Research Review

Senescent Sweetening

Ref: R442

Report Authors:

Richard Colgan and Debbie Rees, Natural Resources Institute (NRI)

Adrian Briddon, Sutton Bridge Crop Storage Research (SBCSR)

Research Review No. 2012/6

The Potato Council is a division of the Agriculture and Horticulture Development Board.

Additional copies of this report and a list of other publications can be obtained from:

Publications

Potato Council
Agriculture & Horticulture Development Board
Stoneleigh Park
Kenilworth
Warwickshire
CV8 2TL

Tel: 02476 478785
Fax: 02476 478902

Our reports, and lists of publications, are also available at www.potato.org.uk

CONTENTS

1. PREFACE

During discussions of the research needs relating to potato storage, senescent sweetening was identified as a topic that has not been extensively studied in recent years. It was thought that the availability of techniques to study the molecular and biochemical changes that occur in plants over time may have generated new information relevant to the problem. As a result, a review of currently available information was commissioned. The aim of the review was to summarise the relevant information, identify research gaps and recommend approaches to the study of the problem. Therefore, the review does not provide new practical recommendations on the management of crops during storage. The intended use of the review is to support the development of research proposals to address the problem of senescent sweetening. However, it does provide an overview of the factors considered to affect the development of senescent sweetening and the methods for assessing tuber status during storage.

Potato Council, 2012

2. SUMMARY

For potato tubers destined for crisping and chipping, accumulation of the reducing sugars glucose and fructose must be avoided as it leads to deterioration in processing quality, and the risk of acrylamide production. If potato tubers are stored at temperatures below approximately 6-8°C, the concentration of glucose and fructose tends to increase due to a process termed *low temperature sweetening*. If potato tubers are stored at a moderate temperature (above approximately 6-8°C), sugar levels are maintained at a relatively low concentration for a period before another form of sweetening occurs – termed *senescent sweetening.* The onset of senescent sweetening cannot currently be predicted, and results in losses because changes are irreversible. The objective of this review is to summarise the present understanding of senescent sweetening; to identify research gaps; and to recommend new strategies to study senescent sweetening.

In the preparation of this review certain questions were highlighted that if answered could improve our understanding of the process of senescent sweetening:

- cultivar tendency for senescent sweetening, and seasonal patterns of its occurrence
- comparison of the rate of the process over the range of sprout suppressants, including ethylene
- the ratio of glucose and fructose accumulation.

Cultivars differ in their susceptibility to senescent sweetening. Although the cultivars that are most susceptible to senescent sweetening tend to have short dormancy there are important exceptions to this rule such as Maris Piper and Record. Growth and storage conditions affect timing of sweetening: early planting, stress and warm storage temperatures all speed up its onset. The most widely accepted hypothesis for the mechanisms of senescent sweetening is that tissue senescence in terms of a breakdown of cellular function occurs and that this is responsible for sweetening. Damage at the cellular level, especially membrane damage, facilitates enzyme access to starch granules thereby speeding up starch breakdown resulting in senescent sweetening. Physiological aging of tubers is associated with increasing oxidative damage. The process of oxidative damage, and how tissues may protect themselves

against it, is considered in some detail given its importance in the process of cell senescence. Strategies to assess physiological age and hence storage quality at harvest and during storage are considered.

New strategies to study senescent sweetening are recommended including molecular techniques to follow gene expression patterns. Comparative studies of varieties with different propensities to develop senescent sweetening would be very informative. Moreover, having a greater understanding of the impact of mineral nutrition on tuber health and storage potential may provide novel methods of maintaining potatoes with low reducing sugar content.

It would be very valuable to identify methods that could detect the changes in tuber status before they impact on fry colour. Molecular methods as indicated above have the potential to provide such markers, and this should be a priority area of study. Although potato growers could not carry out the assessments themselves, it is likely that a relatively straightforward and cost-effective laboratory test could be developed.

A link between senescent sweetening and changes in the ability to protect against oxidative damage (antioxidant capacity) has been established. This provides possible strategies to follow tuber damage and give an early indication of senescent sweetening. Ascorbate concentration in particular should be investigated as a simple indicator of changes in capacity for oxidative protection.

3. INTRODUCTION

Biologically the potato tuber is both a storage organ and a reproductive organ. It enters a state of dormancy at the point of tuber initiation, and at the end of this natural dormancy the tuber sprouts, subsequently forming a new plant. Commercial use of tubers often requires that they be stored for periods beyond their natural length of dormancy. Sprout suppressants may be used to prevent sprout growth. As the tuber goes through different development stages in storage, there are metabolic and compositional changes. The prevention of sprouting and maintenance of the tuber beyond its normal lifetime is likely to impose stresses. Commercially, the maintenance of low sugar concentrations is desirable.

4. THE IMPORTANCE OF SUGAR CONTENT FOR PROCESSING TUBER QUALITY

Although other sugars can be detected in potatoes, the main ones are the disaccharide sucrose, and the monosaccharides, or reducing sugars, glucose and fructose. For potato tubers destined for crisping and chipping, accumulation of the reducing sugars must be avoided as it leads to deterioration in processing quality (darkening of fry colour) (Fuller and Hughes, 1984). More recently, high concentrations of reducing sugars have been shown to be associated with elevated levels of acrylamide (Burch *et al.*, 2008), a process contaminant, occurring as a result of Maillard type reactions between reducing sugars and the amino acid asparagine.

5. SUGAR ACCUMULATION DURING TUBER STORAGE

If potato tubers are stored at temperatures below approximately 6-8°C, sugar levels tend to increase due to a process termed *low temperature sweetening*. There is a

very extensive scientific literature on low temperature sweetening, its causes and strategies to reduce it. Evidence suggests that it is related to inhibition of the glycolytic pathway that metabolises sugars (Dixon and AP Rees, 1980). The sugar accumulation associated with “low temperature sweetening” decreases with storage time, presumably due to sugar metabolism. Potato processors can ameliorate the effects on processing quality to some extent by increasing storage temperature to promote metabolism of sugars (reconditioning).

If potato tubers are stored at a moderate temperature (above approximately 6-8°C), sugar levels are maintained at a relatively low concentration for a period before another form of sweetening occurs – termed *senescent sweetening.* Senescent sweetening cannot be reversed by reconditioning the tubers at higher temperatures. These two processes have been recognised as separate processes for several decades (e.g. Burton, 1989). Senescent sweetening was reported to occur generally after 5-6 months of storage at 10°C in a range of varieties. He reported that sugar accumulation tended to occur earlier at higher storage temperatures, over the range 7-20°C. In some cases it appeared to be associated with the onset of sprouting (especially if the growth of sprouts was inhibited by sprout suppressants) but in other cases it appears to be a senescent process unrelated to sprouting.

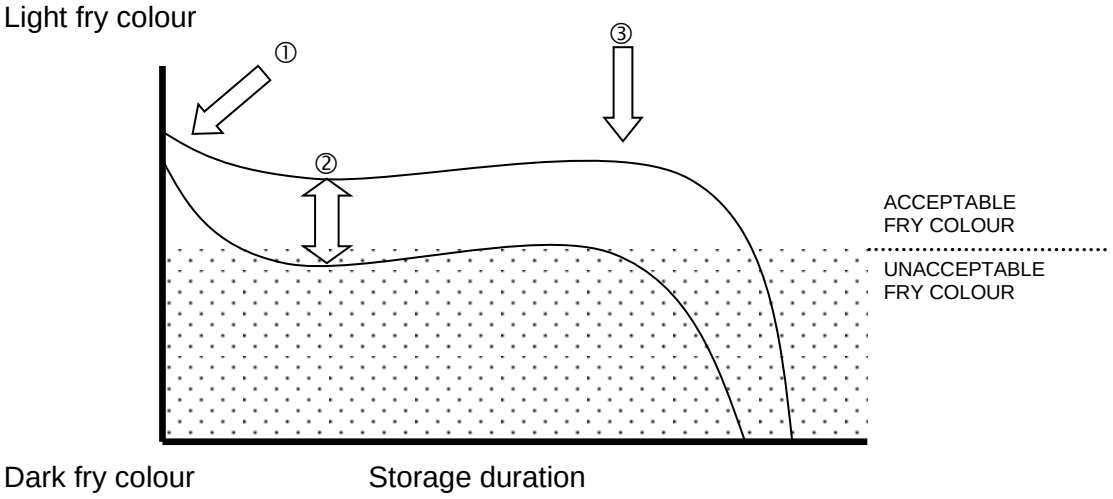


FIGURE 1. SCHEMATIC REPRESENTATION OF FRY COLOUR RESPONSES OF STORED POTATOES.

Figure 1 summarises the behaviour of potato tubers after harvest in terms of processing quality. Under average growing conditions, reducing sugars reach low levels at harvest and crops enter storage with a light, acceptable fry colour and low acrylamide levels (①). Fry colour during storage varies significantly as a result of site, season and agronomy (②). Quality of fry colour during/after storage is influenced by the stage at which the tuber is harvested. Strategies to optimise harvest time include ‘chemical maturity’ (section 13.1) or, with some varieties, molecular tests (PCR) to assess risk of quality deterioration during storage (section 13.1.3). Fry colour during storage is also influenced by storage temperature and this can be used to modify fry colour prior to the onset of senescent sweetening. The required period of storage often exceeds the period of dormancy, so sprout suppressants may be required. Good processing quality can be maintained despite the end of dormancy (although see section 7). (③) Indicates the point at which the tubers start to sweeten through the

process of senescent sweetening. The onset of senescent sweetening cannot currently be predicted, and results in losses because changes are irreversible.

The objective of this review is to summarise the present understanding of senescent sweetening and to identify the research required to improve the industry's ability to control or predict it. Low temperature sweetening is not considered in detail and has been reviewed elsewhere (e.g. Blenkinsop *et al*., 2010.).

6. FACTORS AFFECTING DEVELOPMENT OF SENESCENT SWEETENING

6.1. Cultivars and tendency for senescent sweetening.

Potato cultivars differ considerably in the timing of onset of senescent sweetening. Table 1 shows a range of important UK processing varieties in terms of how long they can be stored before the onset of senescent sweetening. Dormancy characteristics are also included. Although the cultivars that are most susceptible to senescent sweetening tend to have short dormancy there are important exceptions to this rule such as Maris Piper and Record.

TABLE 1: CLASSIFICATION OF POTATO PROCESSING VARIETIES BY ONSET OF SENESCENT SWEETENING

Variety	Main market	Development of senescent sweetening	Length of dormancy
Lady Rosetta	Crisp	Early onset	2
Crisps4all	Crisp	Medium onset	3[1]
Hermes	Crisp	Medium onset	3
Pentland Dell	Chip	Medium onset	3
Cabaret	Chip	Late onset	5
Maris Piper	Chip	Late onset	2
Record	Crisp	Late onset	3
Saturna	Crisp	Late onset	4
Verdi	Crisp	Late onset	6
VR808	Crisp	Late onset	4[1]
Lady Claire	Crisp	Very late onset	6
Markies	Chip	Very late onset	5
Russet Burbank	Chip	Very late onset	8

Dormancy (1-9 scale, 9 = Long, NIAB Pocket Guide, 2008, NIAB , Cambridge.
[1]For newer varieties dormancy periods have been estimated.

6.2. Effects of growth conditions on onset of senescent sweetening

In addition to the effect of cultivar, growing conditions that affect the 'maturity' of tubers at harvest can impact on the timing of the onset of senescent sweetening, and an effect of storage temperature is also evident.

For a given site-variety combination early planting and a warmer holding temperature consistently give rise to earlier senescent sweetening, indicating a relationship with tuber maturity. Groves *et al.* (2005) in a series of experiments trying to elucidate the role of agronomy on processing quality from storage found chitting of seed (physiological aging - Saturna) and early planting (Maris Piper, Lady Rosetta) to hasten the onset of deterioration in processing quality after long term storage (see Figures 2 and 3). Both chitting and early planting lead to an earlier tuber initiation and so tubers were more 'mature' at harvest, suggesting that more mature tubers progress more rapidly to senescent sweetening. Seed maturity itself (i.e. seed produced from short or long seasons) did not significantly affect the onset of senescent sweetening.

