EFFECT OF STORAGE TEMPERATURES ON THE POSTHARVEST PERFORMANCE AND SPROUTING OF SELECTED POTATO CULTIVARS

By

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Declaration

I, Xola Ngceni (Student No.: 218018026), declare that the research reported in this thesis, except where otherwise indicated is my original work. This thesis has never been submitted for any degree or examination at any university.

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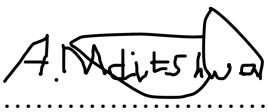

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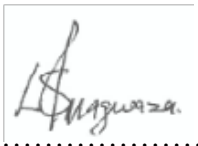

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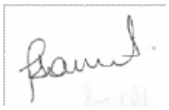

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"Why, my soul, are you downcast? Why so disturbed within me? ***Put your hope in God, for I will yet praise him, my Saviour and my God." Psalms 43:5 NIV***

"The LORD is my light and my salvation; whom shall I fear? the LORD is the strength of my life; of whom shall I be afraid?" Psalms 27: 1 KJV

To God be the Glory. Amen.

Study overview

Sprouting, processing and nutritional quality of potato tubers are cultivar and storage dependent. Due to the extensive choice of potato cultivars currently available in South Africa, there is a limited information on their performance under the wide range of storage temperatures. This research investigated four objectives which were to: i) review the postharvest factors affecting the potato tuber quality; ii) investigate the effect of different storage temperatures on sprouting incidence and processing attributes of the selected potato cultivars; iii) determine the effect of different storage temperatures on nutritional quality parameters of selected potato cultivars; iv) develop prediction models using near infrared spectroscopy (NIRS) for determination of potato quality.

In chapter 2, factors affecting postharvest quality of potato tubers were reviewed. It was observed that factors such as sprouting, mass loss, storage conditions and duration, and postharvest treatments have high influence on the postharvest quality of potato tubers. Sprouting was found to be the major cause of the high postharvest losses occurring in potato industry. Chapter 3 investigated the effect of storage temperatures on sprouting and processing attributes of potato tubers. The results clearly demonstrated that sprouting and processing attributes were both cultivar and storage dependent. For instance, sugars of some potato tubers were highly stimulated by cold storage whereas for sprouting it was vice-versa. The dry matter content and mass loss of potato cultivars slightly increased with storage time and temperature. In chapter 4, the effect of storage temperatures on the nutritional quality of potato tubers was investigated. In this chapter, the findings clearly proved that ascorbic acid and total phenolic content generally decreased while the antioxidant activity was increasing with storage time. Proteins in potato tuber varied based on storage temperature and cultivar. Chapter 5 sought to develop the predictive models for determination of the internal quality of potato tubers. Good models for ascorbic acid, mass loss and total phenolics for all the cultivars were developed. On the hand, poor models were developed for both sucrose and reducing sugars for all the cultivars.

Preface

This thesis is a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

Conference presentations

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Chapter 1

General introduction

1.1. Introduction

The world population is gradually increasing, exceeded seven billion people during 2011 and is also estimated to reach 9.3 billion by 2050. At the same time, more people need to be fed, resulting in a dramatic increase of food demand by 50-70% (Bond et al., 2013). Interestingly, potato (*Solanum tuberosum*) has the increasing potential to counteract the rising demand for food as it can fight against hunger and poverty in the world. In terms of the nutritional content, potato constitutes of 16-20% carbohydrates, 2.5-3.2% crude protein, 0.8-1.2% mineral content, 0.1-0.2% crude fats, 0.6% crude fiber and vitamins(Ali et al., 2016)**.** Furthermore, potatoes contain an assortment of phytochemicals such as carotenoids and polyphenols(Reshi et al., 2017).

Potato has become one of the most important crops based on its annual production. As a result, it is globally ranked as the fourth crop after wheat (*Triticum aestivum L.*), rice (*Oryza sativa* L.) and maize (*Zea mays* L.) (Kingori et al., 2015). China and India are amongst the leading potato producing countries in the world. Both China and India account 74% of the regional output and 77% of the area planted (Scott & Suarez, 2012). However, there is a great variation in potato yields across the world which puts Sub-Saharan Africa below the global average by 75% and less than 30% when compared to the top producing regions(Birch et al., 2012). Remarkably, a dramatic increase in potato production and demand mainly in Asia, Africa and Latin America has been observed. The production output increased from below 30 million tons in the early 1960s to more than 165 million tons in 2007. Hence, for the first time in 2005, the developing world's production surpassed that of the developed world (Devaux et al., 2014).

According to Denner et al. (2012), South Africa (SA) ranks 28th position when compared to other potato producing countries, 0.6% of the world's total production and 0.3% on the country's total surface area. Furthermore, potatoes are mainly cultivated in 16 different regions, differing soils and climatic conditions. These regions vary in terms of the planting dates throughout the year, resulting in agroecological seasons being four, namely a dry or rainy winter season and dry or rainy summer season(Van der Waals et al., 2016). As a result, it can

be deduced that in South Africa there is a continuous supply of potatoes throughout the year. However, the question might arise at the postharvest perspective, regarding the quality maintenance of the existing production of potato tubers and as well their shelf life throughout the food value chains.

Arguably, South Africa lacks enough data regarding the postharvest losses occurring in the potato industry, especially from different interconnected value chains. As a result, it cannot be ignored that postharvest losses occurring over the entire food chain contribute to high losses of resources invested in food production, transport and storage (Beretta et al., 2013). The sad truth is that one-third of the food produced on a global scale end up in waste or used as feed for animals. As a result, it is estimated that approximately 40% of the globally produced food is lost during the early postharvest and processing stages. On that account, reducing postharvest losses play an important role in enhancing the availability of food in the future (Bond et al., 2013). However, one of the greatest challenges in preventing these postharvest losses in developing countries pertains to the understanding of the real extent of losses which makes it difficult to put key measures to prevent them. Moreover, there no real clarifications regarding socio-economic factors contributing to the existence of these losses (Affognon et al., 2015). Hence, there are no agreed standardised consistent measurements for evaluating the postharvest losses (Aulakh and Regmi, 2013). Therefore, the South African potato industry is not exempted to the aforementioned challenges. For, there is less or no statistics on postharvest losses occurring along the potato value chains.

Recently, there has been a significant decline in consumption of table use potatoes globally. In contrast, an increase in consumption of potatoes for food processing such as French fries, potato chips, frozen croquettes, and packed salads has been observed since 1970, increasing by 1356200t (including imported) in 2009 (Mori et al., 2015). At the same time, making the superior quality of processed products is of great priority for the processing industry. As a result, potatoes are expected to meet minimum criteria pertaining to morphological and biochemical parameters. The tuber is expected to be round to oval (45-80mm diameter), high dry-matter content (>20% except for canning products which vary from 18-20%), specific gravity (>1.080), low reducing sugars and low total phenolic compounds(Marwaha et al., 2010). Briefly, the shape and size of the tubers are important for making chips of uniform size. The potato tubers that have high dry matter content and specific gravity use lower oil content

in fried products and therefore longer shelf life. In contrast, for the canned products, they do not require high dry matter content since it results in the sloughing of tubers. Low reducing sugars are recommended since the excessive amounts result in an unacceptably dark colour and bitter taste in fried products. Lastly, less total phenolic compounds are used as an important criterion that indicates the extent of enzymatic discoloration that occurs when the potatoes are peeled, cut or injured(Keijbets, 2008; Marwaha et al., 2010). Hence, the breeding of suitable cultivars for processing industry becomes a priority. Above all, the good post-harvest(storage) performing cultivars are of great importance for the processing industry (Keijbets, 2008).

Potatoes are semi-perishable produce in nature. As a result, they are normally stored in the cold stores of 2-4 °C to reduce postharvest losses (Marwaha et al., 2010). According to Ali and Jansky (2015), storage temperatures below 8 °C are beneficial to potatoes since they prevent bacterial soft rot, water and dry matter loss and prevent sprouting without the use of the sprout suppressants. Although the use of sprout suppressants namely chorpropham and maleic hydrazide is the effective way of controlling sprouting. They turn to be detrimental not only to the environment but to human health (Abbasi et al., 2015; Ahmed et al., 2014; Foukaraki et al., 2016). Hence, the use of low storage temperatures remains advantageous. Despite its benefits, one major drawback of cold storage is that it does not stop the metabolic activities completely within the tuber, but it just reduces the rate at which they occur. As a result, the starch would break down at a slower rate producing sucrose. The production of sucrose results to the subsequent accumulation of reducing sugars namely glucose and fructose. This phenomenon is usually called cold-induced sweetening (Galani et al., 2017). According to Georgelis et al. (2018), the sugars accumulating in non-photosynthetic tissues are usually acquired through starch degradation pathway. Specifically, the enzymes responsible for converting starch into sucrose are UDP-glucose pyrophosphorylase and sucrose-6-phosphate synthase. Furthermore, the one responsible for the conversion of sucrose to reducing sugars is called acid invertase enzyme.

Potatoes that have undergone cold-induced sweetening result in an unacceptable blackening of crisps and chips after processing. The blackening of these fried products usually occurs when the reducing sugars react with free amino acids under high frying temperatures(Ali and Jansky, 2015). Driskill et al. (2007) reported that dark processed fry chips are caused by the accumulation of acrylamide content known as the cancer-causing agent. The increase of

acrylamide in potatoes have caused the global food concern, as a result, more actions need to be taken so as to counteract the increase of the reducing sugars during storage (Clasen et al., 2016; Owolabi et al., 2013; Rezaee et al., 2013).

1.2. Problem statement

Potato is classified as a semi-perishable product that contains high moisture content. Unlike cereals to increase its shelf-life, it cannot be dried, since the absence of moisture would result in shrinkage, weight loss and loss of economic value (Azad et al., 2017). Specifically, postharvest losses are caused by processes such as respiration, sprouting, evaporation of water from the tubers, the spread of diseases, changes in the chemical composition and physical properties of the tubers and as well damage by extreme temperatures (Azad et al., 2017). Hence, the use of proper storage becomes very crucial especially after harvesting for a constant supply of potatoes in the market, even during the offseason periods (Azad et al., 2017; Bhattacharjee et al., 2014). After harvesting, the supply of potatoes becomes available at once. In this case, the supply generally surpasses the current demand (Rastovski et al., 1981). During this time, most farmers would be compelled to sell out most of their produce at low prices (Bhattacharjee et al., 2014). At the same time, they must maintain competition and viability in the market since processors require high-quality potatoes (Kibar, 2012). Briefly, the processors require potato tubers with good appearance (without blemishes), low reducing sugars (0.25% (Fresh weight) fr.wt. for dehydrated products, 0.15% fr.wt. French fries, < 0.1 % fr.wt. for chips and 0.5% fr.wt. canned stuff) , no signs of sprouting, high dry matter content (> 20 % except for canned) and high nutritional composition (Marwaha et al., 2010). During this time maintaining the highest tuber quality throughout the storage by providing good storage condition also becomes very crucial. However, it should be taken into consideration that the storability of potatoes can vary from one cultivar to another. Therefore, intensive selection becomes the best priority for each storage temperature (Bethke, 2014).

Globally, the preferred storage temperature of potatoes varies between 8-12 °C (85–90% RH). Potatoes stored at 8- 12 °C generally last for a longer period (6-9 months) (Paul et al., 2016). In addition, the main advantage of storing potatoes at this range of temperature is to prevent the accumulation of reducing sugars in stored potatoes. Countries experiencing sub-temperate, sub-tropical and tropical climates, short-term (3 to 4 months) storage of potatoes is being practiced using non-refrigerated traditional/on-farm methods (Paul et al., 2016).

Due to the wide choice of cultivars currently available in South Africa, there is a necessity for local cultivar characterisation to investigate the optimum storage guidelines per cultivar (Denner et al., 2012). As a result, there is limited information on whether the effect of postharvest temperature on both storage potential and potato sprouting differs with genotype or not. Therefore, this study seeks to investigate the relationship between the cultivar and the storage conditions (storage temperature) on potato tuber quality.

1.3. Significance of the study

Considering that genotype has an influence on potato development, studying the effect of temperature on storage potential and sprouting in multiple potato cultivars would give a comprehensive understanding of the relationship between these factors. Thus, the knowledge gained from this research will help both the processors and producers to categorize each cultivar based on its potential storability under various temperatures. They will get informed as to which varieties perform the best for the processing quality/ dual purpose along with advanced storage conditions. Such findings will be useful in minimizing the postharvest losses and maintaining the quality of potatoes

1.4. Research questions

- How does postharvest temperature affect the sprouting and processing quality parameters of different potato cultivars during storage?
- What is the effect of storage temperature on the nutritional composition of different potato cultivars?
- How can tuber quality parameters be investigated without being destructed?

1.5. Aim and objectives of the study

This study aimed to evaluate the effect of temperature on the postharvest storage potential and sprouting of selected potatoes cultivars. The specific objectives of the study were:

- To determine the sprouting and processing quality parameters of potato cultivars stored in different temperatures.
- To investigate the protein content and antioxidant properties of potato cultivars stored in different temperatures.
- To develop a predictive model of near infrared spectroscopy as a non-destructive method for quality determination in potato tubers

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Chapter 2

Postharvest factors affecting potato tuber quality: a review

Abstract

Postharvest losses are amongst the major challenges in potato production because huge amounts of potatoes are lost annually due to improper storage conditions. Therefore, this review of literature provides an overview of empirical studies on postharvest challenges and treatments affecting potato tuber quality. Amongst the postharvest challenges affecting potato tuber quality, dormancy, sprouting, mass loss, rotting, mechanical damage, bruising, storage conditions and duration were investigated. On the other hand, chlorpropham (iso-propyl N-(3-chlorophenyl) carbamate, or CIPC), maleic hydrazide, essential oils, ethylene, hydrogen peroxide, ultraviolet-C (UV-C) and gamma irradiation were amongst the postharvest treatments that were investigated in this review. The studies clearly demonstrated that postharvest challenges on tuber quality may vary from one cultivar to another. In a case of mechanical damage and bruising, they may be attributed to handling of potato tubers from harvesting until they reach the target market. CIPC is considered the most effective postharvest treatment in controlling the presence of sprouting in potato tubers. However, there is an increasing concern regarding the use of CIPC and maleic hydrazide as the potential sprout inhibitors since they can persist in the surface of the potato tubers for a long time. As a result, they not only pose problems to the environment but also to human health. Although gamma irradiation is effective in inhibiting sprouting, high concentrations can result to complete tuber breakdown. Furthermore, studies have also reported UV-C, essential oil and hydrogen peroxide as the potential sprout inhibitors that could be used to substitute the use of CIPC in the potato industry.

Keywords: Postharvest challenges, storage conditions and duration, postharvest treatments

2.1. Introduction

Potato production increased by 48% in the developing countries while a decline of 12% in production in the developed countries was observed between 1991 to 2007 (Birch et al., 2012). It was for the first time in 2005 when the potato production of the developing world surpassed that of the developed world (Keijbets, 2008). This alone underlined the importance of potato

as the food security crop to meet the high demands of the growing human populations in developing countries (Birch et al., 2012). Furthermore, potato is ranked as the third most important crop in the world with major production countries being China (25%), India (8%), Russia (6%), Ukraine (6%) and the USA (5%) (Tanios et al., 2018). In Africa, Egypt produces the largest volumes of potatoes followed by Algeria, Malawi, South Africa, and Rwanda. However, South Africa compared to other top African producing countries, has the smallest production area of about 50 000 ha, with highest average yields of 43 t ha^{-1} (Steyn et al., 2016). The most interesting part about potato crop is that it has the capacity to produce more food per unit area and time and is characterised by its high nutritional value (Marwaha et al., 2010).

However, in South Africa, there is very limited information on figures related to postharvest losses of potatoes and as well as value chains under which these losses occur. Aulakh and Regmi (2013) stated that the reason for the lack of information on postharvest losses of crops in general is that approximately 95% of the investments during the past 3 decades concentrated more on enhancing the productivity, while 5% was on reduction of postharvest losses. In the meantime, large amounts of food wasted in sub-Saharan Africa are caused by inappropriate storage measures (Obayelu and Obayelu, 2014).

Postharvest quality over long-term storage remains a concern to both producers and processors. For that reason, ensuring a longer shelf-life, storage conditions must be set correctly so as to reduce sprouting, respiration, loss of water and diseases in potatoes (Copp et al., 2000). For instance, sprouting is one of the major causes of the rapid increase in physiological mass loss of stored tubers. This occurs because the epidermis of the sprout is 100% times more permeable to water compared to the rest of the tuber surface. As a result, a 1% increase of sprouting in the surface of the tuber results in doubling of the moisture loss (Paul et al., 2016). A mass loss of 10% or more leads to a visible shrivelling of the tuber, an undesirable characteristic for consumers, thus losing a number of marketable tubers and as well quality (Ezekiel et al., 2007). Furthermore, postharvest rotting caused by postharvest pathogens results in major losses during storage. In the case of bacterial pathogens such as *Pectobacterium carotovorum* subsp Phytophthora erythroseptica and others function by secreting a wide range of hydrolytic enzymes such as cellulases, pectinases, xylanases and proteases that result to maceration and cell death of the potato tuber (Tanni et al., 2019).

Therefore, it is important to realize that the factors explained above occur at a faster rate under ambient conditions (>8 °C) (Ali and Jansky, 2015; Raigond et al., 2018). As a result, storing

the potato tubers under cold storage (< 8 °C) has been the best way to counteract some of the challenges associated with the postharvest storage. One great advantage of cold storage (< 8 °C) is that it prevents the rate at which postharvest losses caused by sprouting, mass loss and as well as disease infections occur (Ali and Jansky, 2015). However, in terms of the potato processing quality, it causes a rapid increase in reducing sugars, an undesirable characteristic for the processing industry (Galani et al., 2017). It is with this reason the use of synthetic chemicals such as chlorphropham and maleic hydrazide came into place since they prevent some physiological processes (such as sprouting) of the potato tubers even in warm conditions (>8 °C) (Raigond et al., 2018). However, their disadvantage is that they are not environmentally friendly and can cause some complications to human health (Abbasi et al., 2015; Ahmed et al., 2014). Later, essential oils were then developed to reduce the high use of synthetic chemicals that are used during the postharvest storage of potato. Although they are organic, healthy and environmentally friendly, their main drawback is that they are not as effective as compared to synthetic chemicals such as chlorphropham and others. In addition, due to the nature of their degradability, they need to be applied more frequently to improve their efficacy over long-term storage (Owolabi et al., 2013).

Therefore, the aim of this review was to investigate the postharvest challenges and treatments that affect potato tuber quality during storage.

2.2. Postharvest challenges

The shelf life and quality of potatoes are reportedly affected by both pre-and postharvest factors (Hossain and Miah, 2009; Kumar et al., 2004). However, for this review postharvest factors affecting tuber quality are investigated. These factors include dormancy, sprouting, mass loss, postharvest rotting, mechanical damage and bruising, storage conditions and duration as well as postharvest treatments.

2.2.1. Dormancy and sprouting

Dormancy is described as the physiological state in which the tubers are unable to sprout even if the conditions are favourable for germination (Alexandre et al., 2016). Since it is difficult to identify dormancy stage, postharvest dormancy can be used to describe the state from dehaulming to the state in which 80% of the tubers display sprouts of at least 2 mm long. Potato

dormancy varies between 2-3 months depending on the type of the variety (Alexandre et al., 2016; Mani et al., 2014). As a result, potato dormancy is well known as a varietal characteristic (Table 2.1). Although potato dormancy cannot be linked to the earliness of the varieties, there is a possibility of breeding late varieties with relatively short dormancy and early varieties with relatively long dormancy (Mani et al., 2014). The degree of dormancy is usually influenced by the environmental, physiological and hormonal processes. Factors such as soil and weather conditions during growth, maturity, storage conditions and size of the tuber play a major role on potato dormancy (Mani et al., 2014). For instance, in high temperatures, low soil moisture content and fertility during tuber growth period accelerate tuber physiological processes which then reduce the dormancy period (Mani et al., 2014). In addition, the unstable temperatures during storage reduce dormancy more than the constant high temperatures (Mani et al., 2014; Sonnewald and Sonnewald, 2014). Dormancy can be affected by the size of the tuber, with small tubers having longer dormancy than big ones. Abscisic acid (ABA) influences the regulation of tuber dormancy and wound healing. ABA is believed to be at maximum immediately after harvest when meristem dormancy is at deepest. Then it gradually falls during the storage as the dormancy lessens. Hence, the initiation of dormancy is associated with high concentrations of abscisic acid and as well decrease in gibberellins content (Mani et al., 2014; Wróbel et al., 2017). In summary, ABA and ethylene are reported to be linked with onset of dormancy and maintenance of dormancy whereas gibberelins and cytokinins are associated with the release of dormancy and sprouting (Sonnewald and Sonnewald, 2014).

Therefore, for quality purposes, dormancy is divided into three types which include endodormancy, ecodormancy and paradormancy. Endodormancy is the postharvest state that is caused by internal or physiological processes occurring within the tuber. In this state, the tubers do not sprout even if the conditions are ideal for sprouting to occur. During ecodormancy, sprouting is inhibited or delayed by the environmental conditions (Teper-Bamnolker et al., 2010). For instance, tubers stored at lower temperatures have longer dormancy compared to those stored at warm temperatures. In the case of paradormancy, the physiological signal for dormancy would exist in a different location of the tuber from which the dormancy is occurring. This can be explained through apical dominance where the apical meristem or dominant bud/eye inhibits the development of secondary bud or sprout (Mani et al., 2014; Sonnewald and Sonnewald, 2014).

Table 2.1: The effect of dormancy period in different potato cultivars (Denner et al., 2012)

Short Dormancy (40-50days)	**Short- Medium Dormancy (50-70 days)**	**Medium Dormancy (60-80 days)**	**Medium to long Dormancy (70-80 days)**	**Long Dormancy (>90days)**
Hertha	Astrid	Calibra	Avalanche	Aviva
	BP1	Eryn	Columbus	Fianna
	Buffelspoort	Pentland	Darius	Hermes
	Caren	Platina	Fabula	Santana
	Mnandi	Rodeo	Frodo	Vander-plank
	Mondial	Ronn	Lady Rosetta	
	Up-To-Date	Shepody	Sifra	
		Valor		

The end of dormancy can be witnessed by the development of visible bud growth (Aksenova et al., 2013). The loss of dormancy results in the activation of tuber sprouting (Teper-Bamnolker et al., 2010). Sprouting is described as the visible growth of the meristem tissues in the eyes of the potato tubers occurring after the termination of dormancy. The earliest stage of sprouting is observed by the visible white buds often termed as "peeping" or "pipping" (Daniels-Lake and Prange, 2007). Sprouting occurs immediately after the termination of deep dormancy which is also influenced by the extended period of storage (Aksenova et al., 2013; Suttle, 2004; Teper-Bamnolker et al., 2010). Hormones such as ethylene, cytokinins, gibberellins, and auxins are known to play a key role in sprout formation. However, the effect of exogenous application of ethylene is reported to vary depending on the duration of exposure (Sonnewald and Sonnewald, 2014). For instance, the short or periodic application is known to induce sprouting whereas continuous application of ethylene inhibits sprout elongation of some cultivars (Foukaraki et al., 2016; Sonnewald and Sonnewald, 2014). Moreover, hormones such as cytokinins, gibberellins and auxins are known as promoters of sprouting in potatoes (Wróbel et al., 2017).

Sprouting causes a hasty increase in physiological mass loss of the stored tubers. This occurs because the epidermis of the sprout is 100% times more permeable to water compared to the

rest of the tuber surface (Paul et al., 2016). As a result, a 1% increase in the surface of the tuber causes the doubling of the moisture loss from the potato tubers (Paul et al., 2016). During sprouting, processes such as the respiration and evaporation are increased rapidly. Hence, sprouted potatoes result in deterioration in processing quality attributes of the tubers. This is mainly caused by mass loss, reduced turgor, structural changes due to sprout tissue and as well a rise in sugars concentrations caused by the degradation of starch (Paul et al., 2016). The high levels of the starch hydrolysis and accumulation of simple sugars are needed for the supply of the carbon and energy required for the sprout development (de Freitas et al., 2012). Since sprouting is detrimental to the nutritional and processing qualities of the potato tubers, significant losses during storage are then experienced resulting to a smaller number of the marketable tubers (Afek et al., 2000; Knowles and Knowles, 2015; Teper-Bamnolker et al., 2010).

It should be noted that before a potato clone is released to the industry, its dormancy period and sprouting behaviour must be documented. For instance, the dormancy value or duration provides an indication of the time-frame for which the potato will be stored before it initiates sprout development. Hence, the knowledge gained will help on selecting cultivars for short- and long-term storage. Furthermore, the information will also help plan the proper time for applying sprout suppressants and as well as the marketing of the potatoes (Mani et al., 2014). As a result, different markets have different objectives which might be either to stimulate or break tuber dormancy (Vreugdenhil, 2007).

