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**Effects of postharvest treatments on sweetpotato
(*Ipomoea batatas*) storage quality**

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Abstract

After harvest, sweetpotato (*Ipomoea batatas*) storage root quality is reduced due to weight loss, sprouting and rots. There are also hidden quality losses relating to loss of nutritional compounds. In order to maintain sweetpotato quality during storage, sweetpotatoes need to be stored at 13 - 15 °C and 80 - 90% RH. However, controlled temperature methods are difficult to achieve for subsistence farmers in less developed countries who have limited access to electric power. This work was undertaken to determine the potential postharvest techniques that would extend sweetpotato storage life without compromising phytochemical concentration. Postharvest treatments investigated in this work were hot water dipping (with or without coating) and ethylene (with or without 1-MCP) treatments. The work was undertaken using mainly 'Owairaka Red' and 'Clone 1820' sweetpotato cultivars. Following treatments, these sweetpotatoes were stored at 25 °C and 80 - 90% RH for 4 to 12 weeks.

Hot water dipping (HWD) at 51 °C for 11 min delayed sprout growth by 2 weeks but increased weight loss. Coating (carnauba wax 5%) significantly reduced weight loss, but increased sprout growth in 'Owairaka Red'. A combination of HWD and coating was effective in reducing both sprout growth and weight loss. β -carotene content measured in 'Clone 1820' ranged from 17.3 to 25.6 mg/100 g dry weight. The concentration was not affected by HWD or coating, but declined by about 30% during 12 weeks storage. The calculated retinol activity equivalent (RAE) ranged from 363 to 537 RAE, per 100 g of edible portion of sweetpotato. Based on the recommended daily intake for vitamin A, a serve of 100 g would supply more than 25% of daily retinol requirement for all age groups, suggesting that even after storage 'Clone 1820' is a good source of vitamin A. In addition, no treatment adversely affected the phenolic acid and anthocyanin concentrations. Roots that were HWD showed a subtle increase in total phenolic content, phenolic acids and anthocyanin concentration when compared to control roots, but the effect was short-lived.

Previous studies have demonstrated that ethylene is a potential sprout inhibitor, but causes darkening of flesh colour and the development of off-flavours after cooking. Ethylene-induced responses may be inhibited by 1-methylcyclopropene (1-MCP). 1-MCP and ethylene combined effects on sweetpotato physiological, flesh colour and phytochemical variables were assessed during storage. Ethylene treatment with or without 1-MCP inhibited sprout growth, increased root respiration rates by 2-fold, and caused root stem-end split leading to high weight loss and rots. Ethylene treatments also caused flesh darkening, and this was not prevented by a single 1-MCP ($1 \mu\text{L L}^{-1}$) pre-storage treatment. When roots stored in continuous ethylene were subjected to multiple 1-MCP ($1 \mu\text{L L}^{-1}$) treatments, the ethylene-induced root splitting and flesh darkening were delayed/reduced whilst maintaining minimal sprout growth. This implies that ethylene-induced negative responses in sweetpotato can be mitigated with on-going 1-MCP treatment. The sensory results showed that roots stored in air were highly preferred by consumers over roots stored in ethylene; nevertheless, acceptance means scores of all treatments were above five, indicating that ethylene-induced flesh darkening was not severe enough to cause consumer rejection.

Based on these findings, it is proposed that a combination of HWD and coatings can be used to extend non-refrigerated storage life of sweetpotato with no major effect on phytochemical content. The results on ethylene are consistent with previous findings that ethylene suppresses sprout elongation. However, the associated negative effects outweigh the benefits of using ethylene as a sprout control. Future research therefore should focus on finding ways to get the benefit of ethylene for sprout reduction without incurring risk of root splitting.

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List of abbreviations

1-MCP	1-Methylcyclopropene
ANOVA	Analysis of variance
CIP	International potato centre
DW	Dry weight
FeSO ₄ .7H ₂ O	Ferric sulphate
FRAP	Ferric reducing antioxidant power
<i>g</i>	Gravitational acceleration
Gy	Gray
HPLC	High performance liquid chromatography
HSD	Honestly significant difference
HWD	Hot water dipped
HWDC	Hot water dipped plus coating
OD	Optical density
Pa	Pascal
PAL	Phenylalanine ammonia-lyase
POD	Peroxidase
PPO	Polyphenol oxidase
RAE	Retinol activity equivalent
TPC	Total phenolic concentration
WPC	Whey protein concentrate

1 Introduction

1.1 Research Background

Sweetpotato (*Ipomoea batatas* (L.) Lam.), is an important crop for food security in many parts of the developing world. Sweetpotato has a short growth cycle (four to five months); yields well in marginal areas, and is drought tolerant. In addition to these agronomic advantages, sweetpotato roots are a good source of carbohydrates, dietary fibre, vitamins, and minerals (Woolfe, 1992). Sweetpotatoes also contain phytochemicals, which are good for human health. Despite all these nutritional and agronomic benefits, sweetpotato roots are highly perishable after harvesting (Rees et al., 2003). The major causes of product losses are weight loss, sprouting, and rotting (Ravi et al., 1996).

Control of sweetpotato postharvest losses has generally been reliant on the manipulation of storage temperature and relative humidity, with the aim of achieving an ideal storage temperature of 15 °C and a relative humidity of 80% to 95% (Picha, 1985a). In some cases, sprouting and rotting is controlled by the use of synthetic sprout inhibitors and fungicides, respectively (Woolfe, 1992; Afek et al., 1998). However, these control methods are difficult to achieve for subsistence farmers, whose villages are not connected to an electric power network. In addition, the use of chemicals is not only relatively expensive, but chemical residues can be of concern for human health and the environment. Hence, in order for sweetpotato to contribute significantly to food security in developing countries, there is a need to identify low cost storage technologies that might reduce root losses during sweetpotato storage.

1.2 Sweetpotato

1.2.1 Origin and production

Sweetpotato is a dicotyledonous vegetable plant belonging to the *Convolvulaceae* family. Its botanical origin is believed to be South or Central America, but the crop's cultivation is now worldwide. Based on production figures, sweetpotato is ranked as the seventh most important food crop worldwide, after wheat, rice, maize, potatoes, barley and cassava, whereas, in developing countries, sweetpotato is ranked as the fifth most important food crop (Woolfe, 1992). Annual global production is estimated at 105 million metric tonnes, with China producing over 80% of this total. Although

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production is high in China, 50% of the crop is used for animal feed. In contrast, sweetpotato produced in the Sub-Saharan African countries is mainly used for human consumption (Rees et al., 2003; Padmaja, 2009).

In New Zealand, sweetpotato, known as 'kumara' is a minor crop but it has cultural significance, since it was a staple food for Maori people before European contact. The introduction of other food crops such as maize and potato resulted in decline in sweetpotato production. Currently, it is estimated that 17,500 tonnes is produced annually, from 1500 hectares of land (Fresh Facts, 2013). Production is mainly for the domestic market, and the mean annual consumption is estimated at 4 kg/person/year. 'Owairaka Red' is the dominant variety (76% production by weight), and other significant cultivars are 'Beauregard' and 'Toka Toka Gold'. Crops are planted from November to December and harvested between March and April (Bourke, 2009).

In Malawi, sweetpotato is the third most important food crop after maize and cassava (Chipungu et al., 2002). Traditionally, maize has been the most important food crop, but the abolishment of maize input subsidies in the 1990s, as recommended by the World Bank and the International Monetary Fund, led to a steep increase in the retail price of fertiliser. Consequently, farmers could not afford to buy fertiliser for maize production. In response to these increased fertiliser prices and the droughts experienced during the 1991/92 and 1993/94 seasons, the Malawi government adopted a crop diversification strategy, promoting the cultivation of varied crops with higher yields under minimal inputs and having drought tolerance. Currently, sweetpotato is grown on more than 120,000 hectares, with a production of more than 1.2 million tonnes annually (Food and Agriculture Organisation & World Food Programme, 2005) and annual per capita consumption is estimated at 88 kg/person/year (Low et al., 2009). These figures may be on the high side, as FAO statistics appear to combine the figures for sweetpotatoes and potatoes (*Solanum tuberosum*). Although data for sweetpotato alone are not reported, sweetpotato is more widely grown in Malawi than potatoes.

Internationally sweetpotato is mainly grown for its underground storage roots but, in some countries the leaves are also utilised as food (Woolfe, 1992). In Malawi, both the roots and leaves are consumed. Roots are generally consumed after boiling or roasting.

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Sometimes they are processed into dry chips known locally as ‘makaka’ although when compared to cassava (*Manihot esculenta*) processing into dry chips is not a common practice for sweetpotato (Moyo et al., 1998).

Dominating sweetpotato production in Malawi are smallholder farmers, who rely on rain-fed agriculture for a single annual harvest. Planting is undertaken from November to March; and roots are harvested four to six months after planting, depending on each cultivar’s growth characteristics. However, the maintenance of produce quality for off-season markets and consumption is challenging. Farmers store the roots in earth pits treated with ash. In this method, roots are layered within an earth pit, each layer of roots being covered with ash which is gathered from cooking fires (Kwapata, 1983). Although pit storage helps to reduce the temperature and increase relative humidity, there is no accurate control of these parameters, and the pit stored roots are exposed to sub-optimal conditions at one or more periods during storage, consequently suffering increased quantitative and qualitative losses. Root losses of up to 50% have been reported under such conditions in Malawi, when roots were stored for a period of six months. The main losses were due to weight loss, sprouting, weevil damage, and rotting (Kwapata, 1983; Sandifolo et al., 1998).

Despite these storage challenges, sweetpotato production is being promoted, due to the crop’s wide adaptability, drought tolerance, and ability to yield under marginal soils with low external inputs. In addition, the nutritional versatility of the crop makes it a suitable candidate for nutritional programmes. Currently, in Malawi, the use of orange-fleshed sweetpotato is being promoted under the Irish Aid programme ‘Rooting out Hunger in Malawi with Nutritious Orange Fleshed Sweetpotato’ (Sindi et al., 2013) and the Malawi government and several non-governmental organisations (NGOs) are promoting the crop under crop diversification programmes. These efforts towards increased production will be compromised if the problems of storage are not dealt with in the near future. Understanding the general physiological changes that occur in sweetpotato roots during storage would help in the design of more suitable sweetpotato storage methods.

1.3 Phytochemicals in sweetpotato

Sweetpotato storage roots make important dietary contributions of carbohydrates, dietary fibres, minerals (calcium, magnesium, potassium and zinc), and vitamins: (C, B1, B2, E and A). In addition to these compounds, sweetpotato cultivars contain phytochemicals (Woolfe, 1992). Phytochemicals are bioactive non-nutrient compounds found in plants, of which the most widely distributed group consists of polyphenols (Liu, 2004; Scalbert et al., 2005). Apart from being responsible for plant colour, phytochemicals also protect the plant against biotic and abiotic stresses (Harrison et al., 2003; Bednarek & Osbourn, 2009). Based on their chemical structure, phytochemicals can be classified into a number of sub-groups such as carotenoids, phenolics, alkaloids and nitrogen containing compounds (Figure 1.1) (Liu, 2004). The phytochemicals found in sweetpotato (Section 1.3) and their potential human health benefits (Section 1.4) will be discussed in subsequent sections.

1.3.1 Carotenoids

Carotenoids are yellow, orange or red pigments responsible for the colour of many fruits, vegetables, and flowers. Orange- and yellow-fleshed sweetpotato cultivars are excellent sources of carotenoids. β -carotene, α -carotene, and β -cryptoxanthin are the major carotenoids found in sweetpotato, of which β -carotene is the most abundant (Chandler & Schwartz, 1988; Rodriguez-Amaya & Kimura, 2004).

1.3.2 Phenolic acids

Phenolic acids can be divided into two classes: (i) derivatives of benzoic acid, such as gallic acid, and (ii) derivatives of cinnamic acid, such as caffeic and ferulic acid. In sweetpotato storage roots, the cinnamic acid derivatives, for example, chlorogenic acid, caffeic acid, and three isomers of dicaffeoylquinic acids (diCQA): 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 4,5-dicaffeoylquinic acid are the main phenolic acids (Truong et al., 2007; Padda & Picha, 2008c; Champagne et al., 2011; Grace et al., 2014). Of these phenolic acids, chlorogenic acid is found in abundance. For instance, the chlorogenic acid content in roots of the cultivar 'Beauregard' was reported as 205.5 $\mu\text{g g}^{-1}$ DW, which was significantly higher than other acids: 3,5-diCQA (148.3 $\mu\text{g g}^{-1}$ DW), 4,5-diCQA (32.4 $\mu\text{g g}^{-1}$ DW), caffeic (8.9 $\mu\text{g g}^{-1}$ DW), and finally 3,4-diCQA (6.7 $\mu\text{g g}^{-1}$ DW) (Padda & Picha, 2008c). Phenolic acids are found both in the flesh and in the skin, with a higher concentration in the skin than the flesh. In terms of phenolic

acid composition, the skin contains a higher concentration of caffeic acid compared to that of the flesh (Harrison et al., 2003; Padda & Picha, 2008b).

1.3.3 Anthocyanins

Anthocyanins are water soluble flavonoids and are responsible for the blue, red, and purple colour of many fruits and vegetables (Harborne & Grayer, 1988). There are six main groups of anthocyanidins: based on delphinidin, peonidin, cyanidin, pelargonidin, petunidin, and malvidin (Francis & Markakis, 1989). Anthocyanins found in sweetpotato storage root have been characterised by several authors (Tian et al., 2005; Truong et al., 2009; Lee et al., 2013). These researchers found that cyanidin- and peonidin-based anthocyanins are the most abundant in sweetpotato roots. Tian et al. (2005) also found pelargonidin in lesser amounts in cv. Ayamurasaki. Truong et al. (2009) found that reddish-fleshed sweetpotato contain a higher concentrations of peonidin based anthocyanins, while cyanidin anthocyanins are high in purple-fleshed cultivars.

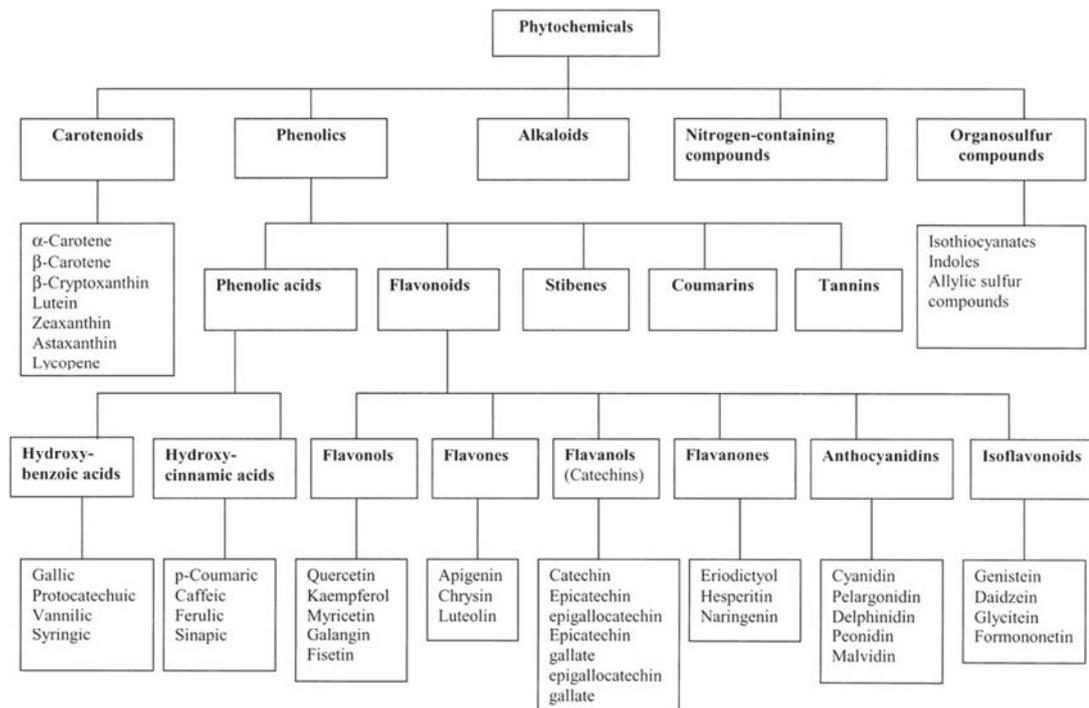


Figure 1.1: Classification of dietary phytochemicals (Liu, 2004)

1.4 Potential health benefits of sweetpotato consumption

Several studies have been conducted to determine the potential health benefits of sweetpotato. These studies have shown physiological functions resulting from both leaves and the storage roots. The focus of this study is on the physiological functions of storage root (Table 1.1).

Table 1.1: Sweetpotato derived compounds with the potential to modify aspects of human health

Health function	Related plant component	References
Antioxidative activity, radical scavenging activity	Polyphenol, anthocyanin, carotenoids	(Philpott et al., 2003; Huang et al., 2004; Philpott et al., 2004; Boo et al., 2005; Huang et al., 2006; Teow et al., 2007; Rautenbach et al., 2010)
Anti-mutagenicity/anti-carcinogenesis	Polyphenol, anthocyanin Purified protein	(Yoshimoto et al., 2001a; Yoshimoto et al., 2001b; Miyazaki et al., 2008; Park et al., 2011) (Li et al., 2013)
Neuroprotective	Polyphenol, anthocyanin	(Wu et al., 2008; Wang et al., 2010)
Reduction of liver injury (hepatotoxicity)	Polyphenol, anthocyanin	(Zhang et al., 2009b; Hwang et al., 2011)
Anti-inflammation	Polyphenol, anthocyanin	(Zhang et al., 2009b; Wang et al., 2010; Grace et al., 2014)
Anti-diabetic	Anthocyanin, polyphenol	(Matsui et al., 2002)

1.4.1 Radical scavenging and antioxidant activity

Free radicals are defined as atoms or molecules having at least one or more unpaired electrons that are therefore very unstable and highly reactive. Examples of free radicals are: hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, and hypochlorite (Halliwell & Gutteridge, 1999; Lobo et al., 2010). Overproduction of free

radicals can cause oxidative damage to biomolecules (lipids, proteins, DNA), eventually leading to many chronic diseases in humans such as atherosclerosis, cancer, diabetics, and cardiovascular diseases (Fang et al., 2002; Uttara et al., 2009). An antioxidant is a molecule stable enough to donate an electron to a charged free radical, and neutralising the free radical and reducing its capacity to damage cells (Lobo et al., 2010). It was hypothesised by Halliwell (1995) that plant dietary antioxidants delay or inhibit cellular damage mainly through their free radical scavenging properties. However, clinical studies involving supplementation of antioxidants such as β -carotene and vitamins C and E failed to prevent chronic diseases and in some cases antioxidant supplementation worsened the conditions (Bjelakovic et al., 2007; Tanvetyanon & Bepler, 2008; Lippman et al., 2009). Recent studies have suggested that the benefits of dietary antioxidants could be indirect by triggering changes in gene expression and up-regulating the body's defence mechanisms (Bohn et al., 2010). In sweetpotato, a significant positive correlation between total phenolic or anthocyanin content and antioxidant activity has been reported by several authors (Teow et al., 2007; Steed & Truong, 2008; Rumbaoa et al., 2009; Rautenbach et al., 2010). For example, Teow et al. (2007) reported R values of 0.937 between Oxygen radical absorbance capacity (ORAC) and Total phenolic content (TPC), and 0.870, 0.690 between antioxidant activity measured by 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) or 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and TPC respectively. Sweetpotato skin has been reported to have higher antioxidant activity than the root flesh (Padda & Picha, 2008b; Steed & Truong, 2008). This implies that sweetpotatoes are probably best consumed with the skin on; in particular food products where sweetpotatoes are being processed after peeling, the skin could be dried and used as a functional ingredient. Different sweetpotato cultivars have varying levels of antioxidant activity; Teow et al. (2007) reported antioxidant values ranging from 2.72 to 29.5 μmol trolox equivalent (TE)/ g FW. Greater antioxidant values were found in purple-fleshed and deep orange-fleshed cultivars, and lowest values were observed in white-fleshed cultivars. Oki et al. (2002), reported higher radical scavenging activity in purple-fleshed cultivars 'Ayamurasaki' and 'Kyushu' than in orange and white cultivars.

1.4.2 Anti-mutagenicity and anti-carcinogenesis

Cells in the body generally follow an orderly path of growth, division, and death. Cancer arises when there is breakdown of this systematic cell growth. Diets rich in fruits and vegetables have been associated with reduced risks of cancer occurrence. The proposed mechanism by which polyphenols or anthocyanins reduce cancer include: scavenging free radicals, suppressing cell proliferation, up-regulating/inducing apoptosis, and modulating activated protein kinase mitogen (MAPK) (Guo et al., 2009; Vauzour et al., 2010; Schauss, 2013). Sweetpotato extracts from the clone ‘P40’, which is rich in anthocyanin, have been reported to have anti-carcinogenic effects from both *in vivo* and *in vitro* studies. ‘P40’ extracts inhibited proliferation of human colon cancer SW480 cells by regulating the cell cycle (Lim et al., 2013). The authors verified their findings in *in vivo* studies, feeding mice with a sweetpotato rich diet. The results showed there was slower tumour (induced by azoxymethane) development in mice fed with a sweetpotato diet compared to the control. Sweetpotato also exhibit anti-mutagenicity properties, for example anthocyanin extracts purified from purple-fleshed sweetpotato ‘Ayamurasaki’ inhibited the mutations induced by tryptophan-pyrolysis (Trp-P)-1, Trp-P-2 (isolated from beef), and 2-amino-3-methylimidazo 4-5-f quinolone (IQ) (isolated from fish) mutagens (Yoshimoto et al., 2001b). In another study, Yoshimoto et al. (2001a) showed that both anthocyanin and organic acids from purple-fleshed sweetpotato inhibited the mutations induced by Trp-1 on *Salmonella typhimurium* TA. The anti-mutagenicity of cyanidin anthocyanidins was stronger than of peonidin. Among the three organic acids tested, caffeic acid showed the strongest anti-mutagenicity, when compared to ferulic and *p*-hydroxybenzoic acids. Apart from polyphenols and anthocyanins, sweetpotato proteins appear to possess anticancer effects. Li et al. (2013) showed that purified protein from sweetpotato roots inhibited proliferation of human colorectal cancer, both *in vitro* and *in vivo* (using an animal model).

1.4.3 Cardiovascular diseases (CVD)

Cardiovascular disease is a collective name referring to the diseases of the heart and blood vessels. Hypertension and atherosclerosis are the main causes of cardiovascular diseases. Atherosclerosis is the condition in which waxy plaque builds up inside arteries leading to narrow arteries and limiting blood flow. Heart attack and stroke are some of the diseases associated with a limited supply of blood to the heart and brain

respectively. There are several biomarkers associated with CVD, examples are: the ratio of low density lipoprotein cholesterol (LDL) to high density lipoprotein (HDL), regulation of blood flow (endothelial function), anti-inflammatory and antioxidant effects, platelet aggregation, blood pressure and gene expression (de Pascual-Teresa et al., 2010; Stoner et al., 2013). This suggests that controlling conditions causing chronic inflammation and blood pressure may also help in lowering the risks of cardiovascular diseases. Miyazaki et al. (2008) found that the area of atherosclerosis plaque in mice on a cholesterol-fat rich diet supplemented with 1% anthocyanin from 'Ayamurasaki' purple sweetpotato, was reduced by half, compared to the plaque area in a control group (without anthocyanin supplementation). In addition, the authors found that 'Ayamurasaki' extracts inhibited LDL oxidation *in vitro*. In another study, Park et al. (2010) showed that purple-fleshed sweetpotato extracts possess anti-diabetic and anti-atherosclerosis properties. Human macrophage cells treated with water or ethanol extracts from purple sweetpotatoes inhibited cupric-ion mediated LDL oxidation, and uptake of oxidised LDL. The authors also reported that purple sweetpotato extracts inhibited fructose-mediated protein glycation.

1.4.4 Anti-diabetic

Diabetes mellitus is characterised by high blood glucose levels. Inhibition of α -amylase activity, an enzyme responsible for the breakdown of starch into maltose, and of α -glucosidase activity, the enzyme responsible for then hydrolysing maltose into glucose, is a strategy to prevent postprandial hyperglycemia (Kim et al., 2008; Kumar et al., 2011). A study by Kusano & Abel (2000) indicated that the oral administration of white skinned sweetpotato extracts to obese Zucker fatty rats reduced their blood glucose, triacylglyceride and free fatty acid lactate levels. Matsui et al. (2002) found that anthocyanins from 'Ayamurasaki' sweetpotato displayed α -glucosidase (maltase) inhibitory activities with inhibitory concentration (IC) values of 200 μ M. The authors also studied the anti-diabetic effects from sweetpotato anthocyanins *in vivo*, showing that the blood glucose in rats administered with 'Ayaramusaki' anthocyanin (100 mg/kg) following maltose (2 g/kg) was 16.5% lower than the blood glucose within the control group. Ju et al. (2011) found that purple sweetpotato extracts showed anti-diabetic effect by suppressing the growth of adipocytes and the secretion of leptin.

1.4.5 Eye health and body immunity

The major carotenoids in sweetpotato are α - and β -carotene, which can be converted in the body to an active form of vitamin A (van Jaarsveld et al., 2005; Maiani et al., 2009). Vitamin A is involved in the immune function, vision, reproduction, and cellular communication (Ross, 2006). The recommended daily intake of vitamin A depend mainly on age and sex, but also people's genetics and lifestyle may be considered (Burri, 2011). The recommended nutrient intake of vitamin A ranges from 300 to 1300 μg retinol activity equivalent (RAE) per day (Table 1.2). Clinical studies in South Africa showed that the consumption of 125 g per day of boiled or mashed 'Resisto' sweetpotato, which contains 9980 μg β -carotene/100 g cooked root, improved the vitamin A status of children under the age of 10 years. At the end of the intervention programme, the proportion of children with a low serum retinol concentration ($< 0.70 \mu\text{mol/L}$) decreased from 71% to 50% (van Jaarsveld et al., 2005).

Table 1.2: Recommended nutrient intakes for vitamin A (μg RAE/day)

Age	Male	Female	Pregnancy	Lactation
0-6 months	400	400		
7-12 months	500	500		
1-3 years	300	300		
4-8 years	400	400		
9-13 years	600	600		
14-18 years	900	700	750	1200
19-50 years	900	700	770	1300
51+ years	900	700		

Source: (Institute of Medicine. Food and Nutrition Board, 2001)

RAE = retinol activity equivalent; 1 retinol activity equivalent = 12 μg β -carotene in food

1.4.6 Reduction of liver injury

Aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) serum activity are some of the biomarkers used in liver injury tests (Luo et al., 2014). Intake of 250 mL sweetpotato beverage (containing 400 mg of anthocyanin) daily for 8 weeks decreased the serum levels of GGT in healthy men with borderline hepatitis (Suda et al., 2007). In an animal model, the anthocyanin fraction

from purple-fleshed sweetpotato reduced dimethyl nitrosamine (DMN)-induced liver injury (Hwang et al., 2011).

1.4.7 Anti-inflammatory

Chronic inflammation is associated with different diseases, such as: rheumatoid arthritis, asthma, bladder cancer, contact dermatitis, and inflammatory bowel disease (Grivennikov et al., 2010; Schauss, 2013). Diets rich in fruits and vegetables have been shown to reduce the severity of some inflammatory related diseases (Giugliano et al., 2006; Lin & Tang, 2008; Guo et al., 2009; Mueller et al., 2010). A study by Wang et al. (2010) investigated the anti-inflammatory properties of extracts from purple coloured sweetpotato. Oral administration of sweetpotato extracts (350 mg/kg/day) in mice for 4 weeks significantly suppressed lipopolysaccharide (LPS)-induced cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expression in mouse brain. Extracts from purple sweetpotato also decreased the overproduction of tumour necrosis factor-alpha (TNF-alpha), interleukin-1 β (IL-1 β), and interleukin-6 (IL-6) in LPS-stimulated mouse brains. In a recent study, Grace et al. (2014) found a reduction in reactive oxygen species (ROS) in human neuroblastoma (SH-SY5Y) cells treated with 100 μ g/mL of sweetpotato extracts from different cultivars: NCPUR06-020, Covington, Yellow Covington, and NC07-847.

Although there is evidence from *in vitro*, animal, and human trials that sweetpotato has several physiological beneficial properties, most of the studies appear to have used extracts from uncooked sweetpotatoes, which is practical if sweetpotato are to be used for nutraceutical products. In addition, some studies have shown that there is a change in the concentration of several phenolic compounds with storage time (Ishiguro et al., 2007; Grace et al., 2014). Currently, there is limited information on how the changes in phenolic compounds during storage may influence sweetpotato physiological functions. Further investigation on physiological properties based on cooked samples is required. The anti-inflammatory properties of sweetpotato extracts from cooked sweetpotato will be investigated in this study.

1.5 Postharvest quality losses

Sweetpotato roots are susceptible to various storage disorders that reduce their quality and shorten their storage life. These disorders can be influenced by both preharvest and postharvest factors. Some of the disorders will be discussed in this section, and their control and prevention measures will be discussed in section 1.8.

1.5.1 Weight loss

Weight loss is the main postharvest loss in sweetpotato (Picha, 1986c; Ray & Ravi, 2005). Weight is lost as a result of respiration and transpiration processes; however, transpiration is the main contributing process to water loss in sweetpotato (Picha, 1986c). Water loss depends on relative humidity, temperature of the product and its surrounding environment, the anatomy and maturity of the product (Wills, 2007). Excessive water loss does not only affect the saleable weight but also makes the roots look unattractive due to shrivel (Ravi, Aked, & Balagopalan, 1996; Ray & Ravi, 2005). Sweetpotato roots can lose up to 10% of their fresh weight without appearing shrivelled (Rico et al., 2007), however in monetary terms this will lead to reduced profits in cases whereby roots are sold on a weight basis. In an effort to reduce water loss in horticultural products, edible coatings, modified packaging and stored at low temperature storage is used. However, use of edible coatings and modified atmosphere has not been extensively researched in sweetpotato .

1.5.2 Sprouting

Unlike potato tubers, the sweetpotato root does not undergo a dormancy period and can sprout any time after harvest if the conditions are conducive (Afek & Kays, 2004). Storage at higher temperatures and relative humidity promote sprout growth. Sprouted roots have a higher respiration rate, leading to greater weight loss and shrivelled roots (Woolfe, 1992; Edmunds et al., 2008). According to the United States sweetpotato standard, when a stored sweetpotato crop contains 10% or more roots with sprouts longer than 19 mm, it is regarded as a defect (United States Department of Agriculture, 2005). In New Zealand, sprouting is considered one of the grading criteria. For roots to be graded as TAG 1 (best quality), the percentage of roots with sprouts longer than 5 mm should be less than 5 % (Turners and Growers & MG Marketing, 2014). This shows that there are economic losses associated with sprouted roots, as they cannot be sold at a premium price.

1.5.3 Postharvest diseases

Sweetpotato roots are susceptible to both bacterial and fungal diseases. The relative importance of specific diseases differs with locality. There are also differences in time of infection; some pathogens affect the roots in the field, others at harvest or during storage. Pathogens may affect the nutritional value and storage life of the root. A detailed review of sweetpotato diseases has been conducted by Ray & Ravi, (2005). The most common postharvest disease, in both temperate and tropical countries, is the fungus *Rhizopus stolonifer* (soft rot) (Ray & Ravi, 2005). Roots may be rotten or have surface discoloration; as a result, their appearance is unattractive for both marketing and consumption. Soft rot infection occurs during harvest and storage. Its occurrence depends on the existence of wounds on the root, which act as pathogen entry points (Clark, 2013). Correct handling to minimise bruising and puncture wounds, then curing the roots before storage are important prevention methods for soft rot. In addition, heat treatments or fungicides (such as Dicloran) are used to control soft rot infection during storage and marketing.

1.5.4 Postharvest pests

Sweetpotato weevil (*Cylas formicarius*) is the most important pest sweetpotato pest worldwide, both in the field and during storage. The occurrence of sweetpotato weevil is localised and this pest has not been reported in New Zealand. Harvested roots infested with sweetpotato weevil may carry weevil eggs or larvae from the field and further root damage may occur after eggs hatch during storage. The larvae cause major damage, as they tunnel into the storage root, depositing waste, and giving roots a bitter taste from terpenes released by the insect. Late harvesting in Indonesia resulted in losses of over 40% due to sweetpotato weevil (Saleh et al., 2003). In Malawi, in pit storage, weevil damage losses of up to 30% have been reported (Sandifolo et al., 1998).

1.5.5 Other disorders

Other disorders include chilling injury, hardcore, and internal breakdown. Sweetpotato is a tropical crop, which suffers chilling injury when exposed to temperatures below 12 °C. Chilling injury symptoms are particularly noticeable when roots are subsequently transferred to higher temperatures. These symptoms include root shrivelling, surface pitting, fungal decay and internal tissue discoloration (Picha, 1987; Afek & Kays, 2004). Internal discoloration has been linked to the synthesis of chlorogenic acid and

other phenolic compounds (Lieberman et al., 1959). The severity of chilling injury depends on the temperature and the duration of exposure to chilling temperatures; it is also dependent on the cultivar (Picha, 1987). Hardcore can be defined as the failure of sweetpotato root tissue to soften during cooking. This disorder depends on the chilling conditions (temperature and exposure time) and the cultivar susceptibility to chilling conditions. Non-cured roots are more susceptible than cured roots (Broadus et al., 1980; Picha, 1987). A further disorder, internal breakdown, is characterised by reduced tissue density and sponginess, which is related to the depletion of storage carbohydrate, since it is used as a respiratory substrate (Afek & Kays, 2004). Exposure of sweetpotato roots to high temperatures and sprouting are two conditions that increase internal breakdown.

1.6 Physiological activities that affect sweetpotato quality during storage

1.6.1 Respiration

Sweetpotato roots have low respiration rates. However, exposure to harsh conditions such as mechanical damage (Ravi, 1994; Rees et al., 2001) and high temperatures (Baishya et al., 1992) can accelerate their respiration rate. For example, uncured roots stored at 25°C had a respiration rate of 54 mg CO₂ kg⁻¹ h⁻¹, and rate of 29 mg CO₂ kg⁻¹ h⁻¹ when stored at 15 to 16 °C. Damaged roots had a higher respiration rate than intact roots (Tomlins et al., 2002). Exposure of sweetpotato to ethylene increased respiration rates during one month of storage at 15 °C (Kitinoja, 1987). A high respiration rate not only shortens the storage life of roots, it also affects the internal and external appearance. The starch stored in the root is used as a substrate for respiration, resulting in declining dry matter content and increases in spongy root texture (Afek & Kays, 2004). Storage at an appropriate low temperature and high relative humidity, coupled with correct handling during harvest (in order to minimise bruising) are ways to reduce the respiration rate of sweetpotatoes after harvest (Ravi et al., 1996).

1.6.2 Transpiration

Transpiration involves the transfer of moisture through the outer layers of the produce to the surface, where it evaporates. Sweetpotato roots contain approximately 60% to 70% water depending on the cultivar (Woolfe, 1992). According to Picha, (1985) transpiration is the main process contributing to water loss during sweetpotato storage.

The rate of transpiration depends on the vapour pressure difference between the product and the surrounding environment (Wills et al., 2007). Therefore, storage at high relative humidity combined with low temperature is one method used to reduce water loss. However, as high humidity also favours fungal development care must be taken to balance the two parameters. Other control measures to reduce water loss include: waxing or coating, and modified packaging.

1.7 Preharvest factors that affect storage quality

1.7.1 Genotype effect

Storage potential of sweetpotato differs among cultivars (Rees et al., 2003). The differences are due to variations in the rate of respiration, transpiration, sprouting ability, and susceptibility to pathogens. Van Oirschot et al. (2006) found that different sweetpotato cultivars vary in their wound healing efficiency during curing. The ability of sweetpotato to heal a wound inflicted during harvesting and handling is vital to prevent excessive water loss and pathogen invasion during storage. Therefore, roots with greater wound healing responses will store longer.

The concentration of phytochemicals in sweetpotato roots is influenced by several factors, genotype being the most important. Based on 19 genotypes with distinctive flesh colours, Teow et al. (2007) found a wide variation in the concentration of β -carotene. White-fleshed cultivars contained very low amounts of β -carotene (less than 1 mg/ 100 g FW) as compared to orange-fleshed cultivars (more than 4.5 mg/100 g FW). Ishiguro et al. (2010), reported a range of 1.3 to 39.9 mg/100g DW for total carotenoids among 8 Japanese cultivars or breeding lines. In addition, the authors found that β -carotene constituted about 80 – 92% of the total carotenoids in orange-fleshed cultivars, while β -carotene 5,8;5',8'-diepoxide (32 – 51%) and β -cryptoxanthin 5,8-epoxide (11 – 30%) were the common carotenoids in yellow-fleshed cultivars/lines. Tomlins et al. (2012) reported a total carotenoid content in range of 0.12 to 21 mg/100 g DW. The authors also observed a decline in total carotenoids with increasing dry matter content.

Huang et al., (2006) analysed six cultivars grown commercially in Taiwan and reported anthocyanin contents ranging from 0.36 to 8.99 mg/100 g DW. Another study by Truong et al. (2009), reported high anthocyanin contents in the purple-fleshed cultivars: 'Stokes Purple' (46.3 mg/100 g FW) and 'NC415' (33.7 mg/100 g FW), and lower

amounts in 'Okinawa' (21.1 mg/100 g FW). Zhu, et al., (2010) reported anthocyanin contents ranging from 78.3 to 695.7 mg/100 g DW amongst the Chinese genotypes analysed; and anthocyanin was not detected in white-fleshed cultivars. Some cultivars have been specifically bred to have higher anthocyanin content, so they can be used as food colourants: examples are 'Radical', a New Zealand cultivar which has an anthocyanin content of 334.1 mg/100 g DW (Joyce et al., 2006) and 'Ayamurasaki', a Japanese cultivar which has an anthocyanin content of 59 mg/100 g FW (Suda et al., 2003). Anthocyanidins present in purple-fleshed sweetpotato have been characterised by several researchers (Yoshimoto et al., 1999; Truong et al., 2009; Lee et al., 2013). Sweetpotato peel also contains anthocyanins, Steed and Truong (2008), reported total anthocyanin of up to 174.7 mg/100 g FW in the peel, and 101.5 mg/100 g FW in the flesh. Philpott et al. (2003) analysed several anthocyanins in New Zealand grown cultivars and clones, and reported significant amounts of anthocyanin in both the peel and flesh of sweetpotato roots.

Phenolic acids are found in white-, orange- and purple-fleshed cultivars, but purple-fleshed cultivars contain more phenolic compounds than white-fleshed ones (Boo et al., 2005). Park et al. (2011) analysed 22 Korean cultivars with different flesh colours and reported a total phenolic content ranging from 4.8 to 128.3 $\mu\text{g/g}$ DW, with higher amounts in purple-fleshed cultivars. However, some non-purple cultivars had a relative high total phenolic content: 'Su' (21.4 $\mu\text{g/g}$ DW), 'Hayanmi' (21.5 $\mu\text{g/g}$ DW) and 'Shinhwangmi' (20.3 $\mu\text{g/g}$ DW). Total phenolic content of Philippine cultivars ranged from 1.93 to 11.59 mg (GAE)/g DW (Rumbaoa et al., 2009). Teow, et al. (2007) reported phenolic contents ranging from 0.003 to 0.949 mg of chlorogenic acid equivalent/g FW, with higher levels in purple-fleshed cultivars. The phenolic content of commercially grown cultivars in the United State, ranged from 1.4 to 4.7 mg/g DW (Padda & Picha, 2008c). These authors also analysed the phenolic acid composition, and identified chlorogenic acid, caffeic acid, 4,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 3,4-dicaffeoylquinic acid as the main phenolic acids found in sweetpotato roots. Similar findings have been reported by Truong et al. (2007).

These studies show that there is a wide variation among cultivars in terms of phytochemicals content, and that colour is an indicator of what type of phytochemicals

are present. However, amongst cultivars of the same colour there are also significant differences in phytochemical concentration and composition.

1.7.2 Cultural practices

Good cultural practices not only enhance crop yield, but also protect roots from pests and diseases. During sweetpotato storage root formation, cracks appear on the soil surface, and act as entry points for sweetpotato weevils. Hilling-up soil around the base of plants and filling soil cracks during root swelling helps control sweetpotato weevil entry, thereby reducing subsequent storage losses. Flooding in sweetpotato fields shortly before harvest increases rotting during curing and storage, raising the occurrence of end rot and internal necrosis (Ahn et al., 1980).

The phytochemical content of sweetpotato storage roots can be influenced by cultural practices. Time of harvest has been reported to affect β -carotene concentration; roots harvested at 12 weeks after planting had less carotenoid content than roots harvested at 16 weeks after planting (K'osambo et al., 1998). Low carotenoid content in early harvested roots has also been reported by Liu et al. (2009). However, in terms of carotenoid composition, there were no differences between early and late harvests. During both harvesting times, β -carotene was the most dominant carotenoid. Yoshinaga et al. (2000) reported that anthocyanin concentration increased with sweetpotato maturity. Roots harvested at three weeks after transplanting had very low anthocyanin levels compared to those harvested at the 9th and 17th weeks after transplanting. These results suggest that carotenoid and anthocyanin synthesis continues during the growing period. An early harvest could lead to less potential benefits through low carotenoid levels, in the case of orange-fleshed cultivars, and reduced anthocyanin levels in purple-fleshed cultivars.

Stress conditions may result in the stimulation of secondary metabolite synthesis as a defence mechanism (Schreiner & Huyskens-Keil, 2006; Wang & Frei, 2011). Drought stressed sweetpotato roots (30 % water deficit) had higher concentrations of β -carotene, vitamin C, chlorogenic acid, and greater antioxidant capacity (Rautenbach et al., 2010). The appropriate exposure of sweetpotato roots to drought/water stress conditions could be used as a tool to enhance phytochemical concentrations in sweetpotato, thus increasing the health benefits through increased antioxidant activity. However practical

applications may be limited, as drought can have negative effect on yield. There is a need for a thorough investigation into the most appropriate time to expose plants to drought/water stress, which will maximise both yield and phytochemicals. In another study, the total anthocyanin concentration of ‘Ayamurasaki’ grown under shade increased slightly, compared to roots from un-shaded plants. The shading effect on anthocyanin concentration was variable among cultivars, as for cultivar Jishu-18 no increase in anthocyanin was observed in roots from shade plants (Hou et al., 2010).

1.8 Postharvest factors that affect storage life and phytochemical concentration

1.8.1 Curing

The sweetpotato periderm is easily damaged during harvest and handling, and this leads to ‘skinning’ and increased susceptibility to decay (Woolfe, 1992; Cantwell & Suslow, 2013). Curing allows healing of wounds that occur during harvest, so curing is recommended for all sweetpotatoes intended for storage. Sweetpotato roots are cured in a controlled temperature room at 28 - 30 °C and relative humidity above 85 to 95% for three to seven days (Cantwell & Suslow, 2013). The optimal curing duration varies among cultivars (van Oirschot et al., 2006). In the tropics and sub tropics, curing is not a common practice, although in some areas curing is done by exposing the roots to sunshine for short periods (Woolfe, 1992). Wound healing in sweetpotato starts with desiccation of cells on the surface, followed by lignification of the underlying cell layers, and finally the formation of the wound periderm (Artschwager & Starrett, 1931). St Amand and Randle (1989) showed that lignification and wound periderm formation were suppressed in roots treated with 2,5-norbornadiene (ethylene action inhibitor) or aminooxyacetic acid (AOA) or cobalt chloride (CoCl₂) (to block ethylene biosynthesis). The authors concluded that ethylene is involved in lignification and wound periderm formation. The benefits of curing during sweetpotato storage include: a reduced rate of water loss and susceptibility to decay (Thompson & Scheuerman, 1993), reduced severity of hardcore disorder in sweetpotato roots exposed to chilling conditions (Broadus et al., 1980), and reduced chilling injury (Picha, 1987).

Curing also enhances sweetpotato flavour by modifying the activity of β - and α -amylase enzymes, which in turn facilitate starch hydrolysis during cooking and the formation of sugars that act as precursors for critical volatile flavour components (Walter, 1987;

Wang et al., 1998). Freshly lifted roots are high in starch and non-starch polysaccharides and low in sugars (Woolfe, 1992). In another study, phenolic compounds of the cultivar 'Beauregard' increased more in uncured roots than in cured roots, when stored at 5 °C. Total phenol content increased from 55.3 to 79.4 mg/100 g in cured roots compared to an increase to 125.8 mg/100 g in non-cured roots (Padda & Picha, 2008a). Lieberman et al., (1959), reported that when roots are exposed to low temperatures, the phenolic content increases as a response to the stress conditions. While exposure of non-cured sweetpotato to low temperature could be used as a tool to enhance sweetpotato health benefit compounds, its application could be limited to the functional food industry, as non-cured roots may not store long and are more susceptible to chilling injury when exposed to low temperatures.

1.8.2 Chemical treatments

Several chemicals have been used to control sweetpotato sprouting. Naphthalene acetic acid (NAA), applied at three different rates: 1, 10 and 100 mg NAA/g talc, inhibited sprouting in sweetpotato during 40 days storage at 25 °C (Paton & Scriven, 1989). Unfortunately, all the NAA treatments increased weight loss (Paton & Scriven, 1989). In another study, sweetpotato treated with different concentrations of sodium hypochlorite (NaClO): 0.33, 1, 3, 9% NaClO by volume with different immersion times: 20, 60 and 180 min, before storage for 4 months at 20 °C and 90% RH, reduced sprouting, however there was a compromise on other quality parameters, as weight loss and surface lesions increased (Lewthwaite & Triggs, 1995).

Dicloran (Botran) is a well-known fungicide that is used to control *Rhizopus* soft rot during storage and transportation of sweetpotato (Woolfe, 1992; Ray & Ravi, 2005; Brash et al., 2010). Welch et al. (1966) reported 1.7% decay in roots treated with Dicloran compared to 28% in the control group 2 weeks after inoculation with *rhizopus* spores. Edmunds and Holmes (2007) also showed that Dicloran was superior in controlling decay compared to Scholar and Pristine fungicides, which are considered to pose a lower risk to environment and human health. Iprodione is another chemical used as a disinfectant; fumigation of the storeroom with Iprodione reduced the root decay of 'Georgia Jet', a rot-susceptible cultivar grown in Israel (Afek et al., 1998).

Although the use of chemicals has been effective in controlling fungal infection and suppressing sprout growth during sweetpotato storage, their use is becoming limited due to increased consumer concerns for the consequences to human health, in relation to both chemical residues and environmental effects. As of January 2008, no detectable residues of Dicloran were allowed on exports to the European Union (EU). In the absence of other control measures to meet EU regulations, farmers will lose access to the EU market. Restrictions on conventional chemical use have revived research into alternate and non-chemical treatments such as heat treatments (Edmunds & Holmes, 2009).

1.8.3 Irradiation

Irradiation doses of 0.10, 0.125, 0.15 and 0.75 kGy suppressed sprout growth in roots stored at 21 °C for one month (Bonsi & Loretan, 1988). In another study, gamma irradiation doses between 0.2 to 1.0 kGy were effective in killing sweetpotato weevils, with no signs of root surface injury or decay in sweetpotato roots stored at 13 °C and 90% RH (McGuire & Sharp, 1995). There have been undesirable effects associated with irradiation; these include increased weight loss and decay, decreased ascorbic acid levels, starch content and discolouration of the flesh colour after cooking (Lu et al., 1986; Bonsi & Loretan, 1988; McGuire & Sharp, 1995; Hallman, 2001). Currently commercial applications of irradiation on sweetpotato are limited to quarantine treatments against the sweetpotato weevil (*Cylas formicarius*). Ionisation irradiation at a dose of 165 Gy was approved for treatment of sweetpotato against sweetpotato weevil in Hawaii (Hallman, 2001). Although, the use of irradiation has the potential to extend sweetpotato storage life, the cost for a facility and treatment, along with consumer acceptability, limits its application in the potato and sweetpotato industry (Bonsi & Loretan, 1988; Kleinkopf et al., 2003).

