

POSTHARVEST DORMANCY AND SPROUT DEVELOPMENT IN POTATO
TUBERS STORED WITH APPLE FRUIT

BY

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DECLARATION

I, Lesibana Sammy Bopape, declare that the mini dissertation hereby submitted to the University of Limpopo, for the degree Master of Science in Horticulture has not been previously submitted by me or anybody for a degree at this or any other university. Also, my work in design and execution, and all materials contained herein have been duly acknowledged.

Bopape LS (Ms)

Date

DEDICATIONS

This study is dedicated to my beloved parents, Attios and Pherly Bopape, my grandmother Johanna Ngwepe and my siblings Levy, Johannes, Ramokone, Molatelo and Seemole Bopape.

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CONFERENCE PRESENTATION

BOPAPE, L.S., SATEKGE, T.K., MAFEO, T.P., 2022. Apple fruit as the biological suppressant for potato tuber sprouting during storage. Oral presentation at the University of Limpopo, 12th Faculty of Science and Agriculture Postgraduate Research Day. Bolivia Lodge (Polokwane, Limpopo), 21-23 September 2022.

BOPAPE, L.S., SATEKGE, T.K., MAFEO, T.P., 2022. Investigating the use of apple fruit as a biological suppressant for potato tuber sprouting during postharvest storage. Oral presentation to be presented at Combined Congress 2023 conference. University of Pretoria, 23-26 January 2023.

ABSTRACT

The potato (*Solanum tuberosum*) is a highly valuable starchy crop, serving as an important staple food. However, the crop postharvest quality is compromised during storage due to sprouting, which normally leads to reduced shelf-life. Thus, the aim of the study was to assess the potential of apple fruit as a biological suppressant against sprouting of potatoes. Potato tubers were collected from four commercial farms, thereafter, stored alone (control) or with apple fruit at ambient temperature (23° C) for 30 days. Analysis of variance showed that storage duration influenced the efficacy of apple treatment on tuber sprouting and dormancy. Tubers stored with apple fruits had significantly ($P<0.05$) reduced mass loss after 30-day storage compared to the control which showed higher mass loss, while tubers stored with apple fruits had significantly reduced sprouting compared with the control. Generally, tuber sprouting, mass loss, decay, starch content, soluble sugars and dry matter accelerated with storage duration. Control tubers for Jamba had 100% sprouting compared to tubers stored with apple fruits, with Elmar obtaining minimum sprouting of 18%. Decay percentage did not differ significantly ($P>0.05$) between tubers stored alone or with apple fruit while reducing sugars significantly ($P<0.05$) increased with an increasing storage duration in untreated tubers as compared to apple treatment. Furthermore, apple treatment had a significantly ($P<0.05$) reduced dry matter and starch content compared to the control in all locations except for Elmar. The study demonstrated that storing potato tubers with apple fruit at ambient temperature can be a biological anti-suppressant in potato tubers. Therefore, the method could be adopted as an alternative to synthetic ethylene gas and various chemicals used to control potato tubers sprouting during postharvest storage.

Keywords: Apples, climacteric fruit, potatoes, shelf-life, soluble sugars, sprouting

CHAPTER 1 GENERAL INTRODUCTION

1.1. Background

The potato (*Solanum tuberosum*) is an important food source ranked third in terms of production and consumption worldwide [Food and Agriculture Organization (FAO), 2018]. The potato tuber is consumed by approximately 1 billion people globally (FAO, 2015) for its macro and micro-nutrients (Abong *et al.*, 2009). With 0.6% of the global production, South Africa is ranked 28th among nations that produce potatoes (Denner *et al.*, 2012). Furthermore, potatoes are widely grown in South Africa mostly in 16 distinct regions with diverse climatic and soil characteristics (Van der Waals *et al.*, 2016). This therefore result in four agroecological seasons in these places as a result of different planting dates throughout the year, including dry or wet winter and dry or rainy summer (Van der Waals *et al.*, 2016). As a result, it could be argued that South Africa has a year-round supply of potatoes. However, concerns about the shelf life and quality maintenance of the current production of these potato tubers across the food value chains may surface from a postharvest viewpoint (Van der Waals *et al.*, 2016). Therefore, tuber quality maintenance during storage is of great importance for the potato industry to fulfil both the local and the global demand for potatoes. However, sprouting affects potato tuber quality during postharvest storage (Lisinska *et al.*, 2009).

Sprouting is a natural physiological process after dormancy whereby a potato tuber starts to develop shoots at the tuber buds base (Aksenova *et al.*, 2013). It is one of the key factors affecting the potato tubers during storage, compromising internal and external quality (Alexandre *et al.*, 2015; Sonnewald and Sonnewald, 2014; Teper-Bamnlker *et al.*, 2010). Once the sprouting becomes excessive during storage, quality and dormancy period of the tubers are reduced increasing mass loss, shrinkage and senescence (Alexandre *et al.*, 2015; Sonnewald and Sonnewald, 2014; Teper-Bamnlker *et al.*, 2010). Therefore, to preserve quality it is important to delay sprout development and maintain tuber dormancy during postharvest storage.

Postharvest methods that potentially delay sprout development have been investigated in potato tubers. These postharvest methods include cold storage (Alamar *et al.*, 2017; Aml *et al.*, 2014), chlorpropham (CIPC) (Terry *et al.*, 2013),

ethylene gas (Prange *et al.*, 2005), maleic hydrazide (Caldiz, 2001), and essential oils (Gomez-Castillo *et al.*, 2013). However, their utilisation has been limited owing to various factors, including unintended negative quality and high costs. For instance, long-term cold storage (2-5°C) results in accumulation of reducing sugars (fructose and glucose), which lead to the darkening of potatoes when fried (Alamar *et al.*, 2017). As a result, the processing industry rejects potato tubers with higher soluble sugars (Dale and Bradshaw, 2003).

Chemical methods such as CIPC and ethylene gas require a long application, often with high concentrations. Moreover, CIPC can be toxic when consumed in large quantities and is environmentally unfriendly (Pringle *et al.*, 2009). These make chemical methods are thus regarded as cost-ineffective and environmentally unacceptable (Balaji *et al.*, 2006; El-Awady Amal *et al.*, 2014). Therefore, it is important to search for cost-effective and eco-friendly sprout-suppressant methods.

The application of essential oils such as peppermint, coriander and eucalyptus oil are reported as biological and cost-effective sprout suppressant in potatoes (Eshel *et al.*, 2008; Teper-Balmnoker *et al.*, 2010). However, essential oils are highly volatile (Teper-Bamnlker *et al.*, 2010), requiring continuous application. Ethylene is also produced biologically by crops. Plants emit ethylene endogenously as a signalling hormone to modulate growth and development (Kumar *et al.*, 2014). The hormone plays a role in response to various biotic and abiotic stresses in plants and is also considered ripening hormone in fruits crops (Kumar *et al.*, 2014). Presently, on-going studies on focus on the effects of exogenous ethylene in facilitating dormancy, senescence mechanism and sprout growth commodities producing low ethylene (e.g., sweet potatoes and onions) (Chope and Terry, 2008; Cools *et al.*, 2011), which have confirmed that continuous ethylene supplementation can be adopted as an alternative to suppress sprouting. Currently, studies on how exogenous ethylene affects dormancy, sprout growth and senescence mechanisms in low-ethylene-producing commodities like sweet potatoes and onions are ongoing. These studies have shown that continuous ethylene supplementation could be a workable alternative to prevent sprouting. (Daniels-Lake *et al.*, 2005; Downes *et al.*, 2010; Cools *et al.*, 2011; Foukaraki *et al.*, 2016). During the ripening of climacteric fruits such as apple, bananas and tomatoes, ethylene gas is emitted abundantly (Cherian *et al.*, 2014). Therefore,

climacteric fruits could be used as an important biological ethylene agent. A recent study by Sun *et al.* (2021) demonstrated that storage of apple fruit with orange (non-climacteric fruit) increased colour development. The results were attributed to ethylene emitted by the fruit as it plays a role in modulating chlorophyll metabolism. Since sprouting in potato tubers was controlled by synthetic ethylene gas (Foukaraki *et al.*, 2016), biological ethylene emitted by climacteric fruit during ripening can also have the same effect of modulating sprouting and dormancy during storage due to their nature of emitting ethylene during ripening . However, there is dearth of information on whether storing potato tubers with climacteric fruits influences dormancy and sprouting. Hence, this study proposes to use climacteric fruit, apples, to control sprouting in potato tubers stored at ambient temperature.

1.2. Problem statement

During postharvest storage of potato tubers, dormancy is broken progressively leading to sprouting development (Pinhero *et al.*, 2009). Potato sprouting is the main postharvest problem that reduces tuber quality by increasing mass loss and shrinkage, thus reducing shelf-life, marketability and consumer acceptability of the produce. Furthermore, sprouted tubers have reduced nutritional and processing quality, producing of toxic compounds (Foukaraki *et al.*, 2016). Methods that were previously tested as the possible sprout-suppressants are cost-ineffective and eco-unfriendly (Dale and Bradshaw, 2003, Teper-Bamnlker *et al.*, 2010, El-Awady Aml *et al.*, 2014).

1.3. Rationale

The potato industry's priority is to preserve tuber quality by maintaining dormancy and delaying sprouting during postharvest storage (Alamar *et al.*, 2017). Currently, the industry relies heavily on synthetic chemicals to control sprouting, which are environmental unfriendly, cost-ineffective and complex to apply (Terry *et al.*, 2013, Caldiz, 2013). Therefore, simple, eco-friendly and cost-effective sprout-inhibiting agents are needed. Climacteric fruit such as apple have the potential to be used as biological sprouting-inhibition agent. This is due to their nature of producing ethylene gas during ripening, which has been found to be effective in inhibiting sprouting (Daniels-Lake *et al.*, 2005; Downes *et al.*, 2010; Cools *et al.*, 2011; Foukaraki *et al.*,

2016). However, there is a dearth of information on the use of climacteric fruits as biological treatment to control sprouting in potato tubers.