2.2.2. Mass loss

Mass loss reduces the quality, shelf life as well as consumer acceptability of potatoes (Mani et al. 2014). According to Ezekiel et al. (2007) mass loss of 10% or more results in visible shrivelling and loss of quality. It is generally caused by water loss occurring in the outer most skin tissues of tubers during respiration and transpiration (Azad et al., 2017). According to Saran and Chhabra (2014), respiration causes a gradual increase in tuber mass loss by converting starch in the presence of oxygen to carbon dioxide, water and heat. The rate of respiration is directly proportional to that of temperature doubling with every 10 °C rise. However, the process of respiration accounts for a small percentage of mass loss than its counterparts (Potato South Africa, 2017). Factors such as physical maturity, sprouting, evaporation, handling (packaging) as well as cultivar/genotype have an enormous effect of tuber mass loss.

The potato tuber is considered physically mature when it has fully developed a thickened periderm layer (skin) below the epidermis (Pinhero et al., 2009)**.** The tuber skin is made up of periderm (figure 2.1) that consists of three types of cells namely phellem, phellogen and phelloderm. The periderm replaces the epidermis during the early tuber development stages. During outward meristematic division the phellogen generates phellem layers with suberized cell walls whereas inward division result to the parerenchyma-like phelloderm (Fogelman et al., 2015). The new skins layers are continuously added through phellogen activity until the tuber is fully expanded. The tuber ceases to expand when the plant reaches senescence or haulm removal stage. During this period the new skin layers are no longer needed, and the periderm undergoes 'skin set', a stage in which the phellogen becomes inactive resulting to the skin becoming strongly attached to the underlying tissues. The periderm is therefore considered mature after skin set whereas before that is considered immature (Fogelman et al., 2015).

Heltoft et al. (2017) indicated that physically mature tubers turn to perform better in storage than the immature tubers. The poor skin-set of immature tubers makes them prone to the permeability of water and disease infections, thus losing more mass due to transpiration. The physical maturity of tubers is attributed to the development of a mature and fully set periderm (Fogelman et al., 2015; Kumar et al., 2004; Pinhero et al., 2009)**.** Therefore, skin maturity development should be considered as the model for predicting storage losses. Moreover, the mass loss occurs differently for dormant and sprouted tubers (Mani et al., 2014). Mass loss of dormant tubers occurs through the periderm and less proportion via the lenticels. It is with this reason that tubers that have thick periderm and fewer lenticels lose less mass than their counterparts (Mani et al., 2014). On the other hand, the sprouted tubers lose more mass than dormant tubers. The degree of sprouting and the number of sprouts per tuber determines the amount of mass loss to occur during storage. The high rate of sprouting increases the permeability of sprout to water vapour. It is estimated that 1% of the sprout increase, results in a 50% increase of respiration rate (Paul et al., 2016). As a result, there is a significant correlation between mass loss and both the length and number of sprouts per tuber (Mani et al., 2014).

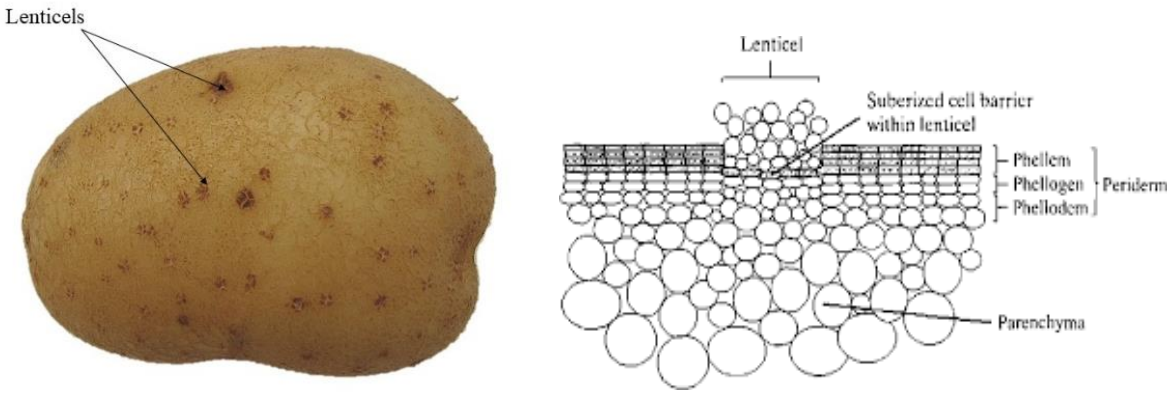

Figure 2.1. The diagrammatic representation of both lenticel and periderm (Tyner et al., 1997) of potato tuber

In addition, the process of evaporation is regarded as the major cause of tuber mass loss during postharvest. In a case where the tubers are harvested with full skin set, the corky cells would help reduce evaporation (Potato South Africa, 2017). Temperature, relative humidity, ventilation/ wind and water vapour pressure deficit are some factors that influence the rate of evaporation occurring in tubers. For instance, the increase in temperature encourages evaporation thereby reducing the mass loss of the tuber. Increasing relative humidity reduces evaporation resulting in less tuber mass loss. Ventilation or wind enhances the rate of evaporation around the tubers (Potato South Africa, 2017). The rate of water loss from any sample of potatoes is directly linked to the water vapour pressure deficit (WVPD) or drying power of the surrounding air. The rate of water loss taking place under the given WVPD is affected by the periderm or outer skin layer of mature potatoes. The periderm restricts the rate of water loss from a tuber to below that of a free water surface, exposed to the same WVPD (Pinhero et al., 2009). According to Scheer (1993) smaller vapour pressure difference between the air in the product and the surrounding air results to less water loss thereby less mass loss (Scheer, 1993).

The mass loss among cultivars is attributed to periderm thickness, a number of cell layers in the periderm and as well the number of lenticels on the tuber surface (Azad et al., 2017; Saran and Chhabra, 2014). In general, the varieties which have thick periderm (a large number of cell layers in the periderm) and as well less lenticels on the tuber surface, tend to lose less mass than those with thin periderm (Saran and Chhabra, 2014). As a result, potato varieties which show minimum mass loss are preferred because they remain firm for a longer period. Due to their longevity, they can be selected specifically for long-distance transportation and export

market (Azad et al., 2017). Ezekiel et al., (2004) observed that Kufri-Chipsona-2 potato cultivar had maximum mass loss (23.7%), with thinner periderm (93 µm), lower number of cell layers in the periderm (5.2) and higher number of lenticels (270/tuber). On the other hand, 'Kufri Chandraamukhi' potato cultivar recorded minimum mass loss with thicker periderm (151 µm), more number of cell layers in the periderm (83) and lower number of lenticels (168/tuber).

Moreover, the degree of permeability and the type of packaging materials are reported to show some significant differences in the physiological mass loss of the tubers during storage and postharvest handling. As a result, the use of polypropylene packaging has been reported to perform better in reducing the mass loss during the postharvest storage of potato tubers (Abbas et al., 2017).

2.2.3. Postharvest rotting

Postharvest diseases (figure 2.2) are the major cause of storage losses of potato tubers. It is estimated that, annually, postharvest diseases contribute about 2-9% of storage losses (Tanni et al., 2019). Soft rot (*Pectobacterium carotovorum* subsp. carotovoru), ring rot (*Clavibacter michiganesis* subsp. sepedonicus) and pink rot (*Phytophthora erythroseptica*) are amongst major diseases that cause rotting of tubers during storage (Sinha et al., 2018). The presence of rotting in these tubers is mainly caused by a wide range of hydrolytic enzymes such as cellulases, pectinases, xylanases and proteases secreted by microorganisms (bacteria) resulting in tissue maceration and cell death (Tanni et al., 2019).

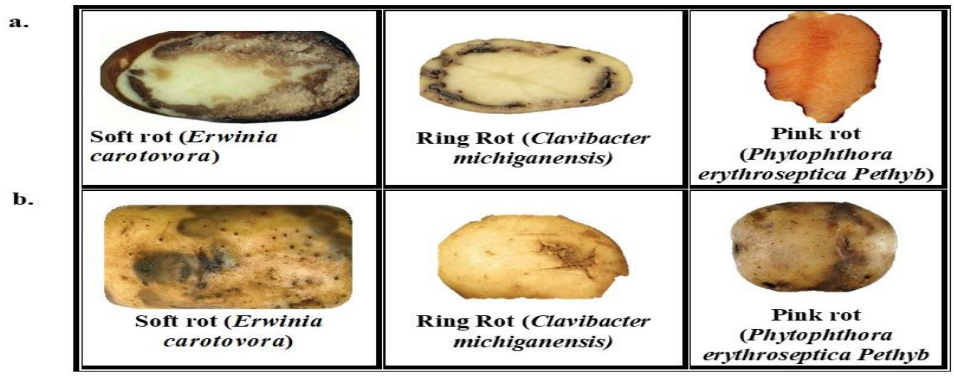

Figure 2.2. The diagrammatic representation of both internal (a) and external view (b) of soft-, ring- and pink rot diseases on potato tubers.

Bacterial soft rot is the most prevalent potato disease found in tropical regions. As a result, it is a major cause of quick and heavy spoilage losses during storage (Tanni et al., 2019). Its name emanates from the characteristic decay of fleshy tissue which results in a watery or slimy mass. The disease does not only affect potatoes but can also be destructive in vegetable and ornamentals at large. As a result, it has a wide host range infecting vegetable species in all families. Soft rot can be detected on crops in the field, in transit, in storage and during marketing resulting in great economic losses. *Erwinia carotovora* sub-sp. *carotovora* and sometimes *Erwinia carotovora* sub-sp. Atroseptic*a* are the primary causes of this disease in tuber crops (Bhat et al., 2010). The bacteria get into the host tissue through injuries. Once the bacteria get inside, they multiply in the intercellular spaces and secret the pectolytic enzymes that macerate the cell walls. This results in a whole tuber being transformed into a soft, watery and rotten mass within 3-5 days. The bacteria adapt in both aerobic and anaerobic conditions over a wide range of temperatures of at least 20 °C. They can survive for weeks in deep wounds since they are protected from desiccation (Bhat et al., 2010; Kushalappa and Zulfiquar, 2001). In bulk storage, soft rot is triggered by the microclimate caused by the potato piles producing localized 'hot spots'. As a result, it then spreads quickly to healthy tubers below such hot spots in the pile, witnessed by high respiration activity and heat released from the rotting tubers (Sinha et al., 2018)

Bacterial ring rot is one of the most serious diseases that affect potato and tubers at large. The disease is known to be caused by *Clavibacter michiganensis subsp.* sepedonicus (Cms) (Stevens et al., 2017). This bacterial pathogen is easily transmitted via the seed tubers. As a result, the seed certifications programs have a zero-tolerance standard for this disease. That means, its detection might lead to the rejection of the whole seed lot. The trick about this bacterium is that the seeds infected might show some symptoms or might not show at all. This bacterium can easily spread and adhere to all types of surfaces that it contacts (Kudela, 2007). The most visible symptom is the distinctive odourless decay restricted first to the immediate vicinity of the vascular ring. This can be witnessed when the tuber is cut crosswise near the stem end. During the initial stages of the decay, the vascular ring looks cream to pale lemon yellow and then changes to the colour of the normal ring of a healthy tuber, finally, the ring becomes brownish at later stages. The affected areas of the tuber can be observed by their soft texture with cheesy consistency. Furthermore, ring rot develops rapidly at a temperature range of 18 to 22 °C and can slightly adapt to 3 °C conditions. The effective management of bacterial

ring rot is through the exclusive use of certified ring rot free seed potatoes and as well as rigorous and proper sanitation(Kudela, 2007; Stevens et al., 2017)**.**

Pink rot (*Phytophthora erythroseptica* Pethyb) is one of the most severe potato tuber diseases because the harvested tubers turn to be asymptomatic after infection and are later realized by their complete rot in storage. The disease can be spread from infected tubers to healthy tubers in the storage or during the handling. Furthermore, pink rot is most detrimental in wet soils with temperature ranging from at least 20 °C (Yellareddygari et al., 2016). The wide range infection can be initiated in temperatures ranging from 5 to 33 °C with an optimum temperature of 20-27 °C. The infected tissues are characterised by a soft, wet, spongy, and rubbery texture. The infection begins at the stem-end of the tuber which can be observed by its creamy to light brown colour, which changes to pink colour when exposed to air for 20-30 minutes (Zhang, 2016). At later stages, the pink colour is then transformed into brown and then black. If the tissue is squeezed, it then expels a clear odourless liquid. All the changes finally lead to wet rot and complete tuber break down. To avoid pink rot disease, it is recommended that disease-free seeds be used and plant the crop in warm and well-drained soils. The use of cultivars that are resistant to pink rot is also recommended. In addition, the elimination of the affected tubers during storage is also important (Yellareddygari et al., 2016; Zhang, 2016).

2.2.4. Mechanical damage and bruising

Potato tuber is mainly parenchymatous because it lacks specialized secondary thickened tissues (McGarry et al., 1996). As a result, it is vulnerable to various forms of damage during commercial production which involves external (shatter, cutting, skinning, cracking) and internal defects (principally blackspot and bruise) (McGarry et al., 1996). Mechanical damage during harvesting and handling results to significant losses of quality and value of potatoes as well as the increase of the of disease incidence during storage (Bentini et al., 2006). According to Denner et al. (2012), the potato harvesting comprises of various activities, which include digging, lifting, dropping, heaping, loading and offloading as well as the transportation of potatoes to storage facilities, these all play a major role in the postharvest losses of potatoes. It is estimated that about 70% of the total damage is due to harvesting and 30% occur during transport and storage (Bentini et al., 2006). The types of tuber damage occurring during harvesting are caused by lifter blade that has not been set at the correct depth which results to complete damage of the tuber. Secondly, the hard objects, chafing and loose skin conditions during harvesting result to abrasions. Bruises are usually caused by stones, chains of the lifter

and trampling on the tubers. Lastly, cracks are mainly caused by the height from which the tubers are dropped. Hence it is important that the lifter must be set at most 50 cm above the soil (Denner et al., 2012).

Mechanical damage can sometimes be unnoticeable during harvesting, transportation and occasionally during grading and marketing after storage (Janick, 2003). As a result, more often consumers become unaware of these injuries at the time of purchase since they are not visible until the tuber is peeled (Mondy and Leja, 1986). Opara and Pathare (2014) defined a bruise as a subcutaneous tissue failure without any visible damage of the skin of fresh produce, where the injured tissue presents itself by discolouration in a damaged spot. These authors further argue that a bruise can become visible to the human eyes after 12 hours of incubation, which means that the affected produce may not be detected until they reach the consumer at the point of purchase or consumption. It is important to note that the visual quality of a potato tuber or any produce plays a significant role (up to 83%) in consumers' perception and can be influenced by the presence of defects. As a result, bruising is one of the significant barriers to purchasing than the price itself (Opara and Pathare, 2014). The symptoms of the internal defects may or may not involve the visible tissue damage but result in colour development in damaged areas which is likely to be yellow, red, brown, blue, grey and black pigmentation to varying degrees. Damaged tissue often involves the development of floury white regions in clear contrast to the cream background of undamaged tissue. In a case where there is no visible fracture, the development of the colour then ends up being black, the syndrome known as internal bruising (McGarry et al., 1996). Furthermore, the enzymatic darkening that happens after bruising greatly reduces the acceptability of fresh potatoes since it does not only cause the dark tissue but as well reduce the nutritional content of the tuber (Mondy and Leja, 1986).

2.2.5. Storage conditions and duration

The potato quality can be maintained for longer periods through alteration of the environmental conditions such as cold storage, adjusted humidity and regulated gas composition (Alamar et al., 2017). Therefore, in this review the storage temperatures as well as controlled atmosphere are discussed.

2.2.5.1. Storage temperatures

Storage after harvest is the major challenge in potato production because huge amounts of potatoes are lost annually due to improper storage conditions (Abbasi et al., 2016). It is undeniable, that storage temperature and relative humidity have a great influence on mass loss, dormancy period, physicochemical properties and potato processing quality (Senkumba et al., 2017). Due to a semi-perishable nature of the potato crop, storing it at low temperatures helps reduce the possibility of bacterial soft rots, dry matter loss and sprouting without the use of chemicals (Bandana et al., 2017). For that reason, this helps encourage the continuous supply of potatoes throughout the market even during the offseason periods (Abbas et al., 2017).

Although low storage temperatures play a significant role in preventing most of the quality parameters of the potato tubers, it results in a process known as cold-induced sweetening. This process is normally caused by an accumulation of sugars during cold storage (<8 °C). Cold-induced sweetening acts as a resistance mechanism of potato from the freezing point by which the tubers prevent the tissue damages which might cause large ice crystals (Dourado et al., 2019; Marangoni, 2017). It is a complex process that encompasses a series of reactions such as changes in gene expression and modulation of the post-translational activity of important enzymes such as sucrose phosphate synthase, the presence of β-amylase isoform, cumulative sucrose synthesis and starch degradation via phosphorolytic and hydrolytic reactions (Dourado et al., 2019). According to Ali et al. (2016), the starch degradation into sucrose is mainly through the UDP-glucose pyrophosphorylase and sucrose-phosphate synthase during low-temperature storage. It is further reported that the increase in hexose sugars is mainly linked to the activity of the acid invertase enzyme. The acid invertase enzyme activity (shown in table 2.2.) is highly correlated to both hexose accumulation and sucrose content of different cultivars exhibiting resistance to cold-induced sweetening. The major drawback of the accumulation of reducing sugars (glucose and fructose) during storage is that it results in an undesirable colour of fried potato products such a potato chips and French-cut fries during processing. This colour is formed when reducing sugars react with amino acids in Maillard reaction to form what is known as acrylamide (Marangoni, 2017).

Table 2.2: Some of the enzymes regulating sugar metabolism in potato tubers (Kumar, 2004)

Enzyme	Outlines of the reaction catalysed
Invertase	Sucrose ⟶ Glucose + Fructose
Sucrose-6-phosphate synthase	Fructose-6-phosphate + UDP-glucose ⟶ Sucrose-6-phosphate
UDP-glucose pyrophosphorylase	Glucose-1-phosphate ⟶ UDP-glucose
Starch phosphorylase	Starch ⟶ Glucose-1-phosphate
ADP-glucose pyrophosphorylase	Glucose-1-phosphate ⟶ ADP-glucose

Although storing potatoes at low temperatures is ascribed to the accumulation of reducing sugars, high temperatures also result in high accumulation of sugars known as senescent sweetening. This process is the consequence of the long-term storage at high temperatures of at least 8 °C (Dourado et al., 2019) and 12 °C (Raigond et al., 2018). The release of these sugars is significant for the growth of the potato plant since they serve as the source of energy (Dourado et al., 2019; Marangoni, 2017). Unlike, cold-induced sweetening that can be mitigated by storing the tubers at high temperatures, senescent sweetening cannot be reversed. Hence, cultivars with later senescent sweetening characteristics are needed for the long-term processing quality (Wiberley-Bradford and Bethke, 2018). Potatoes affected by senescent sweetening become undesirable for both table and processing purposes as they are sweet (Raigond et al., 2018). The potato fries affected by senescent sweetening show a blush of colour close to the centre of the fried chips and this developmental process becomes unacceptable with time thereby impacting the storability of potatoes (Wiberley-Bradford and Bethke, 2018).

Table 2.3: The table showing the effect of storage temperatures on quality attributes of potatoes

Storage temperature (°C)	**Cultivar**	**Findings**	**Reference**
4	Kufri-Pukhraj	Increased reducing sugars and sucrose.	Raigond et al. (2018)
24	Shangi	Lowest reducing sugars (0,89%) after 10 weeks. Increased dry matter content. Rapid sprout development within 2 weeks.	Abong et al. (2015)
8	Agria	High starch, vitamin C content and high firmness. Increased accumulation of reducing sugars.	Şanli (2016)
3	Kuroda	Cold induced sweetening (CIS) due to increased invertase activity.	Amjad et al. (2016)

Table 2.3. clearly demonstrates that storage temperatures have a significant influence on physicochemical as well as nutritional quality attributes of the potato tubers. For instance, the literature consistently demonstrated that low storage temperatures result in an increase of reducing sugars, a decrease of sprouting and as well some significant changes in the nutritional content of potatoes. These biochemical changes occurring within the tuber are reported to vary from one cultivar to another. Therefore, there is a need to evaluate each cultivar based on its suitable storage temperature requirement. The information will be useful for producers in guiding them to reach their target markets.

2.2.5.2. Controlled atmosphere

The temporary anaerobiosis caused by both hypoxic and anoxic conditions can result in the tuber dormancy release irrespective of its physiological maturity (Muthoni et al., 2014). In general, the dormant and well-stored tubers usually have low respiration rate with low carbon dioxide (CO_2) (2-4 mL/kg/h). However, various factors such as warm temperatures, stress, injury, physiological and ethylene can result in a rapid increase of respiration rate. In storage rooms the carbon dioxide concentration that surpasses the conditions of the ambient conditions can be easily witnessed (Daniels-Lake et al., 2013). As a result, in many storages CO_2 contents are monitored and kept below the minimum threshold through ventilation, to maintain the tuber health and as well the processing quality (Bethke, 2014). The common belief that stood many years is that accumulation of carbon dioxide results in the darkening of the processed potato products. As a result, the recommended storage atmosphere has been limited to a concentration below 0.5 to 1% carbon dioxide (Daniels-Lake et al., 2013). On the other hand, low levels of oxygen concentration have been reported to reduce the accumulation of reducing sugars of the stored tubers. For instance, a low concentration of O_2 in the air reduced the accumulation of sugars at 3 °C compared to 5 °C whereas an increase in CO_2 increased the accumulation of sugars at 5 °C compared to 3 °C (Kumar et al., 2004).

Nevertheless, Daniels-Lake et al. (2013) identified some conflicting results on the studies that were previously performed. These studies reported that: (i) 2-5% CO_2 prevents low-temperature sweetening (LTS), (ii) 2-3% of CO_2 has little effect on the potato chip but the increase in concentration (13%) result in high reducing sugars and (iii) the minimum of 0.5% CO_2 results in darkening of the potato chip colour. According to Burton (1958) on the research

where he compared CO_2 and O_2 on sprouting at 10 °C found that the 5 % of oxygen concentration showed rapid sprout growth whereas 10 % stimulated more sprout growth. However, in conditions where 15% CO_2 and 10% O_2 concentrations were applied, sprouting was completely suppressed. Coleman and McInerney (1997) reported that low oxygen concentrations (<10%) for 10 days in the presence of 10 to 60% carbon dioxide or a high carbon dioxide (60%)/oxygen (40%) treatment caused tuber breakdown regardless of cultivar. On the other hand, they found that the most efficient mixtures that improved dormancy release and sprout emergence were 20% CO_2/40% O_2 or 60% CO_2/18-20% O_2 and their effects were improved by 50 ppm ethylene (C_2H_4).

2.3. Postharvest treatments

Sprouting is one of the major causes of potato losses during storage (Finger et al., 2018). These losses manifest themselves through the changes of mass, texture, nutritional content, softening, shrinkage and development of toxic alkaloids in potato tubers (Owolabi et al., 2013). Therefore, postharvest treatments which include chlorpropham, maleic hydrazide, essential oils, ethylene, hydrogen peroxide, ultraviolet-C (UV-C), and gamma irradiation are investigated.

2.3.1. Chlorpropham (iso-propyl N-(3-chlorophenyl) carbamate, or CIPC)

The use of CIPC, a well-known sprout inhibitor in the potato industry, dates back from the mid-1950s (Daniels-Lake et al., 2013). CIPC is a selective and systemic herbicide that can translocate acropetally in plant systems (Paul et al., 2016). As a result, it was primarily known for its herbicidal effect on plants. Currently, it is globally known as one of the best sprout inhibitor (Paul et al., 2014). In South Africa, it is also a registered (Act 36 of 1947) sprout inhibitor (Potato South Africa, 2015). CIPC is mainly applied as a thermal fog, an aerosol, an aqueous spray or dip, or in a dust formulation during the storage (Daniels-Lake et al., 2013; Gouseti et al., 2015). The postharvest application of CIPC inhibits the cell division (mitosis) by preventing the suberization of the harvest injuries as well as inhibiting the formation of the sprouts (Adrian et al., 2013; Alamar et al., 2017; Daniels-Lake et al., 2013; Frazier et al., 2004). During its application, the chemical sublimates from the deposited particles into vapour than to the eyes of the tubers thereby inhibiting sprouting (Adrian et al., 2013). The normal dose that is usually applied in stored potatoes is 18 g per tonne of potatoes, but sometimes can be

doubled to 36 g per tonne of potatoes for long term storage (Paul et al., 2016). However, the single application of the CIPC can maintain long-term sprout control (Frazier et al., 2004).

There is a consensus amongst researchers that CIPC is highly effective in suppressing sprouting when applied to tubers stored at 8-12 °C (Paul et al., 2016). For instance, Şanlı and Karadoğan (2019) demonstrated that CIPC was effective only at cold storage conditions while its efficacy diminished at temperatures above 15 °C. On the other hand, Mehta and Singh (2015) investigated the impact of different CIPC concentrations (15, 20, 25 and 30 mg/kg tuber mass) under ambient conditions (20 to 35 °C, 44 to 86% RH), on potato tubers that were previously stored at 2-4 °C and freshly harvested ones. The authors observed that CIPC of 15 and 20 mg/kg significantly prevented sprouting and losses of freshly harvested potatoes of all the potato cultivars up to 60 days compared to 25 and 30 mg/kg tuber mass. However, the concentrations of 25 and 30 mg/kg were only effective on specific cultivars for up to 30 to 45 days of storage.

