. Debate surrounds the exact temperature at which the alternative pathway is activated in potato tubers although Sherman and Ewing (1982) indicate that it is inactive at temperatures 5 C and higher. Expression of the alternative pathway is known to increase with decreasing temperatures and is also believed to be associated with the increased solubility of oxygen in H_20 at decreased temperatures (Lance, 1981).

Amir *et al.* (1977) also observed an initial increase in sucrose concentration followed by a rapid decrease and subsequent stabilization at 0.3 g/100g fresh weight (Fig. 2b). The respiratory peak (Fig. 2a) corresponded with the peak for sucrose concentration (Fig. 2b). This is in agreement with Solomos and Laties (1975) who suggested that sucrose formation could act as an effective sink for excess ATP via the alternative pathway, *i.e.* the higher the rate of respiration the larger the accumulation of sugars. Further evidence that sugar formation acts as an ATP sink is found in Fig. 2d-f where Amir *et al.* (1977) showed the effects of dinitrophenol (DNP), an uncoupler of oxidative phosphorylation, on tuber respiration(Fig. 2d), sugar content(Fig. 2e) and ATP level (Fig. 2f). Their results indicate that no sugar accumulates in the absence of excess ATP (*i.e.* DNP treatment) although respiratory patterns are similar for both treated and untreated samples (Fig. 2d).

From Fig. 2b we observe that reducing sugars do not begin to accumulate until sucrose reaches its peak concentration (1.1 g/100g f.w.) and respiration rates decline. The levelling of the sucrose concentration corresponds with the plateau of the total sugar concentration implying a plateau of the reducing sugar concentration. This indicates that there is a close association between the amount of sucrose present and the tuber's ability to convert sucrose to reducing sugars. Potential sources for sucrose in the potato tuber are via sucrose phosphate synthetase (SPS) or sucrose synthetase(SS) catalysis. Pressey (1970) reported that sucrose synthetase activities decreased after harvest and

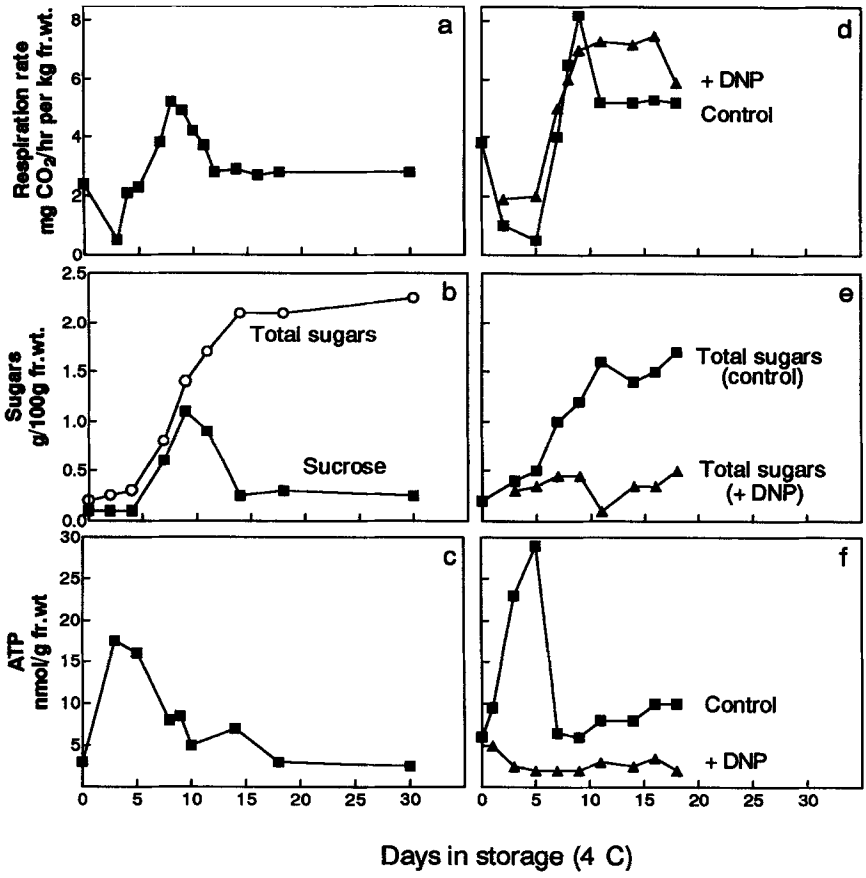

FIG. 2. The effect of 4 C storage on respiration, sugar accumulation and ATP level on freshly harvested tubers stored at 17 C for two weeks then transferred to 4 C (a-c) and freshly harvested tubers stored at 17 C for two weeks and treated with control solutes (■) or DNP (▲) 12 hours prior to being transferred to 4 C (d-f). Time zero refers to placement of tubers at 4 C. Adapted from Amir *et al.*, 1977.

continued to do so under low temperature storage conditions. Sucrose phosphate synthetase activity also decreased if tubers were held at warm temperatures but was found to rapidly increase when tubers were held at low temperatures. This indicates that SPS is the enzyme responsible for sucrose synthesis at low temperatures (Fig. 3).

