Calcium lactate treatment of fresh-cut butternut squash (*Cucurbita moschata*): effect on texture during storage and shelf life

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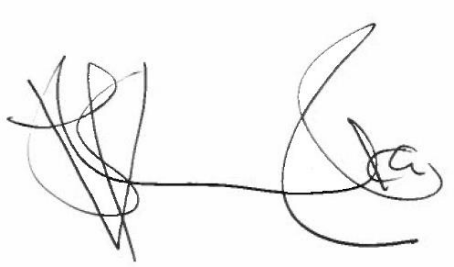
DECLARATION

I Victor Takunda Mhaka hereby declare that this dissertation submitted at the University of Pretoria for the degree MSc Food Science is my own work and has not previously been submitted by me for a degree at this or any other university or institution of higher education.



Victor Takunda Mhaka

August 2020

DEDICATION

This is dedicated to my father whose unrelenting belief in my abilities has carried me through many challenging vicissitudes of life.

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ABSTRACT

Calcium lactate treatment of fresh-cut butternut squash (*Cucurbita moschata*): effect on texture during storage and shelf life

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Supervisor: Dr. N. N. Mehlomakulu

Co-supervisor: Prof. R. de Kock

Butternut squash (*Cucurbita moschata*), originating from Mexico belongs to the *Cucurbitaceae* family and is mainly grown in Mpumalanga and Gauteng provinces of South Africa. Two cultivars of butternut squash, 'Atlas' and 'Pluto' are widely cultivated and used for minimal processing by fresh-cut produce manufacturers in South Africa. Despite the increasing popularity and global market share for fresh-cut produce due their convenience, sensory properties and nutritional profiles, fresh-cut products experience rapid quality deterioration during storage. In this research, exacerbated texture quality deterioration during storage of fresh-cut butternut squash which led to economic losses at a South African based food processing plant was noted. Consequently, the effects of calcium lactate dipping treatment on texture quality and shelf life of fresh-cut butternut squash (*Cucurbita moschata*) cultivars 'Atlas' and 'Pluto' was investigated in this study. The investigation was divided into two subsequent experimental trials: the first was aimed at determining the impact of calcium lactate treatments (0.5% and 3% w/v; for 10 minutes at 50-60°C dipping temperatures) on fresh-cut butternut texture quality during storage (3.5°C) and investigating texture quality differences between 'Atlas' and 'Pluto' fresh-cut butternut. The second experimental trial was aimed at investigating the impact of 10°C storage temperature on the texture quality of fresh-cut butternut squash. Butternut squash cultivars 'Atlas' and 'Pluto' also underwent physical and physicochemical characterization.

In both research experiments fresh-cut butternut sensory quality was evaluated by a trained descriptive panel while the microbiological quality was assessed through testing for total viable counts, *Escherichia coli* (O157:H7), coliforms, *Listeria* species, yeasts and moulds. There

were no significant differences (p>0.05) brought about by calcium lactate dipping treatments in terms of texture quality and shelf life for both ‘Atlas’ and ‘Pluto’ butternut squash in the first experiment. Furthermore, no significant differences (p>0.05) in terms of firmness and shelf life were established between ‘Atlas’ and ‘Pluto’ fresh-cut butternut squash as determined by the descriptive sensory panel. In the second experiment, a significant texture quality (firmness) difference (p<0.05) was observed between the controls and calcium lactate treated (0.5% and 3% w/v) fresh-cut butternut stored at 10°C. This study provided evidence that texture quality retention challenges during storage of fresh-cut butternut squash could better be addressed by adhering to strict cold chain parameters rather than application of calcium lactate dipping treatments at 0.5% and 3% concentrations. In addition, results from this research suggest that there is no variation between butternut squash cultivars ‘Atlas’ and ‘Pluto’ in terms of physicochemical properties, minimal processability, texture quality and shelf life. Further research can explore the application effect of more concentrated calcium lactate solutions (>3% w/v), prolonged dipping times (>10 minutes), higher dipping temperatures (>60°C) and/or a combination thereof in retaining texture quality of fresh-cut butternut squash cultivars.

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LIST OF ABBREVIATIONS

ACC	1-Aminocyclopropane-1-carboxylic acid
AC-EW	Acidic Electrolyzed Water
ACO	1-Aminocyclopropane-1-carboxylic acid oxidase
ACS	1-Aminocyclopropane-1-carboxylic acid synthase
AK-EW	Alkaline Electrolyzed Water
AOS	Active Oxygen Species
AOX	Alternative Oxidase Pathway
CAT	Catalase
CCP	Critical Control Point
EBI	Electron Beam Irradiation
ERH	Equilibrium Relative Humidity
GRAS	Generally Regarded As Safe
JA	Jasmonic Acid
MAL	Microbiological Acceptability Limit
MAP	Modified Atmosphere Packaging
MDA	Malondialdehyde
NHBN	New Hampshire Butternut
OTR	Oxygen Transmission Rate
PAL	Phenylalanine Ammonium Lyase
PME	Pectin Methylesterase
POD	Peroxidase
PPO	Polyphenol oxidase
PPP	Pentose Phosphate Pathway
QCP	Quality Control Point
RH	Relative Humidity
RNA	Ribonucleic acid
RTE	Ready-to-eat
RTU	Ready-to-use
SAL	Sensory Acceptability Limit
SIPK	Salicyclic acid Induced Protein Kinase
TCA	Tricarboxylic Acid Cycle
TSS	Total Soluble Solids
WIPK	Wound-Induced Protein Kinase
WTP	Willingness To Pay
WVPD	Water Vapour Pressure Deficit

1. INTRODUCTION

Butternut squash (*Cucurbita moschata*) is a vegetable crop native from Mexico and its varieties were originally domesticated in South America and eastern Unites States, and are well known for their significant amounts of vitamins, minerals and various beneficial substances to the human diet (Sanjur et al 2002; Jacobo-Valenzuela et al. 2011a; Dari and Yaro, 2016). These beneficial substances include vitamin C, vitamin A, α-carotene, β-carotene, potassium, magnesium, selenium and iron and glucose, fructose, saccharose, cellulose and fibre, respectively. Jacobo-Valenzuela et al. (2011a) mentioned that since ancient times butternut squash has been essential in the diet of rural communities and some urban areas worldwide and is a marginalized crop in terms of cultivation, marketing, industrialization and research. Seroczynska et al. (2019) mentioned that the genus *Cucurbita* has pharmacological effects such as treating gastrointestinal diseases and intestinal parasites due their nutritional and phytochemical composition. Furthermore, Salehi et al. (2019) mentioned that some of the chemical constituents linked with the genus *Cucurbita* include carotenoids, tocopherols, phenols, terpenoids, saponins, sterols, fatty acids and carbohydrates. In South Africa, butternut squash is regarded as a summer crop grown by irrigation farmers mainly grown in the Mpumalanga Highveld and Lowveld and Gauteng (in the Vaal region) (Department of Agriculture, Forestry and Fisheries, 2011).

Global consumption of minimally processed produce has witnessed significant growth over the years leading to a widespread and diversified fresh-cut produce industry (Corato, 2020). In South Africa, the fresh produce business is worth approximately R19 billion possessing a growth rate of 15.5% (Zourides, 2016). An annual growth of approximately 10% in the ready-to-use (RTU) vegetable industry has been witnessed, with produce processing amounting to €300 million in Ireland (Martin-Diana et al. 2005a). According to Ragaert et al. (2004), minimally processed vegetables have had an estimated growth of 10-25% per annum since 1990 in Western Europe. Ragaert et al. (2004) assert further that in Belgium more than 50% of produce turnover at retail level consist of minimally processed produce. The increased demand of minimally processed vegetables on the market can be attributed to shifting consumer attitude towards these foods. Corato (2020) mentioned that a “rich in cash/poor in

time" consumer profile is growing in both developing and developed countries. This consumer profile tends to be satisfied with food products that bring convenience, quality and nutritional value to their plates (Ronquest-Ross et al. 2015). In addition, it has been reported that there is a positive willingness to pay (WTP) for premium cost fresh-cut produce in today's society (Sillani and Nassivera, 2015; Wenban-Smith et al. 2016).

Despite the increased demand in fresh-cut fruits and vegetables globally, the fresh-cut produce industry faces the challenge of retaining quality attributes such as colour, texture, smell, microbiological quality and nutritional value during storage. This has led to numerous research studies on extending the shelf life of minimally processed produce by a plethora of physical, chemical and bio-preservation methods. In this research study, the effectiveness of an organic chemical, calcium lactate, in increasing the shelf life of minimally processed butternut squash (*Cucurbita moschata*) cultivars 'Atlas' and 'Pluto' produced at a South African company was investigated. The company mainly manufactures ready-to-eat foods, minimally processed vegetable products, long life products, juices and snacks to retailers in South Africa and abroad.

Diced butternut is one of the company's top sellers, bringing in revenue of more than R646 000 per annum as documented in the 2019 annual sales report. Minimal processing operations that fresh-cut butternut is exposed to are known to increase texture deterioration, respiration and ethylene production in many foods (Leon et al. 2001). Minimal processing promotes biochemical reactions responsible for changes in colour, flavour, texture and nutritional quality in fresh-cut produce (Ali et al. 2018). These biochemical reactions include demethoxylation, acid hydrolysis and β-elimination that occur to pectin cellular features of plant tissues. In the presence of inherent enzymes, conducive pH and temperatures, these biochemical reactions result in demethoxylation, depolymerisation and solubilisation of pectin (Moelants et al. 2014). Increased customer complaints about diced butternut tissue softening and reduced shelf life have been reported and published in the company's annual customer complaint reports (2015, 2016 and 2017) with each justifiable complaint having resulted in extensive economic losses for the company. This research intended to address the unfavourable and untimely texture deterioration of fresh-cut butternut.

Regardless of various research studies on the textural quality retention properties of calcium lactate in pre-cut vegetables, published work on its effect on the textural quality of minimally

processed butternut squash could not be found at the time of this study. Furthermore, there is no published evidence comparing the texture quality performance of the butternut squash cultivars ‘Atlas’ and ‘Pluto’ when minimally processed. The results of this investigation may help vegetable processors and butternut farmers alike to have an insight on how these cultivars respond to the ascribed processing operations.

2. LITERATURE REVIEW

2.1 Overview

The increased demand for minimally processed vegetables globally has triggered a myriad of research work in areas of quality retention and shelf life extension with regards to fresh-cut produce (Chinnici, D'amico and Di Grusa, 2019; Salam et al. 2020; Yousuf et al. 2020). Minimally processed produce can be defined as raw fruits and vegetables that have gone through washing, peeling, slicing, chopping or shredding processes prior to being packaged for consumption. In addition, minimally processed produce, also known as ready-to-use (RTU), fresh-cut or pre-cut produce, can also be defined as intact fruits and vegetables subjected to processing techniques of lesser magnitude than canning or freezing with the intention of value addition (Barry-Ryan & O'Beirne, 1998; King Jr. & Bolin, 1989). Consumers require minimally processed produce that is of high quality, with reasonable shelf life for home storage and that closely resembles the intact produce raw material (Martin-Diana et al. 2005a). In this chapter, a comprehensive review on the butternut squash background, determinants and factors affecting quality of minimally processed vegetables, shelf life extension and butternut squash minimal processing is presented to further guide this research study. Since the focal point of this research study had much to do with textural quality retention by calcium lactate dipping and its preservation by chemical preservation, research studies on textural quality of minimally processed fruits and vegetables will be thoroughly reviewed. This research also sought to identify knowledge gaps in the area of minimally processed butternut squash texture quality retention by calcium lactate treatment that can be shaped by this study.

2.2 Butternut squash background

Butternut squash (*Cucurbita moschata*) is a member of the *Cucurbitaceae* family along with pumpkin, squash, cucumber and watermelon which are reported to have originated from South America (Lucera et al. 2012). Loy (2004) made an assertion that butternut squash (*Cucurbita moschata*) is part of the *Cucurbita* genus comprising of two other domesticated and commercially successful species of related winter squash *Cucurbita pepo* and *Cucurbita maxima*. The common butternut shape fruit shape came from a spontaneous mutation in a field of crookneck (crooked neck region) winter squash in the late 1920's (Mutschler and Bush,

1987). The first genetically stable (traits passed on from generation to generation) butternut squash cultivar (cultivated variety with human interference via selective breeding), developed in the 1950's, was called New Hampshire Butternut (NHBN) from which most butternut squash cultivars emerged from, including cultivar 'Waltham' that Sakata Southern Africa (Pty) Ltd produce mainly for the southern Africa region (Mutschler and Bush, 1987). Dari and Yaro (2016) mentioned that butternut squash takes 85-140 days to reach maturity with fruit weights ranging from 650g-1kg and that every part of butternut squash can be consumed including tender shoots and leaves. Furthermore, in Ghana butternut squash has gained preference over its *Cucurbitaceae* counterparts due to its early maturity, appealing orange colour and size attractive to producers, traders and consumers (Dari and Yaro, 2016).

2.3 Major determinants of fruit and vegetable quality

Food quality can be defined in various ways depending on the vantage point of the stakeholder involved. Spink (2014) identified four different definitions of food quality from perspectives of four different stakeholders in the food supply chain which were public health professionals, food manufacturing managers, food standards and certification leaders and consumers. From a public health professionals' perspective, food quality is defined as food that does not make people sick. Food quality from a food manufacturing managers' perspective is defined as those food attributes that lead to consistent end product and manufacturing operations. Food quality from a food standards and certification leaders' perspective is defined as food that meets the defined specifications such as viscosity, density, colour and texture. However, of interest in this research study is the consumers' definition which Spink (2014) identified as premium food or quality food acceptable to consumers.

The following section identifies the major determinants of minimally processed fruit and vegetable quality as determined by consumers. These determinants directly influence the consumers' perception of a food product on retailers' shelves and their post-purchase behaviour. These determinants also impact on a finished product's reputation and the willingness of a consumer to continuously purchase the minimally processed product (Kaya, Florkowski and Suh, 2010).

2.3.1 Texture

Fruit and vegetable processing is well known to have a significant impact on the texture of produce. Consumers associate firm and crunchy minimally processed vegetables with freshness and wholeness (Rico et al. 2007; Troyo and Acedo, 2019). Food textural properties are physical characteristics arising from the food's structural elements and are sensed by the feeling of touch. Textural properties are also related to the deformation, disintegration and flow of food under force, and are measured objectively by functions of mass, time and distance (Barrett et al.2010). It is important to note that the term texture is used primarily with reference to solid or semi-solid foods. The textural properties of fruits and vegetables are perceived with the sense of touch during hand picking or chewing in the mouth. Textural profiles of fruits and vegetables are derived from their turgor pressure and the pectic composition of individual plant cell walls and the middle lamella that hold the individual cells together (Barrett et al. 2010).

Textural changes are brought about by a plethora of processing-induced biochemical and enzymatic reactions which can result in exacerbated respiration rates, protein denaturation, senescence, water loss, gelatinization of starch, solubilisation of proteins and acid hydrolysis and demethoxylation of pectin. All these aforementioned biochemical and enzymatic processes are involved in the softening of plant tissues (Cliffe-Byrnes and O'Beirne, 2005; Brennan and Grandison, 2012). For instance, enzymatic protein denaturation may result in loss of plant tissue water-holding capacity while pectin depolymerization softens plant tissue by weakening cell-to-cell integrity and cell turgidity (Brennan and Grandison, 2012; Moelants et al. 2014; Soliva-Fortuny and Martin-Belluso, 2020). Belitz et al. (2009) mentioned that among the plant cell polysaccarides such as cellulose and hemicellolose, pectin has a distinct role in plant tissue firmness, hence its direct relationship with plant texture. Furthermore, Cliffe-Byrnes and O'Beirne (2005) mentioned that plant tissue softening together with loss of chlorophyll, degradation of cell membranes and changes in RNA and protein are characteristics of physiological ageing.

Minimal processing operations often lead to cell disruption which in turn culminates to loss of turgor pressure and softening of plant tissue (Brennan and Grandison, 2012). Furthermore, even though texture is highly dependant on cell turgor, the integrity of cell walls is of necessity to processed fruits and vegetables. A condition known as mealiness, generally perceived as a

loss in textural quality, can develop in some fruits and vegetables like apples and tomatoes because of the breakdown of cellular adhesion in plant tissue (Aked, 2007). Therefore, it is of paramount importance that raw materials ought to be robust enough to withstand mechanical stresses experienced during minimal processing. However, the aforementioned influences of texture are primarily determined by cultivar as well as maturity of the fruit or vegetable.

The firmness of fruits and vegetables has been known as a reliable indicator and is mechanically measurable to determine textural quality (Leneveu-Jenvrin et al. 2020). Even though it is a subjective method, firmness can generally be assessed through the application of light hand pressure by individuals with considerable experience to conduct accurate assessments. As postulated by Ranganathan et al. (2016), texture of vegetables can best be analysed by use of analytical panels. In addition, firmness of fruits and vegetables can be assessed visually e.g. shrivelling, wilting and the flaccidity of leafy vegetables. This subjective method of textural quality determination is also used by consumers in their perception of textural quality of minimally processed vegetables such as fresh-cut butternut (Montero-Calderon et al 2020). Furthermore, various research methods to accurately measure firmness have been developed over the years. For instance, the most common quantitative method of plant tissue firmness assessment is the use of a penetrometer (Lammertyn et al. 2002). Examples of penetrometers include the Magness-Taylor firmness tester and the Effegi penetrometer (Aked, 2007). These machines basically measure the total force required to puncture through a given portion of plant tissue to a standard depth using a standard diameter probe. Other non-commercialised laboratory methods are available to test firmness, for example, vibration tests (Cubeddu et al. 2002). Aked (2007) mentioned that with a sharp tapping of plant tissue, propagation of sound waves occurs in a manner that is detectable by a microphone or piezoelectric sensor. In this case, the variations of sound waves correlate with plant tissue firmness.

2.3.2 Flavour

Barrett et al. (2010) defined flavour as a mingled but unitary experience which includes sensations of taste, smell and pressure, and often cutaneous sensations such as warmth, colour and mild pain. Barrett et al. (2010) also made an assertion that while fruit and vegetable appearance may be the initial quality attributes that attract consumers to a product, flavour may

have the largest impact on acceptability and desire to consume it again. Flavour changes are predominantly common in severe processing methods such as heat treatment, freezing and drying of fruits and vegetables. Brennan and Grandison (2012) reckoned that flavour is rather subjective and difficult to quantify. The main sources of flavour variation include severe processing as well as maturity stage of the produce.

Regardless of the challenges to quantify flavour, human testers and analytical tests can be used. Aked (2007) also mentioned that there are relatively few instrumental tests which can provide results which correlate well with consumer assessment of fresh produce. The use of analytical sensory panels to conduct sensory evaluation has been found to be a comprehensive way of assessing overall quality in products. In these assessments, panels trained in descriptive sensory analyses assess certain quality components in a controlled manner providing detailed information about the sensory attributes of the product, while hedonic assessment is based on preferences of the consumer panel (Lammertyn et al. 2002). Aroma compounds in food are volatile and are primarily perceived by aroma receptors on the olfactory bulb while taste receptors exist in the mouth and are impacted during chewing (Barrett et al. 2010; Glezer and Malnic, 2019).

Flavour is comprised of two components which are taste and aroma (Li et al. 2020). Taste components are usually based on sugar levels (for sweetness) and acidity (for sourness). Kader (2008) and Alvarez et al. (2015) reported that fruit and vegetable processors normally measure sweetness in terms of total soluble solids (TSS) contents in °Brix. The logic behind this approach is that sugar makes up the main component of TSS which can be used as an indicator of percentage sugar levels. Refractometers or hydrometers are used to determine TSS in fruits and vegetables currently in the industry. The former technique is based on the degree of light refraction by the liquid samples while the latter on the sample density (Aked, 2007; Kader, 2008; Alvarez et al., 2015).

In industry, the measurement of aroma is rather informal, as Aked (2007) put it, relying on off-odours in shelf-life samples. However, laboratory measurements have been conducted by headspace analysis using gas chromatography with separated chemicals being identified objectively by use of odourmeters. Kader (2008) mentioned that emphasis should be on

selecting fruit and vegetable cultivars at optimum maturity prior to processing with better flavour and nutritional quality and processing methods that retain quality.

2.3.3 Appearance

Aked (2007) asserted that in fruit and vegetable marketing, appearance is a major factor in influencing consumer preferences. Facets of fruit and vegetable visual quality include hue, colour uniformity, glossiness, absence of defects in shape or skin and freedom from visible disease. Presence of defects is mainly affected by exposure to diseases and insects during cultivation, harvesting and postharvest operations (Barrett et al. 2010). Fruit and vegetable gloss, which is affected by moisture content, wax deposition on surface and postharvest handling, is related to the ability of a surface to reflect light and freshly harvested products are often glossier hence perceived to be of higher quality by consumers. Retailer fruit and vegetable displays are characterised (or try to characterise) uniformity of size, shape and colour to gratify consumer expectations. Rico et al. (2007) also added that consumers often judge quality of fresh-cut fruits and vegetables based on appearance and freshness during purchasing.

It is important to note that final product appearance quality depends mostly on the section or portion of produce being used in the product (Chinnici et al. 2020). Furthermore, in certain products, the peels are removed during processing such that surface blemish consequences will be annulled. In such cases, the flesh colour appearance overrides peel colour. Fruit and vegetable geometrical properties are of more paramount importance to automated processes than manual ones. However, size and shape can be a significant factor even in manual minimal operations like peeling and slicing as uniform units can aid waste reduction and maximisation of yields (Aked, 2007; Brennan and Grandison, 2012). For example, the orange colour of butternut squash rinds can be used to determine harvest maturity by farmers where a bright orange rind colour indicates optimum harvest maturity (Aked, 2007; Brennan and Grandison, 2012; Lucera, et al. 2012). In most cases, colour can be a useful indicator of maturity and eating quality. Colour quality is derived from the natural pigments in fruits and vegetables and these pigments are primarily fat soluble chlorophylls (green), carotenoids (yellow, orange and red), water soluble anthocyanins (red and blue) and betalins (red). Furthermore, formation of water soluble brown, grey and black coloured pigments may be a result of enzymatic and non-enzymatic browning reactions. The enzymes involved in enzymatic browning include

polyphenol oxidase (oxidation of polyphenolic compounds) and phenylalanine ammonia lyase (catalysis of phenolic substrate precursors).

2.4 Quality of fruits and vegetables

The determinants of quality in fresh-cut fruits and vegetables can be influenced, negatively or positively, by various factors that can enhance or mar consumer perceptions. Factors that affect product quality of fresh-cut fruits and vegetables can be divided into two types, pre-harvest and post-harvest factors which are detailed below.

2.4.1 Pre-harvest factors

2.4.1.1 Cultivar selection

Cultivar selection is a major contributor to minimally processed fruit and vegetable quality since plant genotype has a direct influence on the phenotype and physiology of fruits and vegetables (Hodges and Toivonen, 2007; Grisales et al. 2014). It has been proven in a paper by Mutschler and Bush (1987) that even the number and type of mitochondrial DNA plasmids in butternut plant cells varies amongst different cultivars but remain the same within any cultivar. In this study by Mutschler and Bush (1987) different types and numbers of mitochondrial plasmids were found in 'Ponca', 'Ponca-BN', 'Ponca-CR', 'Patriot', 'Waltham', 'New Hampshire', 'Derived Crookneck' and variety (var.) Canada Crookneck. However, the types and numbers of mitochondrial plasmids were found to be similar within the plants of each cultivar mentioned above, including the variety Canada Crookneck. Weston and Barth (1997) made an assertion that genetics and cultivar selection are the major factors involved in the post-harvest quality of fruits and vegetables. Furthermore, due to variation in genetic make-up amongst cultivars of the same plant species, significant differences manifest themselves in size, colour, flavour, texture, pest resistance, eating quality, yield and processing ability (Weston and Barth, 1997; Grisales et al. 2014). Priori et al. (2017) concluded that there was high genetic variability for the synthesis of phenolic compounds, carotenoids, antioxidant activity and minerals (calcium, magnesium, potassium, copper, iron, manganese, zinc and phosphorus) among ten accessions of *Cucurbita moschata* landraces from southern Brazil. Sakata Southern Africa (Pty) Ltd, a seed producer in the southern Africa region refer to the different applicability of various butternut squash cultivars they produce as shown in Table 1 below.

Table 1: Quick reference table for market segment applicability of butternut squash cultivars produced by Sakata Southern Africa (Pty) Ltd (Sakata Southern Africa (Pty) Ltd, 2014)

Cultivar	Days to maturity	Fruit weight (kg)	Fruit shape	Rind colour	Flesh colour	Market segment	Comment
Apollo	85-100	1.5-2.2	Elongated cylindrical and bulbous blossom end	Tan	Orange	Fresh market and processing	Excellent fruit uniformity and yield potential
Atlas	90-105	2-3	Cylindrical and bulbous blossom end	Tan	Deep orange	Fresh market and processing	Industry standard for processing. Very high yield potential. Excellent storage ability and good fruit uniformity.
Barbara	Baby: 45 Mature: 85-95	1-1.5	Cylindrical and bulbous blossom end	Dark green with tan stripes	Deep yellow	Pre-pack as baby and fresh market	Immature fruit very attractive as a baby vegetable.
Cosmos	85-100	0.8-1.2	Cylindrical and bulbous blossom end	Tan	Deep orange	Fresh market	Very uniform, medium size fruit.
Pluto	85-100	1-1.5	Cylindrical and bulbous blossom end	Tan	Deep orange	Fresh market, export and processing	Excellent yield potential. High fruit uniformity. New industry standard for fresh market. Excellent storage ability.