Although, CIPC is reported to be the most effective sprout inhibitor in potato tubers around the world, there are many concerns regarding its use. For instance, the overuse of this chemical may have a negative impact on the environment and non-targeted plants, in cases it enters the food chain, it may result in adverse health effects for consumers. CIPC is known to be a very toxic pollutant, cancer-causing, exhibits cytolytic effects and causes a reduction in ATP synthesis by cell permeability changes (Owolabi et al., 2013; Shukla et al., 2019). Furthermore, it is prohibited to apply CIPC on potato seed tubers or store them in the CIPC containing storerooms (Şanlı and Karadoğan, 2019). A study conducted by Frazier and Olsen (2015) recorded some failures of the seeds treated with 5 and 10 ppm CIPC. They also noticed a severe reduction in yields of about 26% (2.5 ppm CIPC) to 78% (10 ppm CIPC) in 2009 and 36% (1.3 ppm CIPC) to 94% (10 ppm CIPC) in 2010 compared to the control plants. Furthermore, Douglas et al. (2018) came up with the assumption that cross-contamination of grains or potato seeds are caused by residual CIPC in the concrete fabric of the stores. They later proved that the amount of the CIPC left on the floor is influenced by the quantity of the CIPC applied on the physical nature of the concrete. As a result, it can persist in the concrete flooring for decades after the last application. As that was evident in the commercial stores where CIPC was spotted up to a depth of 4 cm, 25 years since the last application (Douglas et al., 2018). Due to increasing concerns regarding the use of CIPC, from July 2017 in the United Kingdom, new legislation was passed which established that the maximum concentration of CIPC applications

must be 36 g per tonne for processing potatoes and 24 g per tonne for fresh market tubers. It further stated that the application of this chemical in the storage must be through active recirculation of fans for its optimization (Alamar et al., 2017). It is very clear that CIPC and other chemicals have either been restricted or may become restricted in several countries due to their environmental concerns (Paul et al., 2014). Therefore, there is a need for alternative postharvest treatment that is less harmful to the environment and as well to humans.

2.3.2. Maleic hydrazide

Maleic hydrazide (1,2-dihydro-3,6-pyridazinedione, MH) is one of the commercially important plant growth regulators in the agricultural industry. It is mainly used to prevent the development of buds of vegetables for long term storage purposes in the market. In Europe, the USA and Canada, it is applied as the preharvest foliar spray to inhibit the growth of sprouting in potatoes (Paul et al., 2014). The maleic hydrazide functions by translocating from the foliage to the tubers thereby preventing sprout growth. Furthermore, it has the potential of preventing sprout growth for at least 1 to 8 months during storage (Daniels-Lake et al., 2013). Caldiz et al. (2001), reported that MH delayed sprout formation and inhibited the sprout growth for a period of 8 months during storage, which in turn prevented mass loss related to it. However, the time of application is very critical. For instance, if it is applied too early it can alter the shape of the tubers and as well resulting in a decline of yields. Again, if it is applied too late, it does not reduce sprouting at all (Daniels-Lake et al., 2013; Paul et al., 2014).

The main concerns for the use of maleic hydrazide is that it is mutagenic, cyto/geno- toxic and as well carcinogenic. As a result, it can be very harmful to humans if it gets consumed. For that reason, the United States Environmental Protection Agency investigated the quantity of MH available in foodstuffs and they found 10–40 ppm in fresh potatoes, 5–7 ppm in onions, 105 ppm in rice and grains, and 20–50 ppm in tobacco (Zhang et al., 2015). Therefore, there is a need for alternatives that are less harmful not only to the environment but to humans.

2.3.3. Essential oils

Currently, consumers are becoming more concerned about their health. Consequently, there is a growing interest in the consumption of organic and as well as healthy food. Limiting the use of artificial preservatives and finding safer alternatives has become very important in the potato industry (Alamar et al., 2017). The use of CIPC, MH and other synthetic chemicals as sprout inhibitors has resulted to some major concerns pertaining to the safety of food as well as that

of the environment (Abbasi et al., 2015). As a result, recent research has focused more on finding the safer alternatives that can be used to control dormancy and growth of sprout in the stored potato tubers. Amongst these safe alternatives, the use of essential oils has been reported to be an effective way of controlling sprouting of tubers in the storage (Finger et al., 2018). They are described as the secondary metabolites that consist terpenoids and phenylpropanoids produced inside epidermal and mesophyll tissues of plants in special morphological modifications like secretory glands, resin ducts, and so on (Abbasi et al., 2015). Essential oils are harmless, derive a nice fragrance from the parent source and can aid as the defense mechanism against the microbial and insect organisms (Abbasi et al., 2015). Terpenoids are derived from different precursors namely isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) during various metabolic pathways. These are present as monoterpenes, diterpenes, triterpenes and tetraterpenes produced during the pyruvate and mevalonate pathways (Abbasi et al., 2015). Carvone is a member of the monoterpene family that is a prominent component of spearmint, caraway and dill weed oil used for potato sprout suppression (Abbasi et al., 2015). In Holland and Switzerland, carvone is normally extracted from caraway oil. It can be effectively applied using cold aerosol application and conventional thermal aerosol techniques (Kleinkopf et al., 2003). Carvone functions by physically burning the bud meristematic tissue, which may require several applications. As a result, it is more efficient in preventing sprout growth after dormancy has ended (Finger et al., 2018). Abbasi et al. (2015), reported that carvone interferes with the growth and elongation of the developing sprout; that is why it is described as a sprout suppressant rather sprout inhibitors. After all, factors such as cultivar, storage temperature, the extent of tuber dormancy, concentration, time and number of applications, and mode of action determine the effectiveness of the essential oils to suppress sprouting (Finger et al., 2018).

Research conducted by Teper-Bamnolker et al. (2010) comparing mint essential oil and synthetic carvone showed that monthly applications of mint essential oil prevented sprouting for up 6 months of storage. Furthermore, they also found that the mint essential oil constituted 73% of the carvone. Lastly, they also reported that the concentration of 4 µl/l of synthetic carvone had similar effects to that of mint. Furthermore, Şanlı and Karadoğan (2019) concluded that caraway and dill oils greatly reduced the chances of mass loss and sprouting under 15 °C compared to CIPC. Caraway was reported to prevent sprouting for a period of about 180 days whereas the dill oils reduced sprouting for 135 days. Lastly, Goodarzi et al. (2016) reported

that a concentration of 2 µl/l of coriander essential oil reduced sprouting for a period of 3 months whereas the concentration above that was ineffective.

Table 2.4: The table showing the efficacy of essential oils on sprouting under various storage temperatures

Treatment	Storage Temperature (°C)	Efficacy of essential oils before sprouting (Days)	References
Caraway	15	180	Şanlı and Karadoğan (2019)
Clove oil	25	63	Abbasi et al. (2016)
Coriander oil	12	30	Goodarzi et al. (2016)
Dill	10	135	Şanlı and Karadoğan (2019)
Eugenol	8	50	Finger et al. (2018)
Menthol	8	50	Finger et al. (2018)
Mint (*Mentha spicata* L.)	8	140	Teper-Bamnolker et al. (2010)
Ruta chalepensis L. essential oil (RCEO)	25	30	Lengliz et al. (2018)
Spearmint	10	120	Şanlı and Karadoğan (2019)
Spearmint	25	54	Abbasi et al. (2015)

2.3.4. Ethylene

Previous studies have reported that ethylene application can either shorten or delay potato dormancy period, but its efficacy can be affected by both treatment duration and concentration (Shi et al., 2018). Furthermore, a continuous application of ethylene is reported to prevent potato sprouting, although it causes the accumulation of reducing sugars (Suttle, 2004). According to Dai et al. (2016), ethylene prevented sprouting but with high accumulation of sugars being observed. They concluded that ethylene suppressed sprouting by affecting the carbohydrate metabolism pathway. Furthermore, a study was conducted by Foukaraki et al. (2016) to investigate whether there was a necessity for continuous application of ethylene throughout the storage. They found that ethylene applied after the first appearance of sprouting showed similar results of sprout suppression when compared to those of continuously applied ethylene. Furthermore, applying ethylene after the first indication of sprouting had little or no effect on the sugar accumulation compared when applied continuously from the harvest.

2.3.5. Hydrogen peroxide

The use of hydrogen peroxide in organic produce is permitted by the National Organic Program standards. However, other hydrogen peroxide products have adjuvants which are restricted to the organic produce (Frazier et al., 2004). Hydrogen peroxide products are applied through humidification in potato storages. The mode of action is like that of essential oils by physically burning or damaging the developing sprout (Daniels-Lake et al., 2013). Its frequent and continuous applications are important for long-term sprout control (Daniels-Lake et al., 2013; Frazier et al., 2004; Kleinkopf et al., 2003). Furthermore, the application of hydrogen peroxide has been found to reduce the presence of pathogens in the lab studies (Kleinkopf et al., 2003). According to the study conducted by Afek et al. (2000) reported that a single application of hydrogen peroxide resulted in a sprouting rate of 61% compared to 58% by Chlorpropham after 6 months storage at 10 ±1 °C.

2.3.6. Ultraviolet-C

A study by Cools et al. (2014) reported that moderate UV-C doses ranging from 5–20 kJ m^{-2} at 9 °C suppressed sprout length and sprout occurrence of different potato cultivars. In addition, there were no DNA damage and cell death detected in response to the selected range of UV-C doses. Periderm DNA damage and programmed cell death were not detected in response to any of the UV-C doses. Another study by Pristijono et al. (2016) observed the significant smaller

number of sprouts and shorter sprouts during the first 20 days after they were exposed to different UV-C intensities (3.4, 7.1, 10.5 and 13.6 kJ m^{-2}) at 20 °C with 80% RH for up to 40 days. The studies showed the potential use of UV-C as the sprout inhibitor during storage.

2.3.7. Gamma irradiation

Previous studies focused more on testing the range of concentrations (0.05-0.5 kGy) of gamma irradiation on their effect on the quality attributes of potatoes under different storage conditions. According to Rezaee et al. (2011), early and 0.15 kGy significantly reduced sprouting, mass loss and a specific gravity of Agria cultivar at 8 and 16 °C after 50 days of storage. Furthermore, Mahto and Das (2015) observed that 0.05-0.5 kGy completely inhibited sprouting and retained high textural parameters. In addition, the sugars increased with the increase in gamma irradiation doses and the absence of losses due rotting after 120 days at 12 °C were observed. However, 0.5 kGy was reported to be detrimental for the potato tubers since they increased the tuber discoloration within two weeks of storage in ambient conditions (Soares et al., 2016). Abdullah et al. (2015) concluded that 0.15 kGy was the most effective concentration in preventing sprouting and microbial count for a period of 14 days in ambient stored potato tubers.

2.4. Conclusion and future prospects

It can be concluded that both dormancy and sprouting of potato tubers are dependent upon cultivar. Sprouting is detrimental to the postharvest quality of potato tubers because it results in high mass loss, reduced turgor, structural changes and rise of sugars. Furthermore, the rate of mass loss in tubers is also cultivar dependent. As a result, cultivars that have thick periderm and less lenticels lose less mass compared to those with thin periderm and more lenticels. Moreover, mechanical damage and bruising negatively affect the potato tuber quality and for that reason the affected potato tuber can be unnoticed during harvesting, transportation and occasionally during grading and marketing after storage. It is with this reason that good handling of potato tubers from the point of harvesting until they reach market becomes very crucial. Although cold storage plays a significant role in preventing the presence of sprouting and postharvest rotting in potato tubers, it results in a process known as cold-induced sweetening. Cold-induced sweetening is an undesirable characteristic for the potato industry since it leads to the darkening of the processed products. On the other hand, storing the potato

tubers under warm temperatures of at least 20 °C not only enhances sprouting but also result in postharvest rotting.

Studies clearly demonstrated that there is an increasing concern regarding the use of CIPC and maleic hydrazide as the potential sprout inhibitors since they can persist in the surface of the potato tubers for a long time. As a result, they not only pose problems to the environment but also to human health. Although gamma irradiation is effective in inhibiting sprouting, high concentrations can result to complete tuber breakdown. Furthermore, studies have also reported UV C, essential oil and hydrogen peroxide to be the potential sprout inhibitors that could be used to substitute the use of chlorphram in the potato industry. Due to the increasing demand for organic and healthy food, there is a need for more research to be conducted specifically on essential oils and as well as other organic treatments. Furthermore, there is also a need to evaluate the substances that could help them to prolong their degradability period.

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Chapter 3

The effect of storage temperatures on sprouting incidence and processing attributes of selected potato cultivars

Abstract

Cold storage is an effective postharvest method used to reduce sprouting of potato tubers. However, high accumulation of reducing sugars experienced during cold storage is the major concern for the processing market. Therefore, this study investigated the effect of different storage temperatures on sprouting incidence and processing attributes of selected potato cultivars. A total number of 8 potato cultivars ('Almera', 'Fandango', 'Ludmilla', 'Markies', 'Panamera', 'Rumba', 'Sifra' and 'Tyson') were harvested from Sesisonke Farm, Harrismith, Free State Province (Republic of South Africa). The cultivars were sorted and put in four storage temperatures set at 4, 8, 12 and ± 25 °C for 70 days. Sprouting rate, mass loss, soluble sugars (glucose, fructose and sucrose), dry matter content, specific gravity and starch content were measured. The results showed that dry matter content, specific gravity, starch, sprouting rate and mass loss of potato cultivars increased with the increase in storage temperature and storage period. 'Rumba', 'Markies', 'Ludmilla', 'Tyson', 'Fandago' and 'Sifra' had consistent dry matter content, specific gravity and starch content of greater that 20%, 1.080 and 13%, respectively, in all storage temperatures qualifying them as suitable cultivars for processing market. In contrast, the sucrose and reducing sugars of potato cultivars increased with the decrease in storage temperatures. 'Markies' potatoes was the best performing cultivar in all storage temperatures compared to other cultivars. This cultivar had very low reducing sugars (< 1 mg/g) and high dry matter content (> 20%). On the other hand, at day 70, 'Panamera' and 'Almera' potato cultivars had the highest reducing sugars at 4 °C recording 8 and 8.08 mg/g, respectively. In conclusion, the experiment clearly demonstrated that storage temperature for potato tubers is cultivar dependent.

Keywords: Cultivar, Temperatures, Sprouting, Starch, Sucrose, Reducing Sugars, Mass Loss, Specific Gravity

3.1. Introduction

Potato (*Solanum tuberosum*) is globally one of the most important food crops. It is ranked as the fourth crop after cereal species namely rice (*Oryza sativa*), wheat (*Triticum aestivum*), and maize (*Zea mays*) (Zaheer and Akhtar, 2016). The Food and Agriculture Organization of the United Nations recommends potato as the food security crop to counteract the rising poverty

challenges (Devaux et al., 2014). The major concern in the potato industry is the large amount of potatoes lost annually due to improper storage conditions (Abbasi et al., 2016). Sprouting is one of major challenges that result to postharvest losses of potato tubers. The presence of sprouting in potato tubers is associated with the breaking down of starch components and rise of reducing sugars that are needed to provide carbon and energy for sprout growth and development (de Freitas et al., 2012). Furthermore, sprouting results to high mass loss, reduced turgor as well as structural changes in potato tubers (Paul et al., 2016b).

Cold storage is one of the effective methods used to prevent sprouting and extend shelf life of potato tubers (Clasen et al., 2016). As a result, without proper cold storage facilities, potato tubers have a maximum shelf life of 6 months after which quality deteriorates very rapidly (Clasen et al., 2016). According to Herman et al. (2016) storing potatoes at temperatures less than 9 °C increases the shelf life by slowing down tuber respiration, mass loss, disease pressure and sprouting. However, management of temperature during storage is dependent upon the intended market. For instance, the tubers destined for fresh market can be stored at temperatures below 7 °C while those destined for processing market are kept at higher temperatures (8–13 °C) in order to preserve their processing quality attributes (Alamar et al., 2017). Regarding the processing quality of potato tubers, cold temperatures result in accumulation of reducing sugars (fructose and glucose) (Galani et al., 2017). These reducing sugars are very detrimental for the processing quality of potato tubers since they result in brown, bitter-tasting products and high levels of acrylamide which is considered carcinogenic, when processed at high temperatures (Clasen et al., 2016; Heltoft et al., 2017). Hence, for potato tubers destined for the processing industry, the maximum reducing sugar levels should range between 0.2 to 0.3% for chips and 0.3 to 0.5% for French fries (Rady and Guyer, 2015).

Lately, there is a rapid shift in the utilization of potatoes from table consumption towards processed products such as French fries, mashed and canned potatoes (Bekele and Haile, 2019; Mori et al., 2015). In developed countries, approximately 60% of potatoes are consumed in a processed form, as this is influenced by the changing of consumer' lifestyles (Bekele and Haile, 2019). Specific gravity and dry matter content are positively correlated with each other. As a result, potato cultivars with a high dry matter content (>20%), specific gravity (>1.080) and starch (> 13%) are highly recommended for the processed products. For that reason, potato tubers with higher specific gravity are best suited for baking, frying, mashing and making French fries (Haque et al., 2018). Furthermore, they are also known to give higher yield of the

processed product with lesser fat absorption during frying thereby improving the texture and taste of the final product (Pereira et al., 2017; Silva et al., 2018).

Various studies have reported that sprouting and processing quality of potato tubers are cultivar dependent (Bethke, 2014; Elmore et al., 2015). The South African potato industry has recently released new cultivars for commercial production. However, there is a limited information on the performance of these newly developed cultivars under the wide range of storage temperatures. Therefore, the aim of this study was to investigate the effect of different storage temperatures on sprouting incidence and processing attributes of the selected potato cultivars.

3.2. Materials and methods

3.2.1. Plant material

The study was conducted in cold storage facilities of the Postharvest Laboratory of the University of KwaZulu-Natal, Pietermaritzburg Campus (29º37′34.8″S, 30º24′12.1″E). A total number of 8 potato cultivars ('Almera', 'Fandango', 'Ludmilla', 'Markies', 'Panamera', 'Rumba', 'Sifra' and 'Tyson') were harvested from Sesisonke Farm, Harrismith, Free State Province (Republic of South Africa). Approximately 3 kg of each cultivar was weighed and put into potato paper bags to track both mass loss and sprouting rate over the storage period. In addition, a total of 60 potato tubers for each cultivar were placed in paper bags for biochemical analysis. All the bags were sorted and put in four storage temperatures set at 4, 8, 12 and room temperature (± 25 °C) for 70 days. A 4×16 factorial experiment in a randomised complete design with 3 replicates for each cultivar was used. During sampling, three tubers were randomly taken from each cultivar for further analysis. The sampling was done on 1, 14, 42 and 70 days, respectively.

3.2.2 Sprouting rate

The sprouting of tubers was expressed in percentage where the number of sprouted tubers was divided by the total number of tubers in a sample and then multiplied by 100. According to Njogu et al. (2015), the tuber was considered sprouted when at least one visible sprout was 2 mm long.

$$Percentage\ Sprouting = \frac{Number\ of\ Sprouted\ tubers}{Total\ number\ of\ tubers\ in\ a\ sample} \times 100 \quad \text{…….. Equation (Eqn) 1}$$

3.2.3. Mass Loss

The tuber mass loss was measured by calculating the difference between the mass after storage and the initial mass before storage. The mass of the sample for each variety was measured using the weighing balance as stated by Rezaee et al. (2013).

$$Mass\ Loss = \frac{Initial\ mass - final\ mass\ after\ storage}{Initial\ mass} \times 100 \quad \text{……. Eqn 2}$$

3.2.4. Dry matter content

The dry matter content (DMC) was determined using the method of Ozturk and Polat (2016) with little modifications. Tubers from each cultivar were peeled and sliced to approximately 100 g sample. The samples were then freeze-dried using Virtis freeze dryer (# 6KBTES-55, SP Industries, USA) over 5 days till they were completely dry. Finally, the tubers from each sample were reweighed and dry matter content (%) was determined as dry weight divided by fresh weight multiply by 100.

$$\%\ Dry\ matter = \frac{Dry\ mass}{Fresh\ mass} \times 100 \text{ …… Eqn 3}$$

3.2.5. Starch content

Starch was estimated by applying the regression equation suggested by Haque et al. (2018). The equation is as follows: $y = 0.942x - 5.099$ ……. Eqn 4.

The estimated starch content was expressed in percentages.

Where: $y = \%\ Starch\ content$ and

$x = \%\ Dry\ matter\ content$

3.2.6. Determination of soluble sugar concentration

The concentrations of soluble sugars were determined according to the method of Tesfay et al. (2016) with little modifications. A sample of 0.20 grams (g) dry weight pulverized potato was mixed with 10 mL of 80% (v/v) ethanol and homogenized for 60 sec. Thereafter, the mixture was incubated in an 80 ^{o}C water bath for 60 min and kept at 4 ^{o}C overnight. After centrifugation at 12 000 g for 15 min at 4 ^{o}C, the supernatant was carefully filtered through glass wool and taken to dryness in a Genevac personal evaporator (EZ-2.3, SP scientific, Genevac Ltd, Ipswhich, England). Dried samples were re-suspended in 2mL of ultra-pure water, filtered through 0.45-mm nylon filters and sugars were analysed using an HPLC (LC – 20 AT,

Shimadzu Corporation, Kyoto, Japan) equipped with a refractive index detector (RID-10 A, Shimadzu Corporation, Kyoto, Japan) and a Rezex RCM–monosaccharide column (300mm_7.8 mm) (8 mm pore size; Phenomenex, Torrance, CA, USA). The concentration of individual sugars was determined by comparison with authentic standards.

3.2.7. Data analysis

The collected data was subjected to the analysis of variance (ANOVA) using GenStat statistical software (GenStat®, 18th edition, VSN International, UK). In cases where significance differences were observed, the means were separated using Tukey mean separation test at $P< 0.05$. Furthermore, the principal component analysis was used to determine the relationship between the variables using Unscrambler X (Unscrambler® X v10.5, CAMO SOFTWARE AS, Oslo Science Park, NORWAY).

3.3. Results and discussion

3.3.1. Sprouting rate and mass loss

The sprouting incidence was significantly ($p<0.001$) affected by the interaction among storage temperature, potato cultivars and storage period. The incidence of sprouting increased with storage temperatures, where 4 °C had low sprouting incidence (Figure 3.1 and Figure 3.2). For instance, the average sprouting rate of all potato cultivars from day 0 to day 70 stored at 4, 8, 12 and ±25 °C were 23, 79, 92 and 100%, respectively, after 70 days of storage (Figure 3.2). Potato tubers stored at ambient temperature (± 25°C) had highest rate of sprouting compared to all other storage temperatures. After two weeks of storage, there was no sprouting observed at 4 and 8 °C, on the other hand, sprouting was observed in tubers stored at 12 and ± 25°C. In terms of the overall sprouting incidence of potato cultivars stored, 'Tyson' (57%) had the highest sprouting incidence followed by 'Rumba' (47%), 'Almera' (44%), 'Fandango' (42%), 'Sifra' (30%), 'Ludmilla' (28%), 'Panamera' (25%) and 'Markies' (24%).

Therefore, the results clearly demonstrated that sprouting is cultivar dependent. These findings are in agreement with those of Abong et al. (2015) who found that sprouting increased significantly with the increase in storage temperature and storage time. Furthermore, they also observed that 'Shangi' cultivar had high sprouting at 12-14 °C reaching approximately 43 mm after 70 days compared to other cultivars. Tuber sprouting is known for causing a hasty increase in physiological mass loss during storage (Paul et al., 2016a). This occurs because the epidermis of the sprout is 100% times more permeable to water compared to the rest of the

tuber surface. As a result, a 1% increase in the surface of the tuber causes the doubling of the moisture loss from the potato tubers (Paul et al., 2016a). During sprouting, processes such as respiration and evaporation increase rapidly. Hence, sprouted potatoes result in deterioration in processing quality attributes of the tubers. This is mainly caused by mass loss, reduced turgor, structural changes due to sprout tissue and as well a rise in sugars concentrations caused by the degradation of starch (Paul et al., 2016a)

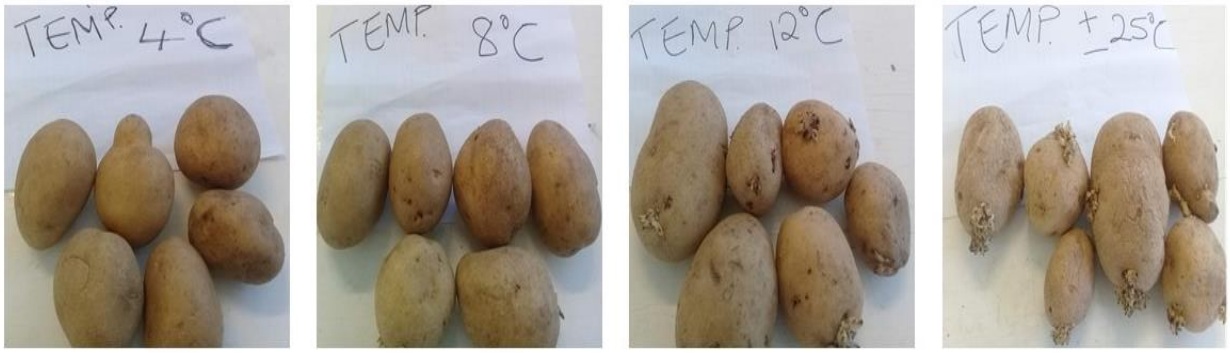

Figure 3.1. The sample of potato tubers stored at 4, 8, 12 and ± 25 °C over 70 days.