1.8.4 Heat treatment

Heat treatments have been developed mainly for insect disinfestations and fungal control (Lurie, 1998). However heat treatment has also been reported to inhibit undesirable postharvest growth such as sprouting during the storage of horticultural crops (Paull & Chen, 1999). There are three main methods of applying heat treatments: hot air (vapour heat and forced hot air treatment); hot water rinsing and brushing and hot water treatment (Fallik, 2004). The use of hot water dipping in sweetpotato has been

studied by Brash et al., (2010); Hu & Tanaka (2007); and Scriven, et al., (1988). Immersing sweetpotatoes in water for 30 min at 50 °C inhibited sprout growth, controlled decay and reduced weight loss (Hu & Tanaka, 2007). In another study, the following temperatures: 90 °C for 2 seconds, 80 °C for 2 seconds, 40 °C for 10 seconds, 70 °C for 10 seconds and 40 °C for 120 seconds, delayed decay of 'L0323' sweetpotato during storage at 25 °C (Scriven et al., 1988). Brash et al. (2010) showed that hot water treatment had the potential to control decay in sweetpotatoes, at a level comparable to that of the fungicide Dicloran. They tested a matrix of temperatures (50, 52.5, 55 and 57.7 °C) and dipping times (15, 30 and 45 seconds). The optimum treatments were 55 °C for 45 seconds. However weight loss was not measured, so the effect on other quality parameters is unknown. Similarly, heat treatment reduced rooting and sprouting in garlic (Cantwell et al., 2003) and in onion (Cantwell et al., 2001).

Heat treatment may lead to undesirable quality changes in fresh produce such as loss of flavour, appearance, firmness, vitamins and minerals (Rico et al., 2007). Sheibani et al. (2012) showed that immersing sweetpotatoes at 60 °C for 34 min, 70 °C for 30 min or 74 °C for 20 min increased the maltose concentration, indicating that at these temperatures the sweetpotatoes were cooked. In contrast, Hu & Tanaka (2007), did not find any significant differences in sugar concentration between the control and sweetpotato dipped in hot water for 30 min at 50 °C. In mango, hot water treatment at 50 °C for 60 min increased carotenoid content (Talcott et al., 2005). Ummarat et al. (2011) showed that hot water treatment at 50 °C for 10 min increased the concentration of ascorbic acid and glutathione in banana stored at 8 or 14 °C.

Overall, these studies have suggested that heat treatment has the potential for extending the storage life of sweetpotatoes, through reduction of fungal decay and sprout suppression. However, most of the studies were undertaken at a low storage temperature (15 °C), but the problem of sprouting is more common in tropical and subtropical conditions where temperatures are relatively high and also farmers may lack the means for temperature control. In addition, prior studies have not examined the effects of hot water dipping on sweetpotato phytochemicals, although studies in other crops have shown that hot water dipping may affect phytochemical concentration. The effects of

heat treatment on sweetpotato phytochemical concentration and root storage life are examined in this work.

1.8.5 Edible coatings

An edible coating is a thin layer of wax or other material applied to the skin of fruit and vegetables to reduce moisture loss and enhance product appearance (Amarante et al., 2001; Baldwin, 2005; Baldwin et al., 2012). Coatings may be composed of natural or synthetic waxes (carnauba wax, beeswax and polyethylene), oils (vegetable + mineral oils), rosin and resins (wood, rosin, and shellac), proteins (whey protein, casein, wheat gluten and zein), fatty acids, emulsifiers, plasticizers and preservatives (Baldwin, 2005; Reinoso et al., 2008; Hassani et al., 2012). The important desired characteristics of coatings are their ability to reduce water loss without creating an anaerobic internal atmosphere (Hagenmaier & Baker, 1993; Chiumarelli & Hubinger, 2014). Different types of coating have different permeability to water and gases. Polyethylene-based waxes have high permeability to carbon dioxide and oxygen, low to medium water vapour permeability, and provide limited gloss (shine) (Bai et al., 2003). In contrast, shellac and rosin-based coatings impart high gloss, but have low permeability to carbon dioxide and oxygen, with moderate water vapour permeability (Hagenmaier & Shaw, 1991). Coatings made with carnauba waxes have low to moderate permeability to oxygen and carbon dioxide, low permeability to water vapour and provide moderate gloss (Hagenmaier & Baker, 1993; Chiumarelli & Hubinger, 2014). Coatings can be applied to the surface of fruit or vegetables by dipping, spraying or brushing (Baldwin, 2005; Dhall, 2012).

Several authors have listed sweetpotato as one of the products that could benefit from coating (Woolfe, 1992; Ravi et al., 1996; Baldwin, 2005); however there is limited published work on the effects of coatings on sweetpotato roots during storage. A study in Nigeria, on sweetpotato coated with Bemul wax (coating developed from cassava starch), reported that coating significantly reduced weight loss after 20 days of storage at ambient temperatures (Afolabi & Oloyede, 2011). In this work effects of edible coating on sweetpotato storage quality will be studied.

1.8.6 Storage environment

1.8.6.1 Controlled temperature and relative humidity storage

Temperature management is the primary technology used to maintain product quality, from the time of harvest to consumption. Other postharvest technologies can be supplementary, but tend not to replace temperature control during storage. Each crop has its own optimal range of storage temperatures, which maximise its storage life. In sweetpotato, the optimal storage temperature is 13 to 15 °C, with a relative humidity of 85 to 95% (Ray & Ravi, 2005). A higher temperature will accelerate respiration and water loss, leading to shrivelled roots. In addition, a high temperature, coupled with high relative humidity promotes the sprouting of roots. On the other hand, low temperatures below 12 °C could lead to chilling injury and hardcore disorders (Picha, 1987).

Storage conditions also affect the root phytochemical content. Carotenoids have been reported to be stable during sweetpotato storage under optimal conditions (Watanabe et al., 1999). However, some scholars have reported an increase in carotenoid concentration. Carotenoid content increased from 9.5 to 11.5 mg/100 g FW, when roots were stored at 15.5 °C (Picha, 1985). It could be argued that the carotenoid increase observed was due to water loss since the results were not corrected to take into account weight loss during storage. Nevertheless, some scholars have also reported an increase, when water loss was taken into consideration. The carotenoid content of cultivar 'Porto Rica' increased by 5 % during storage at 16 °C and 85% relative humidity for 6 months (Ezell & Wilcox, 1948). Similarly, the carotenoid content increased from 82.6 to 172.5 mg/100 g and to 188 mg/100 g DW, when roots were stored in open air and hessian bags respectively (Priyadarshani et al., 2007). These contradictory results could be attributed to differences in the age of the root used in the studies, as it is known that carotenoid levels increase with root development (K'osambo et al., 1998). Therefore it can be suggested that during storage carotenoid concentration may change depending on the age of the roots, with an increase being more likely in roots that are young or roots harvested before reaching optimal carotenoid concentrations, and more stable carotenoid concentrations being likely in roots that are harvested late, when roots has reached maximum carotenoid concentration.

Ishiguro et al. (2007) determined the total phenolic content of four sweetpotato cultivars stored at 5 and 15 °C for 37 days. The phenolic content increased during storage, regardless of the storage temperature. However, the increase was higher in roots stored at 5 °C than those stored at 15 °C. In another study, Padda and Picha (2008a) reported that the total phenolic content increased more in roots that were stored at 5 °C than those that were stored at 15 °C. This increase in phenols when exposed to a lower temperature is linked to cold stress that results in the biosynthesis of phenolics acids. In both cases, an increase in phenolic content resulted in an increase in antioxidant activity. Total anthocyanin content did not change when sweetpotatoes were stored at 13 °C and 95% relative humidity for a period of nine months (Morrison et al., 2004). However, there were changes in terms of individual anthocyanins, but these changes occurred only in the 8th and 9th month of storage.

These studies on storage temperature suggest that sweetpotatoes can be stored at the recommended temperature without any loss of anthocyanin. A low temperature (in this case 5 °C) is good for increasing antioxidant benefits through increased phenolic content. However, this temperature is at odds with the required temperature for maintaining physiological quality, since temperatures below 12 °C eventually lead to chilling injury and internal discoloration.

1.8.6.2 Controlled atmosphere

Controlled atmosphere storage involves manipulating the oxygen and carbon dioxide concentrations in the storage environment (Brecht, 2006). Delate and Brecht (1989) found that sweetpotatoes could be stored for a week at 25 °C under 2% O₂ plus 40% CO₂, 4% O₂ plus 40% CO₂, and 2% O₂ plus 60% CO₂, without adverse effects to postharvest quality. However, increased decay and off-flavours were perceived in roots at 2% O₂ plus 60% CO₂. In another study, sweetpotato weevil were killed in roots stored for 7 days at 25 °C under 8% O₂ and 60% CO₂ treatment (Delate et al., 1990). Chang and Kays (1980), found less weight loss and decay in sweetpotato roots stored in 7% O₂ plus 2 - 3% CO₂. However, roots stored under less than 7% O₂ plus above 10% CO₂ developed an unpleasant flavour. Imahori et al. (2007) reported that no visible signs of injury or decay were observed in sweetpotato roots stored in 0 - 1% O₂ for 7 days at 20 °C. The authors also found that roots stored in 0 - 1% O₂ had higher concentrations of ethanol and acetaldehyde, associated with an increased intensity of

off-flavours. Although CA shows benefits in storage of sweetpotato, the additional cost associated with CA outweighs the beneficial effects (Edmunds et al., 2008).

1.8.7 Effects of ethylene and 1-MCP on sweetpotato

1.8.7.1 Ethylene biosynthesis and perception

Ethylene (C₂H₄) is a plant hormone involved in most plant growth and development stages (Yang & Hoffman, 1984; Abeles et al., 1992). According to Yang and Hoffman (1984) ethylene is produced from L-methionine (Figure 1.1). The first step is the conversion of methionine to S-adenosylmethionine (S-Adomet) by S-adenosylmethionine synthetase (SAM synthase). S-Adomet is then fragmented by enzyme ACC synthase to give MTA (5'-methylthioadenosine) and 1-aminocyclopropane-1-carboxylic Acid (ACC). This step of the reaction is considered a rate limiting step of ethylene biosynthesis (Kende, 1993). Finally, ACC is oxidised by ACC oxidase to form ethylene, CO₂ and cyanide.

For plants to execute an ethylene response first the ethylene molecule has to be perceived and then a signal sent to stimulate explicit physiological responses in the target genes. The mechanism by which plants perceive and transduce ethylene signals has been reviewed by several authors (Bleecker & Schaller, 1996; Binder et al., 2012). Ethylene perception is mediated by a family of receptors, which are located in the endoplasmic reticulum (Chen et al., 2002; Ma et al., 2006). In the model plant, *Arabidopsis* there are five ethylene perception receptors, namely ethylene receptor 1 (ETR1), ethylene receptor 2 (ETR2), ethylene response sensor 1 (ERS1), ethylene response sensor 2 (ERS2) and ethylene insensitive 4 (EIN4) (Hall et al., 2007). In the absence of ethylene, these receptors negatively regulate the ethylene response pathway by up-regulating constitutive triple response 1 (CTR1) activity which, in turn, down-regulates the ethylene signal transduction pathway (Hua & Meyerowitz). When ethylene binds to the receptors, the receptor function is inactivated and therefore stimulates ethylene responses.

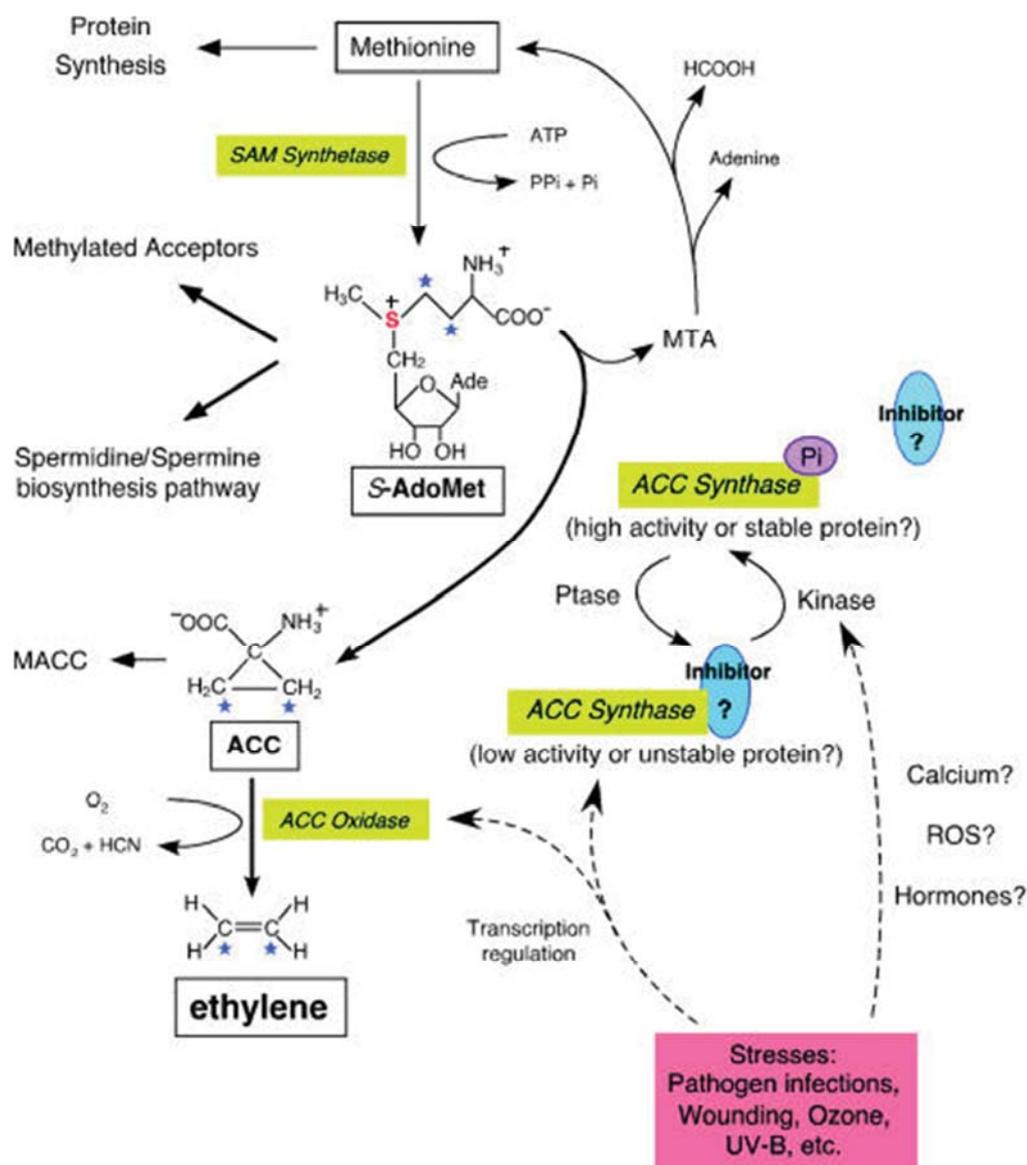


Figure 1.2: Ethylene biosynthetic pathway and signal transduction (Wang et al., 2002). The figure is copyrighted by the American Society of Plant Biologists and is reprinted with permission.

1.8.7.2 Effects of ethylene on sweetpotato storage quality

Responses of sweetpotato to ethylene have been previously studied both by applying exogenous ethylene and also by use of ethylene action inhibitors such as 1-MCP. Some of the sweetpotato responses associated with ethylene application or blockage of ethylene action with 1-MCP are summarised (Table 1.3). Earlier studies on the effects of exogenous ethylene on sweetpotato quality found that exposure to continuous ethylene resulted in a high phenolic content, increased decay and respiration rate. There were also adverse effects on the colour and flavour of baked sweetpotatoes (Buescher et

al., 1975; Kitinoja, 1987). The authors concluded that care must be taken during handling to avoid exposure of sweetpotato to ethylene. Studies by St Amand and Randle (1989) and St Amand and Randle (1991), indicated a role for ethylene in curing, thus a certain concentration of ethylene is required to initiate wound healing and the lignification of sweetpotato periderm. Lignification and wound periderm formation was suppressed when sweetpotato roots were treated with 2,5-norbornadiene (ethylene action inhibitor) or with AOA or CoCl_2 (ethylene synthesis inhibitors) (St. Amand & Randle, 1989). Cheema et al. (2010) found that ethylene concentrations of $100 \mu\text{L L}^{-1}$ inhibited sprout growth of 'Bushbuck' sweetpotato during 4 weeks storage at 25°C , and lower concentrations of 5 and $20 \mu\text{L L}^{-1}$ delayed sprouting by 14 days compared to the control roots which started sprouting by the 7th day in store. The authors also found that ethylene-treated roots appeared to have slightly higher respiration rates and weight loss, although these were not significantly different from those of the control. In another study Cheema et al. (2013) found that exogenous ethylene ($10 \mu\text{L L}^{-1}$) suppressed sprouting, increased respiration and reduced the glucose and fructose concentrations of 'Ibees' and 'Bushbuck' sweetpotato roots stored at 25°C for 4 weeks. Similarly, Amoah (2013) reported reduced sprouting in the North Carolina 'Covington' sweetpotato cultivar stored in continuous ethylene ($10 \mu\text{L L}^{-1}$) for 49 days at 25°C .

Ethylene application has also been reported to inhibit sprouting in potato (Daniels-Lake et al., 2005; Foukaraki et al., 2012b); and onions (Bufler, 2009; Downes et al., 2010; Cools et al., 2012). Just as sweetpotato, exposure of potato to ethylene has adverse effects on the fry colour. An application of 1-MCP ($1 \mu\text{L L}^{-1}$) prevented the darkening of fry colour in ethylene-treated potato tubers, without compromising the sprout inhibition effect (Daniels-Lake et al., 2005; Prange et al., 2005). The effect of 1-MCP treatment prior to ethylene exposure on sweetpotato flesh colour has not been studied.

Table 1.3: Summary of 1-MCP and ethylene effects on sweetpotato storage root quality Symbols (↑) indicates increase, (↓) decrease and (↔) no change

Treatment	Response	Reference
1-MCP (0.625 $\mu\text{L L}^{-1}$)	Respiration, weight loss and sprouting ↓, glucose, & fructose sucrose ↔	(Cheema et al., 2013)
1-MCP (1 $\mu\text{L L}^{-1}$)	Respiration, fructose, glucose & sucrose ↔, sprouting and decay ↓, phenolics concentration on proximal and distal root section ↓, at middle section ↔	(Amoah & Terry, 2013)
Ethylene (10 $\mu\text{L L}^{-1}$)	Respiration ↑, sprouting, glucose & fructose ↓, weight loss ↔, sucrose ↑	(Cheema et al., 2013)
Ethylene (10 $\mu\text{L L}^{-1}$)	Respiration, weight loss, proximal root end decay and splitting, isochlorogenic acid A ↑, sprouting, glucose and fructose, caffeic acid and coumaric acids in the skin ↓,	(Amoah, 2013)
Ethylene (0.1, 1.0, 10 $\mu\text{L L}^{-1}$)	Phenolic, respiration, decay and tissue bursting ↑, colour and flavour rating ↓,	(Kitinoja, 1987)
Ethylene (10 $\mu\text{L L}^{-1}$)	Respiration, POD, catecholase, phenolics ↑, beta-amylase, colour and flavour rating ↓	(Buescher et al., 1975)
1-MCP (0.625 $\mu\text{L L}^{-1}$) + Ethylene (10 $\mu\text{L L}^{-1}$)	Respiration ↑, sprouting, glucose & fructose ↓, weight loss ↔	(Cheema et al., 2013)
Ethylene (10 $\mu\text{L L}^{-1}$) to Air	Sprouting ↑, Respiration, weight loss ↑ when in ethylene, ↓ when moved to air. Decay ↓ when compared to continuous ethylene	(Amoah, 2013)
Air to Ethylene	Sprouting ↓ when moved to ethylene, weight loss and decay ↓ when compared to continuous ethylene	(Amoah, 2013)

1.9 Research opportunity

The literature reviewed in this chapter has shown that both preharvest and postharvest factors can affect the storage quality of sweetpotatoes, and the quality losses may be due to weight loss, sprouting, pests, and diseases. Losses due to pests and diseases were not the focus of this study; in addition the work concentrated on postharvest factors not preharvest factors. Published work has shown the possibility of applying hot water dipping treatments to inhibit sweetpotato sprouting, while other studies have shown that the use of edible coating reduces water loss during storage of fruits and vegetables. To date the effects of coating on other aspects of sweetpotato storage quality are unknown. The effects of hot water dipping on the health-promoting compounds such as carotenoids, phenolic acids and anthocyanin are unknown. Furthermore, the literature reviewed in this chapter has shown that ethylene is a potent sweetpotato sprout inhibitor, but there are also deleterious side effects associated with its use. In potato, ethylene-induced side effects on fry colour can be minimised by prestorage 1-MCP treatment. The effect of 1-MCP on sweetpotato flesh colour, when used in combination with ethylene, has not been studied. In addition, there is limited information on the effects on 1-MCP and ethylene on sweetpotato phytochemical concentrations. In the subsequent chapters, the effects of HWD (with and without coating) and ethylene (with or without 1-MCP) on sweetpotato storage root quality and phytochemicals content will be presented. Overall, this work is aimed at determining potential techniques to extend root storage life without compromising phytochemical concentrations.

Specific objectives

1. To determine if hot water treatment and coating would extend the storage life of sweetpotato roots.
2. To determine the effects of manipulating ethylene action on sweetpotato root storage life and consumer acceptability, using 1-MCP
3. To quantify sweetpotato phytochemical changes during storage and the impact on human health

2 Materials and methods

2.1 Introduction

This chapter describes the plant materials, and common methods used in several experiments in this work. Some methods were specific to individual experiments and will be discussed in the related chapters.

2.2 Plant materials and handling

Five different sweetpotato cultivars were used in this study (Table 2.1). The experiments in 2011 were conducted using ‘Beauregard’, ‘Owairaka Red’, ‘Purple Star’, and ‘Toka Toka Gold’ obtained from Northern Wairoa Vegetable Growers’ Association, Dargaville-Ruawai, New Zealand. Further experiments used ‘Owairaka Red’ and ‘Clone 1820’ sourced from Dargaville and Pukekohe respectively. In all experiments, unless specified in the chapter, the roots were transported to Massey University, Palmerston North, New Zealand, within 24 hours (h) of harvest. Upon receiving the roots in the laboratory, the roots were cured at 30 ± 2 °C and 85 - 95% relative humidity for 4 days in a controlled temperature room.

2.3 Quality assessment

2.3.1 Weight loss

Sweetpotato roots were weighed to 0.001 g precision using a Mettler Toledo balance (Mettler-Toledo PG503s, Greifensee, Switzerland). Weight loss was expressed as a percentage of the initial weight.

2.3.2 Sprouting

Sprouting was assessed in terms of both numbers and length. Roots were individually examined for presence of sprouts, and the number of sprouts per root was recorded. Any sprout growth longer than 1 mm was considered emerged. Sprout length was measured to 0.02 mm accuracy using a digital vernier caliper (Mitutoyo, Japan).

2.3.3 Rots

Rot incidence was evaluated by visually examining each root for visible signs of rots. Roots with visible signs of rots were counted as rotten and expressed as a percentage incidence relative to the initial root number.

Table 2.1: Cultivars and sources for the sweetpotatoes for each year of research

Year	Cultivar	Flesh colour	Sources
2011	Owairaka Red	Cream white	Northern Wairoa Vegetable Growers' Association
	Beauregard	Orange	Northern Wairoa Vegetable Growers' Association
	Toka Toka Gold	Yellow	Northern Wairoa Vegetable Growers' Association
	Purple Star	Purple	Northern Wairoa Vegetable Growers' Association
2012-2014	Owairaka Red	Cream white	Delta Produce, Dargaville,
	Clone 1820	Orange & Purple	Plant and Food Research, Pukekohe,

2.4 Phytochemical analysis

The phytochemicals measured were total phenolic content, carotenoids, phenolic acids, and anthocyanins.

2.4.1 Chemicals and standards

β -carotene (Type II, synthetic, $\geq 95\%$ (HPLC), crystalline), cyanidin 3-O-glucoside chloride (kuromanin chloride), and chlorogenic acid standards, and chemicals: 3,5-di-tert-4-butylhydroxytoluene (BHT) and triethylamine were purchased from Sigma-Aldrich (Auckland, New Zealand). HPLC grade acetonitrile was obtained from Merck KGaA, Darmstadt, Germany. Acetic acid, formic acid, methanol, ethyl acetate, and acetone were purchased from Thermo Fisher Scientific (Palmerston North, New Zealand).

2.4.2 Sample preparation

Four roots per replicate were thoroughly washed with tap water and peeled by hand with a vegetable peeler. Each root was cut into four sections longitudinally and two opposite sections from the roots were further cut into small pieces, weighed to get a composite sample of 250 g. Then the samples were frozen with liquid nitrogen, freeze dried for several days using freeze dryer FD18LT (Cuddon Ltd., New Zealand). Freeze dried samples were stored at $-30\text{ }^{\circ}\text{C}$, until the time for analysis. The dry matter content was calculated by the weight difference before and after freeze-drying. Before extraction, the freeze-dried samples were ground into powder using a grinder (CG2B, Breville Ply. Ltd., Sydney, Australia).

2.4.3 Extraction and quantification of carotenoids

Carotenoids were extracted based on the method described by Bengtsson et al. (2008) and Rodriguez-Amaya & Kimura (2004). In brief, 0.2 g of freeze-dried sweetpotato powder was weighed in centrifuge tube and 1 mL of water was added, to rehydrate the tissue. After 20 min, 2 mL acetone containing 0.1% (w/v) butylated hydroxyl toluene (BHT) was added and the tubes were vortexed for 2 min, followed by centrifugation at 10 °C, 4500 g, for 5 min (Heraeus Multifuge 1S-R Centrifuge, Thermo Fisher Scientific, MA, USA). The supernatant was transferred to new centrifuge tube and the process was repeated four times. Partitioning of the carotenoids was undertaken by adding 3 mL of petroleum ether and 5 mL milliQ water to the sweetpotato extract. Separation of the organic phase and water phase was achieved by centrifuging the extracts at 4500 g for 5 min. The organic phase was then pipetted into a clean glass test tube, this step was repeated twice. The extracts were then evaporated to dryness under a stream of nitrogen using a heating block at 30 - 35 °C. The resultant residue was dissolved in 5 mL of mobile phase (acetonitrile: methanol: ethyl acetate 80:10:10, v/v/v (with 0.05% triethylamine). Approximately 1 mL of sweetpotato extract was filtered through a 0.45 µm regenerated cellulose (RC) membrane single use filter to HPLC vials.

Carotenoids were analysed using a Dionex HPLC instrument equipped with a P680 pump, auto sampler (ASI-100) injector, and thermostatted column compartment (TCC-100) and photodiode array detector operating at 450 nm. The data was acquired and analysed using Chromeleon Software (Dionex Chromatography Data System, MA, USA). Separations were carried out on a C 18 column (Luna® 5 µm C 18 (2) 100 Å, LC Column 150 x 4.6 mm) (Phenomenex, CA, USA.). The mobile phase used for isocratic elution consisted of acetonitrile: methanol: ethyl acetate 80:10:10, v/v/v (with 0.05% triethylamine). The flow rate was 0.7 mL/min and the injection volume was 10 µL. Carotenoids were identified using retention times and UV absorption spectra. Quantification was done using a β-carotene standard calibration curve with 5 concentration points (0.001 to 0.025 mg/mL) and had the correlation coefficient of 0.976. The β-carotene peak was eluted at 35.89 minutes and had a maximum UV absorbance at 455 nm (Figure 2.1).

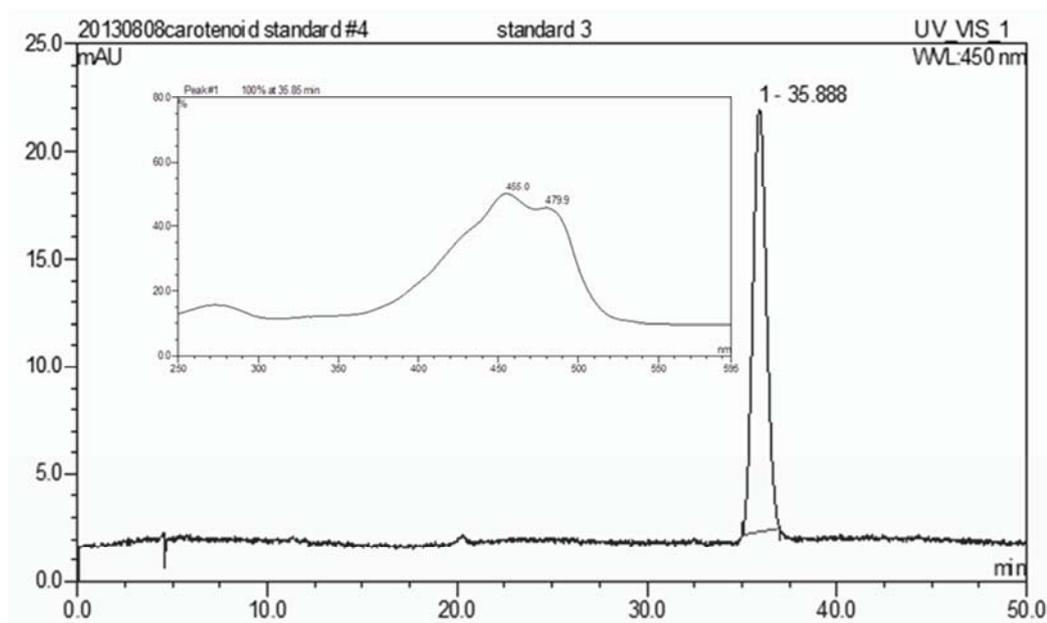


Figure 2.1: Chromatogram of the β -carotene standard determined by HPLC and β -carotene UV spectrum (insert).

2.4.4 Extraction and quantification of phenolic acids and anthocyanin

The phenolic compounds were extracted following methods described by Teow et al. (2007). Five millilitres (5 mL) of acidified methanol solution (methanol: water: acetic acid, 80: 13: 7, v/v/v) was added to 0.5 g sample of freeze dried sweetpotato powder and vortexed for 2 min. In order to facilitate separation of the solid and liquid phase, the mixture was centrifuged at 3200 g, for 5 min using a Thermo Scientific Heraeus multifuge 1S-R Centrifuge (Massachusetts, USA). The resultant supernatant was transferred into another tube, and the extraction process was repeated three times. The pooled extracts were concentrated under stream of nitrogen to a volume of 5 mL. The concentrated extract was used for analysing of total phenolic content, individual phenolic acids, anthocyanins and antioxidant activity. Approximately 1 mL of sweetpotato extract was filtered through a 0.45 μ m regenerated cellulose (RC) membrane single use filter to HPLC vials.

Phenolic acids and anthocyanins were analysed using the method previously described by Champagne et al. (2011). Dionex HPLC instrument equipped with a P680 pump, auto sampler (ASI-100) injector, and thermostatted column compartment (TCC-100) was used. The data was acquired and analysed using Chromeleon Software (Dionex

Chromatography Data System, MA, USA). Separations were carried out on a C 18 column (Luna® 5 µm C 18 (2) 100 Å, LC Column 150 x 4.6 mm) (Phenomenex, CA, USA.). The mobile phase was made up of water and formic acid (solvent A), and water: acetonitrile: formic acid (solvent B). A gradient (Table 2.2), at 0.6 mL min⁻¹ flow rate was used. The total running time was 75 minutes. Column temperature was set at 25 °C and the sample injection volume was 20 µL. The phenolic compounds were monitored at 200 to 595 nm. Identification of phenolic acids and anthocyanin were based on the retention times and absorption spectra of the unknown peaks compared to the reference standards. Anthocyanins were detected at 530 nm, and phenolic acids at 280 nm. Quantification of phenolic acids and anthocyanin was done using standard curves on chlorogenic acid and cyanidin 3-O-glucoside chloride respectively. Chlorogenic acid peak eluted at 7.67 min and had maximum absorbance at 324 nm (Figure 2.2), while cyanidin 3-O-glucoside chloride eluted at 12 min and had maximum absorbance at 514 nm (Figure 2.3).

Table 2.2: The ratio of mobile phase in HPLC operation for phenolic acids and anthocyanin measurement

Time (min)	Solvent A water and formic acid (90/10 v/v)	Solvent B water: acetonitrile: formic acid (60: 30: 10 v/v/v)
0	75%	25%
50	50%	50%
56		100%
61	75%	25%
75	75%	25%

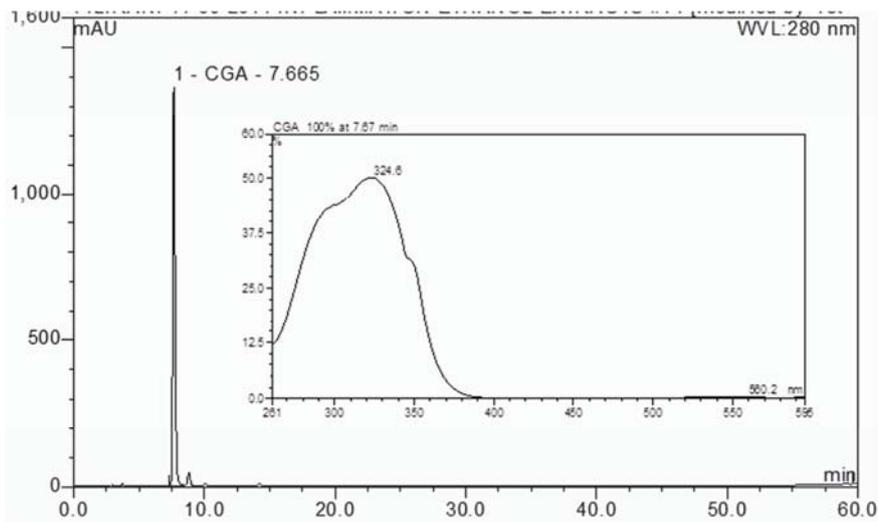


Figure 2.2: Chromatogram of the chlorogenic acid standard determined by HPLC and chlorogenic acid UV spectrum (insert)

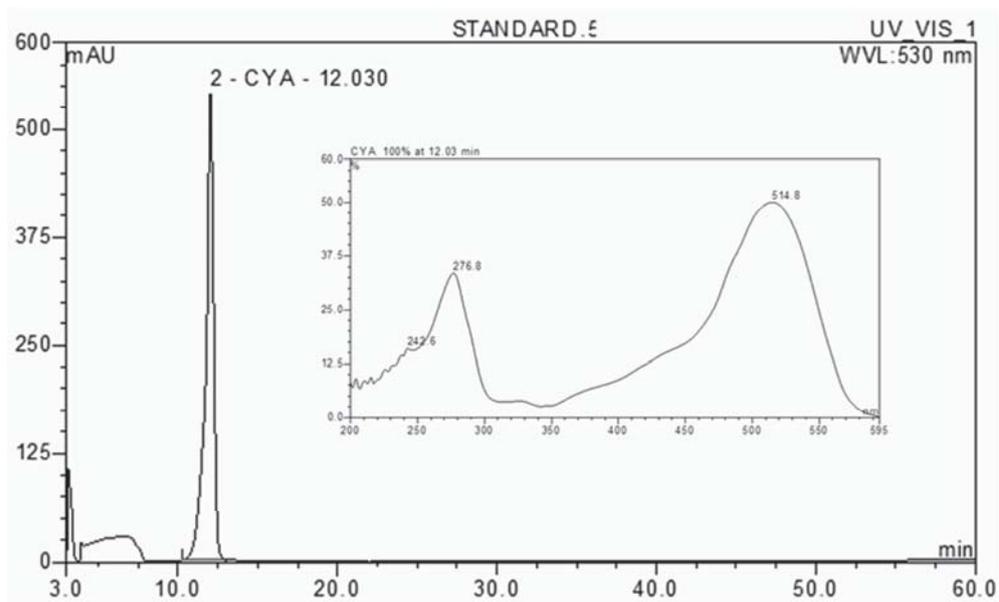


Figure 2.3: Chromatogram of the cyanidin 3-O-glucoside chloride standard determined by HPLC and cyanidin- UV spectrum (insert)

3 Effects of hot water dipping and storage temperatures on sweetpotato storage life and total phenolic concentration

3.1 Introduction

Major postharvest losses in sweetpotato are caused by pest damage, rotting, sprouting, and weight loss (Ray & Ravi, 2005). As discussed in the literature review (Chapter 1), the storage life of sweetpotato roots can be extended by postharvest treatments such as curing, irradiation, heat treatment, fungicides and sprout suppressants. In addition, postharvest storage approaches such as traditional earth pits and clamps, controlled temperature and relative humidity, and modified atmosphere may be used (Woolfe, 1992). However, emphasis in developed countries has been on the use of controlled temperature and relative humidity (15 °C and 80 to 95%), and sprout inhibitors and fungicides to control sprouting and rotting respectively. However, controlled temperature methods are difficult to achieve for subsistence farmers, whose villages may not have access to electric power. The use of chemicals also raises concerns for human health, in relation to application systems, chemical residues and environmental effects.

Non-chemical methods (e.g. hot water treatment) have been found to control sprouting and rotting in many horticultural crops (Fallik, 2004). The effect of hot water treatment on the storability of sweetpotato roots has been studied previously (Scriven et al., 1988; Hu & Tanaka, 2007). These studies were generally conducted in cooled stores, yet sweetpotato is an important food crop in developing countries, where most of the farmers have no access to electric power. In addition, no information is available on the effect of hot water treatments on the phenolic content of sweetpotato, which is important for human health. Studies on other horticultural produce (Kim et al., 2009; Ummarat et al., 2011) have shown that hot water treatment may stimulate the synthesis of phenolic compounds. Therefore, the focus of the study reported in this chapter was to examine the effects of hot water treatment on sweetpotato total phenolic concentration, antioxidant activity, and root storage life following storage at both low (15 °C) and high (25 °C) temperatures.

3.2 Materials and methods

3.2.1 Sweetpotato roots

Four cultivars with varying flesh colours (Figure 3.1) were used in the study. Roots without visible defects were cured at 30 °C and 85 - 95% relative humidity for four days. Thereafter, the roots were divided into two lots: one for hot water treatment and the other as controls.

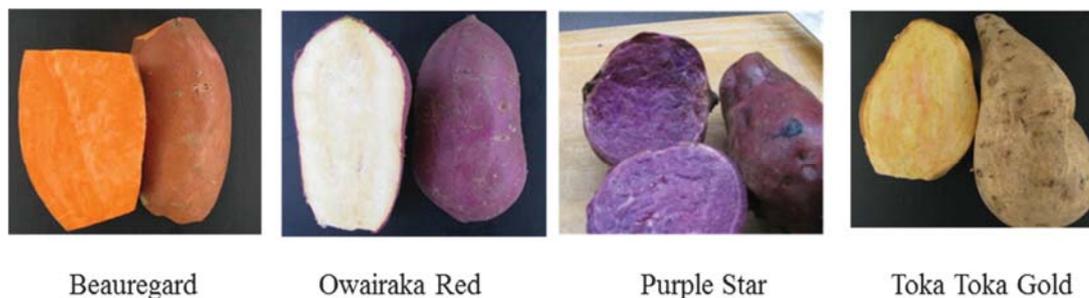


Figure 3.1: Visual appearance of the four sweetpotato cultivars used in the study

3.2.2 Hot water dipping

Hot water dipping was performed following the method described by Hu & Tanaka (2007). Before treatment, all roots were washed with cold water. Roots meant for hot water dipping were placed in a stainless steel wire basket, and immersed for 30 min in water bath containing 40 L of distilled water, preheated to a temperature of 50 °C. The decrease in water temperature on addition of sweetpotato roots was less 2 °C for all treatment batches. After hot water dipping, the roots were cooled under running cold tap water for 10 min., followed by drying at ambient temperature with the aid of an electric fan, until their surfaces were fully dry. Untreated (control) roots were handled in similar way as the hot water dipped samples except that the controls were dipped in cold water.

3.2.3 Storage condition

The roots were stored at two temperatures (15 ± 2 °C or 25 ± 2 °C), for a period of 12 weeks. Relative humidity (RH) was not controlled, to maintain relative higher RH cardboard boxes containing sweetpotato roots were covered with a plastic paper. Both temperature and relative humidity were recorded every 30 min throughout the storage period, using Tiny Tag Ultra data loggers (Gemini, Chichester, UK). The average relative humidity recorded during storage was 76 and 89 % at 25 °C and 15 °C

respectively. Each replication consisted of four similarly sized roots packed in a nylon mesh bag, held in unsealed cardboard boxes placed in temperature controlled rooms. Each treatment was replicated three times.

3.2.4 Quality analysis

3.2.4.1 Weight loss

Roots were weighed before storage and then every 2 weeks during the storage period. Weight loss was expressed as a percentage of the initial fresh weight.

3.2.4.2 Sprout growth

Sprout numbers were assessed on individual roots, and any sprout approximately 1 mm long was considered to be emerged. A scale rating of 1 to 4 was used, where 1 = no sprouts; 2 = 1 to 5 sprouts; 3 = 6 to 10 sprouts; and 4 = >10 sprouts.

3.2.5 Total phenolic concentration and antioxidant activity

3.2.5.1 Reagents

Folin-Ciocalteu phenol reagents, gallic acid, ferrous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), TPTZ (2, 4 6-tripyridyl-s-triazine), acetate buffer, sodium carbonate (Na_2CO_3), and hydrochloric acid were purchased from the Sigma-Aldrich (Auckland, New Zealand).

3.2.5.2 Sample preparation

Samples for dry matter measurement, total phenolic content and antioxidant activity were prepared as described previously in section 2.4.2. Before extraction, the freeze dried samples were ground into powder using a grinder (CG2B, Breville Ply. Ltd., Sydney, Australia).

3.2.5.3 Dry matter determination

Dry matter (DM) content of the sweetpotato from each treatment combination was determined by measuring sample weights before and after freeze drying, and the dry matter percentage was calculated as:

$$DM (\%) = \frac{\text{dry weight}(g)}{\text{initial fresh weight}(g)} * 100\%$$

3.2.5.4 Phenolic extraction

The phenolic compounds were extracted following Teow et al. (2007) as described previously in section 2.4.4.

3.2.5.5 Total phenolic concentration

The total phenolic concentration within the sweetpotato extract was determined, based on the Folin Ciocalteu procedure as described by Molan et al. (2008). Sweetpotato extracts (12.5 μL) were placed in a 96-well microplate, to which 250 μL of 2% sodium carbonate (Na_2CO_3) solution was added, and allowed to react for 5 min at room temperature. Thereafter, 12.5 μL of 50% Folin-Ciocalteu phenol reagent was added to each well of the mixture, followed by 30 min incubation at room temperature. The absorbance was read at 650 nm, with an Elks 808 microplate reader (Bio-Tec Instrument Inc., USA). A standard curve of gallic acid (0 to 1000 $\mu\text{g}/\text{mL}$) was plotted. The total phenolic concentration was expressed as mg gallic acid equivalents (GAE)/g DW.

3.2.5.6 Antioxidant activity

The potential antioxidant activity of the sweetpotato extract was measured using a ferric reducing antioxidant power (FRAP) assay. The FRAP method measures the capacity of a sample to reduce ferric iron to ferrous iron (Benzie & Strain, 1996). The assay was based on the procedures of Molan et al. (2009), which is a modification of the procedure described by Benzie and Strain (1996). The FRAP reagent was prepared by mixing 300 mmol/L acetate buffer (pH 3), 10 mmol/L TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mmol/L hydrochloric acid, and 20 mmol/L ferric chloride solution at the ratio of 10:1:1(v/v/v) respectively. Sweetpotato extract (8.5 μL) was mixed with 250 μL of FRAP reagent. The mixture was incubated at 37 $^\circ\text{C}$ for 30 min. After incubation, the absorbance was read at 595 nm with an Elks 808 micro plate reader (Bio-Tec Instrument Inc., USA). The antioxidant activity was calculated using a standard curve, which was constructed using a ferrous sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) concentration of 0 to 1.5 mg/mL. The antioxidant activity of the sweetpotato extracts was expressed as mg ferrous sulphate equivalent (FSE)/g DW.

3.2.6 Experimental design and statistical analysis

The design consisted of three factors: cultivars (4), storage temperatures (2) and hot water treatments (2). Each treatment combination consisted of three replicates, each comprising four roots. The data were evaluated by factorial analysis of variance, using Minitab 16 (Minitab Inc., State College, Pennsylvania). Means comparisons were undertaken using Tukey's test, at $P < 0.05$.

3.3 Results

3.3.1 Rot incidence and weight loss

There was a low incidence of rots during the entire storage period, only 1.3% of the total roots used in the study were rotten (data not shown). 'Owairaka Red' lost more weight than each of the other three cultivars, throughout the storage period (Figure 3.2A). Significant ($P < 0.05$) differences in weight loss between 'Owairaka Red' and the other three cultivars were observed at 6, 8, 10, and 12 weeks during storage while at 2 and 4 weeks differences were only observed between 'Owairaka Red' and 'Purple Star'. Hot water dipped roots had a significantly ($P < 0.05$) higher weight loss throughout the storage period than the control group (Figure 3.2B). At 12 weeks of storage, weight loss of the hot water dipped roots was 18%, which was 4% more than that of the control group. With regard to storage temperature, storage at 25 °C increased weight loss compared to storage at 15 °C. At 12 weeks, the average weight loss of roots stored at 25 °C was 8% greater than that of roots stored at 15 °C (Figure 3.2C). No interaction effects were observed among the experimental factors.

3.3.2 Effects of hot water dipping on sweetpotato sprouting

The results showed cultivar differences both in sprout numbers and time to initial sprout emergence. 'Owairaka Red' roots started sprouting early at 2 weeks and had more sprouts, while 'Purple Star' was the least prone to sprouting under any treatment. Sprout control by hot water treatment was only observed in 'Owairaka Red', and the effect of hot water treatment was storage temperature dependent (Figure 3.3A-D). 'Owairaka Red' roots that were hot water dipped then stored at 15 °C did not sprout after 12 weeks of storage, whilst the control group at 15 °C and all roots stored at 25 °C started sprouting at two weeks of storage (Figure 3.3B).