Potato tubers are known to possess both alkaline and acid invertases. Schwimmer *et al.* (1961) reviewed the early literature debating the existence of a potato invertase and its potential role in the production of reducing sugars and presented preliminary evidence for the existence of an endogenous inhibitor of the enzyme. The proposed inhibitor was further investigated by

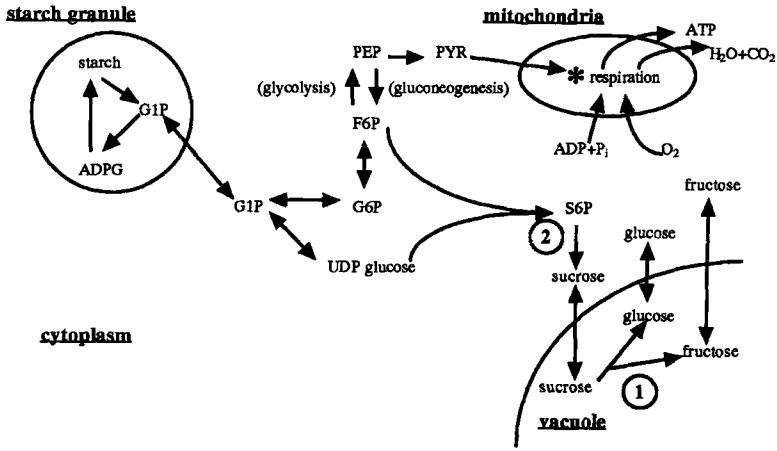

FIG. 3. A theoretical scheme of carbon movement in potato tubers. (*) indicates the presence of both cytochrome mediated and alternative path respiration in the mitochondria. (1) indicates the location of the acid invertase and (2) indicates the location of sucrose phosphate synthetase (SPS) in the pathway. ADPG (ADP-glucose); G1P (glucose-1-phosphate); G6P (glucose-6-phosphate); F6P (fructose-6-phosphate); PEP (phosphoenolpyruvate); PYR (pyruvate); S6P (sucrose-6-phosphate).

Pressey (1966,1967,1969) who developed a technique for removing the inhibitor and monitoring total invertase activity as opposed to the basal activity that could be detected prior to inhibitor removal.

Pressey and Shaw (1966) reported that the inhibitor level decreased upon exposure to low temperatures and increased again with exposure to warm temperatures (Pressey and Shaw, 1966). Invertase levels also responded to changing temperatures but exhibited trends that were opposite to those observed for the inhibitor. This lead to the theory that it was the inhibitor concentration that regulated the invertase's ability to convert sucrose to hexoses.

Anderson and Ewing (1978) determined that the potato tuber invertase was a glycoprotein and improved the technique for separation of the invertase inhibitor. A more recent study by Isla *et al.* (1991b) went on to examine the effects of the proteinaceous inhibitor and found that the invertase experienced greater inhibition from a potato lectin than from its previously identified inhibitor. They found that the proteinaceous inhibitor obtained by Pressey (1967) was non-dissociable and that the potato lectin was a dissociable inhibitor of the invertase and could explain the dissociable curve of inhibition obtained by Schwimmer *et al.* (1961). They concluded that the binding of both the potato lectin and the endogenous inhibitor to the invertase were mutually exclusive. The existence of an endogenous complex between the invertase and the proteinaceous inhibitor remained a mystery as no physiological reason could be found for the presence of that quantity of ineffective enzyme.

Isla *et al.* (1991a) went on to determine that invertase activity could be modulated by its products, fructose and glucose. Inhibition was non-competitive by glucose but regulation by fructose was more complicated and occurred via two interacting sites on the invertase. The high K_i (0.18M) and αK_i (0.33M) obtained for fructose indicated that large amounts would be required for effective inhibition. Under intermediate storage conditions fructose would not reach the concentration required for invertase inhibition therefore respiration may be responsible for maintaining low reducing sugar concentrations (*i.e.* no accumulation). The stabilization of fructose and glucose concentrations that is observed in many kinetic studies could be explained by their regulation of invertase.

In a further study, Isla *et al.* (1992) proceeded to identify the cellular localization of the invertase, the proteinaceous inhibitor and lectin. Invertase (acid β-D-fructofuranoside fructohydrolase) was found to be located in the vacuole where its substrate (sucrose) and products (fructose and glucose) are also located (Fig. 3). Neither the proteinaceous inhibitor nor *S. tuberosum* agglutinin were at a detectable concentration in vacuolar or protoplast preparations indicating that neither plays a role in the *in vivo* regulation of invertase activity (Isla *et al.*, 1992).

Increases in invertase activity in response to low temperatures have been previously reported (Pressey and Shaw, 1966; Pressey, 1967, 1969); however it is accepted that the rate of chemical reaction decreases approximately two-fold for each 10 C decrease in temperature (Avery, 1974). Therefore the observed increase in potato tuber invertase levels may merely be an attempt to compensate for the decreased reaction rate. Invertase activity at decreased temperatures remains unchanged. Activities measured at 37 C and optimum pH (Pressey, 1966, 1967, 1969) are not reflective of the *in vivo* activity.