Table 1 *cont.*: Quick reference table for market segment applicability of butternut squash cultivars produced by Sakata Southern Africa (Pty) Ltd (Sakata Southern Africa (Pty) Ltd, 2014)

Cultivar	Days to maturity	Fruit weight (kg)	Fruit shape	Rind colour	Flesh colour	Market segment	Comment
Quantum	85-100	0.8-1.2	Cylindrical and bulbous blossom end	Tan	Orange	Fresh market export and processing	Excellent yield potential. High fruit uniformity. New industry standard for fresh market. Excellent storage ability and ideal for export and supermarket
Veenas	85-100	0.6-1	Short cylindrical and bulbous blossom end	Tan	Orange	Export, pre-pack and fresh prepared	Ideal for pre-packing and export due excellent uniformity and small fruit size. Excellent yield potential
Waltham	90-100	0.6-1.5	Cylindrical and bulbous blossom end	Tan	Yellow orange	Fresh market, export and processing	Uniform fruit and good yield

In a study by Niewczas et al. (2014) on pumpkin (*Cucurbita maxima* Duch.) cultivars, significant differences (p<0.05) were noted between the conventionally grown 'Bambino' and four novel cultivars ('Karowita', 'Justynka F_1' and experimental hybrids 771 and 774). The novel pumpkin cultivars had higher chemical compositions (dry matter content, saccharides, carotenoid compounds, vitamin C, protein and minerals) than 'Bambino'. Furthermore, all four novel cultivars had smaller fruits which were concluded to make it easier for culinary and industrial utilization. 'Bambino' pumpkin had empty seed cores whereas the novel cultivars had filled seed cores, which was concluded to increase resistance to damages during post-harvest handling and transportation. Armesto et al. (2020) found that the butternut squash cultivar 'Pluto' possessed higher levels of folic acid (24%) and β -carotene (80%) than the cultivar 'Ariel'. On the other hand, the butternut squash cultivar 'Ariel' had higher tocopherol content (approximately 3-fold) than the cultivar 'Pluto'.

Comparisons among cultivars of the same species, under the same treatments, can indicate differences in stress response to fresh-cut processing and/or storage protocols. Hodges and Toivonen (2007) made an assertion that cultivar selection is amongst several factors known to affect wound response during minimal processing operations such as slicing, dicing, chopping, coring, trimming, peeling and/or shredding. This was the case for firmness of fruit slices from five 1-methylcyclopropene (1-MCP) -treated apples (*Malus domestica* Borkh) cultivars, browning on five potato (*Solanum tuberosum* L.) cultivars, and volatile and quality attributes in six cultivars of cantaloupe (*Cucumis melo* L.) (Cantos et al. 2002; Bealieu, 2005; Calderon-Lopez et al. 2005). In the first study, the five apple cultivars treated with 1-MCP and used to produce slices were 'Delicious', 'Empire', 'Idared', 'Law Rome', and 'Mutsu'. 1-MCP treatment reduced ethylene concentration and maintained textural quality of the whole fruit in 'Delicious', 'Empire' and 'Idared' but not for the other two. Apple slices from cultivars that responded to 1-MCP treatment ('Delicious', 'Empire' and 'Idared') also exhibited more firmness and less ethylene concentration that the other two cultivars (Calderon-Lopez et al. 2005).

In another study by Cantos et al. (2002) which investigated the effect of minimal processing on polyphenol oxidase (PPO), peroxidase (POD), phenylalamine ammonia-lyase (PAL) and phenolic compounds in five potato (*Solanum tuberosum* L.) cultivars, the influence of cultivar

selection was also unearthed. A partial correlation between browning and PAL activity was concluded and browning susceptibility of the potato cultivars was in the following order: 'Monalisa', 'Spunta', 'Liseta', 'Cara' then 'Agria'. In a study by Bealieu (2005) on fresh-cut cultivars of cantaloupe which is in the same family, *Cucurbitaceae*, with butternut squash, volatile compounds and quality differences were noted among six different cultivars. These cultivars were 'Athena', 'Sol Dorado', 'Sol Real', 'Gold Rush', 'Mission' and 'Oro Rico'. Significant colour declines during fresh-cut storage were observed in 'Sol Real', and 'Athena' cultivars as measured by Hunter L^* (loss of lightness colour value) and a^* (decline of typical orange hue) colours. All cultivars showed a steady decline in °Brix throughout fresh-cut storage. However, the initial °Brix levels were different with 'Gold Rush', 'Sol Dorado' and 'Sol Real' being <9 °Brix in the mid-season harvest. Furthermore, increasing percentages of acetate compounds was correlated ($\alpha \leq 0.05$, r =0.85) with fruit firmness (as measured with a texture analyser in Newtons) in the cantaloupe cultivars in the following order: 'Mission', 'Oro Rico', 'Sol Real', 'Sol Dorado' then 'Gold Rush'. However, a decreasing percentage of non-acetate esters was negatively correlated (r = -0.86) with cantaloupe firmness in the following order: 'Gold Rush', 'Sol Dorado', 'Sol Real' and then 'Mission'.

2.4.1.2 Climatic conditions

Climatic conditions under which fruits and vegetables are grown are known to be influential on their quality and nutritional value (Conti et al. 2015). This is a phenomenon that has been researched for a while with such claims made in a paper by Weston and Barth (1997). These climatic conditions under discussion include temperature, relative humidity, carbon dioxide concentration, light intensity and water availability which are known determinants of vegetable quality and nutrient content.

A direct relationship has been proven by a wealth of research studies between light intensity and ascorbic acid amount in fruits and vegetables. The lower the light intensity, the lower the ascorbic acid in plant tissue (Kader, 1987; Nagy and Wardowski, 1988; Shewfelt, 1990). In addition, Weston and Barth (1997) asserted that light availability is necessary for the formation of β-carotene which adds to the nutritional quality of any fruit or vegetable. Further studies have concluded that tomatoes produced in full sunlight contained more sugar and dry matter than those grown in shade. The photosynthetic pathway in plants, which requires the presence

of light, is linked with biosynthesis of L-ascorbic acid and transcriptional genes for precursor molecules (GDP-mannose pyrophorylase and L-galactono-1,4-lactone dehydrogenase) involved in its formation (Weston and Barth, 1997; Valpuesta and Botella, 2004; Getinet, Seyoum and Woldetsadik, 2007).

Temperature and relative humidity are beyond any field producers' control but have profound effects on crop quality (Garcia-Jimenez et al. 2018). For instance, lettuce (*Lactuva sativa* L.) is typically grown in regions with cool days and nights to attain firm but mild flavoured heads. Vegetables respond differently to air temperatures especially extremely high temperatures. Beyond the optimum ranges for a specific crop, extremely high temperatures can lead to moisture loss which in turn affect textural quality, especially in leafy vegetables. Most fruits and vegetables exposed to elevated temperatures, such as those experienced during drought, develop bitter flavours (Weston and Barth, 1997; Kader, 2008). Weston and Barth (2007) also reported that B-vitamins accumulate in temperatures of 10-15°C in green vegetables but accumulation of these vitamins is maximal at 27-30°C in tomatoes.

Relative humidity (RH) affects the water potential gradient whose effect can be traced to plant tissue texture in leafy vegetables and poor fruit appearance. No previous studies on the effect of relative humidity on butternut squash (*Cucurbita moschata*) quality could be found at the time of this study. Texture quality in leafy vegetables can be compromised by reduced gloss, wrinkling and wilting due to exacerbated rates of evaporation brought about by water vapour pressure deficit (WVPD). WVPD is defined as the difference between actual vapour pressure (RH) from the saturated vapour pressure and determines the rate of evaporation from a fresh commodity at the same temperature (Paul, 1999). In a study by Brat et al. (2016) they observed that splitting in bananas is a serious physiological disorder associated with an inverse water influx at high relative humidity (RH) through an osmostic peel-to-pulp water flux resulting from more sugar content in the pulp than in the peel.

2.4.1.3 Harvest maturity

The maturity of fruits and vegetables is known to have a direct relationship with stress tolerance levels when subjected to processing operations. Hodges and Toivonen (2007) postulated that stress tolerance changes with produce maturity. A study by Getinet et al. (2007) on the effect of cultivar, maturity stage and storage environment on quality of tomatoes

concluded that maturity stage at harvest was an important determinant of storage life and final fruit quality. In a study by Garcia-Jimenez et al. (2018) it was established that maximum fat concentration, a major quality attribute in seje palm (*Oenocarpus batana*), can be obtained when the fruit is 80% mature. In this case, optimum quality of the seje palm was obtained at 80% maturing as shown during their study.

Processed slices of unripe conference pears (*Pyrus communis* L.) in a study by Soliva-Fortuny et al. (2004), exhibited less browning and softening than those from riper fruit. The fresh-cut industry prefers firmer and less mature fruit, due to their less-susceptibility to textural deterioration and ease of minimal processing, to improve shelf life duration. This was determined by Gorny et al. Kader (1998) where slices from mature green peaches and nectarine demonstrated significantly prolonged shelf life (8 days for both peaches and nectarines) based on visual quality compared to those from overripe fruits (2 days for peaches and 3 to 6 days for nectarines). This phenomenon can be attributed to increased senescence and rate of biochemical reactions linked to quality deterioration in more mature fruits and vegetables (Hodges and Toivonen, 2007). Furthermore, in a study by Soliva-Fortuny et al. (2002), mature green apple slices maintained their initial firmness and colour as opposed to slices from partially ripe and ripe apples.

2.4.1.4 Farming culture

Fruit and vegetable farmers try to optimize yields and quality using selected cultural practices. Nowadays, processors have been involved, especially through contractual farming agreements, in the cultural practices applied to fruits and vegetables due to the relationship between such practices and quality (Brennan and Grandison, 2012). For instance, in 2011 Woolworths Food South Africa published guidelines, dubbed Farming for the Future (FFF), to monitor fruit and vegetable producers (Woolworths (Pty) Ltd, 2011). Such an initiative was fuelled by the undeniable relationship between farming culture with post-harvest quality of fruits and vegetables and sustainability (Woolworths Food, 2011). Armersto et al. (2020) reported that butternut squash cultivars ‘Ariel’ and ‘Pluto’ organically cultivated (with fermented cow manure 25t/ha) had higher contents of minerals potassium (9%), magnesium (67%), sodium (29%), manganese (approximately 3-fold), zinc (approximately 2-fold) and tocopherol (4-fold) than those cultivated conventionally (with commercial fertilizer N:P:K 8:15:15; 750kg/ha

used). Furthermore, the essential amino acids were 1,3-fold higher than non-essential amino acids in organically cultivated butternut squash crop than in conventionally cultivated crop. On the other hand, crops grown under conventional cultivation achieved higher concentrations of folic acid (15%) and β-carotene than in organically cultivated crops. Conti et al. (2015) found that butternut squash cultivar 'Waltham F_1' produced high yield, mean weight, sucrose levels, total protein, ascorbate levels, nitrites and a greater number of fruits under greenhouse cultivation compared to open field cultivation. On the other hand, open field cultivation produced fruits with better values of total acidity, ash, carotenoids content, glucose, fructose and minerals (calcium, potassium, copper and iron).

2.4.2 Post-harvest factors

2.4.2.1 Harvesting to transportation

Harvesting involves produce collection by picking, cutting, digging or shaking and operations that expose the crop to mechanical damage. There are different types of mechanical damages that can be experienced from harvesting to transportation. These are: impact (e.g. produce dropped from harvester to storage crate resulting in cracks or bruises), abrasion (e.g. when produce rubs against each other when packed closely or conveyed at high speeds), compression (e.g. stacking of containers over the other resulting in excessive static load on produce), puncture (e.g. surface punctures due to fingernails or poorly designed equipment), tears and temperature effects (i.e. chilling and freezing injury) (Tabil and Sokhansanj, 2001). During harvesting, intact produce may be thrown or dumped in containers, possibly meeting hard or sharp container edges or colliding with other products resulting in abrasion and impact injuries. Furthermore, mechanical injury may result when under-trimmed products collide with each other while excessive water loss can be a result of over-trimmed produce. According to Tabil and Sokhansanj (2001), in developed countries where harvesting is mechanized, mostly in potato harvesting, losses due to mechanical damage can be high because of poorly designed equipment. It is also important to note that impact injury may be inflicted on mechanically harvested fruits when they are dislodged from trees (Garcia-Jimenez et al. 2018).

During field packaging and transportation to packhouses, it is common practice that harvested produce is placed in containers such as baskets, sacks, wooden boxes and plastic crates for transport to the packinghouse. However, some containers may be too flexible or have rough

insides causing more harm to the produce rather than protecting it from handling storage. Furthermore, the commodities may be packed too tightly or too loosely causing compression damage and bruising. In some instances, when transport to the packing house is not immediately available, commodity containers often wait under the sun. Tabil and Sokhansanj (2001) stated that mechanical injury and temperature stress are the main culprits for quality loss in packaging houses. During transportation, fruits and vegetables are susceptible to mechanical damage due to the vibrating vehicle, sudden stops and starts, rough roads and absence of refrigeration systems in some cases (Pinillos et al. 2018).

Tabil and Sokhansanj (2001) postulated that depending on the physical characteristics of the produce, mechanical damage experienced from harvesting to transportation results in severe quality deterioration and shortened shelf life. Quality deterioration and reduced shelf life manifest due to various factors such as increased respiratory activity and increased transpiration rates culminating from exposed produce surfaces (brought about by cuts, punctures, cracks, abrasion and tears) that serve as avenues for water loss. Due to damaged produce tissue, increased ethylene production has also been reported which triggers deteriorative changes leading to senescence and decay.

2.4.2.2 Processing factors

It is also important to note that minimally processed fruits and vegetables are living tissues subject to metabolic changes. Shelf life of fresh-cut fruits and vegetables depends on the stress tolerance and stress-induced senescence of the raw material during minimal processing operations. Minimal processing of fruits and vegetables often includes wounding of the plant tissue to produce desired cuts or product characteristics. Minimal processing operations of fruits and vegetables include slicing, dicing, chopping, trimming, peeling, coring, and/or shredding, all of which involve tissue wounding. The way plant tissue is affected by wounding (wound response) is influenced by factors such as cultivar, species, maturity, water vapour pressure, temperatures and cutting protocols (Hodges and Toivonen, 2007). Cutting, slicing, dicing, chopping, peeling and shredding result in significant tissue disruption as previously sequestered enzymes and substrates are now mixed. Hydrolytic enzymes are released and signalling-induced wounding responses occur (Hodges and Toivonen, 2007; Kader, 2008).

Abiotic stress sources include storage temperature, humidity, cutting, cutting-knife sharpness and chemical treatments (Hodges and Toivonen, 2007; Lucera et al. 2012). Fresh produce under abiotic stress will either present with the following indicators: browning, increased respiration, increased ethylene evolution, discolouration, loss of flavour, loss of texture, dehydration, development of off-odours, membrane breakdown and tissue softening. Abiotic stress either inhibits or exacerbates manifestation of metabolic characteristics inherent to the fruit and vegetable. These metabolic characteristics include respiration, ethylene production, phenolic compounds biosynthesis and senescence (Corato, 2020).

Wounding of plant tissues initiates wound-induced signalling at the site of injury (local response) which in turn affects non-injured plant tissue in proximity (systemic response) through the production of phenolic compounds. For instance, in wounded lettuce leaf (*Lactuca sativa* L.), wound-induced signalling arises within 30 minutes of injury and moves into unwounded tissue at 0.5cm/hour resulting in increased respiration rate, production of bioactive specialized compounds such as alkaloids, benzoxazinoides, glucosinolates, terpenoids and cyanogenic glucosides that reduce digestibility by predator insects, activation of wound defence signalling pathways and adjusting plant tissue metabolism to the imposed nutritional demands (Ke and Saltveit, 1989). In addition, it has been recorded that tissue wounding processing operations release numerous volatile compounds which include phenylpropanoids, lipoxygenase derived compounds and terpenoids (Myung et al. 2006). Furthermore, auxin, abscisic acid, active oxygen species (AOS) and jasmonic acid are associated with wounding of plant tissues. Jasmonic acid (JA)-mediated wound signal transduction pathway requires the activation of wound-induced protein kinase (WIPK), which can also be accompanied with the activation of salicylic acid induced protein kinase (SIPK) (Seo et al. 1999; Soliva-Fortuny et al. 2002). Plant tissue wounding and pathogenic infection are known to activate both enzymes WIPK and SIPK which are precursors of the JA-mediated wound signal transduction pathway.

Jasmonic acid is a hormonal molecule involved in plant growth and development as well as in plant stress response. Upon its release, JA is attached to specific receptor sites leading to the activation of wound response genes. The JA pathway induces expression of protease inhibiting substances such as glucosinolates, terpenoids and cyanogenic glucosides which reduce digestibility of plant tissue by pests (Leon et al. 2001). In some instances, release of JA is

simulteneous to the release of ethylene; which consequently increases respiration and senescence. Abscisic acid is also a plant hormonal molecule released during plant tissue stress and is involved in altering metabolism to acclamitise wounded plants to stressful conditions. Exacerbated ethylene production following wound-induced signalling, as mentioned above, results in accelarated respiration calminating to increased water loss (Olorunda and Looney, 1977; Cliffe-Byrnes and O'Beirne, 2005; Iqbal et al. 2009; Moelants et al. 2014). Respiration involves the oxidative breakdown of complex substrate molecules such as starch, organic acids and sugars resulting in the production of energy, CO_2 and water (Rojas-Grau et al. 2009). Water loss brought about by wound-induced response and high respiration rates leads to textural quality deterioration in minimally processed vegetables such as diced butternut squash (Leon et al. 2001). Elevation of phenolic compounds levels during wound-induced respiration is also brought about by wound-induction of phenylalanine ammonium lyase (PAL) (the primary enzyme in phenolic biosynthesis), and the phenolic compounds are in turn oxidised by polyphenol oxidase (PPO) and peroxidase enzymes. Oxidation of phenolic compounds by PPO produce quinones which are heavily linked with browning once polymerized, especially in leafy vegetables like lettuce (Hodges and Toivonen, 2007).

Minimal processing has also been linked with other aspects of product metabolism. Gil, Aguayo and Kader (2006) reported on species-specific cutting-induced loss of antioxidant caretonoids (0-25%) and ascorbate (≤5-25%) during storage in whole pineapples, mango, cantaloupe, watermelon, strawberry and kiwifruit. In addition to visual quality loss, firmness and colour attributes also deteriorated during the storage period. However, cutting of fruits and vegetables can significantly enhance total antioxidant capacity primarily due to increases in wound-induced phenolic levels. In that study by Gil et al. (2006), there were vitamin C losses in all fruits and no carotenoid loss in kiwifruit slices and watermelon cubes whereas pineapples, cantaloupe, mango and strawberry pieces experienced 10-25% losses. However, no significant losses in total phenolics were found in any of the fresh-cut fruit products tested after 6 days at 5°C hence suggesting that the antioxidant capacity was maintained.

Other aspects of cutting during minimal processing such as cutting shape and sharpness of cutting blades have been reported to be influential on final fruit and vegetable quality. In a study by Aguayo, Escalona and Artés (2004a), cut cylinders of melon stored for 10 days were

firmer than slices or trapezoidal sections. In a similar study, papaya (*Carica papaya* L.) slices stored at 5°C and 10°C exhibited a better shelf life than papaya cubes treated under the same conditions (Rivera-Lopez et al. 2005). Several studies have indicated the effect of cutting blade sharpness on fruit and vegetable quality. For instance, it was demonstrated that a melon cylinder cut with a blunt blade exhibited elevated ethanol concentrations, electrolyte leakage, greater ethylene production potential and off-odour scores as compared to samples cut with a sharp blade. In addition, the use of sharp blades has been linked with reduced wound response, softening, microbial growth, white blush and lignin accumulation in fresh-cut carrots (Kramer and Wang, 1989; Aked, 2007; Hodges and Toivonen, 2007; Seroczynska et al. 2014). Blunt blades during cutting culminate in greater plant tissue disruption, enhancing the mixing of plant cell enzymes and substrates, that results in more wound responses than sharp blades.

2.4.2.3 Storage factors

It is of paramount necessity to maintain optimal storage conditions at all points of the food chain from material, through processing, distribution, retailers and purchasers. Spoilage becomes imminent under unfavourable conditions which can be influenced by living organisms such as vermin, bacteria, and fungi, as well as biochemical activities and physical processes which bring about changes like dehydration and bruising (Hodges and Toivonen, 2007). The main factors that affect fruit and vegetable quality during storage include temperature, moisture and humidity. One of the main aims of quality preservation during storage is reduction of respiration rate since fruits and vegetables remain metabolically active even after processing (Aked, 2007; Brennan and Grandison, 2012). Respiration rates of fruits and vegetables differ among species and cultivars as steered by their genotypes. For instance, tissues from shoots, green peas and immature fruits have been recorded to possess high respiration rates and shorter storage periods; while mature fruits, roots and storage organs such as bulbs and tubers respire slower thus have longer storage periods (Brennan and Grandison, 2012).

The rate of biochemical reactions has been proven to be related to temperature, with lower temperatures leading to a slower degradation as compared to food exposed to higher temperatures. It has also been reported that spoilage rates double for each 10°C temperature rise, and that shelf-life doubles for each 10°C reduction in fruits and vegetables (Brennan and

Grandison, 2012). Hodges and Toivonen (2007) also asserted that exposure to cold temperatures after harvesting minimises effects of wound stress by reducing the rate of enzymatic reactions that occur during wound response signalling, thus preserving fruit and vegetable quality. Even if 0°C is the most desirable storage temperature for most fresh-cut vegetables, a storage temperature higher than that which would not induce chilling injury would be preferable. This is the case with many fresh-cut vegetables which are shipped and marketed at temperatures ranging from 5-10°C as mentioned by Aguayo et al. (2004b). It is therefore expedient to farmers, processors and retailers alike to maintain cold temperatures during shipping and handling of fresh-cut fruits and vegetables. Duration of cold storage is also known to have a significant effect on final product quality and intact fruits and vegetables (Hodges and Toivonen, 2007). For instance, it has been reported by Aguayo et al. (2004b) that overall quality declined, microbial load (total plate count, yeasts and moulds) increased and sensory quality declined for fresh-cut tomato stored at 0°C and 5°C for over 14 days. Fresh-cut tomato stored at 5°C experienced more rapid decline in overall and sensory quality in comparison to samples stored at 0°C. Temperature (0°C) was found more effective in reducing microbial load than modified atmosphere packaging. In contrast, the same product held at 5°C experienced increased total plate count at the end of the storage period (14 days).

However, reduced temperature (0-5°C) storage of fresh-cut fruits and vegetables can have adverse effects as seen with the manifestation of chilling injuries. In un-cut fruits and vegetables, symptoms of chilling injury include reduction in tissue firmness, increased electrolyte leakage rates, changes in texture, increased soluble solids content, internal browning, increases in ethylene and CO_2 production. Most of the chilling injury symptoms in fresh-cut and un-cut fruits and vegetables are due to lipid membrane separation, weakened hydrophobic bonding in protein-lipid interactions, protein-protein interactions and cell signalling process (Hodges and Toivonen, 2007).

In addition, sub-optimal chilling (0-5°C) has been reported to accelerate oxidatively induced senescence due to disruptions in electron transport chain-associated membranes and can eventually lead to accumulation of AOS. In the case of humidity, it is necessary to maintain the equilibrium relative humidity (ERH) of the fruits and vegetables during storage. If the ERH is not maintained the product will eventually gain or lose moisture during storage. Uptake of

moisture is known to increase susceptibility to microbial growth (since water activity promotes microbial growth) while moisture loss affects fruit and vegetable texture (Hodges and Toivonen, 2007; Arah et al. 2015).

Currently, many fresh-cut products are stored and marketed in modified atmosphere packaging (MAP) together with chilled storage conditions. The essence of MAP is to ensure that the films' oxygen transmission rate (OTR) is equal to the respiration rate of the packaged product (Hodges and Toivonen, 2007; Hailu et al. 2012). When optimal MAP is applied, substantial reductions in oxidative stress, tissues senescence, respiration rates, ethylene sensitivity, low temperature injury and microbial/insect damage are observed (Hodges and Toivonen, 2007). Optimal MAP conditions differ with plant species since respiration rates of tissues vary among fruits and vegetables (Lucera et al. 2012). However, incorrect application of MAP harbours anaerobic conditions within the package that results in anaerobic biochemical processes. These anaerobic biochemical reactions and high CO_2 levels ultimately affect final product quality through production of ethanol, acetylaldehyde, off-flavours and odours (Hodges and Toivonen, 2007). For instance, fresh-cut cilantro leaves placed in film with 1700 mL/day/m^2 as OTR exhibited a rapid decrease in oxygen and increase in carbon dioxide accompanied by decrease in visual quality, off-odour production and electrolyte leakage. Contrary to the above, cilantro leaves placed in packaging film with an OTR of 3500 L/day/m^2 and 6200 mL/day/m^2 better maintained quality of the leaves in terms of visual appeal, reduced electrolyte leakage and less off-odour production (Luo et al. 2004). Furthermore, Ragaert et al. (2007) ascertained that low O_2 or high CO_2 levels exceeding tolerance limits can lead to textural breakdown in minimally processed tissues. In a study by Gimenez et al. (2003) it was observed that O_2 concentrations in air resulted in accelerated textural breakdown of artichokes compared to samples exposed to appropriate MAP conditions (P-Plus 160 film and P-Plus 210 film).