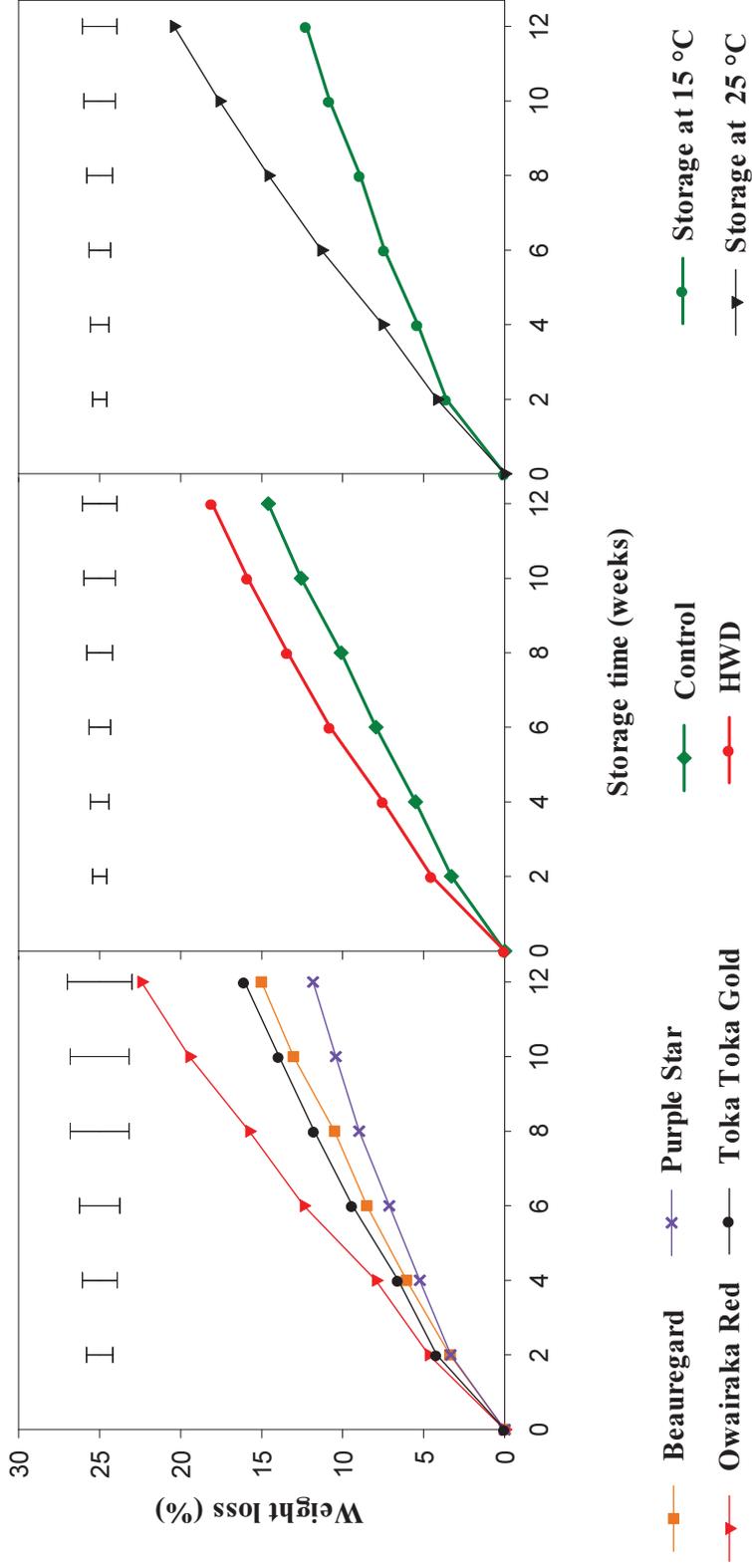


Figure 3.2: Sweetpotato storage root weight loss (%) during 12 weeks of storage as influenced by different factors: cultivar (A), hot water treatment (B) and storage temperature (C). Each data point represents a mean value of 12 replicates in (A) and 24 replicates in (B) and (C). Bars represent HSD_{0.05} (Tukey's test). HWD = hot water dipped at 50 °C for 30 minutes.

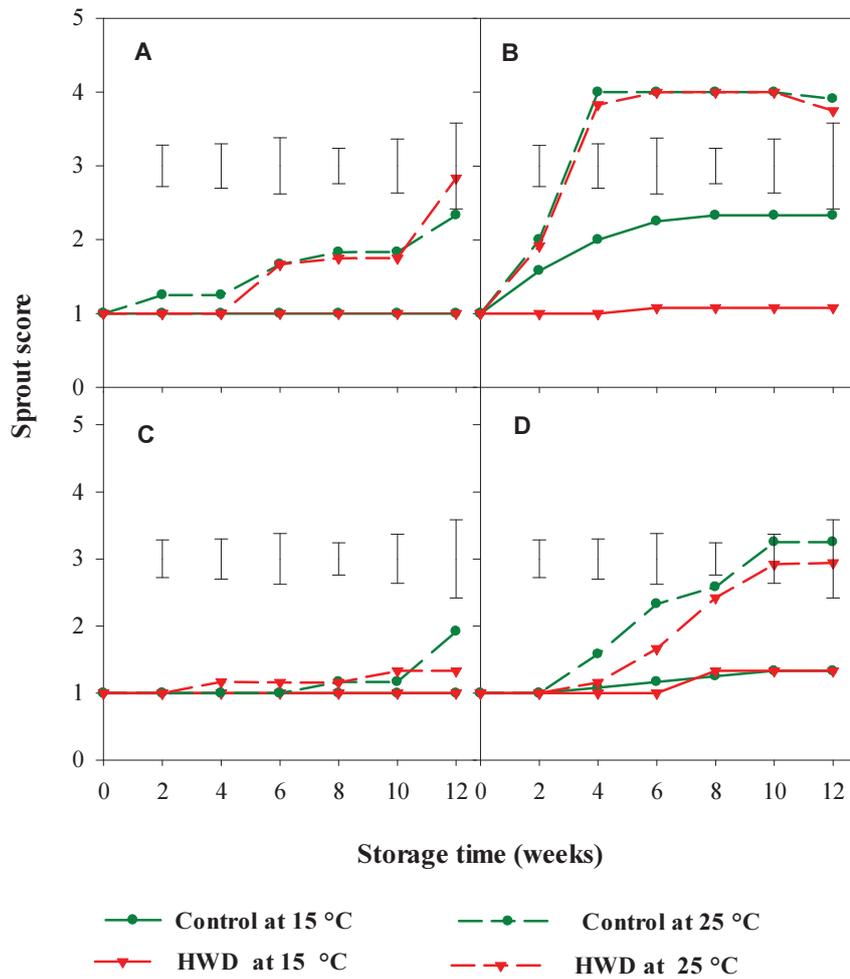


Figure 3.3: Effects of hot water treatment and storage temperature on sprout growth in the storage roots of various sweetpotato cultivars: Beauregard (A), Owairaka Red (B), Purple Star (C) and Toka Toka Gold (D). Each data point represents the mean of 3 replicates, each replicate consisting of 4 roots. HWD = hot water dipped (50 °C for 30 min), whilst control treatments are non-hot water dipped. Vertical bars represent HSD_{0.05} (Tukey's test) at each sampling time. Sprout score scale: 1 = no sprout; 2 = < 5 sprouts; 3 = 6 to 10 sprouts; and 4 = >10 sprouts.

3.3.3 Total phenolic concentration (TPC) at harvest

‘Purple Star’ had the highest TPC (4.8 mg/g DW) and ‘Owairaka Red’ (2.3 mg/g DW) the lowest, but ‘Owairaka Red’ was not statistically different from ‘Toka Toka Gold’ or ‘Beauregard’ (Table 3.1). ‘Beauregard’ storage roots had the lowest dry matter content compared to the other three cultivars.

Table 3.1: Total phenolic concentration and dry matter for four sweetpotato cultivars measured at harvest.

Cultivar	Dry matter (%)	*TPC (mg/g DW)
Beauregard	18.5 ^b	2.6 ^b
Owairaka Red	27.3 ^a	2.3 ^b
Purple Star	27.6 ^a	4.8 ^a
Toka Toka Gold	24.5 ^a	2.4 ^b
P-value	< 0.001	< 0.001

Means followed by the same letter within a column are not significantly different at $P < 0.05$ (Tukey’s test). * TPC expressed as gallic acid equivalence.

3.3.4 Effects of hot water dipping on total phenolic concentration

Hot water dipping effects on TPC were cultivar and storage time dependent. The total phenolic contents of ‘Beauregard’, ‘Owairaka Red’ and ‘Toka Toka Gold’ were not significantly affected by hot water treatment. For ‘Purple Star’, hot water treatment considerably increased TPC by 4 weeks, but there were no significant differences in the last two assessments (Figure 3.4). Storage temperature had no effect on TPC ($P > 0.05$) (Data not shown).

3.3.5 Effects of hot water treatment on antioxidant activity

The antioxidant activity of four cultivars at harvest ranged from 6.7 to 23.4 mg of FeSO₄ /g DW, with the highest antioxidant activity in purple-fleshed cultivar ‘Purple Star’. Hot water treatment and storage temperatures had no significant effects on the levels of antioxidant activity ($P > 0.05$). During storage, the antioxidant activity of the sweetpotato roots either remained unchanged or increased, depending on the cultivar. For ‘Purple Star’, the antioxidant activity increased with storage time. At 12 weeks, the value for antioxidant activity was 34.4 mg/g DW, which was 2-fold higher than the

concentration at 4 weeks (Figure 3.5). The other three cultivars also showed an apparent increase in antioxidant activity with storage time, but the increases were not significant ($P > 0.05$).

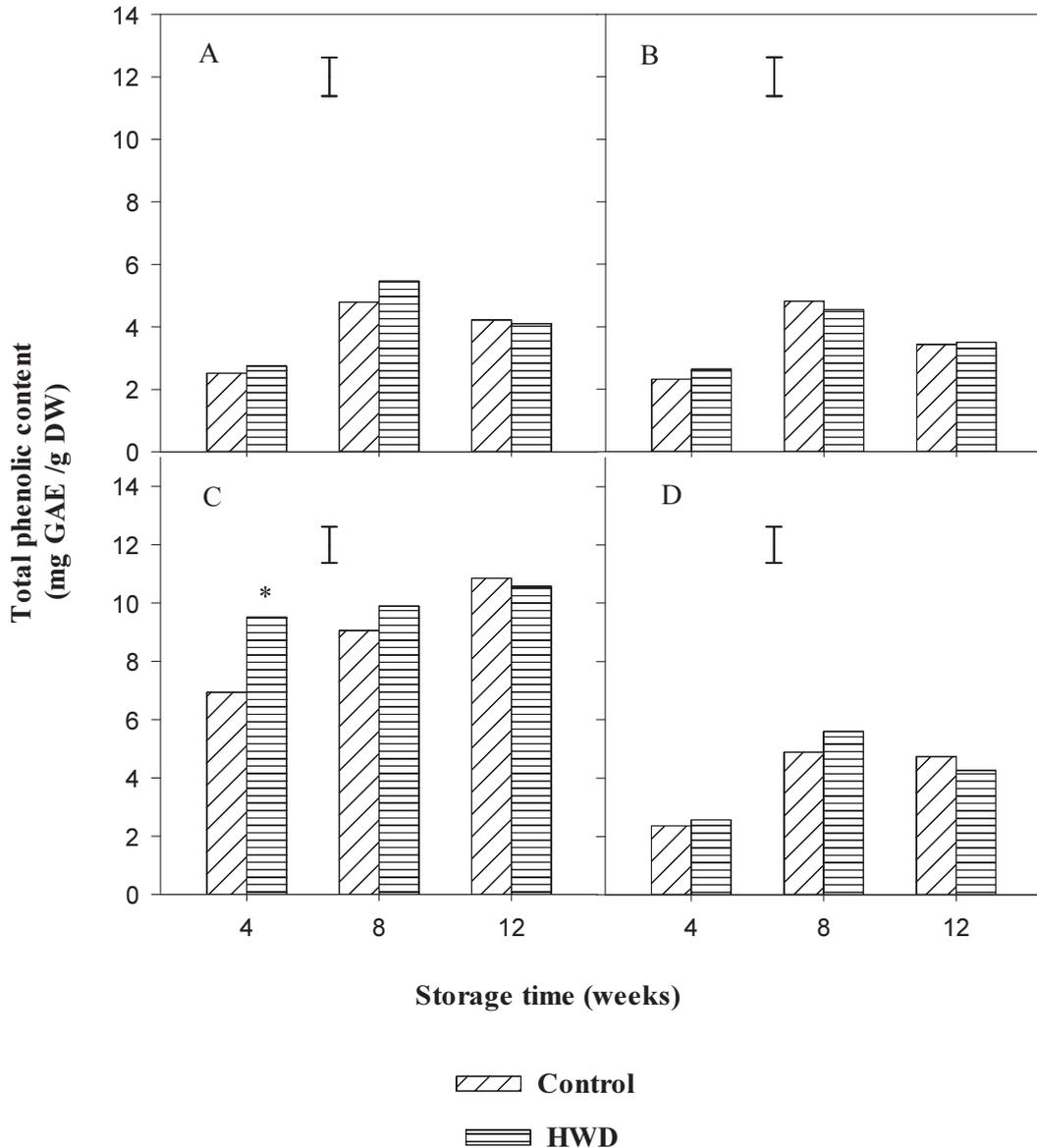


Figure 3.4: Effect of hot water treatment on the total phenolic concentration of four sweetpotato cultivars: Beauregard (A), Owairaka Red (B), Purple Star (C), and Toka Toka Gold (D). Vertical bars represent HSD_{0.05} (Tukey's test). Each data point is the mean of three replicates, each composed of four roots. * indicates a significant difference from the control at $P < 0.05$. GAE = gallic acid equivalent

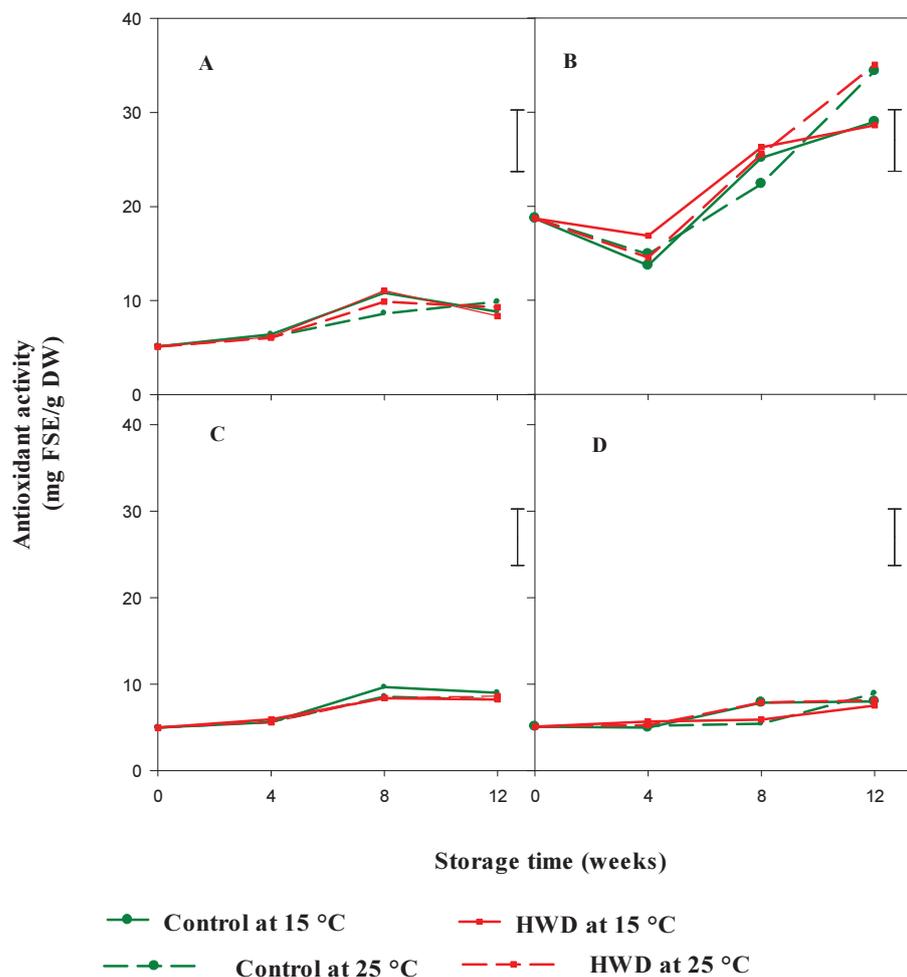


Figure 3.5: Effects of hot water dipping and storage temperatures on the antioxidant activity of four sweetpotato cultivars: Beauregard (A), Purple Star (B), Owairaka Red (C) and Toka Toka Gold (D). Vertical bars represent HSD_{0.05} (Tukey's test). Each data point is the mean of three replicates, each composed of four roots. FSE = ferrous sulphate equivalent.

3.3.6 FRAP and TPC correlation

There was a general trend of increased antioxidant activity with increased total phenolic concentration. For all cultivars tested, there was a positive significant ($P < 0.05$) correlation between total phenolic concentration and antioxidant activity (Figure 3.6). However, a strong correlation was observed in 'Purple Star' ($R^2 = 0.76$, $P < 0.001$)

(Figure 3.6C), and a very weak correlation was observed in ‘Owairaka Red’ ($R^2 = 0.16$, $P < 0.001$) (Figure 3.6B).

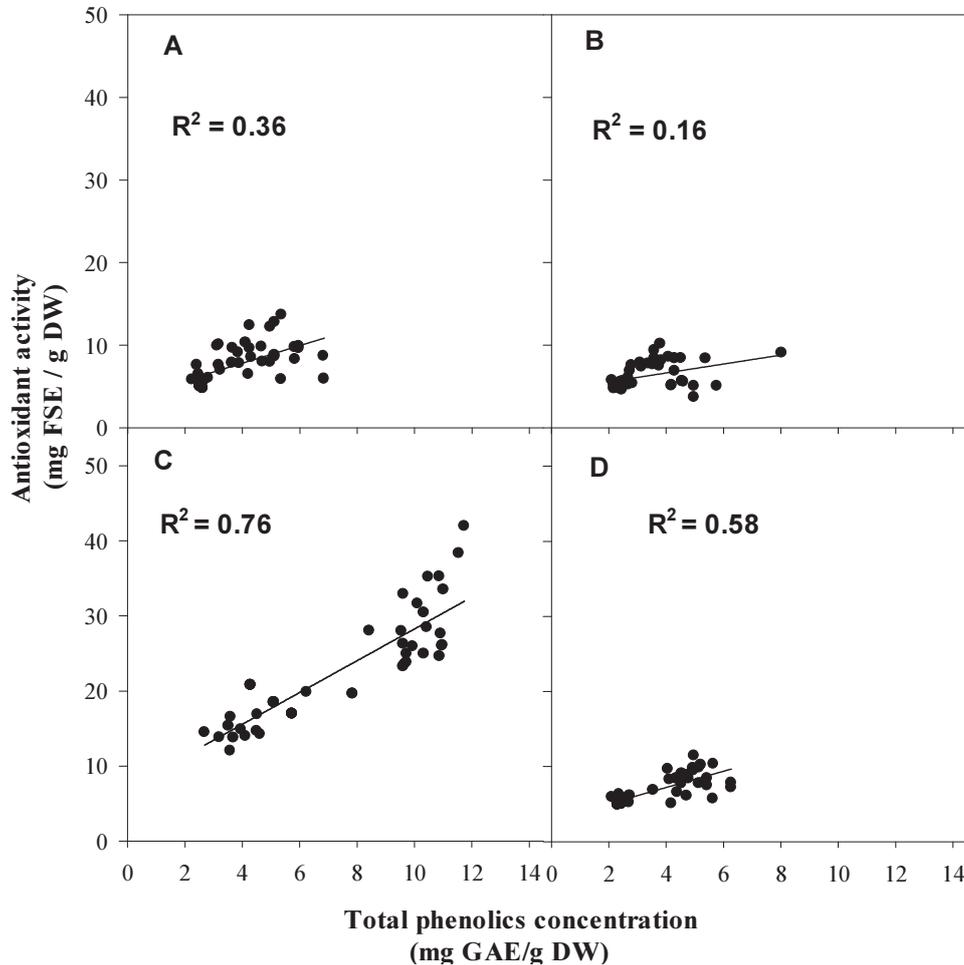


Figure 3.6: Correlation between antioxidant activity (mg FSE/ g DW) and total phenolic concentration (mg GAE/ g DW) in sweetpotato cultivars: Beauregard (A), Owairaka Red (B), Purple Star (C) and Toka Toka Gold (D). FSE = ferrous sulphate equivalent, and GAE = gallic acid equivalent.

3.4 Discussion

Water loss in sweetpotato roots is a function of respiration and transpiration, with the latter contributing more to weight loss (Picha, 1986c). Cultivar differences in rates of weight loss could be related to their specific physical properties and chemical compositions. Cultivars with low dry matter contents and high monosaccharide concentrations tend to lose water at lower rates (Rees et al., 2003). Although sugars

were not measured in this study, a previous study by Lewthwaite et al. (1997) showed that 'Beauregard' had a higher sugar content than 'Owairaka Red'. The reported sugar concentrations (glucose + fructose) in 'Owairaka Red', 'Beauregard' and 'Toka Toka Gold' were 1.05, 3.18 and 0.69 g/100 g fresh weight, respectively. Dry matter (%) in the current study (Table 3.1) showed that 'Owairaka Red' had a higher dry matter content than 'Beauregard'. Therefore, the increased weight loss in 'Owairaka Red' could be related to its relatively higher dry matter content and lower sugar content. The rate of water loss is also influenced by root surface area to volume ratio. Smaller sized roots/fruits have higher surface area to volume ratios, thereby losing more water than large sized fruits, over a similar time period (Wills et al., 2007). The mean root size in the cultivars studied here were 200, 195, 235 and 186 g in 'Beauregard', 'Owairaka Red', 'Purple Star' and 'Toka Toka Gold' respectively, with significant differences being observed between 'Purple Star' and the other three cultivars. Therefore the lower weight loss in 'Purple Star' could be related to its smaller surface area to volume ratio. Finally, increased sprouting could have contributed to the higher weight loss in 'Owairaka Red' (Figure 3.3B). Sprouted roots have an elevated respiration rate and surface area, leading to increased weight loss (Edmunds et al., 2008).

Extreme temperatures during heat treatment have been reported to cause plant tissue injury, resulting in increased weight loss (Lurie & Mitcham, 2007). In this case, exposing roots to a temperature of 50 °C for 30 min may have been too extreme for the cultivars studied. These results are contrary to Hu & Tanaka (2007), who applied the same temperature (50 °C) and exposure time (30 min) in sweetpotato cultivars 'Eicho' and 'Kanoya' followed by storage at 14 °C and 90 - 95% RH for 12 months. The authors reported less weight loss in hot water treated samples compared to untreated control roots. The weight losses observed in this study could be attributed to cultivar differences. Secondly, relative humidity (RH) during storage could also have played a role. A lower RH was used in this study (76 to 89 %) compared to that of 90 - 95% in Hu & Tanaka's study (2007). Higher storage temperatures may increase the rate of weight loss in fresh produce (Wills et al., 2007), as observed in this experiment, where weight loss (%) was higher at 25 °C than during storage at 15 °C.

There was a big difference between ‘Owairaka Red’ and the other cultivars in terms of sprouting responses. ‘Owairaka Red’ started sprouting early and had a higher number of sprouts than the other three cultivars. The differences may be related to the differences in concentration of growth promoters within the four cultivars studied. Hot water treatment was found to be effective in controlling the sprouting of ‘Owairaka Red’ roots subsequently stored at 15 °C. This is in agreement with Hu & Tanaka (2007), who reported that sprouting in the cultivars they evaluated was reduced in hot water treated samples stored at 15 °C. However, these results suggest that hot water treatment alone is inadequate for control of sprouting.

Total phenolic concentration in the sweetpotato extract was determined by the Folin Ciocalteu procedure as described by Molan et al. (2008). It has been suggested that the Folin-Ciocalteu procedure over-estimates the phenolic content of fruits and vegetables that contain high reducing sugars, ascorbic acid, and soluble proteins (Prior et al., 2005). Despite these limitations, the method is simple, reproducible and convenient, and has been widely used in the study of phenolic antioxidants (Grigelmo-Miguel et al., 2009). As expected, there were cultivar differences in TPC with the highest TPC in the purple-fleshed cultivar (Figure 3.4C). In line with these results, Teow et al. (2007) and Huang et al. (2006) indicated higher TPC in purple-fleshed than in orange- or white-fleshed sweetpotato cultivars. Earlier studies have reported an increase in TPC and antioxidant activity after hot water treatment in mangoes (Kim et al., 2009) and in bananas (Ummarat et al., 2011). In this study, the hot water treatment did not consistently influence the TPC of sweetpotato cultivars during storage. There were no significant differences in TPC between HWD and the control for ‘Owairaka Red’, ‘Toka Toka Gold’ and ‘Beauregard’, whereas for ‘Purple Star’ HWD slightly increased TPC for a short time. The increase in ‘Purple Star’ TPC could be related to a defence mechanism and reaction to stress, induced by the hot water treatment (Dixon & Paiva, 1995; Gonzalez-Aguilar et al., 2010). The rise of TPC in HWD samples may allow for additional health benefits, as phenolic acid is associated with antioxidant activity (Schreiner & Huyskens-Keil, 2006). The total phenolic concentration was correlated to antioxidant activity, particularly for ‘Purple Star’, which is similar to results reported by other researchers (Rautenbach et al., 2010; Yang et al., 2010). Anthocyanin concentration has been found to correlate with the antioxidant activity of fruits and

purple-fleshed sweetpotato which contain high levels of phenolic acids and anthocyanin, as opposed to white and orange fleshed cultivars (Zhu et al., 2010; Grace et al., 2014). The high correlation between TPC and antioxidant activity observed for ‘Purple Star’ suggests that this was primarily due to both anthocyanins and phenolic acids, whereas for the other cultivars antioxidant activity was largely dependent on other compounds.

3.5 Conclusion

This study examined the effects of cultivar, hot water treatment, and storage temperature on sweetpotato root quality, TPC, and antioxidant activity. There were cultivar differences in all parameters assessed. The results confirm that low temperature storage (15 °C) is the most important factor in extending the storage life of sweetpotato roots, as evidenced by weight loss and sprouting rates. Hot water dipping (50 °C for 30 min) alone is an inadequate treatment for extending the storage life of sweetpotato roots, and led to higher weight loss, however it did significantly reduce sprouting at 15 °C in ‘Owairaka Red’, and increased TPC in ‘Purple Star’ during early storage. Further studies will explore if combining hot water treatments and edible coatings will retain sweetpotato root quality and phytochemical composition. In addition, details of the hot water treatment conditions (temperature and immersion time) need to be further examined to identify a combination that restricts sprouting without introducing other adverse effects on root quality.

4 Hot water dipping and edible coatings to extend the storage life of sweetpotato roots

4.1 Introduction

In the previous chapter, the effects of hot water treatment on sweetpotato root storage quality were studied. The results showed that hot water treatment alone is inadequate method of extending the storage life of sweetpotato roots, as under elevated storage temperatures it increased the rate of weight loss. Published studies have reported reduced weight loss in fruits and vegetables with the use of edible coatings: Indian jujube fruit (Qiuping & Wenshui, 2007); sweetpotato (Afolabi & Oloyede, 2011); pear (Amarante et al., 2001), and mango (Hoa & Ducamp, 2008). Although several authors have listed sweetpotato as one of the vegetables that could benefit from use of coatings (Woolfe, 1992; Ravi et al., 1996; Baldwin, 2005), none of the published research has compared different edible coatings and the internal tissue responses of sweetpotato roots. In this study, different types of coating were evaluated to enable selection of a suitable coating at an appropriate concentration for sweetpotatoes. Appropriate coatings should provide a barrier, decreasing transpiration and respiration rates with minimal effects on internal gas composition (Baldwin, 2005).

In this chapter, the combined effects of hot water dipping/immersion and edible coating were studied during storage. In addition, the water temperature and immersion duration were redefined to determine a safe zone that could inhibit sprouting with minimal adverse effects on sweetpotato roots.

The specific objectives of this chapter are:

1. To redefine the optimum temperature and immersion time that will control sprouting with minimal adverse effects on weight loss.
2. To determine the most appropriate edible coating for sweetpotato that will reduce weight loss without negative effects on internal gas atmosphere.
3. To determine the combined effects of hot water dipping and the use of edible coating on sweetpotato storage quality (sprouting, weight loss and phytochemical concentration).

4.2 Materials and methods

4.2.1 Plant samples

Commercial cultivar ‘Owairaka Red’ and advanced breeding line ‘Clone 1820’ were used for the experiments. ‘Clone 1820’ is an advanced clone under evaluation at Plant and Food Research (New Zealand); it was selected from segregating sweetpotato seed populations provided by the International Potato Centre - Peru (CIP). ‘Clone 1820’ has a unusual flesh colour combination of both orange and purple. ‘Owairaka Red’ roots were sourced from Delta Produce, Dargaville, whereas ‘Clone 1820’ was sourced from Plant and Food Research, Pukekohe, New Zealand. The roots were transported to Massey University within 24 h of harvest. ‘Owairaka Red’ was used for experiments 1 and 2, whereas for experiment 3, both ‘Clone 1820’ and ‘Owairaka Red’ were used. Upon receiving the roots in the laboratory, roots with visible defects were removed, and those without defects were cured at 30 °C and 85 - 95% relative humidity for 4 days in a controlled temperature room.

4.2.2 Experiment 1: Selection of optimal hot water temperature and immersion time

Hot water temperatures between 40 and 55 °C, and immersion times of 2 to 32 min, were evaluated in a full factorial experiment with 2 replicates, each replicate contained 5 roots. Replicates were stratified according to root size, replicate 1 comprising large roots (mean 400 g) and replicate 2 small roots (mean 150 g). Hot water treatment was performed as described in section 3.2.2. The roots were immersed in a thermostatically controlled water bath for the designated time and temperature combinations. After treatment the roots were cooled under running tap water for 10 min, and then they were air dried at ambient temperature, assisted by an electric fan, until their surfaces was visually dry. Thereafter, roots were stored at 25 ± 2 °C and 85 - 90% RH for 2 weeks. Measurements of water vapour permeance were taken before storage (details in section 4.2.6). Percentage of sprouted roots and rot incidence were recorded after 2 weeks of storage.

4.2.3 Experiment 2: Selection of edible coatings

Two types of edible coatings: Apple Clear (Carnauba wax, Castle Chemicals, Australia) and Whey Protein Concentrate (WPC) (Fonterra, New Zealand) were tested for their effect on sweetpotato root water vapour permeance, weight loss and internal gas modifications during 2 weeks storage at 25 °C and 85 - 90% RH.

4.2.3.1 Coating preparation

WPC solution was prepared, based on the method by Hassani et al. (2012). Briefly, 10 g powder of whey protein (90% protein) was added to 100 mL water and the mixture was blended using hand-held blender (Black and Decker Model SB 3000). The protein in WPC solution was denatured by heating the WPC solution in a water bath with continuous stirring at 90 °C for 30 min. Thereafter the WPC solution was cooled to room temperature, followed by addition of 3 g glycerol per gram of the whey protein powder used, and either 0.1 or 0.2 g of rice bran oil per 100 mL of WPC solution. Carnauba wax was prepared by diluting the concentrated carnauba wax in distilled water at room temperature to a final concentration of 5, 10 or 20%.

4.2.3.2 Coating application

Sweetpotato roots were washed using tap water and allowed to dry at room temperature. Coatings were applied by dipping the roots in the coating solution for 5 min. After coating, sweetpotatoes were allowed to dry at room temperature, with frequent turning of the roots to avoid accumulation of the coating on one side. The control roots were subjected to the same procedure, except that they were dipped in water instead of a coating. Each treatment contained 15 roots, all of which were used for measuring water vapour permeance before storage. A sub-sample of 5 roots was then used for internal gas measurement and the remaining 10 roots were used for measuring weight loss after 2 weeks of storage. Coated and uncoated roots were stored at 25 °C and 85 - 90% RH for 2 weeks.

4.2.4 Experiment 3: Combined effects of hot water treatment and coating

Cured roots of 'Clone 1820' and 'Owairaka Red' were subjected to the following treatments: Hot water dip (HWD), Hot water dip plus coating (HWDC), coated (but not dipped in hot water) and control (uncoated and cold water dipped). Based on the results from experiment 1 and 2, a hot water treatment was conducted at 51 °C for 11 min, and carnauba wax (5%) was applied as the coating. In the combination treatment, the

coating was applied after 10 min cooling in running cold water of HWD samples. Following treatment, sweetpotatoes were stored at 25 ± 2 °C and 85% RH for 8 weeks. A total of 51 roots were used per treatment, 15 roots were used for non-destructive measures of weight loss and sprouting, with repeated measures over time, and 36 roots (4 roots/replicate x 3 replicates x 3 sampling times) were used for phytochemical analysis at 2, 5, and 8 weeks of storage.

4.2.5 Physical properties

Root density, volume, and surface area were required to allow calculation of water vapour permeance of sweetpotato roots.

4.2.5.1 Sweetpotato root volume and density

The root volume was measured by a displacement method. Sweetpotato roots of known weight were immersed in graduated beaker containing water. The root volume was calculated as the difference between the initial volume of the water in the beaker and the combined volume of the water and the immersed root. Root volume and weight showed a strong linear relationship ($R^2 = 0.99$, $P < 0.001$) (Figure 4.1). Root density was calculated by dividing the mass of the root by its corresponding measured volume.

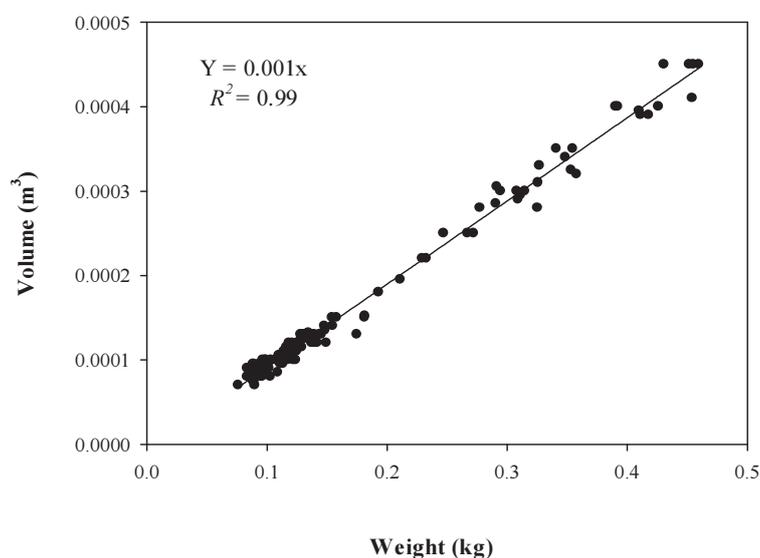


Figure 4.1: Relationship between sweetpotato storage root weight (kg) and volume (m³) for cultivar 'Owairaka Red'. Datum points for individual roots are plotted, along with a continuous line representing the fitted linear relationship.

4.2.5.2 Surface area

The surface area of sweetpotato roots was measured following the procedure described by Banks (1985). Sweetpotato roots of varying sizes were selected and covered with electrical insulation tape, which was later cut and peeled off the root. Flattened pieces of the tape were pasted on A4 paper and scanned. Scanned images were analysed using ImageJ (Image processing and analysis in Java), as described by Jansasithorn (2012). The surface areas of roots of known weight were calculated, using a standard calibration curve. The curve was based on the root surface area established by using tape and the scanned image pixel counts. Thereafter the relationship between weight and surface area were established (Figure 4.2).

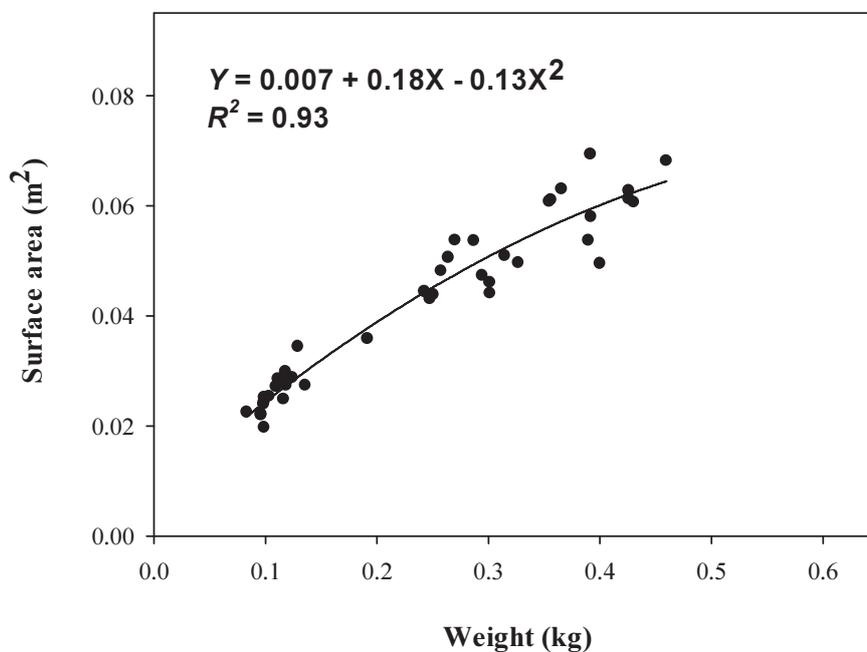


Figure 4.2: Relationship between surface area (m²) and weight (kg) for sweetpotato storage roots cultivar 'Owairaka Red'. Datum points for individual roots are plotted, along with a continuous line representing the fitted relationship.

4.2.6 Water vapour permeance

Water vapour permeance was measured at the start of the experiment, following the procedure described by Maguire (1998). After hot water dipping, sweetpotato roots were allowed to surface dry, and then individual roots were weighed using a precision balance (0.001 g Model P503S, Mettler Toledo, Australia). The roots were placed in a cabinet (Figure 4.3) designed for permeance measurement, fitted with four fans to

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achieve an airflow of approximately 3 m s^{-1} . The rate of weight loss from each individual root was determined by weighing the roots at 0 and 16 h after placement in the cabinet. Relative humidity in the cabinet was determined by using wet and dry bulb temperature readings recorded using thermistor probes (CM types, U bead, $\pm 0.2 \text{ }^\circ\text{C}$; Grant Instrument, Cambridge, U.K). Root periderm temperature was measured by inserting thermistor probes (FF type, U bead, $\pm 0.2 \text{ }^\circ\text{C}$; Grant Instrument, Cambridge, U.K) under the root periderm and temperature data was logged on a Grant Squirrel data logger (1200 series Grant Instrument, Cambridge, U.K.). The surface area of sweetpotato roots used for water vapour permeance calculations was estimated as described previously (Section 4.2.5.2).



Figure 4.3: Cabinet used for water vapour permeance measurements

Rearranging Fick's first law of diffusion (Nobel, 2009).

$$P'_{\text{H}_2\text{O}} = \frac{\dot{r}_{\text{H}_2\text{O}}}{A \cdot \Delta p_{\text{H}_2\text{O}}} \quad \text{Equation 4.1}$$

Where:

$P'_{\text{H}_2\text{O}}$ = Water vapour permeance of the root surface ($\text{mol} \cdot \text{s}^{-1} \text{ m}^{-2} \text{ Pa}^{-1}$)

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r'_{H_2O} = Rate of water loss ($\text{mol}\cdot\text{s}^{-1}$)

A = Surface area of the root (m^2)

Δp_{H_2O} = The difference in partial pressure of water vapour between the environment and the root (Pa)

According to the laws of diffusion, water moves from regions of high concentration to those of low concentration. Therefore the driving force for loss of water in plants is the difference in partial pressures of water vapour between the environment and the plants:

$$\Delta p_{H_2O} = p_{H_2O}^f - p_{H_2O}^e \quad \text{Equation 4.2}$$

Where:

$p_{H_2O}^f$ = partial pressure of water vapour in the root (Pa).

$p_{H_2O}^e$ = partial pressure of water vapour of the environment (Pa)

The partial pressure of water at the root surface was estimated based on the properties of air as presented on a psychrometric chart using the Tetens equation for saturated water vapour pressure (Tetens, 1930):

$$p_{H_2O}^{sat}(T) = 611 \exp\left(17.27\left(\frac{T}{T+237.3}\right)\right) \quad \text{Equation 4.3}$$

Where:

$p_{H_2O}^{sat}(T)$ (Pa) is the saturated water vapour pressure at a given temperature (T) ($^{\circ}\text{C}$)

The partial pressure of water vapour for the environment was calculated using the following equation:

$$p_{H_2O}^e = 611 \exp\left(17.27\left(\frac{T_e}{T_e+237.3}\right)\right) \times \frac{RH}{100} \quad \text{Equation 4.4}$$

Where:

RH = relative humidity (%)

T_e = temperature of environment ($^{\circ}\text{C}$)

Relative humidity

$$RH = \frac{P_{H_2O}^e}{P_{H_2O}^{sat}(T_e)} \times 100 \quad \text{Equation 4.5}$$

$p_{H_2O}^{sat}(T_e)$ = saturated water vapour pressure at temperature T_e (Pa).

4.2.7 Measurement of internal gases

A sub-sample of 5 roots per treatment was used for measurement of root internal atmospheric changes. The internal atmospheres were measured by cannulation (Banks, 1983). Briefly, the surface of the sweetpotato root was cleaned using absolute ethanol. A plug of tissue was removed from the root using hollow 14 gauge needle. Thereafter, an approximately 25 mm long hollow stainless steel needle (14 gauge) without a tip end was inserted into the created hole (Figure 4.4). The outside end of the cannula was glued onto the root surface. A cut-down syringe tip with a septum was tightly pressed into the end of the cannula, and filled with 1 mL water.

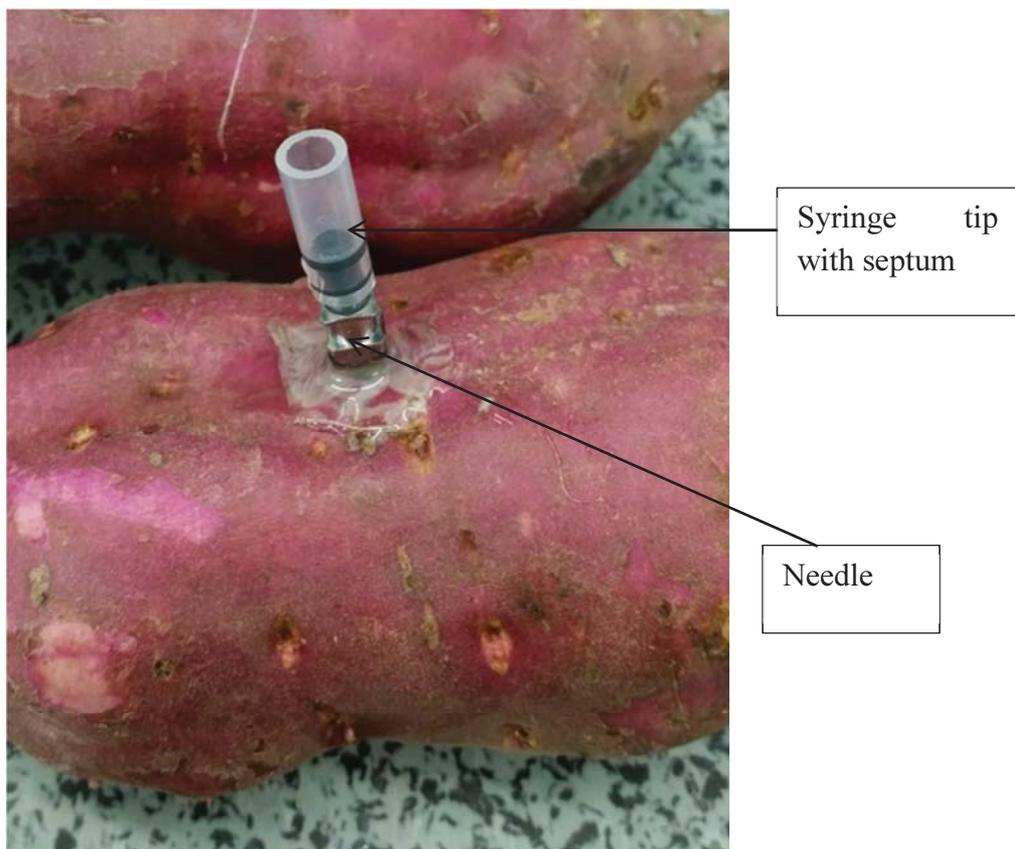


Figure 4.4: Cannulated sweetpotato storage root for internal gas measurements

Using a gas tight glass syringe (Hamilton), 100 μL of gas was taken from each sweetpotato sample. The oxygen and carbon dioxide concentrations were analysed simultaneously using an oxygen electrode (Citicell C: S type, City Technology Ltd., London, UK) in series with a miniature infrared carbon dioxide transducer (Analytical Development Company, Hoddesdon, UK). Nitrogen gas, free of oxygen, was used as a carrier gas (flow rate 35 mL min^{-1}). Output signals were analysed by an integrator (Hewlett Packard, Model 3394A). The internal root concentration for oxygen and carbon dioxide was measured every 2 days, up until the 14th day of storage.

4.2.8 Quality evaluation

4.2.8.1 Sprout growth

Sprouting was assessed in terms of both numbers and length. Sprout length was measured to 0.02 mm accuracy using a digital vernier caliper. Details are described in section 2.3.2. For screening of hot water parameters, number of sprouted roots was counted and expressed as a percentage.

4.2.8.2 Weight loss

Sweetpotato roots were weighed individually to 0.001 g precision using a Mettler Toledo balance. Weight loss was expressed as a percentage of the initial weight prior to hot water dipping for hot water dipped roots and after coating for the coated roots.

4.2.9 Phytochemical concentration

Carotenoids, individual phenolics and anthocyanins were measured in ‘Clone 1820’ at 0, 2, 5, and 8 weeks of storage. Sample preparation, extraction and HPLC conditions were as described previously in section 2.4.3 for carotenoids and section 2.4.4 for phenolic acids and anthocyanins.

4.3 Data analysis

A General Linear Model (GLM) analysis was conducted in order to determine if there were significant differences amongst the treatments (Minitab version 16.0, Minitab Inc., State College, Pennsylvania). Data for percentage weight loss and sprout length and number were analysed as repeated measures over time, and data on sprout number and length were transformed using square root and log transformations respectively. Mean comparisons were carried out using Tukey’s test, at $P < 0.05$. All transformed means

were back transformed for presentation purposes. For experiment 1, the data were analysed by plotting contour plots.

4.4 Results

4.4.1 Selection of optimal hot water temperature and immersion time

Exposure of sweetpotato roots to temperatures of 40 to 55 °C, and immersion times of 2 to 32 min had little or no effect on the water vapour permeance (Figure 4.5 A & D). In smaller sized roots (Figure 4.5A), temperatures above 46 °C and immersion times above 20 min caused water vapour permeance to rise to 150 nmol s⁻¹ m⁻² Pa⁻¹. For larger roots, a similar trend was observed, higher water vapour permeance with higher temperature and longer immersion times (Figure 4.5D). The contour plots for sprouting (Figure 4.5B & E) show that 1 to 30 % sprout growth inhibition was achieved with higher water temperatures (> 52 °C) and longer immersion times (> 20 min). For smaller roots, water temperatures slightly above 50 °C, at both short and longer immersion times reduced sprouting by 75%, while for bigger roots, a combination of high temperatures above 50 °C and short immersion times (< 7 min) were not effective in reducing sprouting, but become effective when applied for longer immersion times (> 7 min). All temperatures below 48 °C were ineffective in controlling sprouting regardless of the immersion time and root size. Based on these results, a combination of high temperatures (> 50 °C) and immersion times (> 7 min) was selected as the best combinations for sprout control. The minimum (< 15%) rot incidence was attained at temperatures below 53 °C combined with short immersion times (< 12 min) (Figure 4.5C & F). Hot water temperatures above 51 °C for 27 to 32 min resulted in over 30% rot incidence. The immersion temperature and time combinations which results in higher rot incidence should be avoided. An optimum treatment was defined as temperature and immersion time combinations that reduced sprouting by 70% and kept rot incidence below 15%. In this case, temperatures between 50 and 52 °C and immersion times between 7 and 12 min were the most favourable ranges.