Zrenner *et al.* (1996) studied the effect of soluble acid invertase activity in relation to the hexose-to-sucrose ratio in 24 different potato cultivars. They found a strong correlation between the hexose/sucrose ratio and the extractable soluble invertase activity by monitoring the invertase and sugar concentrations in the different cultivars. They also isolated a cold-inducible acid invertase cDNA from the potato cultivar Desirée and developed clones expressing the cDNA in an antisense orientation. Analysis showed that inhibition of the soluble acid invertase activity lead to decreased hexose and increased sucrose contents when compared to controls. The hexose/sucrose ratio was again found to decrease with decreasing invertase activities. The total amount of soluble sugars did not significantly change in either study and they concluded that invertases do not control the total combined amount of glucose, fructose and sucrose in cold stored potato tubers, but are involved in the regulation of the ratio of hexose to sucrose.

It has been reported that glucose concentrations are frequently higher than fructose concentrations in stored potato tubers (Davies and Viola, 1994). The reported glucose:fructose ratios range from 1:1 to as high as 10:1 and Davies

and Viola (1994) suggested that this may be a result of genotype, partial or complete starch breakdown by amylases and the starch synthesizing potential of the stored tuber. Fructokinase activity has also been indicated as a possible enzyme responsible for the variation in the ratio (Renz *et al.*, 1993). Zrenner *et al.* (1996) evaluated the glucose to fructose ratio of 24 different cultivars and found that the ratios were between 1.1 and 1.6. These ratios are a strong indicator that invertase is the key enzyme involved in sucrose to hexose conversion.

It is apparent that acid invertase is responsible for the conversion of sucrose to hexose in potato tubers. What remains in question is the cause of the sucrose accumulation, although the work of Amir *et al.* (1977) (Fig. 2d-f) indicates that it is closely associated with ATP production. In this review we have explored the potential role of the alternative pathway in the starch to sucrose conversion (summarized in Fig. 4). If the alternative pathway is responsible for the rapid increases in sucrose accumulation, then decreasing its activity in tubers could result in a low sucrose accumulating tuber. Such a tuber would have decreased substrate levels for the invertase and therefore a decreased potential for reducing sugar accumulation. Hiser *et al.* (1996) have isolated the alterative oxidase Aox1 gene and over-expressed it in transgenic tubers. Their results indicate that changing the alternative oxidase protein level by genetic engineering can effectively change the alternative pathway capacity.

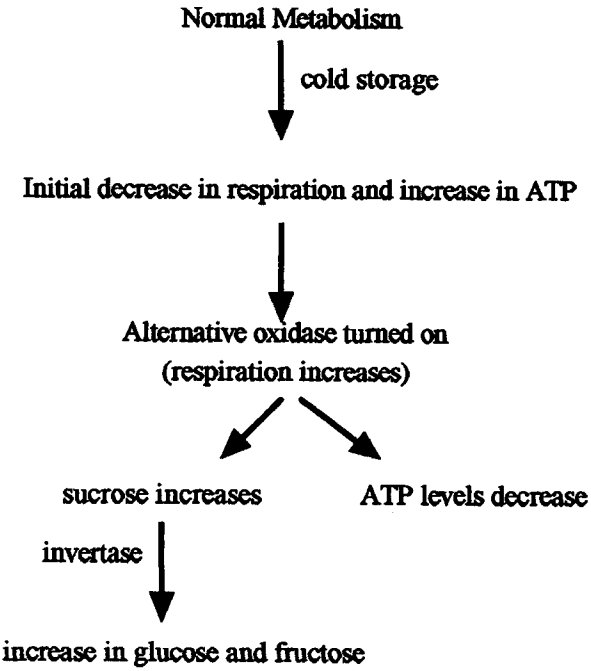

FIG. 4. Proposed pathway for the induction of low temperature sweetening in potato tubers.

Expression of the Aox1 gene in the antisense orientation would be expected to decrease the respiratory peak and minimize the sucrose produced upon transfer of tubers to low temperature conditions. If such a reduction in the alternative pathway did not result in detrimental effects when tubers were placed under cold storage conditions it could have the potential to allow breeders to develop cultivars which could be chipped directly out of cold storage conditions. Increasing antioxidant levels (*e.g.*, ascorbic acid) in tubers may also be necessary if down-regulation of the alternative pathway allowed free radical levels to increase. If tubers still produced excess amounts of sucrose through additional pathways then decreasing invertase activity as discussed by Zrenner *et al.* (1996) may also be required.

Control of the alternative pathway on its own, or in combination with acid invertase activity, may provide insight into the phenomenon of low temperature sweetening in stored potato tubers. The development of a cultivar suitable for cold chipping would result in improved industry efficiency and could lead to increased storage periods for potato tubers grown in the temperate regions.

Acknowledgments

The authors acknowledge the financial assistance of the Ontario Ministry of Agriculture, Food and Rural Affairs (Ontario Food Processing Research Fund), The Ontario Potato Growers' Marketing Board, the Ontario Potato Cultivar Evaluation Association, and the Canadian Potato Chip and Snack Food Association.

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