2.4.2.4 Microbial spoilage

Fruits and vegetables used in the production of ready-to-use (RTU) fresh-cut products are known to harbour indigenous microflora that originate from various sources. Sources of microflora on fruits and vegetable include activities and methods involved in farming, harvesting, handling, processing, packaging and storage in addition to the indigenous populations found on their surfaces (Francis et al. 1999). Psychrotrophic and mesophillic

microbes; both pathogenic and spoilage, are known to be capable of maintaining contamination potential under mild preservation regimes of minimally processed vegetables as noted by Francis et al. (1999). Mild preservation techniques typical to fruit and vegetable processing include strict temperature regimes, modified atmosphere packaging and use of antimicrobial dipping treatments. Oliveira et al. (2011) views minimal processing techniques which include mild preservation regimes as not efficient in eliminating contamination of ready-to-eat (RTE) vegetables with spoilage microorganisms and food-borne pathogens, whereas packing under modified atmosphere can provide a protective barrier against insects, microorganisms (spoilage and pathogenic) and rodents besides extending the shelf life of RTE vegetables

Particular attention has been drawn to psychrotrophic and mesophillic pathogenic and spoilage microorganisms during minimal processing of fresh produce. *Listeria monocytogenes*, *Aeromonas hydrophila* and *Clostridium botulinum* are among the pathogenic psychrotrophs linked with foodborne outbreaks in the past (Francis et al. 1999; Oliveira et al. 2011). Mesophillic pathogens associated with fruits and vegetables include species of the *Salmonella* genus and *E. coli* O157:H7 (Francis et al. 1999). Tournas (2005) mentions that a plethora of bacterial and fungal species are involved in vegetable spoilage after harvesting. Bacterial species include *Erwinia carotovora, Pseudomonas* spp., *Corynebacterium*, *Xanthomonas campestris* and lactic acid bacteria with *Erwinia caratovora* being the most common among vegetables. Fungal species include *Botrytis cinerea*, *Ceratocystis fimbriata* and *Rhizoctonia solani* and species of the genera *Alternaria*, *Aspergillus*, *Cladosporium*, *Colletotridium, Phomopsis*, *Fusarium*, *Penicillium*, *Phoma*, *Phytophthora*, *Pythium* and *Rhizopus*. Among these, *Botrytis cinerea*, *Colletotrichum, Alternaria*, *Cladosporium*, *Phytophthora* and *Rhizopus* spp., affect a wide range of vegetables and fruits.

Devasting economic losses can culminate from microbial deterioration of fruits and vegetables (Tournas, 2005; Raybaudi-Massilia et al. 2009). Economic losses due to activity of spoilage microorganisms emanate from customer complaints charges, rejection of finished products internally or by retailers and brand defamation. Spoilage microorganisms related to short shelf-life and textural deterioration have been reported in a study by Bruno et al. (2005). In that study, high populations of psychrotrophic aerobic bacteria were found in spinach, kale, watercress, cabbage, chicory, spring onion and arugula. Coliforms were detected in most of

the samples which also contributed to decreased shelf life of the products. The high bacterial population was attributed to poor hygiene quality of the raw material, lack of good hygienic practices as well as inadequate storage conditions. According to Ragaert et al. (2007), different microorganisms are able to produce pectinolytic enzymes involved in degradation of the middle lamella and primary cell walls hence influencing textural changes. The most isolated pectinolytic bacteria regarding minimally processed fruits and vegetables are *Erwinia* and *Pseudomonas* species (Ragaert et al. 2007). The range of pectinolytic enzymes include pectin methyl esterase (PME), pectate lyase, polygalacturonase and pectin methyl esterases.

It is of paramount importance to achieve optimum conditions when using mild preservation techniques to reduce or inhibit microbial growth. Francis et al. (1999) highlighted that ill-use of MAP and antimicrobial solutions in conjuction with recommended storage and processing temperatures can have adverse effects. For instance, misguided use of an antimicrobial solution can ineffectively reduce or inhibit growth of pathogenic microbes but, at the same time, effectively reducing populations of spoilage microorganisms. Use of such mild preservation could therefore promote pathogenic microbes even if shelf life is significantly extended. It is therefore necessary for fruit and vegetable processors to optimise or use multiple preservation techniques in light of the aforementioned possibilities (Francis et al. 1999).

2.5 Quality retention in fresh-cut fruits and vegetables

A number of technologies on every stage from harvesting, through processing, to packaging and storage can be applied to retain quality or extend the shelf life of fruits and vegetables. This section focuses on the novel and traditional shelf-life extension techniques that apply to fresh-cut fruit and vegetable processing. These techniques can be grouped into three categories; physical, chemical and biological methods (Rico et al. 2007; Ma et al. 2017).

2.5.1 Physical treatments

2.5.1.1 Modified atmosphere packaging

Modified atmosphere packaging (MAP) is one of the most successful fresh-cut fruit and vegetable preservation methods which is currently used in the industry that involves packaging products in altered gaseous compositions (Rico et al. 2007; Ma et al. 2017). Reduced O_2 levels and increased CO_2 in comparison to atmospheric levels of 20.95% and 0.04% respectively, normally work against the inevitable respiration rates of fresh-cut produce. This phenomena is

common among fresh-cut products that are more tolerant to higher CO_2 concentrations than their intact counterparts (Rico et al. 2007). Even though increased concentrations of CO_2 are known to be both bacteriostatic and fungistatic, it is necessary to maintain low temperatures for the carbon dioxide to be effective (Ohlsson, 1994). Ohlsson (1994) also asserted that most fresh and prepared (RTU and RTE) foods are distributed and stored at about 20-100% CO_2 concentrations at a 1-5°C temperature range. However, uncontrolled CO_2 levels and low O_2 levels can have adverse effects on quality due to fermentation brought about by anaerobes. To counter this, MAP normally uses perforated films to allow controlled escape of CO_2 produced from plant tissue respiration (Ohlsson, 1994; Rico et al. 2007; Ma et al. 2017).

An adequate O_2 concentration inside packages is required to limit aerobic respiration without triggering anaerobic processes (fermentation). In a study by Soliva-Fortuny and Martin-Belloso (2003) an increased growth of mesophillic anaerobic microorganisms was observed at low O_2 levels (0 kPa O_2) than at higher oxygen levels (2.5kPa O_2 + 7kPa CO_2) during modified atmosphere packaging of fresh-cut conference pears. It is also essential to note that different materials for MAP yield varied shelf life results in fresh-cut fruits and vegetables due to differences in oxygen transmission rates that determine gasesous conditions. This was evidenced in research by Hailu, Workneh and Belew (2014) when they observed that using high density and low density polyethylene bags for bananas resulted in 43.0% and 41.2% decay loss at day 36. Unpackaged banana fruits (control) experienced a 16% decay loss at day 15. It was concluded that packaging banana fruits in high density and low density polyethylene bags results in longer shelf life and improved quality followed by the other two packaging materials, dried banana leaf and teff straw, respectively.

2.5.1.2 Active packaging

Active packaging can be defined as an intelligent system that involves the chemical interaction between package or package components and food or internal gas atmosphere. Active packaging also ought to comply with consumer quality and safety demands (Ozdemir and Floros, 2004). Ozdemir and Floros (2004) also ascertained that active packaging systems are mostly concentrated in the fresh produce industry offering a promising alternative to passive MAP in shelf-life extension of fresh-cut fruits and vegetables. The commonly used active packaging method involves use of O_2 adsorbents or scavengers such as Fe^{2+} ions. These agents,

available in sachets or tablets, reduce headspace O_2 levels by oxidation reactions, thus reducing proliferation of certain pathogenic and spoilage aerobic microorganisms (Ohlsson, 1994). Antimicrobial chemical preservatives can be used in active packaging where organic acids and their salts (sorbates, benzoates and propionates), epoxides, alcohols, ozone, hydrogen peroxide, antibiotics and bacteriocins can be applied as multi-layer films. These multi-layer films consist of four layers which include an outer layer, barrier layer, matrix layer and a control layer where the antimicrobial is released from unto the food surface as illustrated in Figure 1 (Ozdemir & Floros, 2004).

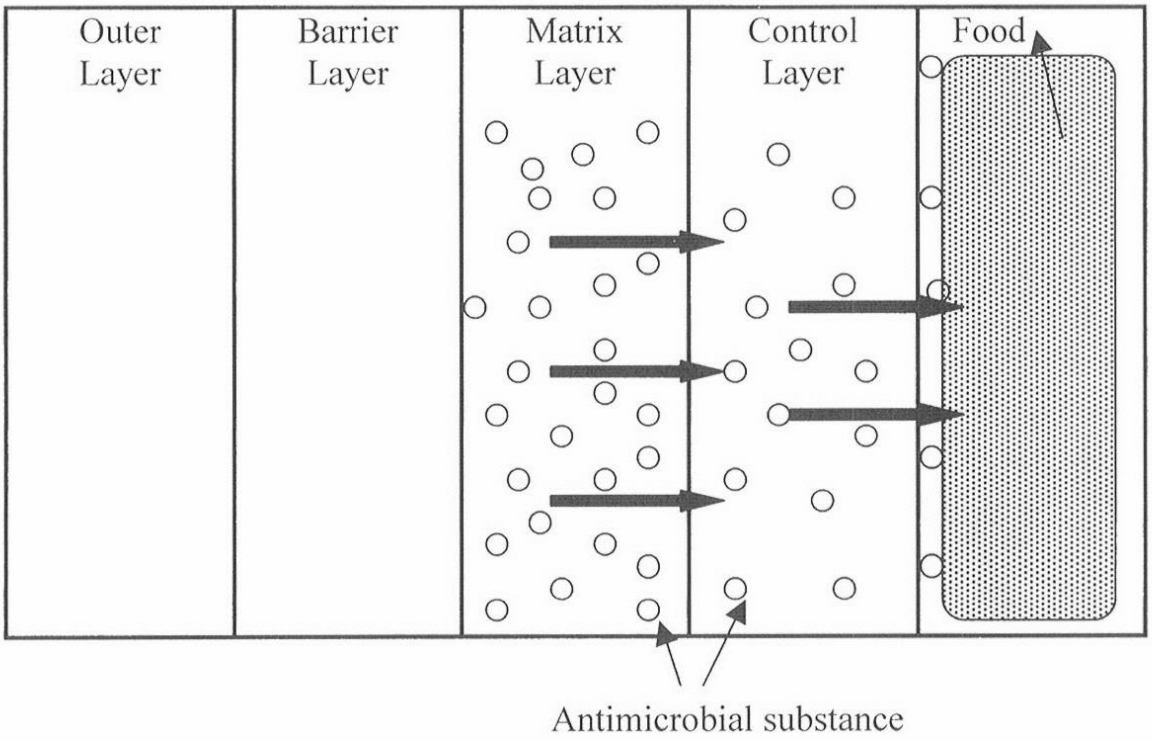

Figure 1: A structure of typical antimicrobial multi-layer active film (Ozdemir and Floros, 2004).

2.5.1.3 Pressurized inert gases

During application of pressurized inert gases, noble gases dissolve in water resulting in hydrophobic hydration, hence restraining intracellular water, and inhibition of enzymatic reactions, consequently restraining fruit and vegetable metabolism, therefore, prolonging shelf life. Inert gases used include argon (Ar), nitrogen (N_2), xenon (Xe), neon (Ne) and krypton (Kr), and their pressurization in water forms ice-like crystals called clathrate hydrate (Purwanto et al. 2001). These ice-like crystals of clathrate hydrate (stable above 0°C) are formed by the

entrapment of gas molecules in a cage-like structure by water molecules through van der Waals forces bonding (Ma et al. 2017). In a study by Zhang et al. (2008), shelf life of fresh-cut green asparagus spears was extended from 3-5 days to 12 days at 4°C using a mixture of Ar and Xe under 1.1MPa for 24 hours. Furthermore, ascorbic acid, chlorophyll, yeast and mould growth, cellular deterioration and water loss were significantly reduced in a study by Meng, Zhang and Adhikari (2012) when fresh-cut green peppers were pressurized with Ar at 4 MPa for an hour. The pressurized Ar achieved this by inhibiting activities of catalase (CAT), peroxidase (POD) and malondialdehyde (MDA) which indicates oxidative stress in the fresh-cut green peppers. Since water loss is strongly associated to textural quality deterioration in fresh-cut produce (Cliffe-Byrnes and O'Beirne 2005), the aforementioned study by Zhang et al. (2008) implicitly concludes that use of pressurized inert gas can preserve textural quality of fresh-cut produce.

2.5.1.4 Ultraviolet light (UV)

UV light is a type of non-ionizing radiation with wavelenghts ranging from 100nm-400nm and is classified into UV-A (315-400nm), UV-B (280-315nm) and UV-C (100-280nm) (Ma et al. 2017). UV-C at 254nm has been found to provide the maximum antimicrobial effects in comparison to the other two which are hardly used in food processing. Quantum energies from UV-A, UV-B and UV-C, which are 4-3.3 eV, 4.4-4 eV and 6-4.4 eV respectively, are not sufficient to cause ionisations but could lead to selective absorption and excitation when interacting with mattter. Essential biomolecules like DNA are known to absorb UV radiation and the consequential excitement leads to photochemical alterations causing biological effects like cell inactivation or mutations (Keifer, 2007). The germicidal effects of UV radiation can either be direct or indirect. The direct effect is due to the DNA damage that it brings to microbial cells. As a consequence of DNA damage, photoproducts such as cyclobutane pyrimidine dimers and purimidine 6-4 pyrimidone are formed after exposure to UV light. These susbtances inhibit transcription and replication and eventuallly lead to mutagenesis and cellular death (Ma et al. 2017).

Advantages include inhibitory effects against most pathogenic and spoilage microorganisms, wide applicability to fresh produce, relatively inexpensive and the easy-to-use equipment needed (Rico et al. 2007; Ma et al. 2017). However, high UV doses are associated with tissue damage and compromise on fresh-cut produce sensory and nutritional traits culminating from

cellular DNA damage (Rico et al. 2007). Allende and Artes (2003a) concluded in their study that UV-C (254nm) reduced microbial load in minimally processed lettuce but resulted in increasesd respiration rate, stress and possibly induced a lignification-like process. The lignification-like process was attributed to plant tissue stress response biochemical reactions leading to production of lignin, which in turn decreased the textural quality of lettuce. Research by Manzocco et al. (2011) found that total viable counts of fresh-cut apples treated with 1.2 kJ/m^2 UV-C dose had about 2 log units lower than the untreated control for up to 8 days at 6°C.

In terms of textural quality retention, a study by Rodoni et al. (2015) showed that firmness of UV-C treated peppers was 50% higher after 12 days at 4°C due to reduction of spoilage microorganisms in comparison to untreated peppers. However, there have been disadvantages associated with application of UV-C treatment in some fresh-cut fruits and vegetables. In a study by Pan and Zu (2012), it was discovered that extended exposure of fresh-cut pineapples (*Ananas comosus* L. Merr. cv. Comte de Paris) to UV-C treatment from 60s to 90s resulted in accelerated browning and significant vitamin C losses. Accelerated browning was attributed to the induction of browning enzyme PPO by UV-C exposure. Similarly, Kasim et al.(2008) reported increased eletrolyte leakage and weight loss in fresh-cut green onion (*Allium cepa* L.) at high UV-C dosages (10 minute and 15 minute UV-C exposures respectively) in comparison to no UV-C exposure (control).

2.5.1.5 Irradiation

Rico et al. (2007) reported that application of low dose gamma radiation is very effective in reducing bacterial, parasitic and protozoan pathogens. This method was approved by the FDA for use in the fruit and vegetable industry at a maximum level of 1.0 kGy (Rico et al. 2007). The frequently applied food irradiation involves exposure of cobalt-60 or casium-137 isotopes (Ma et al. 2017). Irradiations' mode of action towards microorganism inactivation is mainly attributed to destruction of DNA structure together with enzyme and membrane protein denaturation, resulting in loss of reproductive and metabolic capabilities. This method of shelf life extension is known to cause minimal modification on the flavour, colour, nutrients, taste and other quality properties of foods since the radiation levels minimally change the structure

and physiology of plant tissues (Ma et al. 2017). However, carcinogenic potential and public misunderstanding of this technique are challenges currently being faced (Ma et al. 2017).

Another method called electron beam irradiation (EBI) was developed to address the sceptism on the use of radioactive isotopes in conventional irradiation applying gamma radiation (Ma et al. 2017). Electron beam irradiation does not use radioactive isotopes to eliminate microbial contamination but rather, uses machines that generate electron beams at speeds close to that of light at high energy levels and inactivates microorganisms in the same manner as in application of gamma irradiation (Mami et al. 2014).

2.5.1.6 Pulsed light (PL)

This non-thermal method has gained much attention in rapid surface decontamination of food and packaging material. Pulsed light inactivates microorganisms through short-duration and high-power pulses. The power pulses are generated from an inert gas lamp, mainly xenon, and involves a broad spectrum of white light. The spectrum of white light generated during the short outbursts of pulses ranges from UV to near infrared (Ma et al. 2017). The mechanism of microbial inactivation lies in its photochemical effect on structural changes in the cell and DNA of microorganisms similar to the UV radiation mode of action described above (Heinrich et al. 2015). However, this method can result in texture, nutritional and sensory deterioration in fruits and vegetables since its effectiveness has been reported to depend on the type of vegetable or fruit under treatment. Possible changes in sensory and nutritional quality are attributed to the effects brought about by the power impulses on plant cell membranes through structural disruptions (Ma et al. 2017).

2.5.1.7 Cold plasma

This is a non-thermal antimicrobial treatment method that uses a quasi-neutral ionized gas comprised of photons, free electrons, positive and negative ions, excited or non-excited atoms and molecules (Fernandez et al. 2012). This quasi-neutral ionized gas is the plasma and is generated by application of energy forms such as microwaves, electricity, magnetic field, alternating and direct current, on mixtures of gases (Niemira, 2012). The current gas mixture frequently used is composed of air, oxygen, nitrogen, helium, argon and neon. Active or ionized gas particles in plasma react with microorganisms present on food surfaces (Niemira,

2012). Consequently, cellular damage and DNA strands breakage of microorganisms occur, limiting their chances of survival (Niemira, 2014). Since cold plasma treatment is a waterless, contact-less and chemical-free technique to inhibit microbiological growth, it has been successfully applied to different food products (Smeu and Nicolau, 2014). Critzer et al. (2007) successfully determined the antimicrobial effects of cold plasma treatment against *E.coli* O157:H7, *Salmonella* spp. and *Listeria* spp. inoculated on apple, cantaloupe and lettuce surfaces respectively. Reduction of spoilage microorganisms on fruit and vegetable surfaces leads to better shelf life consequently.

2.5.2 Chemical treatments

2.5.2.1 Calcium-based solutions

Use of calcium-based solutions such as calcium lactate, calcium carbonate, calcium citrate, calcium chloride, calcium phosphate, calcium propionate and calcium gluconate is known for texture preservation (Martin-Diana et al. 2007). Calcium fortification in foods has gained popularity globally due to increased consumer nutritional awareness and evidence linking osteoporosis, hypertension and cancer to calcium deficiency (Martin-Diana et al. 2007a). Calcium carbonate and calcium citrate are the main calcium salts added to foods in order to enhance the nutritional value and the selection of the appropriate calcium source depends on bioavailability, solubility, flavour change and interaction with food ingredients. Calcium treatment of minimally processed fruits and vegetables results in functional value-added products through the incorporation of high calcium quantities to the fruit and vegetable matrix. It is also important to note that adequate calcium intake is associated with reduced risk of osteoporosis, hypertension, colon cancer, kidney stones and lead absorption (Pereira et al. 2010).

Calcium lactate, calcium propionate and calcium gluconate are known to improve firmness in fresh-cut fruits and vegetables and avoid disadvantages brought about by calcium chloride such as bitterness and residual flavour (Martin-Diana et al. 2007). Calcium chloride (E509) is commonly used in the food industry as a firming agent for canned tomatoes, whole apples, whole hot peppers, whole and sliced strawberries, diced tomatoes, whole peaches and cucumber pickles (Luna-Guzman and Barrett, 2000). However, due to residual amounts of calcium chloride on product surfaces following dipping treatment, there is an increased

likelihood of bitterness detection by the consumer. In a study by Luna-Guzman and Barrett (2000) comparing the effectiveness of calcium chloride and calcium lactate in maintaining shelf life stability of fresh-cut cantaloupes, it was concluded that the former imparted undesirable bitterness to the fruit pieces. Luna-Guzman and Barrett (2000) also point out that the use of calcium lactate as an alternative to calcium chloride is commendable with its successful use at 0.5–2% concentrations as a firming agent in strawberries having been reported. Calcium lactate as a firming agent was affirmed as generally regarded as safe (GRAS) and can be used with no level limitation other than good manufacturing practices (Food and Nutrition Board, 1981; U.S.Food and Drug Administration, 2019; Electronic Code of Federal Regulations, 2020). Good manufacturing practices encourage the use of a food substance or additive at the minimal amount known to invoke the intended purpose (U.S. Food and Drug Administration, 2019). In a study by Pereira et al. (2010), compression tests on guavas treated with 15 g/L calcium lactate showed a firming effect on fruit texture. The same study also concluded that calcium lactate treatment reduced the respiration rate of guava products, showing O_2 and CO_2 concentrations around 18% and 3% respectively, inside the packages. Calcium salts (chloride, citrate, lactate, ascorbate, tartrate, silicate and propionate) were used in a study by Silveira et al. (2011) as dipping treatment (0.4% at 60°C) for fresh-cut 'Galia' melon quality retention and positive results were obtained. At the end of the 10 day storage at 5°C, calcium ascorbate, chloride and lactate provided melon pieces with a lower respiration rate, increased tissue total calcium content and maintained an acceptable firmness.

The rate of senescence in fruits and vegetables can be attributed to tissue calcium status and increasing calcium salts, e.g. during calcium treatment, results in alteration of various senescence parameters such as respiration, protein content, chlorophyll content and membrane fluidity. Post-harvest calcium treatment of apples has been found to reduce senescence-associated microviscosity of tissue cell membranes hence delaying senescence (Poovaiah, 1986). Changes in cell wall structure, membrane permeability and enzyme activation influence cell physiology. Calcium is essential for the structure and function of cell walls and membranes as evidenced by three types of mechanisms. Firstly, profound deterioration of cell membranes occurs under calcium deficient conditions and secondly, calcium alters the architecture of membranes by changing fluidity and permeability. Thirdly, calcium turns on the active

transport of ions through membranes therefore altering an array of physiological activities associated with membrane function (Lamikanra and Watson, 2004; Martin-Diana et al. 2007).

Dipping treatments for calcium-based solutions are commonly used for fresh products but other sectors of industry use impregnation techniques. Ca^{2+} ions (0.1 nm) passively diffuse through the cell wall structure since plant cell porosity is approximately 3.5nm–9.2nm (Ngamchuachit et al. 2014). The calcium component in these solutions maintains fruit and vegetable cell wall integrity by cross-linking with the cell wall and middle lamella (Rico et al. 2007). Toivonen and Brummel (2008) further explained that calcium acts in two ways, firstly by ionic bridges formed by calcium ions between demethylesterified pectin molecules, specifically on the free carboxyl groups, to produce cross-linked polymer networks in the middle lamella, thus improving cell-to-cell adhesion (Figure 2). Even though slower, the second way relates to Ca^{2+} attraction to plasma membrane phospholipids. Ngamchuachit et al. (2014) postulated further that the initial firming effect of calcium dipping treatments culminates from cross-linking of Ca^{2+} ions to the homogalacturonan in the middle lamella and cell walls, while the subsequent firming during storage can be accounted to interaction of Ca^{2+} ions with negetively charged plasma membrane phospholipids and proteins.

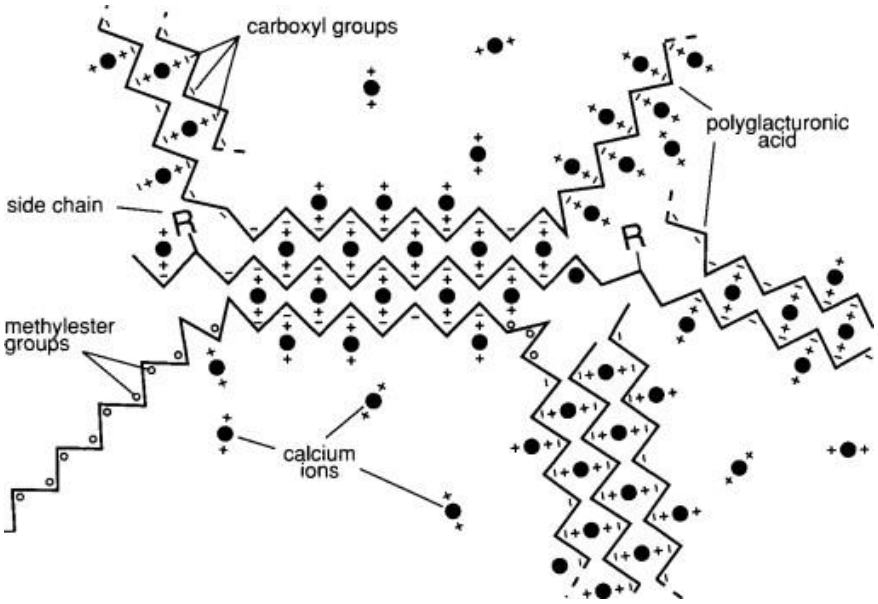

Figure 2: Formation of pectin ionic cross-bridges in the presence of Ca^{2+} ions.