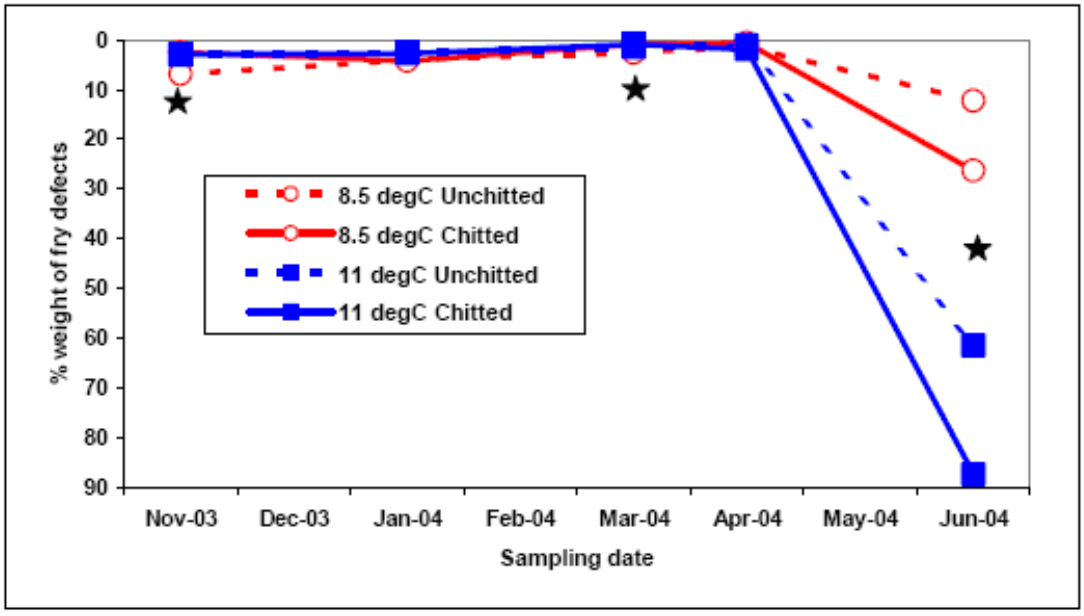


FIGURE 2: EFFECT OF CHITTING ON TUBER FRY QUALITY (% WEIGHT OF FRY DEFECTS) DURING STORAGE OF SATURNA AT TWO TEMPERATURES. * INDICATES SIGNIFICANT FIELD TREATMENT DIFFERENCES AT THE ASSESSMENT DATA MARKED ($P<0.05$) (FROM GROVES *ET AL.* 2005)

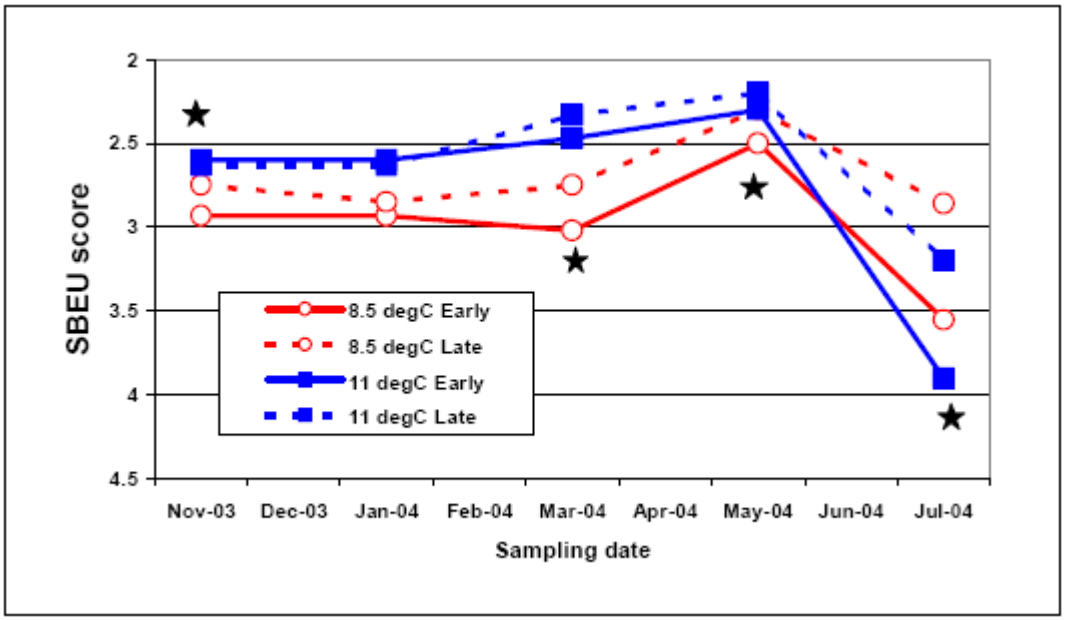
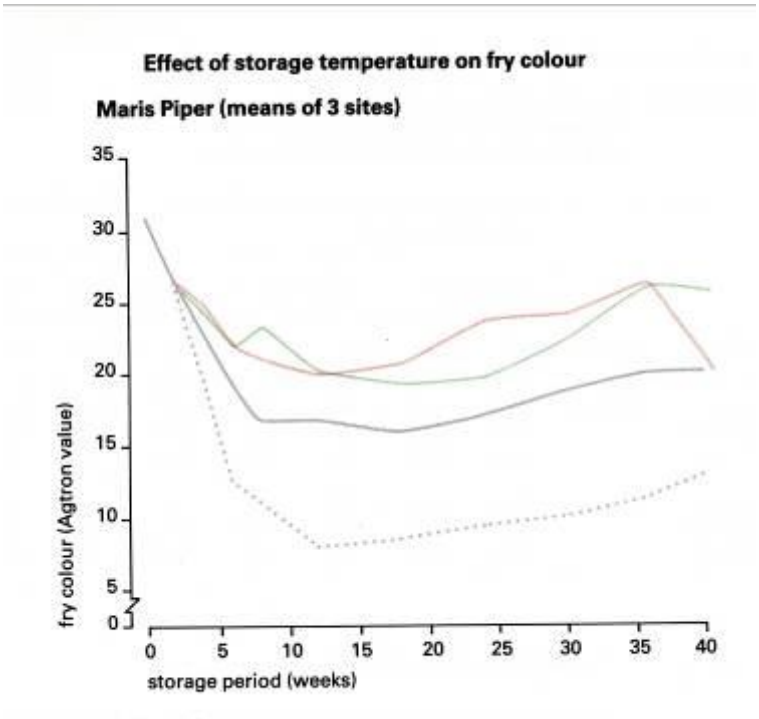


FIGURE 3: EFFECT OF PLANTING DATE ON TUBER FRY QUALITY (FRY COLOUR, SUTTON BRIDGE SCORE) DURING STORAGE AT TWO TEMPERATURES (MARIS PIPER) * INDICATES SIGNIFICANT TREATMENT DIFFERENCES AT THE ASSESSMENT DATE MARKED (P<0.05) (FROM GROVES *ET AL.* 2005)

Stresses during tuber production (drought, heat and nutrient deficiencies) increase sugar accumulation in potato tubers during storage (Knowles *et al.,* 2009).

Figure 4 indicates how storage at higher temperatures can speed up the onset of senescent sweetening. The data shows fry colour for Maris Piper and for Pentland Squire. Both cultivars show deterioration in fry colour (decrease in Agtron value) after ~35 weeks storage at the warmest storage temperature. This was particularly marked in cv Maris Piper.



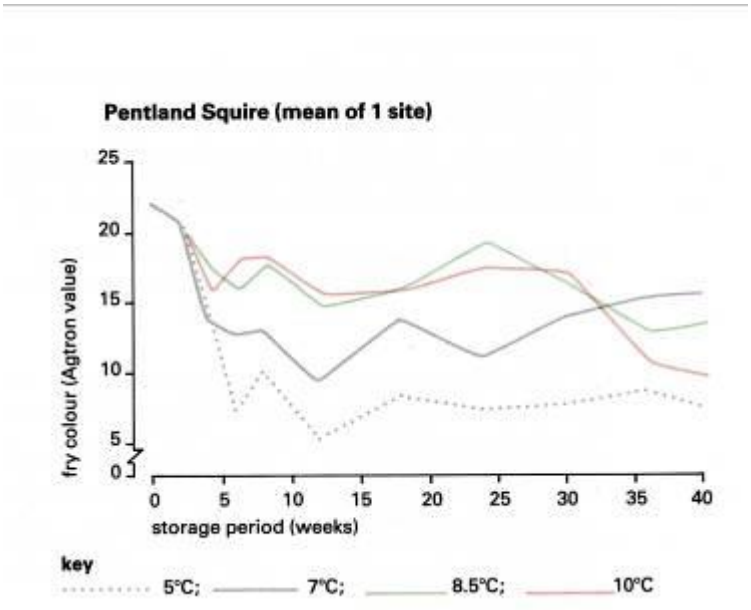


FIGURE 4: FRY COLOUR OF MARIS PIPER (MEAN OF THREE SITES) AND PENTLAND SQUIRE DURING LONG-TERM STORAGE AT 5°C, 7°, 8.5° AND 10°C. FROM SUTTON BRIDGE ANNUAL REVIEW, 1988.

7. THE PHYSIOLOGICAL/BIOCHEMICAL BASIS OF SENESCENT SWEETENING

In some cases it has been observed that sweetening occurs in tubers at the point when dormancy breaks, and sugars are mobilised for sprouting, but application of sprout suppressants prevents sprout growth. In this scenario, where the metabolism of mobilised sugars is inhibited, there will be an accumulation. This form of sweetening has been observed by a number of investigators including Hertog *et al.* (1997). These authors studied sugar accumulation in four varieties over three seasons over a range of storage temperatures in the presence of sprout inhibitors (CIPC or carvone). One interesting observation was that in seasons where the sprout suppressants were unable to prevent sprout growth there was less sugar accumulation. Although this form of sweetening has been called “senescent sweetening” it is distinct from the process usually described, that occurs well after dormancy break. Thus dormancy breaks before January in most cultivars, and yet crops are stored well into the next year and respond to reconditioning well after dormancy break.

The most widely accepted hypothesis for the mechanisms of senescent sweetening is that tissue senescence in terms of a breakdown of cellular function occurs and that this is responsible for sweetening. Damage at the cellular level, especially membrane damage, facilitates enzyme access to starch granules thereby speeding up starch breakdown resulting in senescent sweetening. The biological mechanisms involved are described in more detail below.

8. PATHWAYS OF STARCH BREAKDOWN FOR SUGAR ACCUMULATION IN POTATO

Starch breakdown is the main factor in tuber quality, and so this section summarises our knowledge of the biological mechanisms involved.

Starch consists of large molecules comprising chains of glucose; either as straight chains (amylose) or branched chains (amylopectin). Within tuber cells, the glucose chains are rolled up and packed tightly into a ball, which is stored in a membrane bound cellular compartment called an amyloplast. For plant cells this is a very efficient way to store carbohydrates. With respect to starch breakdown an important issue is that it is not easy for enzymes to access the starch molecule. The process of starch breakdown (mobilisation) depends on some mechanism for improving enzyme access. In the case of senescent sweetening, it has been suggested that as a result of cellular senescence a breakdown of amyloplast structure could contribute to sugar accumulation, and the evidence for this will be examined later in this review.

The mechanisms of starch breakdown are very complex, involving a large number of enzymes. The process has been reviewed by Smith *et al*. (2005). They indicate that the mechanisms involved differ between tissues, and that the mechanism in sprouting potato tubers is not fully understood. More recently, Kotting *et al*. (2010) have summarised the current level of knowledge on the enzymes of starch metabolism and their control mechanisms. Although the mechanisms have not been verified for potato tubers, they are likely to be similar. Chemical modification by phosphorylation of the surface of the starch granule occurs, catalysed by the enzymes glucan water dikinase (GWD) and phosphoglucan water dikinase (PWD). This alters the conformation of the granule surface allowing access of other enzymes involved in the starch breakdown. The activity of GWD itself is controlled by redox state and phosphorylation. Starch granule phosophorylation does appear to be involved in sprouting potato tubers.