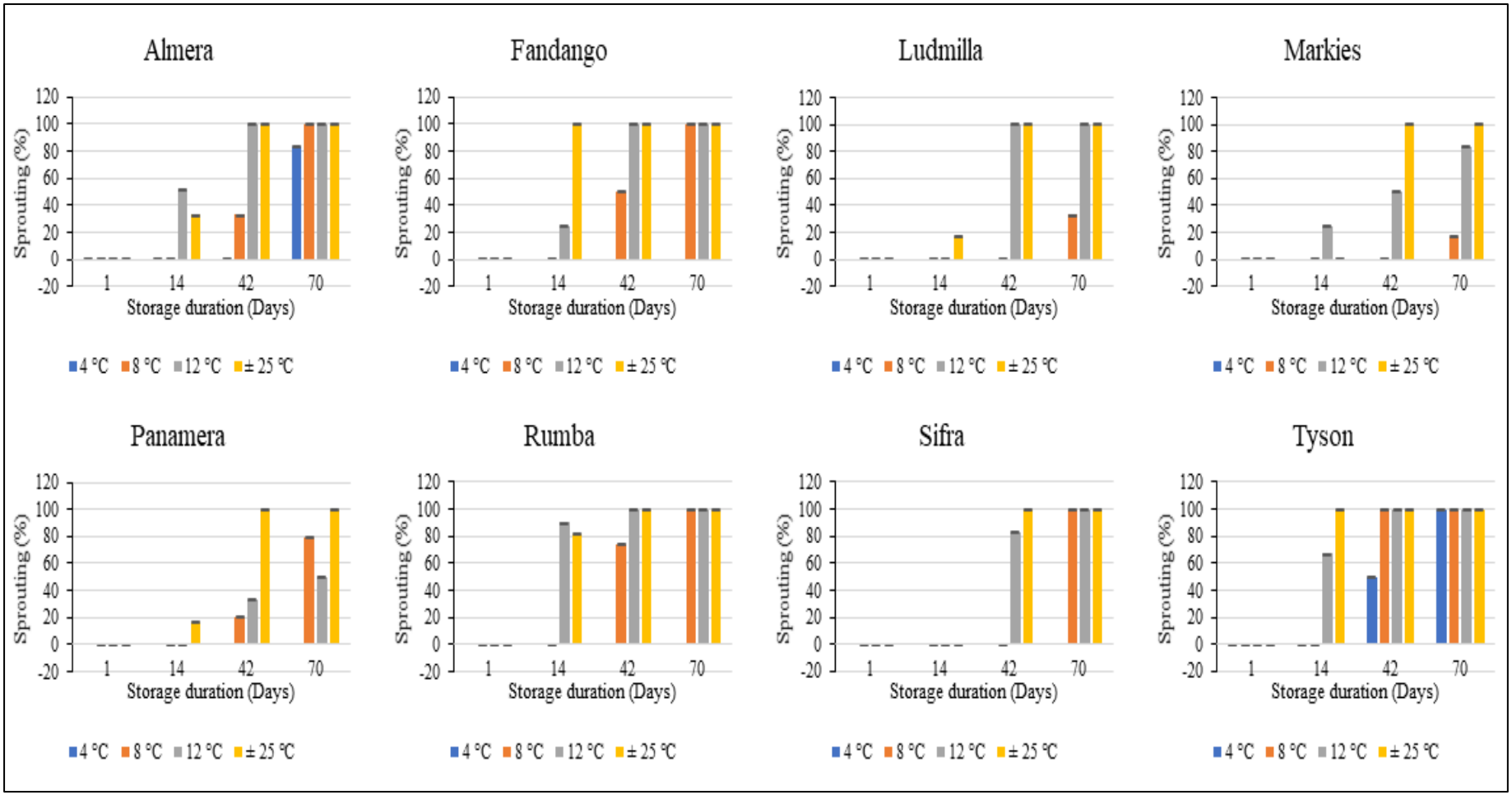

Figure 3.2. The sprouting rate of potato cultivars stored at 4, 8, 12 and ± 25 °C for 70 days. Bars represent means ± standard error (n = 3). Main effects: C: $p<0.001$; D: $p<0.001$; T: $p<0.001$; C*D: $p<0.001$; C*T: $p<0.001$; D*T: $p<0.001$; Interaction effects: C*D*T: $p<0.001$ where: C is cultivar; D is storage duration; T is storage temperature

Similarly, higher mass loss was recorded at ± 25 °C compared to all other storage temperatures, whereas 4 and 8 °C had lowest mass loss observed (Figure 3.3). Furthermore, the average mass loss of all potato cultivars, from day 0 to day 70, stored at 4, 8, 12 and ± 25 °C were 3.52, 3.20, 5.02 and 14,08 % which clearly demonstrated that mass loss being dependent on the change of storage temperature. Notably, the average mass loss of the potato cultivars stored at 8 °C was lower than those stored at 4 °C. Pinhero et al. (2009) argued that decreasing temperature to 3 ℃ results to a dramatic increase in respiration due to high accumulation of reducing sugars formed through starch degradation. They further argued that the respiration rate taking place at 0 ℃ is equal to that occurring at 20 ℃. Therefore, the high mass loss observed at 4 ℃ as compared to 8 ℃ can be attributed to the high respiration rate that took place during the starch degradation process resulting to high accumulation of sugars.

The 4, 8 and 12 ℃ storage temperatures managed to reduce mass loss of all potato cultivars below 10%. Of the potato cultivars stored at ± 25 °C, 'Sifra' (9%) had the lowest mass loss observed after 70 days of storage. According to Ezekiel et al. (2007), mass loss of 10% or more results to visible shriveling and loss of quality. The mass loss is generally caused by water loss occurring in the outer most skin tissues of tubers during respiration and transpiration (Azad et al., 2017). As a result, high rate of sprouting is reported to enhance the permeability of sprout to water loss. Therefore, from these results, it can be argued that the mass loss occurred to the stored potato tubers was also dependent on periderm thickness, number of cell layers in the periderm and as well the number of lenticels on the tuber surface of each potato cultivar (Azad et al., 2017; Saran and Chhabra, 2014).

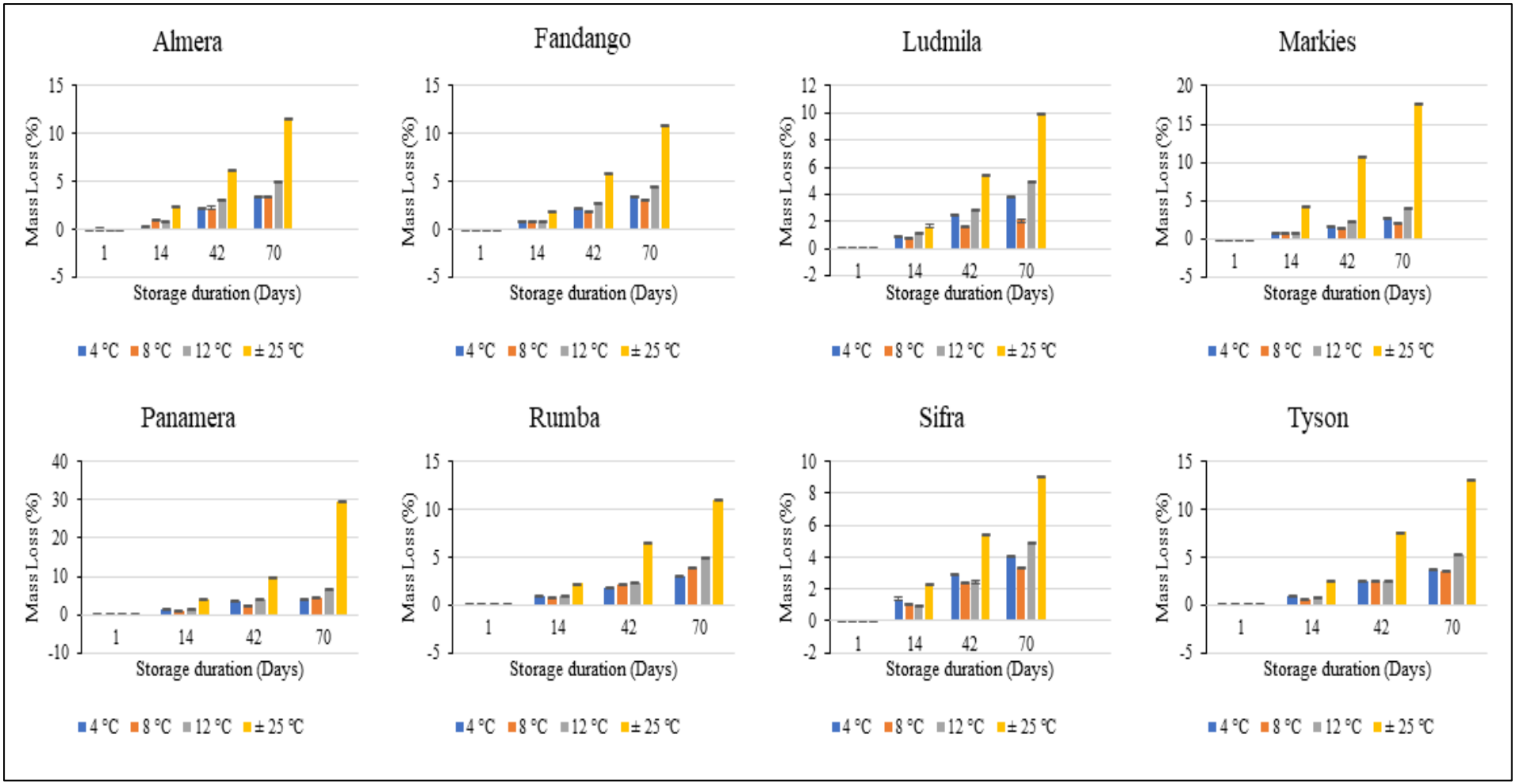

Figure 3.3. The mass loss of the potato cultivars stored at 4, 8, 12 and ± 25 °C for 70 days. Bars represent means ± standard error (n = 3). Main effects: C: $p<0.001$; D: $p<0.001$; T: $p<0.001$; C*D: $p<0.001$; C*T: $p<0.001$; D*T: $p<0.001$; Interaction effects: C*D*T: $p<0.001$ where: C is cultivar; D is storage duration; T is storage temperature.

3.3.2. Dry content and starch

Potato tubers with higher dry matter content are considered more appropriate for the processing industry (Silveira et al., 2017). Tubers with high dry matter content are also suitable for higher number of processed products as well as reduced oil consumption in fried products (Silveira et al., 2017). In this present study, the results showed that there was a significant ($p<0.001$; Figure 3.4) interaction between cultivar, storage temperatures and storage period. The average dry matter content of potato cultivars from day 0 to day 70 increased from 20.63 % to 20.96, 20.88, 22.83 and 22.54% stored at 4, 8, 12 and ±25 °C, respectively. According to Rahman et al. (2016), potato tubers with dry matter content greater than 20% are highly recommended for the processing market. In this study, 'Rumba' (23.65 %), 'Markies' (23.10%), 'Ludmilla' (22.63 %), 'Tyson' (22.10 %), Fandago (21.06), 'Sifra' (20.44%) potatoes had consistent dry matter content of greater that 20% in all storage temperatures that qualifies them as suitable cultivars for processing market.

Similarly, Rahman et al. (2016) reported that 'Diamant', 'Raja', 'Lady Rosetta', 'Dheera', 'Elgar', 'Cardinal', 'Ailsa', 'Omega', 'Endeavour', 'Caruso', 'Forza', 'Belarossa', 'Amanda', 'Ludmila', 'Tomensa', 'Rumba' and 'Jam Alu' potato cultivars had higher dry matter over 20% and were classified suitable for processing market. They further argued that the dry matter content is cultivar dependent. A study by Azad et al. (2017) demonstrated an increase in dry matter content from 15.40 to 20.50% at the end of 120 days of storage in tested varieties of Bangladesh. This implied that the possible increase of dry matter content in our results was also influenced by storage period. Furthermore, Abong et al. (2015) reported the increase of dry matter content in from 18.9 to 28.09% , 26.43%, 23.33% and 23.02% at ambient, 12-14 °C, 8-10 °C and 4-6 °C, respectively, in 'Shangi' potatoes stored for 10 weeks. According to Broćić et al. (2016), the increase in dry matter content under ambient temperature is due to the fact that water loss caused by transpiration is often higher than the losses caused by respiration in such storage conditions.

Starch constitutes 65 to 80% of the total dry weight of the potato tuber. Starch is known to be directly proportional to dry matter content and specific gravity and it is influenced by the genotype (Rahman et al., 2016). The results showed that there was a significant ($p<0.001$; Figure 3.5) interaction among cultivar, storage temperatures and storage period. The results showed that average starch content of potato tubers from day 0 to day 70 increased from 14.33 to 14.64, 14.57, 16.41 and 16.14%, respectively. High starch content was observed in potato

tubers stored at 12 and 25 °C. Our results were comparable to those of Abbasi et al. (2016) who reported that potato tubers stored at 15 °C had high starch contents (17.07 g/100 g) followed by 25 °C (15.29 g/100 g) and 5 °C (13.73 g/100 g) after 84 days. Therefore, it can be deduced that cold storage results to low starch content compared to ambient storage. According to Ali et al. (2016a), during cold storage, starch hydrolyzes into sucrose and hexose sugars. The increase of the reducing sugars can therefore be justified by the degradation of starch content occurring due to cold storage.

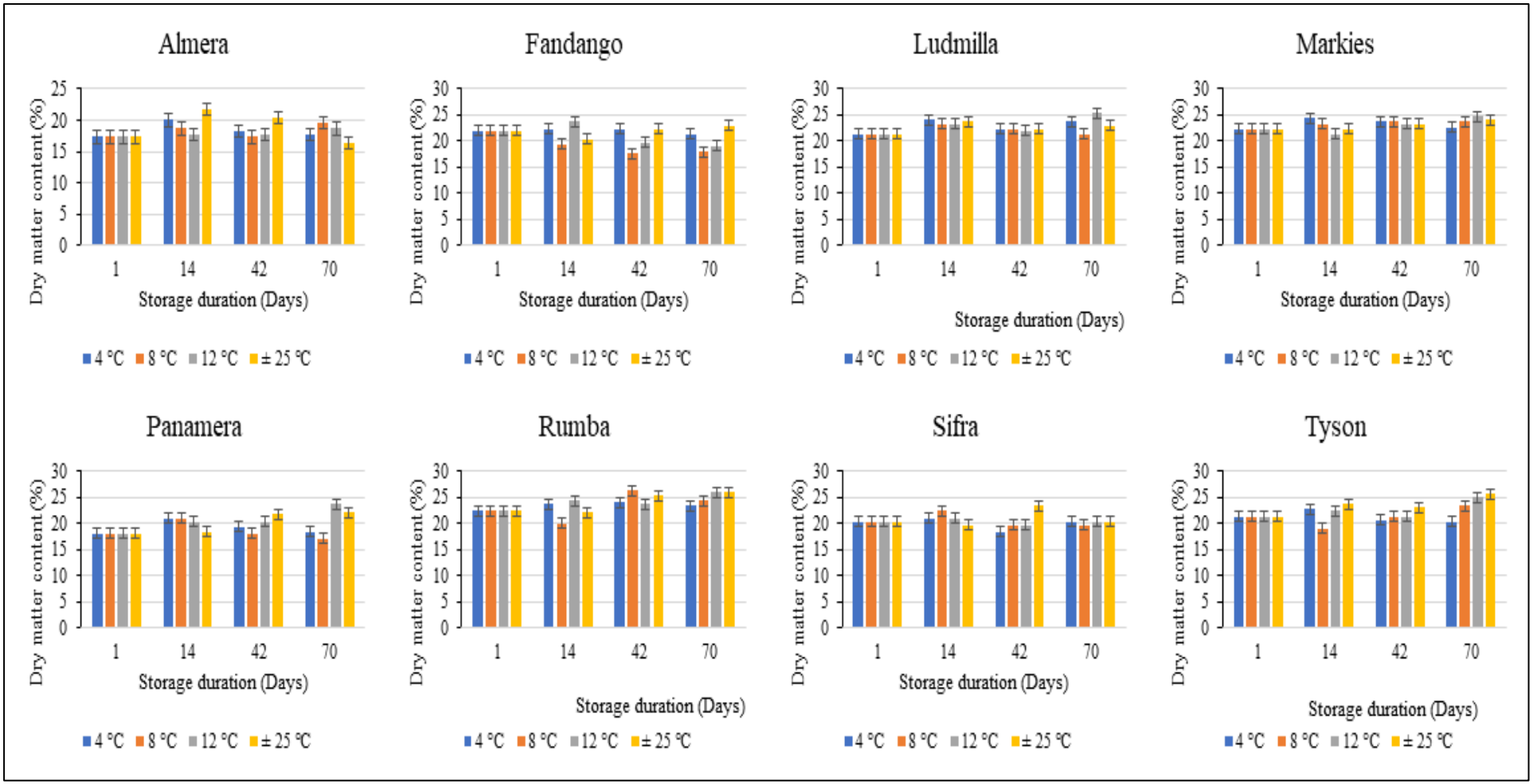

Figure 3.4. The dry matter content of the potato cultivars stored at 4, 8, 12 and ± 25 °C for 70 days. Bars represent means ± standard error (n = 3). Main effects: C: $p<0.001$; D: $p<0.001$; T: $p<0.001$; C*D: $p<0.001$; C*T: $p<0.001$; D*T: $p<0.001$; Interaction effects: C*D*T: $p<0.001$ where: C is cultivar; D is storage duration; T is storage temperature.

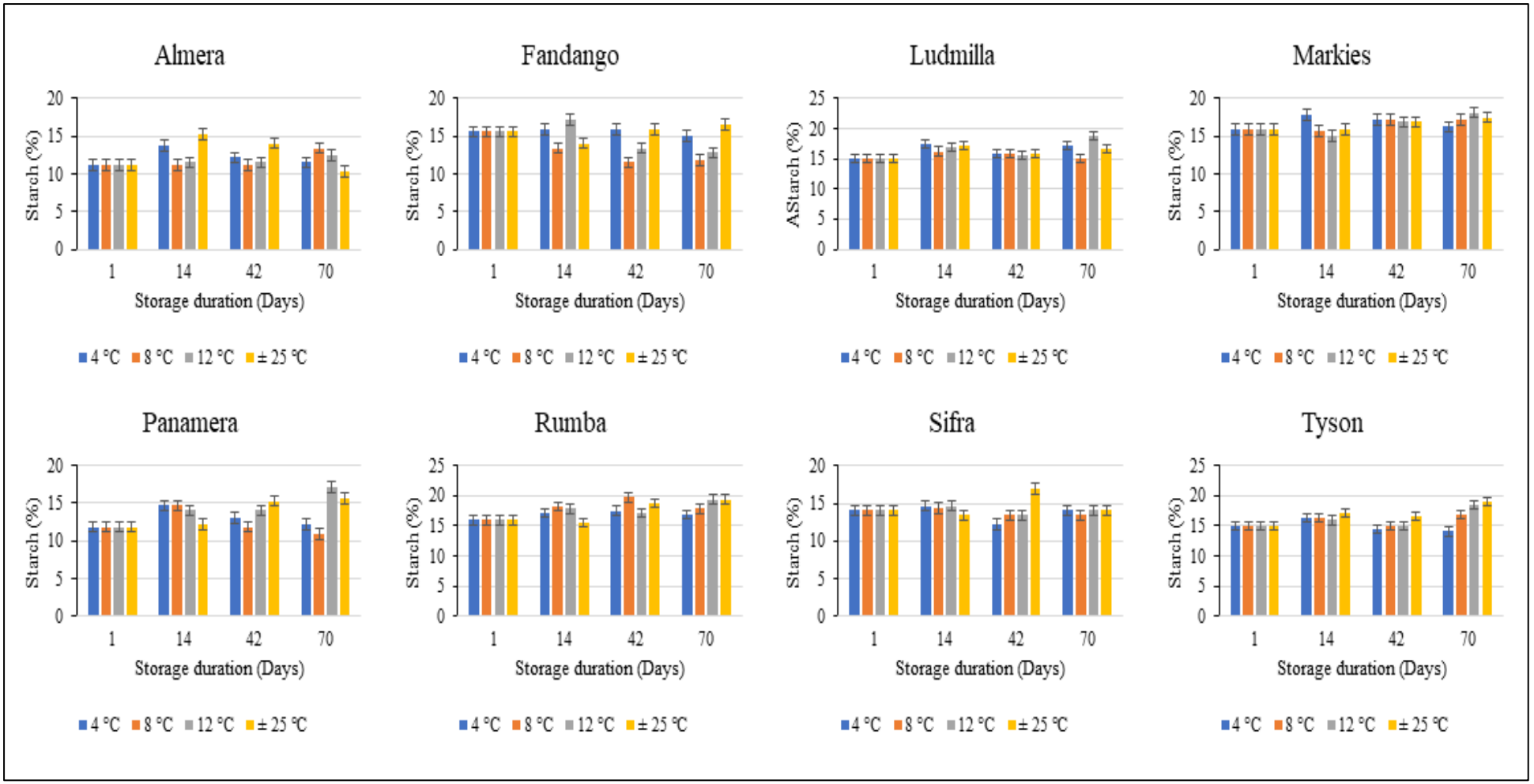

Figure 3.5. The starch content of the potato cultivars stored at 4, 8, 12 and ± 25 °C for 70 days. Bars represent means ± standard error (n = 3). Main effects: C: $p<0.001$; D: $p<0.001$; T: $p<0.001$; C*D: $p<0.001$; C*T: $p<0.001$; D*T: $p<0.001$; Interaction effects: C*D*T: $p<0.001$ where: C is cultivar; D is storage duration; T is storage temperature.

3.3.3. Non-reducing (Sucrose) and reducing (Fructose and Glucose) sugars

3.3.3.1. Non-reducing sugar

The results showed that there was significant interaction ($p<0.001$) among cultivar, storage temperature and storage days (Figure 3.6)**.** The average sucrose of potato tubers from day 0 to day 70 increased from 1.67 mg/g to 2.74 and 2.34 mg/g at 4 °C and 8 °C, respectively. On the other hand, potato tubers stored at 12 and ± 25 °C showed reduction of sucrose from 1.67 mg/g to 1.23 and 1.55 mg/g after 70 days, respectively. It was observed that the sucrose content of the potato tubers decreased with the increase in storage temperatures. For instance, the potato tubers stored at 4, 8, 12 and ± 25 °C had the sucrose content of 1.76, 1.42, 1.19 and 1.13 mg/g, respectively. Based on the interaction between storage temperature and duration, 'Almera' (1.91 mg/g) had the highest sucrose content followed by 'Fandango' (1.68 mg/g), 'Panamera' (1.45 mg/g), 'Tyson' (1.40 mg/g), 'Ludmilla' (1.32 mg/g), 'Sifra' (1.30 mg/g), 'Rumba' (0.99 mg/g) and 'Markies' (0.95 mg/g). Considering the storage duration, the highest sucrose content was observed in 'Panamera' cultivar stored at 8 °C after 70 days of storage.

Sucrose does not directly participate in the Maillard reaction, but it can serve as the source of reducing sugars and some reducing sugars may be produced during the frying process. Hence, lower levels of sucrose are preferred (Rosen et al., 2018). In case of our results, the average sucrose was <1.5 mg/g at day 0 which was a good indication of the frying quality of the most potato cultivars, especially after harvest. Furthermore, low sucrose content after harvest is also the good indication of chemical maturity and storage potential of potato tubers (Heltoft et al., 2017; Kumar et al., 2004). In our results, high sucrose content was observed in potato cultivars stored at 4 °C and 8 °C while low sucrose content was observed 12 °C and 25 °C over 70 days. Therefore, these results were consistent with those of Galani et al. (2016) who reported high amount of sucrose in potatoes stored at 4 °C after 105 days compared 15 °C and room temperature. It can be deduced from our results that the decrease of storage temperature results in high sucrose accumulation over long term storage. Furthermore, the amount of sucrose at tuber maturity is cultivar dependent. It can be argued that the sucrose accumulation is attributed to the number of factors occurring in the field such as growing season, water stress, heat stress and fertility management (Sabba et al., 2007). On the other hand, Rahman et al. (2016) also demonstrated that sucrose among cultivars differed significantly during storage where 'Amanda' had 5.3 mg/g while 'Almerah' recorded the lowest amount of 0.9 mg/g sucrose.