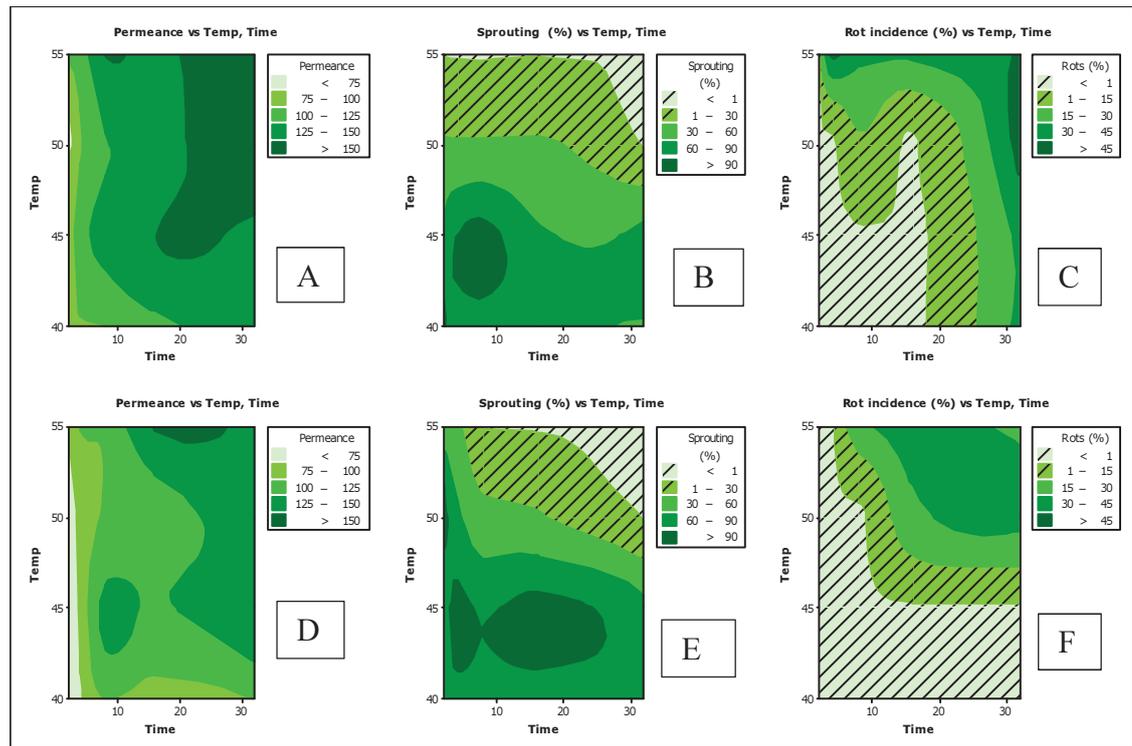


Figure 4.5: Contour plots for water vapour permeance (A & D), sprouting (%) (B & E) and rot incidence (%) (C & F) of sweetpotato roots as affected by hot water temperature (40 - 55 °C) and immersion time (2 - 32 min). Roots were stratified according to size: Small roots (A, B & C) and large roots (D, E & F). Water vapour permeance measurements were taken at 16 h after hot water dipping, while weight loss (%) and sprouting were assessed after 2 weeks of storage at 25 °C

4.4.2 Effect of edible coatings on sweetpotato weight loss and internal gas concentration

The water vapour permeance of coated sweetpotatoes was 10 to 27% less than the average water vapour permeance of untreated control roots (Table 4.1). Among the coating formulations, carnauba 20% showed a significantly ($P < 0.001$) lower water vapour permeance than the other coating formulations, except WPC + 0.2 g oil. As expected, the beneficial effects of coating in reducing water vapour permeance were also observed as reduced weight loss. After 14 days of storage, weight loss (%) of coated roots was approximately 20% lower, when compared to the control treatments (Table 4.1). Comparing the three carnauba based formulations, significant differences in weight loss reduction were only observed between carnauba 20% and carnauba 5%. Similarly, no differences were observed between WPC + 0.1 g oil and WPC + 0.2 g oil.

This suggests that the addition of more rice bran oil to whey protein concentrate had no added advantage in reducing weight loss.

At the start of the experiment, the internal carbon dioxide (CO₂) concentrations in all the treatments, excluding the control, were above 9% but were not significantly different among the treatments (Table 4.1). With an increased storage duration, the internal CO₂ slightly increased. At 14 days of storage, the lowest internal oxygen concentrations were found in roots coated with carnauba 20%, WPC + 0.1 g oil and WPC + 0.2 g oil, and the highest internal oxygen levels were observed in uncoated roots and roots coated with 5 or 10% carnauba. With regards to internal CO₂ concentration, WPC + 0.2 g oil had the highest internal CO₂ concentration, followed by WPC + 0.1 g oil and carnauba 20% (Table 4.1). When compared to the control, the CO₂ concentration in WPC + 0.2 g oil, WPC + 0.1 g oil and carnauba 20% were higher by 78, 38 and 36% respectively.

Table 4.1: Water vapour permeance, weight loss (%) and internal gas atmospheres of coated and uncoated sweetpotato ‘Owairaka Red’ roots following storage at 25 °C and 85-90% relative humidity.

Treatment	Permeance (nmol s ⁻¹ m ⁻² Pa ⁻¹) n = 15	Weight loss (%) n = 10	O ₂ % 2 days n = 5	CO ₂ % 2 days n = 5	O ₂ % 14 days n = 5	CO ₂ % 14 days n = 5
Control	55 ^a	10.5 ^a	14.6 ^a	8.5 ^b	14.2 ^a	10.3 ^c
Carnauba 5%	49 ^b	7.3 ^b	14.4 ^a	9.0 ^{ab}	13.7 ^a	9.9 ^c
Carnauba 10%	44 ^{cd}	6.9 ^{bc}	13.8 ^{ab}	9.5 ^{ab}	14.2 ^a	9.7 ^c
Carnauba 20%	40 ^d	6.5 ^c	13.2 ^{ab}	9.6 ^{ab}	10.2 ^b	14.0 ^b
WPC + 0.1 g Oil	45 ^c	6.8 ^{bc}	10.7 ^b	12.5 ^a	10.0 ^{bc}	14.2 ^b
WPC + 0.2 g Oil	42 ^{cd}	7.3 ^{bc}	11.6 ^{ab}	10.6 ^{ab}	7.0 ^c	18.3 ^a
P-value	< 0.001	< 0.001	0.006	0.043	< 0.001	< 0.001

Means followed by same letters within a column are not significantly different at $P < 0.05$ (Tukey's test). Water vapour permeance was measured 16 h after coating. Weight loss (%) was measured after 14 days of storage.

Taking into account their effects on weight loss, water vapour permeance and internal gas modifications; carnauba 5% and carnauba 10% were chosen as the best coating types to be used in the main experiment. These coatings were effective in reducing weight loss, while their internal oxygen and carbon dioxide concentrations were not significantly different ($P > 0.05$) from the control

4.4.3 Effect of hot water dipping and coating on sweetpotato storage quality

4.4.3.1 Sprout growth

HWD and HWDC delayed sprout growth by 2 weeks in both cultivars (Table 4.2). Throughout the storage period, HWD treatment had the least sprout number per root and shortest length compared to the control ($P < 0.05$); whereas in HWDC treatment sprout growth was similar to the control at 5 weeks of storage suggesting that addition of coating reduced the effectiveness of heat treatment in controlling sprouting. Surprisingly, coating without a hot water dip increased the sprout growth both in terms of numbers and length in ‘Owairaka Red’ roots, but this did not occur in ‘Clone 1820’. At 8 weeks of storage the sprout number and length in coated roots without hot water dipping was about 1.6 times the sprout numbers and length of the control roots. Cultivar ‘Owairaka Red’ had more vigorous sprout growth than ‘Clone 1820’.

4.4.3.2 Distribution of sprouts following various treatments in ‘Owairaka Red’ sweetpotatoes

Sweetpotatoes marketed in New Zealand are graded into three categories: TAG 1, 2 and 3; where TAG 1 is the first quality grade, TAG 2 is of secondary grade and TAG 3 is the minimum quality grade for acceptable produce (Turners and Growers & MG Marketing, 2014). One of the grading criteria is sprout condition, both the percentage of sprouted roots and sprout length are used as quality criteria. For roots graded as Tag 1, the percentage of roots with sprouts greater than 5 mm should be less than 5 %. Tag 2 could have 5 to 10 % roots with sprouts greater than 5 mm and Tag 3 could have < 20% with sprouts greater than 10 mm. Mean sprout length was calculated for each root, then the number of roots in each category were counted and converted into a percentage. A histogram was prepared for mean root lengths in the following categories 0 - 5, 6 - 10 and > 10 long (mm). At 2 weeks after storage, HWD and HWDC were the best treatments, as 100% of the roots were in TAG 1 (0 - 5 mm) category, compared to only

47 and 33% in the control and coated treatments respectively (Figure 4.6). At the end of the storage period, 30% of roots in HWD treatment were still acceptable as TAG 1.

Table 4.2: Mean sprout number/root and sprout length (mm) of sweetpotato ‘Owairaka Red’ and ‘Clone 1820’ during storage at 25 °C and 85-90% relative humidity

Cultivar	Treatment	Mean sprout number/root			Mean sprout length (mm)		
		2 weeks	5 weeks	8 weeks	2 weeks	5 weeks	8 weeks
Owairaka Red	Control	7.34	17.99 ^a	18.86 ^b	5.31	24.11 ^a	38.8 ^b
	HWD	0.00	8.50 ^b	9.11 ^c	0.00	6.94 ^b	9.00 ^c
	Coated	8.50	27.59 ^a	31.99 ^a	9.00	49.11 ^a	62.00 ^a
	HWDC	0.00	17.99 ^a	17.14 ^b	0.00	30.62 ^a	24.11 ^{bc}
Clone 1820	Control	1.00	2.00 ^a	2.00 ^a	2.00	18.95 ^a	30.6 ^a
	HWD	0.00	1.00 ^b	1.00 ^c	0.00	2.00 ^b	9.00 ^b
	Coated	1.00	1.20 ^a	1.50 ^{ab}	2.00	14.84 ^a	18.95 ^a
	HWDC	0.00	1.00 ^b	1.20 ^{bc}	0.00	6.94 ^{ab}	14.84 ^{ab}

Data were repeated measures so were analysed separately at each time point. Data at 2 weeks were excluded from the statistical analysis because of the large number of roots with no sprouts. Means followed by same letters in a column are not significantly different at $P < 0.05$ (Tukey’s test). All values are means of 15 roots. HWD were hot water dipped roots, while HWDC roots were both hot water dipped and coated.

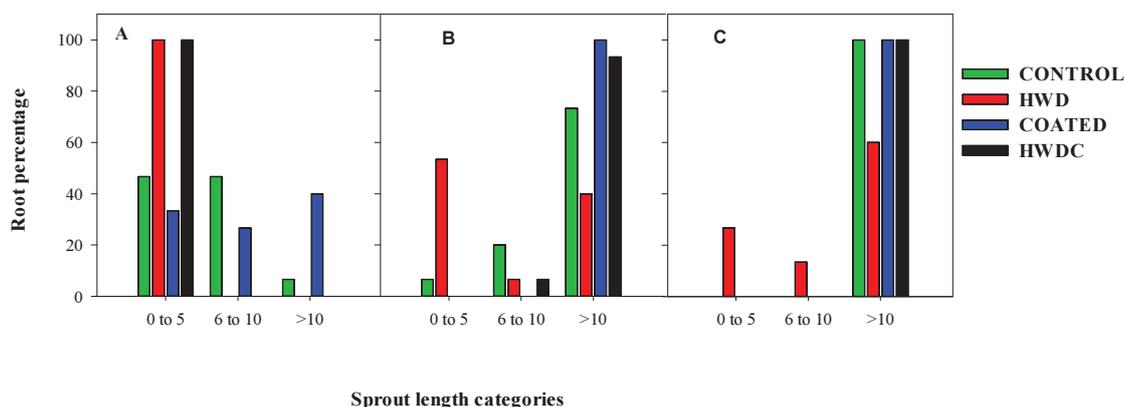


Figure 4.6: Distribution of sweetpotato cv. Owairaka Red sprout length categories per treatment at each sampling time: 2 weeks (A), 5 weeks (B) and 8 weeks (C) during storage at 25 °C and 85 % RH. HWD: hot water dipped, HWDC: hot water dipped and coated.

4.4.3.3 Weight loss

As expected, root weight loss increased in all treatments with increasing storage time. The application of edible coating, with or without prior hot water dipping, significantly ($P < 0.05$) reduced weight loss during storage in both cultivars (Figure 4.7). For ‘Owairaka Red’, the effect of coating on weight reduction was more pronounced throughout the storage period in roots that were hot water dipped compared to undipped roots (Figure 4.7A), whereas for ‘Clone 1820’, the interaction effects of hot water treatment and coating were only observed at 8 weeks (Figure 4.7B).

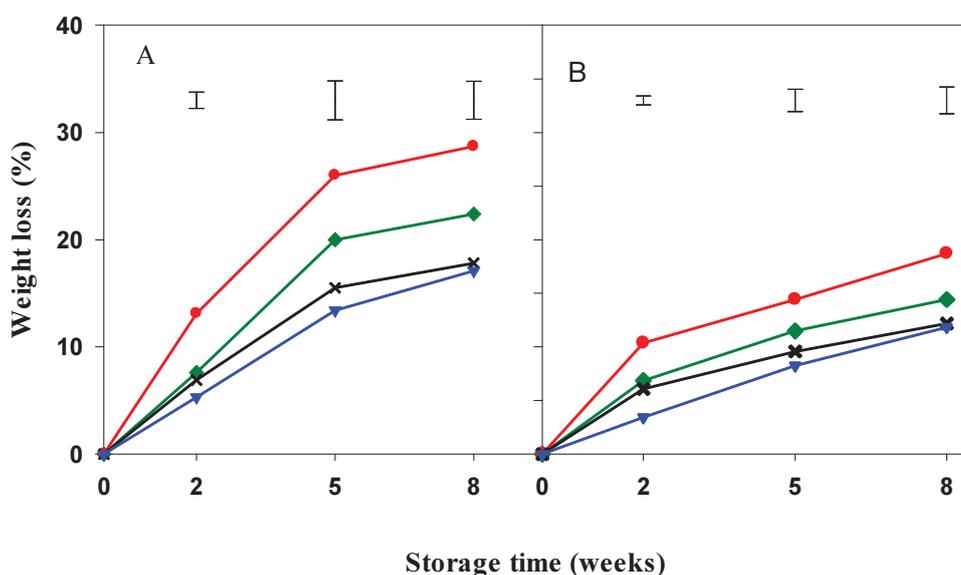


Figure 4.7: Weight loss (%) of sweetpotato ‘Owairaka Red’ (A) and ‘Clone 1820’ (B) subjected to various treatments : Control (♦), HWD: Hot water dipped (●), Coated (▼) and HWDC: hot water dipped and coated (x). The roots were stored for up to 8 weeks at 25 °C and 85-90% RH. Each data point represents a mean of 15 roots. Vertical bars represent $HSD_{0.05}$ (Tukey’s test) at each sampling point.

4.4.3.4 Identification of phytochemicals in ‘Clone 1820’

4.4.3.4.1 Total carotenoids and β -carotene content

Three major carotenoid peaks were eluted at 5.18, 19.89 and 34.64 min (Figure 4.8). All peaks had maximum absorbance at 455 nm. The peak that eluted at 34.7 min was identified as β -carotene by comparison with an external standard. Concentrations of all carotenoid peaks were quantified using a β -carotene standard curve and the concentration were expressed as mg β -carotene equivalent /100 g DW.

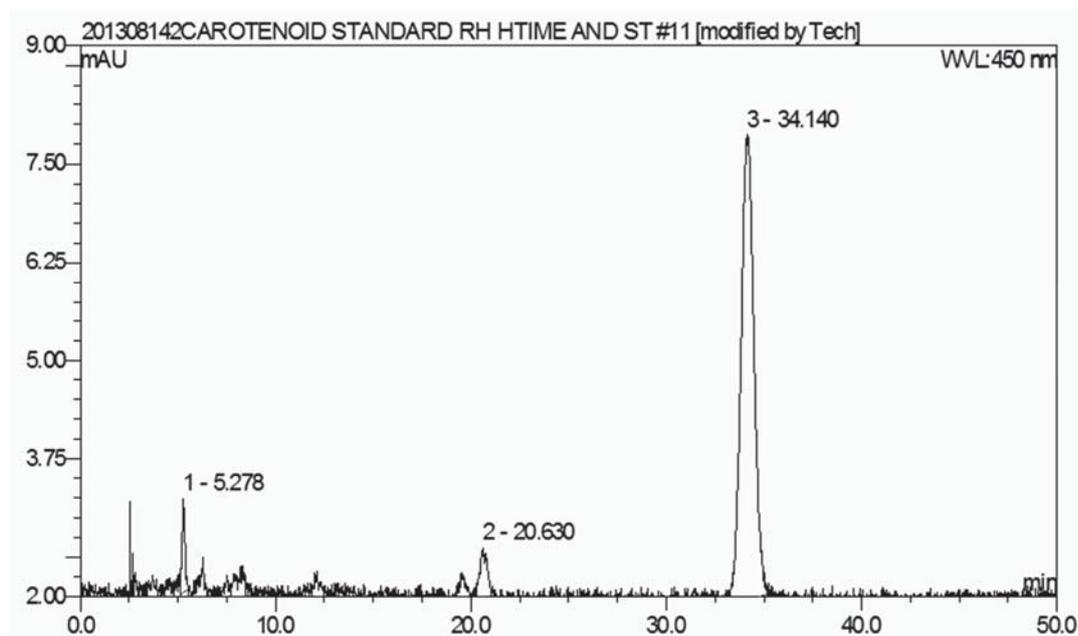


Figure 4.8: Example of HPLC chromatography separating carotenoids in ‘Clone 1820’ sweetpotato roots

4.4.3.4.2 Phenolic acids

There were 8 main phenolic acid peaks eluted (Figure 4.9), and all peaks had maximum absorbance at around 322 to 324 nm (Table 4.3). Peaks 2, 4, 5, and 6 were identified as chlorogenic acid, 3,5-dicaffeoylquinic acid, 3,5-dicaffeoylquinic, and 4,5-dicaffeoylquinic acid respectively. Chlorogenic acid (561 mg/100 g DW) and 3,5-dicaffeoylquinic (1059 mg/100 g DW) were present in the highest concentrations at harvest, representing 90% of the total phenolic acid measured in ‘Clone 1820’ (Table 4.3).

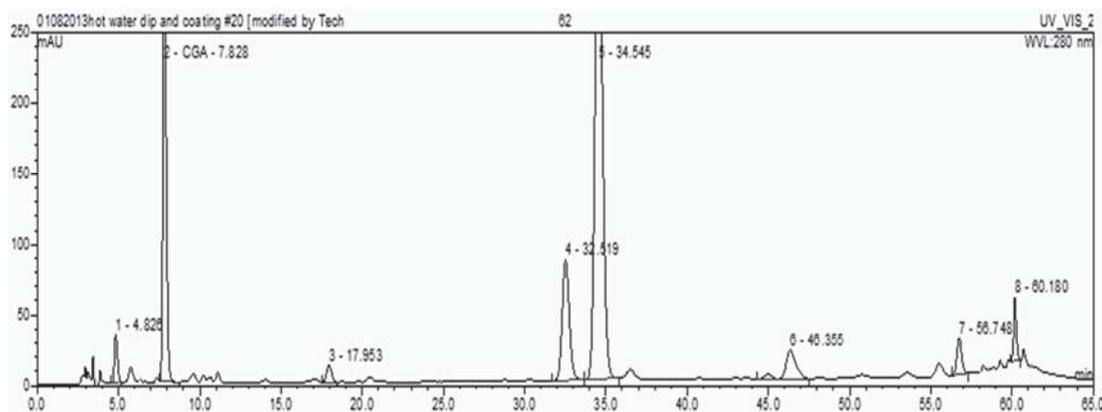


Figure 4.9: A representative HPLC chromatogram of phenolic acids in a root extracts of sweetpotato cv. ‘Clone 1820’

Table 4.3: Identification of phenolic compounds in ‘Clone 1820’ sweetpotato storage root and their concentrations at harvest

Peak no.	Retention time (mins)	λ Max.	Identification	Amount at harvest* (mg /100 g DW)
1	4.85	322	Chlorogenic acid isomer	33.2
2	7.88	323	Chlorogenic acid	561
3	17.95	323	unknown	18.1
4	32.56	322	3,4-dicaffeoylquinic acid	23.3
5	34.55	324	3,5-dicaffeoylquinic acid	1059
6	44.6	324	4,5-dicaffeoylquinic acid	31.5
7	56.74	324	unknown	38.5
8	59.95	323	unknown	39.8
Total phenolic acid				1804.4

*Amount at harvest expressed as mg chlorogenic acid equivalent /100 g DW. Each data point represents a mean of three replicates.

4.4.3.4.3 Anthocyanins

Identification of anthocyanin was undertaken by Liquid Chromatogram-Mass Spectrometry (LC-MS). There were nine anthocyanin peaks that eluted in the LC-MS chromatogram, the first peak eluted before the chlorogenic isomer, the second peak eluted between the chlorogenic acid isomer and the chlorogenic acid peak, and 7 peaks were observed between chlorogenic acid and the three dicaffeoylquinic acids. No anthocyanin peak eluted after the three dicaffeoylquinic acids. By contrast to the HPLC chromatogram (Figure 4.10), showed only eight individual anthocyanins with no anthocyanin peak observed on the HPLC chromatogram at a retention time before the chlorogenic acid isomer. Another difference was that the anthocyanin peaks were observed after the three dicaffeoylquinic acids. The peaks were identified based on the formula detected, measured mass to charge ratio (m/z) for molecular ions, the fragmentation ions and comparing the sequence of elution of the compounds with existing relevant literature (Table 4.4). Anthocyanins were quantified as cyanidin 3-O-glucoside chloride equivalent (mg/100 g DW).

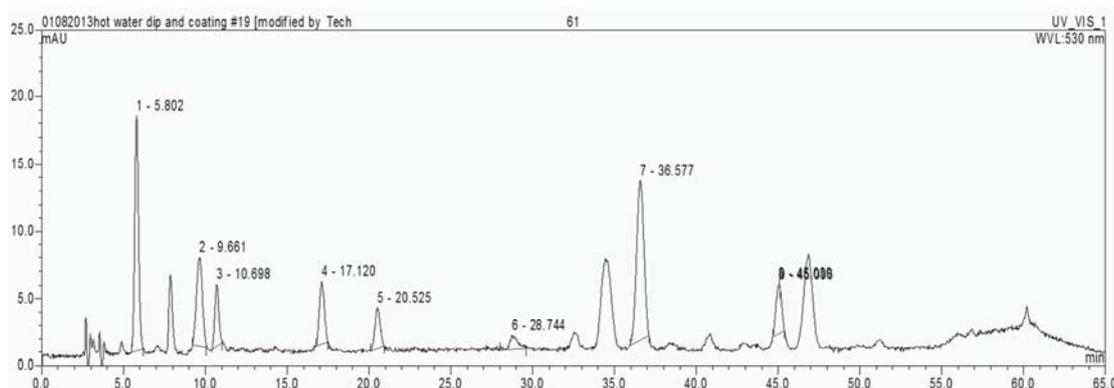


Figure 4.10: HPLC chromatogram for anthocyanins in a root extract of sweetpotato ‘Clone 1820’.

All anthocyanins identified in ‘Clone 1820’ had a basic structure of either cyanidin 3-sophoroside-5-glucoside or peonidin 3-sophoroside-5-glucoside, and were acylated with caffeic, ferulic acid or *p*-hydroxybenzoic acid. The major anthocyanins identified in ‘Clone 1820’ extracts were peonidin-3-sophoroside-5-glucoside (35 mg/100 g DW), unknown peak 7 (31.40 mg/100 g DW) and peonidin-3-*p*-hydroxybenzoyl sophoroside-5-glucoside (23 mg/100 g DW), the other anthocyanins were found in moderate

amounts (Table 4.4). The concentration of peonidin based anthocyanin was 4-times higher than the concentration of cyanidin based anthocyanins.

Table 4.4: Molecular ions, fragmentation ions, tentative identification of anthocyanins in a root extract of sweetpotato ‘Clone 1820’.

Peak	Retention time	M-H (m/z)	Aglycon	Fragmentation ion (m/z)	Proposed identification	*Amount at Harvest (mg/100 g DW)
1	5.73	788	301	463,625	Peo-3-sopho-5-glu	35.0
2	9.59	908	301	463,745	Peo-3- <i>p</i> -hydroxybenzoyl sopho-5-glu	23.0
3	10.67	950	287	449,787	Cya-3-(6"-feruloyl sopho)-5-glu	8.40
4	17.12	935	287	449,773	Cya-3-caffeoyl sopho-5-glu	8.31
5	20.5	949	301	787,463	Peo-3-caffeoyl sopho-5-glu	8.28
6	28.74	1069	301	907,461	Peo-3-caffeoyl- <i>p</i> -hydroxybenzoyl sopho-5-glu	3.72
7	36.6	**		unknown	Unknown	31.46
8	45.07	**		unknown	Unknown	20.80
Total anthocyanin						138.97
Peonidin-based anthocyanin						70.00
Cyanidin-based anthocyanin						16.71

* Values expressed as mg of cyanidin 3-O-glucoside chloride equivalent (mg/100 g DW). Peo = peonidin, Cya = cyanidin, sopho = sophoroside, glu = glucoside ** Peaks not detectable by LC-MS.

4.4.3.5 Effect of hot water dipping and edible coating on the phytochemical concentrations

Principal component analysis (PCA) was undertaken to identify possible patterns in the data related to pre-storage treatments and storage effects. Data for all the anthocyanins, phenolic acids and β -carotene peaks were used for the PCA. Four principal components with eigenvalues values greater than one were retained, and these four PCAs accounted for 75% of the total variance of the data set. The score plot (Figure 4.11A) shows that principal component 1, which accounted for 41% of the variation in the data separates the data according to storage duration, and principal component 2 which explains 15% of the variation in data, separates the freshly harvested and the sequential results of the stored samples. There were no clear separation according to the treatments, but there was slight separation between HWDC and the other treatments at 2 and 5 weeks of storage.

Plotting data for each individual storage period shows a clear pattern on how the data were separated according to treatments (Figure 4.11B-D). The principal component score plot at 2 weeks (Figure 4.11B) shows that there was a clear separation of the samples according to treatments. Principal component 1 separated the hot water dipped samples and non-hot water dipped, and principal component 2 separates the coated and uncoated treatments. Score plots for other storage period did not show any particular pattern (Figure 4.11C and D). This result suggests that with progression in storage time any differences between the treatments diminished. Henceforth only data at 2 weeks of storage will be presented in detail. The loading plot (Figure 4.12) for PCA at 2 weeks after storage was examined to identify phenolic compounds associated with the observed pattern. Principal component one was associated with following compounds: chlorogenic acid isomer, 3,5-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid, cyanidin-3-(6''-feruloyl sophoroside)-5-glucoside and peonidin-3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside, whereas principal component two was associated with 3,4-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, peonidin-3-*p*-hydroxybenzoyl sophoroside-5-glucoside, and cyanidin-3-caffeoyl sophoroside-5-glucoside.

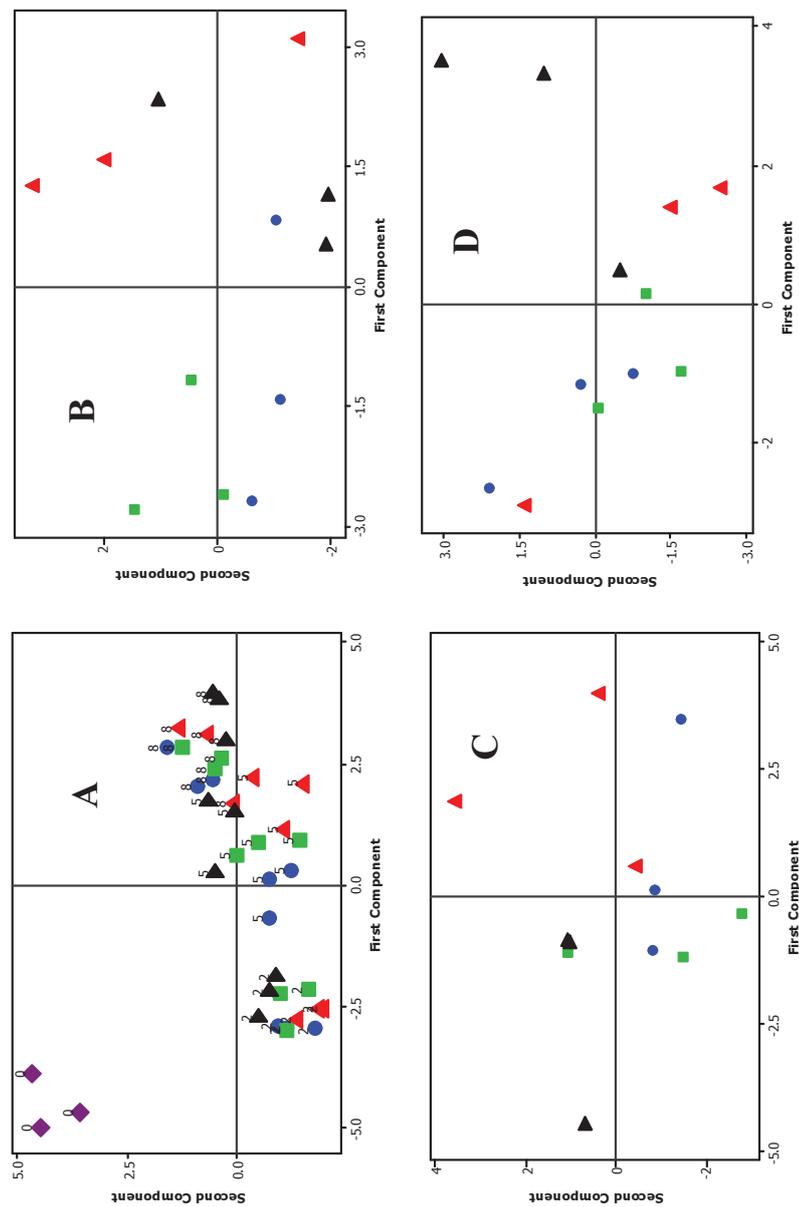


Figure 4.11: Principal component analysis of phenolic compounds and β -carotene of sweetpotato ‘Clone 1820’; subjected to different prestorage treatments: HWDC: Hot water dipped and coated, HWD: Hot water dipped, Coated and Control (untreated). Plots represent scores using at harvest data and storage data (A), 2 weeks data (B), 5 weeks data (C) and 8 weeks data (D). Each data point represents a replicate. Symbols represents: At harvest (◆), Control (■), HWD (▲), Coated (▼) and HWDC (●).

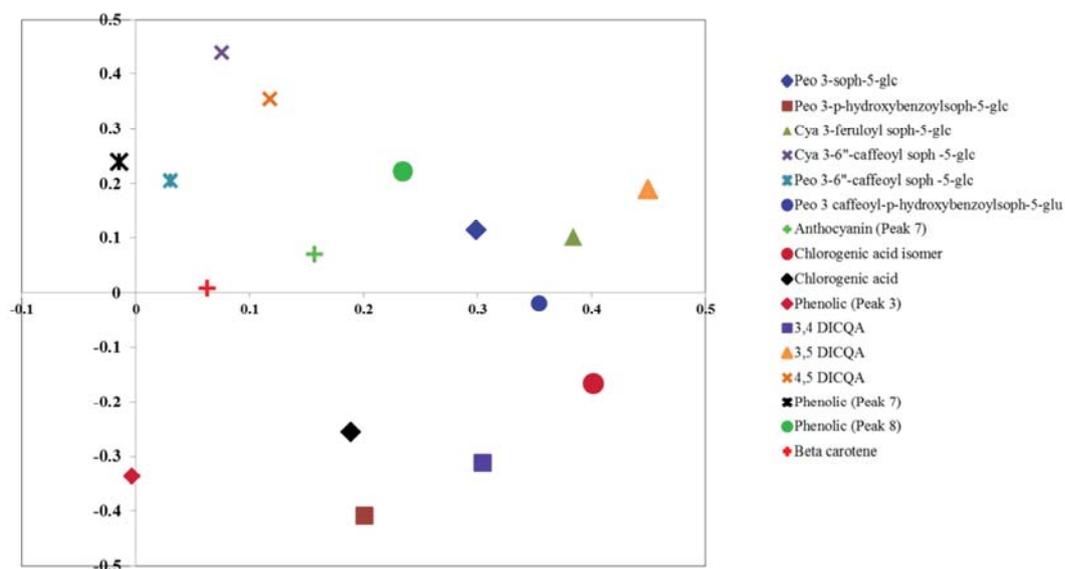


Figure 4.12: Loading plot for principal components analysis based on phenolic compounds and β -carotene in 'Clone 1820' sweetpotato root at 2 weeks of storage

4.4.3.6 Effect of hot water dipping on selected phenolic compounds

Changes in phenolic compound concentrations due to hot water dipping were only analysed for peaks that showed close association with PC 1, as presented in figure 4.11. These peaks were: chlorogenic acid isomer, 3,5-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid, cyanidin-3-(6"-feruloyl sophoroside)-5-glucoside and peonidin-3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside. To determine how heat treatment affected the phenolic compounds, one way analysis of variance (ANOVA) was conducted on the concentration of these peaks using hot water treatment as the factor. Concentrations of cyanidin-3-(6"-feruloyl sophoroside)-5-glucoside, chlorogenic acid isomer, 3,5-dicaffeoylquinic acid and 3,4-dicaffeoylquinic acid slightly increased (7 to 10%) with hot water treatment (Table 4.5), but for peonidin-3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside there was no significant difference between the hot water dipped and non-hot water dipped roots.

Table 4.5: Effect of hot water treatment on the concentration of selected phenolic compounds in ‘Clone 1820’ sweetpotato root after 2 weeks of storage at 25 °C and 85% RH

Treatment	Antho Peak 3	Antho Peak 6	Phenolic Peak 1	Phenolic Peak 4	Phenolic Peak 5
Non-HWD*	7.0 ^b	24.6	35.2 ^b	112 ^b	1022 ^b
HWD**	8.7 ^a	25.8	39.8 ^a	130 ^a	1159 ^a
P-value	0.012	ns	0.013	0.012	0.001

Data expressed as mg of chlorogenic acid equivalent and mg of cyanidin 3-O-glucoside chloride equivalent per 100 g of dry weight for phenolics and anthocyanin respectively. *Non-HWD includes the control group and non-dipped coated roots, **HWD include the HWD and HWDC. Means followed by the same letters within the column are not significantly different at $P < 0.05$ (Tukey's test). ns = not significantly different. Anthocyanin peak **3** = cyanidin-3-(6"-feruloyl sophoroside)-5-glucoside and **6** = peonidin-3-caffeoyl-*p*-hydroxybenzoyl sophoroside-5-glucoside, phenolics peaks **1** = chlorogenic acid isomer; **4** = 3,5-dicaffeoylquinic acid and **5** = 3,4-dicaffeoylquinic acid.

4.4.3.7 Effect of coating on selected phenolic compounds concentration

Peaks associated with PC2 in section 4.4.3.5 were used in the analysis to find how these peaks changed with application of coating. Four peaks showed close association with separation of the samples into coated and uncoated groups. However, the analysis of variance showed that there were no significant phenolic peak differences between the coated and uncoated roots (Table 4.6).

Table 4.6: Effect of edible coating on selected phenolic compound concentrations in ‘Clone 1820’ sweetpotato root after 2 weeks storage at 25 °C and 85% RH

Treatment	Phenolic Peak 1	Phenolic Peak 3	Phenolic Peak 6	Phenolic Peak 8
Uncoated*	38.4	25.4	15.0	66.1
Coated**	36.6	22.6	14.0	59.4
P-value	ns	ns	ns	ns

Data expressed as mg of chlorogenic acid equivalent per 100 g of dry weight. Each data point is a mean of 6 replicates. *Uncoated include the control group and HWD. **Coated include the coated and HWDC. Peaks identification: Phenolic peaks: **1** = chlorogenic acid isomer, **3** = unknown, **6** = 4,5-dicaffeoylquinic acid, and **8** = unknown. ns = not significantly different.

4.4.3.8 Changes of selected phytochemicals in ‘Clone 1820’ during storage

4.4.3.8.1 β -carotene and other carotenoids

β -carotene concentration significantly ($P < 0.05$) reduced during storage. Compared with the concentration at harvest, there was a 30% decline in β -carotene concentration after 2 weeks of storage. Thereafter, no further significant reductions in β -carotene concentration were observed (Table 4.7). Concentrations of the other carotenoids were lower at 2 weeks than at the other sampling times. The calculated vitamin A content ranged from 363.4 to 536.7 RAE per 100 g of edible portion of sweetpotato.

Table 4.7: β -carotene concentration and calculated vitamin A value in a root extract of sweetpotato ‘Clone 1820’ during 8 weeks of storage at 25 °C and 85 - 90%

Storage time	β -carotene (mg/100 g DW)	Other carotenoids (mg/100 g DW)	Vitamin A value (RAE/100 g FW)
0 weeks	25.6 ^a	3.3 ^a	536.7 ^a
2 weeks	19.2 ^b	1.4 ^b	402.5 ^b
5 weeks	17.8 ^b	3.0 ^a	373.3 ^b
8 weeks	17.3 ^b	2.5 ^a	363.4 ^b
P-value	< 0.001	< 0.001	< 0.001

Vitamin A value calculated on fresh weight basis, the dry matter of ‘Clone 1820’ was 25%. RAE = Retinol Activity Equivalent (12 μ g β -carotene and 24 μ g of other carotenoids = 1 μ g retinol = 1 RAE). Means followed with same letters within the column are not significantly different at $P < 0.05$ (Tukey’s test). All values are means of three replicates.

4.4.3.8.2 Phenolic acids

The various individual phenolic acids changed independently during storage, with increases in concentrations of 3,4- and 4,5-dicaffeoylquinic acids, while chlorogenic and 3,5-dicaffeoylquinic acids declined with storage time (Table 4.8). At 8 weeks, the concentration reduction in chlorogenic and 3,5-dicaffeoylquinic acids were 21 and 20% respectively. Concentrations of 3,4-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid increased by 17 and 12-fold during the storage period.

Table 4.8: Effect of storage duration on individual phenolic acid concentrations (mg/100 g DW) in ‘Clone 1820’ sweetpotato root.

Storage time (weeks)	Chlorogenic acid	3,4-diCQA	3,5-diCQA	4,5-diCQA
0	560 ^a	23.3 ^d	1059 ^a	13.2 ^c
2	529 ^b	122 ^c	1091 ^a	13.6 ^c
5	511 ^c	280 ^b	1030 ^a	124 ^b
8	440 ^d	405 ^a	852 ^b	163 ^a
P-value	< 0.001	< 0.001	< 0.001	< 0.001

Data expressed as mg of chlorogenic acid equivalent per 100 g of dry weight. Mean values with the same letters within the column are not significantly different at $P < 0.05$ (Tukey's test). All values are the mean of three replicates. diCQA = dicaffeoylquinic acid

4.4.3.8.3 Anthocyanin

Anthocyanin peaks 1, 2, 7 and 8 were the most abundant anthocyanins in ‘Clone 1820’ at harvest. Changes in concentrations of these individual anthocyanins during storage differed, with some increasing and others decreasing (Table 4.9). There was 17% increase in the concentration of peak 1 with storage time, while concentration for peaks 7 and 8 declined by 41% and 78 % respectively when compared the concentration at harvest.

Table 4.9: Effect of storage duration on the amount of selected anthocyanins within a root extract of sweetpotato ‘Clone 1820’

Storage time (weeks)	Peak 1	Peak 2	Peak 7	Peak 8
0	33.3 ^{bc}	24.7 ^a	33.8 ^a	26.5 ^a
2	32.7 ^c	18.8 ^c	25.8 ^b	7.7 ^b
5	36.3 ^b	21.2 ^b	20.8 ^c	7.4 ^b
8	38.9 ^a	22.5 ^{ab}	20.0 ^c	5.9 ^b
P-value	< 0.001	< 0.001	< 0.001	< 0.001

Data expressed as mg of cyanidin 3-O-glucoside chloride equivalent per 100 g of dry weight. Means followed by same letters within the column are not significantly different at $P < 0.05$ (Tukey's test). All values are mean of three replicates. Peak identification: **1** = peonidin-3-sophoroside-5-glucoside, **2** = peonidin-3-*p*-hydroxybenzoyl sophoroside-5-glucoside, **7** and **8** unknown.

4.5 Discussion

4.5.1 Sprout growth and length

The inhibition of sprouting by hot treatment is dependent on both the temperature and duration of the hot water treatment (Ranganna et al., 1998; Sheibani et al., 2012). In the present study, more than 70% sprout suppression was achieved at water temperature above 51 °C and immersion time longer than 28 min (Figure 4.5). Unfortunately, this combination also increased rot incidence. This observation supports the idea of Ranganna et al.; 1986, who showed that temperature required to achieve 100% sprout inhibition in potato (*Solanum tuberosum*) was beyond the thermal tolerance of potato, causing plant tissue damage. Therefore in this study water temperatures between 50 °C to 52 °C and immersion time 7 to 12 min were identified as optimal combinations to reduce sprouting in sweetpotatoes with minimal adverse effects. This range is close to results of Sheibani et al. (2012) who recommended temperature between 53 and 56 °C and an immersion time of below 10 min for sprout and spoilage control in ‘Beauregard’ sweetpotato.

In experiment 3, hot water treatment (51 °C for 11 min) delayed sprouting up to 2 weeks in both ‘Owairaka Red’ and ‘Clone 1820’ sweetpotatoes (Table 4.2). Sprout suppression with hot water dipping has been previously reported in sweetpotato (Tanaka, 2001; Hu et al., 2011) and potato (Ranganna et al., 1998). Ranganna et al., (1998) proposed that hot water cauterised the potato eyes (axillary buds), thereby delaying sprout elongation. It can thus be suggested that the same mechanism is involved in sweetpotato sprout control. By applying hot water, the meristems developing within the sweetpotato root are damaged. The increased sprout growth in coated roots was unanticipated. This increase may be related to an increased ethylene concentration within coated roots. Studies in apples (Drake & Nelson, 1990; Lau & Yastremski, 1991) have shown that edible coating increases internal ethylene concentration. In sweetpotato, ethylene is required for sprout initiation (Amoah, 2013; Cheema et al., 2013). In Cheema’s study, application of pre-storage 1-methylcyclopropene (1-MCP) suppressed sprout growth of ‘Bushbuck’ and ‘Ibees’ sweetpotatoes during 4 weeks of storage at 25 °C, supporting the hypothesis that ethylene is required for sprout initiation. Although sweetpotato internal ethylene concentrations were not measured in this study, it can be hypothesised that the internal

ethylene concentration of coated sweetpotatoes was higher than that of other treatments, consequently coated roots initiated greater levels of sprouting. The effect of ethylene on sweetpotato sprouting and other quality measures will be tested in the following chapters.

4.5.2 Weight loss and water vapour permeance

According to Picha (1986c), transpiration is the main process contributing to water loss in sweetpotatoes. The weight loss data in this current study showed that all the edible coatings evaluated reduced weight loss during storage, as reported previously (Hoa & Ducamp, 2008; Reinoso et al., 2008). With low water vapour permeance in coated roots, one may anticipate a similar trend for internal gas modification. But this is not always the case as water, oxygen and carbon dioxide have different permeance characteristics. Hagenmaier and Baker (1993) showed that there are two possible ways by which coatings may reduce gas exchange in fruits; firstly by forming an additional barrier on the fruit surface through which gases must diffuse, alternatively the coating plugs existing openings in the fruit epidermis. Using different types of edible coatings on 'Valencia' oranges, Hagenmaier and Baker concluded that the resistance to gas exchange of coated fruits is strongly influenced by the coating's ability to block pores on the surface of the fruit, while for water vapour the restriction depends more on the coating's thickness. This may explain why the trend for reduction in water vapour permeance is different from the trend in internal gas modification for this study. Despite roots coated with carnauba 20% having low water vapour permeance compared to roots coated with WPC + 0.2 g oil, the internal CO₂ in roots coated with carnauba 20% was 24% lower compared to the internal CO₂ in roots coated with WPC + 0.2 g oil, suggesting that WPC + 0.2 g oil had the highest capacity of blocking openings on the sweetpotato root periderm. The effect of carnauba wax was concentration dependent. By applying a high concentration of carnauba wax the coating may be thicker, thereby blocking periderm openings, resulting in reduced internal O₂ concentrations. Despite the high variation in data, the internal gas concentrations for carnauba 20%, WPC + 0.1 g oil and WPC + 0.2 g oil suggest possible internal gas modifications. The optimum coating type and concentration was selected based on 3 factors: water vapour permeance, weight loss reduction, and internal gas modification. Based on these criteria, carnauba wax at 5 and 10% concentration were selected as the coatings to minimise sweetpotato weight loss.

Hot water dipping on its own increased the weight loss in ‘Owairaka Red’ and ‘Clone 1820’ sweetpotato roots during storage at 25 °C. In contrast, Hu & Tanaka (2007) did not find increased weight loss with hot water dipping (50 °C for 30 min) of ‘Eicho’ and ‘Kanoya’ sweetpotato cultivars stored at 14 °C for 12 months. Similarly, El-Sayed et al. (2013) found less weight loss in hot water dipped sweetpotatoes compared with the control after 4 months storage at 13 °C. These disparate results may be due to cultivar and the storage temperature differences. It has been stated that different cultivars have different heat tolerances (Lurie & Mitcham, 2007). In this case, ‘Owairaka Red’ and ‘Clone 1820’ seem more sensitive to heat. The negative effects of HWD on weight loss were reduced when hot water treatment was combined with edible coatings. The reduction in weight loss in HWDC samples might be related to the additional barrier provided by the edible coating, thereby reducing the rate of transpiration (Baldwin, 2005; Baldwin et al., 2012).

4.5.3 Phytochemicals

4.5.3.1 β -carotene

The β -carotene concentration in ‘Clone 1820’ extracts ranged from 17 to 25.6 mg/100 g DW which is within the range observed in orange-fleshed sweetpotato cultivars as reported by other authors (Huang et al., 1999; Teow et al., 2007). Nevertheless, the concentration was low when compared to commercial cultivars in the United State: ‘Covington’ and ‘Beauregard’, which contained 9.1 and 9.5 mg/100 g FW of β -carotene respectively. On a dry weight basis, this translates to 45.5 and 50.8 mg/100 g DW (Yencho et al., 2008). Despite, the low concentration, ‘Clone 1820’ is a good source of pro vitamin A, as the calculated retinol activity equivalent (RAE) ranged from 363.4 to 536.7 RAE per 100 g of sweetpotato. According to the Institute of Medicine, (2001), the recommended daily intake of RAE for children below 13 years ranges from 300 to 500 RAE. This implies that a 100 g serve of ‘Clone 1820’ sweetpotato would be sufficient to meet the daily retinol requirements for children under 13 years old.