2.5.2.2 Acidic electrolyzed water (AEW)

This method involves the use of electrolyzed water to inactivate pathogenic and spoilage microorganisms in food. Acidic electrolyzed water is produced by passing a diluted solution of either sodium chloride or potassum chloride through an electrolytic cell. The anode side of the cells produces compounds and ions such as HOCl, OCl^- and Cl_2 gas (Ma et al. 2017). Eletrolytes of 0.1% sodium chloride solution generates electrolyzed water, producing acidic EW (electrolyzed water) (AC-EW) and alkaline EW (AK-EW) in separate anode and cathode compartments, respectively. Contrary to the known bacteriocidal activity of AC-EW (pH < 2.5), AK-EW (pH>11.5) has not been reported to have any sanitizing properties (Park et al. 2009). Acidic electrolyzed water effectively inactivates *E.coli* O157:H7, *Salmonella enteritidis*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Campylobacter jejumi*, *Enterobacter aerogenes*, *Staphylococcus aureus* and *Bacillus cereus*. It was concluded in a study by Bari et al. (2003) that treatment with 200 ppm chlorine water and AC-EW reduced the number of tested pathogens (*E.coli* O157:H7, *Salmonella enteritidis*, *Salmonella typhimurium* and *Listeria monocytogenes*) by 4.69log CFU-4.87log CFU and 7.46log CFU-7.85log CFU per tomato respectively. In addition, AEW offers minimal negetive effects on human health and sensory and nutritional properties hence being widely used as an alternative to chlorine decontamination in fresh-cut fruits and vegetables (Park et al. 2009). This method is also non-corrosive to skin, mucous membranes and organic material (Ma et al. 2017).

2.5.2.3 Nanotechnology

This technology involves the use of materials with one or more dimensions in the range of 1-100 nm in length (Ma et al. 2017). Silver nanoparticles are well known for their antimicrobial properties against a wide variety of microorganisms as reported by Kuorwel et al. (2015). Their antimicrobial mechanism involves silver ions which can disrupt or kill microorganisms through DNA damage, binding of protein, activation of antioxidant enzymes and structural changes in the cell wall and nuclear membrane (Mastromatteo et al. 2015). However, Ma et al. (2017) emphasized that nano-sized particles may cause sensory changes in foods and potential toxicity risks are associated with this technique. Saifullah et al. (2019) mentioned that to increase flavour compound availability and limit flavour degradation in food, nanoencapsulation is the most effective technique and does so by protecting flavour

compounds from oxidative and thermal degradation. Flavour encapsulation is common in the manufacture of flavour powders which are in turn used in confectionery products, instant beverages, instand desserts, extruded snacks and baked foods. Very little is known on the effects of nanotechnology on fresh-cut produce sensory and nutritional quality with research material still scarce.

2.5.2.4 Ozone

This has been successfully used as a decontamination alternative to chlorine in minimal processing of fruits and vegetables (Ma et al. 2017). In addition to being a successful alternative to chlorine decontamination, application of ozone is better than chlorine decontamination which tends to leave chlorine flavours due to residues but no differences have been discovered in terms of textural and visual quality between the two treatments. Its success in the minimal fruits and vegetable industry was more evident especially when it was granted generally regarded as safe (GRAS) in 1997 by FDA (Tzortzakis et al. 2007). Its antimicrobial action involves the reaction of ozone (oxidation by hydroxyl free radicals) with intracellular enzymes, nucleic material, spore coats and viral capsids of microorganisms. Hydroxyl radicals, which are the principal reactive oxidizing agents are generated when ozone decomposes impulsively during water treatment and inhibit bacteria and viruses (Krasaekoopt and Bhandari, 2010).

A number of studies have been successfully performed on the microbial reduction of applying ozone to fresh-cut produce. For instance, tomatoes, strawberries, table grapes and plums were inoculated with *Botrytis cinerea* (grey mould) in a study by Tzortzakis et al. (2007) and treated with low-level ozone enrichment (0.1 µmolmol.$^{-1}$). Results of the research showed a substantial decline in spore production. Furthermore, Karaca and Velioglu (2014) demonstrated that gaseous ozone treatment (950 µL/L for 20 minutes) contributed to 1.0log–1.5log per gram reduction of *E.coli* and *Listeria innocua* inoculated on fresh-cut lettuce and spinach. Regardless of its success, this method has been reported to be associated with headaches, irritation, lung damage and chronic respiratory diseases, with its increased exposure and concentration (De Candia et al. 2015).

2.5.2.5 Chlorine and chlorine dioxide (ClO_2)

Chlorine compounds are usually used at concentrations of 50-200 ppm with contact times less than 5 minutes (Rico et al. 2007). Rico et al. (2007) asserted that chlorine decontamination, in forms such as liquid chlorine and hypochlorite, is probably the most widely used sanitizing agent for decontamination of fresh produce. ClO_2 has a high oxidation capacity which is 2.5 times greater than that of Cl_2 (Rico et al. 2007). This has been shown to inactivate microorganisms such as *Listeria monocytogenes* and *Salmonella typhimurium* in lettuce leaves (Lee et al. 2004). A study by Guo et al. (2013) made a conclusion that treatment of fresh-cut melon fruit with chlorine dioxide results in decreased respiration and ethylene synthesis which in turn extends the overall shelf life of the product.

Total respiration in ClO_2 treated fresh-cut produce is reduced by inhibition of alternative respiration and cytochrome pathway respiration. Alternative respiration in plant tissue involves the alternative oxidase (AOX) pathway which is also an aerobic respiration pathway together with the glycolysis pathway, tricarboxylic acid cyle (TCA) pathway, pentose phosphate pathway (PPP) and the electron transport system. ClO_2 reduces ethylene production by suppressing the expression of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO) genes (*CmACS2*,*CmACO1* and *CmACO3*) in melon fruit. ACS catalyses the reaction from S-adenosylmethionine to ACC which is a key rate limiting step in ethylene production while ACO catalyses ACC to ethylene (Guo et al. 2013).

2.5.2.6 Organic acids

These include acids such as lactic acid, citric acid, acetic acid and tartaric acid and they have been reported to have strong antimicrobial activity against psychrotrophic and mesophilic microorganisms in fresh fruits and vegetables (Bari et al. 2005). Their mode of action involves pH reduction, thus disrupting cell membrane permeability, reducing intracellular pH and resulting in ion accumulation in cells of microorganisms. Through their aforementioned mode of action when used as treatments in fresh-cut produce processing, growth and survival of spoilage microorganisms responsible for overal quality and textual deterioration such as species of *Erwina* and *Pseudomonas* was retarded (Rico et al. 2007).

2.5.3 Biopreservation

2.5.3.1 Bacteriophages

This method involves the use of microorganisms to infect and multiply in the respective host bacterial cells to decontaminate food (Ma et al. 2017). The microorganisms are environmentally-friendly and harmless to humans and animals. Leverentz et al. (2001) indicated that a bacteriophage mixture significantly reduced *Salmonella* counts in fresh-cut honey melon stored at 5, 10 and 20°C, respectively. In addition, a significant reduction in *L. monocytogenes* counts was observed when a mixture of bacteriophages was used in a study by Leverentz et al. (2003). Ma et al. (2017) added that much research has focused on the antimicrobial effects of bacteriophages but less has been discovered on their effect on sensory (texture, colour and flavour) and physiochemical properties of fresh-cut fruits and vegetables.

2.5.3.2 Bacteriocins

Bacteriocins are antimicrobial peptides or proteins produced by bacteria capable of reducing survival of pathogenic and spoilage microorganisms (Balciunas et al. 2013; Ma et al. 2017). For instance, lactic acid bacteria are widely known for producing bacteriocins (Oliveira et al. 2014). At the moment, two bacteriocins are commercially available; which are nisin, produced by *Lactococcus lactis* and carnocyclin A, from *Carnobacterium maltoramaticum* UAL307 (O'Connor et al. 2015). Nisin has been used commercially for over 50 years against Gram positive bacteria and carnocyclin A is an approved biopreservative in the USA and Canada developed to inhibit *Listeria monocytogenes* in ready-to-eat meat products only. Nisin acts on the cell membrane forming pores that result in cellular death (Meireles et al. 2016). Bari et al. (2005) used 50 ppm nisin on mung bean and broccoli resulting in *L. monocytogenes* reduction by 2.20 and 4.35 log CFU/g, respectively. O'Connor et al. (2015) mentioned that the main advantage of bacteriocin use in fresh-cut fruits and vegetables quality preservation lies in their ability to preserve without affecting sensory qualities. However, research is still being done in order to further identify and use bacteriocins that are more specific to spoilage and pathogenic microorganisms in the fruit and vegetable industry.

2.5.3.3 Bioprotective microorganisms

This technique involves the introduction of competitive microorganisms to reduce growth or survival chances of spoilage and/or pathogenic microbes (Meireles et al. 2016). These

microorganisms are not harmful to humans such as lactic acid acid bacteria and have a GRAS status. Ma et al. (2017) mentioned that bioprotective microorganisms isolated from lactic acid bacteria have shown great potential in their applicability in the fresh-cut fruits and vegetables industry. For example, in a study by Russo et al. (2015), growth of *L. monocytogenes* was inhibited by strains of B2 *Lactobacillus plantarum* and PBCC11.5 of *Lactobacillus fermentum* in fresh-cut cantaloupe. Antagonistic traits of lactic acid bacteria can be ascribed to production of antibiotics or substances such as acidophilin, lactic acid, in addition to nutrient depletion and a decrease in reduction-oxidation potential resulting in competitive antagonism (Visser et al.1986).

2.5.4 Sensory quality determination in fresh-cut produce`

Nicoli (2012) postulated that food quality is a dynamic state constantly moving to reduced levels and suggested the use of well-defined acceptability levels on a quality decay curve to determine shelf life as exemplified in Figure 3. As illustrated in Figure 3, the quality levels intersect with the acceptable level at day 4 (secondary shelf life = 2 days) and 5 (primary shelf life = 5 days) for the opened and unopened pack respectively. According to Nicoli (2012) food shelf life and safe life are different even though inextricably linked, with the former expected to be shorter than the latter.

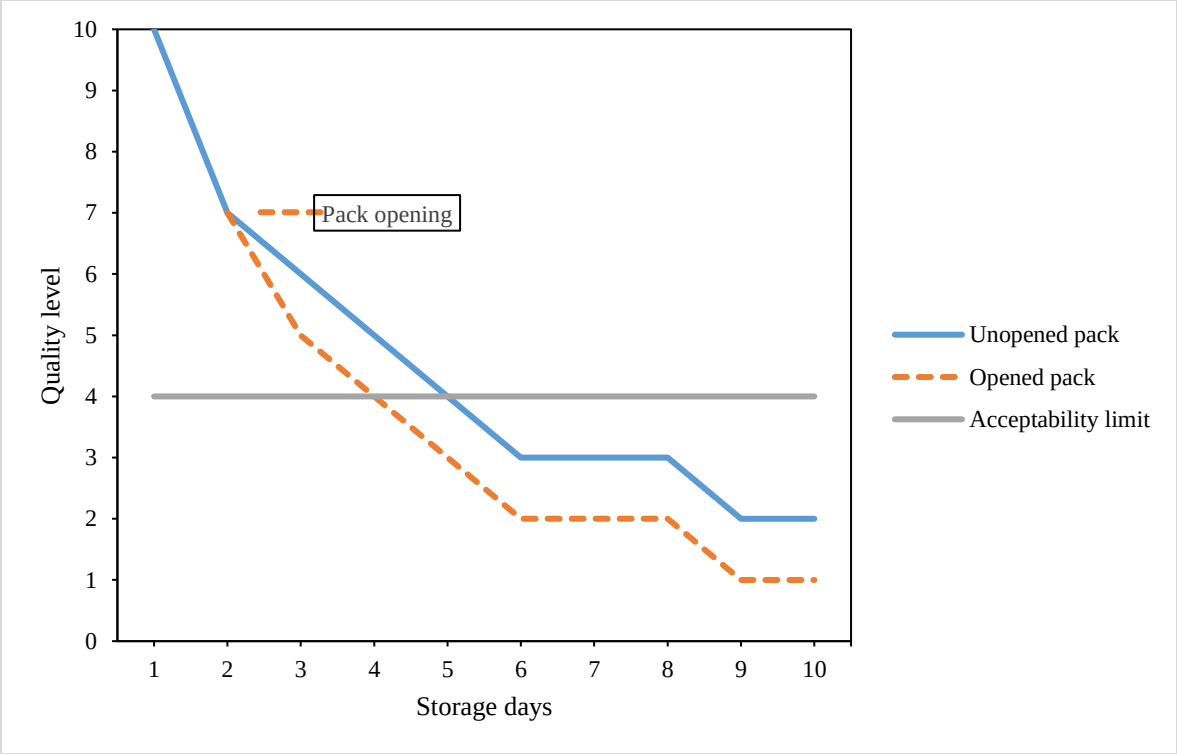

Figure 3: Quality decay curves with acceptability level used for primary shelf life determination

2.5.5 Conclusion

Throughout the years, various methods have been developed, with ongoing research, to preserve the shelf life and safety of fresh-cut fruits and vegetables, even though some are yet to be commercially successful. In spite of these developments, some of the shelf life preservation techniques discussed are expensive and not feasible for a typical vegetable processing plant in a developing country. Furthermore, much of the studies on shelf life preservation of minimally processed vegetables, as shown in the above literature review, have unearthed knowledge mostly on leafy vegetables and less on vegetables of the *Cucurbitacaea* family. In particular, use of mild preservation techniques on fresh-cut butternut squash (*Cucurbita moschata*) on a commercial scale has yet to be fully explored. In the following chapters, the researcher investigated how calcium lactate treatment impacts the textural quality and shelf life of fresh-cut butternut squash cultivars. In South Africa, calcium lactate is readily available, fairly affordable to food processors, has a GRAS status and the minimal processing techniques elaborated in this study are typical of the basic unit processes found in a vegetable processing plant.

3 HYPOTHESES AND OBJECTIVES

3.1 Hypothesis 1

Dipping fresh-cut butternut squash (*Cucurbita moschata*) cultivars 'Atlas' and 'Pluto' in calcium lactate solutions will extend final product texture quality. Furthermore, there will be a difference in final product firmness between 'Atlas' and 'Pluto' fresh-cut butternut squash determined by a descriptive sensory panel. Pectin is a polysaccharide which provides mechanical strength and tissue firmness in plant cells, existing within the cell walls and middle lamella of plant cells in a cross-linked network. Calcium ions form ionic bridges with the free carboxylic groups on demethylesterified pectin molecules resulting in a cross-linked pectin network, therefore, increasing plant tissue mechanical strength by improving cell-to-cell adhesion and the strength of cell wall constituents (Rico et al. 2007; Toivonen and Brummell, 2008). Due to variation in genetic make-up amongst cultivars of the same plant species, significant differences have been shown to manifest in texture quality as well as the content of bioactive compounds in fresh-cut produce (Weston and Berth, 1997; Priori et al. 2017). Fresh-cut produce texture quality can vary due to differences in texture-related metabolic rates (e.g. demethylesterification) and plant tissue physical anatomy (cell size, shape and packing) between cultivars emanating from genetic variations (Toivonen and Brummel, 2008).

3.2 Hypothesis 2

Fresh-cut butternut squash stored at 3.5°C will retain texture for a longer period than fresh-cut butternut squash stored at 10°C. Storage temperature has been shown to affect the quality of fresh-cut produce. Higher storage temperatures accelerate physiological activity within plant tissue. At higher temperatures, exacerbated respiration rates, transpiration rate, tissue softening, enzymatic browning and wound signalling losses have been reported in fresh-cut produce due to increased intracellular metabolic rates. Increased intracellular metabolic rates are linked with exacerbated rates of pectin breakdown by pectinolytic enzymes whose activity is directly proportional to storage temperatures, consequently resulting in plant tissue softening due to loss of pectin related mechanical strength (Tsouvaltzis et al., 2006; Bett-Garber et al. 2011; Benitez et al.2012).

3.3 Objective 1

To determine the effect of calcium lactate dipping treatments of 0.5% (w/v) and 3% (w/v) on the texture of fresh-cut butternut squash and to determine the preferred butternut squash cultivar between ‘Atlas’ and ‘Pluto’ for minimal processing with the aim to reduce economic losses and customer complaints arising from textural deterioration and guide vegetable processors towards procurement of a process-suitable cultivar.

3.4 Objective 2

To determine the effect of storage temperature on the texture of fresh-cut butternut squash with the aim to address possible cold chain integrity deviations that result in rapid fresh-cut butternut textural deterioration.

The objectives of this study are reported as individual research chapters and are presented as such. The overall findings of the study are discussed together as general discussion where respective conclusions and recommendations are also presented.

4 RESEARCH

4.1 Effect of calcium lactate dipping treatment on texture quality of fresh-cut butternut squash cultivars

4.1.1 Abstract

Fresh-cut butternut squash faces the challenge of texture retention during storage due to rapid pectin degradation culminating from disrupted plant cell integrity and intracellular decompartmentalization brought about by peeling, cutting, coring and dicing. In this research section, the effect of calcium lactate dipping treatments (0.5% and 3% w/v) on the texture quality of fresh-cut butternut squash cultivars 'Atlas' and 'Pluto' were investigated. There were no significant differences (p>0.05) identified during physical (fruit size dimensions and texture) and physicochemical (pH, °Brix, % moisture and pectin staining intensity) characterization between 'Atlas' and 'Pluto' butternut squash. 'Atlas' and 'Pluto' butternut squash were identified to have the shapes "buchona" (maw shape) and "bule" (bottle shape) respectively during morphological characterization. There is a possibility that lack of significant differences during characterization could have been due to closely related genetic codes responsible for physical and physicochemical traits of 'Atlas' and 'Pluto' butternut squash.

In addition, the effect of calcium lactate dipping treatments on fresh-cut butternut texture quality was not significant (p>0.05) and no interaction between cultivar and calcium lactate treatment could be established. The reported texture enhancing effect of calcium lactate on fresh-cut produce could not be experienced on fresh-cut butternut probably due to insufficient dipping temperatures that can lead to reduced polymethylesterase activity and Ca^{2+} ion diffusion in butternut tissue, inadequate dipping concentrations and dipping times during the experiment. Furthermore, there was no significant difference (p>0.05) between fresh-cut butternut treated with calcium lactate and the controls in terms of microbiological quality during storage. However, all fresh-cut butternut samples in this research achieved two days longer shelf life in comparison to the shelf life ascribed by the processing company on the product. The improved shelf life obtained can possibly be attributed to an optimal storage temperature (3.5°C) used in this research which is lower than the recommended retail and home refrigeration temperature (5°C) on the packaging label.

4.1.2 Introduction

Together with the diverse global product range of fresh-cut produce, fresh-cut butternut squash demand has increased over the years due to shifting consumer attitudes in favour of convenient and nutritious foods (Ragaert et al. 2004; Martina-Diana et al. 2007). Fresh-cut butternut is also associated with pharmacological effects such as treatment of gastrointestinal diseases (Seroczynska et al. 2019). However, fresh-cut butternut squash is susceptible to texture deterioration during storage that can lead to economic losses for fresh-cut produce manufacturers, retailers and consumers, if not controlled. A 500g fresh-cut butternut squash product manufactured at a produce processing plant for one of the South African major food retailers was subject of customer complaints that led to economic losses at the time of this study. Customers were complaining that the fresh-cut butternut product was losing firmness prematurely. There are various pre-harvest and post-harvest techniques used to approach the challenge of firmness loss in fresh-cut fruits and vegetables which include suitable cultivar selection and calcium lactate dipping treatment.

Calcium lactate has been reported to improve firmness of fresh-cut vegetables due to the behaviour of Ca^{2+} ions in plant tissues. As postulated by Ngamchuachit et al. (2014), Ca^{2+} ions form ionic bonds with the carboxylic groups of demethylesterified pectin molecules, resulting in pectin cross-bridges that account for improved plant tissue firmness. The mode of action of Ca^{2+} ions during calcium lactate dipping to impact the firmness of fresh-cut butternut squash was investigated. Selection of favourable butternut squash cultivars for firmness retention during storage can potentially address the firmness loss challenge raised by customers. It has been suggested that some butternut squash cultivars resist undesirable minimal processing stresses better than others because of genetic variation among cultivars (Emadi et al. 2011; Grisales et al. 2014). Two butternut squash cultivars ‘Atlas’ and ‘Pluto’ were investigated during this research chapter to identify any possible texture quality retention differences during storage of fresh-cut butternut.

This research chapter sought to explore the impact of calcium lactate dipping (0.5% and 3% w/v) on improving the texture of fresh-cut butternut during storage. Furthermore, amongst several butternut squash cultivars in South Africa, ‘Atlas’ and ‘Pluto’ have been reported to be conducive for commercial processing due to their availability and favourable yields (Sakata Southern Africa (Pty) Ltd, 2014). This research also sought to characterize ‘Atlas’ and ‘Pluto’ butternut squash and identify any differences in terms of texture quality retention and response to calcium lactate treatment. Physical (fruit size dimension and

texture) and physicochemical (% moisture, pH, °Brix and pectin staining intensity) characteristics of 'Atlas' and 'Pluto' butternut squash were also determined before minimal processing activities.

4.1.3 Materials and methods

Butternut squash cultivars 'Atlas' and 'Pluto' were sourced from Mjejane Farm in the Mpumalanga lowveld region of South Africa. These cultivars were bred by Sakata Seed Southern Africa's Seeds (Pty) Ltd and grown on a farm with an average temperature of 18°C and 4.6mm rainfall during cultivation (May 2018) and an average temperature of 19°C and 2.95 mm rainfall during harvesting (August 2018) (World Weather Online, 2019). The butternuts were harvested at 105 days and 100 days after cultivation for 'Atlas' and 'Pluto', respectively and were delivered at the processing facility in Boksburg, Gauteng a day after harvesting with a truck delivery temperature of 26°C.

4.1.3.1 Experimental design

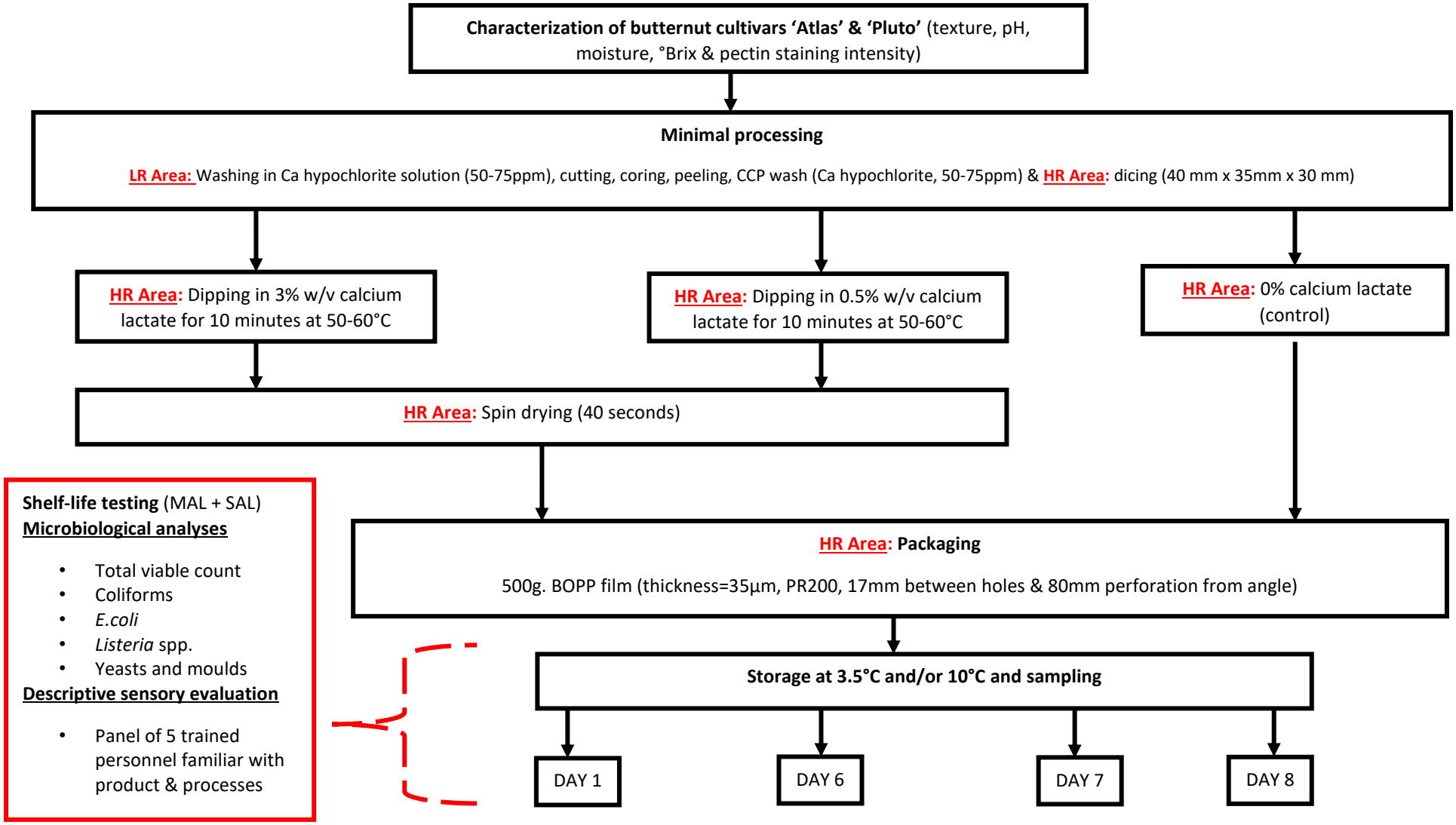

Figure 4: Experimental design showing butternut squash minimal processing steps and quality assessments during storage

4.1.3.2 Sample preparation and minimal processing

Butternut received from the farm were immediately subjected to quality control checks (size, maturity, °Brix, rind integrity and assessed for pest infestation and diseases). The checked butternuts were stored under a controlled storage temperature (3.5°C) in separate perforated lugs (containers) for the experiment as per the minimal processors' receiving and storage procedure. On the same day of receipt, intact butternut squash was sampled from the experiment-dedicated lugs and transported at 24 to 27°C for characterization at the University of Pretoria. The rest of the butternut samples were stored at the processing facility at 3.5°C and processed the following day.