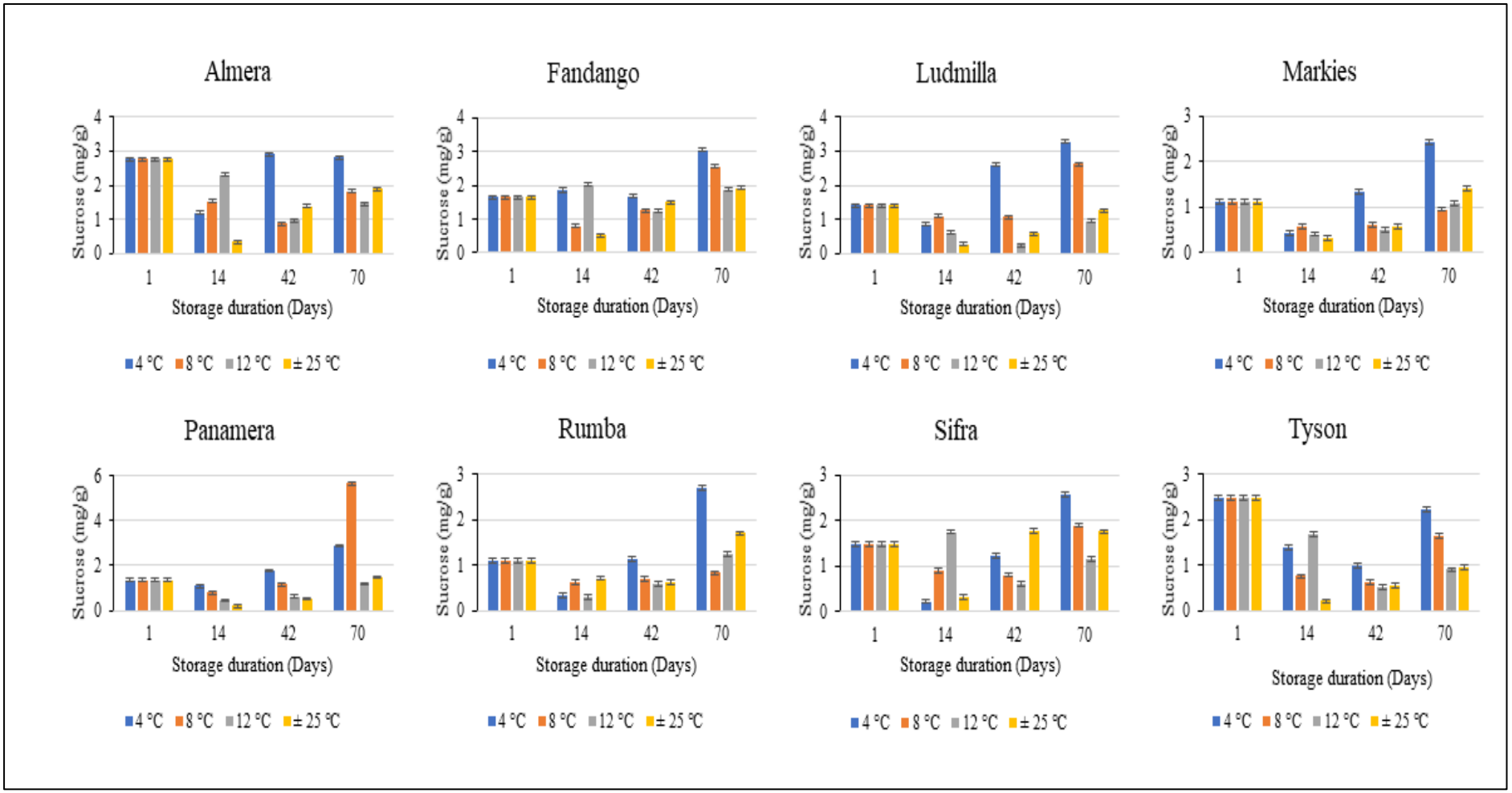

Figure 3.6. The sucrose content of the potato cultivars stored at 4, 8, 12 and ± 25 °C for 70 days. Bars represent means ± standard error (n=3). Main effects: C: $p<0.001$; D: $p<0.001$; T: $p<0.001$; C*D: $p<0.001$; C*T: $p<0.001$; D*T: $p<0.001$; Interaction effects: C*D*T: $p<0.001$ where: C is cultivar; D is storage duration; T is storage temperature.

3.3.3.2. Reducing sugars

The results showed that there was significant interaction ($p<0.001$; Figure 3.7) among cultivar, storage temperature and storage duration. The average glucose content of potato tubers from day 0 to day 70 increased drastically from 1.025 mg/g to 3.89 and 2.27 mg/g at 4 and 8 °C, respectively. At 12 and ± 25 °C, it drastically reduced from 1.025 mg/g to 0.55 and 0.26 mg/g, respectively. The storage temperature that had highest glucose was 4 °C followed by 8, 12 and ± 25 °C with 1.86, 1.4, 0.51 and 0.34 mg/g, respectively. In terms of the overall performance of potato cultivars in all the storage temperatures, 'Almera' (2.24 mg/g) recorded the highest glucose content followed by 'Panamera' (1.71 mg/g), 'Sifra' (1.15 mg/g), Fandago (1.12 mg/g), 'Tyson' (0.89 mg/g), 'Rumba' (0.53 mg/g), 'Ludmilla' (0.31 mg/g) and 'Markies' (0.29 mg/g). All the potato cultivars had low glucose content at 12 and ± 25 °C compared to those stored at 4 and 8 °C. The best performing cultivars were 'Rumba', 'Ludmilla' and 'Markies' with exceptionally low glucose levels in all storage temperatures. On the other hand, 'Almera' and 'Panamera' were the most impacted cultivars when stored in cold storage.

The average fructose content of potato cultivars from day 0 to day 70 increased from 0.27 mg/g to 3.04, 2.15 and 0.46 mg/g at 4, 8 and 12 °C, respectively, after 70 days (Figure 3.8). However, at ± 25 °C it reduced by almost half (0.18 mg/g) compared to the initial fructose content which was 0.27 mg/g. Almost all the cultivars had low manageable fructose at 4, 8, 12 and ± 25 °C. At day 70, 'Panamera' and 'Almera' had 7.56 and 6.86 mg/g, respectively, recording the highest fructose content when stored at 4 °C. At the same time, 'Markies' (0.69 mg/g) had lowest amount of fructose compared to other potato cultivars, stored at 4 °C.

According to Rady and Guyer (2015), the maximum reducing sugar levels for potato tubers destined for processing are expected to range between 2 – 3 mg/g for chips and 3 – 5 mg/g for French fries. Cold storage generally causes an increase of reducing sugars in potato tubers, resulting in a process known as cold induced sweetening (Dourado et al., 2019). Cold induced sweetening is known to be very detrimental to the quality of the processed potato products since it leads to darker and bitter tasting products after being processed at high temperatures. The colour is formed when reducing sugars react with amino acids in a Maillard reaction to form acrylamide which might be carcinogenic to human health (Dourado et al., 2019; Marangoni, 2017). In our results the increase in reducing sugars varied from one cultivar to another. Furthermore, Ali et al. (2016b) also observed higher concentrations of glucose in 'Oscar' and 'Kuroda' potato cultivars stored at 4 °C. The reason for high reducing sugars in

potato tubers stored at cold storage might be attributed to the fact that some cultivars have high acid invertase activity than others. As result, acid invertase enzyme is reported to be highly correlated to the hexose accumulation and sucrose (Marangoni, 2017). According Amjad et al. (2016), invertase enzyme play a major role in the sucrose hydrolysis to glucose and fructose. Hence, this might justify the reason why the fructose, glucose and sucrose were all strongly correlated to each other after 70 days of storage.

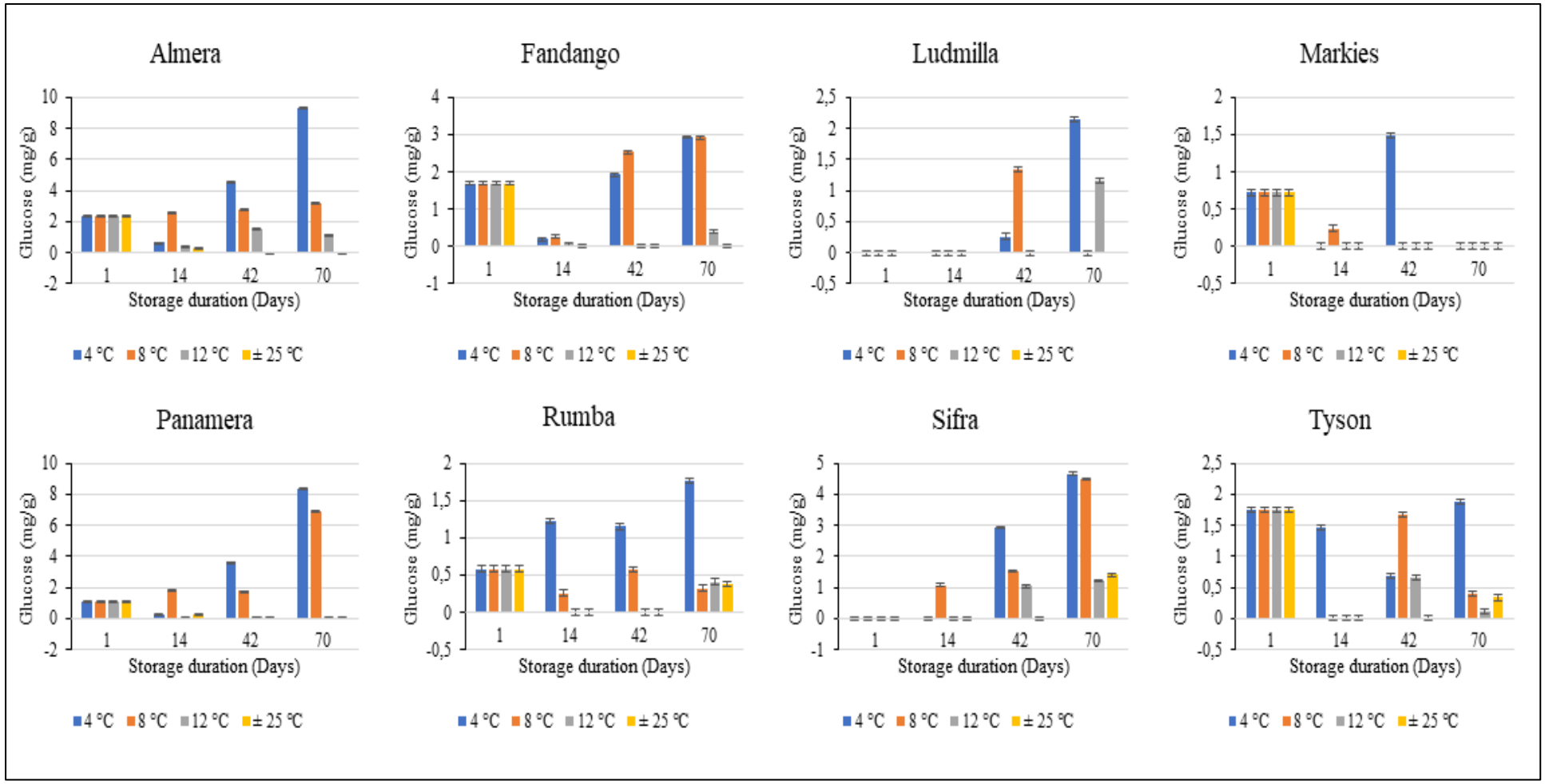

Figure 3.7. The glucose content of the potato cultivars stored at 4, 8, 12 and ± 25 °C for 70 days. Bars represent means ± standard error (n=3). Main effects: C: $p<0.001$; D: $p<0.001$; T: $p<0.001$; C*D: $p<0.001$; C*T: $p<0.001$; D*T: $p<0.001$; Interaction effects: C*D*T: $p<0.001$ where: C is cultivar; D is storage duration; T is storage temperature.

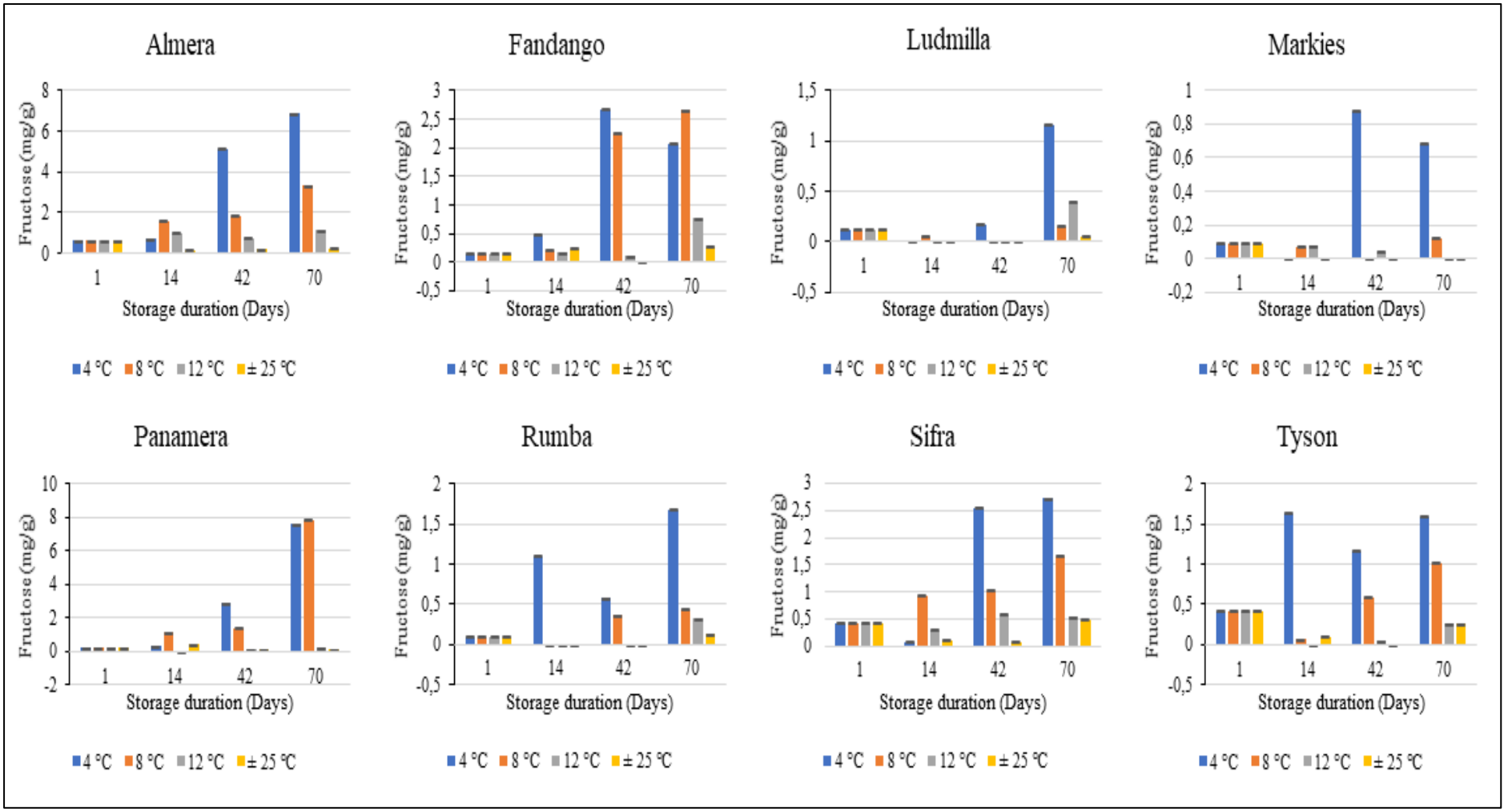

Figure 3.8. The fructose content of the potato cultivars stored at 4, 8, 12 and ± 25 °C for 70 days. Bars represent means ± standard error (n =3). Main effects: C: $p<0.001$; D: $p<0.001$; T: $p<0.001$; C*D: $p<0.001$; C*T: $p<0.001$; D*T: $p<0.001$; Interaction effects: C*D*T: $p<0.001$ where: C is cultivar; D is storage duration; T is storage temperature.

3.3.4. Principal component analysis (PCA)

PCA biplot of potato cultivars after the potato tubers were stored at 4, 8, 12 and ± 25 ℃ (Figure 3.9) explained total variability of 74% for the first two PCs, where PC1 and PC2 explained 51% and 23% of the variance.

The sucrose and the reducing sugars were all strongly correlated to each other (Figure 3.9). These sugars highly accumulated at 4 °C compared to other storage temperatures. Coffin et al. (1987) also observed that potato tubers stored at 5 °C had highest sugar accumulation compared to those stored at 10 and 20 °C. The potato tubers stored at 4 °C were characterized by low starch content, dry matter content as well as specific gravity compared to those stored in other storage temperatures. This might be attributed to the degradation of starch that occurred during cold storage 4 °C being converted to sucrose and reducing sugars (Ali et al., 2016a). Hence, resulting to a scenario where the increase of reducing sugars was followed by the decrease of overall dry matter content of potato tubers.

As shown in Figure 3.9, an increase in mass loss was strongly correlated to the tuber sprouting. The tubers that had the least mass loss were observed at 4 and 8 °C with highest accumulation of sugars and low sprouting rate. These findings are comparable to those of Abong et al. (2015) who demonstrated that sprouting was dependent on storage time and storage temperature. Furthermore, Amjad et al. (2016) also observed that high storage temperature (11 °C) increased sprouting as well as mass loss of potato cultivars.

Although, the sprouted tubers had high dry matter content and specific gravity, the PCA revealed that sprouting incidence was negatively correlated with dry matter content. Therefore, an increase in sprouting incidence generally decreased the dry matter content of potato cultivars. The results agreed with those of Abong et al. (2015) who reported that potato tubers stored at ambient temperature have high dry matter content compared to cold storage temperatures.

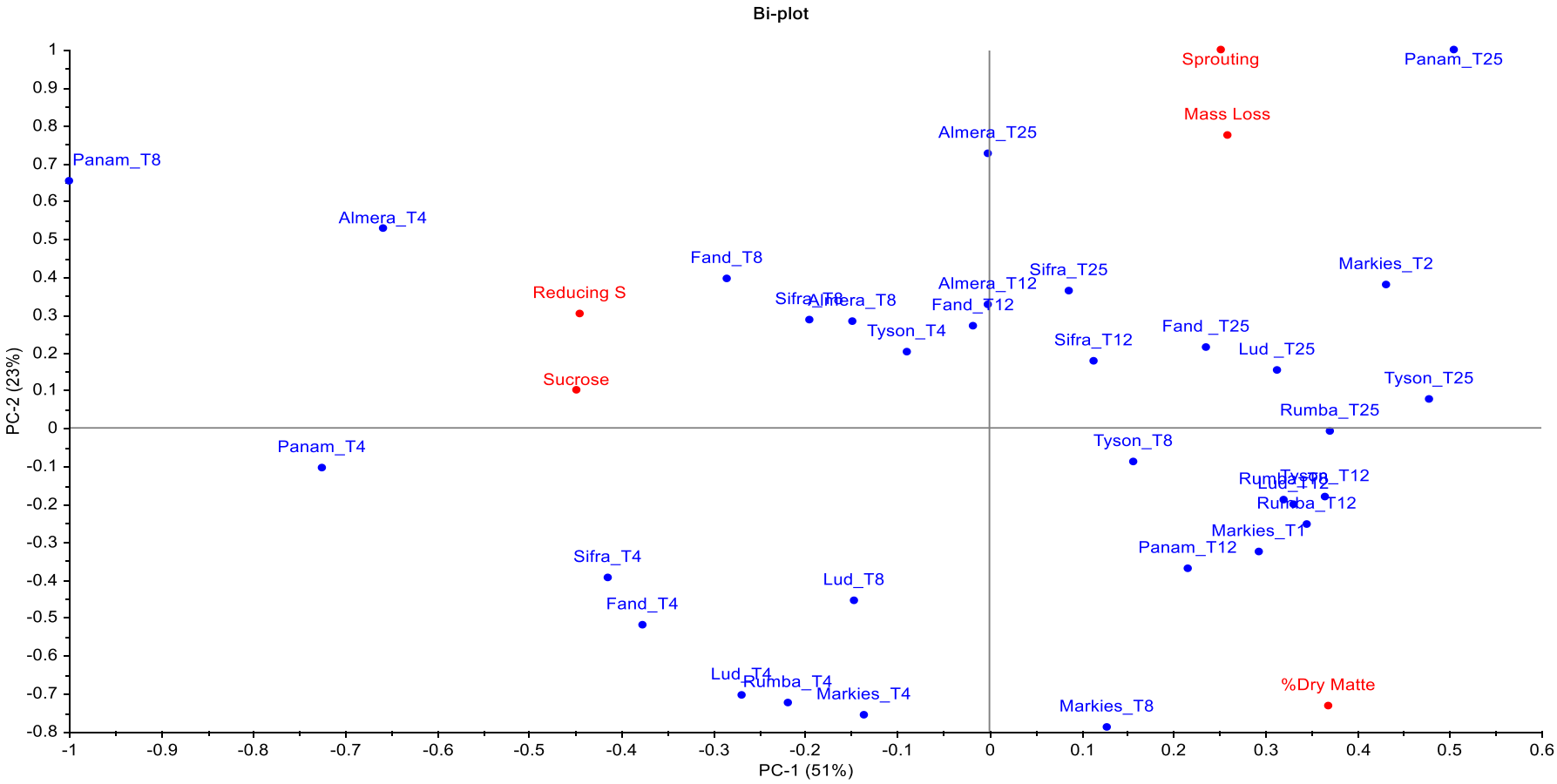

Figure 3.9. Principal component analysis (PCA) bi-plot showing sprouting, mass loss, dry matter content, reducing sugars and sucrose of the selected eight cultivars stored at 4, 8, 12, and 25 ^{0}C for 70 days. Dry matt is Dry matter, Reducing S is Reducing sugars, Lud is Ludmilla, Fand is Fandango, Panam is Panamera, Markies_T1 is Markies_T12, T4 is 4 °C, T8 is 8 °C, T12 is 12 °C and T25 is 25 °C.

3.3.5. Conclusion

The study showed that dry matter content, specific gravity, sprouting rate and mass loss of potato cultivars increased with the increase in storage temperatures over the storage period. In contrast, sucrose and reducing sugars increased with the decrease in storage temperatures. Furthermore, the storage temperature of 4 °C can be the best alternative for inhibiting sprouting and rotting of potato tubers. At 4 °C, high accumulation of sugars was experienced compared to other storage temperatures, the characteristic that cannot be recommended for the processing industry. According to this study, 8 -12 °C maintained low reducing sugars and high dry matter content. Compared to other potato cultivars, 'Markies' was the best performing cultivar in all storage temperatures since it had very low reducing sugars and high dry matter content in all storage temperatures. On the other hand, 'Panamera' and 'Almera' had the highest reducing sugars at 4 °C. The experiment clearly demonstrated that storage temperature for potato tubers is cultivar dependent.

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Chapter 4

Comparisons of total phenolics, ascorbic acid, proteins and antioxidant activity of selected potato cultivars at different postharvest temperatures

Abstract

The South African potato industry has recently released new cultivars for commercial production. However, there is a limited information on the performance of these newly developed cultivars under the wide range of storage temperatures. Therefore, the study investigated the nutritional and antioxidant properties of potato cultivars stored in four different temperatures. Eight potato cultivars (Almera', 'Fandango', 'Ludmilla', 'Markies', 'Panamera', 'Rumba', 'Sifra' and 'Tyson') were split and assigned to four storage temperatures which were 4, 8, 12 and ± 25°C (room temperature). The concentrations of total phenolics, ascorbic acid, protein and total antioxidant activity were evaluated. A decline of total phenolic content was observed in potato tubers stored at 4, 8, 12 °C and room temperature from 2.56 g/kg to 1.41, 1.42, 1.36 and 1.18 g/kg, respectively, after 70 days. The ascorbic acid decreased from 0.76 g/kg to 0.13, 0.26, 0.56 and 0.48 g/kg at 4, 8, 12 °C and room temperature, respectively, after 70 days of storage. The antioxidant activity observed at 4, 8, 12, and room temperature were 18.45, 11.78, 8.22 and 10.80%, respectively. A very strong relationship (r = 0.90) between total phenolics and antioxidant activity of the selected potato cultivars was observed. On the other hand, there was weak negative correlation (r = - 0.29) between the ascorbic acid and antioxidant activity observed in our results. At 4 °C, the total protein content increased from 10.20 to 10.43 % whereas for 8, 12 and room temperature a slight reduction of total protein content to 9.92, 8.8 and 9.7%, respectively. In all storage temperatures, 'Almera' potatoes retained highest total phenolic content (3.63 g/kg) and antioxidant activity (17.94%) whereas highest ascorbic acid (0.58 g/kg) and total protein (11.5%) were recorded in 'Ludmilla' cultivar. In conclusion, the storage temperature had an enormous effect on nutritional and antioxidant attributes of potato cultivars.

Keywords: Cultivar, temperatures, total phenolic content, total antioxidant activity, ascorbic acid, total protein content

4.1. Introduction

Potato (*Solanum tuberosum*) is one of the world's most important non-grain starchy crops. It is ranked third after cereal species; rice (*Oryza sativa*) and wheat (*Triticum aestivum*)

(Raymundo et al., 2018). In less developed countries, it is the most staple food crop and is grown by many subsistence farmers. Its high nutritional value has rendered it popular in the less developed countries (Fernie and Willmitzer, 2001). As a result, it consists of diverse functional ingredients such as polyphenols, vitamins and proteins which are health beneficial to humans (Gumul et al., 2011; Pinhero et al., 2016). Antioxidant phytochemicals such as polyphenols and vitamin C are known to prevent the occurrence of cardiovascular disease and cancer as well as reducing levels of blood cholesterol (Gumul et al., 2011). Therefore, more attention has been given in identifying potato cultivars that are rich in antioxidant phytochemicals for human health benefits (Pinhero et al., 2016).