The main sugars found in potato tubers are glucose, fructose and sucrose. In theory the pattern of these sugars might change depending on the mechanism of starch breakdown. Following cold induced sweetening glucose and fructose have been observed to be present in equal (equimolar) amounts (Hertog *et al.*, 1997). From knowledge of the biochemical pathways of sugar metabolism this implies that sugars released from starch breakdown are synthesised into sucrose which is subsequently broken down. Hertog *et al.* (1997) found this to be true for senescent sweetening as well. If there are two or more mechanisms of senescent sweetening this observation might not always be true, but we are not aware as to whether this has been tested.

9. OXIDATIVE STRESS/DAMAGE AND ITS POSTULATED ROLE IN SENESCENT SWEETENING

The theory of senescent sweetening suggests that as the tubers age breakdown of cellular structure, specifically the membrane surrounding the amyloplast, facilitates access of enzymes to the starch granule. The most common theory is that the membrane breakdown occurs as a result of "oxidative damage" (Kumar and Knowles, 1993 b; Sowokinos *et al*., 1987). This section summarises present knowledge on oxidative damage/stress, and cellular mechanisms to protect against it. *A more detailed description of the studies referred to in this section is provided in Appendix I.*

9.1. Oxidative stress and antioxidants

Although most forms of life are dependent on oxygen for respiration, oxygen can be a damaging chemical especially when in certain chemical forms such as the superoxide radical anion ($O_2^{-\cdot}$), the hydroxyl radical ($OH^{\cdot}$), hydrogen peroxide (H_2O_2) and singlet oxygen. These reactive forms are collectively known as Reactive Oxygen Species

(ROS). They can react with and damage many components of the cell. Cells have therefore devised methods for protecting themselves against ROSs which involve “antioxidants” which react with and destroy ROSs. The same processes occur in animal cells and plant cells, which is why for health reasons the population is encouraged to increase its intake of antioxidants.

9.2. The role and levels of antioxidants in potato tubers

In potato tubers the main antioxidants are phenolics, especially quercetin and chlorogenic acid. Other antioxidants are vitamin E, carotenoids, flavonoids and Vitamin C. Biochemically it is possible to make an overall measurement of anti-oxidant capacity. Phenolics provide the main protection but Vitamin C can contribute up to 13%. Interestingly although potato varieties with orange or purple flesh contain higher levels of carotenoids and flavonoids it has been reported that their antioxidant capacity is not significantly higher than white fleshed potato (Wang, 1997).

9.3. Effect of stress on antioxidant content

Under oxidative stress, the amounts of different antioxidants strongly increase in plants, and it is believed that this results in an increased capacity to scavenge reactive oxygen species (Noctor and Foyer, 1998). Stress during the growing season, such as drought, would be expected to increase oxidative stress, and therefore plants should respond by increasing antioxidant capacity. The reality is a bit confusing, with contrasting observations. It is possible that in some cases under stress the plant cells become incapable of maintaining a sufficiently high antioxidant capacity. Thus tubers subject to heat, drought or herbicides can have altered balance between free radical production and the quenching ability of cells resulting in tubers with elevated sugar content.

9.4. Evidence of increases in antioxidant capacity during ageing related to increased lipid peroxidation

As tubers age in storage there appears to be an increase in antioxidant capacity (superoxide dismutase, catalases and vitamin E) which occurs in parallel with an increase in oxidative damage to membranes. This suggest that the level of ROSs increase with ageing, and the tuber tissues are attempting to protect against this. Ascorbate is an important component of the antioxidant system, and it has been found that treatments leading to an increase in ascorbate levels lead to a reduction in lipid peroxidation. However, ascorbate levels decrease during tuber storage, which is a common phenomenon in senescing tissues.

9.5. Membrane permeability and how this affects sweetening

Peroxidation of the lipids in the membranes (Kumar and Knowles, 1993 b) that occurs during ageing in turn leads to loss of membrane integrity. Loss of amyloplast membrane integrity in particular is thought to lead to increased hydrolysis of starch (Knowles and Knowles, 1989, Sowokinos *et al*., 1987). Similar changes to the structure of amyloplast membranes has also been reported in tubers subject to low-temperature stress (O’Donoghue *et al*., 1995) and it has been proposed that the mechanism of damage is the same in both forms of physiological disorder. However, there are conflicting views on this; amyloplast breakdown does accompany starch breakdown in some tissues (e.g. sprouting grain (Smith *et al*., 2005)). However,

although there are reports of amyloplast breakdown in low temperature sweetening Smith *et al*. 2005 discount this idea on the basis that low temperature sweetening is reversible. Smith *et al*. also appear to believe that starch breakdown in sprouting tubers occurs in intact amyloplasts.

The contribution of lipid peroxidation to senescent sweetening could be determined to some extent by determining whether there is a relationship between propensity for senescent sweetening and rate of antioxidant decline during storage. Vitamin C content in tubers has been reported to decline rapidly during storage at 4°C (Dale, 1987).

Fauconnier *et al.* (2002) reported that the role of membrane breakdown in senescent sweetening is less than clear and found that sucrose content accumulated in varieties Brintje and Désirée at 20°C to the same extent in tubers exhibiting senescence (sprouting tubers characterised by loss of apical dominance and presence of daughter tubers) and those treated with sprout suppressants. In addition although a degree of saturation of fatty acids in cell membranes increases with time this was not correlated with the activity of the enzymes (lipoxygenase) that are often associated with the peroxidation of polyunsaturated fatty acids, but it was evident that the process of sprout development does influence the integrity of cell membranes and this warrants further investigation in relationship between tubers propensity to sprout and development of senescent sweetening.

9.6. Membrane permeability and adaptation to lower temperatures and electrolyte leakage to detect changes

Loss of membrane integrity and increase in leakiness can be assessed by a relatively quick and easy laboratory measurement of “electrolyte leakage”. This method has been used to follow membrane state in a number of studies.

Tubers respond to storage in low-temperatures by increasing the amount of desaturated fatty acids in cell membranes which can help them acclimatize to low temperatures. The viscosity of membranes appears to be regulated by the degree of saturation of lipids in cell membranes. Spychalla *et al.* (1990) found that in four North American processing varieties ((Monona, Red Pontiac, Norchip, Russet Norgold) total fatty acid unsaturation was correlated (poorly) negatively with electrolyte leakage indicating that the more unsaturated (less double bonds) the less electrolyte leakage occurred. Knowles and Knowles (1989) found a strong negative correlation between the double bond-index of fatty acids (the degree of saturation) and electrolyte leakage.

The amount of free fatty acids (FFA) in cell membranes can increase several fold in tubers subject to low temperatures and artificial aging. Generally increases in FFA reduce membrane fluidity leading to a breakdown in membrane permeability. This in turn increases fry colour. Spychalla *et al.* (1990) found that changes to the FFA content of lipids during storage had little bearing on permeability. However, the amount of linolenic acid in membrane lipids appears to confer potatoes with better processing quality.

10. PHYSIOLOGICAL AGEING OF TUBERS AND THE ONSET OF SENESCENCE

Understanding how to quantify the physiological age of a tuber has benefits beyond the processing sector and is also pertinent to the seed potato industry where excess physiological age tends to produce plants of weaker vigour than those derived from younger seed stock.

There have been attempts to define the process of aging in many ways. Reust (1986) refers to physiological age in terms of the influence on productive capacity of the tubers regenerative ability: the same loss of regenerative processes may lead to senescent sweetening. The precise definition of tuber aging is unclear as many of the processes that occur are not fully elucidated.

The aging process is both cultivar specific and temperature dependant. The length of storage at a particular temperature after the break of dormancy has been used to calculate thermal scales for ageing of tubers. Wurr (1978 a; 1978b) correlated tuber behaviour with the number of degree-days tubers were stored post dormancy above a basal temperature of 4°C. Although such studies have had some success in determining aging it was not widely applicable to other cultivars. Others (Hartmans and Van Loon, 1987) have used an 'incubation period' defined as the time between the onset of sprouting and tuber formation on the new sprouts, when stored at 15-20°C which was considered to be consistent between years and specific to cultivar and storage temperature.

A physiological age index (PAI) for tubers was proposed by Cadiz (2001) that tries to reconcile physiological age of tubers with chronological age of seed potatoes. The PAI is based on the haulm killing date (T-0) and the end of the incubation period of seed tubers. The index is calculated as T-1/T-2, where T-1 is calculated from the time from haulm killing data (T-0) to possible planting date, and where T-2 is the time from T-0 to the end of incubation period. The PAI index expresses the physiological age of tubers from physiologically young (0) to old tubers (1). If tubers are stored at relatively high storage temperatures such measures are indicators of tuber age but at temperatures of 4°C or below such relationships are less well correlated with tuber behaviour.

As potatoes undergo aging during storage other physiological changes occur due to metabolic fluxes within the tuber. Loss of apical dominance followed by a progressive loss of sprout vigour occurs. Older potatoes contain elevated indole acetic acid (IAA) oxidase activity; responsible for catabolising native auxin IAA and in addition the sprouts developing from older tubers have a reduced ability to translocate IAA basipetally leading to a loss of apical dominance and a greater chance of stimulating lateral meristem growth.

Aging tubers also undergo a rise in respiration. Increases in respiration are often associated with sprout formation, however other mechanisms must be involved as increased rates of respiration are common to tubers that have been desprouted (Kumar and Knowles, 1996a).

Another effect of aging appears to be that the soluble protein content of tubers decreases due to a combination of increased rates of protein breakdown (proteolysis) by protein degrading enzymes (proteases) and reduced synthesis of new proteins.

These include enzymes involved in sugar movement, and also those that prevent the breakdown of proteins such as patatin. Patatin forms the largest (20-40 %) soluble protein content of tubers (Paiva *et al*. 1983) and has many roles in the tuber including protection against oxidative damage. Thus loss of patatin may lead to increase in membrane breakdown (Knowles and Knowles, 1989). Mikitzel and Knowles (1989) suggested that through loss of ability to synthesise proteins, potato aging inhibits sugar utilisation hence leading to an increase in sugar levels. Profilng protein content is one method considered for assessing tuber status during storage (see section 13.2.5).

11. MINERAL NUTRITION, TUBER QUALITY AND SENESCENCE

Studies on potato tubers and analogies with other plant organs suggest the importance of mineral nutrition, notably calcium, for delaying senescence. For example Dyson and Dygby (1975) found that the application of calcium salt solutions reduced the loss of apical dominance which is associated with tuber senescence. Similarly Davis (1984) reported reduced sprout vigour in dark stored potatoes was caused by a lack of calcium rather than reduced sugar supply. Low calcium content in tubers leads to internal necrosis often referred to as russet spot or internal heat spot, often this is accentuated in drought conditions where increased canopy transpiration reduces deposition of calcium in the tuber. There is also evidence of gradients of minerals within tubers with implications for heterogeneity of quality. In other reproductive organs (apple fruit) low calcium affects the movement and distribution of sugars in tissues. The role of minerals in tuber quality maintenance is an area that may warrant further investigation. *A more detailed description of studies relating to the role of mineral nutrition is provided in Appendix II.*

12. SPROUT SUPPRESSANTS AND EFFECTS ON TUBER QUALITY

Where the mechanisms of sprout suppressants has been studied, findings suggest that they do not prevent dormancy break, but merely sprout development (CIPC) or growth (ethylene). On the other hand 1,4-dimethylnaphthalene is considered in the USA as a dormancy enhancer. Work at Sutton Bridge Crop Storage Research (SBCSR) suggests there is usually no difference in fry colour (after long term storage) between CIPC and DMN suppressed crops.