1.4. Aim

The main aim of this study is to establish if apple fruit could be used as a biological agent to delay sprouting development in potato tubers.

1.5. Objective

The objective of this study is to assess the effect of combined storage of apple fruit+ potatoes on dormancy period, sprout development and overall quality of potato tubers.

1.6. Null Hypothesis

The apple fruit would have no effect on dormancy period and sprout development on stored potato tubers.

1.7. Format of mini dissertation

Chapter 1 outlines the detailed description of the research problem followed by Chapter 2 which reviews the previously utilised methods on the research problem and research gap. Chapter 3 outlines the method and materials used in conducting the study followed by Chapter 4 which addresses the results and discussion of the study. Finally, in Chapter 5, the findings from every chapter are compiled and summarized to explain their relevance and offer suggestions for more study, followed by conclusions. The citation in text and listing of references were adopted according to the Harvard style as approved by the Senate of the University of Limpopo. The plagiarism policy of the University of Limpopo allows a maximum similarity index of 15%, thus; the similarity index for this research project is 14%, according to Turnitin.

CHAPTER 2 LITERATURE REVIEW

2.1. Introduction

Potato tubers are an important food source for human consumption (Burgos *et al.*, 2020; Devaux, 2020). They are highly appreciated for their nutritional quality, offers many health benefits (Teper-Bamolker *et al.*, 2010). However, their postharvest quality and shelf-life during storage are compromised due to sprouting (Teper-Bamolker *et al.*, 2010). Sprouting of potato tubers negatively affects the potato industry, especially the processing sectors (Nyankanga *et al.*, 2018). Sprouted tubers have reduced external and internal quality, with a saleable weight mostly affected and tuber appearance, reducing produce acceptability by consumers (Nyankanga *et al.*, 2018). In addition, tuber sprouting reduces processing qualities, remobilisation of starch and proteins, loss of water and tuber shrinkage (Shukla *et al.*, 2019). Therefore, for optimum utilisation of tubers, it is important to have effective sprout inhibition during storage. Several studies have been conducted on several biological and chemical methods to inhibit sprouting and to maintain quality during storage (Kleinkopf *et al.*, 2003; Afek *et al.*, 2000; Sorce *et al.*, 2005; Shukla *et al.*, 2019).

Chemical treatments such as chlorpropham (CIPC) (Terry *et al.*, 2013), ethylene gas (Mahajan *et al.*, 2008; Paul *et al.*, 2016a) and biological methods such as plant extracts (Boivin *et al.*, 2021), essential oils (Gomez-Castillo *et al.*, 2013) and edible coatings (Maftoonazad *et al.*, 2008) have been used to control sprouting. However, there has been an alarming negative impact of the application of these methods on the post-harvest quality of the produce, environment and human health. This included the accumulation of reducing sugars (Hou *et al.*, 2017) and build-up of acrylamide (Paul *et al.*, 2016b; Wiberley-Bradford and Bethke, 2017) and remains of toxic chemical residues. Therefore, the introducing environmentally safe, less costly and non-toxic sprout suppressant is important during storage of potato tubers. Thus, the aim of this chapter is to review literature on the postharvest methods of controlling potato tuber sprouting.

2.2. Overview of potato sprouting and dormancy

Potato dormancy is a physiological state and condition in which tubers respond to a series of unfavourable environmental conditions by entering a state of growth suspension (Campbell *et al.*, 2008). It consists of the endodormancy, ecodormancy

and paradormancy. Potato tubers enter endodormancy when the internal physiological factors affect the outgrowth of the bud. Ecodormancy; whereby environmental factors (e.g., light) affect the bud. Paradormancy; where the physiological factors (e.g., apical dominance) arrest the bud growth that is linked with physiological aging in potato tubers (Vreugdenhil, 2007). Potato tuber dormancy starts at the first tuber initiation and ends when the first sprout becomes visible (Aksenova *et al.*, 2013). Sprouting is described as the visible growth of meristem tissues in the potato eyes that occurs after the termination of dormancy (Teper-Bamnolker *et al.*, 2010). It is observed by white peeping buds from the potato eye (Figure 2.1) (Daniels-Lake and Prange, 2011). According to Pinhero *et al* (2009), sprouting occurs during postharvest storage after 7 days of storage in untreated tubers. Furthermore, the commencement of sprouting in potato tubers alters the potato quality leading to a deterioration in processing quality due to mass loss (Figure 2.2), decrease in nutritional value caused by sprout tissue growth and increased sugar concentration due to hydrolysis of starch (Daniel-Lakes *et al.*, 2005).

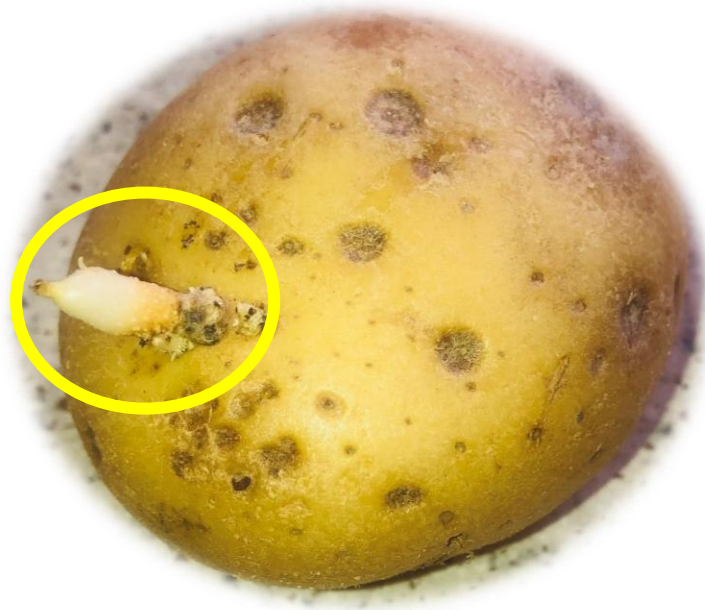


Figure 2.1: White peeping buds (sprouts) from the potato eye after 7 days of storage at ambient conditions



Figure 2.2: Potato sprouting during storage which led to mass deterioration.

2.3. Methods used to alleviate potato sprouting

Chlorpropham

Chlorpropham (CIPC) is widely used postharvest treatment for controlling potato tuber sprouting. It has been found to be an effective anti-sprouting agent due to its ability to delay mitosis in plant cells (Campbell *et al.*, 2010). However, the efficacy of CIPC to suppress sprouting is application duration dependent. For instance, Paul *et al.* (2016b) reported that the continuous application of CIPC at 18-36 g per tonne of potatoes during the storage period is able to allow the potatoes without sprouting for 5 to 12 months. Furthermore, Briddon *et al.* (2018) found that 23-25% and 5-10% of input CIPC, respectively, was retained as a residue on tubers after storage at application rates of 12- 14 g per tonne for potatoes intended for fresh market (stored at 3-4°C) and 23-26 g per tonne for potatoes intended for processing (stored at 7-9°C). Although CIPC is an efficacious anti-sprouting agent, environmental and health concerns have been raised regarding its use (Paul *et al.*, 2016; Gockener *et al.*, 2020). Furthermore, the application of CIPC as hot fog on harvested potatoes during storage causes deposition of solid residues on the potatoes thereby affecting consumer acceptability (Gouseti *et al.*, 2015). Therefore, there is ongoing research seeking an alternative to CIPC compound as it was reported to be carcinogenic to humans (Paul *et al.*, 2016).

2.3.1. Ethylene

Ethylene Production

Ethylene is a naturally occurring hormone that plays a vital role in how plants react to diverse biotic and abiotic stressors (Kumar *et al.*, 2014). The hormone is emitted abundantly during the ripening of climacteric fruit (Table 2.1) (Cherian *et al.*, 2014), leading to green colour loss (hue angle), reduced firmness and increased total soluble solids (Sun *et al.*, 2021). However, the role of ethylene in non-climacteric food like potatoes is less clear, even though it is a well-known ripening hormone. However, it is also approved as a sprout suppressant during storage (Daniels-Lake *et al.*, 2005; Downes *et al.*, 2010; Cools *et al.*, 2011; Foukaraki *et al.*, 2016).

Table 2.1: Measured levels of ethylene inside several climacteric and non-climacteric fruit

Fruit	Ethylene ($\mu\text{L/L}$)
<i>Climacteric</i>	
Apple	25–2500
Pear	80
Peach	0.9–20.7
Avocado	28.9–74.2
<i>Non-climacteric</i>	
Lemon	0.11–0.17
Lime	0.30–1.96
Orange	0.13–0.32

Source: Burg and Burg (1962).

Effect of ethylene gas on sprouting

Ethylene gas has long been identified and used as a sprout suppressant. For instance, the application of ethylene gas at $10 \mu\text{L/L}^{-1}$ in onions remarkably delayed sprouting (Cools *et al.*, 2011). Dai *et al.* (2016) reported that a higher concentration of exogenous ethylene increases the carbohydrate metabolism in potato tubers thereby inhibiting sprouting. However, the application of ethylene gas on potato tubers had an undesirable effect, such as increasing the soluble sugar accumulation (Daniels-Lake *et al.*, 2005). These sugars lead to colour darkening of fried potatoes, and acrylamide production which can be carcinogenic (Daniels-Lake *et al.*, 2005; Prange *et al.*, 2005). A study by Suttle, (2004) on potato tubers showed that the application of ethylene

decreased sprouting and increased soluble sugars accumulation. These studies indicate that ethylene is a good sprout suppressant. However, the increased carbohydrate metabolism associated with ethylene gas on tubers may limit its utilisation. Most research involved synthetic ethylene gas, with no research on the biological ethylene. Therefore, investigating the effect of biological ethylene from fruits on sprouting and sugar metabolism is warranted.