Butternut delivered at the processing facility underwent minimal processing as shown in Figure 4, from the low risk (LR) area to the high risk (HR) area. The intact butternut samples were washed at the quality control point (QCP) with 50 to 75 ppm calcium hypochlorite (Arch Water Products South Africa, Kempton Park, South Africa) solution at 2.5-3°C for 5 minutes. This was followed by cutting ("neck" region), coring (removal of seeds and soft tissue from the bulb area), washing at the critical control point (CCP) with a 50 to 75 ppm calcium hypochlorite solution at 5-10°C for 25 seconds while on a conveyor belt at a speed of 0.1 ms^{-1}. The samples (the neck region only) were manually chopped into 40 mm x 30 mm butternut blocks (dices) with sharp knives.

Following chopping the samples were dipped in calcium lactate (Savannah Fine Chemicals (Pty), Bedfordview, South Africa). Three dipping treatments were used in this investigation: a control (0% calcium lactate), 0.5% calcium lactate and 3% calcium lactate. Dipping treatments were conducted in insulated water baths to minimise heat loss at 50°C to 60°C followed by spin-drying in the high-risk area (HR). Five hundred grams ± 2 g of diced butternut were packed in perforated bags from Packaging World (Pinetown, South Africa) made from biaxially oriented polypropylene film (BOPP) of thickness 35 µm, 17 mm distance between perforation holes and an 80 mm perforation position from angle. Packaged samples were stored (3.5°C) in the dispatch area where samples for texture quality determination were sampled from. Sampling for microbiological acceptability limit (MAL) and sensory acceptability limit (SAL) determination was done on day 1, day 6, day 7 and day 8. Three bags were analysed for both MAL and SAL determinations on every sampling day. The experiment was conducted once after two pre-trial runs of the experiment without duplicates.

4.1.3.3 Characterization of butternut squash cultivars

Texture analysis

Manually peeled and diced, using a hand knife, butternut from the two cultivars were subjected to a penetration test using a texture analyser (Shimadzu EZ-L, Shimadzu Corporation, Kyoto, Japan) to determine the texture of the "neck region". The dimensions for each sample were L = 40 mm, W = 30 mm and H = 35 mm and a 200 N load cell was used with a 2 mm diameter stainless steel probe. A stainless-steel fixing base with a capacity of 500 N and a fixable plate diameter of 25 to 60 mm was used. The probes' stroke or depth was 20 mm with penetration and return speeds of 33 mm/s and 50 mm/s, respectively. Three butternut fruits were sampled per cultivar and each fruit was divided into three diced units during characterization.

Pectin staining intensity

The pectin staining intensity of butternut squash samples was determined (in triplicate) using a chroma meter (Konica Minolta CR – 400, Konica Minolta Sensing Americas, New Jersey, USA). The same samples used for texture analysis were immediately stained after the penetration test using ruthenium red stain (Sigma-Aldrich, Darmstadt, Germany) by dipping each sample in a separate beaker containing 0.02% (w/v) aqueous ruthenium red for 15 minutes. On the chroma meter the International Commission on Illumination/Commission internationale de l'éclairage (CIE) scale L^* a^* b^* setting was used which measures brightness, red or green hue intensity and blue or yellow hue intensity respectively (Hunter Associates Laboratory, 1996; Luo, 2002). The $L^*a^*b^*$ scale is illustrated in Figure 5. Values of a* positive are for red colour intensity while negative values are for green colour intensity (Luo, 2002). According to Hornatowska (2005), the higher the ruthenium red staining intensity, the higher the amount of unesterified (acidic) pectin, therefore, this method was applied to determine the amount of pectin in the plant tissue.

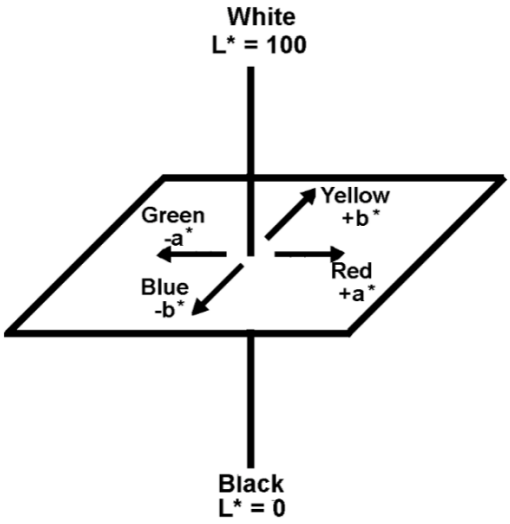

Figure 5: Illustration of the CIE L*a*b* colour scale axes

°Brix

The excess tissue from the butternut fruits from both cultivars used for texture and pectin staining intensity analyses were blended to a paste using a blender (Hamilton Beach Blender HBB250SR, Southern Pines, USA). Blended butternut squash pastes were placed into three labelled beakers and °Brix was measured using a pocket refractometer according to the standard AACC Method 80-51.01 (Atago Pocket PAL-1, Atago Co., Tokyo, Japan).

pH

A pH meter (Hanna Instruments pH 211, Padova, Italy) was used to determine the pH of all butternut squash pastes of both cultivars in triplicates at 25°C ± 1°C. The pH meter probe was placed in labelled beakers containing the same blended butternut squash created for °Brix analysis. Samples from three labelled beakers containing blended butternut squash tissue were analysed per cultivar.

Moisture content

The moisture content of peeled butternut squash fruit cultivars was determined according to the AACC International (1999) air oven method 44-15.02. Peeled butternut fruits used for the analyses were cut manually using a knife and accurately weighed to 5 g and dried in a forced draught oven at 103°C for 4 hours.

Physical characterization

Butternut external length, bulb diameter, neck diameter, %pulp, %seed, %rind and fruit weight were measured for each cultivar in triplicate. External length, bulb diameter and neck diameter were measured before knife cutting and peeling. After cutting and peeling, %pulp, %seed and %rind was calculated by weighing each component and expressing it as a percentage of the respective fruit weight. However, rind thickness was not determined in this study. Butternut morphology was determined by placing cultivars against a 1cm x 1cm grid paper to observe any differences in shape, size and colour.

4.1.3.4 Sensory quality of minimally processed butternut squash

Packed minimally processed 500 g butternut squash samples were stored at 3.5°C in the processing facility's chilling units for a maximum period of 8 days. It was observed that the texture of all samples had drastically deteriorated on the 8th day hence no further analyses were conducted thereafter. This textural deterioration on the 8th day was observed in the two initial pre-trials prior to the final experiment from which results were recorded and analysed. Samples for analyses (microbiological and sensory) were randomly selected in sets of three units on the day of production (day 1), day 6, day 7 and on the day 8. The primary shelf life was determined through the establishment of the time needed to reach the quality level matching the acceptability limit. To determine the sensory acceptability limit (SAL) of the packed sample treatments over the storage period, descriptive sensory evaluation was used as a tool. The panel consisted of 5 (3 females and 2 males; aged 27 to 35 years) trained staff members familiar with the product and its quality specifications. Secondary shelf life (the period after pack opening during which a food product does maintain an acceptable quality level) was not investigated during this study.

Three randomly selected samples of each treatment were assessed on day 1, day 6, day 7 and day 8 and the evaluations were conducted in the sensory evaluation facilities of the processing plants' New Product Development department. The panellists were guided by a document internally known as a "quality contract" which was compiled during the development stages for the product. The sensory attributes assessed were "fresh butternut aroma", "off butternut aroma", "other unidentified aromas", "chlorine aroma", "degree of firmness", "moist texture", "sliminess", "soft edges", "orange colour intensity" and "dehydration". These attributes were measured on a 0-10 scale where 0 represented no detection and 10 was high intensity as shown in Appendix I. Panellists conducted their

assessment individually without the interference of other panellists while recording their findings on a form shown in Appendix I. A blind test was deployed as the way sensory evaluation was conducted with each sample presented separately on labelled white trays. Sample coding and identification was employed in a format which was designed to mitigate any bias from the panel while helping the researcher to easily identify them. The coding format used combined alphabetical letters and Arabic numerals for identification. A prefix of "A" or "P" was used to denote the cultivars 'Atlas' and 'Pluto', respectively. For instance, trays labelled PA1, PA2 and PA3 meant 3 randomly selected 'Pluto' samples of a particular treatment (e.g. 0% calcium lactate), followed by AB1, AB2 and AB3 which would be randomly selected 'Atlas' samples of another treatment (e.g. 0.5% or 3% calcium lactate) for each sampling day.

4.1.3.5 Microbial quality of minimally processed butternut squash

MAL was determined by microbiological analyses to detect *Listeria* species, coliforms, *Escherichia. coli* O157:H7, total viable counts (TVC), yeasts and moulds. A mass of 25 g diced butternut samples was placed in 225 ml of One Broth *Listeria* at 30°C for 72 hours which were pipetted (1000 µL) onto plates containing 15 mL Brilliance *Listeria* Agar at 37°C for a further 24 hours were used to test for *Listeria* species. Twenty-five grams of diced butternut samples were placed in 225 mL of Ringer's solution (4 Ringer's tablets in 2L distilled water) for 30 minutes and pipetted onto plates containing 15 mL of the respective agar for coliform, *E. coli*, TVC, yeast and moulds analyses. Rapid *E. coli* Agar at 37°C for 24 hours was used to test for both coliforms and *E. coli*. Plate Count Agar at 30°C for 72 hours was used for TVC while Chloramphenicol Agar at 25°C for 5 days was used for both yeasts and moulds. The methods used for microbiological analysis were SANS ISO 7954 (1987), SANS ISO 4833, ISO 11290-1 (2017), ISO 11290-2 (2017) and ISO 16649-2 (2001) for yeast and moulds, TVC, *Listeria* species detection, *Listeria* species enumeration and *E. coli* and coliforms, respectively.

4.1.3.6 Statistical analyses

Firstly, characterization data (pH, °Brix, moisture content, pectin staining intensity and texture) was statistically analysed to determine whether there were significant differences between 'Atlas' and 'Pluto' butternut squash cultivars using a Mann-Whitney U Test on IBM® SPSS® version 25 software. Data concerning the effect of the independent variables cultivar selection, storage temperature, storage time and calcium lactate treatments (0%,

0.5% and 3%) on shelf life of minimally processed butternut squash was analysed using Mann-Whitney U Test and the Independent-Samples Krusal-Wallis Test using IBM® SPSS® version 25 software.

4.1.4 Results

4.1.4.1 Characterization of butternut squash cultivars

Chemical characterization of the cultivars 'Atlas' and 'Pluto' showed that they were both high moisture content cultivars (± 90.82%), acidic (± pH 6.13) and with 5.2°Brix level (Table 2). There were no significant differences (p>0.05) between 'Atlas' and 'Pluto' noted during the chemical characterization. The pectin staining colour intensity, as shown in Table 2, reflected the intensity of pectin in butternut samples while the penetration texture analysis results determined the firmness of both cultivars. There were no significant differences (p>0.05) in colour pectin intensity for the a* values of the International Commission on Illumination/Commission internationale de l'éclairage (CIE) *L*a*b** scale (±28.88) and tissue firmness (±12.85N) between 'Atlas' and 'Pluto' (Table 2). The positive a* values indicated that both 'Atlas' and 'Pluto' cultivars had a red hue intensity due to pectin availability. Figure 6 shows the morphological characterization of 'Atlas' and 'Pluto' butternut squash cultivars and one of the visible differences lie in the bulb region pronunciation and overall fruit length. The cultivar 'Atlas' had a more pronounced bulb region as well as longer overall length than those of 'Pluto'. However, as indicated in Table 3, there were no significant differences (p>0.05) between 'Atlas' and 'Pluto' butternut squash in terms of fruit weight, rind percentage, seed percentage, pulp percentage, neck diameter, bulb diameter and external length.

Table 2: Physicochemical characterization of butternut squash cultivars 'Atlas' and 'Pluto'

		Cultivar		
		'Atlas'	'Pluto'	*p*-value
% Moisture ± SD		91.48 ± 0.96	90.16 ± 2.00	1.00
pH ± SD		6.20 ± 0.05	6.06 ± 0.26	1.00
°Brix ± SD		6.00 ± 0.10	4.40 ± 0.84	1.00
Pectin staining intensity	L* ± SD	40.65 ± 1.57	42.09 ± 1.87	1.00
	a* ± SD	29.62 ± 0.89	28.14 ± 1.34	1.00
	b* ± SD	19.02 ± 2.20	20.89 ± 3.91	1.00

Table 3: Physical characterization of butternut squash cultivars 'Atlas' and 'Pluto'

	Cultivar		
	'Atlas'	'Pluto'	*P*-value
External length (cm)	27.47 ± 1.89	21.10 ± 2.26	0.10
Bulb diameter (cm)	12.60 ± 0.97	11.40 ± 0.59	0.20
Neck diameter (cm)	8.96 ± 0.47	9.48 ± 1.12	1.00
Pulp %	90.06 ± 1.10	91.05 ± 0.78	0.40

Seed %	2.70 ± 0.21	2.30 ± 0.31	0.40
Rind %	7.24 ± 1.01	6.66 ± 0.52	0.40
Fruit weight (kg)	2.01 ± 0.44	1.59 ± 0.47	0.40
Texture (N) ± SD	11.43 ± 0.69	14.27 ± 5.12	1.00

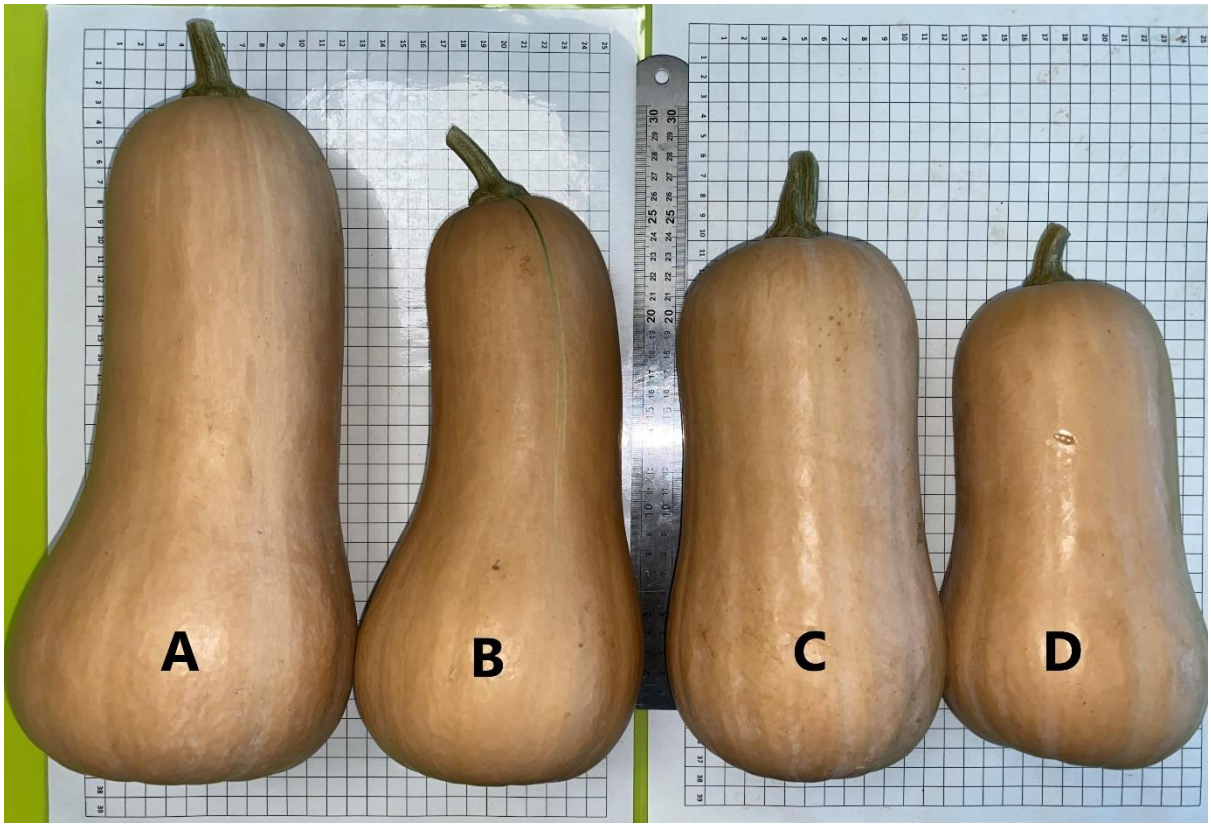

Figure 6: Morphology of butternut squash (*Cucurbita moschata*) cultivars 'Atlas' (A and B) and 'Pluto' (C and D) used in this research study

4.1.4.2 Effects of calcium lactate dipping treatments on fresh-cut butternut squash cultivars

The cultivar selection, calcium lactate treatments applied and the interaction between cultivar and calcium lactate treatments showed no significant differences (p>0.05) on the sensory attributes of fresh-cut diced butternut through the storage period (Tables 4 and 5). There were no significant differences (p>0.05) between 'Atlas' and 'Pluto' minimally processed butternut for all sensory attributes. Fresh-cut butternut squash dipped in 0.5% and 3% calcium lactate experienced a higher dehydration effect (2.54 and 2.51, respectively) in comparison to the more favourable hydrated of the control (1.18) as determined by the descriptive sensory panel (Table 4). There were significant differences (p<0.05) among the storage days noted in the sensory intensities of all sensory attributes of the minimally processed butternut squash.

Table 4: Effect of butternut squash cultivars, calcium lactate dipping treatment and their interaction on the sensory properties of fresh-cut butternut squash throughout storage as evaluated by a trained descriptive sensory panel

Comparison	Attribute						
	Fresh butternut aroma	Degree of firmness	Moist texture	Sliminess	Soft edges	Orange colour intensity	Dehydration
Effect of cultivar							
'Atlas'	8.7 ± 0.2	7.6 ± 0.3	2.4 ± 0.3	1.4 ± 0.3	3.2 ± 0.4	7.2 ± 0.1	2.0 ± 0.2
'Pluto'	8.7 ± 0.2	7.5 ± 0.3	2.4 ± 0.3	1.3 ± 0.3	3.0 ± 0.4	7.2 ± 0.1	2.1 ± 0.2
P-value	0.86	0.82	0.89	0.80	0.83	0.91	0.85
Effect of calcium lactate treatment							
0%	8.7 ± 0.2	7.3 ± 0.3	2.7 ± 0.3	1.6 ± 0.3	3.7 ± 0.4	7.3 ± 0.1	1.2[a] ± 0.2
0.5%	8.8 ± 0.2	7.8 ± 0.4	2.3 ± 0.4	1.2 ± 0.4	2.9 ± 0.5	7.2 ± 0.2	2.5[b] ± 0.3
3%	8.7 ± 0.2	7.7 ± 0.4	2.2 ± 0.4	1.2 ± 0.4	2.8 ± 0.5	7.2 ± 0.2	2.5[b] ± 0.3
P-value	0.95	0.47	0.49	0.69	0.23	0.83	0.00

Results are expressed as the Means ± Standard Error. [abc] means in an attribute's column in a subsection with different letters differed significantly ($p<0.05$). A ten-point sensory scale used where 0 = not intense; 10 = very intense.

Table 5: Effect of butternut squash cultivars, calcium lactate dipping treatment and their interaction on the sensory properties of fresh-cut butternut squash as evaluated by a trained descriptive sensory panel

Comparison	Attribute						
	Fresh butternut aroma	Degree of firmness	Moist texture	Sliminess	Soft edges	Orange colour intensity	Dehydration
Interaction effect of cultivar and calcium lactate treatment							
'Atlas' x 0% calcium lactate	8.6 ± 0.2	7.2 ± 0.4	2.8 ± 0.4	1.6 ± 0.4	3.8 ± 0.5	7.3 ± 0.2	1.1 ± 0.3
'Atlas' x 0.5% calcium lactate	8.8 ± 0.3	8.0 ± 0.6	2.2 ± 0.6	1.2 ± 0.6	2.7 ± 0.7	7.1 ± 0.3	2.6 ± 0.4
'Atlas' x 3% calcium lactate	8.8 ± 0.3	7.7 ± 0.6	2.3 ± 0.6	1.4 ± 0.6	3.0 ± 0.7	7.2 ± 0.3	2.5 ± 0.4
'Pluto' x 0% calcium lactate	8.7 ± 0.2	7.3 ± 0.4	2.7 ± 0.4	1.5 ± 0.4	3.6 ± 0.5	7.2 ± 0.2	1.3 ± 0.3
'Pluto' x 0.5% calcium lactate	8.7 ± 0.3	7.6 ± 0.6	2.4 ± 0.6	1.3 ± 0.6	3.1 ± 0.7	7.2 ± 0.3	2.5 ± 0.4
'Pluto' x 3% calcium lactate	8.7 ± 0.3	7.7 ± 0.6	2.1 ± 0.6	1.0 ± 0.6	2.6 ± 0.7	7.2 ± 0.3	2.6 ± 0.4
P-value	0.90	0.89	0.96	0.91	0.87	0.89	0.84

Results are expressed as the Means ± Standard Error. A ten-point sensory scale used where 0 = not intense; 10 = very intense.

4.1.4.3 Texture quality

Fresh-cut butternut texture quality was found acceptable by the DSP up until day 7 of storage as shown in Figure 7, which illustrates the effect of storage time on the degree of firmness of cultivars 'Atlas' and 'Pluto' dipped in different calcium lactate concentrations (control = 0%, 0.5% and 3%). There was a drastic decrease in firmness on day 8 which was below the firmness acceptability limit (7) hence the texture quality life obtained was 7 days.

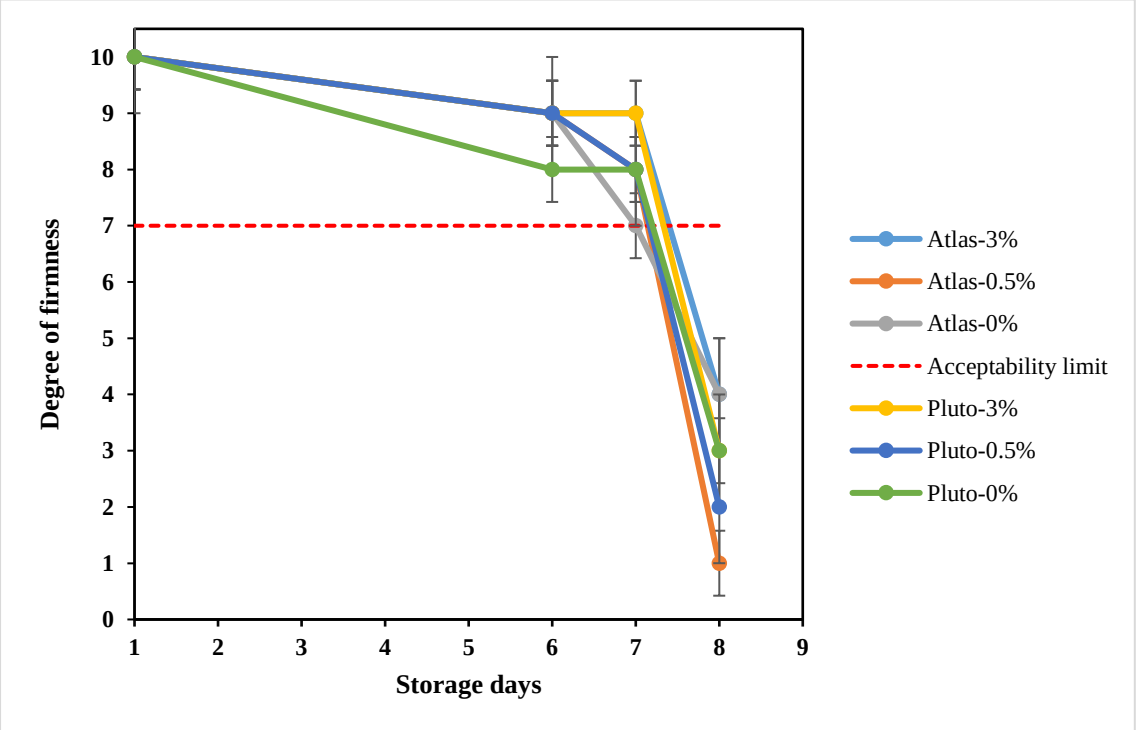

Figure 7: Effect of storage period (days) on the degree of firmness of fresh-cut butternut squash cultivars 'Atlas' and 'Pluto' dipped in 0%, 0.5% or 3 % calcium lactate

4.1.4.4 Microbiological quality

Coliforms, *E. coli* O157:H7, *Listeria* species and moulds were below the limit of detection (<1 CFU/g) (Figure 7). The total viable count and yeasts were more dominant in samples from all treatments and increased with time during storage as shown in Figures 8 and 9. Results for TVC, coliforms, *E. coli* O157:H7, *Listeria* species, yeasts and moulds were within limits stipulated in Woolworths Foods' microbiological guideline (Woolworths (Pty) Ltd, 2018) and international microbiological guidelines highlighted by Francis et al. (1999) (Table 7) throughout the 8-day refrigerated storage period.