13. PREDICTING STORAGE QUALITY AT HARVEST AND DURING STORAGE

Tuber processing quality and how this changes during storage is related to the state of the tuber at harvest, in particular the ‘maturity’. It was established in section 6.2 that tubers that were more ‘mature’ (i.e. had been allowed to develop in the field for longer, because of early planting or because they had been through a chitting process) had a tendency to develop senescent sweetening earlier in the storage season. This section examines various strategies that have been considered firstly to determine tuber “maturity” at harvest, and secondly to determine tuber maturity during storage.

13.1. Strategies applied at harvest

13.1.1. Dry matter content

Hogan et al. (1969) found no relationship of specific gravity (dry matter content) with fry colour. This has been confirmed by other research including Groves *et al.* (2005) and Wiltshire *et al*. (2004) amongst others.

13.1.2. Canopy senescence

Wiltshire *et al*. (2004) in a series of field trials attempted to find predictors of processing quality from storage using assessments at or close to harvest time. Assessments around harvest time included measures of tuber quality as well as measures of the canopy. Correlations were not generally successful but were greatest for crop cover. However, with important differences between cultivars and seasons, even this relationship was not considered sufficiently robust to be used predicatively. The authors concluded that for crops destined for processing from storage, defoliation should only take place once canopy senescence was underway.

13.1.3. Chemical Maturity Monitoring

The Chemical Maturity Monitoring model is in commercial use as a management tool to help ensure good, acceptable processing quality from storage. The model is based on the observation that sucrose declines in concentration relatively slowly, after tuber initiation (Burton and Wilson, 1970), and can contribute to changes in fry colour during storage. The principles were initially published as the Sucrose Rating system which indicated potatoes capable of reaching low sucrose levels at harvest (1.0-2.8 mg/g) demonstrated good processing quality from long term storage. Values >2.8 mg/g were only suitable for short term storage (Sowokinos, 1978). These principles were included in a more complete model, Chemical Maturity Monitoring. The model is based on regular sampling and analyses of tubers for glucose and sucrose concentrations. The initial aim of the model is to delay defoliation/harvest of crops until a minimum tuber concentration of sucrose is achieved (chemical maturity). During storage, changes in sugar concentration are used to optimise storage conditions and may be used to indicate the onset of senescent sweetening (increasing sucrose concentration) before this becomes visible as a change in fry colour.

13.1.4. Thermal scale

In the absence of physiological indicators of tuber maturity, efforts were made to develop a thermal scale for ageing. This approach has been applied to seed tubers to see if maturity in terms of thermal history affects subsequent yield. Initial studies suggested that sprout growth and tuber yields were positively related to the number of degree-days above a base temperature of 4°C accumulated by seed tubers after the end of dormancy (Wurr, 1978b; Allen *et al*., 1979; O'Brien *et al.*, 1983; Allen and O'Brien, 1986). However, attempts to relate seed tuber vigour (e.g. shoot DW) in six cultivars to accumulated storage temperature throughout the storage period were not successful (Scholte,1987). Additional studies suggest that accumulated degree-days after the completion of dormancy may not be completely effective in characterising physiological ageing (Gillison *et al.* 1987; Jenkins *et al.*, 1993). In conclusion, so far thermal scales have not been successful either to predict yield, or quality of stored tubers.

13.1.5. Molecular diagnostics

In some varieties of apples the change in expression of key genes during development, as assessed using the molecular technique of real time Polymerase Chain Reaction (PCR), is being used to select optimum harvest time, and is being investigated as a strategy for predicting storage potential. Research is on-going to apply the same approach to potato tubers. The Nsure test is a QC procedure for potatoes to indicate suitability for processing. Sap extracts (immobilised on paper discs) obtained by growers/store managers at harvest are posted to an analytical facility where assays are performed and the suitability of a crop for processing, after storage, predicted using a traffic light system. The system is based on PCR testing of gene expression, previously identified in empirical storage trials as being associated with storability. Kits are currently available for predicting susceptibility to sugar accumulation during storage of the cultivars Agria and Russet Burbank. It is thought the test could also be suitable for predicting the onset of senescent sweetening (personal communication, M. van Wordragen, Nsure BV, Wageningen, The Netherlands)

13.2. Strategies applied during storage

13.2.1. Sugar concentrations

It is normal practice to measure fry colour during storage. Sometimes sugars may also be measured. For practical reasons the analysis is usually restricted to glucose and sucrose. As soon as concentrations are seen to increase (an increase on two consecutive sampling occasions may be used), the tubers must be used, as this may indicate the onset of senescent sweetening, that cannot be reversed by conditioning.

13.2.2. Respiration rate

Fry colour of crisps of several cultivars was statistically linked to respiration rate (Copp *et al.*, 2000). An increase in respiration rate was linked to deterioration in fry colour, in long-term storage and it was suggested that change in respiration rate could be used to predict the point at which processing quality will start to deteriorate. However, changes in respiration rate were reported that did not subsequently exhibit deterioration in colour. Therefore it is not clear what advantage respiration rate has over direct assessment of fry colour.

13.2.3. Electrolyte leakage

Rate of electrolyte leakage which is a relatively simple measurement (see section 9.6) is generally considered to be a good measure of membrane permeability. Few studies have followed electrolyte leakage during storage to the point of senescent sweetening, with most studies using electrolyte leakage to study the effect of temperature and how tubers adapt to changes in temperature. However, Turnbull and Cobb (1992) citing Williams and Cobb (1992) suggest a link between changes in electrolyte leakage and changes in reducing sugar concentrations, respiration rate and numbers of mitochondria during extended storage of the cv Pentland Dell, a cultivar considered particularly susceptible to senescent sweetening. Results indicate that there may be potential to use electrolyte leakage to follow maturity changes during storage, although fatty acid composition may be a more accurate method.

13.2.4. Physiological age index

Recent work to seek maturity indices in seed potato by using a proteomic approach (Delaplace *et al.*, 2009) have provided indications of maturity indicators that could be investigated for assessing processing potatoes either at harvest or during storage. Thus many components of the antioxidant system (catalase, superoxide dismutase, ascorbate), enzymes involved in starch breakdown and stress response proteins increase while there is a decrease in patatin and chlorogenic acid.

Delaplace *et al*. (2009) found that during storage potatoes undergo physiological aging and to quantify this process a physiological age index (PAI) was proposed. Storage for 270 days at 4°C led to an increase in the PAI from 0.4 to 0.83. Using 2-dimensional electrophoresis it was possible to look at the proteomic profile of tubers during storage. During aging a marked increase in the proteolysis of patatin was observed at PAI 0.6. Concomitantly an increase in the glycolytic enzymes involved in oxidative stress responses occurred. It appeared these changes allowed tubers to maintain their radical scavenging capacity and no effect of oxidative damage was observed suggesting cellular changes during storage prevented membrane damage.

13.2.5. Protein profile

The soluble protein content of tubers decreases with age due to a combination of increased rates of protein breakdown (proteolysis) by protein degrading enzymes (proteases) and reduced synthesis of new proteins. Monitoring changes in the soluble protein profile using 2-D electrophoresis have identified changes to the protein content of aging potatoes that have been correlated with tubers physiological age index which appear to relate to degradation of patatin content (see above).

Patatin forms the largest (20-40 %) soluble protein content of tubers (Paiva *et al*. 1983) and has many roles in the tuber from modifying lipids in membranes (Galliard 1971; Racusen 1986) through to acting as a defence mechanism against fungal attack (Tonón *et al*., 2001). In other species (cowpea) patatin-like proteins have also been implicated in mitigating the effects of drought stress (Matos *et al.,* 2001). Patatins have also been reported to have strong antioxidant activity (Al-Saikhan *et al*., 1995) and while no direct evidence exists on the consequence of patatin loss during storage, Kumar and Knowles (1999) suggested that the loss of patatin's antioxidant activity may lead to increased membrane breakdown.

14. CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

Senescent sweetening is an important economic problem for the potato processing industry. In the case of low temperature sweetening although undesirable, a batch of potatoes can be conditioned and thereby made more suitable for processing, however, as soon as a consignment starts to exhibit senescent sweetening it must be used almost immediately, or the consignment is lost. A better understanding of the mechanism of senescent sweetening would enable us to identify strategies to predict, and possibly to delay its onset.

Metabolic and compositional changes occur in stored tubers during storage as they progress beyond the end of dormancy in the presence of sprout suppressants. The prevention of sprouting and maintenance of the tuber beyond its normal lifetime

imposes physiological stress, and one of the symptoms is the progressive inability of tissues to protect themselves against oxidative stress. At some point these stresses reach a level where the tissues can no longer be maintained, the cellular system starts to break down and sugars start to accumulate. For the potato industry it is economically very import to understand more about this process, critically to understand why varieties differ in the rate at which they reach this point, and to identify early indicators to allow more efficient management of the crop.

14.1. Collection of (existing) information to elucidate the mechanism of senescent sweetening

In the preparation of this review certain questions were highlighted that if answered could improve our understanding of the process of senescent sweetening. Some of this information may already exist, but has not been collected together in one place.

- It would be very valuable to collate more information on cultivar tendency for senescent sweetening, and also on seasonal patterns of its occurrence. Some information on cultivar tendency has been presented, but an expansion of this information would be useful. There is anecdotal evidence that some growing seasons result in more cases of senescent sweetening, but to the knowledge of the authors this information has never been collated.

- Senescent sweetening occurs after dormancy break, and therefore practically can only ever be observed in the presence of sprout suppressants. The occurrence of senescent sweetening does not appear to be specific to particular sprout suppressants, but to the knowledge of the authors there have never been comparative studies of the rate of the process over the range of sprout suppressants and mechanical removal of sprouts. It would be particularly interesting to determine the effect of continuous exposure to ethylene, as used for control of sprout growth, on the rate of development of senescent sweetening.

- The ratio of glucose and fructose accumulation can give information about the biochemical mechanism of starch breakdown, but there is very little information on this ratio in the case of senescent sweetening.

14.2. New strategies to study senescent sweetening

- With advances in molecular biology, using techniques such as microarrays and real-time PCR, it is now relatively straightforward to follow gene expression patterns. A study of gene expression patterns at tuber harvest and during storage right to the point of senescent sweetening, would enable the key gene changes preceding the tissue breakdown to be identified. This would provide a very valuable route for assessing storage potential and progression to senescence. This approach is already being applied to identify susceptibility to low temperature sweetening (see Section 13.1.5). Comparative studies of varieties with different propensity to develop senescent sweetening would be very informative.

- The impact that mineral nutrition has on tissue senescence and sugar accumulation in varieties susceptible to senescent sweetening also merits study.

14.3. Methods for the early prediction of senescent sweetening

This review considered a range of methods currently used to assess tuber status during storage. In practice fry colour is the method of choice for the industry. Assessing the symptom however may give little or no notice of processing problems and will result in crop losses. It would be very valuable to identify methods that could detect the changes in tuber status before they impact on fry colour. Molecular methods as indicated above have the potential to provide such markers, and this should be a priority area of study. Although potato growers could not carry out the assessments themselves, it is likely that a relatively straightforward and cost-effective laboratory test could be developed.

A link between senescent sweetening and changes in the ability to protect against oxidative damage (antioxidant capacity) has been established. This provides possible strategies to follow tuber damage and give an early indication of senescent sweetening. Ascorbate concentration in particular should be investigated as a simple indicator of changes in capacity for oxidative protection. Chlorogenic acid balance in tubers may signal the onset of senescence and also deserves further investigation. Both ethane and ethylene have been used as indicators of aerobic lipid degradation in plant tissues (Konze and Elstner, 1978). Detection at concentrations below 10 ppb is now possible providing a more accurate indicator of tuber health.

APPENDICES

The appendices provide a more detailed description of the studies summarised in some of the sections above.

15. APPENDIX I

The theory of senescent sweetening suggests that as the tubers age breakdown of cellular structure, specifically the membrane surrounding the amyloplast, facilitates access of enzymes to the starch granule. The most common theory is that the membrane breakdown occurs as a result of “oxidative damage” (Kumar and Knowles, 1993 b; Sowokinos *et al*., 1987). Other physiological changes that occur as potato tubers undergo aging lead to increased respiration and changes in soluble protein content. All these aspects are discussed in further detail below.