Phenolic compounds are secondary metabolites produced in plants that have common structure based on the aromatic ring with one or more hydroxyl substituents. These compounds are grouped according to their chemical structure such as flavonoids, phenolic acids, tannins, stilbenes, coumarins, and lignans (Akyol et al., 2016; Ezekiel et al., 2013). As a result, their presence greatly contributes to the organoleptic properties of the plant-derived processed foods (Akyol et al., 2016). The phenolic compounds can be found in the peel and flesh of the potato tuber, with the peel reported to have the highest concentrations (Akyol et al., 2016). In terms of phenolic acids, chlorogenic acid is the most dominating followed by caffeic acid, gallic acid, protocatechuic acid and quercetin (Külen et al., 2013).

Generally, the total phenolic content is reported to increase with storage duration, but other studies have reported a little change or a decrease of phenols after storage (Ezekiel et al., 2013). Ezekiel and Singh (2007) observed an increase in the phenolic content of potato cultivars (‘K. Lauvkar’, ‘K. Jyoti’, ‘K. Chipsona-1’ and ‘K. Chipsona-2’) stored at 4 and 8 °C after 180 days of storage. On the other hand, Külen et al. (2013) reported the decline in the total phenolics of potato cultivars (‘CO97226-2R/R’, ‘CO99364-3R/R’, ‘CO97215-2P/P’, ‘CO97216-3P/P’, ‘CO97227-2P/P’, ‘CO97222-1R/R’, ‘Purple Majesty’, ‘Mountain Rose’, ‘All Blue’, ‘Yukon Gold’ , ‘Russet Nugget’ and ‘Russet Burbank’) stored at 4 °C after 2 months, which later increased to harvest level after 7 months. In their results, there were no significant differences observed between the total phenolics recorded after harvest and those recorded after 7 months in cold storage. Galani et al. (2017) also reported that the total phenolic content of some potato cultivars such as ‘K. Sutlej’, ‘K. Sadabahar’, ‘K. Himsona’, and ‘K. Badshah’ continued to increase at room temperature whereas for other cultivars the phenolic content declined. In this

regard, the study clearly demonstrated that storage duration, temperature as well as cultivar are amongst the several factors affecting the total phenolics content in potato tubers.

Potato consists of secondary compounds with high antioxidant activity. The levels of antioxidants are reported to differ with flesh colour of potato tubers (Ezekiel et al., 2013). Although, potatoes contain high levels of antioxidant compounds, phenolics contribute up to 58-82% of the total antioxidant activity (Galani et al., 2017). During storage, Galani et al. (2017) could not observe any significant differences in the DPPH- antioxidant activity on potato tubers stored at room temperature (25–32 °C), in the incubator (15 °C) and in cold storage (4 °C) after 90 days of storage. However, Madiwale et al. (2011) reported a strong correlation between storage duration and antioxidant activity. In addition, Abbasi et al. (2016) observed a slight increase of the antioxidant activity which then followed by a gradual decline during storage.

Vitamin C (or ascorbic acid) is one of the significant antioxidants that prevent the oxidative stress and is also involved in several cell functions such as cell division and growth. It is one of the important ingredients in the human diet since its deficiency leads to scurvy disease. Although, vitamin C is high in potatoes, storage can significantly influence its availability (Külen et al., 2013). Galani et al. (2017) reported fluctuation of ascorbic acid in potato tubers stored at 4 and 15 °C as well as at room temperature over the storage duration of 90 days. They further reported that its content remained above the initial levels. Moreover, they also observed that ascorbic acid content was cultivar dependent in all the storage temperatures. In contrast, some studies have reported the significant decrease of ascorbic acid during storage (Abbasi et al., 2016; Külen et al., 2013; Mazza et al., 1983). The decrease of ascorbic acid is reported to be more experienced during cold storage (Külen et al., 2013). For instance, Külen et al. (2013) observed a significant decline of vitamin C of potato tubers stored in cold storage (4 °C), decreasing by 24% 45% and 52% after 2, 4 and 7 months, respectively.

Generally, potatoes are known to be a high carbohydrate source, but they also contain a very good quality protein which is comparable to that of the egg protein. The high biological value and consumption of potatoes as a staple makes it a good nutritional source of proteins (Pinhero et al., 2016). During storage, Mazza et al. (1983) reported a slight increase of true and crude protein. However, there is a limited information on the effect of storage temperature on total protein of potato tubers over the storage duration.

The South African potato industry has recently released new cultivars for commercial production. However, there is a limited information on the performance of these newly developed cultivars under the wide range of storage temperatures. Therefore, the aim of the study was to investigate the effect of different storage temperatures on ascorbic acid, total phenolics, antioxidant activity and protein content of the selected potato cultivars.

4.2. Materials and methods

4.2.1. Plant materials

The study was conducted in cold storage facilities of the Postharvest Laboratory of the University of KwaZulu-Natal, Pietermaritzburg Campus (29º37′34.8″S, 30º24′12.1″E). Eight potato cultivars ('Almera', 'Fandango', 'Ludmilla', 'Markies', 'Panamera', 'Rumba', 'Sifra' and 'Tyson') were harvested from Sesisonke Farm, Harrismith, Free State Province (Republic of South Africa). A total of 60 potato tubers for each cultivar were placed in paper bags for postharvest storage experiments. All the bags were sorted and put in four storage rooms with temperatures set at 4, 8, 12 and room temperature for 70 days. A 4×8 factorial experiment in a randomised complete design with 3 replicates for each cultivar was used. During sampling, three tubers were randomly taken from each cultivar for further analysis. The sampling was done at 1, 14, 42 and 70 days, respectively.

4.2.2. Chemicals and reagents

The chemicals used for the experiments including metaphosphoric acid, 2,6-Dichlorophenolindophenol, Folin-Ciocalteu reagent, ferulic acid, sodium carbonate, methanol and hydrochloric acid were all of analytical grade, procured from Monitoring and Control Laboratories (Durban, South Africa), Prestige Laboratory Supplies CC (Durban, South Africa) and Sigma Aldrich (Johannesburg, South Africa).

4.2.3. Determination of total phenolics (TPC)

A method by Ah-Hen et al. (2012) with little modifications was used to measure TPC**.** The total phenolic compounds were determined by Folin-Ciocalteu photo-colorimetric method. A freeze-dried sample of 0.2 g was weighed accurately on an analytical balance. A volume of 4 mL of an aqueous solvent of 80% methanol acidified with 1% HCL was added to the sample to extract the phenolic compounds. The mixture was then homogenized in a horizontal shaker at 200 rpm for 2 h at room temperature. Thereafter, the mixture was centrifuged for 10 min at

7000 rpm. The supernatant was kept in the dark from which the aliquot of 100 µL was taken and mixed in a test tube with 750 µL Folin-Ciocalteu reagent and left to stand for 5 min. Then, 750 µL of aqueous sodium carbonate solution (60 g L^{-1}) was added and the mixture was allowed to stand for 90 min in the dark at a controlled temperature of 22 °C. Finally, the absorbance was measured at 725 nm with a UV-visible spectrophotometer (UV-1800, Shimadzu Corporation, Japan) and compared to a standard curve concentration between 0-40 µg /100 µL ferulic acid to calculate the actual concentration of the sample extract (R^2 = 0.994).

4.2.4. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) activity

The total antioxidant activity was measured using the method described by Abbasi et al. (2016) that involves electron transfer reaction-based assay by employing free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH). A sample of 5 mg of freeze-dried potato extract was incubated with 1.5 mL of DPPH solution (0.1 mM in 95% ethanol). The reaction mixture was properly shaken and allowed to stand for 20 min under ambient temperature. Absorbance of the resultant mixture was determined at 517 nm against blank. The radical scavenging activity was determined as decrease in the absorbance of DPPH using the following equation:

$$Total\ antioxidant\ activity\ (\%) = \left[1 - \left(\frac{Asample\ 517\ nm}{Ablank\ 517nm}\right)\right] \text{x } 100$$

4.2.5. Determination of ascorbic acid (AA)

A method by Boonkasem et al. (2015) with little modifications was used for measuring ascorbic acid. A 0.1 g of freeze-dried sample was extracted using 5 ml of 3% metaphosphoric acid (v/v). The mixture was then shaken at 200 rpm using the horizontal shaker (KS 130 C, Laboratory Equipment, Germany) for 30 min. Thereafter, the mixture was then centrifuged for 10 min using GenVac personal evaporator (EZ-2.3, SP scientific, Genevac Ltd, Ipswhich, England) at room temperature. Then, 1 mL of the supernatant was taken for further analysis. Into 1 mL of the supernatant, a volume of 5 mL of 0.05 mM of 2,6-Dichlorophenolindophenol (DCIP) solution was added and mixed for 5 sec. The absorbance of the solution was read at 515 nm against blank immediately before 15 sec using the spectrophotometer (UV-1800, Shimadzu Corporation, Japan). The results were then calculated using the standard curve prepared at concentration between 0-20 ug/mL of ascorbic acid (R^2 = 0.987).

4.2.6. Determination of protein concentration

The Leco TruSpec N Nitrogen Determinator instrument (software version 1.6x, LECO Corporation, United States of America) was used to determine total nitrogen and then converted by multiplying with the factor of 6.25% to determine the total protein content in a potato sample and was expressed in percentage. For total protein analysis, due to financial constraints, only six cultivars namely ‘‘Fandango’’, ‘‘Ludmilla’’, ‘‘Markies’’, ‘‘Panamera’’, ‘‘Rumba’’ and ‘‘Tyson’’ were used (AOAC., 1990).

4.2.7. Data analysis

The collected data was subjected to the analysis of variance (ANOVA) using GenStat statistical software (GenStat®, 18th edition, VSN International, UK). In cases where significant differences were observed, the means were separated using Tukey mean separation test at $P< 0.05$.

4.3. Results and discussion

4.3.1. Total phenolic content

Total phenolic compounds are amongst the antioxidants that are beneficial to the human health due to their ability to deactivate reactive oxygen species (Rojas-Padilla and Vásquez-Villalobos, 2016). In plants, polyphenols are linked to defense mechanisms against pest and pathogens. They are also involved in the sealing of injured plant surface by initiating the healing process (Valcarcel et al., 2015). In this present study, for all cultivars, the highest total phenolics concentration was observed after two weeks of storage with the average concentration of 5.40 g/kg (Figure 4.1). Thereafter, total phenolics declined, reaching the minimum concentration after 70 days and this trend was observed in all storage temperatures. After two weeks of storage, the average total phenolic content of potato cultivars stored at 4, 8, 12 and room temperature increased from 2.56 g/kg to 3.82, 6.62, 4.22 and 6.86 g/kg, respectively. After 70 days, there was a significant decline in total phenolic content of potato tubers stored at 4, 8, 12 and room temperature to 1.41, 1.42, 1.36 and 1.18 g/kg, respectively. The total phenolic content reduced with the increase in storage duration. In terms of the overall total phenolics of potato cultivars, it was observed that room temperature had highest concentration of 3.22 g/kg followed by 8, 4 and 12 °C with 3.08, 2.54 and 2.47 g/kg, respectively. However, the concentration of total phenolics varied amongst the potato cultivars with ‘Almera’ (3,63 g/kg) having the highest followed by ‘Fandango’ (3.09 g/kg), ‘Sifra’ (2.93

g/kg), ‘Panamera’ (2.87 g/kg), ‘Markies’ (2.74 g/kg), ‘Rumba’ (2.70 g/kg), ‘Ludmilla’ (2.44 g/kg) and ‘Tyson’ (2.22 g/kg).

Previous studies have shown that storage time and temperature have an enormous effect on total phenolic content. For instance, Abbasi et al. (2016) reported the highest increase in total phenolic content of ‘Lady Rosetta’ potato cultivar after the 1st month of storage at 25 °C which was followed by major decline on the 84th day of storage. Furthermore, Külen et al. (2013) observed a decrease in the total phenolic content of potato tubers stored at 4 °C after 2 months of storage. Similarly, our results showed a maximum concentration of total phenolics after two weeks, then a notable decrease was observed in all storage temperatures after 2 months. The increase in total phenolics during the first 2 weeks might be attributed to the response mechanism of potato tubers to the storage environment. According to Nayak (2011), polyphenols can be stimulated by the presence of stress. Furthermore, an activation of phenylalanine ammonia-lyase (PAL) enzyme which plays a significant role in the biosynthesis of polyphenols might have been stimulated by the storage condition resulting to the increase in the phenolic content observed after two weeks (Madiwale et al., 2011).

However, some studies reported an increase in the concentration of phenolics in potato tubers stored at 4 and 8 °C over the long-term storage (120 and 180 days) (Ezekiel and Singh, 2007; Lewis et al., 1999). In contrast, our results showed a significant decrease of the concentration of total phenolics during storage of 70 days. Furthermore, it can be argued that the different storage duration from our study might also be a contributing factor to the results obtained. According to Akyol et al. (2016), genotype and storage condition may play an important role on the TPC of potato tubers. It is with this reason that differences might be attributed to the cultivars used in the previous studies which were different from our study.

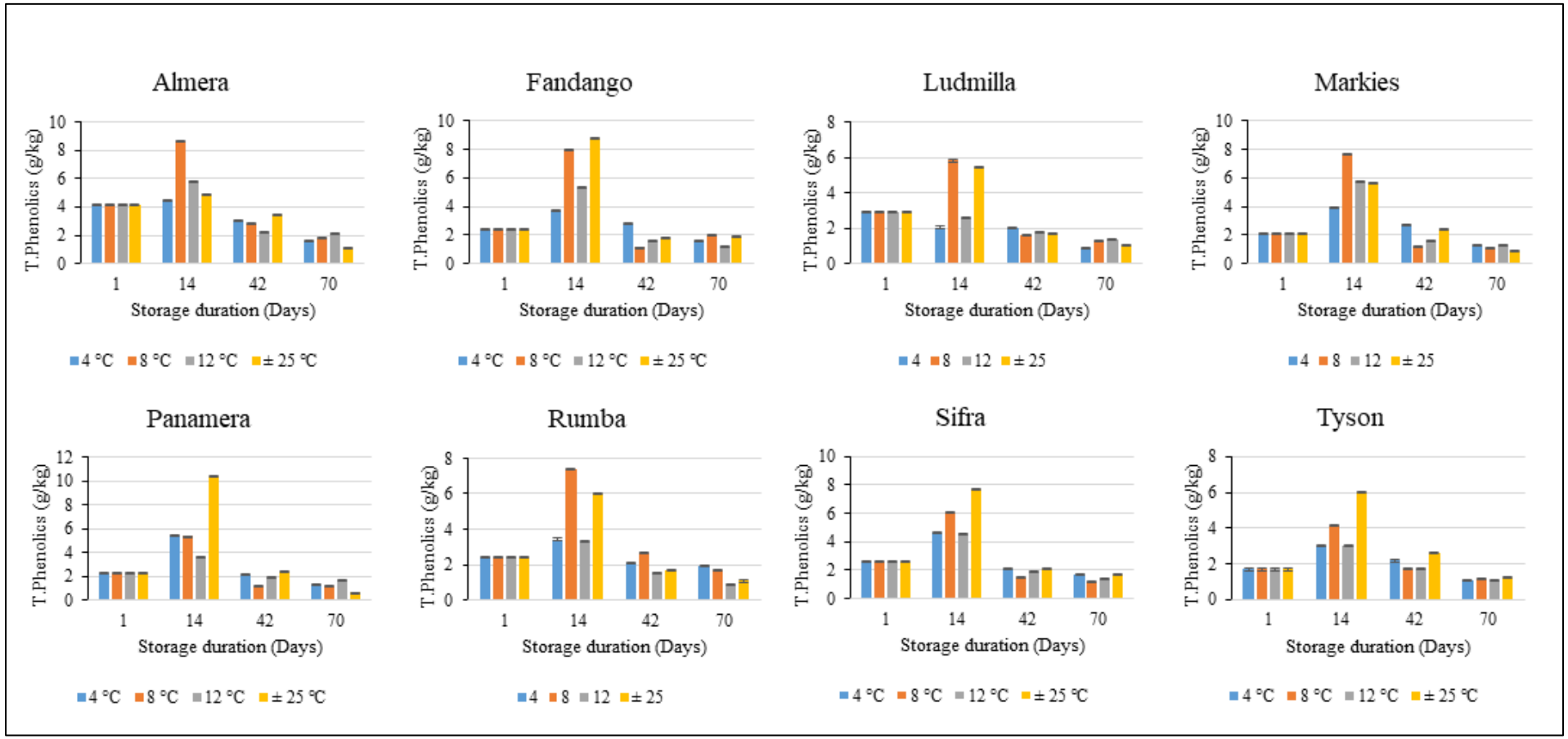

Figure 4.1. The total phenolics of the potato tubers stored at 4, 8, 12 and room temperature for 70 days. Bars represent means ± standard error (n=3). Main effects: C: $p<0.001$; D: $p<0.001$; T: $p<0.001$; C*D: $p<0.001$; C*T: $p<0.001$; D*T: $p<0.001$; Interaction effects: C*D*T: $p<0.001$ where: C is cultivar; D is storage duration; T is storage temperature

4.3.2. Total antioxidant activity

Antioxidant activity increased in all storage temperatures from 3.75 % to 23.72, 29.13, 14.14 and 7.48% at 4, 8, 12 and ± 25 °C, respectively, over the 70 days of storage (Figure 4.2). The antioxidant activity increased with storage duration. The highest antioxidant activity was observed at 4 and 8 °C recording 23.72 and 29.13%, respectively, after 70 days of storage. Overall, the total antioxidant activity decreased with the increasing temperature. For instance, the antioxidant activity found at 4, 8, 12 °C and room temperature were 18.45, 11.78, 8.22 and 10.8%, respectively. At 4 °C, the antioxidant activity fluctuated over storage with highest antioxidant activity observed after two weeks of storage, then slightly reduced yet retaining high antioxidant activity compared to 0 day. At 8 and 12 °C, there was an increasing trend of antioxidant activity over storage duration. However, at room temperature the highest total antioxidant activity was observed after two weeks thereafter reduced drastically but retained high antioxidant activity compared to the initial activity. In terms of cultivars, the highest antioxidant activity was found in 'Almera' (17.94%), 'Fandango' (14.75%), 'Sifra' (14.01%), 'Panamera' (13.93%), 'Rumba' (10.48%), 'Tyson' (9.78%), 'Markies' (9.13%) and 'Ludmilla' (8.58%). Figure 4.3 revealed a very strong relationship (r = 0.90) between the total phenolics and antioxidant activity of the selected potato cultivars. On the other hand, there was weak negative correlation (r = -0.29) between the ascorbic acid and antioxidant activity observed in our results. Therefore, ascorbic acid had little or no contribution to the antioxidant activity of the selected potato tubers.

According to Galani et al. (2017), total phenolics contribute up to 58-82% of the total antioxidant activity in potato tubers. Even though they are found in relatively low amounts in potato tubers, yet their antioxidant activity can be greater than that of other fruits and vegetables (Ezekiel et al., 2013). Madiwale et al. (2011) reported a strong positive correlation between storage duration and antioxidant activity. Similarly, our results showed that the antioxidant activity of the potato tubers stored at 8 and 12 °C increased exponentially with the storage duration. In addition, Abbasi et al. (2016) observed a slight increase of the antioxidant activity which was followed by a gradual decline during storage. Furthermore, Külen et al. (2013) reported the fluctuation of the antioxidant activity during cold storage (4 °C) but remained high compared to the initial activity. Likewise, our results showed that the antioxidant capacity of the potato tubers stored at 4 °C and room temperature varied during storage duration but was higher after 70 days of storage compared to 0 day.

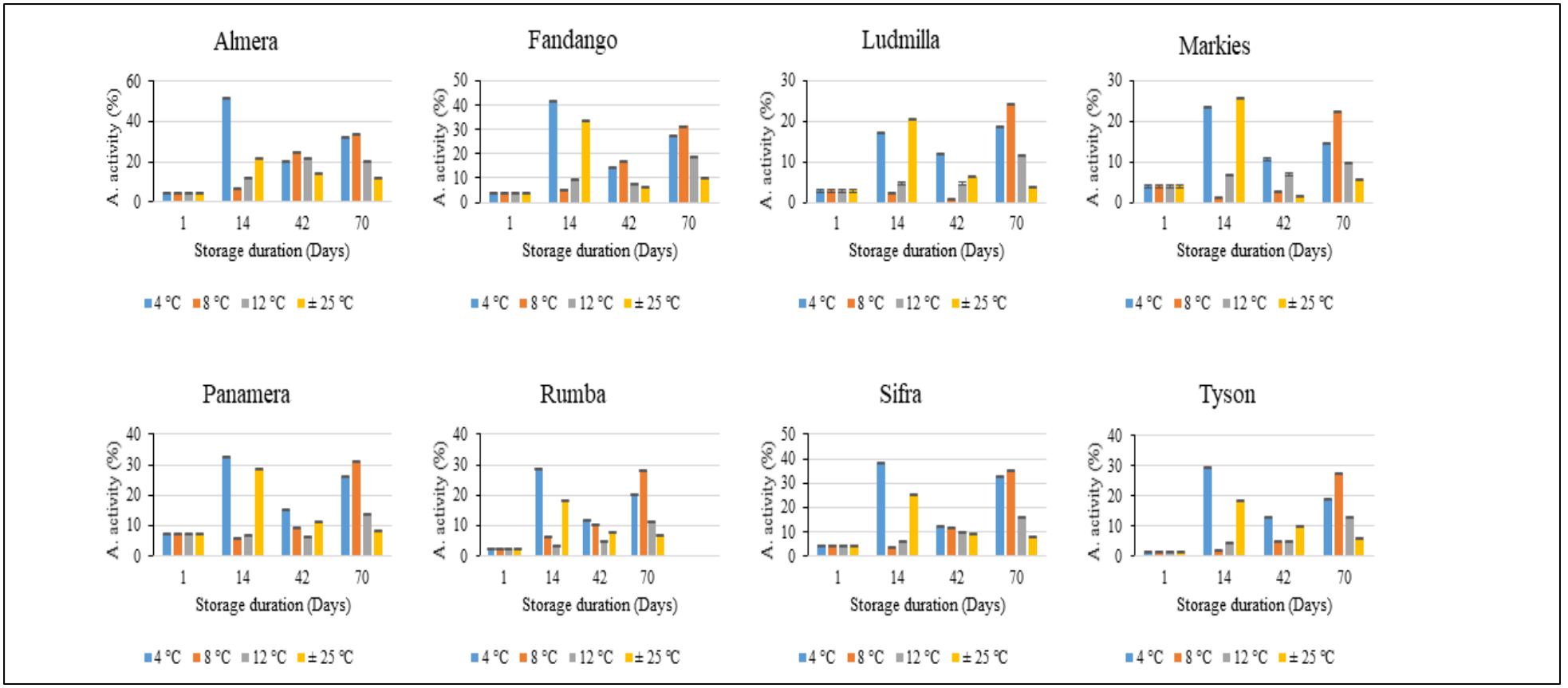

Figure 4.2. The total antioxidant activity of the potato tubers stored at 4, 8, 12 and room temperature for 70 days. Bars represent means ± standard error (n=3). Main effects: C: $p<0.001$; D: $p<0.001$; T: $p<0.001$; C*D: $p<0.001$; C*T: $p<0.001$; D*T: $p<0.001$; Interaction effects: C*D*T: $p<0.001$ where: C is cultivar; D is storage duration; T is storage temperature

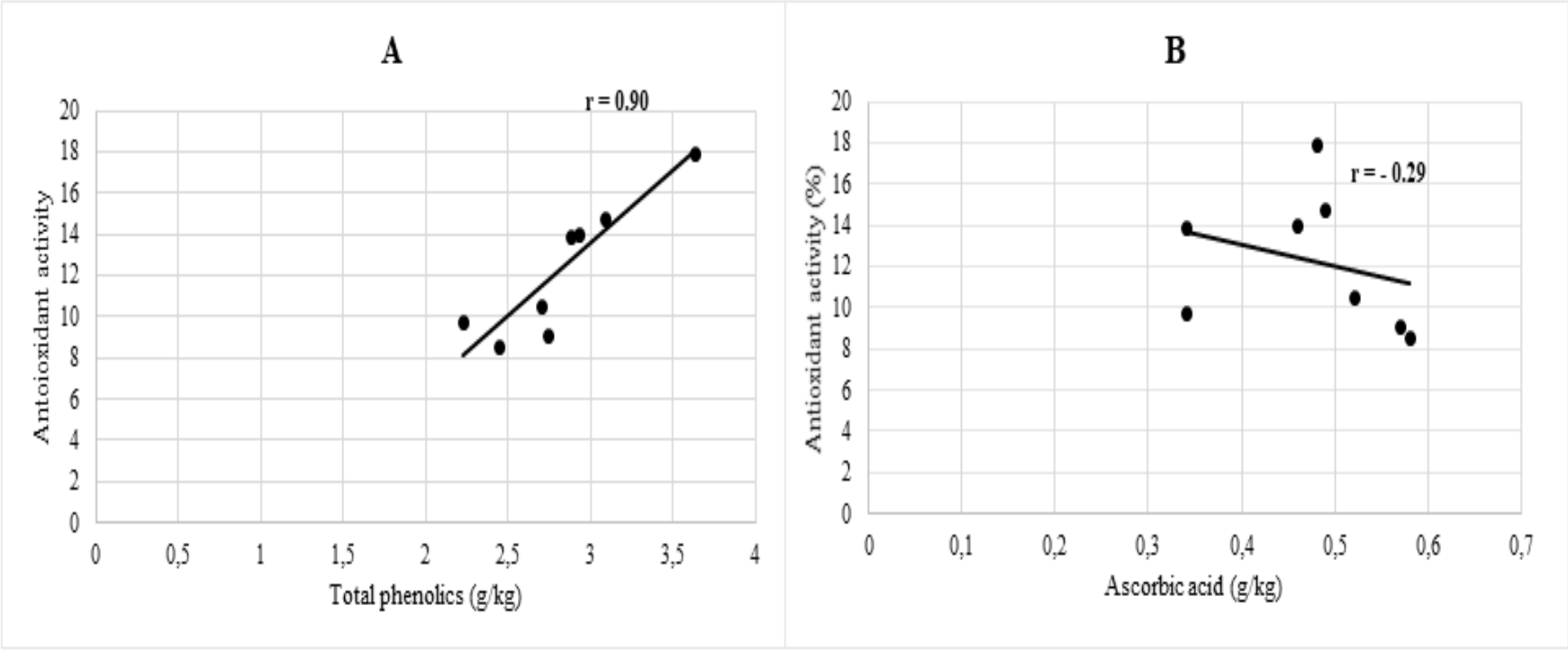

Figure 4.3. The relationship between antioxidant activity with total phenolics (A) and ascorbic acid (B) of selected potato cultivars.