2.3.2. Plant extracts

Various plant extracts have been used as organic sprout suppressant (Boivin *et al.*, 2021). Many plant extracts were effective in delaying sprouting and maintaining postharvest quality of potato tubers (Song *et al.*, 2008; Tartoura *et al.*, 2015). The use of extract from *Azadirachta indica* delayed sprout development in potato tubers (Song *et al.*, 2008). The application of carvon naturally found in *Mentha* essential oil has also been reported to be an effective sprout suppressant during storage (Farooqi, 2001). However, according to Giri *et al.* (2020), *Mentha* essential oil causes damage to the vascular tissue resulting in necrosis of the emerging bud while inhibiting sprouting, leaving black necrotic symptoms in potato tubers. The application of black spruce (*Picea mariana*) extracts reduced sprouting of 'colomba' potato (Boivin *et al.*, 2021). However, these plant extracts required continuous application for their efficacy to suppress undesirable sprouting and damages of potato tubers during storage.

2.3.3. Essential oils

Essential oils (EOs) are safe sprout suppressant developed to reduce synthetic chemical use during postharvest storage of potato tubers (Finger *et al.*, 2018). The EOs can also reduce decay in a stored potato tuber (Abbasi *et al.*, 2015). The application of natural EOs such as garlic, coriander, spearmint and peppermint are utilised as acceptable alternate sprout inhibitors with low toxicity to humans and minimum adverse effects on potato tubers (Alamar *et al.*, 2017). For instance, thermal fogging with spearmint (*Mentha spicata* L.) essential oil has been globally utilised as a commercial anti-sprouting treatment in postharvest potato storage (Teper-Bamnolker *et al.*, 2010).

In a study comparing mint essential oil and synthetic carvone, Teper-Bamnolker *et al.* (2010) discovered that mint essential oil inhibited sprouting for up to 6 months of

storage. They also discovered that 73% of the carvone was present in the mint essential oil. Finally, they noted that the effects of synthetic carvone at a concentration of 4 µl/l were comparable to those of mint. Additionally, compared to CIPC, caraway and dill oils significantly reduced the likelihood of mass loss and sprouting under 15°C, according Şanlı and Karadoğan (2019). Caraway was said to prevent sprouting for 180 days or more, whereas dill oils reduced sprouting for 135 days. Gomez-Castillo *et al* (2013) reported that the treatment of coriander essential oil on 'Kennebec' potato resulted in soft watery rotting potato tubers. The same authors also reported that eucalyptus oil induced rotting and white moulds in 'Agrida' cultivar. This indicates that the efficacy of the essential oils is cultivar dependent. Also, essential oil from coriander led to strong rotten odour in the tuber (Gomez-Castillo *et al.*, 2013). Numerous essential oils have been discovered to prevent infections and to sprout in potatoes that have been preserved. But the main drawback of using these oils is their chemical instability and volatility in the presence of air, light, moisture, and high temperatures, which can cause some active components to rapidly evaporate and degrade (Owolabi *et al.*, 2010). Therefore, it is preferable to manage how these formulations are released so that after a single application, a gradual evaporation of the active ingredient can start early in the storage and last for the needed amount of time.

2.3.4. Edible coatings

Edible coatings are soluble formulations applied directly on the produce surface to maintain and extend their postharvest life (Bourtoom, 2008; de Azeredo, 2012). Edible coatings improve food appearance while at the same time providing safety to the food by its environmentally friendly nature (Sharma *et al.*, 2019). Recent studies have demonstrated that edible coatings reduce respiration rate, thereby limiting mass loss and extending produce shelf life of the treated produce (Hasan *et al.*, 2021; Jodhani *et al.*, 2021). Maftoonazad *et al* (2008) reported that edible coatings (sodium alginate and methyl cellulose) had been used to improve moisture and gas barriers, microbial protection and mechanical properties and extend the shelf life of some produce such as peaches. It has also been reported that chitosan-based coating reduced weight loss and maintained firmness in stored potato tubers (Saha *et al.*, 2014). Also, Karanisa *et al.*, (2019), reported that paraffin wax coating delayed potato tuber sprouting and extended tuber dormancy at 15°C. However, these edible coatings have

limited effect on potato quality during storage as they increased accumulation of reducing sugars (Maftoonazad *et al.*, 2008).

2.4. CONCLUSION AND FUTURE PERSPECTIVES

Sprouting is a major postharvest problem in potato tubers, affecting their shelf life and purchase pattern. This review is aimed to critically describe chemical and biological methods used to control sprouting in potato tubers. Chemical methods such as CIPC and ethylene gas are very good sprout suppressant. However, their use is limited due to their residual effect and ability to induce sweetening. Biological methods such as plant extracts and edible coatings can serve as an alternative to chemical methods. However, research is still required to develop proper treatment protocols due to their volatility, they can be cost-ineffective as continuous application will be required to be efficacious. Therefore, an alternative is needed to reduce the application levels of agrochemicals and to rather increase the reliance on essential oils but to instead emphasis on the development of biological sprout suppressant agents that do not pose potential hazard to human livelihoods and are less costly.

CHAPTER 3

MATERIALS AND METHODS

3.1. Experimental sites and plant materials

Fresh uniform potato tubers (*cv. Mondial*) without sprout and visible defects were obtained from four commercial farms namely; Elmar, Jamba, Leeubult and Solly boerdery, located in Indermark (23°.04,14" S; 29°.07,10" E), Dendron (23° 19"49.01" S; 29° 19"01.39" E), Bandelierkop (23° 20"34.64" S; 29° 48"24.09" E) and Bochum (23°.19, 11" S; 29°.07,17" E), respectively in Limpopo Province. The tubers were thereafter transported to Plant Production Laboratory (23° 88"06" S; 29° 73"39" E), University of Limpopo (Mankweng, Limpopo Province, South Africa).

3.2. Experimental design, treatments and procedure

The study was carried out in a 2x3 factorial experiment, arranged in a completely randomised design. Factors included tubers stored with apple fruit (Granny smith) at two levels (tubers stored alone or stored with four apple fruit) and storage duration at three levels (0, 15 or 30-days). The treatments were replicated seven times.

A total of 896 potato tubers were used in the study. Each location comprised of 224 potato tubers, with 112 tubers per treatment. Sixteen tubers were stored with 4 apples or alone (control) in a brown paper bag at ambient temperature ($\pm 23^{\circ}\text{C}$) to simulate supermarket conditions. Only 4 apples per bag were used to ensure consistent space in all the bags throughout the experiment. During storage, ten potato tubers per replicate were used for non-destructive assay (mass, sprouting and decay), whilst 6 tubers were used for destructive assay (dry matter, sucrose, fructose and glucose) at 15-day intervals for 30 days.

3.3. Data collection

3.3.1. Mass loss

The mass of each tuber was measured using a weighing scale (Model: HBC1002, Adam Equipment, South Africa) at a 15-day interval. The tuber mass loss was determined using equation 1 (Rezaee *et al.*, 2013)

$$\text{Mass loss (\%)} = \frac{\text{Initial mass} - \text{Final mass after storage}}{\text{Initial mass}} \times 100 \dots\dots\dots (1)$$

3.3.2. Sprouting percentage

Tubers with ≥ 3 mm sprout were considered having sprouted (Gomez-Castillo *et al.*, 2013). The number of sprouted tubers were divided by the total number of tubers stored and multiplied by 100%. Thereafter, the sprouting percentage was determined according to equation 2 (Njogu *et al.*, 2015):

$$\text{Sprouting (\%)} = \frac{\text{No of sprouted tubers}}{\text{Total No of tubers}} \times 100 \dots\dots\dots (2)$$

3.3.3. Dry matter

Potato tubers were peeled and sliced to approximately 50 g and oven dried at 65° C until constant mass. Thereafter, the dry matter was determined according to equation 3 (Maraphum *et al.*, 2021):

$$\text{Dry matter (\%)} = \frac{\text{Final weight}}{\text{Initial Weight}} \times 100 \dots\dots\dots (3)$$

3.3.4. Soluble sugars

The soluble sugars were determined according to the methodology described by Ngobese *et al.* (2017). Briefly, 10 mL of 70 % ethanol was added to 0.5 g of fresh potato flesh. The samples were submerged in an 80° C water bath for 1 hr 30 minutes with occasional agitation using a vortex and left to cool overnight at 4° C. Thereafter, the supernatant was evaporated at 60° C using the oven (Model: 279, Ecotherm, Labotec, South Africa). The evaporated samples were re-suspended in 2 mL of HPLC water and then put into glass vials for high performance liquid chromatography (HPLC) analysis after being filtered by 0.45 m syringe nylon filters. A HPLC binary pump with a refractive index detector was used to measure the concentrations of sucrose, fructose, and glucose. The extracts were injected into a Rezex™ RHM-monosaccharide H+ (8 %) column of 7.8 mm diameter \times 300 mm in length. Fluoride distilled water was used as mobile phase and analysis was performed at a flow rate of 0.6 μ L/min and column temperature of 80° C. The presence and concentration of individual samples were calculated using the peak area of samples against those of fructose (0.5-3 mg/mL; $Y = 111409x - 10693$; $R^2 = 0.99$), Sucrose (0.5-3 mg/mL; $Y =$

37432x + 3313.4; R² = 0.99) and glucose (0.5-3 mg/mL; Y= 79323x + 1143.3; R²= 0.99) standard concentration curves and expressed in mg/mL fresh mass basis.