15.1. Antioxidants and free radical scavengers

Antioxidants are well known for their scavenging capabilities of reactive oxygen species (ROS), including the superoxide radical anion ($O_2^{-}\cdot$), the hydroxyl radical ($OH\cdot$), hydrogen peroxide (H_2O_2) and singlet oxygen thus preventing damage to plant cell membranes maintaining cellular compartmentalisation.

Under conditions of oxidative stress, the amounts of different antioxidants strongly increase in plants, and it is believed that this results in an increased capacity to scavenge reactive oxygen species (Noctor and Foyer, 1998). Potato varieties (Norchip and Monona) with higher antioxidant content appear to be more resistant to damage having the lowest amount of electrolyte leakage and sugar content compared to varieties Russet Norgold and Red Pontiac that are considered to be low in antioxidants (Spychalla *et al.,* 1990).

Antioxidants can be divided on the basis their hydrophilic and lipophilic properties. The hydrophilic antioxidant capacity of tubers is approximately ten fold higher (10-15 µM g^{-1} FW) than the lipophilic antioxidant capacity (0.2-0.5 µmol g^{-1} FW) Chu *et al*., (2002). Hydrophilic antioxidants are predominated by phenolic acids, flavonoids including anthocyanins and ascorbate, while the main component of the lipophilic fraction is comprised of various forms of vitamin E and carotenoids. A range of antioxidant contents for potato have been reported and are highly specific to cultivar. A list of some of the major antioxidant classes are detailed below:

15.1.1. Phenolics

The hydrophilic proportion of antioxidants is correlated highly with the content of polyphenols quercetin and chlorogenic acid of potatoes (Al-Saikhan *et al*., 1995). Chlorogenic acid predominates making up 80% of the total phenolic acids in tubers, but red and purple fleshed potatoes are also high in scopolin, caffeic, cryptochlorogenic and ferulic acid. White fleshed varieties range in phenolics between 60 and 394 µg 100 g^{-1} FW with the biggest chlorogenic acid component of white flesh potatoes ranging from 26 to 329 µg100 g^{-1} FW (Blessington, 2002).

15.1.2. Vitamin E

The lipophilic antioxidant component is largely made up of vitamin E, carotenoids and alpha lipoic acid. Vitamin E encompasses four forms each of tocopherol (α,β,δ and γ

tocopherol) and tocotrienol. Tocopherols appear to be synthesised in the inner membranes of chloroplasts and its is suggested that its role in plants is to protect pigments and proteins associated with photosynthesis (Kanwischer *et al*., 2005).

15.1.3. Carotenoids

Carotenoids are a major source of lipophilic antioxidant in potato flesh and range in concentration from 50-100 µg $100g^{-1}$ FW (Blessington, 2002) in white flesh tubers and 150-250 µg $100g^{-1}$ FW in yellow flesh tubers. Xanthophylls (violaxanthin, zexanthin and lutein) make up the largest component of carotenoids, however, very little pro-vitamin A (α-carotene or β-carotene) is present in tubers. The carotenoid content is partly regulated by the activity of carotenoid cleavage dioxygenase 4 enzyme, down regulation of this enzyme increases carotenoid content (predominately violaxanthin) 2- to 5 fold (Campbell *et al*., 2010).

15.1.4. Flavonoids including anthocyanins

Red and purple fleshed Andean potatoes have high amounts of flavonoids; a family of antioxidants containing anthocyanins, catechins and epicatechins. Flavonoids are synthesised via phenylalanine through the phenylpropanoid pathway. Red and purple fleshed potatoes constitute up to 60 µg $100g^{-1}$ FW of catechin and epicatechin but only half that amount is found in white fleshed potatoes. Anthocyanins are the predominant flavonoids in potato and are responsible for the red and purple pigmentation that is either found in the skin and (or) flesh. Total anthocyanin content in unpeeled red potatoes ranges between 8 to 38 mg 100 g^{-1} FW (Rodriguez-Saona *et al.,* 1998). Anthocyanins in red fleshed potatoes contain predominately glycosides (3-glucosidase sugar-linkages) of pelargonidin and peonidin, the former has only weak antioxidant activity (Wang, 1997), while purple fleshed varieties are also high in acylated glycosides of malvidin and petunidin and delphinidin (Lachman, 2005). It is therefore unsurprising that red and purple fleshed potato varieties are generally high in antioxidant capacity but content is highly specific to cultivar. Studies comparing the total antioxidant capacity (µmol of TE:Trolox equivalents per gram FW) of unnamed red versus white potato varieties (Wang, 1997) found total antioxidant capacity similar to white potatoes (10.98 and 10.59 µmol of TE:Trolox equivalents/g FW, respectively), while Jemison (2009) reports that white fleshed potato variety Kennebec have half the total antioxidant activity of purple fleshed variety Purple Majesty. The skin of purple fleshed varieties have on average twice the total antioxidant capacity (µmol of TE) than the corresponding flesh.

15.1.5. Vitamin C

Vitamin C (L-Ascorbic acid) is a primary metabolite in potato that functions both as an antioxidant, an enzyme cofactor and modulator of cell-signalling events in a range of physiological processes. Dale *et al*., (2003) investigated the ascorbic acid content of European potato varieties and selected breeding lines and found that ascorbic acid provided up to 13% of the total antioxidant capacity of the tuber with concentrations ranging from 13 to 36 mg 100 g^{-1} FW. The distribution of vitamin C is not uniform with higher concentrations in the apical end compared to the stem end (Sweeney *et al*., 1969).

15.1.6. The role of antioxidants during aging and stress

The antioxidant systems capable of defending tissue against free radical oxidation during storage have been investigated by Spychalla and Desborough (1990b) who observed that superoxide dismutase, catalases and vitamin E (α tocopherol) increased during tuber aging after 40 weeks of storage at 9°C and in response to low-temperature storage at 3°C. The rise in antioxidant systems occurs at the same point as increases in lipid peroxidation in tubers suggesting damage can occur before *de novo* synthesis of scavenging compounds. Dipierro and De Leonardis (1997) reported similar increase in catalase activity during prolonged storage and low-temperature storage, moreover the degree of lipid peroxidation was elevated at low temperature storage (3°C) but not at 9°C suggesting that greater damage to cell lipids occurs during low-temperature storage than occurs during senescence.

Dipierro and De Leonardis (1997) investigated the role of ascorbate (AsA) and AsA peroxidase activity and observed that conditions that induce AsA biosynthesis and its accumulation resulted in a decrease in lipid peroxidation. While increased synthesis of antioxidants can occur under stress over long-term storage concentrations decline. AsA content decreased during storage. A number of European potato varieties were observed to lose up to 43% AsA during 4 months storage at 4°C (Dale *et a*l., 2003). Long-term storage of potatoes at 20°C in the absence of sprout inhibitors led to a rapid decline in AsA during the first month of storage followed by a gradual decline thereafter (Delaplace, 2008). Such decreases in AsA content are observed in many plant systems undergoing senescence (Borraccino, 1984). Physical stress such as bruising and mis-handling of potatoes can accelerate AsA loss during storage (Mondy *et al*., 1987), while AsA breakdown appears to be even greater during low-temperature stress.

Total free radical scavenging capacity can be maintained throughout the storage period by increased activity of other scavenging compounds: superoxide dismutase, catalases and glutathione transfersases (Dipierro and De Leonardis, 1997). But the activity of individual scavengers appears to fluctuate throughout the storage period.

Some of the highest antioxidant content in potato have been attributed to pigmented tubers belonging to Native Andean potato (*S.tuberosum L*. and *S.phureja*) cultivars. These exhibit a diverse range of flesh and skin colours and antioxidant content which has been linked to drought tolerance (Andre, 2009a). While the antioxidant content of yellow fleshed tubers ‘Sipancachi’ was weakly effected by drought, other pigmented varieties; red fleshed ‘Sullu’ and purple fleshed ‘Guincho Negra’ showed varying degrees of antioxidant and polyphenols reduction in response to drought conditions. Higher carotenoid content appears to confer greater resistance to drought than those high in anthocyanins. During periods of drought polyphenol content can decline unlike p-carotene, vitamin E and vitamin C content which are less affected by drought conditions (Andre, 2009a). Altered sucrose flux induced by the drought stress is partly responsible for the changes in gene expression of key genes in the anthocyanin pathway (Andre, 2009b). A large variation in antioxidants can occur under stress conditions and deficiencies in certain antioxidants (glutathione, ascorbate or tocopherol) can lead to compensatory increases in other antioxidants (Kanwischer *et al*., 2005).

15.2. Changes in cell membrane integrity

The double walled membrane of the amyloplast provides selective separation of the starch granule and the cytoplasm, the inner membrane is considered the site of transport proteins that control the movement of sugar-phosphate intermediates and inorganic phosphate. Sowokinos *et al*. (1987) investigated the ultra structure of amyloplasts of the variety Norchip stored over a 10 month period at 9°C. Loss of the amyloplast bi-layer was only observed at the end of the 10 months storage period while age related senescent sweetening was detected between 8-10 months storage. Stress caused by mechanical handling of potatoes also increased the amount of sucrose and glucose present in tubers without gross physical disruption of the amyloplast membrane integrity until late (10 months) into the storage season however, changes to the fine structure of membranes beyond the detection of Electron Micrographs may be sufficient to increase access to starch degrading enzymes before any physical differences is observed.

Similar changes to the structure of amyloplast membranes has also been reported in tubers subject to low-temperature stress (O'Donoghue *et al*. 1995) but since low-temperature sweetening is reversible they result from different mechanisms. The loss of membrane integrity was reported by Kumar and Knowles (1993b) to be initiated by free radical induced lipid peroxidation that lead to increase in electrolyte leakage within cells and increase starch and sucrose breakdown (Kumar and Knowles, 1993b). Moreover, loss of cell membrane integrity can alter the mobility and kinetic properties of membrane bound proteins such as transport proteins and ion channels forming electrochemical gradient and ionic imbalances (Carruthers and Melchior, 1986). Reduction in enzyme activity may lead to accumulation of reducing sugars caused by an imbalance between the extent of starch hydrolysis and the rate of utilization of the glucose and fructose pool through respiration or in starch synthesis.

The rate of electrolyte leakage can be seen as a measure of membrane permeability. The viscosity of membranes appears to be regulated by the degree of saturation of lipids in cell membranes. Spychalla *et al*. (1990) found that in four North American processing varieties (Monona, Red Pontaic, Norchip, Russet Norgold) total fatty acid unsaturation was correlated negatively with electrolyte leakage indicating that the more unsaturated the (less double bonds) the less electrolyte leakage. While an early study by Knowles and Knowles (1989) found a stronger negative correlation between the double bond-index of fatty acids (the degree of saturation) and electrolyte leakage.

The amount of free fatty acids (FFA) in cell membranes can increase several fold in tubers subject to low temperatures and artificial aging. In general increases in FFA reduce membrane fluidity leading to a breakdown in membrane permeability. This in turn increases fry colour. Spychalla *et al*. (1990) reported that changes to the amount of linolenic acid rather than overall FFA content of membrane lipids was a better indicator of potatoes storage potential and processing quality.

Lipid membrane peroxidation produces lipid-hydroperoxidases through hydroxyl radical formation; these enzymes often yield ethane, malondialdehyde and lipofuscin-like fluorescent compounds that accumulate in tissues as tubers age (Kumar and Knowles, 1993) and monitoring the evolution of these breakdown products may provide a measure of tissue stress and senescence.

Later studies by Fauconnier *et al*. (2002) reported that the role of membrane breakdown in senescent sweetening is less than clear and found that sucrose content

accumulated in vars. Brintje and Désirée at 20°C to the same extent in tubers exhibiting senescence (sprouting tubers characterised by loss of apical dominance and presence of daughter tubers) and those treated with sprout suppressants. Moreover, although the degree of fatty acid saturation increased with time it was not correlated with the activity of lipoxygenesase enzymes that are often associated with the hydroperoxidation of polyunsaturated fatty acids. However, the evidence suggests that the process of sprout development had a large influence on the integrity of cell membranes. This warrants further investigation in the relationship between tubers' propensity to sprout and development of senescent sweetening.