4.3.3. Ascorbic acid

Storage temperature had a significant influence on the ascorbic acid content of potatoes (Figure 4.4). Ascorbic acid content decreased from 0.76 to 0.13, 0.26, 0.56 and 0.48 at 4, 8, 12 and ± 25 °C, respectively, after 70 days of storage. The storage temperatures of 4 and 8 °C had drastic decrease of ascorbic acid while 12 and 25 °C maintained high ascorbic acid. Furthermore, the ascorbic acid decreased with the increase in storage duration. For instance, the average ascorbic acid at day 0 was 0.76 g/kg then decreased to 0.6, 0.17 and 0.36 g/kg, after 14, 42 and 70 days of storage, respectively. The overall performance of storage temperatures revealed the lowest reading of ascorbic acid of 0.37 g/kg of potato tubers stored at 4 °C, compared to those stored at 8, 12 and ± 25 °C which recorded 0.48, 0.56 and 0.50 g/kg, respectively. The highest ascorbic acid was recorded in 'Ludmilla' with 0.58 g/kg, followed by 'Markies', 'Rumba', 'Fandango', 'Almera', 'Sifra', 'Panamera' and 'Tyson' with 0.57, 0.52, 0.49, 0.48, 0.46, 0.34 and 0.34 g/kg, respectively. The findings clearly demonstrated that cultivars vary in their ability to retain ascorbic acid under different storage temperature and over the storage duration.

Our results were similar to the previous studies which reported a drastic decrease of ascorbic acid during cold storage (Abbasi et al., 2016; Külen et al., 2013; Mazza et al., 1983). The degradation of ascorbic acid during storage, especially in low temperatures, might be due to oxidation to dehydroascorbic and later to diketo-gluconic acid (Rivero et al., 2003). Külen et al. (2013) observed a significant decline of vitamin C of potato tubers stored in cold storage (4 °C), decreasing by 24%, 45% and 52% after 2, 4 and 7 months, respectively. In this study, the decrease of ascorbic acid was not only observed at 4 °C but also in 8 °C. Nevertheless, ascorbic acid of the potato tubers stored at 12 and ±25 °C fluctuated over storage and was slightly high yet below the initial content after 70 days of storage. Similarly, Galani et al. (2017) reported the variation of ascorbic acid in tubers stored at 15 °C and room temperature after 90 days of storage. They also hypothesized that the ascorbic acid might have been released as way of response mechanism to stress caused by cold storage.

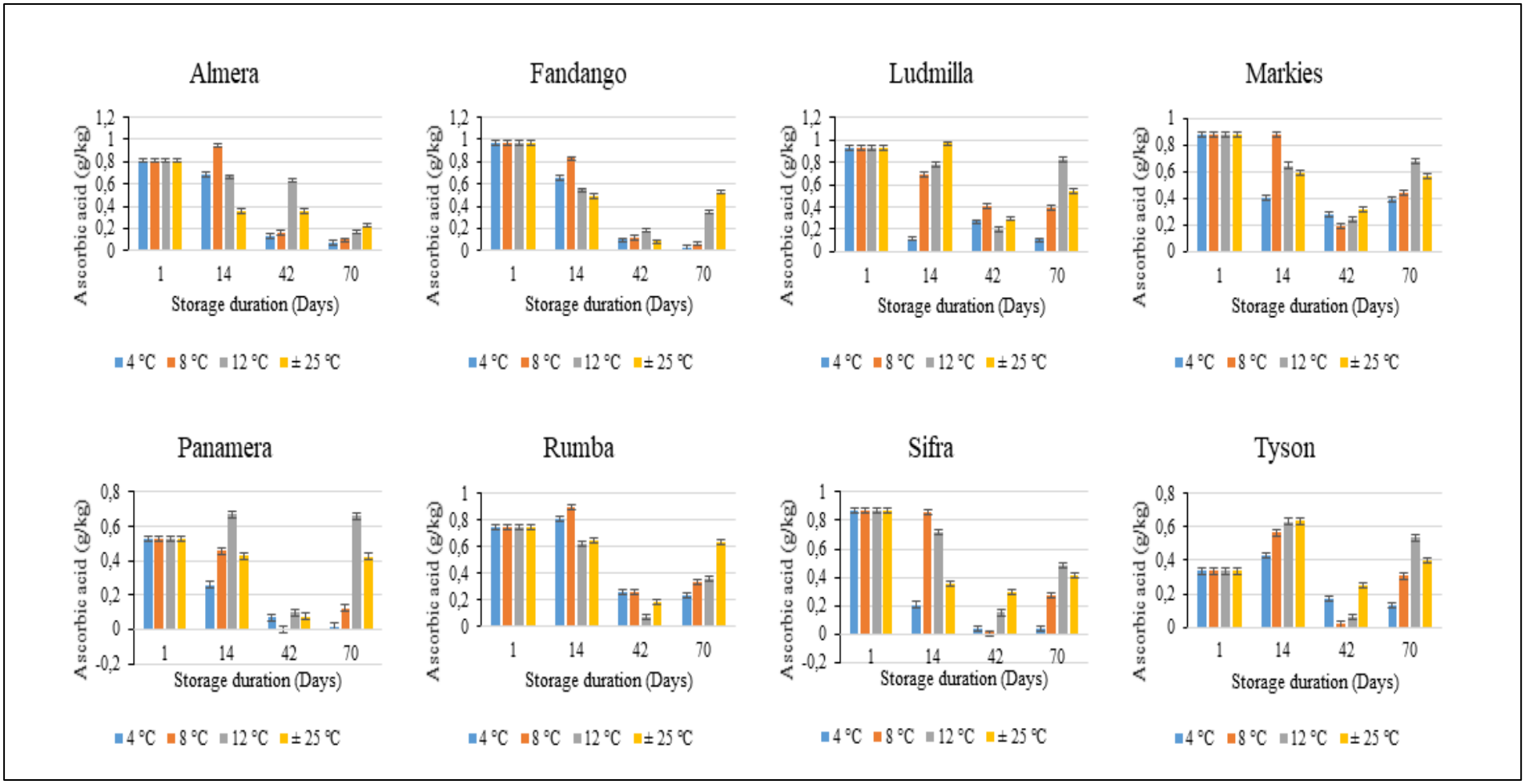

Figure 4.4. The ascorbic acid of the potato tubers stored at 4, 8, 12 and room temperature for 70 days. Bars represent means ± standard error (n=3). Main effects: C: $p<0.001$; D: $p<0.001$; T: $p<0.001$; C*D: $p<0.001$; C*T: $p<0.001$; D*T: $p<0.001$**;** Interaction effects: C*D*T: $p<0.001$ where: C is cultivar; D is storage duration; T is storage temperature

4.3.4. Total protein content

Nutritionally, the quality potato protein is comparable to that of eggs and consists of higher lysine compared to other vegetables and cereals (Gumul et al., 2011). This study showed that storage temperature and duration had a significant effect on total protein content (Figure 4.5). At 4 °C, the total protein content increased from 10.20 to 10.43% whereas for 8, 12 and room temperatures a slight reduction of total protein content to 9.92, 8.80 and 9.7%, respectively, was observed after 70 days of storage. The results also showed that total protein fluctuated over the storage duration with high average concentration observed at 42 days followed by 0 and 70 days with 10.12, 10.02 and 9.75%, respectively. The highest protein content was observed at 25, 4, 8 and 12 °C with 10.12, 10.06, 9.9 and 9.8%, respectively. In terms of the cultivar, the highest protein content was observed in 'Ludmilla' followed by 'Rumba', 'Fandango', 'Panamera', 'Tyson' and 'Markies' with 11.50, 10.44, 9.73, 9.52, 9.34 and 9.29%, respectively.

Our results are comparable to those of Mazza et al. (1983) who reported a slight increase of true and crude protein. Ozturk and Polat (2016) reported an increase of the protein content of most potato cultivars stored at 4-6 °C. Similarly, in this study, the potato tubers stored at 4 °C showed a slight increase in total protein content over 70 days. Furthermore, studies observed an increase of amino acids in numerous potato cultivars when stored at low temperatures contributing to the increase of the total protein of potatoes (Davids et al., 2004; Matsuura-Endo et al., 2006). However, at 8, 12 and room temperature, there was a slight decrease of total protein observed over 70 days. The potato cultivars also differed amongst themselves over the storage duration in all storage temperatures.

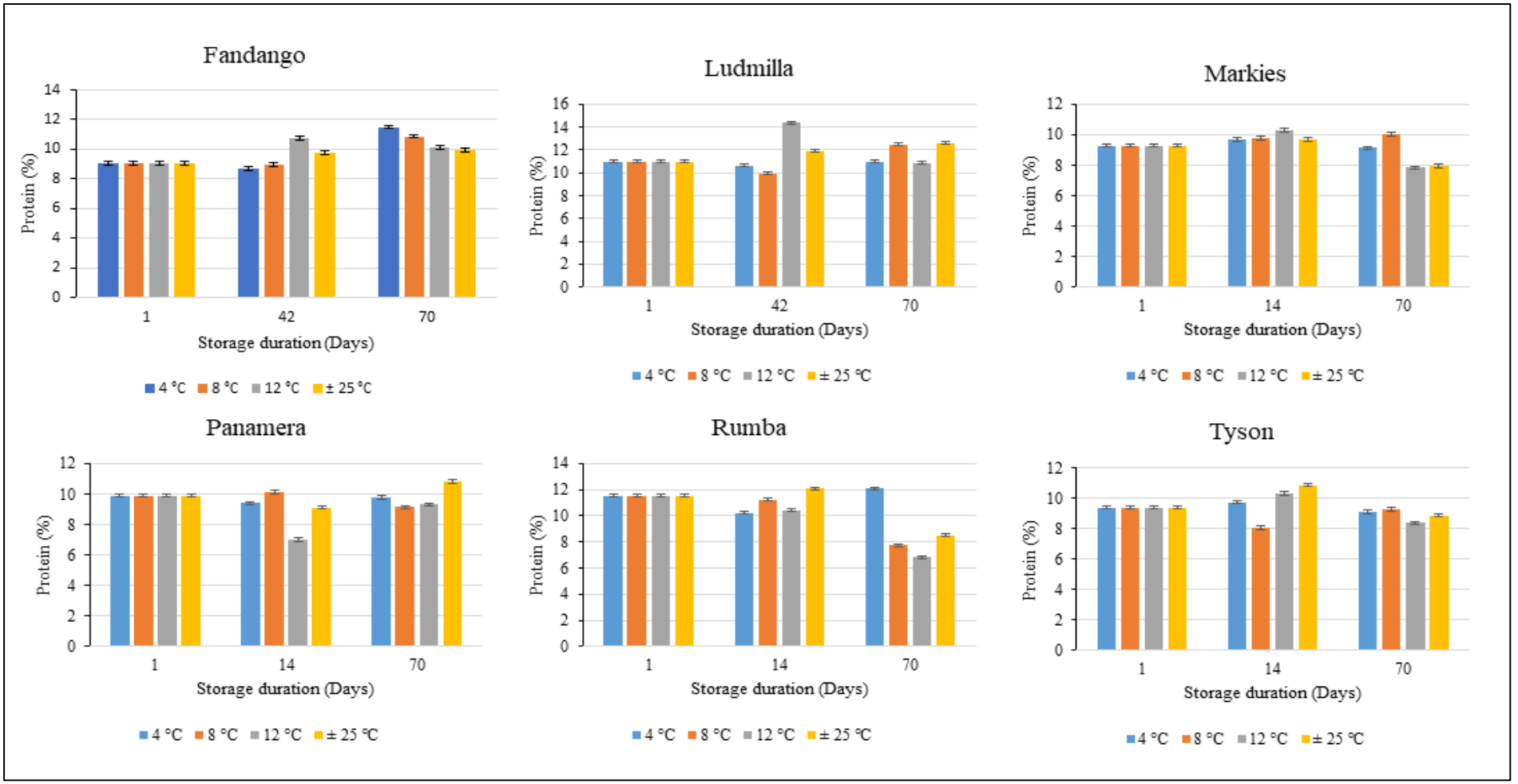

Figure 4.5. The total protein of the potato tubers stored at 4, 8, 12 and room temperature for 70 days. Bars represent means ± standard error (n=3). Main effects: C: $p<0.001$; D: $p<0.001$; T: $p<0.001$; C*D: $p<0.001$; C*T: $p<0.001$; D*T: $p<0.001$; Interaction effects: C*D*T: $p<0.001$ where: C is cultivar; D is storage duration; T is storage temperature

4.4. Conclusion

The findings showed that the highest retention of protein and total phenolic content was observed at room temperature, while the highest antioxidant activity and ascorbic acid was observed at 8 and 12 °C, respectively. Furthermore, total phenolic content and ascorbic acid decreased significantly with the increase in storage duration. Unlike the total phenolic content, the total antioxidant activity increased with the increase in storage duration. The total protein content fluctuated over storage duration. 'Almera' retained highest total phenolic content and antioxidant activity in all storage temperatures whereas highest ascorbic acid and total protein were recorded in 'Ludmilla' potatoes. It was observed that different potato tuber quality parameters were both temperature and cultivar dependent during storage. Therefore, further research aimed at understanding the role storage temperatures on postharvest quality and nutritional attributes of various potato cultivars is warranted.

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Chapter 5

Development of predictive models for the determination of potato quality using visible and near infrared spectroscopy

Abstract

Diffuse reflectance near infrared (NIR) spectroscopy was explored as a non-destructive method for detecting internal quality of potato tubers. The study compared predictive models developed in spectra acquired in different storage temperatures. Various potato quality attributes were measured including mass loss, soluble sugars as well as ascorbic acid and total phenolic concentration. Partial least squares regression was applied to spectral data to develop prediction models for each quality parameter and by randomly dividing the data into calibration and validation sets. The best models were developed in the spectra acquired at 8 °C. For instance, this spectra showed a good prediction for ascorbic acid (R^2p = 0.81; RMSEC = 0.15 g/kg; RMSEP = 0.137 g/kg ; RPD= 2.33), mass loss (R^2p = 0.81; RMSEC = 0.42 %; RMSEP = 0.50 %; RPD= 2.35) , reducing sugars (R^2p = 0.65; RMSEC = 0.53 g/kg; RMSEP = 0.66 g/kg ; RPD= 1.75), sucrose (R^2p = 0.58; RMSEC = 0.30 g/kg; RMSEP = 0.40 g/kg ; RPD= 1.60) and total phenolic content (R^2p = 0.79; RMSEC = 1.14 g/kg; RMSEP = 1.19 g/kg ; RPD= 2.25). The best models were only applicable for rough quantitative predictions which could be useful for screening of potato tubers whether for their processing or nutritional characteristics. However, the models developed for sucrose and reducing sugars were only suitable to discriminate high from low concentrations found in potato tubers. This study clearly demonstrated the potential of near infrared spectroscopy as a non-destructive technique for determining quality attributes of potato tubers.

Key words: Potatoes**,** near infrared spectroscopy, ascorbic acid, total phenolic content, mass loss, sugars

5.1. Introduction

Recent trends have shown a significant increase in global consumption of potatoes. The potatoes are consumed fresh and also processed into a wide range of products such as par-fried potato strips, French fries, potato chips, potato starch, potato granules, potato flakes, and dehydrated diced potatoes (Pedreschi et al., 2016). However, the accumulation of reducing sugars (glucose and fructose) during storage is one of the major problems in the processing industry since they result in an undesirable colour of fried potato products. This colour is formed when reducing sugars react with amino acids in a Maillard reaction to form acrylamide

(Marangoni, 2017). Furthermore, sucrose indirectly participates in the Millard reaction by serving as the source of reducing sugars which some may even be produced during the frying process. Hence, lower levels of sucrose and reducing sugars are preferred (Marangoni, 2017; Rosen et al., 2018).

Potatoes serve as excellent source of diverse functional ingredients such as polyphenols, vitamins and proteins which are beneficial to human health (Gumul et al., 2011; Pinhero et al., 2016). Antioxidant phytochemicals such as polyphenols and vitamin C are known to prevent the occurrence of cardiovascular disease and cancer as well as reducing the levels of blood cholesterol (Gumul et al., 2011). Therefore, more attention has been given in identifying potato cultivars that are rich in antioxidant phytochemicals for human health benefits (Pinhero et al., 2016).

The analytical methods that are normally used to determine the potato quality are gas liquid chromatography, HPLC and UV–vis spectrophotometry (Escuredo et al., 2018). Even though they provide accurate information, their major drawback is that they are destructive, manual, time consuming and laborious (Su et al, 2017; Rady and Guyer, 2015). Unlike the traditional analytical methods, near infrared spectroscopy (NIRS) is the rapid and inexpensive method that simultaneously analyses many parameters without the use of chemical reagents (Bonierbale et al., 2009). NIRS works by revealing the chemical information that is closely linked with the vibrational behaviour of the molecular bonds and tell the available molecules within the tested product (Lebot et al., 2013). Although the use of NIR to assess internal quality of various agricultural crops has gained popularity in recent years, very little research has been conducted on developing reliable and accurate models for evaluating the quality of potatoes during postharvest handling. Therefore, the objective of the study was to develop a predictive model for determining the quality of the potato tubers using near infrared spectroscopy (NIRS).

5.2. Materials and methods

5.2.1. Plant materials

Eight potato genotypes (‘Almera’, ‘Fandango’, ‘Ludmilla’, ‘Markies’, ‘Panamera’, ‘Rumba’, ‘Sifra’ and ‘Tyson’) were harvested from Sesisonke Farm, Harrismith, Free State Province. The potato cultivars were split and stored in four storage temperatures set at 4, 8, 12 °C and ± 25 °C room temperature for 70 days. The sampling was performed at 14, 42 and 70 days, respectively where potato cultivars were first scanned using vis-near infrared spectroscopy and

then destructed for determination of the internal quality. A total number of 288 samples were used with each storage temperature containing 72 samples.

5.2.2. NIRS spectral acquisition

The spectral data of the whole potato tuber samples were acquired using a method described by Magwaza et al., (2016) with slight modification. Reflectance NIR spectra was acquired using a laboratory bench-top monochromator NIR Systems Model XDS spectrometer (Foss NIR Systems, Inc., Silver Spring, MD, USA) equipped with a quartz halogen lamp and PbS detector. Before scanning and after each 30 min, the NIR spectrometer was calibrated by scanning a 100% white reference tile. The spectra were acquired with a circular sample cup with a quartz window (38 ¼ mm in diameter and 10 mm in thickness). All samples were carefully scanned by placing them on a sample cup in an enclosed box, specifically designed to prevent light leakage. The NIR system was operated with Vision software (Vision TM, version 3.5.0.0, Tidestone Technologies Inc., KS, USA). Reflectance spectra were obtained at 2 nm intervals from 400 to 2500 nm wavelength range. Each spectrum consisted of 32 scans which were automatically averaged and saved as absorbance intensity [log (1/R)]. The integration time was less than 500 ms per spectrum collected.

5.2.3. Reference measurements

5.2.3.1. Determination of ascorbic acid (AA)

A method by Boonkasem et al. (2015) with little modifications was used for measuring ascorbic acid. A 0.1 g of freeze-dried sample was extracted using 5 ml of 3 % metaphosphoric acid (v/v). The mixture was then shaken at 200rpm using the horizontal shaker (KS 130 C, Laboratory Equipment, Germany) for 30 min. The mixture was then centrifuged for 10 min using GenVac personal evaporator (EZ-2.3, SP scientific, Genevac Ltd, Ipswhich, England) at room temperature. Then, 1 mL of the supernatant was taken for further analysis. Into 1 mL of the supernatant, a volume of 5 mL of 0.05 mM of 2,6-Dichlorophenolindophenol (DCIP) solution was added and mixed for 5 sec. The absorbance of the solution was read at 515 nm against blank immediately before 15 sec using the spectrophotometer (UV-1800, Shimadzu Corporation, Japan). The results were then calculated using the standard curve prepared at concentrations between 0-20 ug/mL of ascorbic acid ($R^2 = 0.987$).

5.2.3.2. Mass loss

Tuber mass loss was measured by calculating the difference between the mass after storage and the initial mass before storage. The mass of the sample for each variety was measured using the weighing balance as stated by Rezaee et al. (2013).

$$\text{Mass Loss (\%)} = \frac{Initial\ mass - final\ mass\ after\ storage}{Initial\ mass} \times 100$$

5.2.3.3. Determination of soluble sugar concentration

The concentrations of soluble sugars were determined according to the method of Tesfay et al. (2016) with little modifications. A sample of 0.20 g pulverized potato sample was mixed with 10 mL of 80% (v/v) ethanol and homogenized for 60 sec. Thereafter, the mixture was incubated in an 80 ^{o}C water bath for 60 min and kept at 4 ^{o}C overnight. After centrifugation at 12 000g for 15 min at 4 ^{o}C, the supernatant was carefully filtered through glass wool and taken to dryness in a Genevac personal evaporator (EZ-2.3, SP scientific, Genevac Ltd, Ipswhich, England). Dried samples were re-suspended in 2 mL of ultra-pure water, filtered through 0.45-mm nylon filters and sugars were analysed using an HPLC (LC – 20 AT, Shimadzu Corporation, Kyoto, Japan) equipped with a refractive index detector (RID-10 A, Shimadzu Corporation, Kyoto, Japan) and a Rezex RCM–monosaccharide column (8-mm pore size; Phenomenex, Torrance, CA, USA). The concentration of individual sugars was determined by comparison with authentic standards. Sucrose (R^2 =0.999), glucose (R^2 =0.999) and fructose (R^2=0.999) standards were used to determine the concentration of each sugar.

5.2.3.4. Determination of total phenolic concentration

A method by Ah-Hen et al. (2012) with little modifications was used to measure total phenolics (TPC)**.** The total phenolic compounds were determined by Folin-Ciocalteu photo-colorimetric method. A freeze-dried sample of 0.2 g was weighed accurately on an analytical balance. A volume of 4 mL of an aqueous solvent of 80% methanol acidified with 1% HCL was added to the sample to extract the phenolic compounds. The mixture was then homogenized in a horizontal shaker at 200 rpm for 2 h at room temperature. Thereafter, mixture was centrifuged for 10 min at 7000 rpm. The supernatant was kept in dark from which the aliquot of 100 µL was taken and mixed in a test tube with 750 µL Folin-Ciocalteu reagent and left to stand for 5 min. Thereafter, 750 µL of aqueous sodium carbonate solution (60 g L-1) was added and the mixture was allowed to stand for 90 min at 22°C. Finally, the absorbance was measured at 725

nm with a UV-visible spectrophotometer (UV-1800, Shimadzu Corporation, Japan) and compared to a standard curve concentration between 0-40 µg /100 µl ferulic acid to calculate the actual concentrations of the sample extract ($R^2 = 0.994$).

5.2.4. Chemometric analysis

Chemometric analysis of data was carried out using The Unscrambler® X chemometric software (The Unscrambler® X v10.5, CAMO SOFTWARE AS, Oslo Science Park, NORWAY). Various pre-processing methods including multiplicative scatter correction (MSC), Savitzky-Golay first derivative and standard normal variate (SNV) were used to correct light scattering and reduce the changes of light path length. Outlier samples were selected based on F-residuals and Hotelling T^2 outlier detection at 5%. PLS regression models were developed using Kernel algorithm. The best models were developed using the selected wavelength ranging from 850 – 2500 nm.

Each storage temperature consisted of 72 samples which were split into calibration (48) and validation set (24). The performances of the developed model were evaluated based on the regression statistics described by coefficient of determination (R^2) for calibration (R^2c) and prediction (R^2p), root mean square error of cross validation (RMSEC), root mean square error of prediction (RMSEP) and the residual predictive deviation (RPD) (Olarewaju et al., 2019). A good predictive model was judged based on its high R^2p and RPD values with low RMSEC and RMSEP values.