3.3.5. Starch content

The starch content was estimated using a regression equation 4 (Haque *et al.*, 2018):

Where Y=% starch content; X= % dry matter content

3.3.6. Decay incidence

Potato tubers showing brown copper lesions or white sporulation on the tuber surface during storage were considered as decayed. The decay percentage was calculated according to equation 5 (Ma *et al.*, 2016):

$$\text{Decay (\%)} = \frac{\text{No of decayed Tubers}}{\text{Total No of Tubers}} \times 100 \dots\dots\dots (5)$$

3.3.7. Apple fruit ripening parameters

To determine whether apple fruit (Granny Smith) were ripening and emitting sprout suppressant, ethylene gas (Cherian *et al.*, 2014), fruit firmness, mass loss, total soluble solids and colour (Hue and chroma) were determined at 7-day interval during storage for 2 weeks. Briefly, firmness was measured using a penetrometer (fruit pressure tester, FT 327) and results were expressed in Newton (N). Total soluble solids were measured using a handheld refractometer (Master-T, Model: 0229305). Hue (h*) and Chroma (c*) were measured using a Chroma meter (CR-400, Minolta Corp. Osaka, Japan). Mass loss was calculated according to equation 1.

3.4. Statistical analysis

Two-way analysis of variance (ANOVA) was used to determine whether apple fruit had a significant effect on dormancy period and sprout development in potato tubers during storage. The significant treatment means were achieved through least significant difference (LSD) at 5% level of significance. Statistical analysis was performed using GenStat, version 21th, VSN International, UK.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Effect of apple treatment on sprouting of potato tubers during storage

Sprouting differed significantly ($P < 0.05$) on tubers stored with apple fruit compared to the control amongst the three production sites (Elmar, Solly and Jamba) (Figure 4.1-4.3). Tubers obtained from Leeubult did not differ ($P > 0.05$) according to the analysis of variance hence means separation by LSD was not performed and the results were not illustrated. Tubers harvested from Elmar sprouted at day 30 with apple treatment significantly ($P < 0.05$) reducing the sprouting percentage (4%). Tubers from Jamba boerdery started sprouting at 15 days in control and apple treatment. However, tubers stored with apple fruits had significantly reduced sprouting (45%) compared with the control (70%). The same trend was observed for tubers harvested from Solly boerdery. However, at 30 days, tubers stored with apple fruit had significantly higher sprouting percentage (90%) compared to the control (80%). These results indicate that storing apple fruits with potato tuber delay sprouting and were consistent throughout the three production sites, except Leeubult. Climacteric fruit like apples emit ethylene during ripening at storage (Sun *et al.*, 2021), therefore ethylene might have diffused into tubers and delayed sprouting. The effect of ethylene on sprouting was observed in potato tubers treated with synthetic ethylene gas (Daniels-Lake *et al.*, 2005; Shi *et al.*, 2018). Prange *et al.* (2005) showed that short-term application of ethylene (72 h at concentration of 0.02- 20 μL) induced dormancy while continuous ethylene application (2 μL for 6 months) inhibited sprouting completely. In the present study, the complete inhibition of sprouting was not observed, probably because apples were removed 14 days after storage (short application) as they started rotting. Nevertheless, small sprouts are usually an inconsequential issue in the commercial processing industries, unlike large sprouts (>50 mm), which are undesirable (Daniels-Lake *et al.*, 2005). The mechanism of how ethylene gas delays sprouting is unclear. However, it was postulated by Shi *et al.* (2018) that potato tubers should be exposed to ethylene continuously during storage to prolong the storage life of potatoes by suppressing sprouting.



Figure 4.1: Mondial tubers stored alone at day 9 of storage showing sprouts at ambient temperature.



Figure 4.2: Mondial Tubers stored with apple fruit showing reduced sprouting on day 9 of storage at ambient temperature

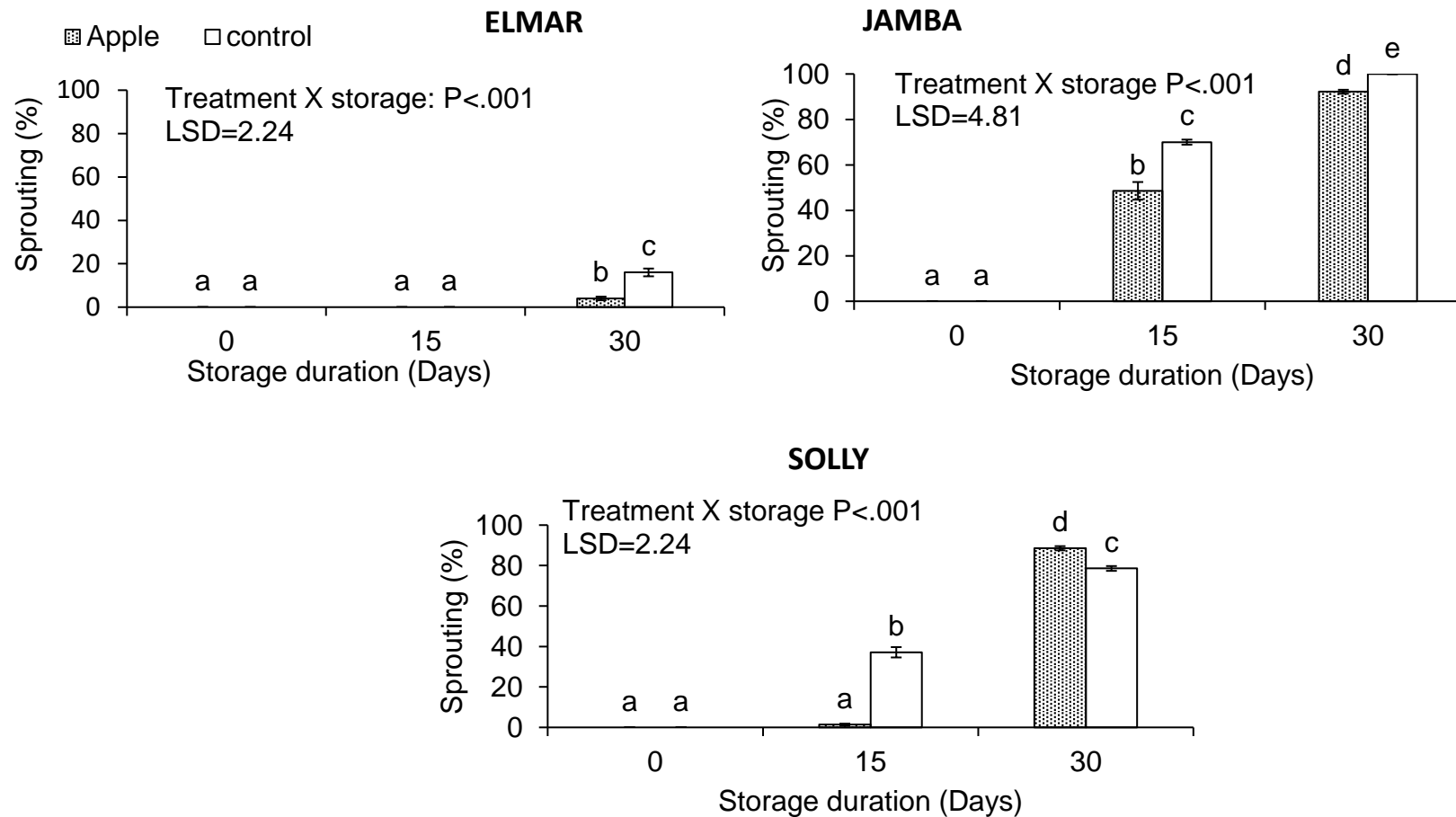


Figure 4.3: Effect of apple treatment on tuber sprouting percentage in Mondial potatoes stored under ambient temperatures for 30 days. Different letters indicate the statistical significance difference ($P \leq 0.05$) among treatments during storage duration.

4.2. Effect of apple treatment on mass loss of potato tubers during storage

Mass loss was significantly ($P < 0.05$) reduced in tubers stored with apple fruit compared to the control treatment (Figure 4.4). The tubers stored alone significantly lost 2.5% mass at 15 days storage compared to apple treatment (2%) from Jamba. Tubers stored alone from Solly showed a significantly higher mass loss (4.02%) as compared to the apple treatment potato tubers (1.269%). The same trend was observed in tubers harvested from Leeubult. However, in tubers from Elmar, apple treatment increased mass loss (4.85%) compared to the control (2.43%).

Sprouting increases physiological mass loss in tubers (Azad *et al.*, 2017; Paul *et al.*, 2016a). Furthermore, the commencement of sprouting in potato tubers alters the potato quality leading to a deterioration in processing quality due to mass loss, decrease in nutritional value caused by sprout tissue growth and increased sugar concentration due to starch hydrolysis (Daniel-Lakes *et al.*, 2005). Present study also observed this, whereby mass loss increased with sprouting percentage. Our findings support the observations made by Paul *et al.* (2016a) and de Freitas *et al.* (2012) that sprouting is one of the main issues that contribute to postharvest losses of potato tubers and that the presence of sprouting in potato tubers is related to the breakdown of starch components and rise of reducing sugars, which are required to provide carbon and energy for sprout growth and development (Paul *et al.*, 2016b; Saran and Chhabra, 2014). In the present study, apple fruit reduced the sprout growth as compared to the control, thereby reducing the respiration rate responsible for causing a gradual increase in mass loss.