15.3. Age-induced proteolysis

The soluble protein content of tubers decreases with age due to a combination of increased rates of proteolysis and reduced synthesis of new proteins. Seed tubers stored at 4°C (95% RH) for up to 32 months exhibited a decrease in soluble protein content which was correlated with an increase in free amino acid content suggesting in part an increased rate of proteolysis (Kumar and Knowles, 1993).

The rate of proteolysis is governed by cell membrane integrity: loss of cellular compartmentalisation allows free access of proteases to membrane bound proteins. Furthermore, structural modifications to proteins can confer increased access of proteases or may increase protease activity (Dalling, 1987). In response for the need to identify indices of physiological age (Delaplace, 2009) used two-dimensional electrophoresis to determine changes to the proteosome during aging in seed potatoes stored at 4°C for 270 days where changes in the physiological age index (PAI) of tubers had increased from 0.14 to 0.83. Once aging of potatoes had reach a PAI of 0.6 marked changes in the rate of proteolysis of patatin was observed. Moreover, changes to the gylcolytic pathway occurred concomitantly and enzymes responsible for combating oxidative stress. It appears that increases in the antioxidant potential of tubers allows for increased capacity to deal with free radical production associated with the aging process.

Patatin is a glycoprotein that forms the largest (20-40%) soluble protein component of tubers (Paiva *et al*., 1983). The protein is conjugated with small complex glycans: xylose, fucose, mannose and N-acetylglucosamine and is primarily expressed within the vacuole of tubers. Patatin has multiple roles in the tuber and was first reported to have acyl hydrolase activity by Galliard (1971) who demonstrated patatin's ability to catalyse the deacylation of a range of lipids. Later, patatin's function as an esterase was identified by Racusen (1986). Patatin's role has since expanded to include defence against fungal pathogens through its ability to act as an acidic β-1,3-glucanase (Tonón *et al*., 2001) and patatin-like proteins from cow-pea have been induced during periods of drought stress (Matos *et al.,* 2001) which suggests that patatins may have a wider role in responding to stress (Shewry, 2003). Patatin is also reported to have strong antioxidant activity (Al-Saikhan *et al*., 1995). The consequence for patatin loss during storage is not known however, Knowles and Knowles (1989) suggested that the loss of patatin's antioxidant activity may lead to increased membrane breakdown.

The extent of proteolysis is affected by potatoes' ability to synthesise protease inhibitors. Apart from patatin potato multicystatin (PMC), a cys-proteinase inhibitor, is a protein that declines with tuber age (Weeda *et al*., 2007; 2009). PMC binds and inhibits multiple proteinases simultaneously (Walsh and Strickland, 1993). If

proteolyses causes the age-induced decline in protein content as Kumar *et al*., (1999) suggest then the loss of PMC may result in increased proteolysis.

15.4. Age-induced rise in respiration

It is often observed that an increase in respiration rate occurs during the initial stages of sprout formation (Kumar and Knowles 1993c) and this rise in respiration is common to tubers that have been desprouted (Kumar and Knowles, 1996a). Respiration rates of old seed tubers (18 months old) are higher (53%) than those stored for 6 months (Kumar and Knowles, 1996a). Older tubers produce and utilise more ATP and the increase in respiration is associated with an intact coupled cytochrome pathway which rules out the respiration rise resulting from cellular dysfunction (Kumar and Knowles, 1996a). Higher respiration rates in older tubers may be a consequence of tubers struggling to maintain an adenylated energy charge (AEC) similar to younger tubers and therefore utilising more ATP (Kumar and Knowles 1996a). Moreover, increases in oxidative stress and loss of cell membrane integrity lead to greater electrolyte leakage allowing access to membrane bound ATPase enzymes thus providing a greater sink for ATP and elevating respiration. Raised respiration rates of older tubers are often accompanied by increased activity of glutathione scavenging of free radicals (Kumar and Knowles 1996 b).

15.5. Starch breakdown during aging

Starch consists of large molecules comprising chains of glucose; either as straight chains (amylose) or branched chains (amylopectin). Within tuber cells, the glucose chains are rolled up and tightly packaged into an amyloplast. The process of starch breakdown (mobilisation) depends on some mechanism for improving enzyme access. In the case of senescent sweetening, it has been suggested that as a result of cellular senescence a breakdown of amyloplast structure could contribute to sugar accumulation. The mechanisms of starch breakdown are very complex, involving up to 40 isozymes. The process has been reviewed by Smith *et al*. (2005). They indicate that the mechanisms involved differ between tissues, and that the mechanism in sprouting potato tubers is not fully understood. More recently, Kotting *et al*. (2010) have summarised the current level of knowledge on the enzymes of starch metabolism and their control mechanisms. Although the mechanisms have not been verified for potato tubers, they are likely to be similar. Chemical modification by phosphorylation of the surface of the starch granule occurs, catalysed by the enzymes glucan water dikinase (GWD) and phosphoglucan water dikinase (PWD). Phosphate groups attached to glucose molecules within the amylopectin structure are involved in the tight packaging of the molecule. Therefore the phosphorylating activity of GWD and PWD alters the conformation of the granule surface allowing access of other enzymes involved in the starch breakdown. The activity of GWD itself is controlled by redox state and phosphorylation. Starch granule phosophorylation does appear to be involved in sprouting potato tubers.

Most of the evidence for senescent sweetening has been correlated with an age-related decline in cell membrane integrity, however, there is limited evidence for increase in phosphorylase activity in long-term stored potato. In seed potatoes increased activity of alpha-1,4-glucan phosphorylase (EC 2.4.1.1) was investigated by Kumar and Knowles (1997). A 60% increase in the phosphorylase activity of potatoes

stored between 5 to 30 months was reported and this was attributed to the L- isoform of alpha-1,4-glucan phosphorylase.

16. APPENDIX II

16.1. Calcium nutrition, tuber quality and senescence

Calcium nutrition has been linked to a number of physiological traits in potato. Dyson and Dygby (1975) identified that the application of calcium salt solutions reduced the loss of apical dominance. Russet spot, internal heat necrosis internal browning and chocolate spot all refer to physiological disorders caused by a lack of calcium (Collier *et al*., 1987; Yencho *et al*., 2008).

Calcium is readily transported in xylem cells and is responsible for cell to cell cohesion: divalent calcium ions form cross-bridges with carboxyl groups on non-esterified regions of homogalacturonan molecules (Figure 5) to form 'Junction zones' (Morris *et al.*, 1982). Calcium cross-bridges, bind polymers together in the middle lamella to form a rigid gel that enhances cell-to-cell cohesion (Carpita and Gibeaut, 1993; McNeil *et al.*, 1984; Seymour and Gross, 1996; Siddiqui and Bangreth, 1996).

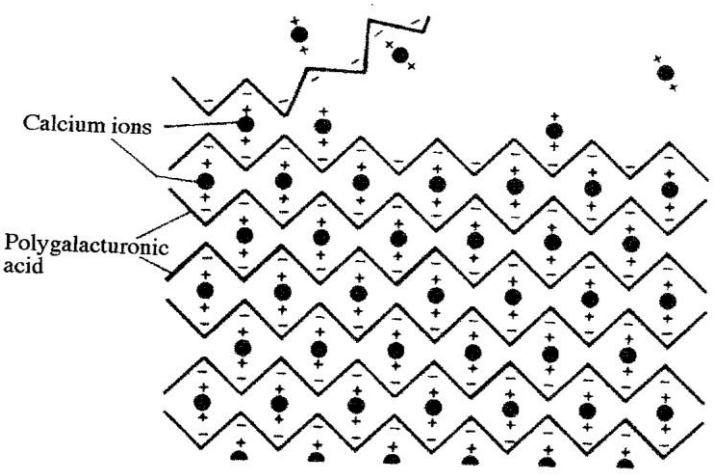


FIGURE 5. THE EGG-BOX MODEL OF PECTIN-CALCIUM INTERACTIONS (MORRIS *ET AL*., 1982).

The egg box pectin/ Ca^{2+}-configuration offers a spatial arrangement that allows certain proteins to bind. The greater the number of calcium cross-bridges, the greater the cementing ability of pectin (Satoh, 1998). Pectate lyase, a non-structural cell wall protein, is also able to bind to calcium (Scavetta *et al*., 1999).

16.1.1. Importance of calcium nutrition in combating senescence

In other reproductive organs (apple and pear) a wealth of research has been conducted on the role of calcium and other nutrients in storage potential and its particular role in maintenance of cell to cell cohesion and the impact on tissue firmness and senescence. In apple (Hipps and Perring, 1989) fruits low in calcium are prone to a range of senescent breakdown type disorders and the development of bitter bit, a form of localised cell death (Perring and Pearson, 1987). In apple the distribution of calcium in not uniform with zones of depletion around the calyx end (Perring, 1981) of the fruit which is prone to the onset of senescence. Moreover during storage calcium re-mobilises and moves towards the central cortex leading to zones of depletion in the core and outer cortex; regions susceptible to developing senescence

(Perring, 1984b; 1985). The effectiveness of calcium in maintaining cell to cell cohesion and as a secondary messenger is also affected by other divalent and monovaliant ions (Stow, 1989). Excess potassium or magnesium competes with calcium for binding sites leading to displacement of calcium. During storage not only is a high calcium content (5 mg/100g) important for maintenance of quality during long-term storage, but the ratio of potassium to calcium to must not exceed 30:1 (150 mg/100g FW).

16.1.2. Evidence for calcium's role in sugar accumulation

Calcium has also been linked to sugar unloading in cells. In apple sorbitol is the primary product of photosynthesis. Sugars are transported from leaves through sieve elements within the phloem network and unloaded in sink tissues (fruits or storage organs). Unloading of sorbitol in apple occurs in the apoplast before it is transported across the plasmallema where conversion to fructose by sorbitol dehydrogenase takes place. Apples low in calcium, and specifically the calyx end of fruit prone to low-calcium, are at greater risk of sorbitol accumulating within the apoplast and interstial air spaces (Perring *et al*., 1984; Perring 1984a). This causes a glassy appearance often referred to as water-core. A similar low calcium water core disorder associated with sugar accumulation has been reported in melon (Serrano *et al*., 2002).

16.1.3. Interaction of calcium with other mineral nutrients and organic acids

Calcium is affected by other mineral nutrients, magnesium and potassium, that displace calcium from the pectin matrix between cell walls leading to weaker cell to cell cohesion (Stow, 1989) and accelerated rates of tissue senescence. Moreover, conjugation of calcium by oxalate ions can also lead to loss of cell wall strength and is implicated in increasing in the flexibility of cell walls (McNeil *et al*., 1984), which is required during expansion of shoots and roots. Oxalate is formed through the oxidation of ascorbic acid which initially leads to the formation of the monodehydroascorbate (MDA) radical. MDA forms ascorbate and dehydroascorbic acid (DHA). The latter is unstable and breakdowns to form oxalic acid (Smirnoff, 1996).

16.1.4. Factors affecting calcium uptake and distribution

Calcium content of storage and reproductive tissue can be affected by competition with shoot growth. Summer pruning of apple shoots increases the amount of calcium in fruits, either through reducing the amount of calcium in new shoot growth or reducing gibberellin content. Gibberellins have been implicated in inhibiting calcium translocation (Saure, 2005). In potato calcium is transported to tubers via the xylem cells linked to stolon roots during tuber development, but is not transported through the periderm (Busse *et al*. 2006). Calcium content of tubers is generally low due to the low transpiration rate and calcium deposition in tubers is influenced strongly by competition with transpiring shoots. Under hot dry conditions less calcium is translocated into tubers leading to an increase in necrotic lesions (Busse *et al*., 2006).

Recently (2011), Subramanian *et al.,* investigated the 3-dimensional distribution of mineral content in potato tubers and reported that mineral content was not homogenous throughout the tuber. The skin contained 17% of the tubers' zinc, 34% of the calcium and 55% of the iron. More importantly, in the flesh mineral content was

higher at the stem end than the bud. Calcium was higher in the outer regions of the flesh but lower in the cortex. These findings are consistent with lack of movement of certain minerals in the tuber after vascular unloading.