5.3. Results and discussion

5.3.1. Reference and spectral characteristics

The mean, range and standard deviation of ascorbic acid, mass loss, reducing sugars, sucrose and total phenolic content are provided to give an idea of the structure of the samples used in this study (Table 5.1). Based on the descriptive statistics provided in Table 5.1, a wide variation in both calibration and validation sets of the measured parameters was observed, rendering them useful for the development of the predictive model. According to Magwaza et al. (2014), the accuracy of the calibration models is mainly dependent upon the precision of the measured physical and biochemical parameters as well as enough variation in both calibration and validation sets. In our study, factors such as cultivar, storage temperature and duration highly

contributed to the variation observed in the measured parameters as this was clearly demonstrated by high coefficient of variation.

Potato cultivars stored at 4, 8, 12 and room temperature appeared similar and all had six broad absorption peaks around 476, 856, 970, 1194, 1464 and 1920 nm regions (Figure 5.1). The peaks at 970 and 1464 nm were associated with second and first vibrational overtones of OH stretching related to water absorption, while the peaks at 1274 and 1920 nm were related to the second and first overtones of CH stretching as well as the third overtone of OH, CH and CH_2 deformation associated with sugar solution as previously reported by Magwaza et al. (2013).

Table 5.1. Statistical summary of calibration and validation sets of ascorbic acid, mass loss, reducing sugars, sucrose and total phenolic content of the whole potato tubers stored at 4, 8, 12 and ± 25 °C (room temperature). RT is room temperature

Parameter	Temperature	Calibration					Validation				
		Mean	Min	Max	$SD_{cal.}$	$CV_{cal.}$ %	Mean	Min	Max	SD_{pred}	$CVp_{red.}$%
Ascorbic acid (g/kg)	4	0,250	0,014	0,807	0,209	83,379	0,229	0,014	0,682	0,204	89,072
	8	0,414	0,002	0,949	0,306	73,968	0,413	0,002	0,973	0,319	77,301
	12	0,440	0,061	0,834	0,247	56,204	0,436	0,061	0,738	0,260	59,607
	RT	0,418	0,080	0,636	0,154	36,838	0,385	0,072	0,960	0,229	59,464
Mass loss (%)	4	2,432	0,322	4,038	1,087	44,695	2,632	0,894	4,000	1,089	41,387
	8	2,002	0,611	4,590	1,169	58,381	1,993	0,591	4,563	1,174	58,911
	12	2,897	0,728	6,741	1,777	61,339	3,018	0,718	6,736	1,861	61,676
	RT	7,955	1,633	29,550	6,141	77,196	7,985	1,682	29,525	6,217	77,866
Reducing sugars (g/kg)	4	2,309	0,035	8,093	2,261	97,884	2,190	0,030	8,094	2,165	98,864
	8	0,883	0,000	3,072	0,783	88,621	1,263	0,035	3,238	1,157	91,593
	12	0,313	0,000	1,180	0,388	123,968	0,307	0,000	1,133	0,381	124,291
	RT	0,110	0,000	0,833	0,183	166,127	0,124	0,000	1,161	0,244	196,446
Sucrose (g/kg)	4	1,875	0,220	3,283	0,862	45,953	1,758	0,228	3,355	0,976	55,506
	8	1,162	0,584	2,633	0,601	51,714	1,188	0,610	2,633	0,640	53,876
	12	1,030	0,246	2,314	0,580	56,325	1,019	0,245	2,312	0,580	56,939
	RT	1,024	0,201	1,918	0,604	58,933	0,950	0,200	1,918	0,607	63,862
Total phenolics (g/kg)	4	2,463	0,860	5,534	1,161	47,141	2,578	0,911	5,350	1,240	48,119
	8	3,366	1,056	8,664	2,649	78,708	3,057	1,062	8,561	2,675	87,506
	12	2,477	0,817	5,835	1,481	59,793	2,449	0,874	5,717	1,493	60,951
	RT	3,438	0,545	10,414	2,731	79,433	3,409	0,676	8,726	2,675	78,490

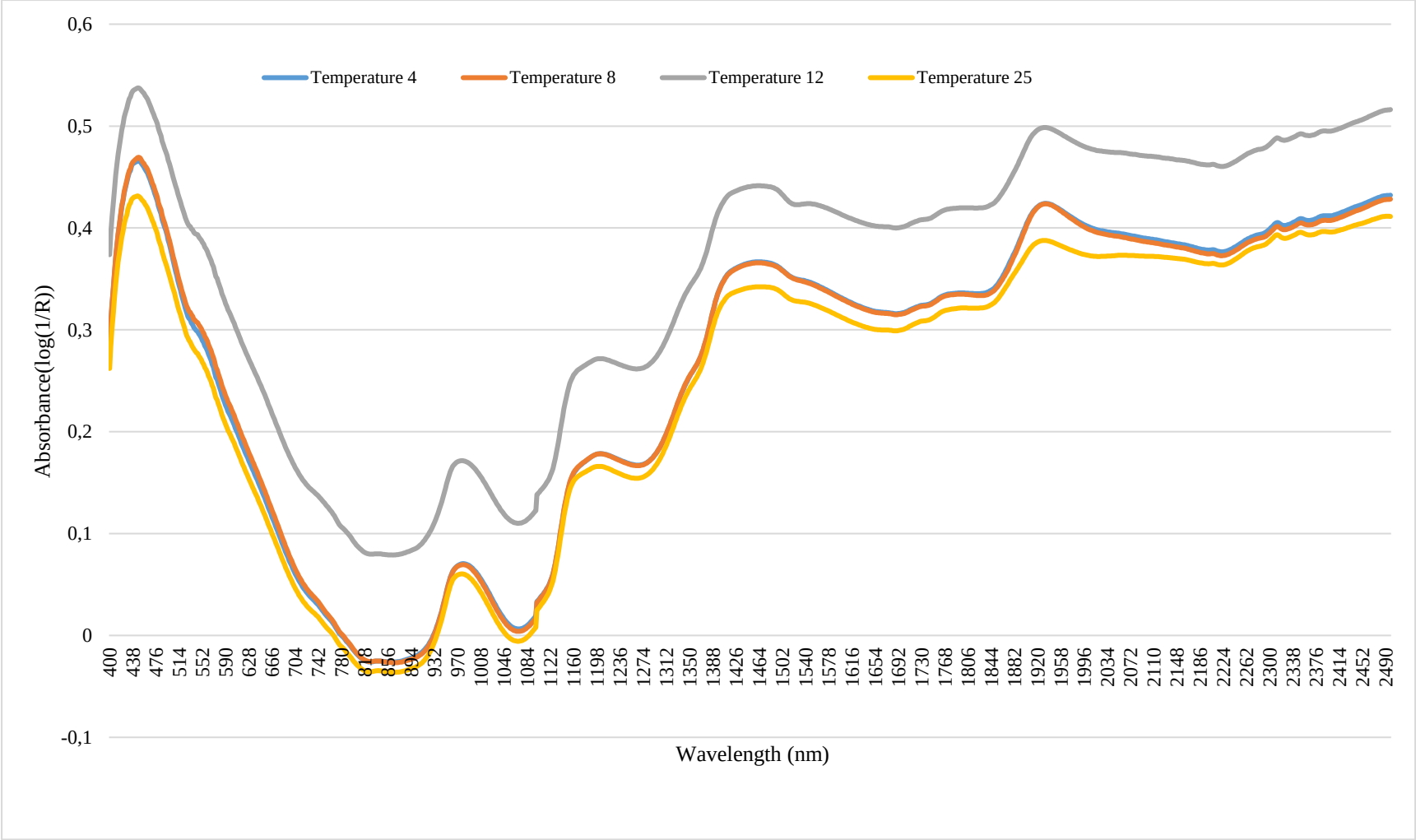

Figure 5.1. Reduced to average raw spectra of potato cultivars stored in four storage temperatures namely 4, 8, 12 and ± 25 °C.

5.3.2. Development of PLS models

The prediction models were developed using the PLS multivariate analysis. Several mathematical pre-processing methods were explored, including no pre-processing to achieve the good models for determination of internal quality parameters of the potato tubers. Furthermore, the optimum number of latent variables was determined to develop the best and stable calibration models. Ascorbic acid, mass loss, reducing sugars, sucrose, and total phenolic content had 6, 7, 10, 9 and 8 latent variables, respectively, used to develop the calibration models. Sucrose and mass loss models for spectra at 12 °C and room temperature, respectively could not be developed due to the lack of linearity between the measured and spectral data. Moreover, the measured data for reducing sugars at 12 °C and room temperature was not enough to be used in the development of the calibration and predictive models. Hence, these models were not represented in table 5.2. Special attention was given to the best model statistics which include high R^2p and RPD as well as low RMSEC and RMSEP (Table 5.2). Therefore, in our results the best models were developed from the spectra acquired at 8 °C. In this spectra, the ascorbic acid ($R^2p = 0.81$; RMSEC = 0.15 g/kg; RMSEP = 0.137 g/kg ; RPD= 2.33), mass loss ($R^2p = 0.81$; RMSEC = 0.42%; RMSEP = 0.50%; RPD= 2.35) , reducing sugars ($R^2p = 0.65$; RMSEC = 0.53 g/kg; RMSEP = 0.66 g/kg ; RPD= 1.75), sucrose ($R^2p = 0.58$; RMSEC = 0.30 g/kg; RMSEP = 0.40 g/kg ; RPD= 1.60) and total phenolic content ($R^2p = 0.79$; RMSEC = 1.14 g/kg; RMSEP = 1.19 g/kg ; RPD= 2.25) had good prediction ability compared to other spectra acquired in other storage temperatures (Figure 5.2). According to Olarewaju et al. (2019), the models with RPD greater than 3 are regarded as excellent, those between 2 and 2.5 are suitable for quantitative predictions, those between 1.5 and 2 are fit for differentiating high from low values while those less than 1.5 are not applicable in the real world. The models developed for ascorbic acid, mass loss and total phenolic content had the RPD ranging from 2.25 to 2.35, meaning that they were only useful to give the rough estimation of their concentrations in the potato tubers but not really the exact values. On the other hand, the sucrose and reducing sugars had the lowest RPD ranging from 1.60 - 1.75 useful for rough predictions in potato tuber. Sugars are reported to be more concentrated on the vascular ring than other parts of the tuber. Hence, the diffuse reflected light might not have detected the concentration on the pith compared to the concentration found closer to the skin (Rady and Guyer, 2015). Haase (2011) reported prediction models with RPD of 1.5 and 1.7 for 'Frito Lay' and 'Russet Norkotah' potato cultivars which he considered poor to predict the reducing sugars. Rady and Guyer (2015) developed NIR models for predicting glucose and sucrose in two potato

cultivars. In their study, the models developed for glucose had RPD value of 1.46 and 2.95 for ‘Frito Lay’ and ‘Russet Norkotah’ potato cultivars, respectively. For sucrose, the RPD values were 1.13 and 1.02 for ‘Frito Lay’ and ‘Russet Norkotah’ respectively. Therefore, our results were not unique to the above mentioned, since we reported the RPD values of 1.75 and 1.60 for models developed for reducing sugars and sucrose, respectively. The periderm thickness of the potato cultivars used in our study might be one of the factors that might have limited the diffuse reflectance signal (Rady and Guyer, 2015). Camps and Camps (2019) noted that the models developed from peeled potatoes for reducing sugars were most accurate compared to those developed from the unpeeled and cut potato tubers.

Table 5.2. The performances of the calibration and predictions models of potato cultivars developed from spectra acquired at 4, 8, 12 and ± 25 °C (room temperature).

Parameter	Temperature	Calibration			Validation		
		LV	R^2c	RMSEC	R^2p	RMSEP	RPD
Ascorbic acid (g/kg)	4	10	0.70	0.05	0.73	0.103	1.98034
	8	6	0.76	0.15	0.81	0.137	2.330058
	12	8	0.74	0.13	0.70	0.137	1.89873
	RT	10	0.73	0.08	0.71	0.119	1.922588
Mass loss (%)	4	6	0.77	0.53	0.74	0.54	2.017094
	8	7	0.87	0.42	0.81	0.50	2.348446
	12	8	0.76	0.87	0.76	0.89	2.091518
Reducing sugars (g/kg)	4	10	0.38	1.81	0.41	1.61	1.344519
	8	10	0.77	0.53	0.65	0.66	1.753305
Sucrose (g/kg)	4	10	0.67	0.5	0.66	0.55	1.774533
	8	9	0.76	0.3	0.58	0.4	1.600203
	RT	10	0.66	0.36	0.69	0.33	1.838155
Total phenolic content (g/kg)	4	6	0.74	0.62	0.76	0.59	2.102473
	8	8	0.81	1.14	0.79	1.19	2.247792
	12	7	0.73	0.78	0.76	0.72	2.073132
	RT	9	0.69	1.54	0.68	1.42	1.884125

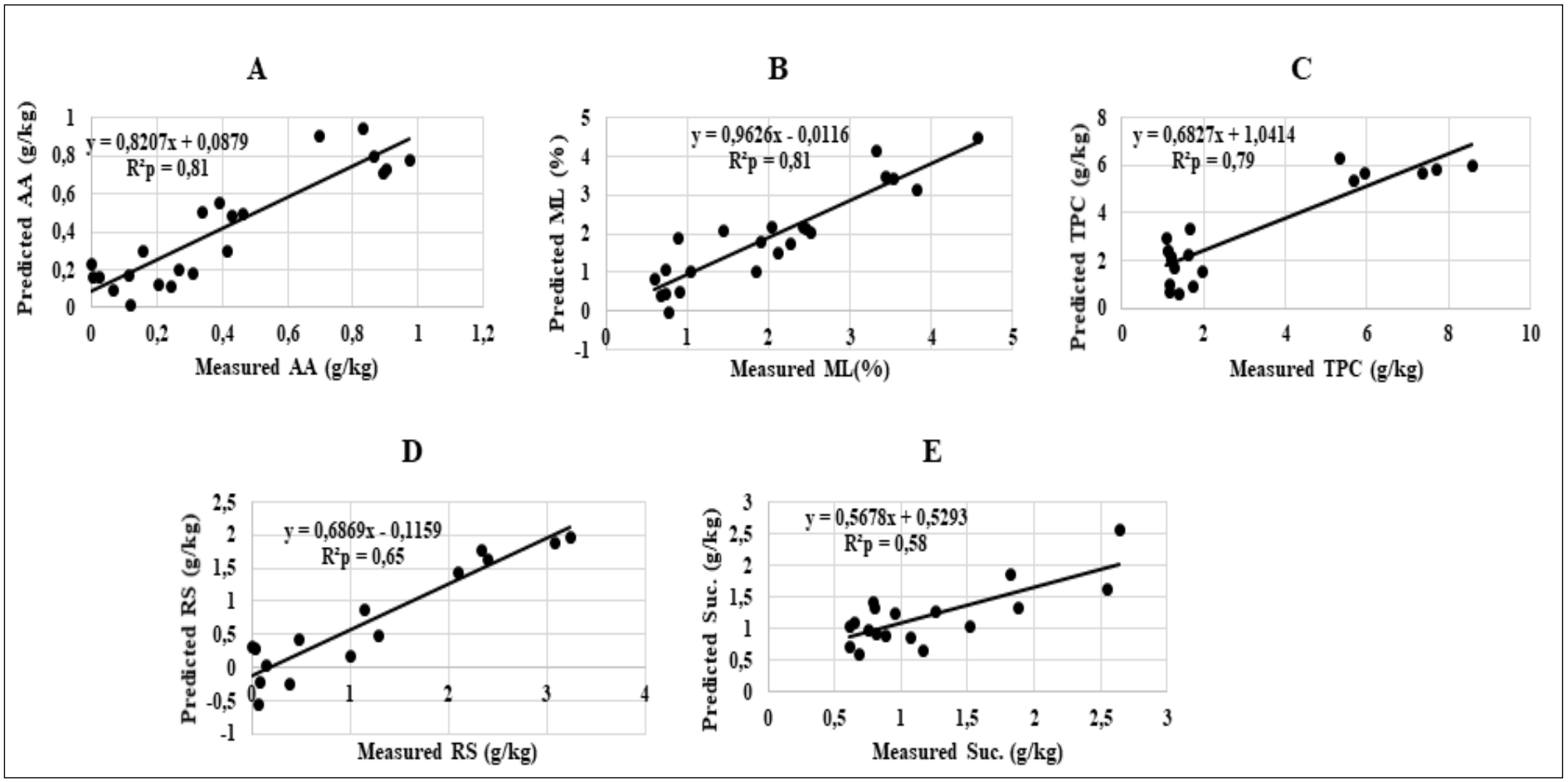

Figure 5.2. Scatter plots of NIR predicted ascorbic acid (A), mass loss (B), total phenolic content (C), reducing sugars (D) and sucrose (E) against conventionally measured parameters of potato tubers.

5.4. Conclusion

The study showed that there is a potential of developing models for predicting quality parameters of potato tubers using NIR technology. However, more improvements need to be done to develop models that are more stable and suitable for all applications and storage conditions. In this study, the best models developed for predicting ascorbic acid, mass loss and total phenolic content of potato tubers were only applicable to predict their rough estimations, meaning that they were not suitable to be used to predict their exact concentrations. However, the models developed for sucrose and reducing sugars were only suitable to discriminate their high from low concentrations in potato tubers.

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Chapter 6

General discussion and conclusion

6.1. Introduction

Potatoes are semi-perishable produce in nature, as a result, they are normally stored at 2-4 °C to reduce postharvest losses (Marwaha et al., 2010). According to Ali and Jansky (2015), storage temperatures below 8 °C are beneficial to potatoes since they prevent bacterial soft rot, water and dry matter loss and prevent sprouting without the use of the sprout suppressants. Although sprout suppressants such as chorpropham and maleic hydrazide are very effective, these treatments are known to be detrimental both to the environmental and human health (Abbasi et al., 2015; Ahmed et al., 2014; Foukaraki et al., 2016). Hence, the use of low storage temperatures remains advantageous.

Various studies have reported that sprouting incidence, processing and nutritional quality of potato tubers are cultivar dependent (Bethke, 2014; Elmore et al., 2015). The South African potato industry has recently released new cultivars for commercial production. As a result, there is a limited information on the performance of these newly developed cultivars under the wide range of storage temperatures. Therefore, the aim of this research was to investigate the effect of storage temperatures on the postharvest performance and sprouting of the selected potato cultivars. This chapter highlights the objectives of this study followed by concise major key findings of each objective. Moreover, the implications of the study as well recommendations are also highlighted.

6.2. Key research findings

6.2.1. Postharvest factors affecting potato tuber quality: A review

Postharvest losses, often resulting from improper storage conditions, are amongst the major challenges facing the potato industry. Therefore, this section of the dissertation reviewed postharvest challenges and treatments affecting potato tuber quality.

The literature clearly demonstrated that sprouting is well-known as a varietal characteristic. It is one of the major physiological processes that contribute to the postharvest losses of potatoes.

It results to high mass loss, reduced turgor, structural changes due to sprout tissue and as well rapid rise in the concentration of sugars caused by starch degradation (Paul et al., 2016). Mass loss due to sprouting negatively affects the quality, shelf life as well as consumer acceptability of potatoes (Mani et al. 2014).). It is mainly influenced by the occurence of evaporation and respiration taking place in the outer skin of the potato tubers as well as its physical maturity (Fogelman et al., 2015; Kumar et al., 2004; Pinhero et al., 2009) (Saran and Chhabra, 2014).

As abovementioned, the use of sprout suppressants is the effective way of controlling sprouting even through these treatments are losing popularity to their negative effects on human health (Abbasi et al., 2015; Ahmed et al., 2014; Foukaraki et al., 2016). Although, the use of essential oils and other organic sprout suppressants is the best alternative to replace the harmful chemicals, they are expensive since they have to be applied more frequently to increase their efficacy (Daniels-Lake et al., 2013; Frazier et al., 2004; Kleinkopf et al., 2003). Cold storage remains the best alternative since it does not only prevent sprouting but also reduce the possibility of bacterial soft rots without the use of chemicals (Bandana et al., 2017). The major challenge is that the incidence of bacterial soft rots tends to increase at storage temperatures between 20 to 27 °C. Hence, the use of low storage temperatures remains advantageous. Although low storage temperatures play a significant role in preventing most of the quality parameters of the potato tubers, such temperatures often lead to cold-induced sweetening. The major drawback of the accumulation of reducing sugars (glucose and fructose) during storage is the development of undesirable colour of fried potato products such a potato chips and French-cut fries during processing. Hence, this study assessed the effect of wide range of cold storage temperatures on the performance of different potato genotypes.

6.2.2. The effect of different storage temperatures on sprouting incidence and processing attributes of selected potato cultivars

This study was set up to investigate the effect of different storage temperatures on sprouting incidence and processing attributes of selected potato cultivars. Sprouting rate, mass loss, soluble sugars (glucose, fructose and sucrose), dry matter content, specific gravity and starch content were measured. The findings showed that sprouting and processing attributes are cultivar dependent. Notably, ‘Markies’ potatoes was the best performing cultivar in all storage temperatures compared to other cultivars. This cultivar had very low reducing sugars and high dry matter content. On the other hand, ‘Panamera’ and ‘Almera’ potato cultivars had the highest reducing sugars at 4 °C after 70 days of storage. These findings are in agreement with

the observations made on sprouting (Abong et al., 2015), dry matter content (Rahman et al., 2016), sucrose (Galani et al., 2016) and reducing sugars (Ali et al., 2016) by various researchers. The increase of sucrose and reducing sugars on potato tubers stored at 4 °C has been reported by Ali et al. (2016), respectively. According Amjad et al. (2016), an invertase enzyme which play a major role in the sucrose hydrolysis to glucose and fructose during cold storage might be the possible explanation of the increase of sugars during the cold storage observed in our findings. Lastly, the high sprouting incidence at high temperatures might be due to the stimulation of gibberellins in relation abscisic acid during storage (Mani et al., 2014; Wróbel et al., 2017).

6.2.3. Comparisons of total phenolics, ascorbic acid, proteins and antioxidant activity of selected potato cultivars stored at different postharvest temperatures

This research chapter assessed nutritional properties of potato tubers stored in four different temperature. The assessed nutritional and phytochemical attributes included total phenolics, ascorbic acid, proteins as well as antioxidant activity. The findings showed that, in all storage temperatures, 'Almera' potatoes retained highest total phenolic content and antioxidant activity whereas the highest ascorbic acid and total protein content were recorded in 'Ludmilla' cultivar. Notably, at 4 °C, the total protein content increased from 10.20 to 10.43 % whereas for 8, 12 and room temperature a slight reduction of total protein content to 9.92, 8.8 and 9.70%, respectively. Overall, the study showed that storage temperature had an enormous effect on nutritional and antioxidant attributes of potato cultivars. The observed decreasing trend of total phenolics during storage might be due to the degradation of the polyphenolic compounds, particularly chlorogenic acid (Madiwale et al., 2011). On the other hand, the degradation of ascorbic acid during storage especially cold storage might be due to oxidation to dehydroascorbic and later to diketo-gluconic acid (Rivero et al., 2003). Since polyphenols contribute about 80% of the antioxidant activity in potato tubers, it is possible that they might have been triggered by storage temperatures which in turn affected the antioxidant activity of the potato tubers (Nayak, 2011). The high protein content in potato tubers stored in low temperatures could be linked to the increased accumulation of amino acids in such storage environments (Davids et al., 2004; Matsuura-Endo et al., 2006; Ozturk and Polat, 2016).

6.2.4. Development of predictive model for the determination of the quality using near infrared spectroscopy

Diffuse reflectance near infrared (NIR) spectroscopy was explored as a non-destructive method for detecting internal quality of potato tubers. Various potato quality attributes were measured including mass loss, soluble sugars, ascorbic acid and total phenolic concentration. The models developed for predicting ascorbic acid, mass loss and total phenolic content of potato tubers were only applicable to predict rough estimations of these parameters in potato tubers not really the concise values. On the other hand, the models developed for sucrose and reducing sugars were only suitable to discriminate high from low concentrations of these parameters in potato tubers. Our results were similar those of Rady and Guyer (2015) and Haase (2011) concerning sucrose and reducing sugars. The periderm thickness of the potato cultivars used in our study might be one of the factors that might have limited the diffuse reflectance signal (Rady and Guyer, 2015). Hence, future studies should consider using peeled potatoes for the development of the models for predicting sucrose and reducing sugars (Camps and Camps, 2019). There was a lack of the supporting literature concerning the models developed for ascorbic acid, mass loss and total phenolic content. Therefore, more research aimed at developing prediction models for the abovementioned parameters is warranted.

6.3. Implications of the study

The knowledge gained from this research will help both the processors and producers to categorize each cultivar based on its potential storability under various temperatures. They will get informed as to which varieties perform the best for the processing quality/dual purpose along with advanced storage conditions. Additionally, these findings will be useful in minimizing the postharvest losses and maintaining the quality of potatoes.

6.4. Conclusions and recommendations

In conclusion, the study clearly demonstrated that sprouting and storage performance are cultivar dependent. Thus, it is critical to develop proper storage protocols for each potato cultivar. This will ensure longer storage life and high quality of processed products. Concerted efforts should be made to develop models that are more stable and suitable for quick assessment of internal tuber quality.

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