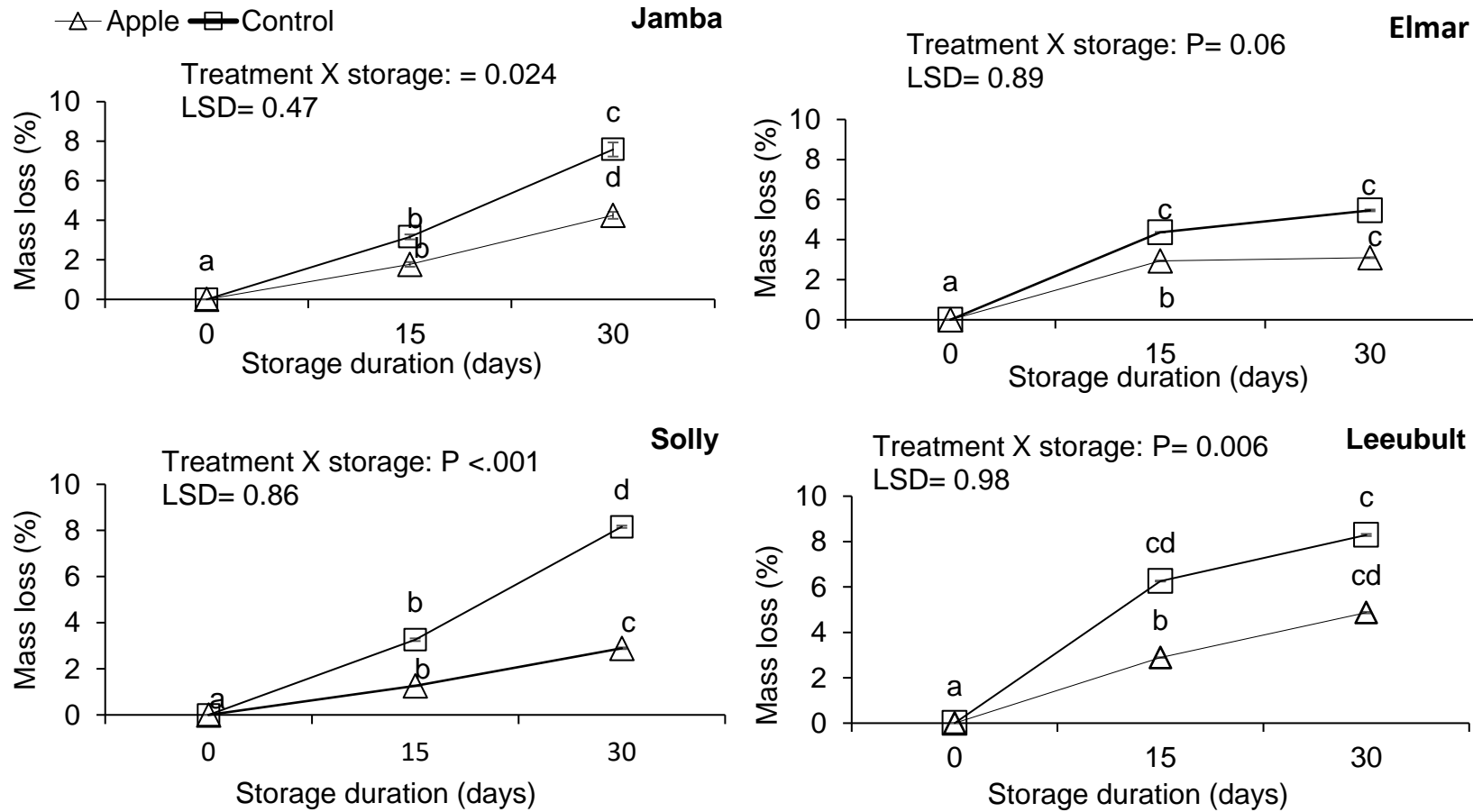


Figure 4.4: Effect of apple treatment on mass loss percentage of Mondial potatoes stored for 30-days at ambient temperature. Tubers were obtained from Jamba, Elmar, Leeubult and Solly's Boedery farm. The vertical bars represent standard error (n=7). Different letters indicate the statistical significance difference ($P < 0.05$) among treatments during storage duration ($P \leq 0.05$).

4.3. Effect of apple treatment on decay of potato tubers during storage

Table 4.1 illustrates the effect of storing potato tubers with apple fruit on decay incidence. Potato tubers started showing signs of decay at 15-day storage in both control and the apple treatment. This decay incidence was only observed in tubers harvested from Jamba and Leeubult because the apples started rotting which as a result affect the tubers stored with the fruits. For both production sites, decay incidence was significantly ($P < 0.05$) higher in tubers stored with apple fruit compared to the control. It is unclear how apples induce decay in the potato tubers. However, the apples started rotting at 15 days storage and were removed. This might have caused the tubers to decay. Arancibia *et al* (2013) also found that ethephon (ethylene) increased the incidence of root tip rot in sweet potato. Therefore, further studies could determine whether ethylene induces potato decay and the mechanistic mode of action. It could be that apples during ripening emit substrates that are used by microorganisms for proliferation. Nevertheless, our results suggest that this decay is production sites dependent, as tubers from other locations were not affected. Therefore, cultural practices in Jamba and Leeubult boerderies might have played a role in the susceptibility of the tubers to postharvest decay (Sinha *et al.*, 2018)

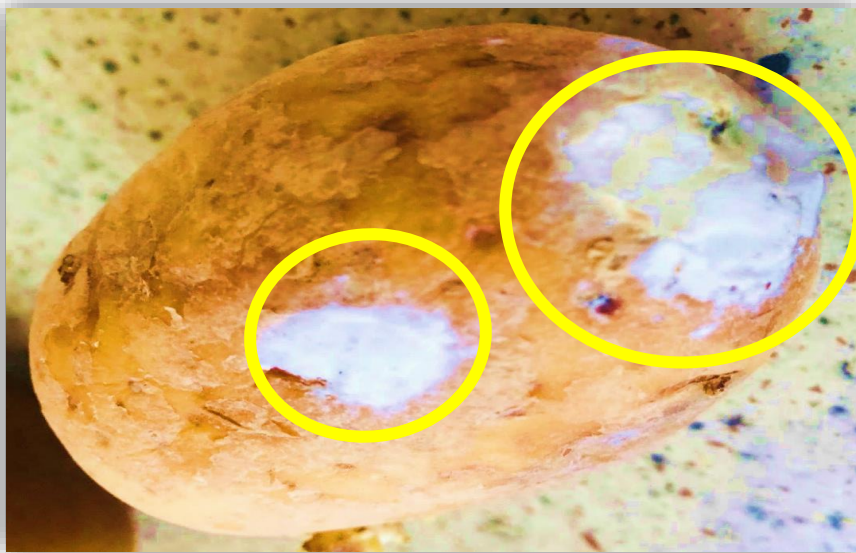


Figure 4.5: Mondial potato showing white sporulation during storage at ambient temperature.



Figure 4.6: Mondial potatoes showing Tubers brown copper lesions during storage at ambient temperatures.

Table 4.1: Effect of apple treatment on decay incidence of Mondial potato tubers during 30-day ambient storage

Location		Storage duration			P-value	LSD _{0.05}
		0	15	30		
Jamba	Control	0 ± 0 ^a	0 ± 0 ^a	21.67 ± 2.54 ^b	<.001	3.03
	Apple	0 ± 0 ^a	0 ± 0 ^a	47.85 ± 1.32 ^c		
Leeubult	Control	0 ± 0 ^{ab}	1.44 ± 0.42 ^{ab}	7.59 ± 1.05 ^c	<.001	1.29
	Apple	0 ± 0 ^{ab}	1.43 ± 0.42 ^{ab}	17.29 ± 1.05 ^a		
Elmar	Control	<i>ns</i>	<i>ns</i>	<i>ns</i>		
	Apple	<i>ns</i>	<i>ns</i>	<i>ns</i>		
Solly	Control	<i>ns</i>	<i>ns</i>	<i>ns</i>		
	Apple	<i>ns</i>	<i>ns</i>	<i>ns</i>		

Different letters indicate the statistical significance difference among treatments during storage duration ($P \leq 0.05$). Each value is a mean of replicates ($n=7$) \pm standard error.

4.4. Effect of apple treatment on dry matter content during storage

The dry matter content was significantly ($P < 0.05$) lower in tubers stored with apples throughout the 30-day duration compared to the control. This effect was observed in tubers harvested from Jamba and Solly boerdery, with Leeubult and Elmar tubers having higher dry matter content (Figure 4.7). Potato tubers showing high dry matter content are considered more appropriate for processing industry (Silveria *et al.*, 2017). According to Chope and Terry (2008), the high dry matter is due to delays in sprout growth that might have also delayed the breakdown and catabolism of sugars resulting in high dry matter content as fructose, glucose, and sucrose accounts for over 65 % of dry matter content. Evidently, apple treatment delayed sprout growth during storage; this is seen by high dry matter content in tubers stored with apple fruits compared to the control. Azad *et al.*, (2017) study demonstrated a high dry matter content during storage. Downes *et al.*, (2010) also observed the results in onion bulbs, who reported that high dry matter has been linked to increased storability.