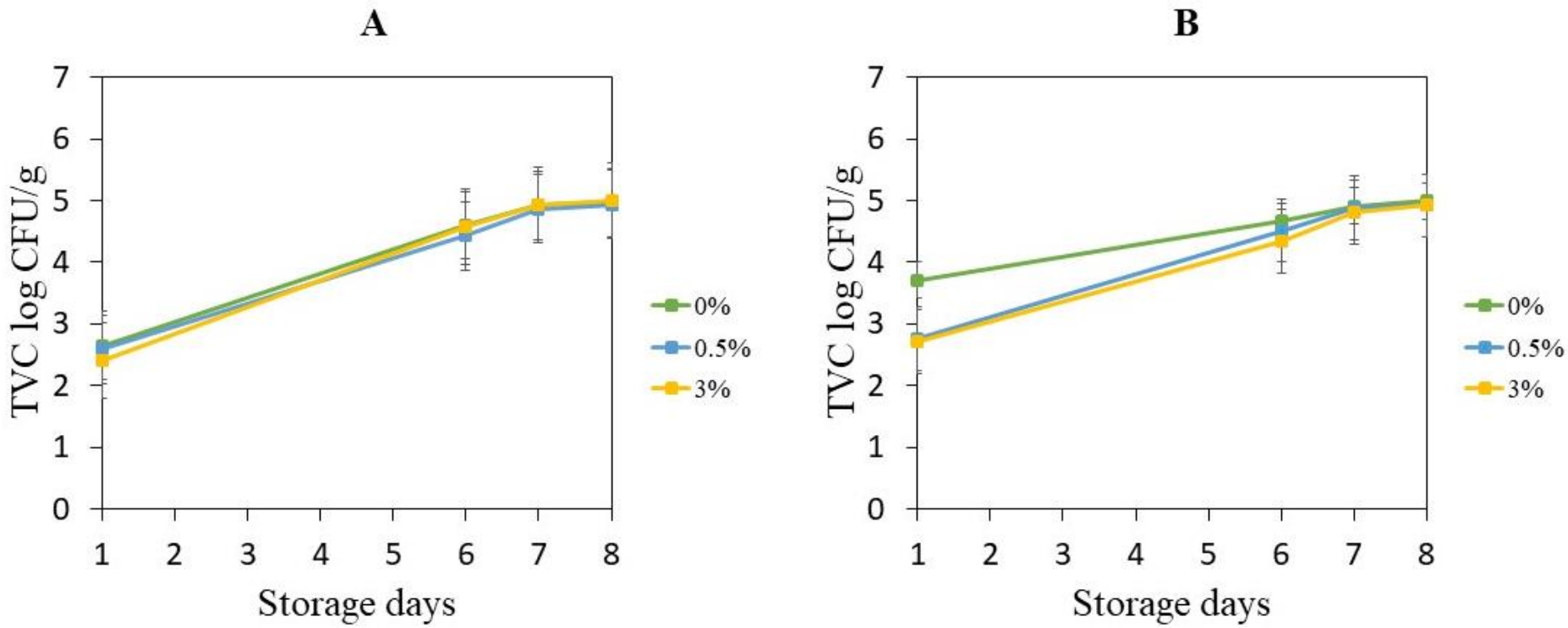

Figure 8: Effect of storage period on total viable counts (TVC) of fresh-cut 'Atlas' (A) and ‘Pluto’ (B) butternut squash dipped in 0%, 0.5% and 3% calcium lactate

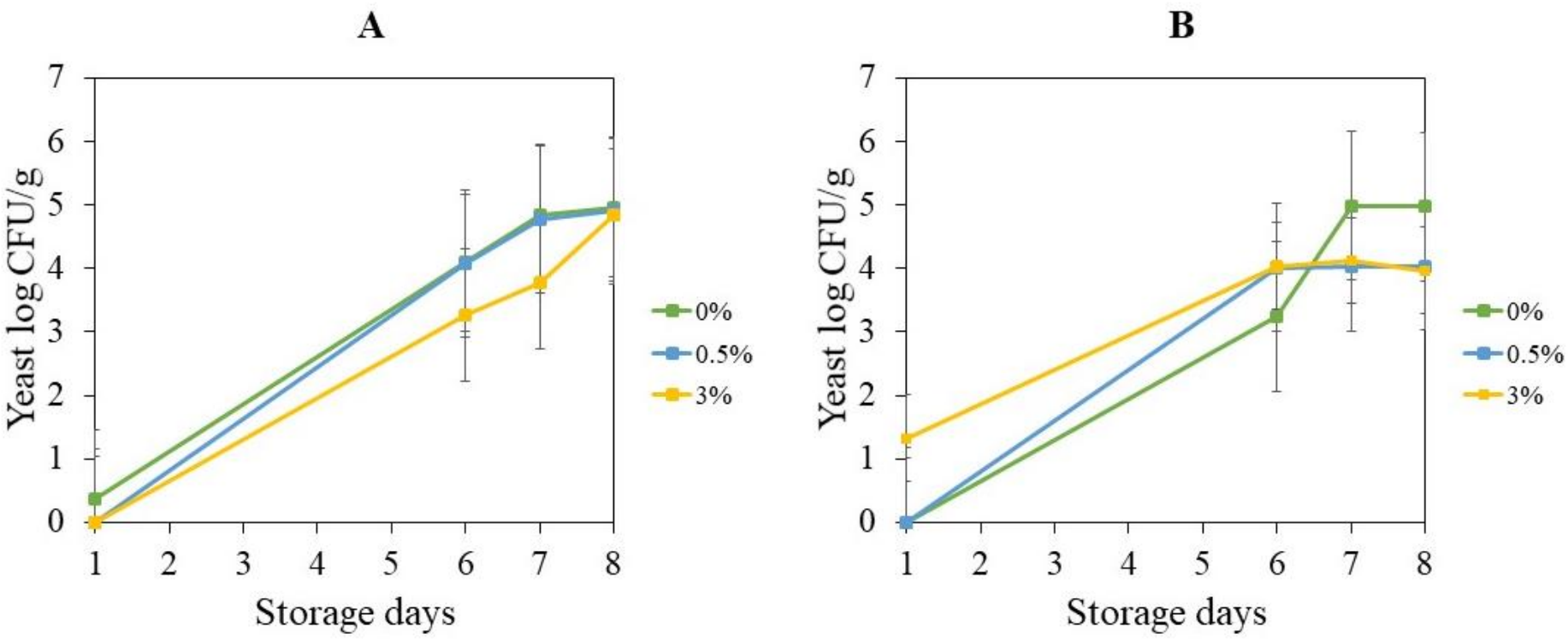

Figure 9: Effect of storage period on yeast counts of fresh-cut 'Atlas' (A) and 'Pluto' (B) butternut squash dipped in 0%, 0.5% and 3% calcium lactate

4.1.5 Discussion

4.1.5.1 Characterization of butternut squash cultivars ‘Atlas’ and ‘Pluto’

Jacobo-Valenzuela et al. (2011b) classified the shapes of butternut squash into four main categories: “ovalada” (oval), “bule” (bottle shape), “buchona” (maw shape) and “herradura” (horseshoe shape) (Figure 10). Findings of morphological characterization of butternut squash cultivars suggested that ‘Atlas’ and ‘Pluto’ were more consistent with the shapes “buchona” and “bule”, respectively. Findings for both cultivars on fruit length, fruit weight, bulb diameter and seed (%) were consistent with those reported by Jacobo-Valenzuela et al. (2011a). Brown (2016) also reported similar results to the findings of this research for ‘Atlas’ butternut squash regarding fruit length and fruit weight. However, findings of the current research on ‘Atlas’ butternut squash were inconsistent with those reported by Brown (2016) regarding bulb diameter which was larger (24 cm). Jacobo-Valenzuela et al (2011a) reported higher butternut rind (%) values (8.20–13.89%) while their pulp (%) had lower values (71.75–86.06%) than in both findings for ‘Atlas’ and ‘Pluto’ butternut squash.

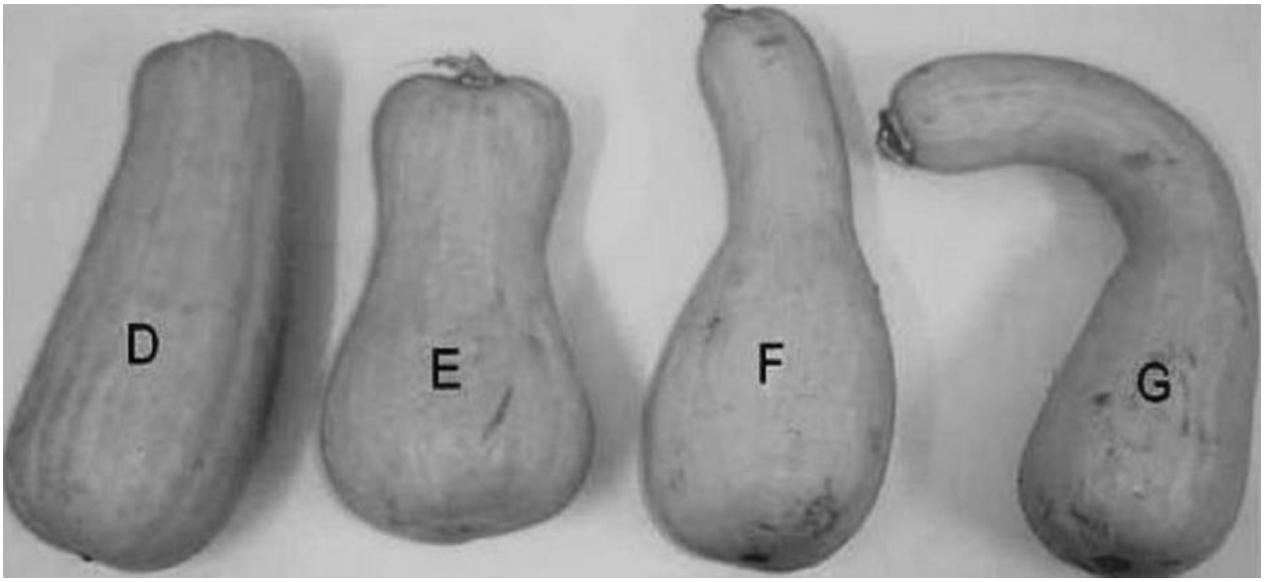

Figure 10: Butternut squash (*Cucurbita moschata*) shape types: "ovalada" (D), "bule" (E), "buchona" (F) and "herradura" (G) (Jacobo-Valenzuela et al., 2011b)

The variations in the above research results from other authors with the findings of the current research can be attributed to differences emanating from farming culture which has been reported to impact butternut squash yield, physical and chemical properties (Armersto et al., 2020). No

published research results on butternut squash pectin colour intensity were found during this study to compare with. However, similar research work used methods that quantified pectin, unlike the determination of pectin staining intensity used in this current research study. Jacobo-Valenzuela et al. (2011b) quantified pectin content in butternut squash cv. Cehualca which had an average of 7.34% using a 1975 AOAC technique. Other quantitative chemical (Zhang et al. 2014) and enzymatic (Pitchkina et al. 2008) pectin methods have been used to characterize butternut squash cultivars but no published work could be found on pectin quantities of 'Atlas' and 'Pluto' butternut squash.

As summarized in Tables 2 and 3, there were no significant differences (p>0.05) noted during physicochemical and physical characterization of 'Atlas' and 'Pluto' butternut squash cultivars in this study. Findings of this research regarding pH and moisture (%) of 'Pluto' butternut squash were consistent with results obtained by Armersto et al. (2020) during characterization of 'Pluto' and 'Ariel' butternut squash. 'Atlas' and 'Pluto' butternut cultivars were found to be more acidic (±6.20 and ±6.06, respectively) than butternut squash cv. 'Celhualca' (±6.77) characterized by Jacobo-Valenzuela et al. (2011a). However, butternut squash samples (cultivars not specified) characterized by Roura et al. (2007) were more acidic (pH range of 5.8 to 6.0) than the butternut used in this research study. Characterization results (cultivars not specified) obtained by Jacobo-Valenzuela et al. (2011a) also indicated higher moisture (±91.55%) and °Brix (±6.42) than both 'Atlas' (±91.48% moisture and ±6.00 °Brix) and 'Pluto' (±90.16% moisture and ±4.40°Brix) butternut squash used in this study. However, butternut squash characterization results obtained by Dari and Yaro (2016) showed lower moisture levels (82.15%) in comparison to the butternut squash in this study. In addition, research results from Noseworthy and Loy (2008) on two different butternut squash cultivars 'New Hampshire Baby Butternut' (NHBBN) and self-selected 'Puritan Butternut' obtained higher °Brix levels (both cultivars had an average of 8.70 °Brix each) than those of both cultivars used in this study. Butternut sample studies by Roura et al. (2007) had a wide moisture content range (79-93%) while the °Brix (8-11) was also higher than that found in this research.

The evidence presented above, showing that the physicochemical properties of butternut squash from different studies vary, showed that differences in climatic conditions from which butternut squash fruits were sampled, might have had a major impact. Similarly, Belgis et al. (2016) noted

the variability in the total soluble solids (TSS) content between lai (*Durio kutejensis*) and durian (*Durio zibethinus*) cultivars, cultivated under the same conditions, indicated variability in sugar, organic acids, soluble pectins, anthocyanins and ascorbic acid. In addition, Belgis et al. (2016) postulated that both sensory and physicochemical parameters of produce are determined by metabolites whose existence is influenced by genetics and growth conditions. It is known that climatic conditions (e.g. soil profile, temperature, relative humidity and light intensity) contribute to metabolite quantities and plant physiology, therefore, differences in growth conditions result in metabolite and physiological variations among cultivars (Weston and Barth, 1997; Valpuesta & Botella, 2004; Getinet, Seyoum and Woldetsadik, 2007). Differences in pH and °Brix levels between the butternut cultivars and those from other research studies could have emanated from the fact that different cultivars, which are fundamentally different genetically, have variable metabolite quantities. It is also important to note that the difference in pH values also culminates from variability in organic acid (e.g. citric acid and malic acid) content amongst cultivars. Furthermore, variabilities in pH level (due to organic acid content) and TSS levels lead to differences in fruit and vegetable taste since TSS and pH levels are linked to sweetness and sourness, respectively.

4.1.5.2 Effect of calcium lactate dipping treatments on fresh-cut butternut squash cultivars

Calcium lactate showed to have a pronounced dehydration effect on fresh-cut butternut compared to the control. This can possibly be attributed to the effect of calcium lactate on osmotic dehydration kinetics where water loss and water activity reduction are significant in fresh-cut fruits and vegetables exposed to solutions containing calcium (Rastogi et al., 2002). Hypertonic solutions, in this case 0.5% and 3% calcium lactate, possess high osmotic pressure that facilitates water mass transfer from vegetable tissue into the solution and diffusion of solutes (e.g. Ca^{2+} ions) from the osmotic solution into the tissue with relatively lower osmolarity (Rastogi et al., 2002). Consequently, reduction in plant tissue moisture content leads to dehydration. This possibly resulted in the visual dehydration observed by the sensory panel in calcium lactate treated butternut squash (Table 4). This trend was observed by Silva, Fernandes and Mauro (2014) where different calcium lactate concentrations (2% and 4%) resulted in higher water loss and reduced water activity when fresh-cut pineapples were dipped therein as compared to a 0% calcium lactate treatment.

Tables 4 and 5 indicate that both calcium lactate treatments (0.5% and 3%) did not significantly improve texture quality of fresh-cut butternut squash in comparison to the control. However, calcium lactate has been shown to improve firmness in fresh-cut produce due to the ability of the calcium component of cross-linking with the pectin located in plant cell walls and middle lamella thereby enhancing the textural quality (Rico et al. 2007). Figure 2 illustrates the possible mechanism by which calcium ions were expected to be involved in the formation of pectin cross-linkages that could have resulted in improved textural quality in fresh-cut butternut after dipping for 10 minutes in both 0.5% and 3% calcium lactate. There was no published work at the time of this study on the recommended surface area for optimum Ca^{2+} ion diffusion into fresh-cut butternut and associated butternut compositional factors. Fresh-cut butternut dices had a surface area of approximately 24.3 cm^2/g and the cut and exposed surfaces possibly would have facilitated Ca^{2+} ion diffusion within the tissues. However, even though the Ca^{2+} ion cross-bridge formation principle was thought to be at hand during this current study, no significant texture quality improvement was observed. This could have been as a result of the factors that affect Ca^{2+} diffusion such as temperature, dipping time and concentration of calcium lactate. For instance, in the study by Rico et al. (2007) on the effect of calcium lactate dipping (1.5% w/v) and heat-shock (25°C and 50°C) on ready-to-eat carrots, firmness improvement ($p<0.05$) culminated from the use of calcium lactate dipping and heat treatment (50°C) together, as shown in Figure 11 below. In reference to the current study; calcium lactate concentrations and dipping times shown in Table 6 were used for guidance during the research study since no previous published material was available on fresh-cut butternut squash.

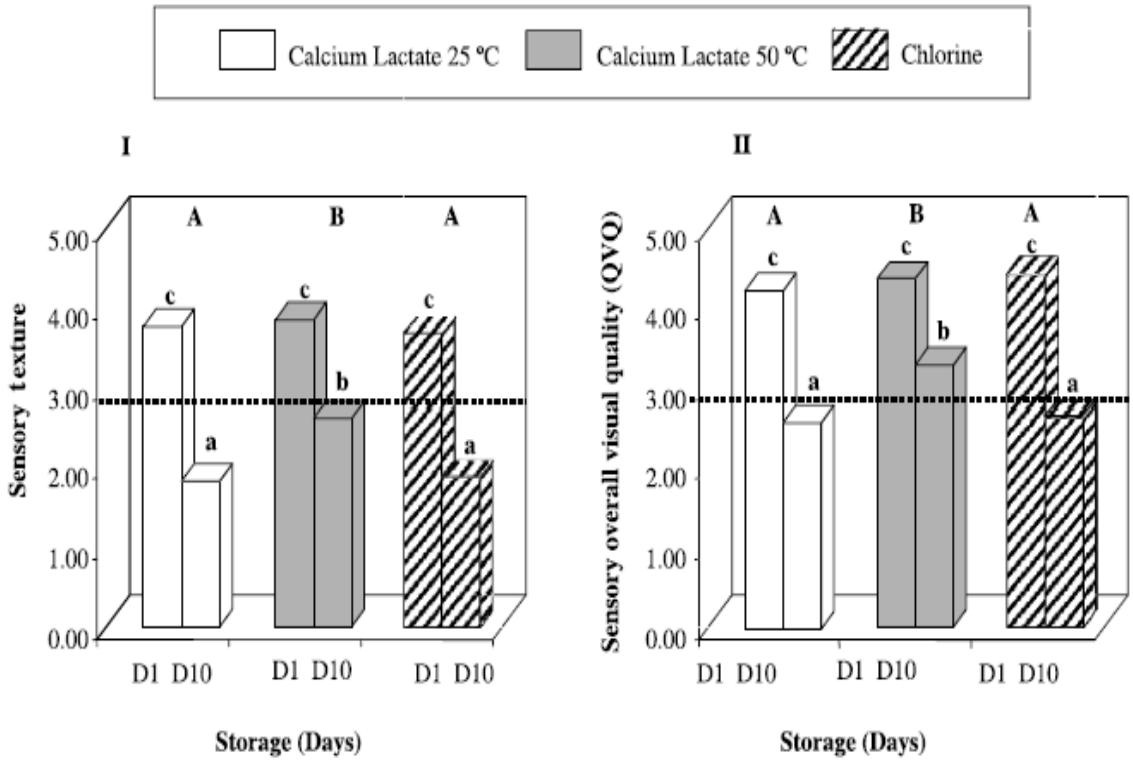

Figure 11: Sensory texture (I) and sensory overall visual quality (II) after treatment (day 1 = D1) and at the end of the storage (day 10 = D10) in sliced carrots treated with chlorine (0.012%), calcium lactate (1.5%) at 25°C and calcium lactate at 50°C. The dashed line indicates acceptable texture for saleability. Sensory texture (I) and Sensory overall visual quality (II) scores represented firmness intensity and overall sliced carrot visual quality as determined by a descriptive sensory panel, 5.00 being the highest score. (Rico et al. 2007).

Table 6: Calcium lactate concentrations and dipping times used for fresh-cut fruits and vegetables textural retention

Fruit or vegetable	Calcium lactate concentration	Calcium lactate dipping temperature	Dipping time	References
Cantaloupe	2.50%	60°C	1 minute	Luna-Guzman and Barrett, 2000
Carrots	1.50%	50°C	1 minute	Rico et al. 2007
Melon	1.42%	60°C	1 minute	Silveira et al. 2011

Dipping temperature is one of the factors that affect the effectiveness of calcium salt dipping treatments on fresh-cut produce and might have significantly influenced results in this study (Rico et al. 2007). Ca^{2+} ions (0.1 nm) can passively diffuse within the cell wall structure since cell wall porosity is between 3.5 to 9.2 nm (Ngamchuachit et al. 2014). Furthermore, a synergistic relationship between heat treatment and calcium salt dipping has been proven to aid Ca^{2+} ion diffusion in the apoplast of parenchyma cells. This relationship between heat treatment and calcium treatment was postulated by Rico et al. (2007) when they concluded that temperatures of 40°C to 60°C increase Ca^{2+} ion diffusion which in turn improves texture quality and calcium levels in plant tissues. A 50°C to 60°C dipping temperature range was used in this study and no significant impact (p>0.05) on texture quality was observed on fresh-cut butternut exposed to 0.5% and 3% calcium lactate solutions. A higher temperature (>60°C) could have possibly facilitated optimum Ca^{2+} ion diffusion, enough to significantly improve fresh-cut butternut texture quality by establishing ionic pectin molecule cross-bridges, that enhance plant tissue mechanical strength. Additionally, lack of texture quality improvement could have been caused by lower than optimal butternut dice core temperatures after dipping to initiate thermal breakdown of cellular membranes, especially cell vacuoles, which contain calcium. Imaizumi et al. (2019) mentioned the importance of intracellular calcium movement to cell wall regions to increase pectin crosslinking, hence higher structural stability, brought by destruction of cellular membranes. Fresh-cut vegetables, like potato tuber slices, have been reported to experience low thermal conductivity during heating to initiate intracellular calcium movements (Imaizumi et al. 2019).

Butternut from all treatments achieved a textural quality life of 7 days in cold storage (3.5°C), two more days than what is expected (5 days) of the product by the company. Complaints of texture deterioration of the commercial product in retailer's refrigerators were mostly on the 4th and last day of shelf life (day 5) (personal communication based on internal company records). Results of this study have shown an improved overall shelf life than those ascribed by the processing company. The key to fresh-cut fruit and vegetable shelf life is storage and delivery temperatures (Hodges and Toivonen, 2007). Sensory assessment of the samples was done on samples from the processing facility's dispatch storage with a constant temperature of 3.5°C. The final product reaches the retail refrigerators (<5°C) via strictly controlled refrigerated (<5°C) trucks. However, the risk of a broken cold chain resulting in premature texture deterioration due to enzymatic and microbiological breakdown of plant tissue pectin structure in fresh-cut butternut cannot be

completely ruled out. Possible broken cold chain during handling of fresh-cut butternut can explain the difference in texture quality between samples used in this study and those exposed to the long supply chains where a cold chain can be broken.

4.1.5.3 Microbiological quality of minimally processed butternut squash cultivars

There were no significant differences (p>0.05) among treated and control fresh-cut 'Atlas' and 'Pluto' butternut squash microbiological counts which were also within limits throughout the 8-day refrigerated storage period. The total viable and yeast counts were dominant while coliforms, *E. coli* O157:H7, *Listeria* species and moulds were below the limit of detection (Figures 8 and 9). Table 7 shows the fresh-cut produce standards used to determine the microbiological acceptability limit of fresh-cut butternut squash in this study.

Table 7: Microbiological results at day 8 in comparison to microbiological standards for fresh-cut fruits and vegetables (Francis et al. 1999; Woolworths (Pty) Ltd, 2018)

Microorganism	0.5% calcium lactate treatment average log CFU/g)		3% calcium lactate treatment (average log CFU/g)		Control (average log CFU/g)		Standard
	'Atlas'	'Pluto'	'Atlas'	'Pluto'	'Atlas'	'Pluto'	
Listeria species	ND	ND	ND	ND	ND	ND	<2 log CFU/g
Listeria monocytogenes	ND	ND	ND	ND	ND	ND	<2 log CFU/g
Escherichia coli O157:H7	ND	ND	ND	ND	ND	ND	<2 log CFU/g
Total viable count (TVC)	4.94 ± 1.32	4.92 ± 1.12	4.99 ± 1.26	4.92 ± 1.33	4.97 ± 1.37	4.97 ± 1.19	<5 x 7 log CFU/g
Coliforms	ND	ND	ND	ND	ND	ND	<3 log CFU/g
Yeasts	4.91 ± 2.53	4.04 ± 2.10	4.84 ± 2.28	3.97 ± 2.11	4.96 ± 2.58	4.97 ± 2.59	<5 log CFU/g
Moulds	ND	ND	ND	ND	ND	ND	<3 log CFU/g

*ND- not detectable

4.1.5.4 Effect of storage days on texture quality of minimally processed butternut squash

Figure 7 illustrates the effect of storage time on the degree of firmness of fresh-cut butternut squash cultivars 'Atlas' and 'Pluto' which indicates a decline in firmness during storage for all treatments. Furthermore, there were significant differences noted in the intensities of fresh butternut flavour, sliminess, moist texture, soft edges and dehydration of all minimally processed butternut squash samples during storage. For instance, it was noted that the degree of firmness along the cut edges significantly decreased as the storage period progressed as illustrated in Figure 7. This is due to

the gradual breakdown of pectin in the middle lamella and membranes of parenchyma cells (Martin-Diana et al. 2007) of the minimally processed butternut squash over time. Since pectin in plant tissue is hugely linked with plant tissue firmness, its breakdown translates to texture loss as witnessed in this research during the storage period.