There was a reduction in β -carotene concentration over storage time (Table 4.7). This is in agreement with the findings of Tumuhimbise et al., (2010) who found that sweetpotato β -carotene declined during storage at room temperature. In contrast, Ezell and Wilcox (1948), Grace et al. (2014) and Picha (1985a) reported an increase in sweetpotato β -carotene concentration during storage at 15 - 16 °C. The observed

differences could be due to the disparity in storage temperature. Higher temperatures may lead to a reduction in β -carotene, whereas storage at low temperature (15 to 16 °C) may lead to an increase in β -carotene concentration. Another possibility is that the roots used in the studies could differ in age, as it is known that carotenoid levels increase with root development (K'osambo et al., 1998). It can be proposed that the carotenoid concentration changes observed during storage depend on the age of the roots, with an increase in roots harvested early before reaching their optimal carotenoid concentration, and a decline in roots that are harvested late, after the root has reached its maximum carotenoid concentration. The reduction in β -carotene content during storage has an implication on the sweetpotato daily intake required to meet the recommended daily RAE. While consumption of 100 g of freshly harvested roots of 'Clone 1820' sweetpotato could supply 537 RAE; after 8 weeks of storage one has to consume 30% more sweetpotato to achieve the same RAE. According to the Australia and New Zealand Food Standards Code (2014), a good source of vitamin should be able to supply 25% of the recommended daily intake, suggesting that a 100 g serve of 'Clone 1820' even after storage is a good source of vitamin A. Hot water treatment and coating had no effect on the β -carotene concentration of 'Clone 1820' throughout the storage period, implying that hot water treatment and coating can be used as a postharvest treatment in sweetpotato without a negative effect on carotenoid concentration.

4.5.3.2 Phenolic acids and anthocyanins

Phenolic acids have been previously studied in sweetpotato roots (Yoshinaga et al., 2000; Teow et al., 2007; Truong et al., 2007). The concentrations of phenolic acids in 'Clone 1820' sweetpotatoes presented in Table 4.3 are within the range of the reported values. Chlorogenic and 3,5-dicaffeoylquinic acids were the most abundant phenolic acids, consistent with previous studies (Ishiguro et al., 2007; Teow et al., 2007; Padda & Picha, 2008a). Coating applications had no effect on phenolic acid concentrations (Table 4.6). The concentrations of chlorogenic isomer (peak 1), 3,4-dicaffeoylquinic (peak 5) and 3,5-dicaffeoylquinic (peak 4) acids increased significantly after hot water treatment (Table 4.5). The increased concentration of some phenolic compounds after hot water treatment is presumably related to a plant stress response. Abiotic stress induced polyphenol biosynthesis has been reported in plants (Dixon & Paiva, 1995; Cisneros-Zevallos, 2003; Schreiner & Huyskens-Keil, 2006) as a result of increased phenylalanine ammonia-lyase (PAL) activity, which consequently increases the

accumulation of phenolic compounds. Although PAL activity was not measured in this study, other researchers have reported increased PAL activity after exposure to a hot water dip (Qin et al., 2003). These results indicate that hot water dipping does not only help in inhibiting sprouting but can also increase phenolic acids concentrations, thereby increasing potential health benefits. However, the increased phenolic acid concentration was temporary, being only up to two weeks, as thereafter there were no differences in phenolic concentration between stored hot water dipped and the control roots.

Changes in phenolic acid concentrations during storage have been reported by several authors (Ishiguro et al., 2007; Padda & Picha, 2008a; Grace et al., 2014). Likewise, in this study, there were significant changes in phenolic compound concentrations due to storage time effects (Table 4.8). The various phenolic compounds behaved differently with storage. Consistent with the results of Grace et al., (2014), the chlorogenic acid concentration in this study declined with storage. On the contrary, Ishiguro et al. (2007) and Padda & Picha (2008a) found an increase in chlorogenic acid during storage. These differences could be attributed to sampling methods, as in Ishiguro's study the whole sweetpotato root was used for analysis, unlike in this case where the skin was removed. Therefore, the increase in chlorogenic acid in Ishiguro's study could be more from the skin portion, not from the flesh. This assumption is supported by the results of Padda and Picha (2008a), who separately analysed the concentration for the skin, cortex, cambium and pith, and found higher increases of chlorogenic acid in the skin than in other parts. On the other hand, storage temperature also played a role. In the two studies, the increase in chlorogenic acid was observed when roots were stored at a low temperature of 5 °C but not at 15 °C. This increase in phenolics when exposed to a lower temperature may be linked to cold stress, which results in biosynthesis of phenols as a response to stress conditions (Lieberman et al., 1959).

Purple-fleshed sweetpotato contain anthocyanins that are mainly peonidin or cyanidin glycosides acylated with caffeic acid, ferulic acid or *p*-hydroxybenzoic (Yoshimoto et al., 1999; Terahara et al., 2004; Kim et al., 2012). This concurs with the findings in this study where only peonidin- and cyanidin- based anthocyanins were identified in 'Clone 1820' extracts. There is great variation in total anthocyanin concentration among cultivars (Yoshimoto et al., 1999; Truong et al., 2009; Lee et al., 2013). Joyce et al.

(2006) measured the concentrations of total anthocyanin in four sweetpotato cultivars grown in New Zealand, the observed concentrations were 334.1, 55.9 and 6.8 mg kuromanin chloride equivalents/100 g DW in 'Radical', 'C42' and 'Owairaka Red' respectively. The total anthocyanin concentration of 'Clone 1820' in this study was 139 mg cyanidin glucoside equivalent/100 g DW, which is higher than that of 'C42' and 'Owairaka Red', but almost half the concentration found in 'Radical'.

Changes of anthocyanin during storage have been studied previously (Morrison et al., 2004; Grace et al., 2014). Grace et al. 2014, found a reduction of both total anthocyanin and individual anthocyanins in 'NCPUR06-020' sweetpotatoes during 8 months storage at 15 °C and 80 - 85% RH. In contrast, Morrison et al. 2004 showed that the total anthocyanin levels in 'Radical' were stable during storage at 13 °C. In this study, both an increase and decrease in individual anthocyanins were observed suggesting that degradation and biosynthesis were taking place. Several studies have shown that anthocyanin can be degraded in plant tissue by β -glycosides, polyphenol oxidase and peroxidase enzymes (Shi et al., 1992; Zhang et al., 2005). The increase in concentration of some of the anthocyanins need further investigation, to establish whether the increase is due to the accumulation of degraded products from other phenolic compounds or an actual synthesis.

4.6 Conclusions

The present study was designed to determine the effects of hot water dipping/immersion and coating on sweetpotato root quality (weight loss, sprouting and phytochemical composition). From the preliminary studies, temperatures between 50 °C and 52 °C and immersion times between 7 to 12 min were determined as the best range to use in sweetpotato for sprout control with minimal negative effects. Carnauba wax at concentrations of 5 or 10% was the optimum coatings to reduce weight loss without internal gas modification. A combination of hot water treatment and coating was equivalent to coating alone in controlling weight loss, but HWDC was less effective in sprout control when compared to hot water treatment. This information can be used to develop targeted interventions aimed at solving individual sweetpotato storage problems. In cases where weight loss is the main problem, the use of a combined treatment (hot water immersion and coating) is recommended as it controlled sprouting to a certain extent and was as good as coating alone in minimising weight loss, whereas

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hot water dipping is recommended in cases where sprouting is the main cause of concern. In addition, hot water dip is a potential strategy to enhance concentrations of polyphenols in sweetpotatoes, thereby increasing their possible health benefits.

5 Effects of ethylene and 1-methylcyclopropene on sweetpotato sprout growth, flesh colour, and phytochemical composition

5.1 Introduction

Sprouting is one of the factors limiting the postharvest life of sweetpotato roots especially at elevated temperatures and high relative humidity (Afek & Kays, 2004). Sprouted roots have a raised respiration rate, leading to increased weight loss and the development of pithy or shrivelled roots (Edmunds et al., 2008). In previous chapter coating application increased sprout growth in ‘Owairaka Red’ roots. It was hypothesised that the increase could be linked to increased internal ethylene concentration. Measuring internal ethylene concentration in a dense produce like sweetpotato without cavity is difficult as such an attempt was made to correlate ethylene production of sweetpotato roots coated with different coatings and sprout growth. However the results did not show any correlation between ethylene production and sprout growth (see Appendix A).

Ethylene gas has also be used as a sprout inhibitor in potato (Foukaraki et al., 2012b), onion (Cools et al., 2012), and sweetpotato (Cheema et al., 2013). Cheema et al. (2013) found that applications of exogenous ethylene ($10 \mu\text{L L}^{-1}$) suppressed sprouting of ‘Ibees’ and ‘Bushbuck’ sweetpotato roots stored at 25 °C for 4 weeks. Similarly, Amoah (2013) reported reduced sprouting in the North Carolina sweetpotato cultivar ‘Covington’ when stored in continuous ethylene ($10 \mu\text{L L}^{-1}$) for 49 days at 25 °C. Whilst these sprout inhibiting results are promising, the treatment’s effects on market quality and sensory attributes require further study. The use of ethylene may cause a darkening of flesh colour and the development of an off-flavour in cooked sweetpotatoes (Buescher et al., 1975; Kitinoja, 1987). Although the darkened flesh does not lead to nutritional loss, it may be undesirable for consumers.

There is evidence that ethylene-induced responses may be inhibited by using ethylene inhibitors, such as 1-methylcyclopropene (1-MCP) (Sisler & Serek, 1997; Blankenship & Dole, 2003). An application of 1-MCP ($1 \mu\text{L L}^{-1}$) prevented the darkening of flesh in fried ethylene-treated potato (*Solanum tuberosum*) tubers, without compromising the sprout inhibition effect (Daniels-Lake et al., 2005; Prange et al., 2005). The effect of 1-

MCP on sweetpotato flesh colour, when used in combination with ethylene, has not been studied. Based on the existing information on potato, it was hypothesised that 1-MCP treatment, prior to ethylene exposure, would reduce sweetpotato flesh darkening while retaining any sprout inhibition effects. In addition, the sensory acceptability of ethylene-treated and untreated sweetpotatoes requires evaluation.

Specific objectives for this chapter are:

1. To determine the combined effects of ethylene and 1-MCP on sweetpotato sprouting, weight loss and internal flesh colour.
2. To determine the effects of ethylene and 1-MCP treatments on carotenoids, phenolic acids and the anthocyanin concentration.
3. To measure the acceptability of ethylene-treated and untreated sweetpotatoes to consumers.

5.2 Materials and methods

5.2.1 Plant material

Three sets of experiments were conducted as summarised in Table 5.1. In experiments 1 and 3, ‘Owairaka Red’ roots were sourced from Delta Produce, Dargaville, New Zealand. In experiment 2, roots of ‘Clone 1820’ were used. ‘Clone 1820’ was selected because it contains substantial amounts of phenolic acids, carotenoids, and anthocyanin. The ‘Clone 1820’ roots were sourced from Plant and Food Research, Pukekohe, New Zealand. All cultivars were harvested in April 2013. Roots were transported to Massey University, Palmerston North, within 24 h of harvest. Roots without visible defects were cured at 30 °C and 90 - 95% relative humidity, for four days. ‘Owairaka Red’ roots were stored for 4 weeks and sampling was conducted every week as repeated measures. ‘Clone 1820’ roots were stored for a longer period (8 weeks) to allow an extended period in which to monitor any phytochemical changes due to exposure to ethylene and 1-MCP treatments. All the measures for ‘Clone 1820’ were non-repeated. Sensory evaluations (experiment 3) were undertaken using ‘Owairaka Red’ roots. By the time of the experiment, the roots had been in store at 14 - 15 °C for about 7 months.

Table 5.1: Sweetpotato cultivars used and parameters measured in the three experiments

Parameters	Exp.1	Exp.2	Exp.3
Weight loss	✓	✓	-
Sprout numbers & length	✓	✓	-
Respiration rate	✓	✓	-
Rot incidence	✓	✓	-
Internal colour	✓	✓	-
Sugars	✓	✓	-
Carotenoids	-	✓	-
Phenolic acids	✓	✓	-
Anthocyanin	-	✓	-
Sensory test	-	-	✓
Cultivar	Owairaka Red	Clone 1820	Owairaka Red
Storage duration	4 weeks	8 weeks	4 weeks

5.2.2 1-MCP treatment

1-MCP was applied according to Agro Fresh guidelines; in brief, 86.9 mg of 0.14% 1-MCP powder (Smartfresh™, Agro fresh Inc., USA) was placed in an airtight 100 mL syringe fitted with a 3-way Luer stopcock. Warm milliQ water (2 mL) was injected into the syringe to dissolve the powder. Following vigorous shaking (2 min), all the contents of the syringe were injected into closed high-density polyethylene barrels, each containing 5 kg of sweetpotato roots. Control samples were treated in the same manner, excluding the application of 1-MCP. Following 24 h of incubation at 20 °C, the barrels were vented outdoors. Thereafter, the roots were divided into the designated treatments, and stored in a flow-through system, with either continuous air or ethylene (10 µL L⁻¹), for 4 and 8 weeks using ‘Owairaka Red’ and ‘Clone 1820’ respectively. Two green-breaker tomatoes were included in each of the 1-MCP treatment barrels as positive biological indicators of 1-MCP responses. The treated and untreated tomatoes were stored at room temperature (20 °C), and their ripening was monitored for 3 weeks. Ripening of 1-MCP treated tomatoes was significantly delayed (Figure 5.1) indicating the efficacy of applied 1-MCP.

Before 1-MCP treatment



Control tomatoes



Tomatoes intended for 1-MCP treatment

After 2 weeks @ 20 °C



Control tomatoes



1-MCP treated

Figure 5.1: Visual appearance of tomatoes before treatment (+/- 1-MCP) and after 2 weeks storage at room temperature (20 °C).

5.2.3 Ethylene application

Ethylene ($10 \mu\text{L L}^{-1}$) was applied continuously at a constant flow rate of 720 mL min^{-1} into the 56 L airtight barrels in a flow-through system, for the entire storage period. The desired concentration of ethylene was obtained by mixing $5000 \mu\text{L L}^{-1}$ ethylene (in 20.7% oxygen in nitrogen gas) (BOC Ltd, Auckland, New Zealand) and compressed air using a purpose built gas mixer with control needle valves and a pressure gauge to regulate the flow rate. The gas mix was passed through an 1800 mL

jar containing 750 mL of 43% glycerol in water to humidify the gas to around 85 % RH before continuing to each barrel. The concentrations of the mixed gases were measured on a weekly basis using a gas chromatograph (GC) (Shimadzu GC-2014, Shimadzu Scientific Instruments, Auckland, New Zealand). The GC was calibrated using a $10.1 \pm 0.5 \mu\text{L L}^{-1}$ ethylene standard (BOC Ltd., Auckland, New Zealand). The data were acquired using Shimadzu GC Solutions software.

5.2.4 Respiration measurement

Respiration was measured as the amount of carbon dioxide accumulated over time. Sweetpotato roots (3 roots/replicate/treatment) were placed in 1800 mL glass jars with an airtight lid fitted with a rubber septum. Using a 1 mL syringe, gas was withdrawn from the headspace just after sealing the jars and again after 30 min. Carbon dioxide concentration was determined using an infra-red CO₂ transducer (Analytical Development Company, Hoddesdon, UK), with O₂-free Nitrogen as a carrier gas (flow rate 35 mL min⁻¹). The output signal was analysed by an integrator (Hewlett Packard, Model 3394A). Respiration rates were calculated taking into account accumulation time, sample weight, and the free volume of the jar. The final respiration rates were a mean of three replicates, each replicate consisting of three roots. The equipment was calibrated with β -standard $0.49 \pm 0.01\%$ CO₂ (BOC Ltd, Auckland, New Zealand).

5.2.5 Weight loss

Weight loss (%) during storage was measured as previously described in section 2.3.1.

5.2.6 Sprout growth

Sprout number per root and length (mm) was assessed as previously described in section 2.3.2.

5.2.7 Rot incidence

Roots with visible signs of rots were counted as rotten and expressed as a percentage incidence relative to the initial root number.

5.2.8 Colour measurement

At each sampling time, six roots were randomly selected from each treatment and halved longitudinally. One half was used for raw colour measurement, and the other half was cooked for 10 min (total quantity of sweetpotato in the oven 550 to 600g) using a domestic microwave (850 W Panasonic Model NN-7852, Matsushita

Electrical Industrial co., Japan) and allowed to cool before measurement. Colour measurements were performed using a reflectance spectrophotometer (Model CM-2600D, Konica Minolta Sensing Inc., Japan) equipped with an 8 mm diameter measuring head and D65 as the light source. The spectrophotometer was calibrated with a standard white colour surface. Three measurements were taken from each piece of root and averaged to give one reading per root.

5.2.9 Phytochemical analysis

The phytochemicals measured were carotenoids, phenolic acids, and anthocyanins. Samples were prepared and analysed as described previously in section 2.4.

5.2.10 Analysis of non-structural carbohydrates

The soluble sugars were extracted as described by Cheema et al. (2013). Freeze dried sweetpotato powder (1.0 g) was extracted with 20 mL of water for 1 h at room temperature. Thereafter the extracts were centrifuged at 4500 g for 10 min. The supernatant was filtered through a 0.45 µm filter into HPLC vials. Sugar analysis was performed using a Dionex HPLC system equipped with a P680 HPLC pump; an ASI-100 automated sample injector, and a Thermostated Column Compartment TCC-100. Sugars were separated using a Varian Metacarb H Plus (7.88 x 300 mm) column, and detected using a refractive index detector (Shodex RA 101, Showa Denko Europe GmbH, Munich, Germany). MilliQ water at a flow rate of 0.3 mL/min was used as the mobile phase. Identification of each sugar was based on HPLC retention times and confirmed with external standards. Quantification was undertaken using external standards of glucose, sucrose, and fructose dissolved in water.

5.2.11 Sensory evaluation (Experiment 3)

A consumer acceptability study was conducted on 5th December 2013 at Massey University. Before sensory evaluation, sweetpotato roots were subjected to one of four storage regimes: Air (control), 1-MCP (1 µL L⁻¹) + air, Ethylene (10 µL L⁻¹) and 1-MCP (1 µL L⁻¹) + ethylene (10 µL L⁻¹). The roots were stored in a flow-through system at 25 °C and 85% RH for a period of 4 weeks. Fifteen (15) roots from each treatment were randomly selected and boiled intact in a covered pot for a period of 45 min. Cooked roots were kept intact in closed coded plastic containers until serving time. Sensory acceptance was measured using the method of Stone & Sidel (2004). Sweetpotato slices (approximately 20 g) from each treatment were presented in a

random order and coded with three digit random numbers. All samples were served at room temperature. A cup of water was provided for cleansing the mouth between tasting in order to reduce the overlap of flavours. Participants were requested to taste all the samples from the four treatments. Following each tasting, participants rated the sweetpotato sample using a 9 point hedonic scale, where 9 represented extremely liked and 1 extremely disliked. Overall acceptance, colour, sweetness and texture were four additional sensory attributes evaluated. Participants were also asked to rank the four samples on a 4 point scale, 1 representing the best liked sample and 4 the least liked sample, detailed questionnaire in appendix B.

5.3 Statistical analysis

Data were analysed using Minitab 16 (Minitab Inc., State College, Pennsylvania). For Experiment 1, data were analysed as repeated measures and for Experiment 2 the phytochemical effects data were analysed as non-repeated measures. Data on sprout number and length were transformed using square root and log transformation methods respectively. Means were compared by Tukey's test, at $P < 0.05$. All transformed means were back-transformed for presentation. Rotten roots were excluded for weight loss and respiration rate statistical analysis. Sensory data were analysed using analysis of variance (ANOVA) and the Kruskal-Wallis test for nonparametric data using PASW statistics 18 (SPSS, Inc., 2009, Chicago, IL, USA).

5.4 Results

5.4.1 Respiration

Respiration rates before exposure to ethylene and 1-MCP treatment were 128 and 133 $\text{nmol kg}^{-1} \text{ s}^{-1}$ in 'Owairaka Red' and 'Clone 1820' respectively. Respiration rates in ethylene or 1-MCP + ethylene treatments increased rapidly, and reached the maximum values of an almost two-fold increase in both cultivars (Figure 5.2A and B). In addition, the respiration rates of ethylene and 1-MCP + ethylene treated roots remained significantly ($P < 0.05$) higher than those of air (control) and 1-MCP + air treatments from the time of exposure until the end of storage for 'Owairaka Red' and for most assessments for 'Clone 1820'. 1-MCP treatment had no effect on respiration rate irrespective of the storage conditions, as no differences in respiration rates were found between the 1-MCP + ethylene and the ethylene treatment alone, or between the air (control) and 1-MCP + air treatments ($P > 0.05$). Storage duration effects were only

observed in the control and 1-MCP + air treatment of ‘Owairaka Red’, where the respiration rate appeared to drift upwards during 4 weeks of storage, statistically the rate at 4 weeks was not higher than that at the start.

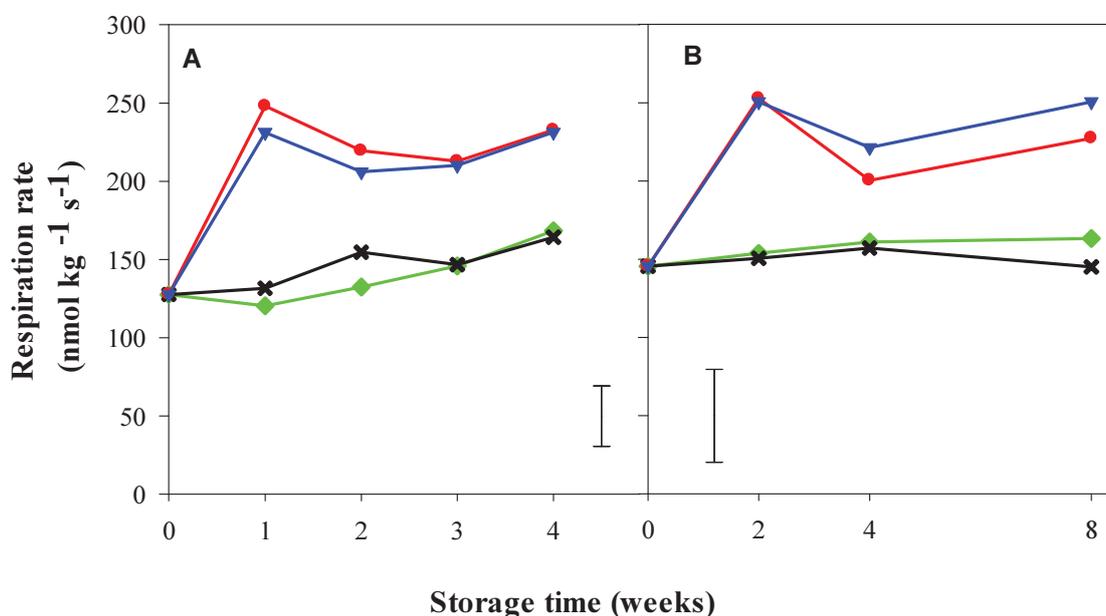


Figure 5.2: Respiration rate ($\text{nmol kg}^{-1} \text{s}^{-1}$) of sweetpotato cvs. Owairaka Red (A) and Clone 1820 (B) roots subjected to different treatments: Air (\diamond), 1-MCP + Air (\times), $10 \mu\text{L L}^{-1}$ ethylene (\bullet) and 1-MCP + Ethylene (\blacktriangledown). 1-MCP ($1 \mu\text{L L}^{-1}$) was applied before storage for 24 h at 20°C . Each data point is the mean of three replicates, each replicate consisting of three roots. Vertical bars represent $\text{HSD}_{0.05}$ (Tukey's test) for all samples after time zero

5.4.2 Weight loss

Root weight loss occurred throughout the storage period, in all treatments. In both cultivars, weight loss (%) was higher in roots stored in continuous ethylene compared to roots stored in continuous air (Figure 5.3 A and B) irrespective of 1-MCP treatment. Significant ($P < 0.05$) differences were observed at 3 and 4 weeks for ‘Owairaka Red’ (Figure 5.3A) and at 8 weeks in ‘Clone 1820’ (Figure 5.3B). At 4 weeks the increased percentage of weight loss in ‘Owairaka Red’ and ‘Clone 1820’ was 38 and 48% respectively, relative to the values of air (control) treated roots. The weight loss (%) of 1-MCP treated ‘Owairaka Red’ roots was slightly lower than the air (control) treated

roots, with significant differences observed at 2 and 3 weeks; thereafter the weight loss no longer significantly differed.

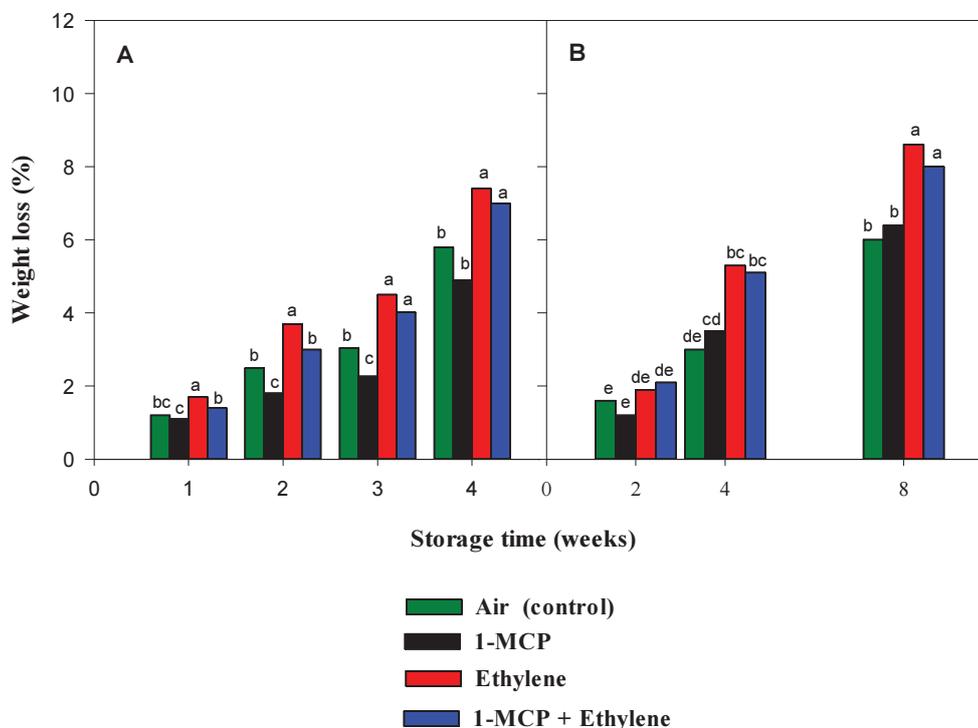


Figure 5.3: Average weight loss (%) of sweetpotato cvs. **Owairaka Red (A)** and **Clone 1820 (B)** roots subjected to different treatments during storage at 25 °C : 1-MCP (1 $\mu\text{L L}^{-1}$) was applied for 24 h at 20 °C before storage. Rotten roots were excluded from analysis; each data point is the mean of at least 17 roots for ‘Owairaka Red’ and three replicates (4 roots/replicate) for ‘Clone 1820’. Values with same letters within the graph are not significantly different at $P = 0.05$ at each sampling time for ‘Owairaka Red’ and across the storage period for ‘Clone 1820’.

5.4.3 Sprout growth and length

Ethylene alone or 1-MCP + ethylene treatments suppressed sprouting in both cultivars (Figure 5.4A-D). In both cultivars air-treated roots had sprouts by 2 weeks of storage. Throughout the storage period no sprouts were observed in ‘Owairaka Red’ roots that were treated with ethylene or 1-MCP + ethylene (Figure 5.4A & C). On the other hand, ‘Clone 1820’ roots treated with 1-MCP + ethylene and ethylene started sprouting at 4 and 8 weeks respectively (Figure 5.4 B& D). Compared to air storage, continuous ethylene storage inhibited the elongation of sprouts in ‘Clone 1820’ by approximately 2.5 times. At the end of the storage period, the corresponding

maximum sprout lengths in ‘Clone 1820’ were 55, 62, 18, and 20 mm in air (control), 1-MCP + air, ethylene and 1-MCP + ethylene respectively (Figure 5.4D). No significant differences were found between the air (control) and the 1-MCP + air treatments throughout the storage period.

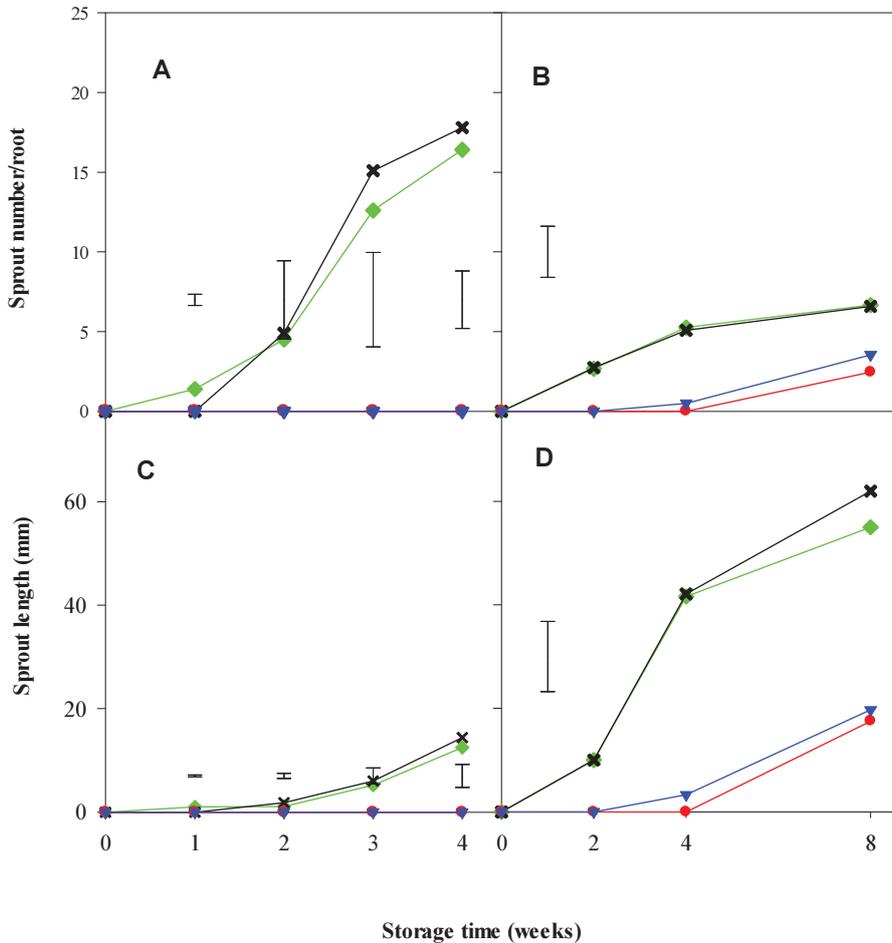


Figure 5.4: Average sprout number per root and sprout length (mm) of sweetpotato cvs. Owairaka Red (A, C) and Clone 1820 (B, D) roots subjected to different treatments during storage at 25 °C : Air (♦), 1-MCP + Air (x) 10 μL L⁻¹ ethylene (●) and 1-MCP + Ethylene (▼). 1-MCP (1 μL L⁻¹) was applied for 24 h at 20 °C before storage. Each data point is the mean of at least 17 roots for ‘Owairaka Red’ and three replicates (4 roots/replicate) for ‘Clone 1820’. Vertical bars represent HSD_{0.05} (Tukey’s test) at each sampling time for ‘Owairaka Red’ and across the storage period for ‘Clone 1820’.

5.4.4 Rot incidence and other disorders

Ethylene alone or 1-MCP + ethylene treatments caused tissue bursting at the proximal end of the root. This occurrence was more intense in ‘Owairaka Red’ than in ‘Clone

1820'. In addition to tissue bursting, the skin of 'Clone 1820' roots was bumpy and could be easily peeled off (Figure 5.5). The tissue bursting was associated with a high incidence of rots. By the end of the storage period, the percentage of rotten roots was about 40% and 13 % in 'Owairaka Red' and 'Clone 1820' respectively (Table 5.2).



Figure 5.5: Visual appearance of sweetpotato cvs. Owairaka Red (A & B) and Clone 1820 (C & D) roots stored in continuous air (A & C) and in 10 µL L⁻¹ ethylene (B & D).

Table 5.2: Percentage of rotten roots of sweetpotato cvs. Owairaka Red and Clone 1820 subjected to different treatments during storage at 25 °C for 3 and 4 weeks in ‘Owairaka Red’ and 4 and 8 weeks in ‘Clone 1820’.

Treatments	‘Owairaka Red’		‘Clone 1820’	
	3 weeks	4 weeks	4 weeks	8 weeks
Air	0	3.3	0	0
1-MCP	0	0	0	0
Ethylene	37	43	0	17
1-MCP + Ethylene	23	40	0	8

5.4.5 Internal flesh colour

The internal flesh colour of cooked ‘Clone 1820’ roots was yellow-red, with a hue angle (°h) ranging from 57 to 62, and lightness (L*) ranging from 34 to 37. Ethylene treatment had no effect on the internal colour of cooked ‘Clone 1820’ sweetpotato roots (data not shown). For ‘Owairaka Red’, the internal cream colour of raw sweetpotato roots was not affected by the ethylene treatment ($P > 0.05$), but cooked samples showed a significant ethylene effect ($P < 0.05$). In all treatments, the internal colour of the cooked ‘Owairaka Red’ samples was yellow, with an average hue angle of 94° (data not shown). However, colour lightness, showed that ‘Owairaka Red’ roots stored in ethylene were darker than those stored in air, regardless of 1-MCP pre-treatment (Figure 5.6) and the darkness became progressively deeper over the first three weeks of storage.

5.4.6 Non-structural carbohydrates (sugars)

Sucrose, glucose and fructose concentrations were measured at the end of the storage period. Roots treated with ethylene only, had a lower concentration of glucose and fructose compared to the other treatments (Table 5.3). The reduction in glucose and fructose concentration was 1.9 to 4 times those observed in air or 1-MCP + air treated samples. The sucrose concentration for ‘Clone 1820’ roots exposed to ethylene alone was significantly ($P = 0.003$) higher than air and 1-MCP + air treatments. This trend was not observed in ‘Owairaka Red’, but air-treated ‘Owairaka Red’ roots had a lower sucrose concentration than 1-MCP + ethylene-treated roots.

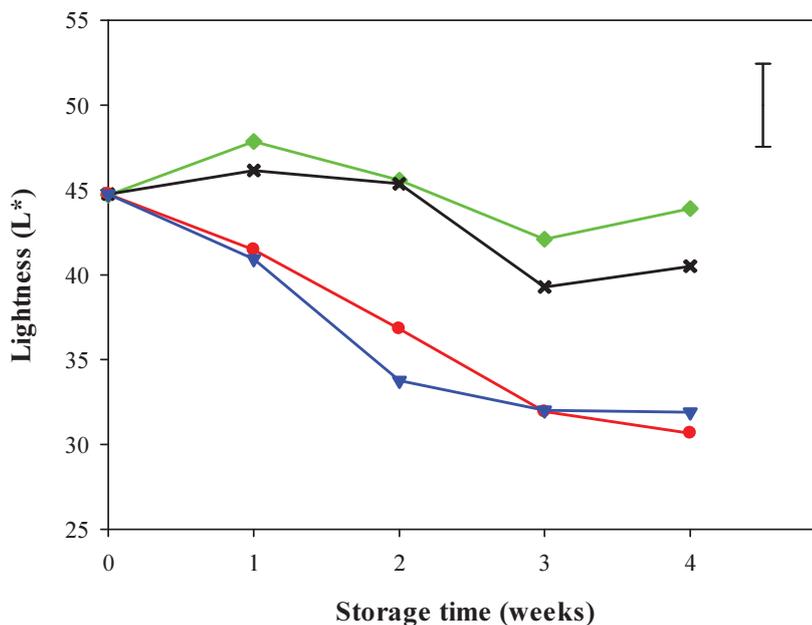


Figure 5.6: Internal flesh colour lightness (L^*) of cooked sweetpotato cv. Owairaka Red roots subjected to different treatments: Air (♦), 1-MCP + Air (×), $10 \mu\text{L L}^{-1}$ ethylene (●) and 1-MCP + ethylene (▼). 1-MCP ($1 \mu\text{L L}^{-1}$) was applied for 24 h at 20°C before storage. Lightness values of 0 = black and 100 = white. Each data point is the mean of six roots. Vertical bars represent $\text{HSD}_{0.05}$ (Tukey's test).

Table 5.3: Sugar concentrations (mg/g DW) of sweetpotato cvs. Owairaka Red and Clone 1820 roots treated with or without 1-MCP ($1 \mu\text{L L}^{-1}$) stored at 25°C in continuous air or ethylene ($10 \mu\text{L L}^{-1}$).

Treatment	'Owairaka Red'			'Clone 1820'		
	Sucrose	Glucose	Fructose	Sucrose	Glucose	Fructose
Air	79.1 ^b	9.9 ^a	4.1 ^b	145.9 ^b	50.9 ^a	18.3 ^b
1-MCP + Air	93.5 ^{ab}	13.1 ^a	5.7 ^a	152.3 ^b	57.3 ^a	24.0 ^{ab}
Ethylene	88.4 ^{ab}	3.7 ^b	1.4 ^c	218.2 ^a	26.4 ^b	9.7 ^c
1-MCP + Ethylene	101.5 ^a	10.5 ^a	4.0 ^b	179.5 ^{ab}	55.9 ^a	25.9 ^a
P-value	0.021	< 0.001	< 0.001	0.003	< 0.001	< 0.001

N = 3, means with the same superscript letter in a column are not significantly different at $P = 0.05$ (Tukey's test). Sugar measurements were done at 4 and 8 weeks for 'Owairaka Red' and 'Clone 1820' respectively.

5.4.7 Phenolic acid concentrations in ‘Owairaka Red’

Chlorogenic, 3,4-dicaffeoylquinic, and 3,5-dicaffeoylquinic acids were the main phenolic acids found in ‘Owairaka Red’ storage roots. Concentrations of chlorogenic acid and 3,5-dicaffeoylquinic acid in roots treated with ethylene or 1-MCP + ethylene were higher than the concentrations in roots treated with air and 1-MCP + air) (Table 5.4). The concentration increase of chlorogenic acid and 3,5-dicaffeoylquinic acid were respectively 3.8 and 2.0 times the value of the air treatments. In addition, the concentration of 3,5-dicaffeoylquinic acid in roots treated with 1-MCP + Air was slightly higher than the concentration in roots stored in continuous air. The concentration of 3,4-dicaffeoylquinic acid was higher in roots stored in ethylene or 1-MCP + ethylene when compared to air (control) treatment but were not significantly different from 1-MCP + Air.

Table 5.4: Phenolic acids concentration (mg/100 g DW) in sweetpotato ‘Owairaka Red’ roots subjected to different treatments and stored for 4 weeks at 25 °C.

Treatment	Chlorogenic acid	3,4-dicaffeoylquinic	3,5-dicaffeoylquinic
Air	7.7 ^b	20.3 ^b	6.5 ^c
1-MCP + Air	7.9 ^b	23.4 ^{ab}	7.6 ^b
Ethylene	29.5 ^a	27.0 ^a	16.4 ^a
1-MCP + Ethylene	27.7 ^a	27.2 ^a	16.1 ^a
P-value	< 0.001	0.001	0.001

N = 3, means followed by the same superscript letter in a column are not significantly different at $P = 0.05$ (Tukey’s test). All concentrations measured as chlorogenic acid equivalent.

5.4.8 Sensory evaluation of ‘Owairaka Red’ sweetpotato storage roots

Seventy two (72) panellists (48 female and 24 male), were recruited to evaluate the sweetpotato samples from all four treatments. The participants had prior knowledge of sweetpotato, as 99 % of the panellists indicated that they had consumed sweetpotatoes before. The sensory data were analysed with non-parametric test-Kruskal-Wallis and parametric test using analysis of variance (ANOVA). The results from Kruskal-Wallis and ANOVA were similar, in that both analyses rejected the null hypothesis; so means based on ANOVA are presented here, according to Montgomery’s (2013) recommendations.

1-MCP + air treated samples had the highest mean colour acceptance score (7.47), and ethylene treated samples had the lowest acceptance score of 6.25 (Table 5.5). Statistical differences ($P < 0.05$) were only observed between 1-MCP + air and the other three treatments. The sweetness of the air and 1-MCP + air treated samples were highly preferred, with mean acceptance scores of 7.26 and 7.20 respectively, which were significantly ($P < 0.05$) higher than the mean scores for roots stored in ethylene or 1-MCP + ethylene. No differences were found in texture scores ($P > 0.05$). In general, samples from all treatments were acceptable as the mean scores for all attributes were above five ('neither liked nor disliked'). About 35% and 31% of the participants regarded 1-MCP + air and air treated samples as the most preferred samples respectively (Figure 5.7). Almost quarter of the respondents regarded the ethylene and 1-MCP + ethylene samples as the worst samples.

Table 5.5: Mean scores for acceptability of cooked sweetpotato cv. Owairaka Red roots subjected to different treatments

Treatment	Sensory attributes			
	Colour	Sweetness	Texture	Overall liking
Air (control)	6.75 ^b	7.26 ^a	6.97	7.17 ^a
1-MCP + air	7.47 ^a	7.20 ^a	6.89	7.15 ^a
Ethylene	6.25 ^b	6.46 ^b	6.26	6.43 ^b
1-MCP + Ethylene	6.42 ^b	6.68 ^b	6.50	6.58 ^b
P-value	< 0.001	0.003	ns	0.006

All values are means of 72 observations. Sensory ratings were on a scale of 1 to 9, where a rating of 9 is 'extremely like' and 1 is 'extremely dislike'. 1-MCP ($1 \mu\text{L L}^{-1}$) was applied at 20°C for 24 h before storage. Ethylene ($10 \mu\text{L L}^{-1}$) was applied throughout the storage period of 4 weeks. Means followed by same superscript letter within a column are not significantly different at $P = 0.05$ (Tukey's test). ns = not significantly different.

5.4.9 Correlations between sensory and instrumental data

Lightness had a strong negative correlation with chlorogenic acid ($r = -0.882$, $P < 0.001$), 3,4-dicaffeoylquinic acid ($r = -0.872$, $P < 0.001$), and 4,5-dicaffeoylquinic acid ($r = -0.923$, $P < 0.001$). Equally, colour acceptance scores were negatively correlated with chlorogenic acid ($r = -0.827$, $P = 0.001$) and 4,5-dicaffeoylquinic acid ($r = -0.780$, $P = 0.001$) (Table 5.6). These results are consistent with post-cooking darkening in 'Owairaka Red' being due to the increased concentration of phenolic

acids. Sensory scores for sweetness were positively correlated with fructose ($r = 0.789$, $P = 0.002$) and glucose ($r = 0.763$, $P = 0.004$). Surprisingly sensory colour acceptance was not correlated with lightness values ($P > 0.05$).

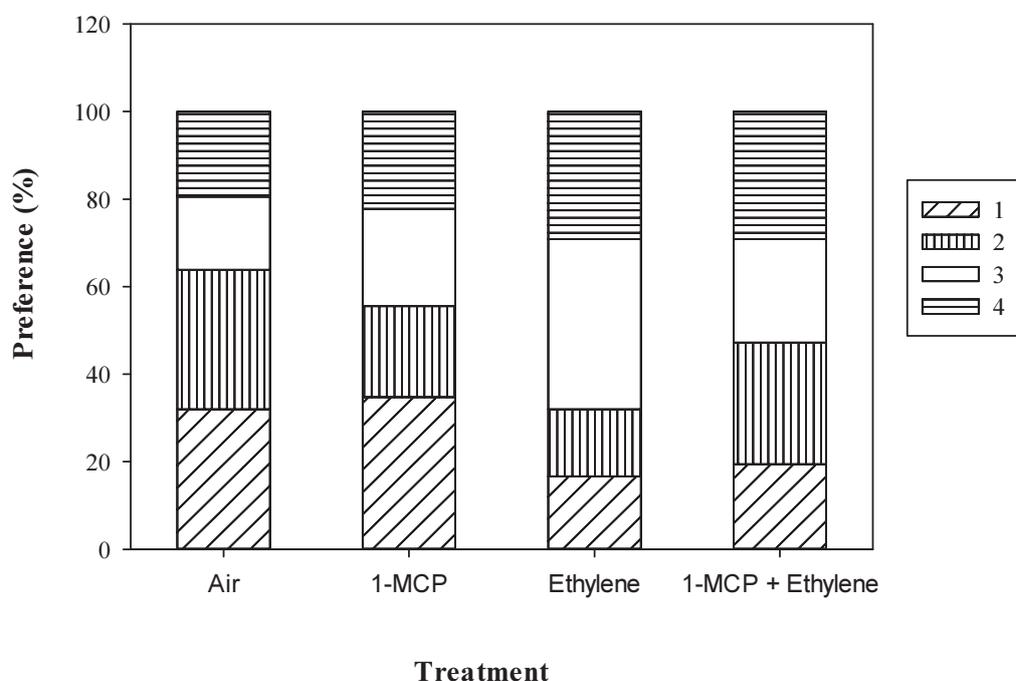


Figure 5.7: Diagrammatic presentation of best-preferred sweetpotato samples. Ranking was done on 1 to 4 scale: 1 = most preferred and 4 = the least preferred sample (ties were not allowed). N = 72

5.4.10 Effects of ethylene and 1-MCP application on phytochemical concentration in ‘Clone 1820’ sweetpotato

The carotenoid concentration in ‘Clone 1820’ at harvest was 22.94 mg/ 100 g DW and ranged from 17 to 22 mg/ 100 g DW during storage (data not shown). 1-MCP and ethylene application had no effect ($P > 0.05$) on carotenoid concentration (data not shown). The changes observed were due to storage, with a reduction on carotenoid concentration during storage when compared to the concentration at harvest.

Storage duration effects on individual phenolic acid concentrations were more apparent than 1-MCP or ethylene application effects. Chlorogenic acid and 3,5-dicaffeoylquinic acid concentrations declined with storage time, while concentrations for 3,4 and 4,5-dicaffeoylquinic acid increased with storage (Figure 5.8A-D).

Table 5.6: Pearson correlations of sensory data and instrumental data of cooked sweetpotato cv. Owairaka Red roots

	CGA	3,4 diCQA	Glucose	Fructose	Sucrose	Lightness	4,5 di CQA	Sweetness	Texture	Colour
3,4 diCQA	0.816	1.00								
Glucose	-0.665	-0.343	1.00							
Fructose	-0.736	-0.393	0.963	1.00						
Sucrose	0.371	0.444	0.214	0.216	1.00					
Lightness	-0.882	-0.872	0.448	0.464	-0.622	1.00				
4,5 di CQA	0.994	0.833	-0.619	-0.683	0.441	-0.923	1.00			
Sweetness	-0.973	-0.768	0.763	0.789	-0.352	0.898	-0.974	1.00		
Texture	-0.957	-0.765	0.787	0.799	-0.331	0.896	-0.958	0.997	1.00	
Colour	-0.827	-0.409	0.766	0.855	-0.023	0.498	-0.780	0.801	0.773	1.00

CGA = Chlorogenic acid, 3,4-diCQA = 3,4-dicaffeoylquinic and 4,5-diCQA = 4,5-dicaffeoylquinic acids measured in 'Owairaka Red' roots after 4 weeks of storage at 25 °C and 85% RH. Colour refers to mean colour acceptance scores from sensory evaluation. Values in bold indicate a significant correlation at $P < 0.05$

Ethylene and 1-MCP effects on phenolic acid concentrations were not uniform among the individual phenolic acids. While both chlorogenic acid and 3,4-dicaffeoylquinic acid concentrations in roots treated with ethylene or 1-MCP + ethylene tend to decrease relative to air and 1-MCP + air treated roots, only the decrease in ethylene treated roots was significantly different from the control. Summing all the storage times, the reduction in chlorogenic acid in ethylene samples was about 30% when compared to samples stored in air. For 3,5-dicaffeoylquinic acid, the reduction in concentration was more noticeable in 1-MCP + ethylene treated roots at 8 weeks of storage. There was a slight increase in concentration of 3,4-dicaffeoylquinic acid due to ethylene treatment. When all storage times were averaged, ethylene stored roots had about 20% higher concentration of 3,4-dicaffeoylquinic acid than their counterparts stored in air. Ethylene or 1-MCP application had no effect on concentration of 3,5-dicaffeoylquinic acid.