Application of soluble calcium (69-168 kg ha^{-1}) in the field in the form of calcium chloride, calcium nitrate or a mixture of calcium chloride, urea, calcium nitrate has been successful in reducing necrotic lesions (Ozgen *et al.,* 2006). No difference in the uptake between calcium chloride or nitrate was reported. Studies in apple demonstrated that pre-harvest application of calcium nitrate conferred greater cell wall strength than application of calcium chloride. This was due to increased deposition of nitrogen in the cell wall, rather than improved uptake of calcium (Huxham, 1999). The role of mineral content, the distribution in potato tubers and its effect on sucrose and glucose/fructose content is not clear and would warrant further investigation.

17. REFERENCES

Alsaikhan, M. S., Howard, L. R. and Miller, J. C. (1995). Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum*, L). *Journal of Food Science,* 60(2), 341-348

Allen, E. J. and Obrien, P. J. (1986). The practical significance of accumulated day-degrees as a measure of physiological age of seed potato-tubers. *Field Crops Research,* 14, 141-151.

Allen, E. J., Bean, J. N., Griffith, R. L. and Obrien, P. J. (1979). Effects of length of sprouting period on growth and yield of contrasting early potato varieties. *Journal of Agricultural Science,* 92, 151-163.

Andre, C. M., Oufir, M., Hoffmann, L., Hausman, J.-F., Rogez, H., Larondelle, Y. and Evers, D. (2009a). Influence of environment and genotype on polyphenol compounds and in vitro antioxidant capacity of native Andean potatoes (*Solanum tuberosum* L.), *Journal of Food Composition and Analysis,* 22(6), 517-524.

Andre, C. M., Schafleitner, R., Legay, S., Lefevre, I., Alvarado Aliaga, C. A., Nomberto, G., Hoffmann, L., Hausman, J.-F., Larondelle, Y. and Evers, D. (2009b). Gene expression changes related to the production of phenolic compounds in potato tubers grown under drought stress. *Phytochemistry,* 70(9), 1107-1116.

Blenkinsop, R. W., Copp, L. J., Yada, R. Y. and Marangoni, A. G. (2003). A proposed role for the anaerobic pathway during low-temperature sweetening in tubers of *Solanum tuberosum*. *Physiologia Plantarum,* 118(2), 206-212.

Blessington, T., Hale, A. L. and Miller, J. C., Jr. (2002). Variability for antioxidant activity in potato (Solanum tuberosum) as influenced by genotype and storage. *Hortscience,* 37(5), 737-738.

Borraccino, G., Mastropasqua, L., Deleonardis, S. and Dipierro, S. (1994). The role of the ascorbic-acid system in delaying the senescence of oat (*Avena sativa* l) leaf segments. *Journal of Plant Physiology,* 144(2), 161-166.

Burch, R.S., A. Trzesicka, M. Clarke, J Stephen Elmore, A. Briddon, W. Matthews and N. Webber. (2008). The effects of low-temperature potato storage and washing and

soaking pre-treatments on the acrylamide content of French fries. *Journal of the Science of Food and Agriculture*, Vol. 88, 6 pp989-995.

Burton, W.G., 1989. Post-harvest physiology. In: Burton, W.G. (Ed.), The Potato, 3rd ed. Longman Scientific and Technical, Harlow, pp. 423–522.

Burton, W.G. and A.R. Wilson. (1970). The apparent effect of the latitude of the place of cultivation upon the sugar content of potatoes grown in Great Britain. *Potato Research*, 13, 269-283.

Busse, J. S. and Palta, J. P. (2006). Investigating the in vivo calcium transport path to developing potato tuber using Ca-45: a new concept in potato tuber calcium nutrition. *Physiologia Plantarum,* 128(2), 313-323..

Caldiz, D. O., Fernandez, L. V. and Struik, P. C. (2001). Physiological age index: a new, simple and reliable index to assess the physiological age of seed potato tubers based on haulm killing date and length of the incubation period. *Field Crops Research,* 69(1), 69-79.

Campbell, R., Ducreux, L. J. M., Morris, W. L., Morris, J. A., Suttle, J. C., Ramsay, G., Bryan, G. J., Hedley, P. E. and Taylor, M. A. (2010). The Metabolic and Developmental Roles of Carotenoid Cleavage Dioxygenase4 from Potato. *Plant Physiology,* 154(2), 656-664.

Carpita, N.C. & Gibeaut, D.M. (1993)**.** Structural modules of primary cell walls in flowering plants: consistency of molecular structure with physical properties of the walls during growth. *The Plant Journal* **3,** 1-30

Chu, Y. F., Sun, J., Wu, X. Z. and Liu, R. H. (2002). Antioxidant and anti proliferative activities of common vegetables. *Journal of Agricultural and Food Chemistry,* 50(23), 6910-6916

Carruthers, A. and Melchior, D. L. (1986) How bilayer lipids affect membrane-protein activity. *Trends in Biochemical Sciences,* 11(8), 331-335.

Copp, L. J., R. W. Blenkinsop, R. Y. Yada and A. G. Marangoni. (2000). The relationship between respiration and chip color during long-term storage of potato tubers *American Journal of Potato Research,* 77, 279-287.

Collier, G. F., Wurr, D. C. E. and Huntington, V. C. (1978) Effect of calcium nutrition on incidence of internal rust spot in potato. *Journal of Agricultural Science,* 91(AUG), 24

Dale, M. F. B., Griffiths, D. W. and Todd, D. T. (2003). Effects of genotype, environment, and postharvest storage on the total ascorbate content of potato (*Solanum tuberosum*) tubers. *Journal of Agricultural and Food Chemistry,* 51(1), 244-248.

Delaplace, P., Brostaux, Y., Fauconnier, M. L. and du Jardin, P. (2008a). Potato (*Solanum tuberosum* L.) tuber physiological age index is a valid reference frame in postharvest ageing studies. *Postharvest Biology and Technology,* 50(1), 103-106.

Delaplace, P., Fauconnier, M. L., Sergeant, K., Dierick, J. F., Oufir, M., van der Wal,

F., America, A. H. P., Renaut, J., Hausman, J. F. and du Jardin, P. (2009). Potato (*Solanum tuberosum* L.) tuber ageing induces changes in the proteome and antioxidants associated with the sprouting pattern. *Journal of Experimental Botany,* 60(4), 1273-1288.

Delaplace, P., Rojas-Beltran, J., Frettinger, P., du Jardin, P. and Fauconnier, M. L. (2008). Oxylipin profile and antioxidant status of potato tubers during extended storage at room temperature. *Plant Physiology and Biochemistry,* 46(12), 1077-1084.

Dipierro, S. and DeLeonardis, S. (1997). The ascorbate system and lipid peroxidation in stored potato (*Solanum tuberosum* L) tubers. *Journal of Experimental Botany,* 48(308), 779-783.

Dixon, W.L. and ap Rees, T. (1980). Carbohydrate metabolism during cold induced sweetening of potato tubers. *Phytochemistry*, 19, 1653-1656.

Driskill, E. P., Knowles, L. O. and Knowles, N. R. (2007). Temperature-induced changes in potato processing quality during storage are modulated by tuber maturity. *American Journal of Potato Research,* 84(5), 367-383.

Dyson, P. W. and Digby, J. (1975). Effects Of Calcium On Sprout Growth And Subapical Necrosis In Majestic Potatoes. *Potato Research,* 18(2), 290-305.

Fauconnier, M. L., Rojas-Beltran, J., Delcarte, J., Dejaeghere, F., Marlier, M. and du Jardin, P. (2002). Lipoxygenase pathway and membrane permeability and composition during storage of potato tubers (*Solanum tuberosum* L. cv Bintje and Desiree) in different conditions. *Plant Biology,* 4(1), 77-85.

Fuller, T.J. and J.C. Hughes. 1984. Factors influencing the relationships between reducing sugars and fry colour of potato tubers of cv. Record. International Journal of Food Science & Technology, Vol. 19, 4 pp 455-467

Galliard, T. (1971). Enzymic deacylation of lipids in plants - effects of free fatty acids on hydrolysis of phospholipids by lipolytic acyl hydrolase of potato tubers. *European Journal of Biochemistry,* 21(1), 90-94

Gillison, T. C., Jenkins, P. D. and Hayes, J. D. (1987). Some factors affecting the expression of the physiological age of potato seed tubers. *Journal of Agricultural Science,* 108, 437-451.

Groves, S., J. Wiltshire, A. Briddon and A Cunnington (2005). Managing maturity to improve crop processing quality and storage. *AHDB Potato Council report* 807/236, Kenilworth, Warwickshire, CV8 2TL, GB.

Hartmans, K. J. and Vanloon, C. D. (1987). Effect of physiological age on growth vigor of seed potatoes of 2 cultivars .1. Influence of storage period and temperature on sprouting characteristics. *Potato Research,* 30(3), 397-410.

Hertog, M., Putz, B. and Tijskens, L. M. M. (1997). The effect of harvest time on the accumulation of reducing sugars during storage of potato (*Solanum tuberosum* L.) tubers: Experimental data described, using a physiological based, mathematical model'. *Potato Research,* 40(1), 69-78.

Hipps, N. A. and Perring, M. A. (1989). Effects of soil-management systems and nitrogen-fertilizer on the firmness and mean fruit weight of cox orange pippin apples at harvest. *Journal of the Science of Food and Agriculture,* 48(4), 507-510.

Hogan, J.M. , Vose, L., Illyn, T.N., and Highlands, M.E. (1969). Frying colour of potatoes in relation to total solids and other compositional factors. U.S. Dept. Agr., ARS 73-65, pp. 143-146, In Potato Processing, 4th edition edited by W.F. Talburt and O. Smith, AVI publishing.

Huxham, I.M., Jarvis, C.M., Shakespeare, L., Dover, C.J., Johnson, D., Knox, J.P & Seymour, G.B. (1999). Electron-energy-loss spectroscopic imaging of calcium in the cell walls of apple fruits. *Planta,* 208, 438-443.

Iritani, W. M. and Weller, L. (1978) Influence of low fertility and vine killing on sugar development in apical and basal portions of Russet Burbank potatoes. *American Potato Journal,* 55(5), 239-246.

Jenkins, P. D., Gillison, T. C. and Alsaidi, A. S. (1993a). Temperature accumulation and physiological aging of seed potato-tubers. *Annals of Applied Biology,* 122, 345-356.

Jenkins, P. D., Gillison, T. C. and Alsaidi, A. S. (1993b). Temperature accumulation and physiological aging of seed potato-tubers. *Annals of Applied Biology,* 122, 345-356.

Jemison,J., Camire, M., and Dougherty M. (2009) http://umaine.edu/awaterquality/home/potato-research/potato-antioxidant-research

Kanwischer, M., Porfirova, S., Bergmuller, E. and Dormann, P. (2005) 'Alterations in tocopherol cyclase activity in transgenic and mutant plants of *Arabidopsis* affect tocopherol content, tocopherol composition, and oxidative stress', *Plant Physiology,* 137(2), 713-723.

Kotting, O., Pusch, K., Tiessen, A., Geigenberger, P., Steup, M. and Ritte, G. (2005) Identification of a novel enzyme required for starch metabolism in *Arabidopsis* leaves. The phosphoglucan, water dikinase. *Plant Physiology,* 137(1), 242-252.

Knowles, N.R and Knowles, L.O. (1989). Correlations between electrolyte leakage and degree of saturation of polar lipids from aged potato (*Solanum tuberosum* L) tuber tissue. Annals of Botany, 63, 331-338

Knowles, N. R., Driskill, E. R. and Knowles, L. O. (2009). Sweetening responses of potato tubers of different maturity to conventional and non-conventional storage temperature regimes. *Postharvest Biology and Technology,* 52(1), 49-61.

Kumar, G. N. M., Houtz, R. L. and Knowles, N. R. (1999). Age-induced protein modifications and increased proteolysis in potato seed-tubers. *Plant Physiology,* 119(1), 89-99.