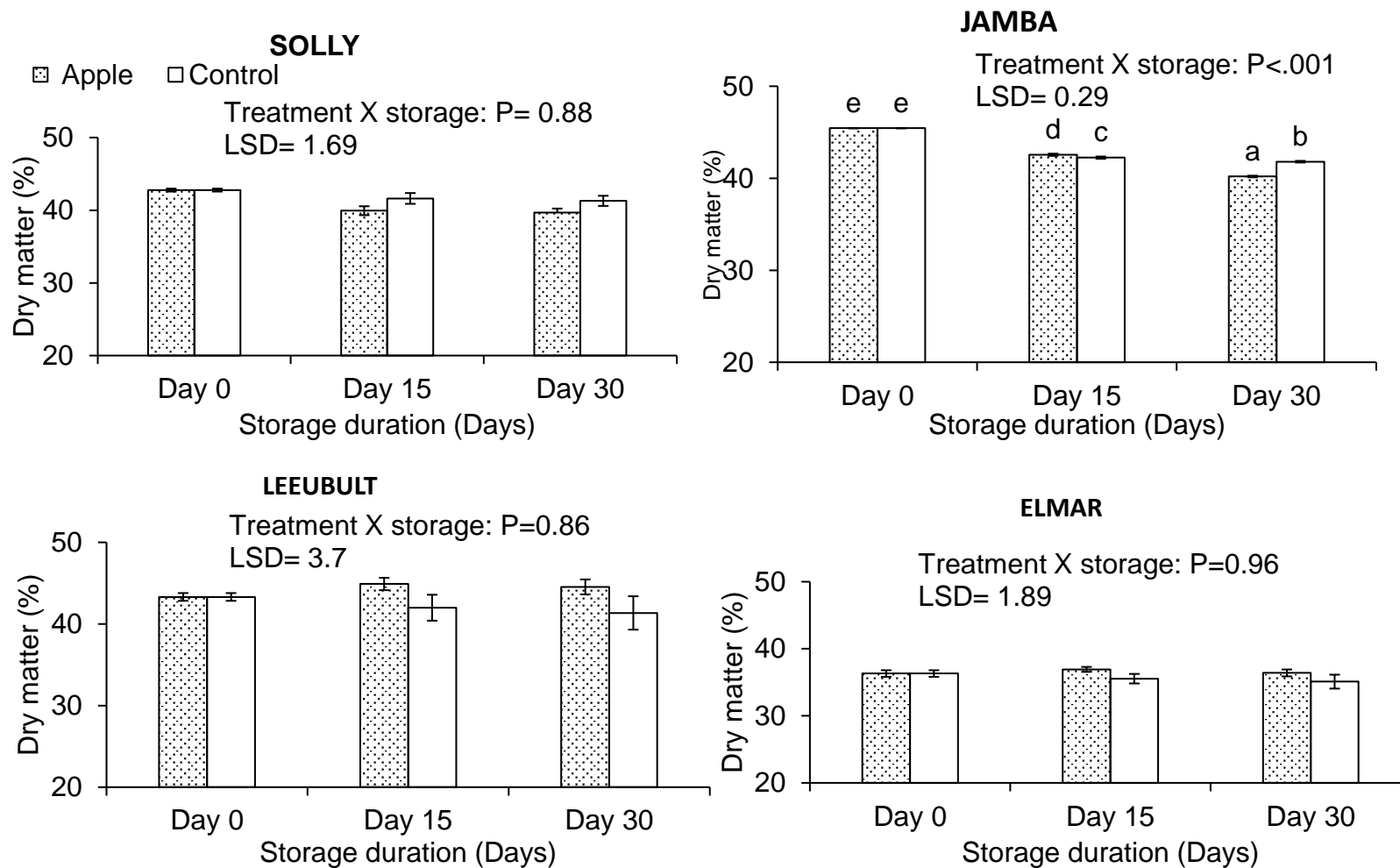


Figure 4.7: Effect of apple treatment on dry matter (%) of potato tubers from Jamba, Solly, Leeubult and Elmar Boerdery stored for 30 days. The vertical bar means represent standard error (n=7). Different letters indicate statistical significance difference among treatments during storage duration.

4.5. Effect of apple treatment on starch content of potato tubers during storage

Potato tubers stored with apples had significantly ($P < 0.01$) lower starch content compared to the tubers stored alone during ambient storage. This effect was observed in tubers harvested from Jamba boerdery. In tubers from the other production sites, there was no significant difference ($P > 0.05$) between tubers stored alone and with apple fruit on starch content (Figure 4.8). Starch constitutes 65 to 80% of the total dry weight of the potato tuber. However, sprouting leads to remobilisation of starch and proteins (Börnke *et al.*, 2007). Furthermore, sprouting in potatoes enhances the starch hydrolysis into reducing sugars (Shukla *et al.*, 2019). In the present study, starch content decreased with increased sprouting in tubers stored alone as compared to apple treatment (Figure 4.8). This might be due to the hydrolysis of starch-by starch degrading enzymes (e.g., alpha-amylase). According to Hajirezaei *et al* (2003), starch degradation is related to the induction of sprouting, and it is a prerequisite for the initiation of sprouting. Therefore, apples reduced sprouting compared to tubers stored alone, resulting in lower starch levels due to starch remobilization. Thammawong and Arakawa (2007) reported that treating fruits (eg., onion, sweet potato) with ethylene gas reduced starch content, and increased respiration rate, suggesting that during sprouting, carbohydrate metabolism increases. It can therefore be inferred that apple fruit retards starch degradation, thus reducing sprouting in potato tubers (Figure 4.3).

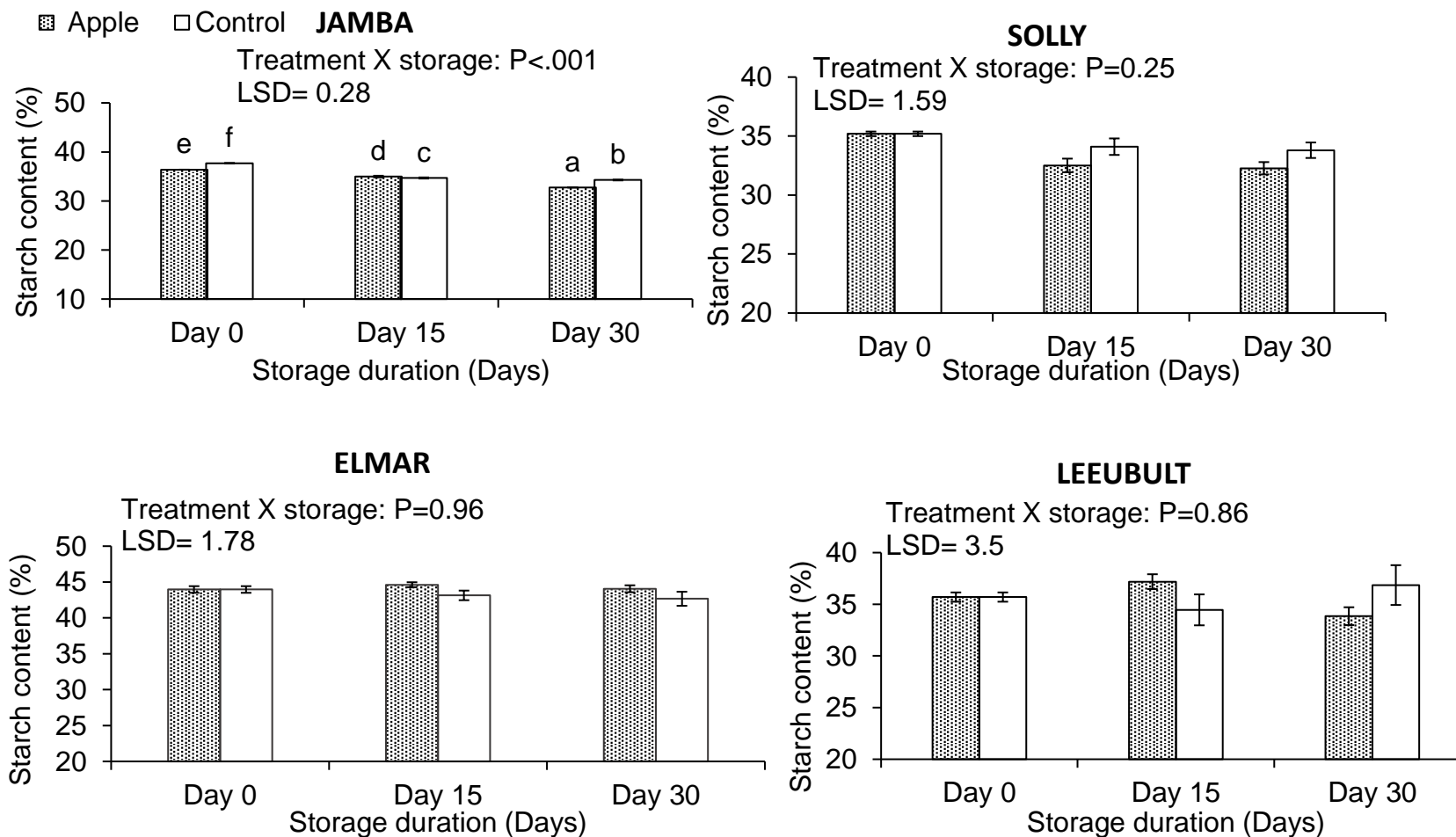


Figure 4.8: Effect of apple treatment on starch content (%) of potato tubes stored for 30 days at ambient conditions. The vertical bars represent standard error (n=7). Different letters indicate statistical significance difference among treatments during storage duration.

4.6. Effect of apple treatment on soluble sugars

Potato tubers stored with apple fruit had significantly ($P < 0.05$) reduced fructose (Figure 4.9) content in tubers harvested from Elmar. Furthermore, the fructose content in tubers stored apple fruits were not significantly ($P > 0.05$) affected by apple fruits in tubers harvested from Jamba, Leeubult and Solly. The sucrose and glucose content (Figure 4.10-4.11) in tubers from other production sites except Jamba were not significantly affected by apple fruit compared to the control ($P > 0.05$). As opposed to the control, which had a high glucose content, the overall results also revealed a quick reduction in glucose content during storage in apple treatment, especially when sprouting commenced (Figure 4.9). The application of apple treatment as a sprout suppressant resulted in reduced sugar concentration compared to the control in tubers obtained from Leeubult, Elmar and Solly. These results suggest that storing potato tubers with apple fruit does not induce sweetening. The changes in sugar content observed in this study also varied according to location. This is because the tubers were grown on different locations. According to Kyriacou *et al.* (2009), tuber sugar accumulation can be affected by crop management.

According to Daniels-Lake *et al.* (2005), the application of ethylene gas on potato tubers had an undesirable effect, such as increasing the soluble sugar accumulation. Furthermore, Tosetti *et al.* (2021), also confirmed that ethylene supplementation suppresses postharvest sprouting but increases reducing sugars. These sugars lead to colour darkening of fried potatoes, and acrylamide production which can be carcinogenic (Daniels-Lake *et al.*, 2005; Prange *et al.*, 2005). These studies indicated that ethylene is a good sprout suppressant. However, the increased carbohydrates metabolism associated with ethylene gas on tubers has limited its utilisation. Most research involved synthetic ethylene gas, with no research on the biological ethylene hence the study aimed at using apple fruit as a biological ethylene agent. In this study, apple treatment as a biological ethylene agent maintained lower soluble sugars in tubers during storage, with reduced sprouting. It was also observed by Cools *et al.*, (2011) that the levels of soluble sugars in onion were decreased by ethylene treatment. This is significant for the processing industry as they need potatoes with low sugar content to avoid the browning of fries.