No consistent changes in anthocyanin concentration were observed due to ethylene and 1-MCP application. 1-MCP + air treatment retained or slightly increased the initial anthocyanin concentrations. Concentrations of peonidin-3-sophoroside-5-glucoside (peak 1), peonidin-3-caffeoyl sophoroside-5-glucoside (peak 5) and unknown (peak 6) were significantly ($P < 0.05$) higher in 1-MCP + air treatments than the at harvest concentrations (Table 5.7). Ethylene application had no effect on the concentration of the anthocyanins measured, as concentrations of individual anthocyanins in ethylene treated roots were not significantly different from the initial concentration.

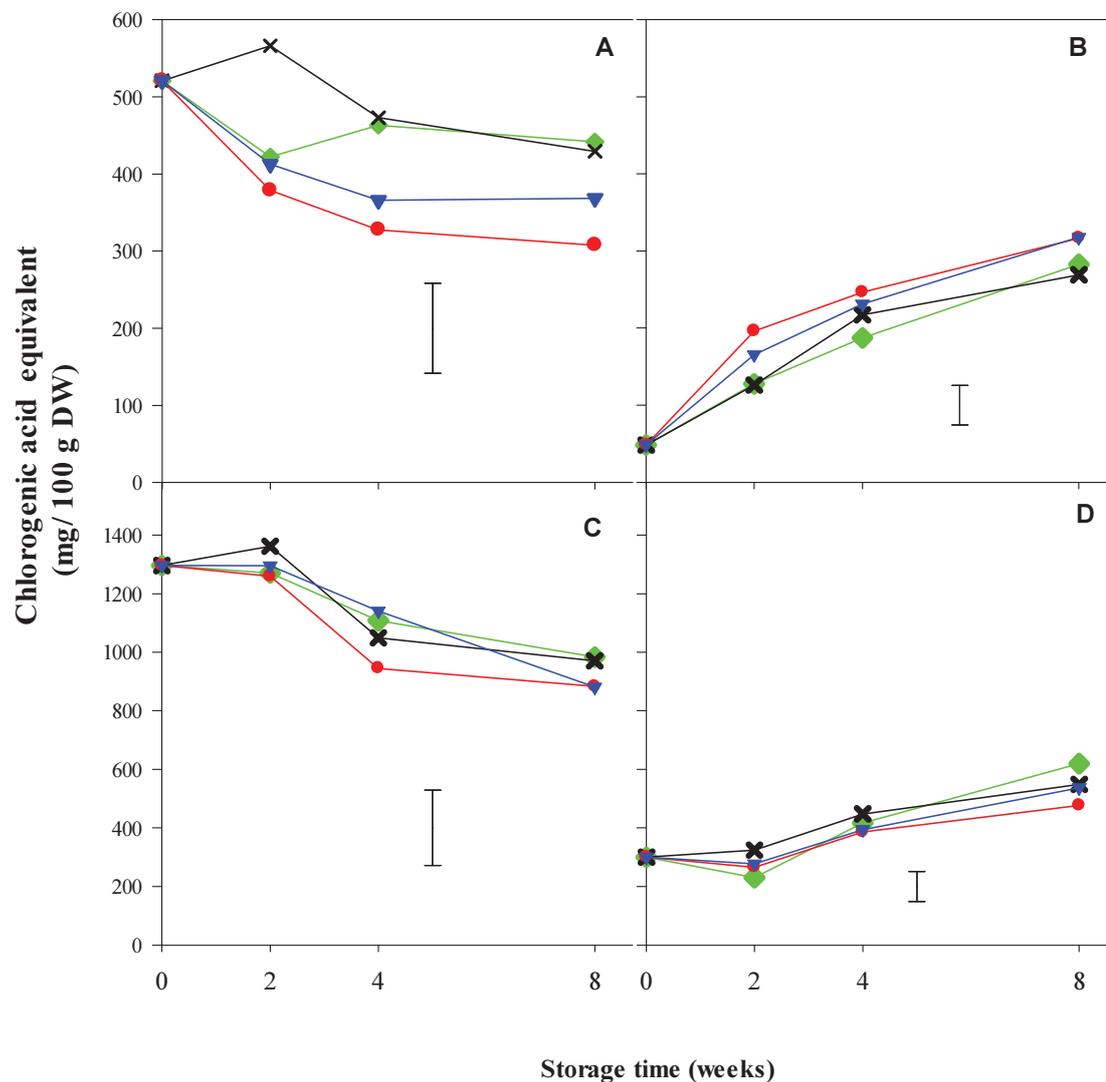


Figure 5.8: Changes in individual phenolic acids: Chlorogenic acid (A), 4,5-dicaffeoylquinic acid (B), 3,5-dicaffeoylquinic acid (C) and 3,4-dicaffeoylquinic acid (D) of sweetpotato cv. Clone 1820 roots treated with Air (♦), 1-MCP + Air (x), 10 µL L⁻¹ ethylene (●) and 1-MCP + Ethylene (▼). 1-MCP (1 µL L⁻¹) was applied for 24 h at 20 °C before storage. Each data point is a mean of three replicates (4 roots/replicate). Bars represent HSD at $P < 0.05$ level as determined by Tukey's test.

Table 5.7: Effects of ethylene and 1-MCP on anthocyanin concentration in sweetpotato cv. Clone 1820 subjected to different treatments

Treatment	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7
At harvest	15.2 ^{bc}	19.2 ^{ab}	9.1 ^{ab}	12.7 ^{ab}	8.6 ^b	17.9 ^b	24.1
Air	14.8 ^c	13.1 ^b	8.6 ^b	13.9 ^a	9.0 ^b	21.2 ^{ab}	21.6
1-MCP + Air	21.8 ^a	23.9 ^a	11.9 ^a	13.1 ^{ab}	13.2 ^a	24.6 ^a	22.6
Ethylene	16.9 ^{bc}	16.7 ^b	9.1 ^{ab}	11.0 ^b	8.8 ^b	17.9 ^b	21.2
1-MCP + Ethylene	18.8 ^b	18.4 ^b	8.5 ^b	15.6 ^a	11.9 ^a	21.0 ^{ab}	21.1
P value	<0.001	<0.001	<0.001	0.006	<0.001	0.002	ns

All data quantified as mg/100 g cyanidin 3-O-glucoside chloride equivalent, N = 3. Peak identification: **1** = Peonidin-3-sophoroside-5-glucoside, **2** = Peonidin-3-*p*-hydroxybenzoyl sophoroside-5-glucoside, **3** = Cyanidin-3-(6"-feruloyl sophoroside)-5-glucoside, **4** = Cyanidin-3-caffeylsophoroside-5-glucoside, **5** = Peonidin -3-caffeyl sophoroside-5-glucoside, **6** and **7** unknown. Peaks identification was carried out by using LC-MS.

5.5 Discussion

5.5.1 Effects of exogenous ethylene on sweetpotato root storage quality

Exogenous ethylene application suppressed sprouting in ‘Owairaka Red’ and ‘Clone 1820’ sweetpotatoes (Figure 5.4). These results are comparable with results from other sweetpotato studies (Amoah, 2013; Cheema et al., 2013). This present study confirms that ethylene is an effective sweetpotato sprout inhibitor. In contrast, Chegeh and Picha (1993) found an increase in sprouting in ‘Beauregard’ and ‘Jewel’ sweetpotato roots exposed to 1, 10, or 1000 $\mu\text{L L}^{-1}$ ethylene for 15 days at room temperature (21 °C), suggesting that the ethylene effect may be cultivar specific. The mechanism by which ethylene inhibits sprouting is not fully understood. Cheema et al. (2013) suggested that ethylene is required for sprout initiation while high concentrations inhibited sprout extension. The results from this study support the hypothesis that high concentration of ethylene inhibit sprout extension but do not provide data to support the suggestion that ethylene is required for initiation, as 1-MCP when applied alone did not inhibit sprouting. Measurements of internal ethylene in sweetpotatoes that are either treated or untreated with 1-MCP would be helpful to understand if internal ethylene plays a role in sweetpotato sprout initiation.

‘Owairaka Red’ roots treated with ethylene had a higher concentration of phenolic acids than roots exposed to air alone (control), (Table 5.4.). Concurrently, ethylene treated roots were darker than the air (control) roots, (Figure 5.6). Ethylene-induced flesh darkening in sweetpotato has previously been reported by Buescher et al. (1975) and Kitinoja (1987). They demonstrated that the darkening was due to increased biosynthesis of phenolic compounds. The results from ‘Owairaka Red’ confirm the reports that ethylene application induces phenolic acid synthesis and are consistent with the hypothesis that post-cooking darkening is exacerbated by an increased concentration of phenolic acids. Likewise, the correlation matrix (Table 5.6) also supports this hypothesis as there were strong negative correlations between the measured lightness values and the concentrations of chlorogenic acid, 3,4-dicaffeoylquinic acid and 3,5-dicaffeoylquinic acid. Surprisingly, no post-cooking darkening was observed in ‘Clone 1820’, yet it has a higher concentration of phenolic acids than ‘Owairaka Red’. The flesh colour differences in the two cultivars might have played a role, flesh darkening being only clearly visible in pale-fleshed cultivars.

There have been extensive studies in changes in sweetpotato sugar concentration during storage (Picha, 1986a, 1986b; Lewthwaite et al., 1997; Zhang et al., 2002). The focus in this study was not on the storage effect for sweetpotato sugars, but rather ethylene effects on reducing sugars, and mainly in relation to post-cooking darkening. In potato, colour darkening is linked to increased reducing sugars (Daniels-Lake et al., 2005; Foukaraki et al., 2012a). However, in this study, ethylene treatment led to a reduction in glucose and fructose concentrations (Table 5.3). It appears that ethylene induced darkening in cooked sweetpotato is not mediated by reducing sugars. Similarly, Amoah (2013) and Cheema et al. (2013) also found reduced glucose and fructose concentration in sweetpotatoes stored in continuous ethylene. Low levels of glucose and fructose could be related to a high respiration rate, as sugars are used as substrates in respiration.

The respiration data showed that the ethylene treatments were associated with higher respiration rates (Figure 5.2A and B). These results are consistent with earlier reports (Buescher et al., 1975; Kitinoja, 1987; Amoah, 2013; Cheema et al., 2013). It is suggested that ethylene causes stress resulting in increased respiration (Reid & Pratt, 1972), a proposition that is supported by the observation of roots bursting in this study. The tissue bursting could be related to ethylene induced radial cell expansion (Apelbaum & Burg, 1971). Tissue splitting was observed only on the proximal end and not on the distal end. Since shoots always arise from the proximal end, it is possible that cells on the proximal end are metabolically more active than cells on distal end.

Exogenous ethylene application can induce disease resistance, susceptibility or have no effect depending on the pathogen and product (Abeles et al., 1992). Ethylene induced tissue bursting was also associated with higher incidence of fungal growth; this effect was more pronounced in ‘Owairaka Red’ than ‘Clone 1820’. The observed visual symptoms were typical of *Rhizopus stolonifer*, characterised by soft watery patches and mycelium (Figure 5.9). Ethylene-induced fungal growth has been reported previously in sweetpotato by Kitinoja (1987). Likewise, preharvest applications of ethephon, an analogue of ethylene, also increased proximal rot incidence during storage in ‘Beauregard’ roots (Arancibia et al., 2013). Differences in levels of rot incidence between ‘Owairaka Red’ and ‘Clone 1820’ could be due to differences in latent infection, as these cultivars were sourced from different locations. It is possible that

‘Owairaka Red’ had a high latent infection, and exposure to ethylene weakened its disease resistance thereby rendering the roots more susceptible to rots. Alternatively, the differences could be due to differences in ethylene sensitivity.



Figure 5.9: Symptoms of stem rot in sweetpotato cv. Owairaka Red exposed to ethylene

In both cultivars, ethylene-treated roots showed greater weight loss than their counterparts stored in air (Figure 5.3). Amoah (2013) also found higher weight loss in ethylene-treated sweetpotatoes. The tissue bursting may have allowed greater water loss, consequently leading to weight loss. In contrast to this result, Cheema et al. (2013) did not find increased weight loss in ‘Ibees’ or ‘Bushbuck’ sweetpotato roots stored in $10 \mu\text{L L}^{-1}$ ethylene for 4 weeks. Furthermore, in Cheema’s study no ethylene induced tissue splitting was observed. These disparities may relate to differences in cultivar sensitivity to ethylene.

Phenolic compounds and carotenoids were measured in ‘Clone 1820’ to find out if ethylene treatment had any detrimental effect on these potentially health-benefiting compounds. Ethylene treatment had no negative effect on carotenoids, whilst

concentrations of anthocyanins and phenolic acids declined or increased due to ethylene exposure. The results for ‘Owairaka Red’ confirms earlier findings by other researchers (Buescher et al., 1975; Kitinoja, 1987) that ethylene can induce phenolic acids. In addition, the result showed that not all individual phenolic acids increase due to ethylene exposure. Amoah (2013) studied the effects of ethylene treatment alone on individual phenolic acid concentrations, but the differences in the analysis procedure make comparison of results between these two studies difficult. In Amoah’s study, concentrations in skin, distal and proximal tissues were analysed separately, whereas in this study the skin was removed and there was no other similar tissue separation. Nevertheless, they also concluded that an ethylene effect on the phenolic acid concentration is specific to individual phenolic compounds.

When ‘Clone 1820’ roots were exposed to ethylene, the ethylene-induced increase in phenolic acid concentration was not observed. This suggests that the effect of ethylene on phenolic acid biosynthesis is cultivar dependent. The cultivar difference might be related to differences in ethylene sensitivity. Accumulation of phenolic compounds has been found to be a necessary step in biosynthesis for the wound healing substances suberin and lignin (Biggs, 1985). In this study, root proximal end split was more intense in ‘Owairaka Red’ roots than in ‘Clone 1820’, concurrently the rot incidence was also high in ‘Owairaka Red’. Therefore, it is possible that the increased concentration of chlorogenic acid and 3,5-dicaffeoylquinic acid in ‘Owairaka Red’ is a normal plant tissue biological response to enhance wound healing or pathogen defence.

5.5.2 Effect of 1-MCP treatment on sweetpotato storage root quality

1-MCP has been used as a tool in demonstrating the effects of ethylene in various plants (Watkins, 2006; Huber, 2008). ‘Clone 1820’ sweetpotato roots treated with 1-MCP ($1 \mu\text{L L}^{-1}$) for 24 h retained higher levels of some anthocyanins than observed in the controls (Table 5.7). In pineapple, 1-MCP treatment decreased browning and hydrolysis of ascorbic acid (Budu & Joyce, 2003). In avocado, 1-MCP treatment reduced polyphenol oxidase (PPO) and peroxidase (POD) activity, consequently reducing flesh discoloration (Hershkovitz et al., 2005). Anthocyanins can be degraded in plant tissue by β -glycosides, PPO, and POD enzymes (Shi et al., 1992; Zhang et al., 2005). If 1-MCP treatment also reduces PPO and POD activities in sweetpotato roots, it can be

suggested that retention of high anthocyanins concentration in roots treated with 1-MCP could relate to reduced activities of PPO and POD enzymes in 1-MCP treated roots.

1-MCP application has been reported to inhibit sprouting in sweetpotato (Cheema et al., 2013). But in the study reported here this did not occur in either cultivar. Effective concentrations of 1-MCP vary with respect to species, cultivars, treatment time and temperature (Blankenship & Dole, 2003). It is possible that the discrepancy was due to cultivar differences. The variation reported among cultivars may be associated with differences in skin permeance, internal ethylene concentrations and ethylene receptor turnover. The diffusion of 1-MCP in plant tissue is affected by tissue structure and cuticle resistance (Nanthachai et al., 2007). Different sweetpotato cultivars have different skin or periderm properties; some have thick and rough skin, others thin skin. Thus, in cultivars with thin skins 1-MCP may diffuse more readily than cultivars with thick skins. 1-MCP application in this study was undertaken after the sweetpotato roots were cured. Curing results in thickened cell walls or lignification (Artschwager & Starrett, 1931) and the thickness of the lignified layer is cultivar and relative humidity dependent (van Oirschot et al., 2006). In the study by Cheema et al. (2013), there is no information on whether the sweetpotatoes were cured or not. It is possible that 1-MCP was applied to uncured sweetpotatoes with a limited lignified layer, thereby reducing resistance to 1-MCP infusion.

High internal ethylene concentration has been reported to reduce 1-MCP efficacy in apples (Watkins & Nock, 2012; Jung & Watkins, 2014) and tomato (Zhang et al., 2009a). Internal ethylene concentrations in the cultivars used in this study were not measured. It is possible that internal ethylene concentrations in ‘Clone 1820’ and ‘Owairaka Red’ are higher than in the cultivars used by Cheema et al 2013. To understand if internal ethylene concentration plays a role in 1-MCP efficacy in sweetpotato, it would be helpful in the future to measure the internal ethylene concentration of different sweetpotato cultivars before 1-MCP treatment, and monitor their response to 1-MCP treatment during storage.

5.5.3 Combined effects of 1-MCP and ethylene treatments

1-MCP inhibits ethylene effects by blocking ethylene receptor binding sites (Sisler & Serek, 1997). Therefore, it was anticipated that ethylene-induced responses would be

blocked when sweetpotatoes were pre-treated with 1-MCP, but that was not the case in this study. 1-MCP when combined with ethylene treatment did not differ from the responses of sweetpotato when treated with ethylene alone; except that 1-MCP + ethylene treated roots had higher glucose and fructose concentration compared to roots treated with ethylene alone (Table 5.3), suggesting that the degradation of glucose and fructose associated with the ethylene treatment was inhibited by 1-MCP. However, the mechanism by which 1-MCP inhibited the ethylene-induced glucose and fructose reduction during storage remains to be investigated. In addition, the concentrations of cyanidin-3-caffeoylsophoroside-5-glucoside and peonidin-3-caffeoyl sophoroside-5-glucoside were higher in roots treated with 1-MCP + ethylene than in roots treated by ethylene alone (Table 5.7), however, the percentage concentration change was very modest to be attributed to treatment effect.

The effects of ethylene on sprout inhibition were not modified by 1-MCP treatment, indicating that ethylene was not inhibited. It is possible that a concentration of $1 \mu\text{L L}^{-1}$ 1-MCP was not adequate to block all the ethylene receptors in 'Owairaka Red' and 'Clone 1820' sweetpotatoes. Even though $1 \mu\text{L L}^{-1}$ is a high dose used for most fruits and vegetables (Blankenship & Dole, 2003; Watkins, 2006), some researchers have reported higher doses to be more effective. Able et al. (2002) showed that 1-MCP concentration of $12 \mu\text{L L}^{-1}$ was optimal in extending the storage life of pak choy leaves and broccoli florets. In tomato, a 1-MCP concentration of $20 \mu\text{L L}^{-1}$ was more effective in extending the storage life of ripe tomatoes (Wills & Ku, 2002). It is possible that if higher dose than $1 \mu\text{L L}^{-1}$ of 1-MCP was used, ethylene responses could have been blocked.

In et al. (2013) and Tassoni et al. (2006) have shown that plant tissues may synthesise new ethylene receptors, thereby regaining sensitivity to ethylene. It is possible that there was ethylene receptor regeneration in the sweetpotato cultivars used in this study, thereby allowing continued ethylene perception, which resulted in ethylene-induced sprout inhibition. Similar observations on regained ethylene sensitivity after 1-MCP treatment have been reported in sugar beet (Fugate et al., 2010), pear (Ekman et al., 2004), and carnations (In et al., 2013).

5.5.4 Sensory acceptability of ethylene treated sweetpotatoes

The instrumental data on lightness (Section 5.4.5) clearly showed that ethylene treated sweetpotatoes were darker than untreated samples. Sensory evaluation was conducted to ascertain if consumers would accept the ethylene-darkened samples. Despite some of the panellists commenting that ethylene samples were ‘dull’, ‘mottled appearance’, ‘blotchy’ and ‘dark’, no significant differences were observed between ethylene treated and air treated samples (Table 5.5). The fact that acceptance means scores were above five, indicates that if ethylene can be commercialised as a sprout inhibitor in sweetpotato, consumers will accept ethylene treated sweetpotatoes.

5.6 Conclusions

Ethylene effects, with or without 1-MCP pre-storage treatment, were tested to determine individual or combined effects on sweetpotato root sprouting, internal flesh colour, weight loss, phytochemical concentrations and consumer acceptability. Ethylene treatment with or without 1-MCP inhibited sprout growth, whereas 1-MCP treatment alone did not significantly differ from the control and sprouting started by the second week of storage in both cultivars. Additionally, ethylene treatment increased root respiration rates and weight loss, whilst also leading to tissue bursting on root proximal/stem ends. Contrary to expectations, ethylene-induced responses were not inhibited by 1-MCP. The flesh colour of cooked sweetpotatoes was darker in ethylene treated roots than the control or 1-MCP + Air. But the flesh darkening due to ethylene was acceptable to consumers. The results confirm that ethylene is a potential sprout inhibitor in sweetpotato. The results also suggest that no generalised conclusion can be made about ethylene induced phenolic acid biosynthesis, flesh darkening in cooked sweetpotatoes and disease incidence, as ethylene effects on these parameters were cultivar dependent. It also appears that a 24 hour exposure to 1-MCP ($1 \mu\text{L L}^{-1}$) is not sufficient to block ethylene-induced responses in sweetpotato.

6 Suppression of ethylene-induced responses in sweetpotato roots by multiple 1-methylcyclopropene applications

6.1 Introduction

1-methylcyclopropene (1-MCP), an ethylene action inhibitor, counteracts ethylene effects by binding to ethylene receptors making the plant tissue insensitive to ethylene (Sisler & Serek, 1997). Therefore plants treated with 1-MCP are not expected to respond to ethylene (Sisler & Serek, 2003). However, this is not always the case as plants treated with 1-MCP may regain sensitivity to ethylene (In et al., 2013). Feng et al. (2004) suggested that the recovery of ethylene sensitivity after 1-MCP treatment is due to synthesis of new ethylene binding sites or sites that are dissociated from the inhibitor after a certain period of 1-MCP treatment. The hypothesis of new ethylene receptor regeneration has been confirmed by some molecular work in tomato (Tassoni et al., 2006) and carnations (In et al., 2013). Results in chapter five showed that a single dose of 1-MCP ($1 \mu\text{L L}^{-1}$) did not alter ethylene-induced respiration, root splitting, internal flesh darkening or sprouting inhibition in ‘Owairaka Red’ and ‘Clone 1820’ sweetpotatoes. With these results it was reasonable to assume that new ethylene binding sites were being produced in ‘Owairaka Red’ and ‘Clone 1820’ sweetpotatoes, thereby allowing continued ethylene perception.

Several studies have shown that plants treated with multiple 1-MCP have prolonged suppression of ethylene responses (Cameron & Reid, 2001; Jayanty et al., 2004; In et al., 2013; Lu et al., 2013; Nock & Watkins, 2013). For instance, colour development and the softening of ‘Bartlett’ pears were slowed when fruit were retreated with 1-MCP (0.2 or $0.4 \mu\text{L L}^{-1}$) after 4 weeks at -1°C (Ekman et al., 2004). Multiple 1-MCP (0.1 or $1.0 \mu\text{L L}^{-1}$) treatments in ‘Redchief Delicious’ apples retained their firmness when stored at 5°C and above, but little effect was observed when fruit was stored at 0°C (Jayanty et al., 2004). With this background, it was hypothesised that multiple 1-MCP treatments would provide better protection against ethylene-induced flesh darkening in cooked sweetpotato. Therefore the objective of this study was to determine the effects of multiple 1-MCP treatments on ethylene production, weight loss, sprouting and the internal flesh colour of ‘Owairaka Red’ sweetpotatoes during storage at 25°C and 85% RH.

6.2 Materials and methods

6.2.1 *Plant materials*

'Owairaka Red' roots were sourced from Delta Produce, Dargaville, New Zealand. After curing the roots were subjected to different storage conditions: air (control), single 1-MCP ($1 \mu\text{L L}^{-1}$) + air, ethylene ($10 \mu\text{L L}^{-1}$), single 1-MCP ($1 \mu\text{L L}^{-1}$) + ethylene ($10 \mu\text{L L}^{-1}$), and multiple (3 doses) 1-MCP ($1 \mu\text{L L}^{-1}$) + ethylene ($10 \mu\text{L L}^{-1}$). Each treatment was applied to 20 roots. At the start of the experiment the roots had been stored at 15°C and 85% RH for 2 months.

6.2.2 *1-MCP application*

1-MCP was applied as described previously in section 5.2.2. For the single 1-MCP treatment, sweetpotato roots were treated with 1-MCP ($1 \mu\text{L L}^{-1}$) for 24 h at 20°C prior to storage and no additional 1-MCP was applied. For the multiple 1-MCP treatment sweetpotato roots were treated with 1-MCP ($1 \mu\text{L L}^{-1}$) prior to storage then followed by two additional 1-MCP ($1 \mu\text{L L}^{-1}$) treatments following 4 and 8 days of storage. The initial 1-MCP treatment was applied at 20°C , and the subsequent treatments were applied at 25°C . For roots stored in ethylene gas, the ethylene gas flow was stopped and the barrels were flushed with air (free of any added ethylene) for 12 h before the additional 1-MCP treatments.

6.2.3 *Ethylene application*

Ethylene ($10 \mu\text{L L}^{-1}$) was applied continuously during storage, except the time of 1-MCP reapplication. Ethylene was applied as previously described in section 5.2.3.

6.2.4 *Measurement of ethylene production*

Ethylene production was measured with an ethylene detector ETD 300 (Sensor sense, Nijmegen, Netherlands) using the stop and flow method. The stop and flow method allows accumulation of ethylene in a cuvette during the period that the cuvette is not being measured (Cristescu et al., 2013). Two to three roots were placed in 1800 mL jars fitted with two septa. To avoid the accumulation of CO_2 gas in the jars, sachets of soda lime, a CO_2 scrubber, were placed in each jar. In addition, before the gas was flushed through the detector, the gas was passed through a column containing soda lime and Drierite, to scrub CO_2 and moisture respectively. The results obtained were analysed with the use of valve controller software and expressed as $\text{nL kg}^{-1} \text{h}^{-1}$.

6.2.5 Weight loss

Weight loss (%) during storage was measured as previously described in section 2.3.1.

6.2.6 Sprout growth

Sprout number per root and length (mm) were assessed as previously described section 2.3.2.

6.2.7 Rot incidence

Roots with visible signs of rots were counted as rotten and expressed as a percentage incidence.

6.2.8 Internal flesh colour

The internal flesh colour of cooked sweetpotatoes was measured at 0, 1, 2, and 3 weeks of storage using a reflectance spectrophotometer (Model CM-2600D, Konica Minolta Sensing Inc., Japan) as described previously in section 5.2.8.

6.2.9 Statistical analysis

Data were analysed using Minitab 16 (Minitab Inc., State College, Pennsylvania). Data on sprout number and length were transformed using square root and log transformation methods respectively. Means were compared by Tukey's test, at $P < 0.05$. All transformed means were back transformed for presentation. Rotten roots were excluded from weight loss statistical analysis.

6.3 Results

6.3.1 Ethylene production

Ethylene production in all treatments ranged from 1.3 to 4.7 nL kg⁻¹ h⁻¹. A single application of 1-MCP alone or in combination with ethylene did not affect ethylene production, while the ethylene production of roots treated with multiple 1-MCP + ethylene was reduced by almost half by the second and third week of storage (Figure 6.1).

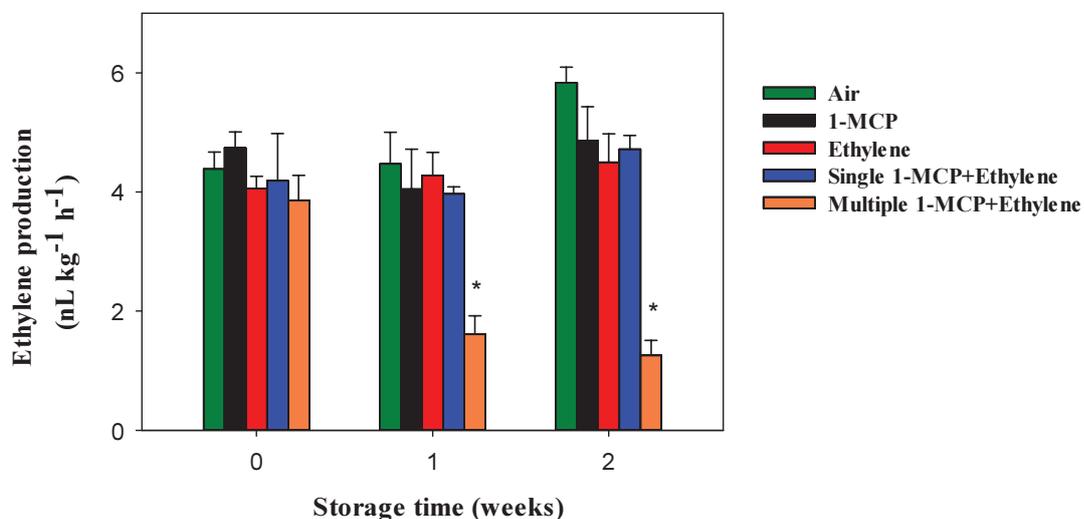


Figure 6.1: Ethylene production of sweetpotato ‘Owairaka Red’ roots treated with air, single 1-MCP ($1 \mu\text{L L}^{-1}$) + air, ethylene ($10 \mu\text{L L}^{-1}$), single 1-MCP ($1 \mu\text{L L}^{-1}$) + ethylene ($10 \mu\text{L L}^{-1}$), and multiple 1-MCP ($1 \mu\text{L L}^{-1}$) + ethylene ($10 \mu\text{L L}^{-1}$) during storage at 25°C and 85% RH. Multiple 1-MCP treatments: 1-MCP applied at 0, 4, and 8 days of storage. An asterisk (*) indicates a significant difference compared to the other treatments at $P < 0.05$.

6.3.2 Weight loss

Weight loss in all treatments increased with storage duration (Figure 6.2). Roots treated with ethylene or single 1-MCP + ethylene had a significantly higher weight loss than the roots given other three treatments at 2 and 3 weeks of storage. Multiple 1-MCP application significantly ($P < 0.05$) reduced the ethylene-induced weight loss, but weight loss was not different from air and 1-MCP + air treatments. At 3 weeks, the weight loss (%) was: 8, 8, 12, 13 and 10 % in air, single 1-MCP + air, ethylene, single 1-MCP + ethylene and multiple 1-MCP + ethylene respectively.

6.3.3 Sprout growth

No sprouts were observed in roots treated with ethylene alone or single 1-MCP + ethylene (Figure 6.3 A & B). In contrast, roots treated with multiple 1-MCP + ethylene started sprouting at 2 weeks but the sprout growth was not significantly ($P > 0.05$) different from roots treated with ethylene or single 1-MCP + ethylene. When compared

to the sprout growth in roots stored in air, sprouts in multiple 1-MCP + ethylene were about 1.8 times fewer and 4.8 times shorter than the sprouts in roots treated with air or single 1-MCP. Looking at the number of sprouted roots per treatment, air and single 1-MCP + air had the highest number of sprouted roots compared to multiple 1-MCP + ethylene (Figure 6.3 C).

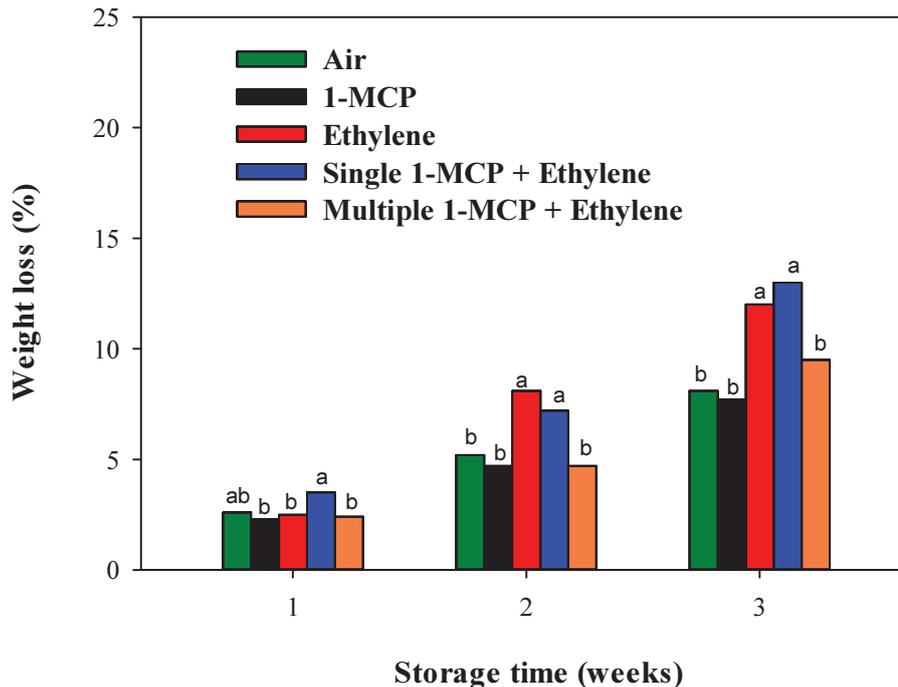


Figure 6.2: Weight loss (%) of sweetpotato 'Owairaka Red' roots treated with air, single 1-MCP ($1 \mu\text{L L}^{-1}$) + air, ethylene ($10 \mu\text{L L}^{-1}$), single 1-MCP ($1 \mu\text{L L}^{-1}$) + ethylene ($10 \mu\text{L L}^{-1}$), and multiple 1-MCP ($1 \mu\text{L L}^{-1}$) + ethylene ($10 \mu\text{L L}^{-1}$) during storage at 25°C and 85% RH. Multiple 1-MCP treatments: 1-MCP applied at day 0, day 4, and day 8 of storage. Values with the same letter at each sampling time are not significantly different from each other. Values are averages of at least 12 roots.

6.3.4 Root stem end disorder and rot incidence

The multiple 1-MCP + ethylene treatment delayed ethylene-induced root splitting by 2 weeks compared to roots treated with ethylene or single 1-MCP + ethylene (Figure 6.4). Roots treated with multiple 1-MCP + ethylene only split at 3 weeks while roots treated with ethylene or single 1-MCP + ethylene started splitting at 1 week. Rot incidence (%) also followed the same trend, with a high rot incidence in roots treated with ethylene

alone or single 1-MCP + ethylene compared to roots with multiple 1-MCP + ethylene. No rots were observed in roots treated with air and single 1-MCP + air. At 3 weeks, the rot incidence was 56, 59, and 36 % in ethylene, single 1-MCP + ethylene and multiple 1-MCP + ethylene respectively (Figure 6.5).

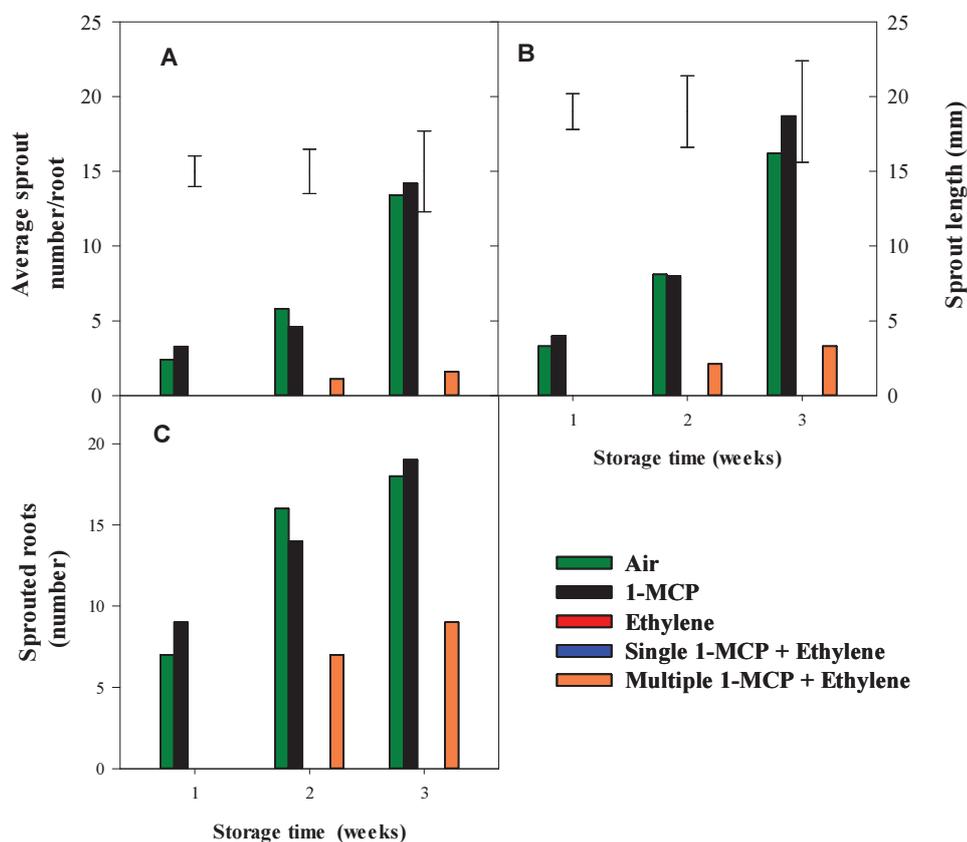


Figure 6.3: Average sprout number/root (A), sprout length (mm) (B), and number of sprouted roots (C) in sweetpotato cv. 'Owairaka Red' roots treated with air, single 1-MCP ($1 \mu\text{L L}^{-1}$) + air, ethylene ($10 \mu\text{L L}^{-1}$), single 1-MCP ($1 \mu\text{L L}^{-1}$) + ethylene ($10 \mu\text{L L}^{-1}$), and multiple 1-MCP ($1 \mu\text{L L}^{-1}$) + ethylene ($10 \mu\text{L L}^{-1}$) during a 3 week storage period at $25 \text{ }^\circ\text{C}$ and 85% RH. Multiple 1-MCP treatments: 1-MCP applied at 0, 4, and 8 days of storage. Vertical bars represent HSD_{0.05} (Tukey's test). Values are averages of at least 12 roots.

6.3.5 Internal flesh colour

The hue angle of cooked roots ranged from 93° to 96° significant differences were only observed between air and single 1-MCP + ethylene (Table 6.1). Roots treated with air were more yellow than roots treated with single 1-MCP + ethylene. The measured lightness values ranged from 37 to 52, the lowest values were observed in roots treated with ethylene or single 1-MCP + ethylene, and the highest values were observed in the

roots stored in air. This indicated that roots treated with ethylene or a single 1-MCP + ethylene treatment were darker than roots stored in air. The lightness value of multiple 1-MCP + ethylene treated roots was intermediate between air stored samples and ethylene or single 1-MCP + ethylene treated roots, suggesting that the ethylene-induced flesh darkening was reduced with multiple 1-MCP + ethylene treatments. No significant interaction effects were observed between storage time and the treatments.

At 2 weeks



Single 1-MCP + ethylene



Multiple 1-MCP + ethylene

At 3 weeks



Single 1-MCP + ethylene



Multiple 1-MCP + ethylene

Figure 6.4: Visual appearance of sweetpotato ‘Owairaka Red’ roots treated with a single 1-MCP ($1 \mu\text{L L}^{-1}$) + ethylene ($10 \mu\text{L L}^{-1}$) treatment or a multiple 1-MCP ($1 \mu\text{L L}^{-1}$) + ethylene ($10 \mu\text{L L}^{-1}$) treatment at 2 and 3 weeks of storage at 25 °C and 85% RH. Multiple 1-MCP treatments: 1-MCP applied at 0, 4, and 8 days of storage.

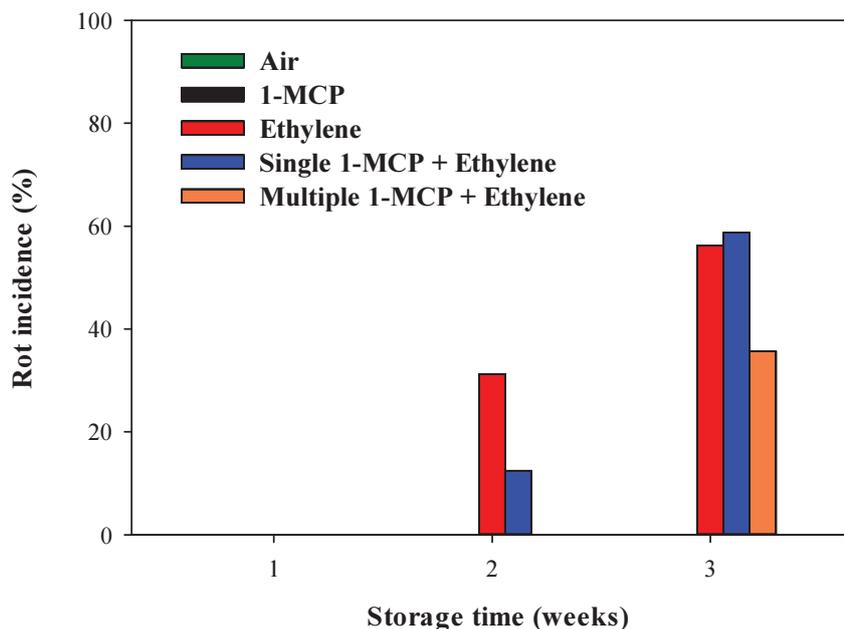


Figure 6.5: Rot incidence (%) in sweetpotato ‘Owairaka Red’ roots treated with air, single 1-MCP ($1 \mu\text{L L}^{-1}$) + air, ethylene ($10 \mu\text{L L}^{-1}$), single 1-MCP ($1 \mu\text{L L}^{-1}$) + ethylene ($10 \mu\text{L L}^{-1}$), and multiple 1-MCP ($1 \mu\text{L L}^{-1}$) + ethylene ($10 \mu\text{L L}^{-1}$) during storage at $25 \text{ }^\circ\text{C}$ and 85% RH. Multiple 1-MCP treatments: 1-MCP applied at 0, 4, and 8 days of storage.

Table 6.1: Flesh hue angle and lightness for cooked sweetpotato ‘Owairaka Red’ roots subjected to various treatments and stored for 3 weeks at $25 \text{ }^\circ\text{C}$ and 85% RH.

Treatment	Hue angle (h°)	Lightness
Air	94.8 ^a	51.5 ^a
Single 1-MCP + air	95.5 ^{ab}	49.0 ^a
Ethylene	93.3 ^{ab}	36.5 ^c
Single 1-MCP + ethylene	92.9 ^b	38.1 ^c
Multiple 1- MCP + ethylene	93.3 ^{ab}	44.0 ^b
P-Value	0.013	< 0.001

Lightness values of 0 = black and 100 = white. Means followed by the same letter within a column are not significantly different at $P < 0.05$ (Tukey’s test).

6.4 Discussion

Sweetpotatoes are classified as non-climacteric and produce low levels of ethylene $\sim 0.1 \mu\text{L kg}^{-1}\cdot\text{h}^{-1}$ (Cantwell & Suslow, 2013). As expected for non-climacteric fruits and vegetables exogenous ethylene did not induce further ethylene production (Kende, 1993). A single 1-MCP treatment, whether in the presence or absence of ethylene, did not induce additional ethylene production (Figure 6.1). This is contrary to observations in other non-climacteric fruits and vegetables. For instance, 1-MCP treatment induced ethylene production in parsley (Ella et al., 2003) and coriander (Jiang et al., 2002). In addition, the multiple 1-MCP treatment significantly reduced ethylene production at 2 and 3 weeks of storage. The reason for the decline in ethylene when roots were treated with multiple 1-MCP is not clear, but similar observations have been found in strawberries (Jiang et al., 2001) and pepper (Tian et al., 2004) after single 1-MCP treatments. These results suggest that the effect of 1-MCP on ethylene production in non-climacteric produce varies among plant tissues.

1-MCP blocks ethylene receptors, thereby blocking ethylene effects (Sisler & Serek, 1997). A single dose of 1-MCP in the presence of ethylene did not suppress ethylene-induced weight loss and flesh darkening. These results confirm the results in Chapter five, that a single dose of 1-MCP ($1 \mu\text{L L}^{-1}$) is inadequate to counteract ethylene-induced responses in ‘Owairaka Red’ sweetpotatoes. Multiple 1-MCP treatments significantly reduced the ethylene-induced weight loss and flesh darkening. It would seem possible that ethylene receptors were regenerated and by re-treating the sweetpotatoes with 1-MCP the new receptors were in turn blocked, thereby reducing the ethylene-induced responses. Prange et al. (2005) found similar results in potato where more than a single dose of 1-MCP ($1 \mu\text{L L}^{-1}$) was required to counteract ethylene-induced fry colour darkening in ‘Shepody’ potatoes stored for more than 4 weeks, although one dose was adequate for ‘Russet Burbank’. The effect of the multiple 1-MCP on root splitting was temporary. At 3 weeks, the roots treated with multiple 1-MCP started splitting, indicating that the roots had regained ethylene sensitivity. The ability to regenerate new receptors after 1-MCP treatment differs among plant tissue (Blankenship & Dole, 2003). It appears that ‘Owairaka Red’ sweetpotatoes have high receptor turnover, but this can only be confirmed with molecular work. Storage temperature also plays a role in the rate of receptor turnover (Cameron & Reid, 2001;

Macnish et al., 2010). Cameron and Reid (2001) found that the half-life of 1-MCP activity in *Pelargonium peltatum* flowers was 2, 3, or 6 days at 25, 20.7, or 12 °C, indicating there was a slower recovery rate at low temperatures than at higher temperatures. The ideal storage temperature for sweetpotatoes is 15 °C, so it is possible that the high temperature (25 °C) used in this study accelerated the ethylene receptor regeneration in ‘Owairaka Red’.

Even though the multiple 1-MCP treatment reduced the flesh darkening of cooked sweetpotato roots, it did not interfere with the ethylene sprout inhibition effect (Figure 6.3). The few sprouts that were observed in roots treated with multiple 1-MCP + ethylene could have emerged due to the intermittent ethylene application during the first few days of storage, as ethylene application had to be stopped for 36 h (12 h for flushing out ethylene and 24 h for 1-MCP treatment) to allow second and third 1-MCP applications. The lack of interference of 1-MCP application on ethylene sprout inhibition while reducing flesh darkening is similar to results found in potato (Prange et al., 2005; Daniels-Lake et al., 2008). Prange et al. 2005 suggested that the potato eyes (axillary sprouts) are more metabolically active than the pith and cortex tissues, so new binding sites will be synthesised and become available for ethylene binding, in that way reducing the sprouting. In contrast, the site for fry colour darkening is in the pith and cortex of the tuber where cells are less active metabolically, so there is less ethylene receptor regeneration to replace those blocked with 1-MCP. It can be suggested that there is a similar scenario in sweetpotatoes, that the tissues where sprouts originate may have higher receptor turnover than the pith and cortex.

6.5 Conclusion

The multiple 1-MCP treatment has potential to delay ethylene-induced root splitting and reduce ethylene-induced flesh darkening in ‘Owairaka Red’ sweetpotatoes while maintaining minimal sprout growth. However, the benefit of multiple 1-MCP application was short-term, suggesting that ‘Owairaka Red’ has a high ethylene receptor turnover. In conclusion, applying ethylene, even when accompanied by multiple 1-MCP treatments, is unlikely to be a useful storage technique for ‘Owairaka Red’ sweetpotatoes.