Kumar, G. N. M. and Knowles, N. R. (1993a) Age of potato seed-tubers influences

protein-synthesis during sprouting. *Physiologia Plantarum,* 89(2), 262-270.

Kumar, G. N. M. and Knowles, N. R. (1993b) Changes in lipid-peroxidation and lipolytic and free-radical scavenging enzyme-activities during aging and sprouting of potato (*Solanum tuberosum)* seed-tubers. *Plant Physiology,* 102(1), 115-124.

Kumar, G. N. M. and Knowles, N. R. (1993c). Involvement of auxin in the loss of apical dominance and plant-growth potential accompanying aging of potato seed tubers. *Canadian Journal of Botany-Revue Canadienne De Botanique,* 71(4), 541-550.

Kumar, G. N. M. and Knowles, N. R. (1995) Nature and role of enhanced respiration during sprouting of aged potato seed-tubers. *Plant Physiology,* 108(2), 117-117.

Kumar, G. N. M. and Knowles, N. R. (1996a). Nature of enhanced respiration during sprouting of aged potato seed-tubers. *Physiologia Plantarum,* 97(2), 228-236.

Kumar, G. N. M. and Knowles, N. R. (1996b). Oxidative stress results in increased sinks for metabolic energy during aging and sprouting of potato seed-tubers. *Plant Physiology,* 112(3), 1301-1313.

Kumar, G. N. M. and Knowles, N. R. (1997). Changes in carbohydrate metabolism during aging of potato seed-tubers. *Plant Physiology,* 114(3), 402-402.

Lachman, J. and Hamouz, K. (2005). Red and purple coloured potatoes as a significant antioxidant source in human nutrition - a review. *Plant Soil and Environment,* 51(11), 477-482.

McNeil, M. Darvill, A.G., Fry, S.C. & Albersheim, P. (1984). Structure and function of the primary cell walls of plants. *Annual Review of Biochemistry,* 53, 635-663.

Matos, A. R., d'Arcy-Lameta, A., Franca, M., Petres, S., Edelman, L., Kader, J. C. and Zuily-Fodil, Y. (2001). A novel patatin-like gene stimulated by drought stress encodes a galactolipid acyl hydrolase. *FEBS Letters,* 491(3), 188-192.

Mikitzel, L. J. and Knowles, N. R. (1987). Effect of seed-tuber age on mobilization of carbohydrate reserves during sprouting. *Hortscience,* 22(5), 1126-1126.

Mondy, N. I., Leja, M. and Gosselin, B. (1987). Changes in total phenolic, total glycoalkaloid, and ascorbic-acid content of potatoes as a result of bruising. *Journal of Food Science,* 52(3), 631-633.

Morris, E.R., Powell, D.A., Gidley, M.J. & Rees, D.A. (1982**).** Conformations and interactions of pectins I. Polymorphism between gel and solid states of calcium polygalacturonate. *Journal of Molecular Biology,* 155, 507-516.

Noctor, G. and Foyer, C. H. (1998). Ascorbate and glutathione: Keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology,* 49, 249-279.

Obrien, P. J., Allen, E. J., Bean, J. N., Griffith, R. L., Jones, S. A. and Jones, J. L. (1983). Accumulated day-degrees as a measure of physiological age and the relationships with growth and yield in early potato varieties. *Journal of Agricultural*

Science, 10, 613-631.

Odonoghue, E. P., Yada, R. Y. and Marangoni, A. G. (1995a). Low-temperature sweetening in potato-tubers - the role of the amyloplast membrane. *Journal of Plant Physiology,* 145(3), 335-341.

Ozgen, S., Karlsson, B. H. and Palta, J. P. (2006). Response of potatoes (cv Russet Burbank) to supplemental calcium applications under field conditions: tuber calcium, yield, and incidence of internal brown spot. *American Journal of Potato Research,* 83(2), 195-204.

Paiva, E., Lister, R. M. and Park, W. D. (1983). Induction and accumulation of major tuber proteins of potato in stems and petioles. *Plant Physiology,* 71(1), 161-168.

Perring, M. A. (1980). Uptake of nutrients by the apple fruit. *Acta Horticulturae (Wageningen),* (92), 223.

Perring, M. A. (1981). Calcium and magnesium in the apple fruit - their distribution at harvest and redistribution during storage. *Journal of the Science of Food and Agriculture,* 32(10), 992-993.

Perring, M. A. (1984a). Lenticel blotch pit, watercore, splitting and cracking in relation to calcium-concentration in the apple fruit. *Journal of the Science of Food and Agriculture,* 35(11), 1165-1173.

Perring, M. A. (1984b). Redistribution of minerals in apple fruit during storage - preliminary investigations with the variety Spartan. *Journal of the Science of Food and Agriculture,* 35(2), 182-190.

Perring, M. A. (1985). Redistribution of minerals in apple fruit during storage - effects of late summer pruning, calcium sprays and low-temperature breakdown. *Journal of the Science of Food and Agriculture,* 36(5), 333-342.

Perring, M. A. and Pearson, K. (1987). Suppression of bitter pit by calcium injected into the core cavities of harvested apples. *Journal of Horticultural Science,* 62(3), 303-304.

Perring, M. A., Pearson, K. and Martin, K. J. (1984). The distribution of calcium in apples with watercore. *Journal of the Science of Food and Agriculture,* 35(12), 1326-1328.

Racusen, D. (1986). Esterase Specificity Of Patatin From 2 Potato Cultivars. *Canadian Journal of Botany-Revue Canadienne De Botanique,* 64(9), 2104-2106.

Rodriguez-Saona, L. E., Giusti, M. M. and Wrolstad, R. E. (1998). Anthocyanin pigment composition of red-fleshed potatoes. *Journal of Food Science,* 63(3), 458-465.

Rowe, R. C. and Powelson, M. L. (2002). Potato early dying: Management challenges in a changing production environment. *Plant Disease,* 86(11), 1184-1193.

Reust W. 1986. EAPR working group `Physiological age of the potato'. *Potato Research* 29: 268-271

Sabba, R. P., Bussan, A. J., Michaelis, B. A., Hughes, R., Drilias, M. J. and Glynn, M. T. (2007). Effect of planting and vine-kill timing on sugars, specific gravity and skin set in processing potato cultivars. *American Journal of Potato Research,* 84(3), 205-215.

Satoh, S. (1998). Functions of the cell wall in the interactions of plant cells: analysis using carrot cultured cells. *Plant Cell Physiology,* 39(4), 361-368.

Saure, M. C. (2005). Calcium translocation to fleshy fruit: its mechanism and endogenous control. *Scientia Horticulturae,* 105(1), 65-89.

Scavetta, R.D., Herron, S.R., Hotchkiss, A.T., Kita, N., Benen, J.A.E., Kester, H.C.M., Visser, J., & Jurnak, F (1999). Structure of a plant cell wall fragment complexed to pectate lyase C. *The Plant Cell ,*11*,* 1081-1092.

Scholte, K. (1987). The effect of crop-rotation and antigranulocytes nematicides on the incidence of *Rhizoctonia solani* in potato. *Potato Research,* 30, 187-199

Seymour, G.B. & Gross, K.C. (1996). Cell wall disassembly and fruit softening. *Postharvst News and Information* **7**, 45-52.

Serrano, M., Amoros, A., Pretel, M. T., Martinez-Madrid, M. C., Madrid, R. and Romojaro, F. (2002). Effect of calcium deficiency on melon (*Cucumis melo* L.) texture and glassiness incidence during ripening. *Food Science and Technology International,* 8(3), 147-154.

Shewry, P. R. (2003). Tuber storage proteins. *Annals of Botany,* 91(7), 755-769.

Shock, C. C., Holmes, Z. A., Stieber, T. D., Eldredge, E. P. and Zhang, P. (1993). The effect of timed water-stress on quality, total solids and reducing sugar content of potatoes. *American Potato Journal,* 70(3), 227-241.

Siddiqui, S. & Bangreth, F. (1995). Effect of preharvest application of calcium on flesh firmness and cell wall composition of apples - influence of fruit size. *Journal of Horticultural Science,* 70, 263-269.

Smirnoff, N. (1996a). The function and metabolism of ascorbic acid in plants. *Annals of Botany,* 78(6), 661-669.

.

Smith, A.M., Zeeman, S.C., and Smith, S.M. (2005). Starch degradation*. Annual Reviews of Plant Biology*.56: 73-98.

Sowokinos, J.R. 1978. Relationship of harvest sucrose content to processing maturity and storage life of potatoes. American Potato Journal, 55, 333-344.

Sowokinos, J. R., Orr, P. H., Knoper, J. A. and Varns, J. L. (1987) Influence of potato storage and handling stress on sugars, chip quality and integrity of the starch (amyloplast) membrane. *American Potato Journal,* 64(5), 213-226.

Spychalla, J. P. and Desborough, S. L. (1990a). Fatty-acids, membrane-permeability,

and sugars of stored potato-tubers. *Plant Physiology,* 94(3), 1207-1213.

Spychalla, J. P. and Desborough, S. L. (1990b). Superoxide-dismutase, catalase, and alpha-tocopherol content of stored potato-tubers. *Plant Physiology,* 94(3), 1214-1218.

Stow, J. (1989). The involvement of calcium ions in maintenance of apple fruit tissue structure. *Journal of Experimental Botany*, 40, 1053-1057.

Subramanian, N. K., White, P. J., Broadley, M. R. and Ramsay, G. (2011). The three-dimensional distribution of minerals in potato tubers. *Annals of Botany,* 107(4), 681-691.

Sweeney, M. R., McPartlin, J., Weir, D. G. and Scott, J. M. (2003). Measurements of sub-nanomolar concentrations of unmetabolised folic acid in serum. *Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences,* 788(1), 187-191.

Takeda, T. and Fry, S. C. (2004). Control of xyloglucan endotransglucosylase activity by salts and anionic polymers. *Planta,* 219(4), 722-732.

Tonon, C., Daleo, G. and Oliva, C. (2001). An acidic beta-1,3 glucanase from potato tubers appears to be patatin. *Plant Physiology and Biochemistry,* 39(10), 849-854.

Turnbull, N.D. and Cobb, A.H. (1992). A study of tuber cell integrity and ultrastructure during prolonged potato storage. Aspects of Applied Biology 33. Production and protection of potatoes.

Walsh, T. A. and Strickland, J. A. (1993). Proteolysis of the 85-kilodalton crystalline cysteine proteinase-inhibitor from potato releases functional cystatin domains. *Plant Physiology,* 103(4), 1227-1234.

Wang, H., Cao, G. H. and Prior, R. L. (1997). Oxygen radical absorbing capacity of anthocyanins. *Journal of Agricultural and Food Chemistry,* 45(2), 304-309.

Weeda, S. M., Kumar, G. N. M. and Knowles, N. R. (2007). Potato multicystatin may facilitate protein accumulation by regulating protease activity in developing potato tubers. *Hortscience,* 42(4), 919-919.

Weeda, S. M., Kumar, G. N. M. and Knowles, N. R. (2009). Developmentally linked changes in proteases and protease inhibitors suggest a role for potato multicystatin in regulating protein content of potato tubers. *Planta,* 230(1), 73-84.

Wiltshire, J., S. Groves and A. Briddon (2004). Pre-harvest fctors influencing tyber quality for post storage processing. *AHDB Potato Council report* 807/196, Kenilworth, Warwickshire, CV8 2TL, GB.

Wurr, D. C. E. (1978a). Effects of date of defoliation of seed potato crop and storage temperature of seed on subsequent growth .1. Sprout growth', *Journal of Agricultural Science,* 91, 737-745.

Wurr, D. C. E. (1978b). Effects of date of defoliation of seed potato crop and storage

temperature of seed on subsequent growth .2. Field growth', *Journal of Agricultural Science,* 91, 747-756.

Yencho, G. C., McCord, P. H., Haynes, K. G. and Sterrett, S. B. R. (2008). Internal heat necrosis of potato - A review. *American Journal of Potato Research,* 85(1), 69-76.