4.1.6 Conclusions

The results of this research work uncovered that the two readily available butternut squash cultivars in South Africa, 'Atlas' and 'Pluto', are both suitable to use for the minimally processed value-added fresh-cut diced butternut product. It can be also concluded from the data represented in Figures 5, 7 and 8 that the fresh-cut butternut squash from all treatments had a shelf life of 7 days. There were no major sensory differences between 'Atlas' and 'Pluto' minimally processed butternut squash as determined by the sensory panel except for the negative dehydration effect that was brought by calcium lactate dipping. Minimally processed samples from both cultivars had a similar 7-day microbiological acceptability limit (MAL) and sensory acceptability limit (SAL) in all treatments (0%, 0.5% and 3% calcium lactate dipping treatments). Since no significant texture quality differences were noted between calcium lactate treated and non-dipped fresh-cut butternut it can be concluded that the minimal processing techniques and parameters used in this study were not suited to significantly improved textural quality as hypothesized.

4.2 Effect of storage temperature on texture quality of minimally processed butternut squash

4.2.1 Abstract

Texture quality deterioration in fresh-cut butternut squash (*Cucurbita moschata*) can be brought about by different post-processing factors such as storage temperature, which tends to affect plant tissue metabolism rates and proliferation of pathogenic and spoilage microorganisms. The effect of storage temperatures (3.5°C and 10°C) on texture of minimally processed butternut squash cultivars 'Atlas' and 'Pluto' was investigated in this section. The investigation was motivated by the effort to reduce processor economic losses brought about by fresh-cut butternut squash texture deterioration possibly emanating from inconsistent cold chain integrity (temperatures above 5°C) during transit, at the retailer or caused by the intended user. Prior to controlled temperature storage, fresh-cut butternut squash cultivars were subjected to calcium lactate dipping treatments (0.5% and 3% w/v) whose impact at the different storage temperatures was also investigated.

Findings suggested that storage of minimally processed butternut cultivars at 3.5°C favourably retains texture quality (7 days) compared to storage at 10°C (2 days). Furthermore, it was observed that calcium lactate treatments had a significant (p<0.05) firming effect on texture quality of fresh-cut butternut cultivars stored at 10°C in contrast to those stored at 3.5°C where calcium lactate treatments did not impact texture quality significantly (p>0.05). The microbiological quality of fresh-cut butternut stored at 3.5°C and 10°C was acceptable during the storage period as guided by microbiological standard guidelines in terms of total viable counts and counts for *Listeria* species, *E. coli* (O157:H7), coliforms, yeasts and moulds. The findings of this research indeed suggested that fresh-cut butternut texture loss can be caused by non-optimal 10°C storage temperature and that, in contrast, a consistent storage at 5°C can possibly retain fresh-cut butternut firmness.

4.2.2 Introduction

Unacceptable loss of firmness in minimally processed butternut has been shown to result in economic losses for processors mainly due to customer complaints and tarnished brand integrity (Raybaudi-Massilia et al. 2009). Regardless of minimal processing steps aimed at preserving fresh-cut produce firmness such as calcium lactate dipping treatment, non-optimal temperatures along the cold chain can consequently undo any efforts to curb economic losses brought by premature

firmness loss. Aguayo et al. (2004b) postulated that even though 0°C is conducive for most fresh-cut products, many of them are shipped and marketed at 5°C and sometimes at temperatures as high as 10°C.

Activity of pectinolytic enzymes increases in plant tissue as storage temperatures increase, resulting in reduced tissue mechanical strength that eventually leads to premature texture deterioration in fresh-cut produce (Ragaert et al. 2007; Moelants et al. 2014). In addition, proliferation of texture deteriorating microorganisms such as *Pseudomonas* species in fresh-cut produce has been reported to be prominent at storage temperatures above 5°C (Lamikanra, 2002; Ragaert et al. 2007). Results in Chapter 4.1 suggested that neither calcium lactate dipping nor cultivar selection had a significant impact on improving the texture quality of fresh-cut butternut squash. Consequently, there was a possibility that the texture quality loss customer complaints could have emanated from poor cold chain integrity. The aim of this study was to possibly identify poor cold chain integrity during handling of fresh-cut butternut as the probable root cause of texture quality loss customer complaints that can be addressed by processors, retailers and consumers. Therefore, this research chapter explored the impact of 10°C storage on minimally processed butternut squash firmness compared to the texture quality impact brought by 3.5°C storage.

4.2.3 Materials and methods

4.2.3.1 Materials

Butternut cultivars ‘Atlas’ and ‘Pluto’ (Sakata Seed) sourced from Mjejane Farm in Mpumalanga lowveld region were used during this study. ‘Atlas’ and ‘Pluto’ butternuts were harvested in February 2020 at 105 and 100 days maturity, respectively and delivered at the processing facility on the day of harvest.

4.2.3.2 Sample preparation and minimal processing

Butternuts were stored in a refrigerated area at 1-5°C for a day prior to minimal processing. Minimal processing steps implemented are shown in the experimental design (Figure 5). Storage and minimal processing steps and parameters were identical to those elaborated on the first experiment (4.1.3.1). Sensory and microbiological assessment samples were drawn from controlled storage (3.5°C and 10°C).

4.2.3.3 Microbial and sensory quality of minimally processed butternut

Sensory and microbiological parameters and assessment methods were similar to those used during the first experiment (4.1.3.3). Sensory and microbiological analyses were conducted on day 1, day 2, day 5, day 6, day 7 and day 8.

4.2.3.4 Statistical analyses

Similar statistical methods highlighted in section 4.1.3.4 were used in this trial: Mann-Whitney U Test and the Independent-Samples Krusal-Wallis Test using IBM® SPSS® version 25 software.

4.2.4 Results

4.2.4.1 Effect of storage temperature on minimally processed butternut squash

Minimally processed butternut squash stored at 3.5°C had more favourable perceived intensities of fresh butternut aroma, degree of firmness, sliminess and soft edges than fresh-cut butternut stored at 10°C as shown in Table 8. Fresh-cut butternut squash stored at 3.5°C had more intense fresh butternut flavour (average = 9.13), and was firmer (average = 8.29) than those stored at 10°C. As shown in Table 8, there were significant differences (p<0.05) in the intensities of all sensory attributes measured on different storage days. Table 9 also shows that storage temperature and storage period had a significant interaction (p<0.05), impacting on all perceived attributes except for sliminess, as was determined by the descriptive sensory panel. There was a significant interaction (p<0.05) amongst storage temperature, calcium lactate concentration and storage time on sensory attributes except for sliminess (Table 10).

Table 8 shows that there were significant differences (p<0.05) between fresh-cut butternut squash control (0% calcium lactate) and calcium lactate treatments (0.5% and 3%) in terms of perceived intensities of fresh butternut aroma, degree of firmness, moist texture, soft edges and dehydration when stored at 10°C. Similar to the first round of experiments in section 4.1, there were no significant differences (p>0.05) amongst calcium lactate treatments (0%, 0.5% and 3%) on all perceived attributes of fresh-cut butternut squash when stored at 3.5°C. However, there was no significant difference (p>0.05) between samples treated with 0.5% and 3% calcium lactate, respectively, for all sensory intensities under both storage conditions of 3.5°C and 10°C (Table 9).

Table 8: Effect of storage temperature, storage period, calcium lactate dipping treatment and their interactions on the sensory properties of fresh-cut butternut squash as evaluated by a trained descriptive sensory panel

	Attribute						
Comparison	Fresh butternut aroma	Degree of firmness	Moist texture	Sliminess	Soft edges	Orange colour intensity	Dehydration
Effect of storage temperature							
3.5°C	$8.7^{a} \pm 0.1$	$7.5^{a} \pm 0.0$	$2.5^{a} \pm 0.1$	1.4 ± 0.0	$3.2^{a} \pm 0.0$	$7.2^{a} \pm 0.1$	$2.1^{a} \pm 0.0$
10°C	$6.9^{b} \pm 0.1$	$6.7^{b} \pm 0.0$	$1.9^{b} \pm 0.1$	0.0 ± 0.0	$2.7^{b} \pm 0.0$	$5.7^{b} \pm 0.1$	$2.1^{a} \pm 0.0$
P-value	<0.001	<0.001	<0.001	1.000	<0.001	<0.001	0.02
Effect of storage period							
1 day	$10.0^{a} \pm 0.1$	$9.8^{a} \pm 0.1$	$1.0^{a} \pm 0.1$	$0.0^{a} \pm 0.0$	0.3 ± 0.1	$6.2^{a} \pm 0.1$	$0.5^{a} \pm 0.1$
2 days	$10.0^{a} \pm 0.1$	$9.8^{a} \pm 0.0$	$1.3^{ac} \pm 0.1$	$0.0^{a} \pm 0.0$	0.0 ± 0.1	$5.0^{a} \pm 0.2$	$0.0^{b} \pm 0.1$
5 days	$6.3^{b} \pm 0.1$	$5.0^{b} \pm 0.1$	$2.3^{b} \pm 0.1$	$0.0^{a} \pm 0.1$	$3.9^{a} \pm 0.1$	$5.6^{ab} \pm 0.2$	$3.9^{c} \pm 0.1$
6 days	$5.2^{c} \pm 0.1$	$4.9^{c} \pm 0.1$	$1.6^{c} \pm 0.1$	$0.0^{a} \pm 0.0$	$4.1^{b} \pm 0.1$	$7.0^{ac} \pm 0.1$	$3.9^{cd} \pm 0.1$
7 days	$4.4^{d} \pm 0.1$	$4.1^{d} \pm 0.1$	$1.4^{a} \pm 0.1$	$0.0^{a} \pm 0.1$	2.2 ± 0.1	$7.2^{ad} \pm 0.2$	$4.0^{ce} \pm 0.1$
8 days	$3.4^{ce} \pm 0.1$	$3.3^{e} \pm 0.1$	$6.9^{d} \pm 0.1$	$5.4^{b} \pm 0.1$	8.6 ± 0.1	$7.1^{af} \pm 0.2$	$4.0^{bf} \pm 0.1$
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Effect of calcium lactate treatment							
0%	$6.9^{a} \pm 0.0$	$6.2^{a} \pm 0.0$	$3.5^{a} \pm 0.01$	0.7 ± 0.0	$4.1^{a} \pm 0.0$	6.6 ± 0.1	$0.6^{a} \pm 0.0$
0.5%	$8.2^{b} \pm 0.1$	$7.41^{b} \pm 0.1$	$1.5^{b} \pm 0.1$	0.7 ± 0.0	$2.6^{b} \pm 0.1$	6.4 ± 0.1	$2.7^{b} \pm 0.1$
3%	$8.4^{b} \pm 0.1$	$7.7^{b} \pm 0.1$	$1.5^{b} \pm 0.1$	0.7 ± 0.0	$2.2^{c} \pm 0.1$	6.4 ± 0.1	$2.8^{b} \pm 0.1$
P-value	<0.001	<0.001	<0.001	0.4	<0.001	0.7	<0.001

Results are expressed as the Means ± Standard Error. ^{abc} means in an attribute's column in a subsection with different letters differed significantly ($p<0.05$). A ten-point sensory scale used where 0 = not intense; 10 = very intense. Effect of storage period and calcium lactate treatment are for fresh-cut butternut stored at 3.5°C and 10°C conducted in one experiment.

Table 9: Effect of storage temperature, storage period, calcium lactate dipping treatment and their interactions on the sensory properties of fresh-cut butternut squash as evaluated by a trained descriptive sensory panel

Comparison	Attribute						
	Fresh butternut aroma	Degree of firmness	Moist texture	Sliminess	Soft edges	Orange colour intensity	Dehydration
Interaction effect of storage temperature and calcium lactate treatment							
3.5°C x 0% calcium lactate	8.7 ± 0.1	7.4 ± 0.1	2.6± 0.1	1.4 ± 0.1	3.6 ± 0.1	7.3 ± 0.1	1.2 ± 0.1
3.5°C x 0.5% calcium lactate	8.6 ± 0.1	7.7 ± 0.1	2.4 ± 0.1	1.3 ± 0.1	3.0 ± 0.1	7.2 ± 0.2	2.5 ± 0.1
3.5°C x 3% calcium lactate	8.70 ± 0.1	7.6 ± 0.1	2.3 ± 0.1	1.3 ± 0.1	2.9 ± 0.1	7.2 ± 0.2	2.5 ± 0.1
10°C x 0% calcium lactate	5.1^{a} ± 0.1	5.0^{a} ± 0.1	4.4^{a} ± 0.1	0.0 ± 0.1	4.5^{a} ± 0.1	5.9 ± 0.1	0.1^{a} ± 0.1
10°C x 0.5% calcium lactate	7.8^{b} ± 0.1	7.1^{b} ± 0.1	0.6^{b} ± 0.1	0.0 ± 0.1	2.1^{b} ± 0.1	5.6 ± 0.2	3.1^{b} ± 0.1
10°C x 3% calcium lactate	8.10^{bc} ± 0.1	7.9^{bc} ± 0.1	0.6^{bc} ± 0.1	0.0 ± 0.1	1.4^{bc} ± 0.1	5.6 ± 0.2	3.3^{bc} ± 0.1
P-value	<0.001	<0.001	<0.001	1.000	<0.001	0.01	<0.001
Interaction effect of storage temperature and storage period							
3.5°C x 1 day	10.0^{a} ± 0.1	9.7^{a} ± 0.1	0.8^{a} ± 0.1	0.0 ± 0.1	0.6^{a} ± 0.1	7.4^{a} ± 0.8	1.1^{a} ± 0.1
3.5°C x 2 days	10.0^{a} ± 0.1	9.7^{a} ± 0.1	0.8^{a} ± 0.1	0.0 ± 0.1	0.6^{a} ± 0.1	7.4^{a} ± 0.8	1.1^{a}± 0.1
3.5°C x 5 days	10.0^{a} ± 0.1	9.7^{a} ± 0.1	0.8^{a} ± 0.1	0.0 ± 0.1	0.6^{a} ± 0.1	7.4^{a} ± 0.8	1.1^{a} ± 0.1
3.5°C x 6 days	9.0^{b} ± 0.1	8.9^{b} ± 0.1	0.7^{b} ± 0.1	0.0 ± 0.1	1.3^{b} ± 0.1	7.1^{b} ± 0.2	3.1^{b} ± 0.1
3.5°C x 7 days	9.4^{c} ± 0.1	8.1^{c} ± 0.1	1.4^{c} ± 0.1	0.0 ± 0.1	2.2^{c} ± 0.1	7.2^{c} ± 0.2	4.1^{c} ± 0.1
3.5°C x 8 days	6.4^{d} ± 0.1	3.3^{d} ± 0.1	6.9^{d} ± 0.1	5.4 ± 0.1	8.7^{d} ± 0.1	5.0^{d} ± 0.2	0.0^{d} ± 0.1
10°C x 1 day	10.0^{a} ± 0.1	9.8^{a} ± 0.1	1.3^{a} ± 0.1	0. 0 ± 0.1	0.0^{a} ± 0.1	7.1^{a} ± 0.2	0.0^{a} ± 0.1
10°C x 2 days	10.0^{a} ± 0.1	9.8^{a} ± 0.1	1.3^{a} ± 0.1	0. 0 ± 0.1	0.0^{a} ± 0.1	5.0^{a} ± 0.2	0.0^{a} ± 0.1
10°C x 5 days	4.3^{e} ± 0.1	4.0^{e} ± 0.1	2.3^{e} ± 0.1	5.5 ± 0.1	3.9^{e} ± 0.1	5.6^{e} ± 0.2	3.9^{e} ± 0.1
10°C x 6 days	3.5^{f} ± 0.1	3.0^{f} ± 0.1	2.6^{f} ± 0.1	7.3 ± 0.1	6.8^{f} ± 0.1	6.9^{f} ± 0.2	4.6^{f} ± 0.1
10°C x 7 days	0.0^{g} ± 0.1	0.0^{g} ± 0.1	0.0^{g} ± 0.1	7.4 ± 0.1	9.2^{g} ± 0.1	7.4^{g} ± 0.2	4.6^{f} ± 0.1
10°C x 8 days	0.0^{g} ± 0.1	0.0^{g} ± 0.1	0.0^{g} ± 0.1	7.7 ± 0.1	9.6^{g} ± 0.1	8.0^{g} ± 0.2	4.6^{f} ± 0.1
P-value	<0.001	<0.001	<0.001	1.000	<0.001	<0.001	<0.001

Results are expressed as the Means ± Standard Error. ^{abc} means in an attribute's column in a subsection with different letters differed significantly (p<0.05). A ten-point sensory scale used where 0 = not intense; 10 = very intense.

Table 10: Interaction effect of storage temperature, calcium lactate treatment and storage period on the sensory properties of fresh-cut butternut squash as evaluated by a trained descriptive sensory panel

Comparison	Attribute						
	Fresh butternut aroma	Degree of firmness	Moist texture	Sliminess	Soft edges	Orange colour intensity	Dehydration
Interaction effect of storage temperature, calcium lactate treatment and storage period							
3.5°C x 0% calcium lactate x 1 day	10.0 ± 0.1	9.4 ± 0.1	1.2 ± 0.2	0.0± 0.1	1.0 ± 0.1	7.4 ± 0.2	0.2 ± 0.1
3.5°C x 0% calcium lactate x 2 days	10.0 ± 0.1	9.40 ± 0.1	1.2 ± 0.2	0.0 ± 0.1	1.0 ± 0.1	7.4 ± 0.2	0.2 ± 0.1
3.5°C x 0% calcium lactate x 5 days	10.0 ± 0.1	9.4 ± 0.1	1.2 ± 0.2	0.0 ± 0.1	1.0 ± 0.1	7.4 ± 0.2	0.2 ± 0.1
3.5°C x 0% calcium lactate x 6 days	9.0 ± 0.1	8.80 ± 0.1	0.9 ± 0.2	0.0 ± 0.1	2.0 ± 0.1	7.4 ± 0.2	0.8 ± 0.1
3.5°C x 0% calcium lactate x 7 days	9.4 ± 0.1	7.9 ± 0.1	1.7 ± 0.2	0.0 ± 0.1	2.8 ± 0.1	7.2 ± 0.2	3.9 ± 0.1
3.5°C x 0% calcium lactate x 8 days	6.5 ± 0.1	3.5 ± 0.1	6.8 ± 0.2	5.7 ± 0.1	8.5 ± 0.1	7.1 ± 0.2	0.0 ± 0.1
3.5°C x 0.5% calcium lactate x 1 day	10.0 ± 0.2	10.0 ± 0.2	0.6 ± 0.2	0.0 ± 0.1	0.5 ± 0.2	7.4 ± 0.3	1.5 ± 0.2
3.5°C x 0.5% calcium lactate x 2 days	10.0 ± 0.2	10.0 ± 0.2	0.6 ± 0.2	0.00 ± 0.1	0.5 ± 0.2	7.4 ± 0.3	1.5 ± 0.2
3.5°C x 0.5% calcium lactate x 5 days	10.0 ± 0.2	10.0 ± 0.2	0.6 ± 0.2	0.0 ± 0.15	0.5 ± 0.2	7.4 ± 0.3	1.5 ± 0.2
3.5°C x 0.5% calcium lactate x 6 days	9.0 ± 0.2	9.1 ± 0.2	0.6 ± 0.2	0. 0 ± 0.1	1.0 ± 0.2	7.0 ± 0.3	4.2 ± 0.2
3.5°C x 0.5% calcium lactate x 7 days	9.4 ± 0.2	8.4 ± 0.2	1.3 ± 0.2	0.0 ± 0.1	2.0 ± 0.2	7.2 ± 0.3	4.2 ± 0.2
3.5°C x 0.5% calcium lactate x 8 days	6.3 ± 0.2	3.2 ± 0.2	7.1 ± 0.2	5.3 ± 0.1	8.7 ± 0.2	7.0 ± 0.3	0.0 ± 0.2
3.5°C x 3% calcium lactate x 1 day	10.0 ± 0.2	10.0 ± 0.2	0.6 ± 0.2	0.0 ± 0.1	0.4 ± 0.2	7.5 ± 0.3	1.5 ± 0.2
3.5°C x 3% calcium lactate x 2 days	10.0 ± 0.2	10.0 ± 0.2	0.6 ± 0.2	0.0 ± 0.1	0.4 ± 0.2	7.5 ± 0.3	1.5 ± 0.2
3.5°C x 3% calcium lactate x 5 days	10.000 ± 0.2	10.0 ± 0.2	0.6 ± 0.2	0.0 ± 0.1	0.4 ± 0.2	7.5 ± 0.3	1.5 ± 0.2
3.5°C x 3% calcium lactate x 6 days	9.0 ± 0.2	9.0 ± 0.2	0.6 ± 0.2	0.0 ± 0.1	1.0 ± 0.2	7.0 ± 0.3	4.2 ± 0.2
3.5°C x 3% calcium lactate x 7 days	9.4 ± 0.2	8.1 ± 0.2	1.3 ± 0.2	0.0 ± 0.1	1.8 ± 0.2	7.2 ± 0.3	4.1 ± 0.2
3.5°C x 3% calcium lactate x 8 days	6.4 ± 0.2	3.2 ± 0.2	6.8 ± 0.2	5.3 ± 0.1	8.7 ± 0.2	7.2 ± 0.3	0.0 ± 0.2
10°C x 0% calcium lactate x 1 day	10.0 ± 0.1	10.0 ± 0.1	1.4 ± 0.2	0.0 ± 0.1	0.0 ± 0.1	5.0 ± 0.2	0.0 ± 0.1
10°C x 0% calcium lactate x 2 days	10.0 ± 0.1	10.0 ± 0.1	1.4 ± 0.2	0.0 ± 0.1	0.0 ± 0.1	5.0 ± 0.2	0.0 ± 0.1
10°C x 0% calcium lactate x 5 days	0.0 ± 0.1	0.0 ± 0.1	7.0 ± 0.2	0.0 ± 0.1	9.0 ± 0.1	5.0 ± 0.2	0.0 ± 0.1
10°C x 0% calcium lactate x 6 days	0.2 ± 0.1	0.1 ± 0.1	7.6 ± 0.2	0.0 ± 0.1	9.1 ± 0.1	8.4 ± 0.2	0.3 ± 0.1
10°C x 0% calcium lactate x 7 days	0.0 ± 0.1	0.1 ± 0.1	7.6 ± 0.2	0.0 ± 0.1	9.1 ± 0.1	8.4 ± 0.2	0.3 ± 0.1
10°C x 0% calcium lactate x 8 days	0.0 ± 0.1	0.1 ± 0.1	7.6 ± 0.2	0.0 ± 0.1	9.1 ± 0.1	8.4 ± 0.2	0.3 ± 0.1
10°C x 0.5% calcium lactate x 1 day	10.0 ± 0.2	9.8 ± 0.2	1.2 ± 0.2	0.0 ± 0.1	0.0 ± 0.2	5.0 ± 0.3	0.0 ± 0.2
10°C x 0.5% calcium lactate x 2 days	10.0 ± 0.2	9.8 ± 0.2	1.20 ± 0.2	0.0 ± 0.1	0.0 ± 0.2	5.0 ± 0.3	0.0 ± 0.2
10°C x 0.5% calcium lactate x 5 days	6.0 ± 0.2	5. 0 ± 0.2	0.0 ± 0.2	0.0 ± 0.1	1.9 ± 0.2	5.9 ± 0.3	5.9 ± 0.2
10°C x 0.5% calcium lactate x 6 days	5.0 ± 0.2	3.9 ± 0.2	0.0 ± 0.2	0.0 ± 0.1	6.6 ± 0.2	6.4 ± 0.3	6.2 ± 0.2
10°C x 0.5% calcium lactate x 7 days	5.0 ± 0.2	3.9 ± 0.2	0.0 ± 0.2	0.0 ± 0.1	6.6 ± 0.2	6.4 ± 0.3	6.2 ± 0.2
10°C x 0.5% calcium lactate x 8 days	5.0 ± 0.2	3.9 ± 0.2	0.0 ± 0.2	0.0 ± 0.1	6.6 ± 0.2	6.4 ± 0.3	6.2 ± 0.2
10°C x 3% calcium lactate x 1 day	10.0 ± 0.2	9.8 ± 0.2	1.2 ± 0.2	0.0 ± 0.1	0.0 ± 0.2	5.0 ± 0.3	0.0 ± 0.2
10°C x 3% calcium lactate x 2 days	10.0 ± 0.21	9.7 ± 0.2	1.2 ± 0.2	0.0 ± 0.1	0.0 ± 0.2	5.1 ± 0.3	0.0 ± 0.2
10°C x 3% calcium lactate x 5 days	7.0 ± 0.2	7.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.1	1.0 ± 0.2	6.0 ± 0.3	6.0 ± 0.2
10°C x 3% calcium lactate x 6 days	5.2 ± 0.2	5.1 ± 0.2	0.0 ± 0.2	0.0 ± 0.1	4.7 ± 0.2	6.1 ± 0.3	7.2 ± 0.2
10°C x 3% calcium lactate x 7 days	5.2 ± 0.2	5.1 ± 0.2	0.0 ± 0.2	0.0 ± 0.1	4.7 ± 0.2	6.1 ± 0.3	7.2 ± 0.2
10°C x 3% calcium lactate x 8 days	5.2 ± 0.2	5.1 ± 0.2	0.0 ± 0.2	0.0 ± 0.1	4.7 ± 0.2	6.1 ± 0.3	7.2 ± 0.2
P-value	<0.001	<0.001	<0.001	1.0	<0.001	0.02	<0.001

Results are expressed as the Means ± Standard Error. A ten-point sensory scale used where 0 = not intense; 10 = very intense.