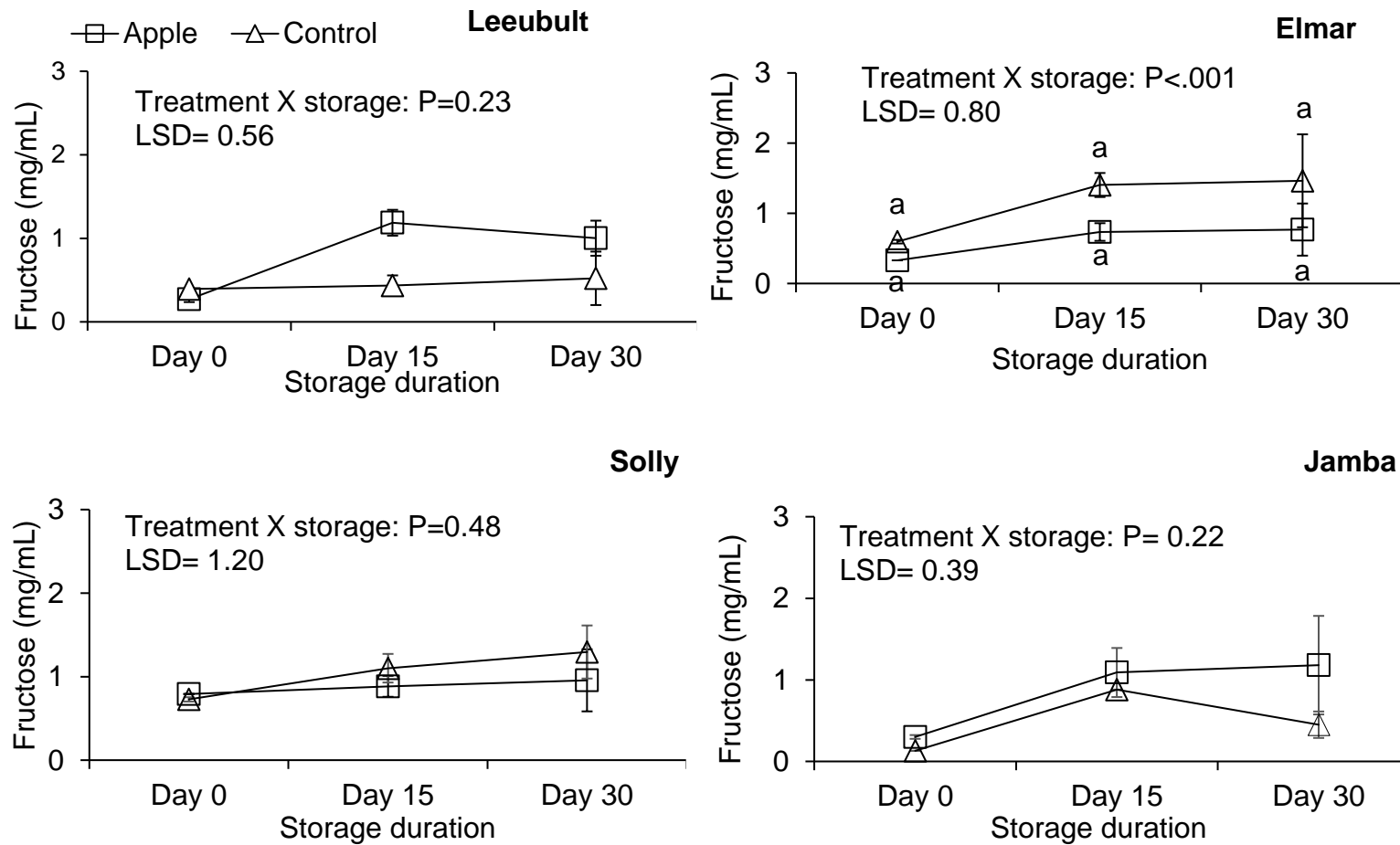


Figure 4.9: Effect of apple treatment on fructose content of Mondial potato tubers stored for 30 days under ambient temperatures. Different letters indicate the statistical significance difference among treatments during storage duration ($P\leq 0.05$) and means with the same letters do not vary from each other.

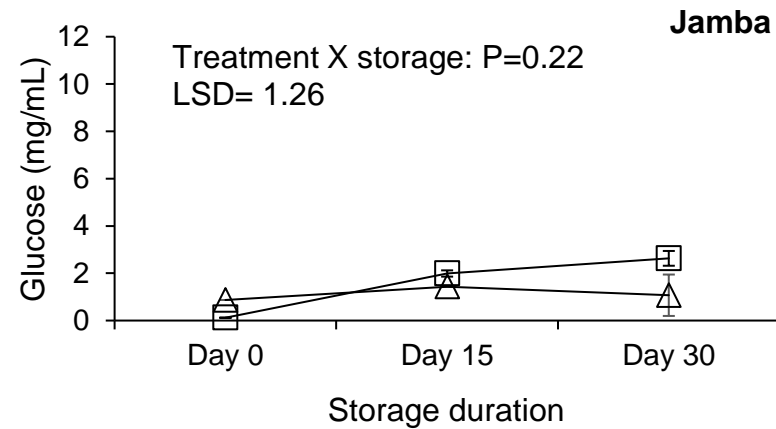
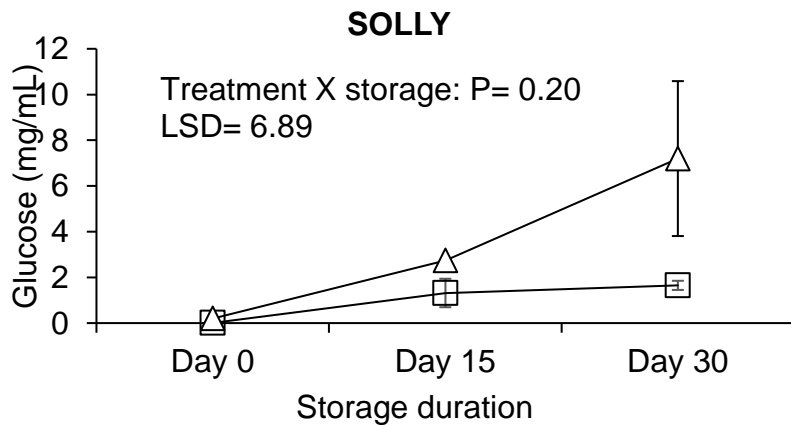
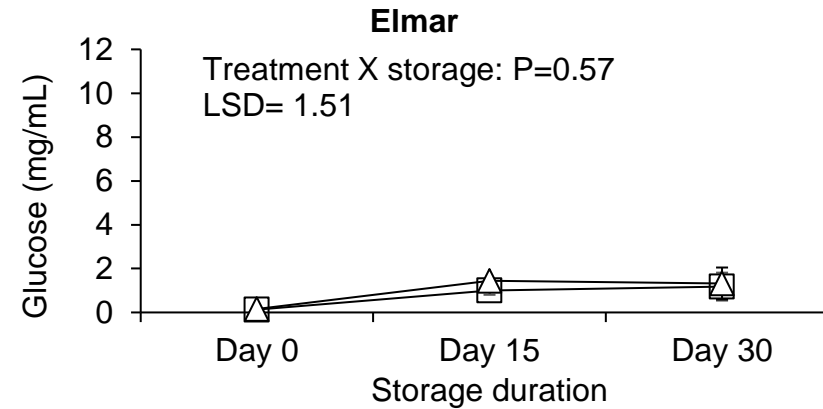
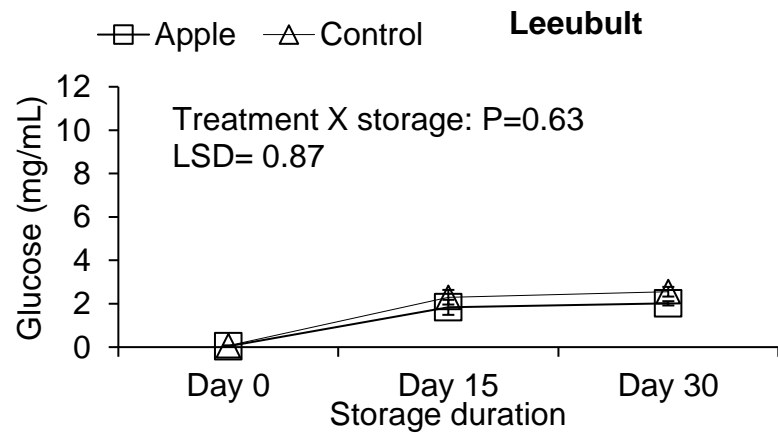


Figure 4.10: Effect of apple treatment on glucose content of Mondial potato tubers stored for 30 days at ambient temperatures. Different letters indicate the statistical significance difference among treatments during storage duration ($P \leq 0.05$) and means with the same letters do not vary from each other.