7 Anti-inflammatory properties of sweetpotato extracts on lipopolysaccharide (LPS)-induced IL-8 production in HT-29 cells

7.1 Introduction

Inflammation is an early immune reaction that is mediated by immune cells and their cytokines. A properly regulated inflammation involves a balanced production of proinflammatory and anti-inflammatory cytokines (Medzhitov, 2010). Proinflammatory cytokines are products that initiate inflammatory process such as Tumour Necrosis Factor (TNF), Interleukin (IL)-6, IL-8, and IL-1 β , whereas anti-inflammatory cytokines are products that suppress the activity of proinflammatory cytokines, hence down regulating the inflammatory response to end the inflammation process (Dinarello, 2000). Prolonged or inappropriate inflammation leads to chronic inflammation. Chronic inflammation is associated with rheumatoid arthritis, asthma, bladder cancer, and inflammatory bowel diseases (Schauss, 2013). Diets rich in fruits and vegetables can reduce the severity of some inflammation related diseases (Giugliano et al., 2006; Lin & Tang, 2008; Guo et al., 2009; Mueller et al., 2010). The reduction in disease severity is associated with the presence of bioactive compounds in fruits and vegetables.

Sweetpotato storage roots are composed of carbohydrates, dietary fibres, minerals (calcium, magnesium, potassium and zinc), and vitamins: (C, B1, B2, E and A). In addition to these compounds, some sweetpotato cultivars contain phytochemicals such as carotenoids, phenolic acids, and anthocyanin (Woolfe, 1992). The anti-inflammatory properties of sweetpotato storage roots have been studied both *in vitro* (Grace et al., 2014) and *in vivo* (Wang et al., 2010). These studies showed the potential anti-inflammatory properties of sweetpotatoes based on uncooked roots, however sweetpotatoes are generally consumed after cooking. Therefore in this chapter, the effect of cooked sweetpotato extracts on the production of the proinflammatory biomarker IL-8, stimulated by lipopolysaccharide (LPS) in the HT-29 ‘intestinal enterocyte cell line’, was studied. IL-8 cytokine was selected as it is known to play a central role in the initiation and maintenance of intestinal inflammatory and immune response (Savidge et al., 2006) and can be produced by HT-29 cells (Kolios et al., 1996). Two cultivars were selected ‘Owairaka Red’ is the main commercial cultivar in

New Zealand and the skin contains considerable amount of anthocyanins, and ‘Clone 1820’, possesses both carotenoids and anthocyanins.

7.2 Materials and Methods

7.2.1 Preparation of sweetpotato extracts

Two sweetpotato cultivars, ‘Owairaka Red’ and ‘Clone 1820’, were harvested in April, 2014 by Delta Produce, Dargaville; and Plant and Food Research, Pukekohe, New Zealand respectively. The sweetpotato roots were divided into two groups: one for measurements before storage and the other for after-storage measurements. The roots designated for after-storage measurements were cured at 30 °C for 4 days and stored at 15 °C and 85 % RH for one month. Roots for before storage measurements were cooked, freeze dried and stored at -30 °C until the time for the extraction. The treatments were:

- ‘Owairaka Red’ before storage (OR-BS)
- ‘Clone 1820’ before storage (CN-BS)
- ‘Owairaka Red’ after 4 weeks storage (OR-AS)
- ‘Clone 1820’ after 4 weeks storage (CN-AS)

Sweetpotato roots were randomly assigned into three replicates (during sampling), each containing 3 kg of roots. The roots were washed, sliced into uniform pieces (10 mm thick) using a vegetable slicer, then steamed for 15 min (Figure 7.1). Thereafter, the cooked samples were freeze dried using a freeze drier model FD18LT (Cuddon Ltd., New Zealand). Before extraction, freeze dried sweetpotato pieces were ground into powder using a blender (CG2B, Breville Pty. Ltd., Sydney, Australia). Sweetpotato extracts were extracted following procedures by Chong et al. (2013) with minor modification. Freeze dried sweetpotato powder (150 g) was mixed with 500 mL 80% ethanol, and left to stand overnight at room temperature with continuous stirring. The crude ethanol extract was filtered under vacuum through a Whatman filter paper no. 1 and evaporated to dryness using the rotary evaporator (Rotavapor R-215, Büchi Labortechnik AG, Flawil, Switzerland). The resultant slurry, free of ethanol, was freeze-dried using a freeze drier model FD18LT (Cuddon Ltd., New Zealand). The dry powder was used for bioassay, and analysis of sugar and phenolic compounds.

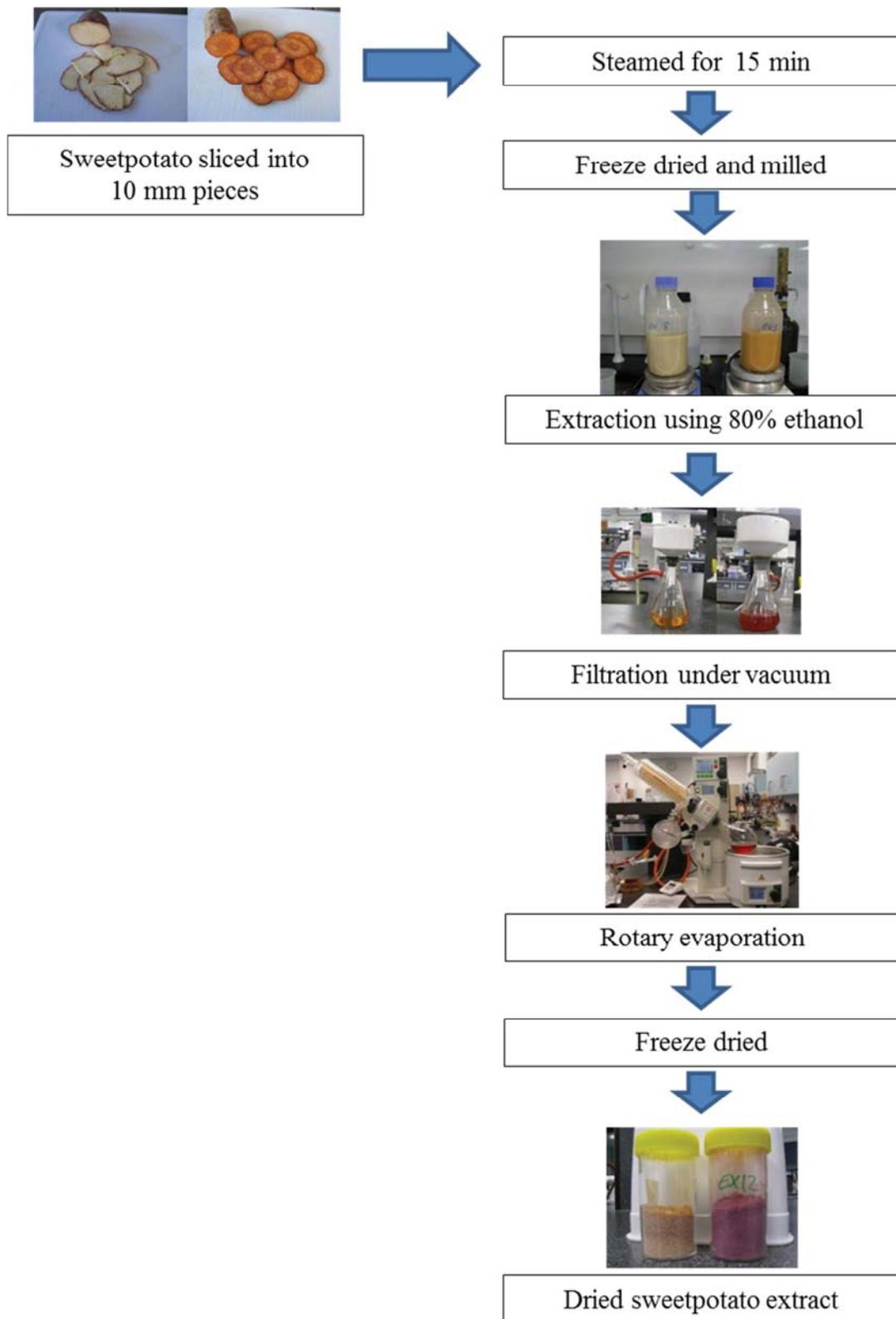


Figure 7.1: Schematic diagram for preparation of sweetpotato extracts

7.2.2 Quantification of phenolic acids and anthocyanins in sweetpotato extracts

The dried sweetpotato extracts (1 g) were mixed with 20 mL mobile phase consisting of acetonitrile: water: formic acid (60: 30: 10 v/v/v) and filtered through a 0.45 µm syringe filter into HPLC vials and analysed using HPLC as explained previously in section 2.4.4. Quantification used chlorogenic acid and cyanidin 3-O-glucoside chloride standard curves for phenolic acids and anthocyanin respectively.

7.2.3 Sugars analysis

The dried sweetpotato extracts (1 mg) were dissolved in water (10 mL), filtered through a 0.45 µm filter into HPLC vials. Sugar analysis was performed using a Dionex HPLC system as described previously in chapter 5 (Section 5.2.10).

7.2.4 Cell line and cell culture conditions

HT-29 colon carcinoma and MDA-MB-231 breast cancer cells were purchased from American Type Culture Collection (ATCC). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 1% L-glutamine, 1% penicillin-streptomycin, and 10% foetal calf serum (D-10) in tissue culture flasks, and passaged once to twice weekly as needed using trypsin-EDTA as recommended by the provider. All tissue culture reagents were purchased from Invitrogen (Invitrogen, Auckland, New Zealand).

7.2.5 Cytotoxicity

Cytotoxicity of the sweetpotato extracts on breast cancer MDA-MB-231 cells and HT-29 cells were performed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The assay detects the reduction of MTT tetrazolium dye (yellow in colour) to insoluble formazan (blue) product by metabolically active cells (Mosmann, 1983). Breast cancer MDA-MB-231 cells were only used for preliminary screening, using four levels of sweetpotato extracts concentrations: 1, 10, 100 and 1000 µg/mL. The tests for HT-29 cells were conducted using two sweetpotato extract concentrations: 10 and 100 µg/mL.

The cells were plated into 96-well plastic tissue culture plates at a concentration of 2×10^4 cells per well in a volume of 0.1 mL D-10 (DMEM containing 1% L-glutamine, 1% penicillin-streptomycin, and 10% foetal calf serum). The cells were allowed to adhere

for 24 hours. On the following day, the medium and unattached cells were removed and replaced with 0.1 mL D-0.4 (medium as for D10 but containing 0.4% FCS) containing sweetpotato extracts or control (medium only) at various concentrations into three replicate wells per treatment on duplicate plates. After 72 hours, 10 μ L of a 5 mg/mL solution of MTT (Sigma-Aldrich) in Phosphate Buffered Saline (PBS) was added to each well. The reaction was stopped after 3-4 hours of incubation with MTT, the medium was decanted, and DMSO (100 μ L) was added to each well to dissolve the formazan crystals that had formed in the cells. The extent of the reduction of MTT to formazan was quantified by measuring the optical density (OD) at 550 nm using the 96-well plate reader (ELX 808, Bio-Tek Instruments Inc.). Optical density of the test extracts was compared to the negative control (medium only).

7.2.6 Effects of the sweetpotato extract co-incubated with LPS on IL-8 production in HT-29 cells

A stock solution of sweetpotato extract was prepared by wetting 100 mg of sweetpotato extract powder in 0.1 mL dimethyl sulfoxide (DMSO) and then dissolving the wetted powder in 9.9 mL phosphate buffer saline (PBS). HT-29 cells were plated at 50,000 cells per well into 24-well plates in a volume of 1 mL DMEM (without L-glutamine), containing penicillin and streptomycin at 1% and foetal calf serum (FCS) at 10%. The cells were allowed to grow for 3 days, and then half the wells were stimulated with LPS (1 μ g/mL) from *Escherichia coli* 055:B5 in the presence of test samples: sweetpotato extracts, maltose, or the controls. Controls were: medium only (negative control), blank (Medium + DMSO), and glucosamine (positive control). Glucosamine (Sigma-Aldrich) was made up in solution in a sterile PBS and used at a final concentration of 5 mM. Each treatment was tested in a single well. After 48 h, the plates were centrifuged and the supernatants collected and stored at -80 °C until analysis.

7.2.7 Effects of pre-incubation of sweetpotato extracts with HT-29 cells before LPS stimulation

Pre-incubation involved pre-treating the cells with sweetpotato extracts for 24 h before challenging them with LPS (1 μ g/mL). HT-29 cells were plated at 10,000 cells per well into 96 well plates and allowed to grow for 4 days. The cells were pre-treated with sweetpotato extracts at 20 μ g/mL for 24 h or the 2 controls; medium only (negative control) and 5 mM glucosamine (positive control), and then half of the wells were stimulated with LPS (1 μ g/mL) for an additional 24 h. The final concentration of extract

in the well was 10 µg/mL. After incubation, the plates were centrifuged and the supernatants collected and stored -80 °C until analysis.

7.2.8 IL-8 measurement

HT-29 supernatants were assessed for IL-8 content using a human IL-8 enzyme-linked immunosorbent (ELISA) kit (Duo-Set, R&D Systems, Minneapolis, MN, USA), following manufacturer's instructions. Briefly, 96-well plates (Nunc) were coated with captured antibody, incubated, washed with PBS containing 0.5% Tween-20, blocked with PBS containing 3% Bovine Serum Albumin (BSA), incubated, washed, and the test samples added into triplicate wells. Plates were incubated, washed, and the biotin-labelled detector antibody added. Plates were incubated, washed, then streptavidin-horseradish peroxidase was added, incubated, and the plates were washed again. Finally tetramethylbenzidine (TMB) was added, the plates incubated, and the reaction stopped with the addition of 1 M sulfuric acid. Plates were read at 450 nm using the 96-well plate reader (ELX 808, Bio-Tek Instruments Inc.). The mean reading of blank wells was subtracted from test wells, and the readings then normalised to a seven-point purified human IL-8 standard curve.

7.2.9 Statistical analysis

One way analysis of variance with post hoc Tukey's multiple comparison test was conducted. These analyses were performed using Minitab (version 16.0, Minitab Inc., State College, Pennsylvania). Results are expressed as mean ± SEM

7.3 Results

7.3.1 Phenolic acids in 'Owairaka Red' and 'Clone 1820' sweetpotato extracts

In both cultivars, seven major peaks were eluted (Figure 7.2) and identified using LC-MS as previously described in section 4.4.3.4. The total phenolic acids ranged from 451 to 3252 mg/100 g DW, 'Clone 1820' extracts had 6 times the concentration of total phenolic acids compared to 'Owairaka Red' (Table 7.1). A similar trend was observed in the concentrations of all phenolic acids, with higher concentration of individual phenolic acids being observed in 'Clone 1820'. Chlorogenic acid and 3,5-dicaffeoylquinic acid were the most abundant phenolic acids before storage. Storage duration had no effect ($P > 0.05$) on individual phenolic acid concentrations in

‘Owairaka Red’ samples, while in ‘Clone 1820’, caffeic acid, 3,4-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid concentrations increased with storage.

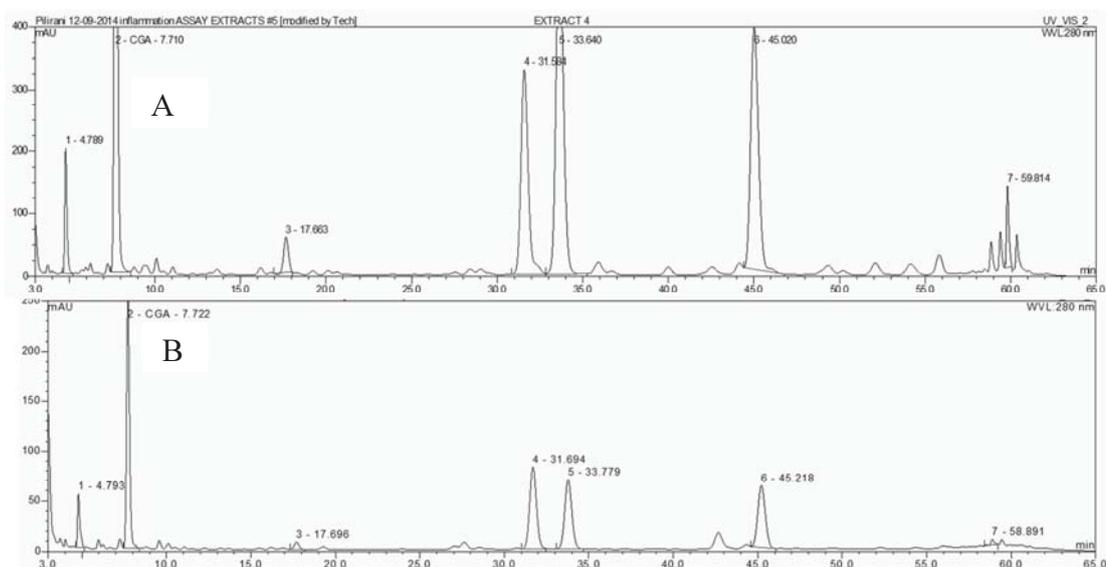


Figure 7.2: Representative HPLC chromatograms of ‘Clone 1820’ (A) and ‘Owairaka Red’ (B). Peak numbers corresponds to peak numbers in table 7.1.

Table 7.1: Phenolic acids concentration (mg/100 g DW) in ‘Owairaka Red’ and ‘Clone 1820’ sweetpotato extracts

Peak No.	Compound	Owairaka Red-BS	Clone 1820-BS	Owairaka Red-AS	Clone 1820-AS	P-value
1	Chlorogenic acid isomer	19.8 ^b	82 ^a	22.8 ^b	118 ^a	<0.001
2	Chlorogenic acid	162 ^b	737 ^a	152 ^b	829 ^a	<0.001
3	Caffeic acid	7.8 ^c	49 ^b	6.2 ^c	61 ^a	<0.001
4	3,4-di CQA	78 ^c	438 ^b	98 ^c	665 ^a	<0.001
5	3,5-di CQA	109 ^b	751 ^a	107 ^b	792 ^a	<0.001
6	4,5-di CQA	74.3 ^c	579 ^b	114 ^c	767 ^a	<0.001
7	unknown	0.7 ^b	17 ^a	0.8 ^b	20 ^a	<0.001
	Total phenolic	451 ^c	2654 ^b	501 ^c	3252 ^a	<0.001

All concentrations measured in chlorogenic acid equivalent. -BS = measurements before storage and -AS indicates after storage measurements. Means followed by the same letter within a row are not significantly different at $P < 0.05$.

7.3.2 Anthocyanin in ‘Owairaka Red’ and ‘Clone 1820’ sweetpotato extracts

Eight (8) peaks of individual anthocyanins were identified in ‘Clone 1820’, and only 3 peaks were observed in ‘Owairaka Red’ (Figure 7.3). As expected, ‘Clone 1820’ had higher total anthocyanin concentration than ‘Owairaka Red’ (Table 7.2). The concentrations were 12 and 17 times higher than the levels in ‘Owairaka Red’ before storage and after storage respectively. In general, there was an increase in the anthocyanin concentrations with storage except for peonidin-3-caffeoyl-p-hydroxybenzoyl sophoroside-5-glucoside (peak 6) and unknown peak 8.

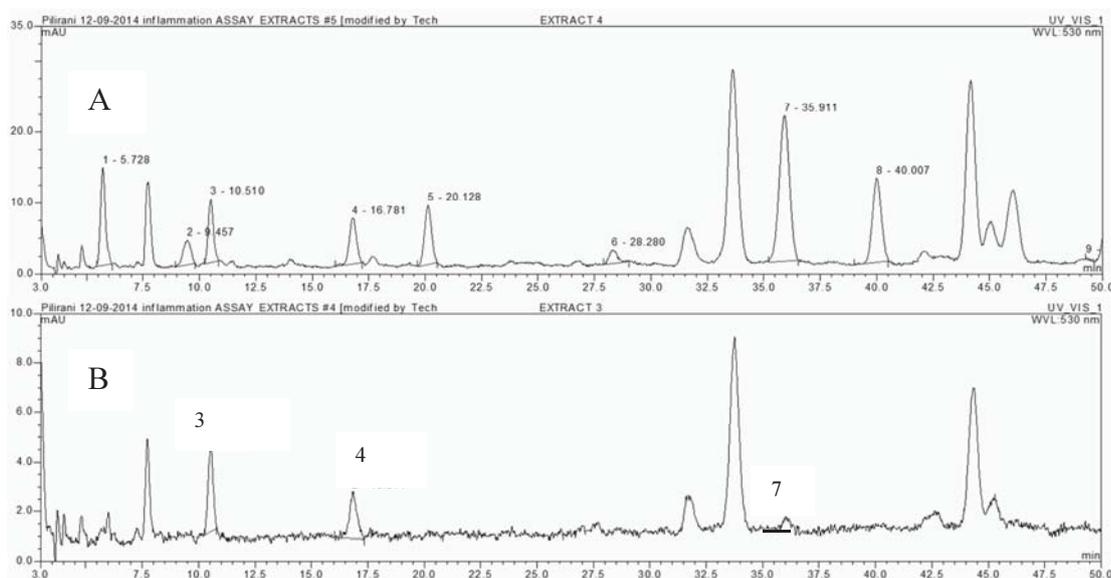


Figure 7.3: Representative HPLC chromatograms of ‘Clone 1820’ (A) and ‘Owairaka Red’ (B). Peaks number in the chromatogram corresponds to peak numbers in the table (Table 7.2).

7.3.3 Sugar concentration in sweetpotato extracts

The carbohydrate column (Meta carb H Plus (7.8 mm x 300 mm)), used in this study could not separate maltose and sucrose peaks, so the maltose and sucrose were quantified as one in maltose equivalent. No differences were observed in total sugar concentration in the two cultivars, but individual sugar concentrations differed (Table 7.3). The concentrations of glucose and fructose in OR-BS were almost half the concentration of glucose and fructose in OR-AS and concentrations in ‘Clone 1820 extracts’.

Table 7.2: Anthocyanin concentration (mg/100 g dry weight) in sweetpotato extracts

Peak No.	Owairaka Red-BS	Clone 1820-BS	Owairaka Red-AS	Clone 1820-AS	P-value
1	0.5 ^c	10.1 ^b	1.1 ^c	27.2 ^a	0.002
2	nd	4.6 ^b	nd	18.1 ^a	<0.001
3	3.6 ^c	7.4 ^b	9.7 ^b	18.3 ^a	<0.001
4	1.3 ^c	7.7 ^b	7.5 ^b	23.5 ^a	<0.001
5	nd	9.4 ^b	nd	13.9 ^a	0.029
6	nd	32.8	nd	35.8	ns
7	7 ^b	18 ^a	8.3 ^b	5 ^b	0.001
8	nd	40 ^a	nd	40 ^a	<0.001
Total	12.4 ^c	130 ^b	27 ^c	182 ^a	<0.001

Abbreviations: nd = not detected, ns = not significant different at $P = 0.05$; -BS = samples before storage, -AS = samples after 4 weeks of storage at 15 °C and 85% RH. All data quantified as mg/100 g cyanidin 3-O-glucoside chloride equivalent. Peak identification: **1** = Peonidin-3-sophoroside-5-glucoside, **2** = Peonidin-3-sophoroside-5-glucoside, **3** = Cyanidin-3-(6"-feruloyl sophoroside)-5-glucoside, **4** = Cyanidin-3-caffeoylsophoroside-5-glucoside, **5** = Peonidin-3-caffeoyl sophoroside-5-glucoside, **6** = Peonidin-3-caffeoyl-p-hydroxybenzoyl sophoroside-5-glucoside, peaks **7** and **8** = unknown. Means followed by the same letter within a row are not significantly different at $P < 0.05$.

Table 7.3: Sugar concentration (g/100 g dry weight) in the sweetpotato extracts

Sample	Maltose + Sucrose	Glucose	Fructose	Total sugars
Owairaka Red-BS	85.7 ^{ab}	1.8 ^c	1.8 ^b	89.3
Clone 1820-BS	71.5 ^{ab}	4.6 ^{ab}	4.2 ^a	80.4
Owairaka Red-AS	87.5 ^a	3.4 ^{bc}	4.5 ^a	95.4
Clone 1820-AS	68.2 ^b	6.8 ^a	4.6 ^a	79.5
P-value	0.047	0.001	<0.001	ns

Means followed by the same letter within a column are not significantly different at $P < 0.05$ (Tukey's test). Abbreviation ns = not significantly different. -BS = samples before storage, -AS = samples after 4 weeks of storage at 15 °C and 85% RH.

7.3.4 Cytotoxicity tests of sweetpotato extracts

The effect of sweetpotato extracts on cell viability of MDA-MB-231 cells and HT-29 cells was investigated using the MTT assay. The sweetpotato extracts at 1000 $\mu\text{g/mL}$ appeared to be cytotoxic on MDA-MB-231 breast cancer cells (Figure 7.4). Based on this outcome, the test on HT-29 cells used two sweetpotato concentrations: 10 and 100 $\mu\text{g/mL}$, and concentrations were not cytotoxic (Figure 7.5).

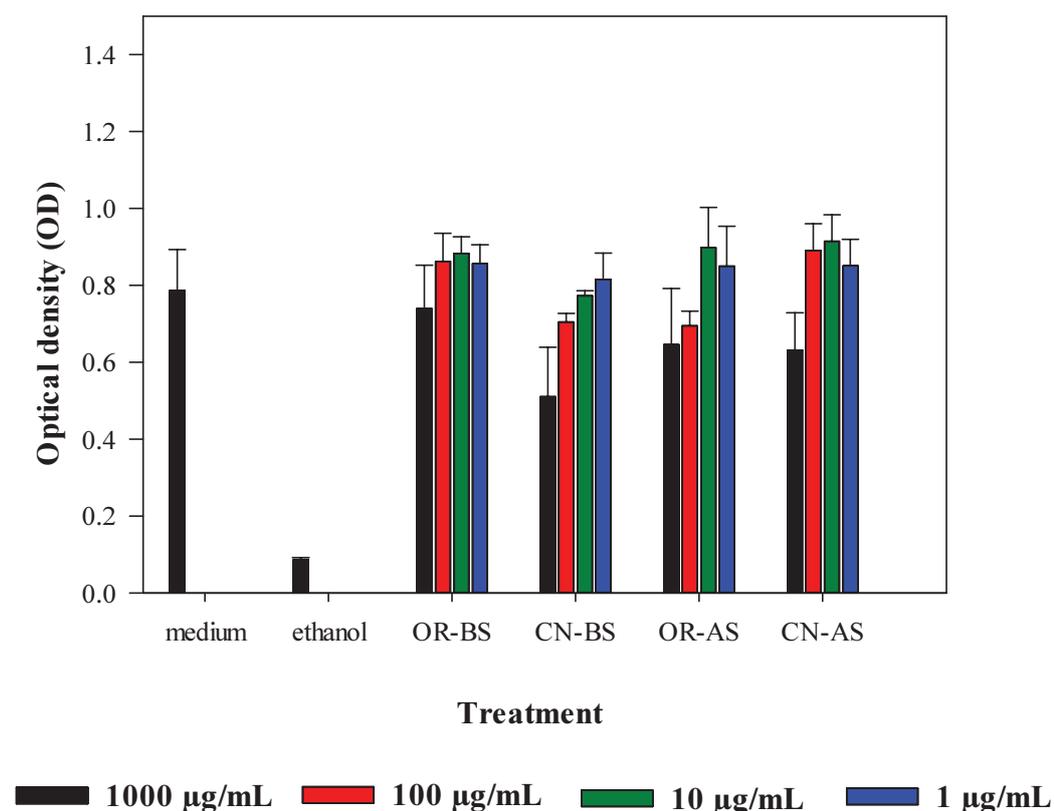


Figure 7.4: Cell viability MDA-MB-231 cells subjected to different treatments: medium only (negative control), blank (DMSO + medium), maltose, sweetpotato extracts: OR = 'Owairaka Red' and CN = 'Clone 1820'; -BS = samples before storage, -AS = samples after 4 weeks of storage at 15 °C and 85% RH. Error bars represent standard error of the mean. Each treatment was measured in triplicate.

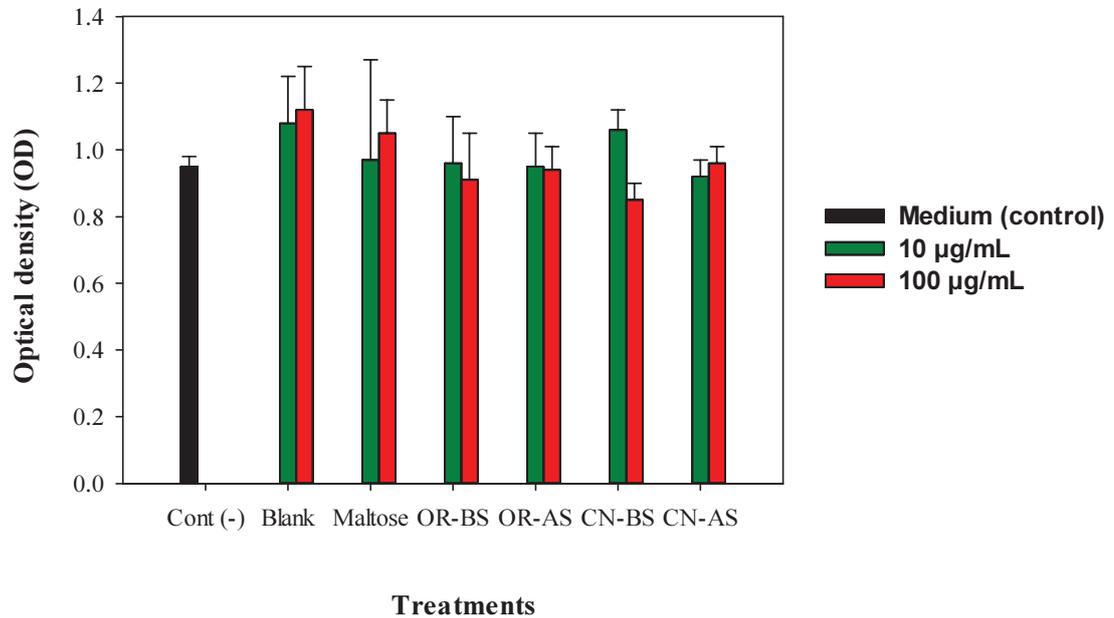


Figure 7.5: Cell viability of HT-29 cells subjected to different treatments: medium only (negative control), blank (DMSO + medium), maltose, sweetpotato extracts: OR = ‘Owairaka Red’ and CN = ‘Clone 1820’; -BS = samples before storage, -AS = samples after 4 weeks of storage at 15 °C and 85% RH. Error bars represent standard error of the mean. Each treatment was measured in triplicate.

7.3.5 Effects of sweetpotato extracts on basal (without LPS stimulation) IL-8 production in HT-29 cells

The basal IL-8 production in HT-29 cells ranged from 221.1 to 443.5 pg/mL (Figure 7.6). The concentration in the two controls were 165.6 and 283.3 pg/mL in medium (negative control) and glucosamine (positive control) respectively. Compared with the negative control, sweetpotato extracts from ‘Owairaka Red’ and ‘Clone 1820’ roots before storage stimulated IL-8 production except for the concentration of 1 µg/mL in ‘Clone 1820’. Similarly, the Blank (DMSO + medium) at 100 µg/mL also enhanced IL-8 production suggesting that there was something in the extract that was acting as an IL-8 stimulant. Maltose did not induce basal IL-8 production.

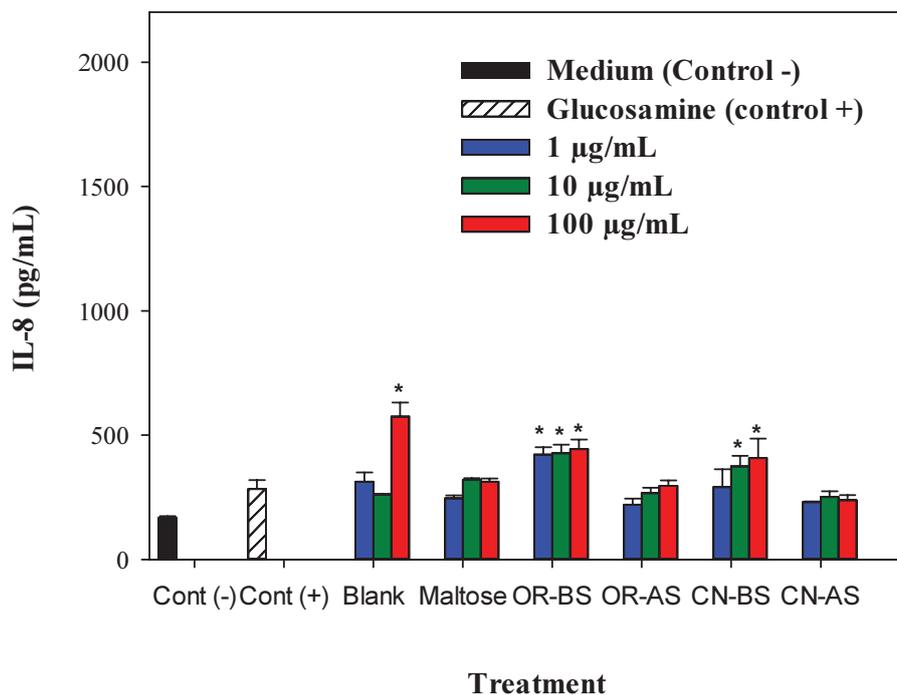


Figure 7.6: Basal Interleukin 8 (IL-8) production in HT-29 cells subjected to different treatments: medium only (negative control), glucosamine (positive control), blank (DMSO + medium), maltose, and sweetpotato extracts. OR = ‘Owairaka Red’ and CN = ‘Clone 1820’; -BS = samples before storage, -AS = samples after 4 weeks of storage at 15 °C and 85% RH. Error bars represent standard error of mean. *denotes significant differences ($P < 0.05$) compared to the negative control (medium only).

7.3.6 Effects of sweetpotato extracts on IL-8 production stimulated with LPS in HT-29 cells

As expected, LPS induced IL-8 production at levels higher than basal production. None of the sweetpotato extracts inhibited production of IL-8 (Figure 7.7). Extracts from ‘Owairaka Red’ before storage at a concentration of 1 µg/mL significantly ($P < 0.05$) induced additional IL-8 production compared to medium (negative control). In addition, low concentrations (1 and 10 µg/mL) of maltose and CN-BS extracts also induced IL-8 production but this was not statistically different from the negative or positive control. Surprisingly, glucosamine (positive control) did not reduce IL-8 production. There was no clear dose response, and there was high variation between replicates.

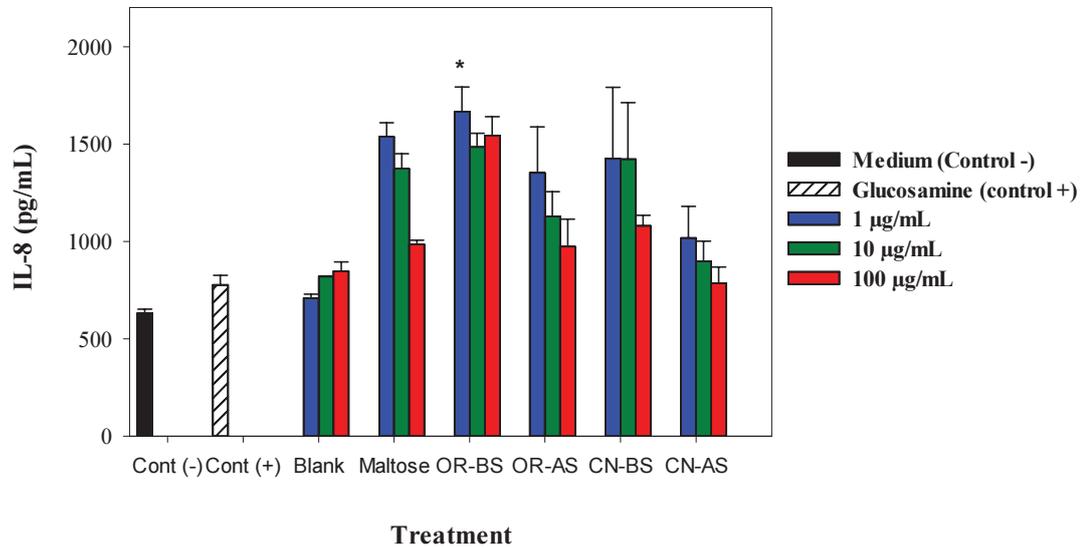


Figure 7.7: Interleukin 8 (IL-8) production 24 h after LPS (1 µg/mL) stimulation. HT-29 cells were subjected to different treatments: medium only or (negative control), glucosamine (positive control), blank (DMSO + medium), maltose and sweetpotato extracts. OR = ‘Owairaka Red’ and CN = ‘Clone 1820’; -BS = samples before storage, -AS = samples after 4 weeks of storage at 15 °C and 85% RH. Error bars represent standard error of mean. *denotes significant differences ($P < 0.05$) compared to the negative control (medium only).

7.3.7 Effects of sweetpotato extracts on IL-8 production in HT-29 cells stimulated with LPS (pre-incubation)

HT-29 cells were pre-treated with sweetpotato extracts and the two controls for 24 h before stimulation with LPS (1 µg/mL). The basal IL-8 ranged from 96 to 131 pg/mL, high in CN-BS extracts and low in negative control. Statistically no differences ($P > 0.05$) were observed in basal IL-8 production among the treatments (Figure 7.8). After stimulation with LPS, none of the extracts reduced IL-8 production, and a positive control was not effective in reducing the IL-8 production. IL-8 production in cells treated with OR-BS extracts was higher than the positive control, but not significantly different from the other treatment.

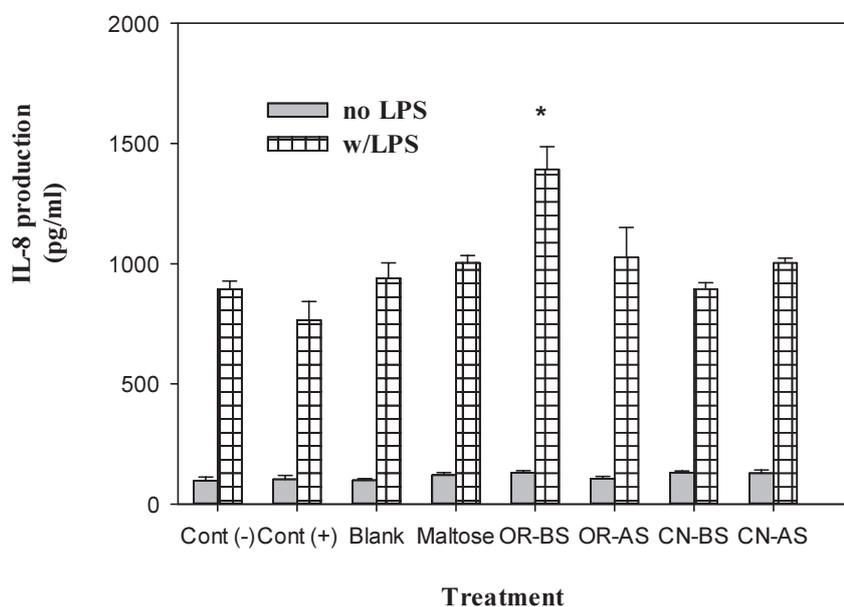


Figure 7.8: Interleukin 8 (IL-8) production 24 h after LPS (1 $\mu\text{g}/\text{mL}$) stimulation. HT-29 cells were pre-incubated with different extracts: medium only (negative control), glucosamine (5 mM) (positive control), maltose and sweetpotato extracts (10 $\mu\text{g}/\text{mL}$) for 24 h, then stimulated with LPS (1 $\mu\text{g}/\text{mL}$) for 24 h. -BS = samples before storage, -AS = samples after 4 weeks of storage at 15 $^{\circ}\text{C}$ and 85% RH. Error bars represent standard error of the mean. *denotes significant differences ($P < 0.05$) compared to the negative control (medium only).

7.4 Discussion

The possibility of extracts from ‘Owairaka Red’ and ‘Clone 1820’ sweetpotatoes showing anti-inflammatory properties by reducing production of cytokine Interleukin 8 (IL-8) was determined in HT-29 cells stimulated with lipopolysaccharide (LPS). Glucosamine (positive control) that was supposed to inhibit the production of the IL-8 was not effective, which makes the interpretation of the results from this study limited. Yomogida et al. (2008) showed that glucosamine concentrations ranging from 2 to 10 mM were effective in suppressing IL-8 production in HT-29 cells. A concentration of 5 mM was used in this study, which is within the effective range reported by Yomogida et al. 2008. Therefore, it is unlikely that the ineffectiveness of glucosamine in this study was due to low concentration. LPS dose of 1 $\mu\text{g}/\text{mL}$ used in this study was tenfold higher than the LPS concentration glucosamine has been reported to suppress

(Yomogida et al., 2008). It is possible glucosamine was ineffective due to the high dose of LPS used. Despite this limitation, there are some trends that were observed which are worth discussing, these are: sweetpotato extracts from cooked ‘Owairaka Red’ and ‘Clone 1820’ did not show anti-inflammatory properties, as production of IL-8 was not reduced in cells treated with sweetpotato extracts.

- OR-BS and CN-BS extracts induced additional IL-8 production when compared to the negative control.
- Maltose, which was included as a standard, induced additional IL-8 production, and the trend showed an inverse relationship between maltose concentration and IL-8 production (Figure 7.7).

These results are contrary to the findings of other researchers who reported that (raw) sweetpotatoes possess anti-inflammatory properties. For instance Grace et al. (2014) found a reduction in reactive oxygen species (ROS) in human neuroblastoma (SH-SY5Y) cells treated with 100 µg/mL sweetpotato extracts from different cultivars: NCPUR06-020, Covington, Yellow Covington, and NC07-847. *In vivo* model, Wang et al. (2010) showed that extracts from purple coloured sweetpotato inhibited production of LPS-induced cyclooxygenase (cox-2) and inducible Nitric Oxide Synthase (iNOS). These two studies used sweetpotato extracts from uncooked sweetpotatoes. One of the differences between cooked and uncooked sweetpotato is the presence of maltose in the cooked sweetpotatoes (Picha, 1985b; Morrison et al., 1993). Therefore, it can be suggested that the ineffectiveness of the sweetpotatoes extracts to reduce IL-8 production was due to the maltose presence. This hypothesis is supported by the results of the maltose standard, which on its own also induced additional IL-8 production (Figure 7.7). In addition, other studies have shown that increased concentrations of glucose in cell media increase the production of IL-8. For example, Urakaze et al. (1996) found an increase in IL-8 production in human aortic and umbilical vein endothelial cells treated with different concentration of glucose: 4, 9.5, 20.5 and 42.8 mM, which translates to 0.72, 1.71, 3.69 and 7.66 mg/mL. Similarly, Kinoshita et al. (2006) found increased IL-8 production in human umbilical vascular endothelial cells (HUVECs) treated with 3 and 4 mg/mL of glucose. It appears unlikely that the concentration of glucose was the cause for the increased IL-8 production in cells treated with OR-BS and CN-BS extracts. The concentrations of glucose per well ranged from

1.48 to 7.86 $\mu\text{g/mL}$, which is lower than the concentrations that have been reported in literature to induce IL-8 production. To determine whether there was correlation between the sugar concentration in the extracts and the measured IL-8 concentration, the sugar concentrations of sweetpotato extracts at 100 $\mu\text{g/mL}$ were correlated with their corresponding IL-8 concentration (Figure 7.9)). There was a weak positive ($r = 0.31$, $P > 0.05$) correlation between maltose and IL-8 production, while glucose ($r = -0.68$, $P = 0.016$) and fructose ($r = -0.82$, $P = 0.001$) showed a negative correlation with IL-8 concentration (Figure 7.9 A-C). Sweetpotato extracts with the highest glucose concentration had low IL-8 production, which is contrary to the theory that high glucose concentration induces IL-8 production; and suggests that the increased IL-8 production in HT-29 cells treated with OR-BS and CN-BS extracts was not due to glucose or fructose concentration.

Human cells grow well in extracellular fluid with an osmolarity value between 280 - 320 mOsm/L (Sherwood, 1993). Several researchers have reported increased IL-8 production in cells exposed to both hyper-osmotic and hypo-osmotic conditions. Németh et al. (2002) found that hyper-osmotic stress stimulated IL-8 production in the HT-29 and Caco-2. In another study, exposure of Caco-2 cells to hyper-osmotic (450 mOsm/L) and hypo-osmotic (150 mOsm/L) conditions induced production of IL-8 (Hubert et al., 2004). By adding sweetpotato extracts to the cell media, the osmolarity of the DMEM could be modified. To explore if osmolarity of the medium plus the sweetpotato extracts contributed to increased IL-8 production, the osmolarity of the cell medium (DMEM) plus sweetpotato extracts was estimated by calculating the osmolarity of the main sugars present in the extracts plus the osmolarity of the cell medium (DMEM). The medium osmolarity was 324 mOsm/L, and increased to 324.3 mOsm/L in the presence of sweetpotato extracts (data not shown). The change in the medium osmolarity was negligible, it is unlikely that osmolarity of the media contributed to increased IL-8 production.

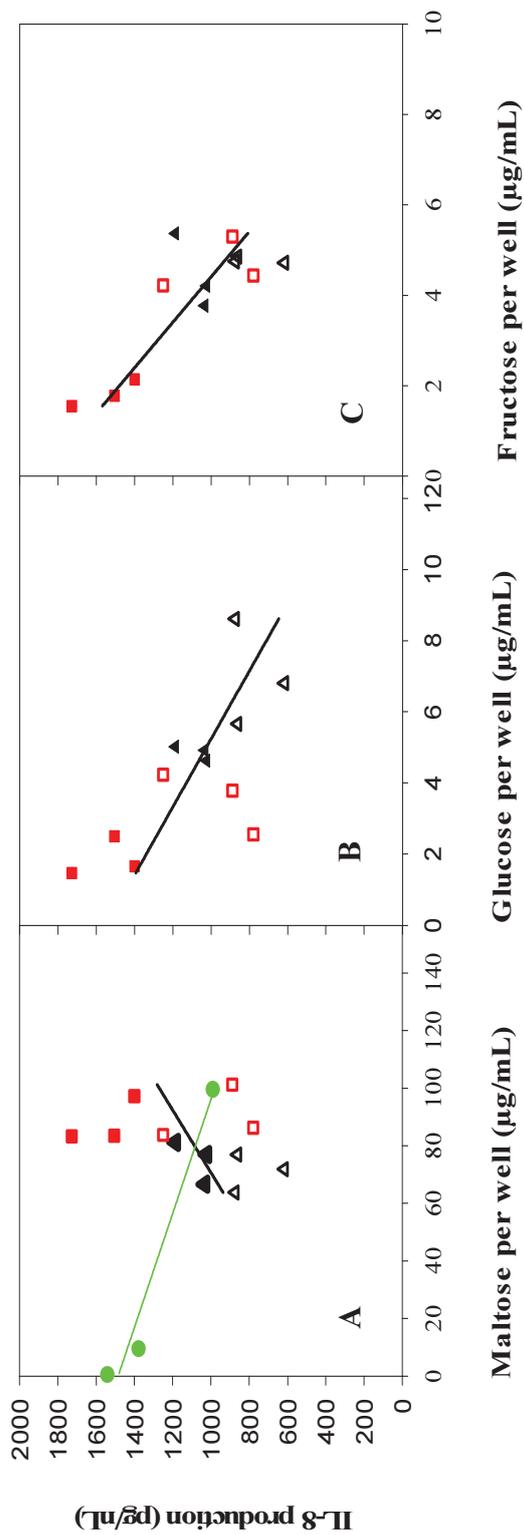


Figure 7.9: Relationship between IL-8 production and concentration (µg/mL) of Maltose (A), Glucose (B) and Fructose (C) in ‘Owairaka Red’ (square symbols) and ‘Clone 1820’ sweetpotatoes, sampled before storage (closed symbols) and after storage measurements (open symbols). Maltose standard fitted line (green colour)

Kenny et al. (2013) found that crude and semi-purified potato peel extracts containing glycoalkaloids did not reduce IL-8 production in Jurkat cells, but pure individual glycoalkaloids reduced IL-8 production significantly. Kenny and colleagues suggested that the difference between semi-purified peel potato extracts and individual glycoalkaloids was due to low concentrations of the individual glycoalkaloids in the semi-purified or crude potato extracts. To establish if low concentrations of phenolic compounds were the cause for ineffectiveness in reducing IL-8 production, the concentrations of some of the main phenolic compounds identified in sweetpotato extracts used in this study were compared with the effective doses that have been reported in the literature (Table 7.4).