4.2.4.2 Microbiological quality assessment

Coliforms, *E. coli*, *Listeria* spp. and moulds were below the detection limits throughout the storage period for all treatments (Table 11). There was a significant difference ($p<0.05$) between minimally processed butternut squash stored at 3.5°C and those stored at 10°C with regards to TVC and yeast counts. Furthermore, there was a significant difference ($p<0.05$) with regards to TVC and yeast counts with increasing storage period, indicating a linear relationship between microbial counts and storage period. Calcium lactate treated fresh-cut butternut stored at 3.5°C and 10°C did not indicate any microbiological difference and it was also noted that all the microbiological findings were within acceptable microbiological limits.

Table 11: Effect of storage temperature, storage period and calcium lactate treatment on total viable count, coliforms, *E. coli*, *Listeria* species, yeast and moulds in fresh-cut butternut squash

	Microbiological assessment (Log CFU/ml)					
Comparison	Total viable counts	Coliforms	*E. coli*	*Listeria* spp.	Yeast	Moulds
Effect of storage temperature						
3.5°C	$4.3^{a} \pm 0.0$	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	$3.3^{a} \pm 0.0$	0.0 ± 0.0
10°C	$3.8^{b} \pm 0.0$	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	$2.3^{b} \pm 0.0$	0.0 ± 0.0
P-value	0.1	1.0	1.0	1.0	<0.001	1.0
Effect of storage period						
1 day	$2.6^{a} \pm 0.1$	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	$0.1^{a} \pm 0.1$	0.0 ± 0.0
2 days	$2.8^{a} \pm 0.1$	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	$0.0^{a} \pm 0.1$	0.0 ± 0.0
5 days	$4.9^{b} \pm 0.1$	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	$4.4^{b} \pm 0.1$	0.0 ± 0.0
6 days	$4.7^{b} \pm 0.1$	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	$4.4^{b} \pm 0.1$	0.0 ± 0.0
7 days	$4.9^{b} \pm 0.1$	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	$4.4^{b} \pm 0.1$	0.0 ± 0.0
8 days	$4.9^{b} \pm 0.1$	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	$4.6^{b} \pm 0.1$	0.0 ± 0.0
P-value	<0.001	1.0	1.0	1.0	<0.001	1.0
Effect of calcium lactate treatment						
0%	4.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.9 ± 0.2	0.0 ± 0.0
0.5%	3.9 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.7 ± 0.3	0.0 ± 0.0
3%	3.9 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.6 ± 0.3	0.0 ± 0.0
P-value	<0.001	1.0	1.0	1.0	<0.001	1.0

Results are expressed as the Means ± Standard Error. ^{abc} means in a column in a subsection with different letters differed significantly ($p<0.05$).

4.2.4.3 Textural quality assessment

Samples stored at 10°C fell below the acceptability limit of a firmness intensity score of 7 earlier during storage than those stored at 3.5°C (Figure 12). Minimally processed butternut achieved 7 days and 3 days acceptable textural quality for 3.5°C and 10°C storage, respectively. As shown in Tables 8-10, minimally processed butternut squash exposed to 10°C experienced more rapid deterioration in the sensory parameters assessed by the descriptive sensory panel compared to those stored at 3.5°C. Overall, minimally processed butternut squash stored at 3.5°C and 10°C achieved a shelf life of 7 days and 2 days, respectively.

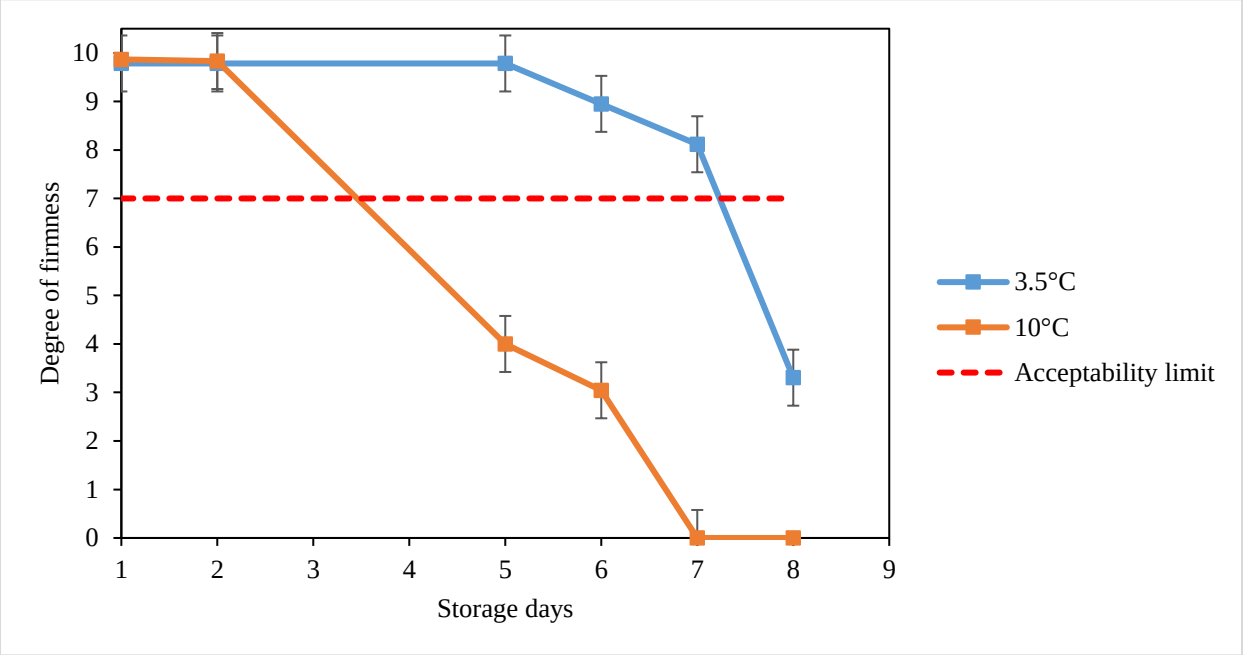

Figure 12: Texture changes for minimally processed butternut squash cultivars 'Atlas' and 'Pluto' stored at 3.5°C and 10°C. Degree of firmness and its acceptability limit was determined by the trained descriptive panel throughout storage of fresh-cut butternut.

4.2.5 Discussion

Minimally processed butternut squash stored at 3.5°C had better retention of perceived quality attributes than samples stored at 10°C - most likely due to the relationship between temperature and metabolic rates of fresh-cut produce. As postulated by Soliva-Fortuny et al. (2002), fresh produce remains metabolically active after being minimally processed into fresh-cut products, resulting in ethylene production, respiration, transpiration and enzymatic browning, at faster rates. Tsouvaltzis et al. (2006), in their work on the effect of storage temperature on leeks (*Allium porrum*

L.) found that there was a linear increase in inner leaf growth (ILG) and fresh weight loss (FWL) and significant colour loss with increasing temperatures of 0°C, 10°C and 20°C.

Bett-Garber et al. (2011) revealed that lower constant storage temperature at 4°C resulted in better fresh-cut cantaloupe quality attribute (aroma, flavour and texture) retention than constant storage at 10°C or transferring fresh-cut cantaloupe from 4°C to 10°C storage after 24 or 48 hours. Bett-Garber et al. (2011) also postulated that lower temperatures near 0°C are ideal for fresh-cut cantaloupe to minimize biochemical changes such as respiration and transpiration and to keep microbial growth as low as possible. Furthermore, as revealed by Lamikanra (2002) on work done on fresh-cut lettuce, samples stored at 12°C had more bacterial contamination than those stored at 5°C, indicated susceptibility to microbial spoilage at higher temperatures in fresh-cut vegetables. One of the principal factors that control the quality of minimally processed produce is how low temperatures (0-5°C) can reduce the effect of wounding stress from processing steps such as cutting, slicing, coring, dicing and peeling. Activity rate of wound-signaling enzymes wound-induced protein kinase (WIPK) and salicyclic acid induced protein kinase (SIPK) has been reported to be inhibited by low temperature storage of fresh-cut produce (Seo et al. 1999; Hodges and Toivonen, 2007). Wounding stress, due to wound-induced signaling in cut plant tissue, can culminate in fresh-cut fruit and vegetable yellowing, enzymatic browning, whitening of cut surfaces and development of off odours at exacerbated rates (Seo et al. 1999; Soliva-Fortuny et al. 2002; Hodges and Toivonen, 2007).

Findings in this research also indicated that calcium lactate treatment of 0.5% and 3% had a significant ($p<0.05$) effect on textural quality life of fresh-cut butternut squash in comparison to the control (0%) when samples were stored at 10°C (Table 10). In contrast, fresh-cut butternut squash exposed to calcium lactate treatments (0%, 0.5% and 3%) and stored at 3.5°C did not experience significant shelf life changes ($p>0.05$) as shown in Table 10. The positive effect of calcium lactate dipping on fresh-cut butternut stored at 10°C aligns with results obtained by Luna-Guzman and Barrett (2000) on fresh-cut cantaloupe (member of the *Cucurbitaceae* family). Luna-Guzman and Barrett (2000) concluded that dipping fresh-cut cantaloupe cylinders at 60°C maintained tissue firmness because of Ca^{2+} ions effects on plant tissue cell-to-cell adhesion that improves mechanical strength. A similar conclusion was drawn by Rico et al. (2007) when ready-to-eat carrots dipped in calcium lactate ($15gL^{-1}$) resulted in significantly higher ($p<0.05$) firmness

and reduced water activity than samples dipped in sodium hypochlorite solution (120 gL^{-1} free chlorine). In addition to the effect of Ca^{2+} ions on cell-to-cell adhesion to produce cross-linked pectin polymer networks in the middle lamella, Ca^{2+} ions can also retain firmness by establishing cell membrane stability through reduction of senescence-associated membrane lipid changes. Lamikanra and Watson (2004) postulated that calcium solutions are effective in inhibiting the lipase enzyme that catalyzes membrane-bound lipids, which results in unstable membrane rigidity, hence tissue firmness.

Calcium lactate dipping (0.5% and 3%) had a significant positive impact ($p<0.05$) on firmness of minimally processed butternut squash when samples were stored at 10°C in comparison to those stored at 3.5°C. Increased pectin methyl esterase (PME) activity has been linked with high temperature (>5°C) storage and minimal processing of fresh-cut fruits and vegetables. Silva et al. (2011) reported that activation of PME at 55°C to 65°C resulted in a positive firming effect in fresh-cut *Cucurbita moschata* slices. Research findings by Rico et al. (2007) reported that heat-shock (50°C) treatment of ready-to-eat carrots together with calcium lactate dipping resulted in increased PME activity. Furthermore, findings by Martin-Diana-et al. (2005) revealed that calcium lactate washing treatments (0.5%, 1.5% and 3%) at 50°C of Iceberg lettuce (*Lactuca sativa* sp.) contributed to reduced activity of polyphenol oxidase and peroxidase in addition to increased activity of PME. PME enzyme activity in plant tissue has a linear relationship with temperature and has been found to retard carrot tissue softening at mild heating temperatures (60-70°C) by Imaizumi et al. (2019). PME increases the rate at which de-esterification (demethylation) of pectin in plant tissue occurs, thus releasing more free carboxylic acid groups that can react with Ca^{2+} ions to form ionic pectin cross-linkages that maintain plant tissue firmness (Yang et al. 2017). Additionally, Imaizumi et al. (2019) reported that demethylated pectin molecules are not easily decomposed by β -elimination. Yang et al. (2017) reported that the use of calcium lactate and PME maximally preserved quality attributes (hardness, colour, weight loss and springiness) using an impregnation technique rather than calcium lactate alone. The increased activity of PME, an enzyme reported to be present in plant tissues (e.g. pumpkin plant tissue) (Silva et al. 2011), at a higher storage temperature (10°C) could have been the reason why the effect of calcium lactate dipping (0.5% and 3%) was more significant ($p<0.05$) than at 3.5°C.

4.2.6 Conclusion

Storage temperature plays a vital role in the quality retention of minimally processed butternut squash, with a lower temperature of 3.5°C achieving more desirable shelf life results than samples stored at 10°C. Furthermore, the effect of calcium lactate treatment was more effective at a higher storage temperature of 10°C than the lower 3.5°C. However, samples stored at 3.5°C demonstrated better overall sensory quality retention regardless of calcium lactate treatment. Textural quality retention challenges for minimally processed butternut squash experienced by the company can possibly be resolved by stricter cold chain integrity measures prior to use by the end user, as opposed to the use of calcium lactate treatment to retain textural quality.

5 GENERAL DISCUSSION

5.1 Methodological considerations

5.1.1 Butternut squash cultivar sampling

Even though published research studies on the effect of farming culture on texture quality of fresh-cut butternut could not be found at the time of this study, absence of information on butternut squash sample cultivation conditions could have limited further interpretation of the findings. Additionally, butternut squash cultivar genetic information and selective breeding history was inaccessible during the study. This information could have possibly provided a wider understanding of how both cultivars were expected to respond to minimal processing since genetic variation between cultivars has been shown to impact fresh-cut produce sensory quality and processability (Hodges and Toivonen, 2007; Grisales et al. 2014; Priori et al. 2017).

Butternut cultivars assessed during morphological characterization were sourced from one farm, Mjejane in Mpumalanga lowveld region, which limits the findings of this research in terms of sample representation. Butternut squash cultivars are commercially grown in other South African regions such as the Mpumalanga highveld and the Vaal region in Gauteng province (Department of Agriculture, Forestry and Fisheries, 2011). Climatic conditions and soil profiles have an impact on fresh-cut produce quality, and these differ depending on the cultivation area/region (Weston and Barth, 1997; Conti et al. 2015). Therefore, compiled morphological findings were not sampled in a manner representative of ‘Atlas’ and ‘Pluto’ cultivars in South Africa.

5.1.2 Characterization

5.1.2.1 Texture and moisture analysis

Textural profiles of butternut squash cultivars were only determined by application of a penetration test which measured the amount of force (Newtons) required to puncture through butternut dices. Even though this was aligned to various squash (*Cucurbita* species) firmness tests in other studies (Suwannarak et al. 2015; Krasnow and Hausbeck, 2016), other textural tests such as compression test, rupture test, shear test and toughness tests could have widened understanding during characterization and storage. Emadi et al. (2011) assessed the mechanical properties of butternut by evaluating toughness, rupture force, shear strength and cutting force in order to determine behaviour during desired and unwanted (compression, impact and vibration) mechanical loading

that occurs during minimal processing. In this study, no texture analysis was conducted on minimally processed butternut squash cultivars during storage. Emadi et al. (2011) mentioned that understanding the physical and mechanical properties of produce is useful in increasing the effects of wanted and decreasing the effects of unwanted mechanical loading. In the study by Emadi et al. (2011), there were comprehensive findings on the physical and mechanical properties of butternut without any penetration/puncture test. One of the findings included compression tests that revealed the mechanical properties of butternut skin, flesh and unpeeled product. Therefore, findings of this study could not be explored further to reveal the physical and mechanical properties such as response to shear and compression forces of peeled 'Atlas' and 'Pluto' butternut squash during minimal processing. There was also a possibility of inaccurate moisture levels (%) in butternut squash samples during cultivar characterization due to the possibility of case hardening that can negatively affect moisture migration during conventional oven drying. Case hardening during drying of fruits and vegetables can be a result of surface hardening mainly due to sugar caramelization (Farias et al. 2020), and this was not taken into consideration during this study.

5.1.2.2 Pectin analysis

Pectin content was determined by indirectly linking it to the intensity of ruthenium red pectin staining on a *L*a*b** scale using a chroma meter when butternut cultivars were being characterized during this research study. This research work did not go further to quantify and analyse the pectin content during characterization and minimally processed butternut storage, which could have provided more detail regarding the nature and behaviour of pectin molecules between the two cultivars ('Atlas' and 'Pluto') and upon subjection to treatments (calcium lactate concentrations, storage period and storage conditions). Instead, an indirect pectin ruthenium red staining intensity technique was used during characterization to identify any significant differences between the two cultivars emerging from the pectin content of the respective butternut squash cultivars. However, more detailed analysis in terms of pectin content and the degree of methyl esterification could have shed more light on the pectin content of both cultivars. For instance, Zhang et al. (2014) quantitively investigated the accumulation of acid soluble pectin (ASP), water soluble pectin (WSP) and oxalate soluble pectin (OSP) in winter squash (butternut squash and pumpkin) by sequential extraction with water (WSP), and then ammonium oxalate (OSP) at room temperature which was followed by extraction with boiling hydrochloric acid (ASP). The ruthenium red staining method used only stains unesterified (acidic) pectins. On the contrary, another staining

technique which uses alkaline hydroxylamine hydrochloride which reacts with methyl esters of pectin is chemically selective and reckoned as superior to the staining obtained by ruthenium red (Hornatowska, 2005), perhaps both staining techniques could have been used alongside each other.

5.1.3 Sample preparation and minimal processing

Findings on the textural quality of minimally processed butternut squash did not consider the impact of calcium hypochlorite (50-75 ppm) application at the QCP and CCP on fresh-cut butternut tissue firmness. Calcium hypochlorite washing at the QCP and CCP is used by the company for its antimicrobial properties. However, a combination of a chlorine-based calcium salt ($CaCl_2$) and chitosan coating at 0.5% and 0.25% concentrations, respectively had a positive impact on firmness because of the action of Ca^{2+} ions on pectin structures of fresh-cut butternut as postulated by Suwannarak et al. (2015). Additionally, the methodology of this research did not consider the necessity of monitoring core temperatures of butternut dices, which have been shown to affect intracellular migration of Ca^{2+} ions to cell wall regions, resulting in a positive plant tissue firming (Imaizumi et al. 2019). There is a possibility that the dipping time of 10 minutes in 50-60°C calcium lactate concentrations was not enough to raise diced butternut core temperatures to optimal temperatures (>60°C) for intracellular Ca^{2+} ion migration, since fresh-cut produce tissue is a poor thermal conductor as mentioned by Imaizumi et al. (2019). There were no experimental replicates done in section 4.1 to verify the data on the findings. On the contrary, there were experimental replicates in the second research (section 4.2), which meant statistical conclusions were drawn from a larger pool of data than the first research section (4.1).

5.1.4 Sensory and microbiological assessment

In this study only a descriptive sensory panel (DSP) was relied on to analyse textural properties of various samples during the storage period. Combining textural analysis results from the DSP and penetration tests throughout the storage period could have brought more objective results. In addition, the coding system (e.g. PA1 = first sample for ‘Pluto’ at 0% calcium lactate; AB2 = second sample for ‘Atlas’ at 0.5% calcium lactate) on trays presented to the descriptive panel was a limitation since there was a possibility that panellists quickly learnt to identify the samples and how they were linked to quality aspects, hence leading to bias. Furthermore, microbiological quality of minimally processed butternut was determined by testing for total viable count, *E. coli* O157:H7, *Listeria* species, yeasts and moulds but specific pectinolytic microorganisms inherent

to the intact fresh produce (e.g. *Pseudomonas* species and *Erwina carotovora*) (Tournas, 2005) were not considered.

Exclusion of the study of pectinolytic microorganisms during processing and storage of minimally processed butternut omitted another major factor in texture quality retention (Ragaert et al. 2007) in fresh-cut produce. For instance, the rapid texture deterioration experienced by butternut stored at 10°C could have been due to action of pectinolytic microorganisms such as *Pseudomonas* species and *Erwina carotovora* and/or a combination thereof with a physiological high temperature-induced cellular pectin degradation. Tournas (2005) postulated that *Erwina carotovora* is widely common in almost all kinds of vegetables and that it can even grow at refrigeration temperatures (0-8°C). *Pseudomonas* species have also been mentioned by Yousuf et al. (2018) to be significantly responsible for fruit and vegetable microbiological spoilage.

5.2 Effect of calcium lactate treatment and storage temperature on the textural shelf life of fresh-cut butternut squash

Even though the effect of calcium lactate treatment has shown a positive impact in retaining textural quality of various fruits and vegetables, its effect was not significant ($p>0.05$) at 3.5°C. It was only at 10°C storage temperature that a significant difference ($p<0.05$) in retaining textural quality was reported, however, an overall shelf life of 2 days was only achieved. It is inconclusive to say calcium lactate treatment does not improve texture quality of fresh-cut butternut squash stored at 3.5°C since a limited number of calcium lactate concentrations (0.5% and 3%) were investigated in this study. The significant impact of calcium lactate dipping solutions (0.5% and 3% at 50-60°C) on the textural quality of fresh-cut butternut stored at 10°C can possibly be attributed to PME activity in the butternut plant tissue. PME activity has been shown to catalyse the formation of Ca^{2+} ionic cross-bridges with de-esterified pectin at favourable storage temperatures (>5°C) (Silva et al. 2011; Yang et al. 2017). Therefore, there is a possibility that the significant impact on the firmness of fresh-cut butternut squash dipped in calcium lactate and stored at 10°C, was due to increased PME activity which in turn facilitated formation of ionic cross-bridges in butternut tissue middle lamella and cell walls, resulting in higher firmness detected by the descriptive sensory panel than in samples stored at 3.5°C.

The effect of calcium lactate above 3% (w/v) concentration on fresh-cut butternut squash can be safely explored since it has an FDA generally regarded as safe (GRAS) status without any

concentration limitations (U.S. Food and Drug Administration, 2019; Electronic Code of Federal Regulations, 2020). However, the use of calcium lactate treatment concentrations by processors ought to be at minimal levels which were known to induce the desired effect (in this case, firmness enhancement) as per good manufacturing practice (GMP) recommendations (U.S. Food and Drug Administration, 2019). There were no published South African legislative restrictions or regulations on the use of calcium lactate on fresh-cut produce found during this study. Recommended dipping temperatures optimum for Ca^{2+} ions diffusion used were (50–60°C) in both experiments of this study. Higher calcium lactate concentrations or longer dipping times can possibly achieve significant differences regarding textural quality. The aim of investigating the effect of calcium lactate treatment was to motivate the use of a calcium-based organic dipping minimal processing step to address textural quality challenges the company was experiencing, with cut edge tissue softening affecting the diced butternut final product. Data from Chapter 4.2 suggested that addressing cold chain integrity can possibly achieve improved texture quality retention than the calcium lactate dipping treatments explored in this study. In all experiments conducted, microbiological stability throughout storage life was within the stipulated requirements.

5.3 Future research

Further research can focus on the effect on butternut squash textural quality that can be brought about by applying both calcium lactate dipping and PME impregnation as minimal processing steps which can increase the effect of calcium ions in improving plant tissue mechanical strength. Additional work can also be done in uncovering the effects of specific pectinolytic spoilage microorganisms such as *Erwina carotovora* and *Pseudomonas* spp., which are known to cause firmness deterioration, on the textural quality of not just ‘Atlas’ and ‘Pluto’ fresh-cut butternut squash, but also include other butternut squash cultivars such as ‘Quantum’, ‘Titan’ and ‘Waltham’ available on the South African market. In an effort to improve butternut squash textural quality the fresh-cut industry and researchers alike can possibly focus more on abiotic stress factors like storage temperature and considering cost-benefit analysis of quality deterioration reduction options available. In terms of characterization, butternut squash cultivars from other regions in South Africa (Mpumalanga highveld, Gauteng Vaal region) can also be investigated (e.g. proximate analysis, pectin content, pH, °Brix, morphology, firmness and PME activity) to edify the knowledge of butternut producers and processors.

6 CONCLUSIONS AND RECOMMENDATIONS

Fresh-cut butternut squash textural shelf life could not be improved by application of calcium lactate dipping treatments of 0.5% and 3% in a way that addressed the textural deterioration challenges that contributed to customer complaints and consequent economic losses in the diced 500 g butternut product. The shelf life of minimally processed butternut squash achieved an extra 2 days of shelf life when stored at 3.5°C, a storage temperature that is not always a reflection of supply chain temperature integrity. Therefore, to address the problem at hand of premature textural deterioration, focus can be thrust on maintaining cold chain integrity post-packing, which includes transportation refrigeration, retail store refrigeration and end user storage conditions. In addition, results obtained in this study suggest that both ‘Atlas’ and ‘Pluto’ butternut squash cultivars have similar physical and physicochemical characteristics and had experienced the same effect of calcium lactate dipping at 0.5% and 3% concentration levels. Therefore, it can be recommended to the broader fresh-cut industry in South Africa that there is no significant difference in textural shelf life that can be exerted when comparing ‘Atlas’ and ‘Pluto’ butternut squash.

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8 APPENDIX I: DESCRIPTIVE SENSORY PANEL EVALUATION FORM

BUTTERNUT, DICED 500G SENSORY EVALUATION

DATE				
LOCATION	**In2Food (Pty) Ltd, 67 Middle road, Bartelett, Boksburg, 1459**			
NAME OF PANELIST				
CAPACITY				
AGE GROUP (please tick)	**18-25** ☐ **26-39** ☐ **40-50** ☐ **≥50** ☐			
SEX (please tick)	**Male**		**Female**	

ATTRIBUTES									
AROMA				**TEXTURE**				**APPEARANCE**	
Fresh butternut	**Off butternut**	**Other unidentified off-flavours**	**Chlorine smell**	**Firm**	**Moist**	**Slimy**	**Soft edges**	**Orange colour**	**Dehydration**

COMMENTS:

SCALE

Intensity of descriptors (e.g. Texture-firm) to be scored between 0 and 10.
0= least intense or pronounced and 10= most intense or pronounced.