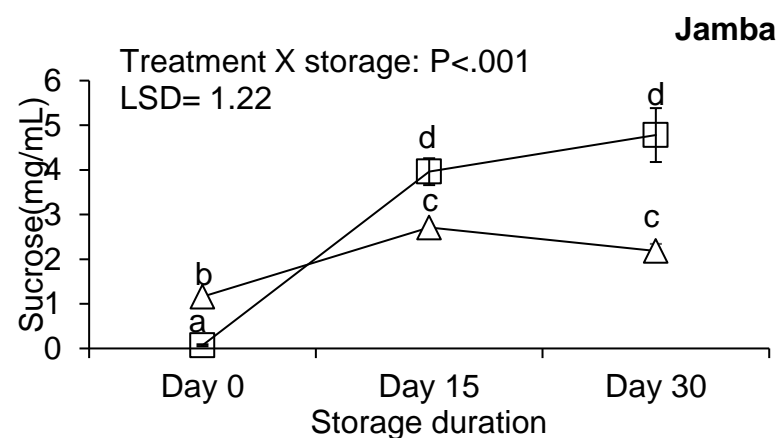
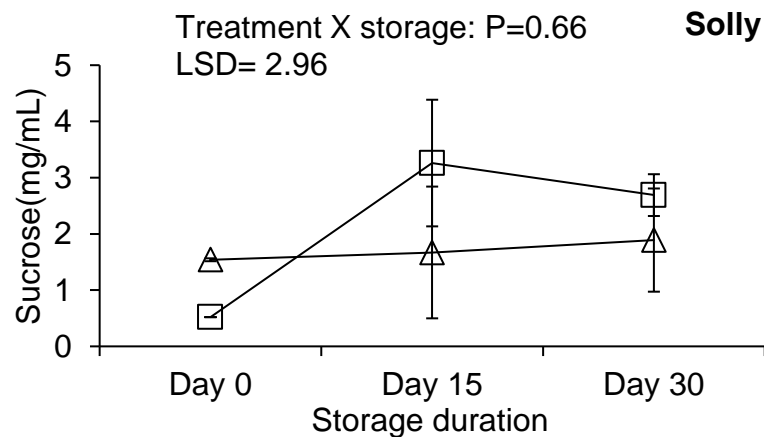
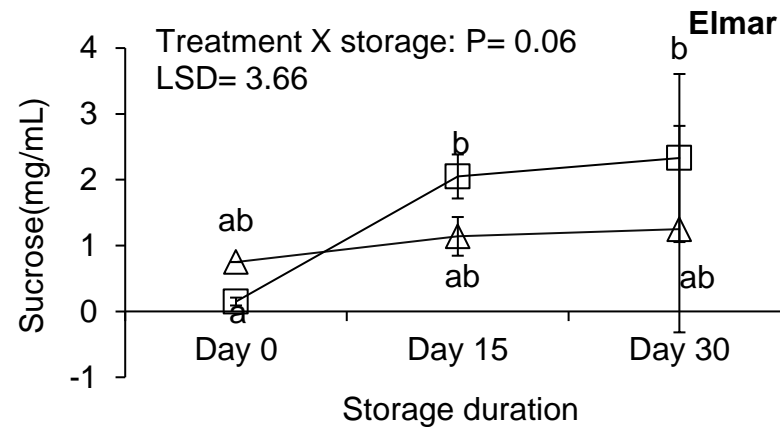
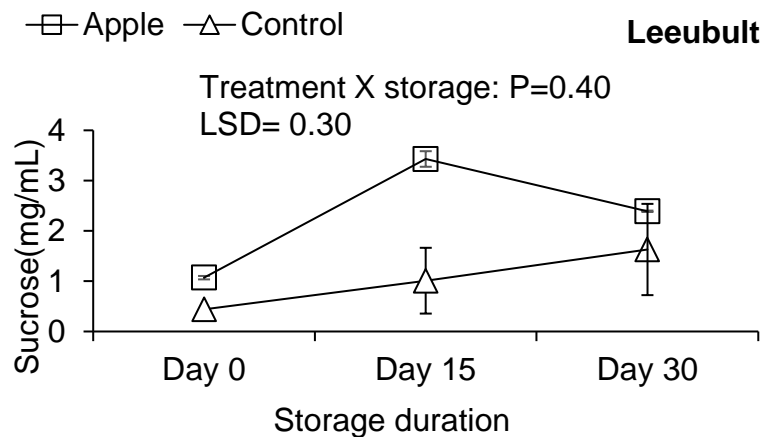


Figure 4.11: Effect of apple treatment on sucrose content of Mondial potato tubers stored for 30 days at ambient temperatures. Different letters indicate the statistical significance difference among treatments during storage duration ($p < 0.05$) and means with the same letters do not vary from each other.

4.7. Peel colour representing the ethylene (C₂H₄) emission in apple fruit during storage

Ethylene is produced in large quantities as climacteric fruit ripens (Cherian *et al.*, 2014), which speeds up the process of ripening since it is essential for the breakdown of chlorophyll, maintaining fruit firmness, and softening (Adams and Brown, 2007). Apples are considered climacteric fruits because they typically exhibit a spike in ethylene production and increased respiration during ripening. This therefore leads to changes in peel color (hue angle), a decrease in firmness, a decrease in mass loss. Furthermore, ripening of apple fruit also causes an increase in total soluble solids (TSS), a reduction in acidity, and the formation of volatile compounds. Generally, ethylene emission typically rises as the hue angle declines. The apple fruit in the study demonstrated a decline in hue angle (Figure 4.12), which was used to quantify apple ripening during storage and to find ethylene emission. This is in line with a study paved by Sun *et al* (2021) who indicated that a decline in hue angle results in an increase in ethylene production.

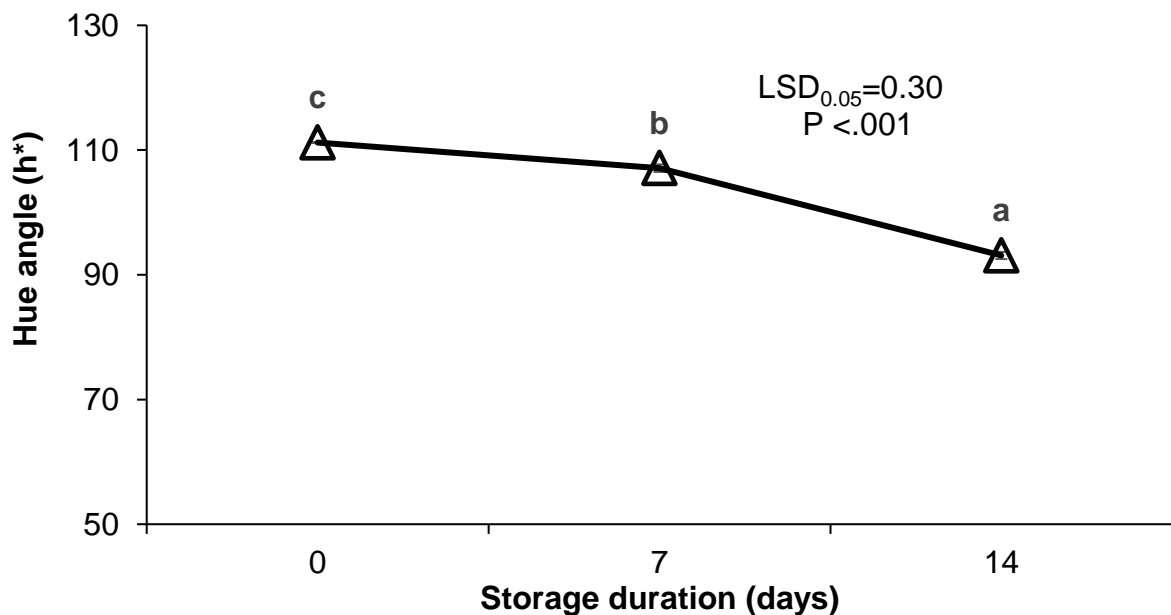


Figure 4.12: Peel colour (hue angle) representing the ethylene (C₂H₄) emission in apple fruit during storage. Means with different letter (s) are significantly different by LSD test at $P \leq 0.05$. And means with the same letters do not vary from other.

CHAPTER 5

SUMMARY, SIGNIFICANCE, RECOMMENDATIONS AND CONCLUSION

5.1. Summary

The study was carried out to determine the effect of apple fruit on sprouting incidence of potato tubers. The treatment was applied for 30 consecutive days and sprouting, mass, decay, soluble sugars, starch, and dry matter content were measured at 15-day intervals. The results showed that storing potato tubers with apples was an effective postharvest technique to delay sprout development. Tubers stored with apples had significantly ($P < 0.05$) reduced mass loss and did not increase soluble sugars during storage. Moreover, tubers stored with apple fruit had significantly reduced sprouting compared with the control. Throughout the storage duration, potato tubers stored with apple fruit had significantly ($P < 0.05$) high dry matter and reduced starch content compared to the control treatment. Furthermore, decay percentage did not differ significantly ($P > 0.05$) between tubers stored alone or with apple fruit while reducing sugars significantly ($P < 0.05$) increased with an increasing storage duration in untreated tubers as compared to apple treatment. Therefore, the apple fruit could prolong the tubers' dormancy period.

5.2. Significance of findings

The present study's findings provide important information on the use of apples as a possible biological sprouting-inhibiting agent, which is environmentally acceptable and non-toxic. Furthermore, this will help reduce the postharvest loss of potato tubers and thereby extend the shelf life, increasing market value and consumer acceptability of the potato tubers.

5.3. Conclusion

There was a reduced sprouting percentage during the tubers' storage with apple fruit compared to the control. Moreover, the study showed that dry matter content, sprouting rate and mass loss of potato tubers increased with increasing storage

duration. Furthermore, there was a significantly reduced sucrose, glucose, and fructose with increased in storage duration. The study revealed that sugar content varied according to production sites. The study also revealed significant effect of apple fruits treatment as biological sprout controlling agent for two weeks but not for promoting sprouting. This method can therefore be a promising practice for the potato processing industry in maintaining post-harvest quality of tubers during storage and for farmers following traditional and modern storage method of preserving potato quality and shelf-life during storage. This method could be adopted as a safer and environmentally friendly alternative to synthetic ethylene gas and various chemicals used to control sprouting of potato tubers during storage.

5.4. Recommended future studies

Apple fruit were stored for a short time with potato tubers with potato tubers. Therefore, future studies have to look on storing apple fruit with potato tubers for longer periods. Furthermore, in order know and recommend the required apple-supplied ethylene concentration for inhibiting sprouting, it is necessary to determine the amount of ethylene emitted by the apple fruit during storage. To achieve this, it might be necessary to develop suitable protocols for ethylene measurements during uninterrupted potato tuber storage.

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