Chlorogenic acid was found in both 'Owairaka Red' and 'Clone 1820' extracts, and was one of the most abundant phenolic acids with an overall mean of 153 and 783 mg/100 g DW in 'Owairaka Red' and 'Clone 1820' respectively (Table 7.1). Anti-inflammatory properties of chlorogenic acid have been reported *in vitro* using different cell lines: mouse leukemic monocyte macrophage (RAW 264.7) (Hwang et al., 2014), Human Peripheral Blood Mononuclear Cells (PBMC) (Krakauer, 2002) Neutrophils (Chauhan et al., 2011) and human intestinal Caco-2 (Shin et al., 2015). The lowest effective doses reported in the literature ranged from 2 to 708 µg/mL (Table 7.4). In this study, 100 µg/mL of 'Clone 1820' extracts supplied 0.81 µg of chlorogenic acid per well. This concentration is 2.5 to 864 times lower than the effective doses reported in Table 7.4. For 'Owairaka Red' sweetpotato extract, 100 µg/mL supplied about 0.16 µg per well, which are about 8 % of the effective dose reported by Krakauer (2002) and less than 1% of the effective doses reported by other researchers (Chauhan et al., 2011; Hwang et al., 2014; Shin et al., 2015). If chlorogenic acid is the main phenolic acid responsible for anti-inflammatory activity in sweetpotato, it can be suggested that a testing regime needs to be developed that will allow screening of extracts at higher concentration. We were quite cautious in using a maximum of 100 µg/mL in this experiment, since preliminary studies on cytotoxicity showed that sweetpotato extracts at concentration a 1000 µg/mL were toxic to breast cancer cells MDA-MB-231. Sweetpotato extract concentrations higher than 100 µg/mL could be considered in future studies.

The caffeoyl derivatives: 4,5 and 3,5-dicaffeoylquinic acids were also present in significant concentrations both in ‘Owairaka Red’ and in ‘Clone 1820’ sweetpotato extracts. A 100 µg/mL sweetpotato extract supplied 0.1 and 0.7 µg (4,5 diCQA) and 0.1 and 0.8 µg (3,5 di CQA) in ‘Owairaka Red’ and ‘Clone 1820’ respectively. Park et al. (2009) showed a significant reduction in nitric oxide production in mouse leukemic monocyte macrophage (RAW 264.7) cells treated with 4,5 (620 µg/mL) and 3,5 (392 µg/mL)-dicaffeoylquinic purified from roots of *Aconitum koreanum*. These effective doses are 490 to 886 times higher than the concentration present in 100 µg/mL of ‘Clone 1820’ extracts, and 3500 to 6200 times higher than levels in ‘Owairaka Red’ extracts (Table 7.4).

Caffeic acid was also found in sweetpotato extracts used in this study. Búfalo et al. (2013) and Park et al. (2009) have studied the anti-inflammatory properties of caffeic acid. They found that caffeic acid concentrations of 5 and 372 µg/mL were effective in reducing nitric oxide production in RAW 264.7 cells. Caffeic acid concentrations in 100 µg/mL sweetpotato extracts were 0.007 and 0.06 µg/well in ‘Owairaka Red’ and ‘Clone 1820’ respectively. These concentrations are 83 to 714 times lower than the lowest effective dose reported by Búfalo et al. (2013).

Cyanidin 3-O-glucoside chloride and peonidin-3-glucoside inhibited IL-8 and IL-6 production in human umbilical cell vein endothelial cells stimulated with CD040 (Shin et al., 2015). The lowest effective dose was 0.5 µg/mL (Table 7.4). This dose is 2.63 times higher than the total anthocyanin amount supplied by 100 µg/mL ‘Clone 1820’ extracts, and 25 times higher than the concentration supplied by 100 µg/mL ‘Owairaka Red’ extracts.

From the comparison of lowest effective doses and concentration of phenolic compounds in sweetpotato extracts (Table 7.4), it is apparent that the concentrations of the polyphenols associated with anti-inflammatory properties were low in ‘Clone 1820’ and ‘Owairaka Red’ extracts. Therefore, it can be suggested that the ineffectiveness to reduce IL-8 production could be related to low concentration of the phenolic compounds.

Table 7.4: Phenolic concentration in sweetpotato extracts used in the study compared with the effective doses reported in the literature for different health properties

Compound	Concentration in sweetpotato extracts (100 µg/well)		Lowest effective doses against inflammatory modulators (µg/ml)	Cell line	Response	References	Effective concentration reported in the references divided by concentration in OR and CN	
	OR	CN					OR	CN
Chlorogenic acid	0.16	0.81	700	RAW 264.7	NO cyclooxygenase-2-inducible	(Hwang et al., 2014)	4375	864
			2	Human PBMC	TNF α , IFN γ	(Krakauer, 2002)	12	2.47
			20	Neutrophils	TNF α	(Chauhan et al., 2011)	125	24.6
			354	Caco2 cells	IL-8	(Shin et al., 2015)	2213	427
			708	Caco2 cells	IL-8	(Shin et al., 2015)	4425	874
Caffeic acid	0.007	0.06	372	Raw 264.7	Nitric oxide	(Park et al., 2009)	53143	6200
			5	RAW 264.7	Nitric oxide	(Búfalo et al., 2013)	714	83
4,5 diCQA	0.10	0.70	620	Raw 264.7	Nitric oxide	(Park et al., 2009)	6200	886

Table 7.4 (Continued)

Compound	Concentration in sweetpotato extracts ($\mu\text{g/mL}$)		Lowest effective doses against inflammatory modulators ($\mu\text{g/mL}$)	Cell line	Response	References	Effective concentration reported in the references divided by concentration in OR and CN	
	OR	CN					OR	CN
3,5 diCQA	0.11	0.80	392	Raw 264.7	Nitric oxide	(Park et al., 2009)	3564	490
Cyanidin-3-glucoside	0.02*	0.19*	0.5	Human umbilical vein	IL-8 IL-6	(Xia et al., 2007)	25	2.63
Peonidin 3-glucoside	0.02*	0.19*	0.5	Human umbilical vein	IL-8 IL-6	(Xia et al., 2007)	25	2.63

*Total anthocyanin concentration $\mu\text{g/well}$

CN = Clone 1820

OR= Owairaka Red

7.5 Conclusion

Extracts from cooked sweetpotatoes did not show anti-inflammatory properties while extracts from cooked freshly harvested sweetpotatoes induced additional IL-8 production. With the absence of an effective result from the glucosamine (positive control), the results from this study are inconclusive. However, it can be concluded that the concentrations of the phenolic compounds in the sweetpotato extracts were too low to inhibit IL-8 production. A repeat of this bioassay with higher sweetpotato dose, an effective positive control, and different biomarkers would be necessary, in order to understand why extracts from freshly harvested sweetpotatoes induced additional IL-8 production.

8 General discussion and conclusions

8.1 Introduction

The ultimate goal of this work was to determine the potential of postharvest techniques to extend sweetpotato storage life without compromising phytochemical content. During the storage of sweetpotatoes, root quality may be reduced due to weight loss, sprouting and rots. There are also less obvious quality losses relating to changes in nutritional compounds. Several studies have been conducted with the aim of maintaining either physiological quality or nutritional compounds. However, in most cases, these two aims have not been combined. The work reported here looked at root quality using both internal and external measures. The internal quality measures included carotenoids, phenolic compounds, sugars, and flesh colour whilst the external variables were weight loss, sprouting and rot incidence. Postharvest treatments investigated in this work were hot water dipping (with or without coating) and ethylene (with or without 1-MCP) treatments. The key findings and the implications of these technologies for extending sweetpotato storage, retaining phytochemicals, and the potential impact on human health will be discussed in this chapter.

8.2 Extension of storage life of sweetpotatoes

8.2.1 Hot water treatment and coating

The effective water temperature range for reducing sweetpotato sprouting, with minimal root damage, was successfully defined using ‘Owairaka Red’ as a model cultivar. Sweetpotatoes could be safely treated with 51 °C for 11 minutes with minimal root damage. Interestingly, there appeared to be a trade-off between increasing sprout control and increasing incidence of rots, as time and temperature were varied. The underlying reason for this trade-off presumably relates to skin damage as dipping temperatures and times are increased. Some confirmation for this deduction comes from the increasing weight loss and water vapour permeance seen after hot water dipping (HWD). The results also demonstrated that the addition of an edible coating to HWD treated sweetpotato roots could be a strategy for avoiding the HWD-induced weight loss. To the best knowledge of this author, this is the first time the use of HWD and coating has been reported in sweetpotato. The potential for coating applications is limited by the associated reduced efficacy of HWD in sprout control (Section 4.4.3.1). However, the effect is cultivar dependent; coating increased sprouting in ‘Owairaka

Red' but not in 'Clone 1820'. Therefore, HWD and coating might be more practical in sweetpotato cultivars that are less prolific in sprouting.

Increased sprouting in coated 'Owairaka Red' roots was unexpected. It is thought that ethylene plays a role in sweetpotato sprout initiation (Cheema et al., 2013). Internal ethylene concentrations of 'Golden Delicious' apples coated with Nutri-save were higher than those of uncoated fruit (Lau & Yastremski, 1991). If edible coating also increases internal ethylene concentration in sweetpotato, this could help explain the increased sprouting in coated sweetpotato roots. Attempts to measure the ethylene production in sweetpotato roots coated with different types of edible coatings were undertaken. Due to the unavailability of 'Owairaka Red' sweetpotato roots at the time of the experiment, roots of 'Clone 1820' were used. This experiment failed to give evidence for a relationship between coating and ethylene production. It is possible that 'Clone 1820' roots are not a good model for such experiments as they are less prolific at sprouting compared to 'Owairaka Red', and therefore did not display a differential ethylene concentration after coating. Alternatively, it is possible that ethylene production is not a good measure of internal ethylene concentration in sweetpotatoes. In apples and cantaloupe melon, internal ethylene concentration has been measured by directly withdrawing air from the fruit cavity (Lyons et al., 1962; Jung & Watkins, 2014). Sweetpotato roots are dense compared to apples and have no distinct cavity, so this method may not be applicable for internal ethylene measurements in sweetpotato. Other methods such as cannulation and fitted chambers have been used in the measurement of internal gases in bananas (Banks, 1983). In this study, the cannulation method was used for assessing internal oxygen and carbon dioxide modifications in coated roots, and there were no indications of rots near the area where the cannula was inserted. Employing such methods, combined with the use of GC, could be an approach for future measurements of all three gases: oxygen, carbon dioxide and ethylene.

Internal gas modification was measured up to 14 days after coating. During this time, there was no indication of carbon dioxide build up in roots coated with 5 or 10 % carnauba wax, whilst roots coated with 20 % carnauba wax and whey protein concentrate showed a slight increase in internal carbon dioxide and a drop in oxygen. It is not known whether this condition gets worse with storage progression, to the point of

causing anaerobic conditions. Under anaerobic conditions, fruits and vegetables accumulate ethanol, acetaldehyde, ethyl acetate, and other volatiles, leading to off-flavours (Baldwin et al., 1995; Seehanam & Boonyakiat, 2010). It has been reported that alcoholic and other off-flavours occur in sweetpotato roots kept in modified air, containing less than 7 % oxygen and carbon dioxide levels above 10% (Chang & Kays, 1980; Delate et al., 1990). In this study, none of the tested coating formulations caused a drop in oxygen below 7%. Despite this is encouraging, it is suggested that the internal gas atmosphere in coated roots should be measured over a longer period to validate this result. In addition, consumer acceptability of coated sweetpotatoes should also be determined.

The present study has demonstrated the possibility of extending the storage life of sweetpotato roots at elevated temperatures by using a combined treatment of HWD and an edible coating. The application of this method in developing countries would be a challenge, with regard to maintaining water temperatures during immersion, as it would require basic equipment with a reliable energy source.

8.2.2 Ethylene and 1-MCP treatments

This work confirms previous findings by Amoah (2013) and Cheema et al. (2013), that ethylene is a potential sweetpotato sprout inhibitor. Unfortunately, ethylene treatment was also associated with tissue splitting, flesh darkening, high rot incidence and high weight loss. The magnitude of these responses differed between cultivars. Differences in cultivar responses could be due to differences in ethylene sensitivity. Ethylene-induced darkening was only observed in cooked samples. During cooking plant cells release iron and phenolic compounds, and when these two compounds combine they form a ferrous-chlorogenic acid complex (colourless) which, on exposure to air, oxidises to form a ferri-dichlorogenic acid complex (bluish-grey in colour) (Friedman, 1997; Wang-Pruski & Nowak, 2004).

Ethylene-induced negative effects are a major drawback to ethylene's potential use as a sweetpotato sprout inhibitor. It is well established that ethylene-induced responses can be blocked by the application of 1-MCP (Sisler & Serek, 2003). Until this study, the combined effects of 1-MCP and ethylene treatments on sweetpotato flesh colour were not known. Neither was the consumer acceptability of ethylene-treated sweetpotatoes

determined. A single dose of $1 \mu\text{L L}^{-1}$ 1-MCP was not effective at reducing ethylene-induced responses (Section 5.4.5 and 6.3.5). It is possible that a single dose of $1 \mu\text{L L}^{-1}$ 1-MCP was not of a high enough concentration to bind all ethylene receptors present at the time of treatment, thus ethylene was still being perceived. Therefore use of a higher 1-MCP dosage could be considered in the future. The actual 1-MCP concentration achieved in the treatment barrel was not measured. Although the delay in ripening of green tomatoes included in the treatment barrels verified the presence of 1-MCP, it does not prove that the intended $1 \mu\text{L L}^{-1}$ was achieved. It is possible that the 1-MCP concentration in the barrels was lower than the intended concentration of $1 \mu\text{L L}^{-1}$. 1-MCP concentrations can be measured using gas chromatography and quantified using isobutylene as a standard (Blankenship & Dole, 2003; Chope et al., 2007). Using Glass Porapak Q GC columns, both 1-MCP and ethylene concentrations can be analysed from the same gas sample (Blankenship & Dole, 2003). Other studies have shown that the presence of ethylene during 1-MCP application reduces 1-MCP efficacy (Macnish et al., 2012). Sweetpotatoes produce low levels of ethylene, so it was assumed that the ethylene concentration in the barrel was minimal. In future studies, measurements of both 1-MCP and ethylene during sweetpotato 1-MCP treatment should be considered.

Apart from the dose effect, the time taken from harvest to 1-MCP treatment may affect 1-MCP efficacy (Watkins, 2006). Generally, the shortest time between harvest and 1-MCP treatment is recommended, to achieve maximum benefits of 1-MCP. Unlike most fruits and vegetables, sweetpotato have to be cured before storage. There is no published information on the 'optimum time' for 1-MCP treatment in sweetpotatoes. In this study, sweetpotatoes were treated with 1-MCP after curing. In another study aimed at investigating whether 1-MCP treatment could reduce flooding-induced sweetpotato breakdown, 1-MCP treatment was applied at three different times: before curing, during curing and after curing. 1-MCP treatment applied during curing was more effective in reducing sweetpotato breakdown than 1-MCP treatment before curing and after curing (Arthur Villordon, Pers. Com.). In onions, 1-MCP treatments applied both before and after curing were effective in suppressing sprout growth (Downes et al., 2010). It is possible that 1-MCP could have been more effective in controlling the ethylene-induced responses observed in this study if it was applied before or during curing. Different 1-

MCP application times, followed by exposure to ethylene may be explored in the future in order to gain insight on the best time for 1-MCP treatment in sweetpotatoes.

When sweetpotato roots were given repeated doses of 1-MCP and stored in continuous ethylene flow-through systems, most of the ethylene-induced responses except sprouting inhibition were either delayed or reduced (Chapter 6). This implies that, if continuous ethylene application is to be considered as a treatment for sprout control in sweetpotatoes, the other negative effects can be mitigated by using repeated doses of 1-MCP. In this study, sweetpotato roots were treated with $1 \mu\text{L L}^{-1}$ 1-MCP at 0, 4, and 8 days of storage, and the sweetpotato roots showed ethylene insensitivity for only 2 weeks, thereafter they regained ethylene sensitivity as evidenced by root splitting. This suggests that sweetpotatoes have a high receptor turnover, details of which will be discussed later (Section 8.3). If the hypothesis of high receptor turnover in sweetpotato is correct, then ethylene-induced negative responses can only be reduced with on-going 1-MCP application throughout the storage period. Reapplying 1-MCP treatments every 3 to 4 days, as in this study, would be difficult for packhouses. Therefore, it may be necessary to adopt use of a slow release 1-MCP source such as 1-MCP in cyclodextrin, which has been tested in cut flowers (Seglie et al., 2011; Seglie et al., 2012). Alternatively, a 1-MCP tablet with a release and fan system as described by Nock and Watkins (2013) for apples, could be used. Ethylene application requires sophisticated equipment; this may only be feasible for farmers in developed countries. In developing countries, use of ethylene analogues such as calcium carbide (Sy & Wainwright, 1990) could be an alternative option for continuous ethylene supply. However, this has to be verified through research, and it must be noted that calcium carbide is associated with health risks (Asif, 2012).

8.3 Sweetpotato responses to ethylene manipulation using 1-MCP

Three key observations have been made from this work on sweetpotato responses to ethylene manipulation using 1-MCP.

- 1-MCP effects on ethylene-induced responses were transitory
- Different sweetpotato tissues responded differently to 1-MCP
- Multiple 1-MCP treatments reduced ethylene production and flesh darkening but did not inhibit ethylene effects on sprout inhibition.

Recovery of plant ethylene sensitivity after 1-MCP treatment has been attributed to synthesis of new ethylene receptors after the 1-MCP treatment (Feng et al., 2004; In et al., 2013). With repeated doses (2 to 4 times) of 1-MCP, 1-MCP has shown prolonged suppression of ethylene responses in apples and pears (Cameron & Reid, 2001; Jayanty et al., 2004; In et al., 2013; Lu et al., 2013; Nock & Watkins, 2013). In this study, after 3 doses of $1 \mu\text{L L}^{-1}$ 1-MCP, sweetpotato still responded to ethylene. This leads to a suggestion that sweetpotato may have a rapid turnover of receptors. A question remains as to what the physiological basis for this rapid receptor turnover might be? If we could understand this, then it might help to determine ways to gain the benefit of ethylene for sprout reduction without incurring risks of tissue splitting and weight loss. Rapid receptor turnover may also be increased by the high storage temperature ($25 \text{ }^{\circ}\text{C}$) used in this study. Cameron & Reid (2001) showed that *Pelargonium peltatum* flowers treated with 1-MCP regained ethylene sensitivity faster when stored at high temperature ($25 \text{ }^{\circ}\text{C}$), and more slowly when were stored at low temperature ($12 \text{ }^{\circ}\text{C}$). Apples and pears are normally stored at lower temperatures (0 to $5 \text{ }^{\circ}\text{C}$), so the rate of receptor turnover may not be as high as in sweetpotato. It is possible that if experiments were conducted at a lower temperature, for example $15 \text{ }^{\circ}\text{C}$, which is the recommended storage temperature for sweetpotato, the responses of 1-MCP could have been different. In onions, treatment of cured dry bulbs with $1 \mu\text{L L}^{-1}$ 1-MCP reduced sprout growth in bulbs stored at $4 \text{ }^{\circ}\text{C}$ or $12 \text{ }^{\circ}\text{C}$, but not when stored at $20 \text{ }^{\circ}\text{C}$ (Chope et al., 2007), which also suggest that onions stored at $20 \text{ }^{\circ}\text{C}$ had a higher ethylene receptor turnover.

It is unclear why sweetpotato cortex and pith tissues partially responded to multiple 1-MCP but sprouting in outer tissues did not. Similar results were shown when potatoes were treated with 1-MCP before exposure to continuous ethylene (Prange et al., 2005; Daniels-Lake et al., 2008). Potato fry colour was significantly reduced by 1-MCP but ethylene-induced sprout inhibition was not blocked. Prange et al. (2005) hypothesised that the basis for the differential response of potato tissue to 1-MCP was due to higher ethylene receptor turn over in potato eyes than the cortex. If this hypothesis also applies for sweetpotato then it could explain why ethylene inhibited sprouting despite repeated doses of 1-MCP. Alternatively, the difference could be due to differences in the capacity for 1-MCP metabolism. Huber et al. (2010) suggested that 1-MCP can be metabolised in the plant. If we assume that the cells in the cortex of sweetpotato root are

less active metabolically than outer tissues, then it is possible there was less 1-MCP metabolism in the cortex, leading to reduced darkening.

The number of ethylene receptors regulates plant tissue sensitivity to ethylene (Klee, 2002). A lower number of receptors would result in greater ethylene tissue sensitivity (Hua & Meyerowitz; Hall & Bleecker, 2003), and increased receptor numbers result in the opposite effect (Ciardi et al., 2000). It is possible that the sweetpotato outer tissues have lower receptor numbers than the cortex and the pith. With advances in molecular biology techniques such as tissue printing, which allows analysis of tissue localised gene expression at both messenger ribonucleic acid (mRNA) and protein levels (Varner & Ye, 1994), it will be possible to test this hypothesis in the future. Finally, the differential responses of tissues to 1-MCP could be due to the presence of unique ethylene receptors that are specific to sweetpotato outer tissues which may not be blocked by 1-MCP, but this would be extraordinary.

Cheema et al. (2013) studied the effects of ethylene, aminoethoxyvinylglycine (AVG), and 1-MCP on sweetpotato sprouting. Roots treated with ethylene, 1-MCP or AVG did not sprout after 4 weeks of storage at 25 °C. The authors then hypothesised that while continuous exposure of sweetpotato to exogenous ethylene leads to inhibition of sprout growth, ethylene is also required for sprouting. By applying 1-MCP, the action of ethylene was blocked, while AVG sprout growth inhibition was thought to be due to reduced ethylene production. However, in their study no data on ethylene production was presented. In this study, roots treated with multiple 1-MCP showed reduced ethylene production at 2 and 3 weeks of storage compared to the control, ethylene or single 1-MCP + ethylene treatments. Ethylene biosynthesis in system 1 is under negative feedback regulation, thus perception of ethylene inhibits further synthesis of ethylene (Barry et al., 2000). By blocking ethylene perception with 1-MCP, the negative feedback regulation is lost, resulting in increased ethylene production (McCollum & Maul, 2007). The decreased ethylene production after 1-MCP treatment, as observed in this study, is contrary to the behaviour of the non-climacteric and pre-climacteric stages of climacteric fruits (System 1), and is consistent with the behaviour of climacteric fruits during the ripening phase (System 2). The regulation of these systems is associated with the differential expression of ACO and ACS isoforms (Nakatsuka et al.,

1998; Tassoni et al., 2006), a molecular study looking for isoforms of ACO and ACS in 1-MCP treated sweetpotato would be a logical extension of this study, to understand how 1-MCP reduced ethylene production in sweetpotato.

In a study by Amoah (2013), swapping sweetpotato roots previously stored in ethylene, to air (free of ethylene) resulted in abundant sprouts. Taken together, these results support the hypothesis that ethylene initiates sprouting. A conceptual model (Figure 8.1) is proposed on the role of ethylene in sweetpotato sprouting and other responses. It appears that there are different internal ethylene induction concentration thresholds for different processes. A moderate internal ethylene concentration may be required for lignification and wound periderm formation during sweetpotato curing. Similarly, the moderate levels could be adequate to initiate sprouts. Nevertheless, the levels may not induce high activity of POD and PAL, as no flesh colour darkening was observed. Exposure of sweetpotato roots to ethylene may increase the internal ethylene concentration, resulting in increased sprout initiation, rapid cell expansion, and POD and PAL activities. By blocking ethylene perception using multiple 1-MCP treatments, the ethylene-induced sprout initiation is prevented, and cell expansion is delayed. Ethylene-induced flesh darkening was reduced, but not totally blocked by multiple 1-MCP application. This suggests that with 1-MCP applications, the activities of POD and PAL were not reduced to the levels of the control roots. It is possible that the response of POD and PAL activity to ethylene exposure is fast, as such the darkening observed in the roots treated with multiple 1-MCP + ethylene could have occurred during the first days of the storage in ethylene, before the roots were given a second and third dose of 1-MCP.

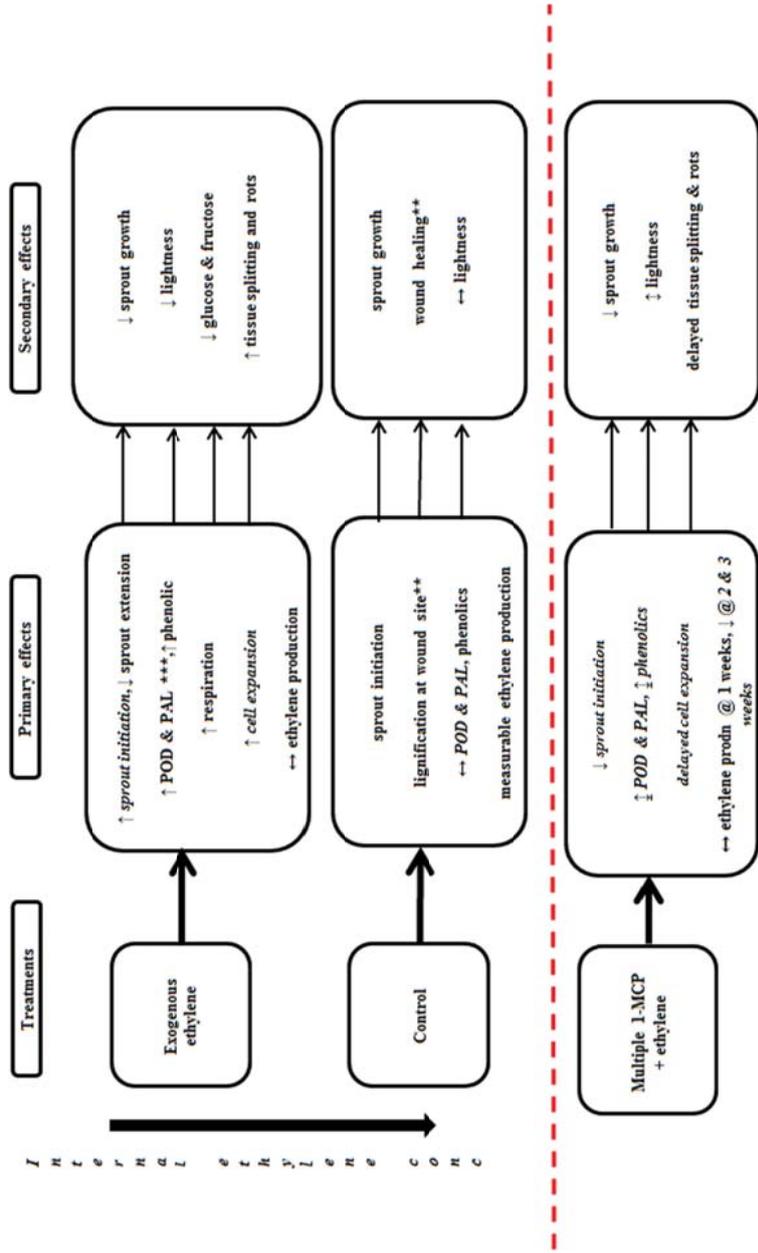


Figure 8.1: Schematic chart showing the responses of sweetpotato roots to exogenous ethylene. An asterisk presents data from other studies: ******(St. Amand & Randle, 1991) *******(Buescher et al., 1975). Arrows (↑) shows an increase and (↓) a decrease, and no effect (↔). Intermediate response to that of the control and ethylene treatments (↕). When sweetpotatoes were treated with a single 1-MCP + ethylene treatment, ethylene-induced responses dominated, and in roots with a single 1-MCP + air treatment the responses were similar to those of the controls (data not included in the chart). Responses in italics are based on assumptions.

8.4 Phytochemical changes and human health

8.4.1 β -carotene

This work has shown that hot water treatment (with or without coating) (Section 4.4.3.4) and ethylene treatment (with or without 1-MCP treatment) (Section 5.4.10) do not affect the carotenoid concentration in ‘Clone 1820’ sweetpotato roots. Thus, these treatments can be applied without negative effects on β -carotene concentration. It was clear from the results in section 4.4.3.4.1 and 5.4.10 that β -carotene concentration declined during storage. The reduction in β -carotene concentration during storage has an implication on the sweetpotato daily intake required to meet the recommended daily retinol activity. While consumption of 100 g freshly harvested ‘Clone 1820’ sweetpotato roots could supply 536.7 RAE, the same serve will supply just 363 RAE after 8 weeks of storage. The recommended daily active equivalent for children below 13 years ranges from 300 to 500 RAE (Institute of Medicine. Food and Nutrition Board, 2001). That implies that a serve of 100 g of ‘Clone 1820’ sweetpotato will meet 75 to 100 % of the daily retinol requirement for children under 13 years old, so regardless of the decline in β -carotene concentration during storage ‘Clone 1820’ is still a good source of provitamin A.

In nature, β -carotene is mostly present as all trans β -carotene, but thermal processing may cause isomerisation of β -carotene from trans- to cis- forms (Chandler & Schwartz, 1988; Britton, 1995; Zepka & Mercadante, 2009; Knockaert et al., 2013). The formation of cis- β -carotene isomers is undesirable because it reduces the provitamin activity and is less available than trans- β -carotene (Britton, 1995). The concentration of cis isomers was not quantified in this study, while the separation of trans- and cis- β -carotene isomers is possible by using a stationary phase C 30 HPLC column (Emenhiser et al., 1995; Kimura et al., 2007), for this study a C 18 HPLC column was used. The quantity of trans to cis- β -carotene isomerisation is related to the heat and length of treatment (Chandler & Schwartz, 1988). Chen et al. (1994) studied thermo-isomerisation of α and β -carotene in a model system, and found that isomerisation of both α and β -carotene was minimal at temperatures of 50 and 100 °C for 30 min. This suggests that the temperature being recommended from this study, of 51 °C for 11 minutes, may not cause isomerisation.

8.4.2 Phenolic acids and anthocyanin

It is of importance that the levels of bioactive compounds are maintained or increased from harvest to consumption. This work has shown for the first time that HWD increases the total phenolic content (Section 3.3.5) and some of the phenolic acids and anthocyanins (Section 4.4.3.6) in sweetpotato. However, the increased polyphenol concentration was short lived; up to 2 weeks of storage. Thus, the HWD benefit of increased polyphenol concentration would only be beneficial when applied 2 weeks before consumption, not at the beginning of the storage period. Sweetpotatoes are generally stored without washing. Before packing the roots for retail, the roots are brushed or washed to remove excess soil. Thus, hot water treatment could be used as part of this cleaning process, consequently enhancing polyphenols. However, the immersion time of 11 min is not easy to integrate into a processing line. It would probably require a batch treatment approach, followed by rapid heat removal, which could be difficult with large product volumes.

Ethylene application increased chlorogenic and 3,5-dicaffeoylquinic acids concentrations (Section 5.4.7). The study showed that ethylene acted differently on individual phenolic acids and the effects were cultivar dependent. The cultivar variation may be related to differences in ethylene sensitivity. Increased concentrations of chlorogenic and 3,5-dicaffeoylquinic acids could be regarded as positive benefits, considering that both are associated with antioxidant activity. However, they have a negative impact on the flesh colour of cooked sweetpotato. Nevertheless, flesh darkening was not severe enough to impact on the consumer acceptability of ethylene-treated samples. That implies that ethylene-treated samples will still be acceptable to consumers, regardless of the flesh darkening.

8.4.3 Health properties

The antioxidant activity (Section 3.3.5) and anti-inflammatory properties (Chapter 7) of the sweetpotato cultivars used in this study were also investigated. With the presence of both carotenoids and phenolic compounds in 'Clone 1820', it was expected that 'Clone 1820' extracts would show a higher inhibition of IL-8 production than 'Owairaka Red' extracts. However, this trend was not observed in this study. Extracts from neither 'Owairaka Red' nor 'Clone 1820' showed anti-inflammatory properties. The assay used for extraction was not good at recovering carotenoids, as after freeze drying it was

noticed that some carotenoids were stuck on the freezer bags. That means the extracts that were used for the inflammation assay were rich in phenolics but perhaps did not contain differential amounts of carotenoids. The concentrations of the sweetpotato extracts tested were highly diluted. Preliminary studies on sweetpotato extract cytotoxicity showed that a concentration at 1000 $\mu\text{g/mL}$ was toxic to breast cancer cells MDA-MB-231. The work reported here used only 100 $\mu\text{g/mL}$, so intermediate doses may need to be tested in future.

The data in sections 7.3.6 and 7.3.7 seem to suggest that cooked sweetpotatoes might be pro-inflammatory. It is worth noting that *in vitro* studies do not reflect real life, as they are test tube based studies, which provide an initial picture of possible effects of the compounds in the body (Rolfes et al., 2015). Furthermore, only the IL-8 biomarker was measured in this study, but the inflammation pathway is complex and several biomarkers are involved such as pro-inflammatory markers: IL-21, IL-1 β and IL-8 and anti-inflammatory markers: tumour necrosis factor- α (TNF- α), IL-1, IL-6 and IL-4 (Medzhitov, 2010). In future, both pro- and anti-inflammatory properties should be tested; in addition, the assay should include extracts from both cooked and uncooked sweetpotatoes. It was also noticed that the glucosamine, which was included as a positive control, did not suppress IL-8 production at 5 μM . Concentrations higher than this could be considered in the future. 5-amino salicylic acid is a well-known anti-inflammatory drug, and it has been used in several anti-inflammatory assays as positive control (Aguzzi et al., 2011; Serra et al., 2013), so alternatively, in future studies glucosamine could be replaced with 5-amino salicylic acid.

In this study, only one cell line HT-29 was used, but as the compilation in Table 7.4. shows there are other cell lines such as Caco-2, Neutrophils and Human PBMC that have been used in studies examining the anti-inflammatory effects of polyphenol rich extracts. These cells could be used to investigate the anti-inflammatory effects of sweetpotato extracts.

Health benefits associated with consumption of a diet rich in polyphenols have been reviewed by Pandey & Rizvi (2009) and Scalbert et al. (2005). Some of the benefits identified include anti-aging, anti-diabetic, anti-carcinogenic and neuro-protection

effects. The fact that sweetpotato did not show anti-inflammatory properties in this study does not forfeit these other potential benefits. The effect of sweetpotato extracts exhibiting anti-diabetic, anti-aging and neuro-protective properties could be explored further.

8.5 Recommendation for future studies

8.5.1 Role of internal ethylene on sweetpotato sprouting

The effects of multiple 1-MCP applications that included a reduction in ethylene production and suppressed sprout growth, could be the basis to test the hypothesis that ethylene is required for the initiation of sprouts. An exchange of ethylene stored roots to storage in air free of ethylene showed dramatic sprout growth (Amoah, 2013), supporting the idea that ethylene is required for sprout initiation. This hypothesis can be tested by treating sweetpotatoes with multiple 1-MCP applications, then storing the roots in air free of ethylene to allow them to sprout. If multiple 1-MCP applications inhibited sprout growth through blocking the ethylene perception required for sprout initiation, then a transfer of roots to ethylene-free air should not show dramatic sprouting. Coupling such a study with microscopic work examining for the presence of pre-existing sprout meristems in roots exposed to ethylene would be useful to understand the role of internal ethylene in sweetpotato sprouting.

8.5.2 Effect of temperature on sweetpotato responses to 1-MCP

Few studies have been undertaken using 1-MCP to control sprouting in sweetpotato. In the present study 1-MCP treated roots were held at 25 °C, which may explain the hypothesised fast receptor turnover. It would be desirable to see if turnover is slower and 1-MCP is therefore more effective, if studies were performed at 15 °C.

8.5.3 Acceptability of coated sweetpotatoes

The consumer acceptability of coated sweetpotatoes should be determined. The internal gas modification was only measured for up to 14 days of storage. Therefore, it is suggested that internal atmosphere modification should be monitored for a longer storage duration (> 14 days), and in addition to oxygen and carbon dioxide; internal ethylene concentration should be monitored. This would allow assessment of the likely risks from coating

8.5.4 Study of hot water treatment and coating in a packhouse

Results from this study suggest there is the potential to use a combined treatment of hot water dipping and coating to extend the storage life of sweetpotato. This result has to be verified in an industrial situation, and using different sweetpotato cultivars.

8.6 Final Conclusion

In this work, HWD and coating, ethylene and 1-MCP treatments were investigated to test their effects on extending the storage life of sweetpotato while maintaining the health benefiting compounds carotenoids, phenolic acids, and anthocyanins. The use of HWD and coatings to extend the storage of sweetpotato might present an attractive technology to less developed countries that are limited in refrigeration facilities to maintain sweetpotatoes at the ideal storage temperature of 15 °C. Coating may have greater potential in cultivars that are less prolific in sprouting.

This work confirmed that ethylene is a sweetpotato sprout inhibitor; however, the associated negative effects outweigh the benefits of using ethylene. Future research should focus on finding ways to get the benefits of ethylene for sprout reduction without incurring the risk of splitting. Furthermore, the data supports the importance of the role of ethylene in non-climacteric produce, as demonstrated in sweetpotato cultivar ‘Owairaka Red’. This cultivar may serve as a model system for understanding the role of ethylene in non-climacteric produce, as it has high receptor turn over and different tissues appear to respond variably to 1-MCP applications. The proposed conceptual model on the role of ethylene in sweetpotato quality responses could be helpful in devising a means of avoiding ethylene-induced negative responses in stored sweetpotato roots.

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Appendix

Appendix (A): Effects of different edible coatings on sweetpotato ethylene production

The results presented in chapter 4 showed that coating application increased sprouting in ‘Owairaka Red’ sweetpotato roots. This appendix briefly discusses the experiment which was carried out in an attempt to investigate if there were possible correlation between coating and ethylene production. Due to unavailability of ‘Owairaka Red’ roots at the time of the experiment, roots of ‘Clone 1820’ were used. After the experiment was set, the Ethylene detector - Sense sensor had a problem, and it took 2 weeks to be fixed, as such ethylene production was measured at 3 and 4 weeks of storage.

Sprout growth

Sprouts were first observed at 2 weeks of storage, but no significant differences were observed among the treatments both in terms of sprout growth and sprout number (Table A1).

Table A1: Average sprout number per root and sprout length

Treatment	Mean sprout number/root			Mean sprout length (mm)		
	week 2	week 3	week 4	week 2	week 3	week 4
Control	0.7	2.8	2.8	10.8	20.6	33.1
Carnauba	1.7	1.9	2.6	22.8	24.3	34
Apple wax	1.8	2.7	4.1	7.4	24.9	26.8
Castle 873	2.8	3.2	3.2	20.5	29.9	46.8
Stone fruit	1.7	3	3.4	23.5	21.1	42.3
P-value	ns	ns	ns	ns	ns	ns

Ethylene production and correlation with sprout growth

Ethylene production of coated and uncoated sweetpotato roots ranged from 1.77 to 3.12 nL kg⁻¹ h⁻¹. No significant ($P > 0.05$) differences were observed among the treatments (Figure A1). The ethylene production data was correlated with sprouting data to see if there was any relationship between sprout growth and ethylene production. No

significant correlation were observed between ethylene production and sprout growth (Figure A2).

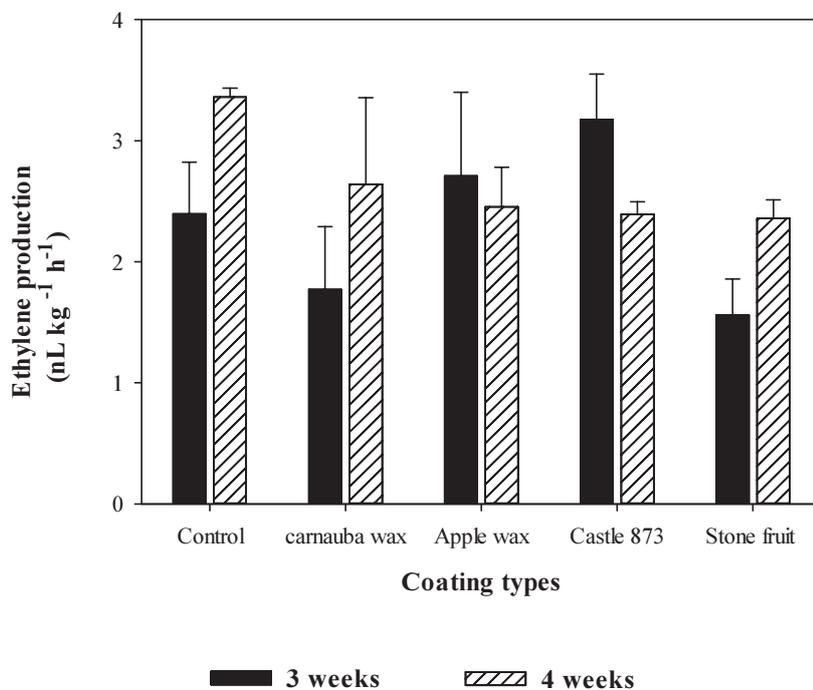


Figure A1: Ethylene production of ‘Clone 1820’ sweetpotato roots coated with different types of edible coating and stored at 25 °C and 80 - 85 % RH for 4 weeks. Each bar is a mean of 3 replicates.

Conclusion

This experiment failed to give evidence for a relationship between coating and ethylene production. Nevertheless this does not rule out the possibility that coating may enhance internal ethylene concentration in ‘Owairaka Red’. It is possible that ethylene production is not a good measure of internal ethylene concentration. Internal ethylene measurements using cannulation method should be considered in the future.

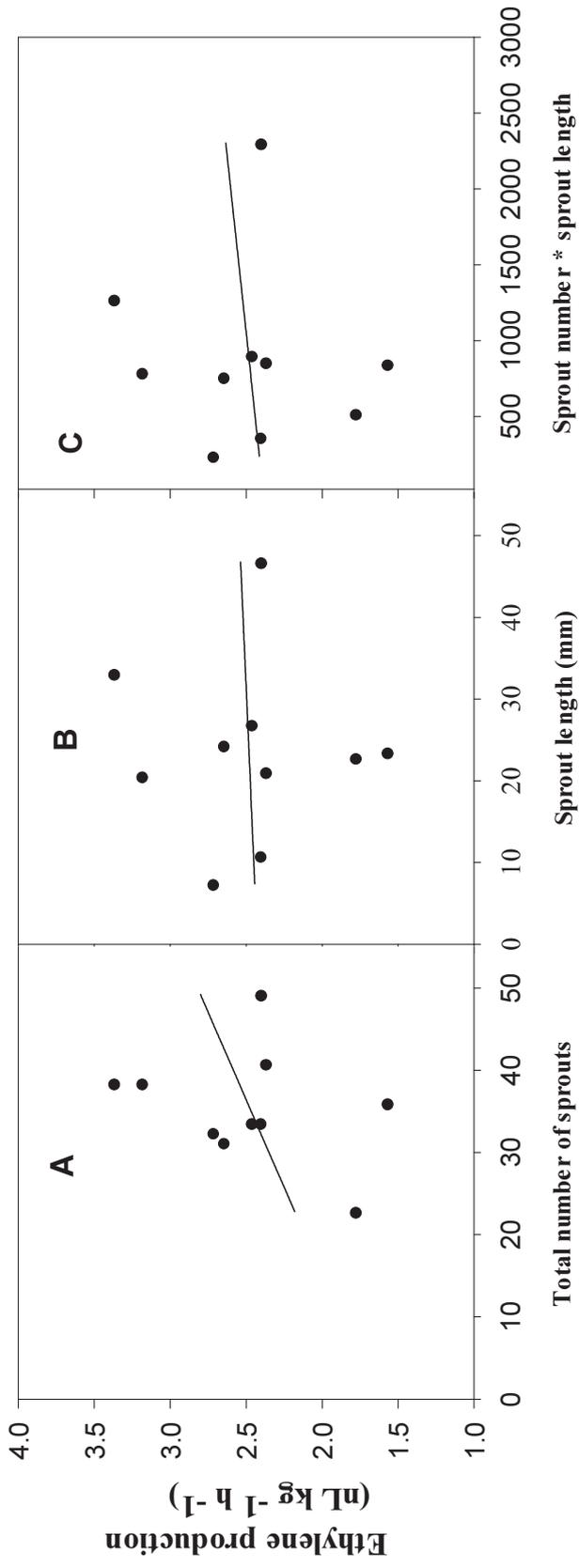


Figure A2: Correlation of ethylene production and total sprout per treatment (A), total sprout length (B) and total sprout growth: length * number (C)

Appendix B: Questionnaire for sweetpotato sensory evaluation

CONSUMER SENSORY EVALUATION OF COOKED SWEETPOTATOES

All information provided will be used for academic purpose only.

Instructions:

- I. You will be given four (4) different cooked sweetpotato samples to evaluate
- II. Please take a sip of water before tasting each sample
- III. Evaluate the product in front of you by looking at it and tasting it (Please evaluate one sample at a time)
- IV. Please tick the box to indicate how much you liked and disliked this product
- V. Finally, rank the samples from most liked to least liked

GENERAL QUESTIONS

Please circle/complete the questions below:

Sex : Male / Female

Age :

How often do you eat sweetpotatoes?

- Every day
- Every week
- Every month
- Rarely
- Never

Sample code: _____

Please indicate by placing a mark in the box your liking on the following attributes of this sample

9-point hedonic scale	Overall liking	Colour	Sweetness	Texture
Like extremely	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Like very much	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Like moderately	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Like slightly	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Neither like nor dislike	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike slightly	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike moderately	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike very much	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike extremely	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Overall comment(s) of this sample:

Sample code: _____

Please indicate by placing a mark in the box your liking on the following attributes of this sample

9-point hedonic scale	Overall liking	Colour	Sweetness	Texture
Like extremely	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Like very much	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Like moderately	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Like slightly	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Neither like nor dislike	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike slightly	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike moderately	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike very much	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike extremely	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Overall comment(s) of this sample:

Sample code: _____

Please indicate by placing a mark in the box your liking on the following attributes of this sample

9-point hedonic scale	Overall liking	Colour	Sweetness	Texture
Like extremely	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Like very much	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Like moderately	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Like slightly	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Neither like nor dislike	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike slightly	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike moderately	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike very much	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike extremely	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Overall comment(s) of this sample:

Sample code: _____

Please indicate by placing a mark in the box your liking on the following attributes of this sample

9-point hedonic scale	Overall liking	Colour	Sweetness	Texture
Like extremely	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Like very much	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Like moderately	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Like slightly	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Neither like nor dislike	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike slightly	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike moderately	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike very much	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dislike extremely	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Overall comment(s) of this sample:

Rank the samples from most liked to least liked, using the following numbers: 1 = most liked and 4 = the least liked, (Ties are not allowed)

Sample	Rank (1 to 4)
383	_____
574	_____
855	_____
985	_____

Thank you very much for participating