KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF SCIENCE

DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY

TOPIC:

ASSESSING THE CHANGES IN QUALITY OF IMPORTED APPLES (*MALUS PUMILA*) ALONG THE POSTHARVEST IMPORT CHAIN

A THESIS SUBMITED TO THE DEPARTMENT OF FOOD SCIENCE AND TECHNOLOGY IN PARTIAL FULFILMENT OF REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN FOOD QUALITY MANAGEMENT

BY

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APRIL 2018

DECLARATION

I declare that this submission is the result of my own research and thus does not contain any previously published material except for some information which the source for each one has been stated clearly.

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DEDICATION

I dedicate this work to some close friends who have been my backbone in this accomplishment. First to Ellikplim Kwawu Anaglate; thanks for pushing me to start this Master's program. I do appreciate the support you gave me on every side too. Secondly to Daniel Apau Osafo and Richard Yinbil Namalteng. Thanks for pushing me to finish this Master's program. I appreciate all the cover ups and great ideas shared. God bless you all.

ACKNOWLEDGEMENTS

My foremost gratitude goes to my mentor and project supervisor, Dr. Faustina Wireko-Manu. I appreciate her great coaching and support through the inception of this research to this point. God bless you for all the sacrifice of time to ensure I do a great work and on time too. I also acknowledge the opportunity given me by Mr. Samuel Amankwah of O. A. Amankwah Enterprise, to work with their imported apple samples and Cold Storage room. My deepest appreciation again goes to Dr. Emmanuel de-Graft Johnson Owusu Ansah for the support given in analysing my data. Thank you all.

Above all, I say thank you to God for seeing me through this program.

ABSTRACT

Changes in quality parameters were studied on two commercially imported apple cultivars in Ghana: 'Golden Delicious' and 'Pink Lady', with the aim of investigating the changes in the apples along the postharvest import chain. Freshly imported apples were sampled and analysed for the following: percentage moisture, weight (and subsequent weight loss), total soluble solids, titratable acidity and pH using standard methods; and antioxidant activity using the DPPH assay. These results were used as control for changes that were recorded along the study. The rest of the samples were stored for a 90-day period under different conditions: Cold Room Storage (0 - 4 ^{o}C), Cold Shelf Storage (10 – 18 ^{o}C) and in Ambient Storage (25 – 28 ^{o}C), each representing a major stage in the postharvest import chain in Ghana. Sampling and analysis were done every 15th day for 90 days. Physical defects were also observed on sampling days. At the end of the 90 day storage period, storage of apples under cold room conditions retained better quality characteristics than all other storage conditions. Golden Delicious apples retained higher moisture content than the Pink Lady apples as storage progressed; even though a significant loss in weight was record on day 15 in both cultivars and in all storage conditions. Fresh samples of Pink Lady apples had 79% DPPH inhibition, as compared with 74% in Golden Delicious. However, a significant drop in the AA of Pink Lady apples occurred by day 30 in storage, whereas this was seen on day 60 for Golden Delicious apples, both in cold room storage. All samples under ambient storage had lost a significant amount of AA by day 15 in storage. Soft rot, Bitter pit and discolouration were also severe in the ambient storage samples. Changes in pH, total soluble solids and titratable acidity still fell within consumer acceptable ranges

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE

INTRODUCTION

1.1 Background

World trade in agricultural products has grown rapidly in recent years. Consequently, import of agricultural produce from different countries has seen an increase over the years in Ghana. Both fruits and vegetables are imported into grocery and wholesale stores across the ten regions of Ghana. Particular among them are imported apples. The growing of apples for commercial gain has advanced in many developed countries. More than 63 million tons of apples are produced every year around the world (FAOSTAT, 2016). Since the year 2014, China has remained the largest apple producing country, representing over 40% of the world's total production (FAOSTAT, 2014). United States, Turkey, Poland Italy, Brazil, Russia, France and Germany are the largest apple producing countries, whereas in Africa, South Africa is the only country that grows and also exports apples.

Apple, *Malus pumila*, is a temperate fruits potentially grown and harvested only in the temperate regions of the world with temperatures below 10 ^{o}C. It is an important fruit desired for its taste, nutritive value and associated health benefits. It is in high demand throughout the year in Ghana. The increased patronage of imported apples can be attributed to a number of reasons among consumers. There has been a general upsurge in the campaign on daily fruit and vegetables consumption to promote health among Ghanaians. Urbanization has also influenced the lifestyle and eating pattern of Ghanaians. Again, apples in Ghana are sold and consumed whole without any reduction in size for market sales; thus many consumers deem it more hygienic than several other fruits which are usually peeled and cut. Apples are also sold in convenient packs and are available at convenient locations including by the roadside and busy streets nationwide. Added to these are the notable high antioxidant activity and vitamin C content associated with apples (Biedrzycka and Amarowicz, 2008; Wolfe *et al*, 2003).

After apples mature and are harvested by farmers, they may be sent directly to the market or stored for future commercial purposes. Fruit quality indices are largely affected by storage (Khan *et al*., 2017; Schrader *et al*., 2009). Thus, there are several technological systems that have been developed to retain quality and improve the shelf-life of apples in storage. These include Controlled Atmosphere storage and Cold Storage warehouses (with temperatures within 0 to 4^{o} C) (Viškelis *et al*, 2011). From various storehouses, packaged apples are then taken through quality and safety inspections prior to shipping.

Imported agro foods are transported under refrigeration conditions into the receiving country. For both climacteric and non-climacteric fruits, temperature may affect their physical, chemical, sensory, nutritional composition and general quality attributes at harvest and after harvest. Hence, rapid cooling after harvest has been studied to significantly improve firmness retention in apple while in storage (Jan *et al*, 2012a).

All over the world, even among global leaders in apple production, there is a constant strive to enhance the quality of harvested apples in storage and to improve shelf-life. Obtaining and maintaining apple fruit firmness and other quality attributes from the orchard through to the consumer, therefore, tends to be one of the major issues facing both apple producers and marketers. So here in Ghana, the uncertainty of the postharvest quality of apples surrounds the following questions. Could there be any quality changes that apples imported into Ghana may be going through? Are there any quality losses that correlate with typical storage temperatures of apples imported in Ghana? Are the temperature changes along the postharvest import chain causing any changes in the quality of imported apples? Are there effects on retail handling practices on apple quality? ‘Golden Delicious’, the most popular and most abundant apple cultivar on the Ghanaian market, may possibly be undergoing major quality changes along the postharvest import chain, attributable to various causes. Therefore this study aims at evaluating the changes in quality attributes that may occur along the postharvest import chain of apples. It will also help determine the optimal storage and handling conditions, which maximizes the quality attributes of imported apples in Ghana.

1.2 Problem Statement and Justification

In Ghana and other tropical countries, apples (*Malus pumila*) are imported from the temperate zones and are sold wholly with no size reduction, peeling or additional packaging. Thus, to some consumers, apples are more hygienic and safe for consumption; as compared with fruits like pawpaw and watermelon. Yet, the conditions under which imported apples are transported and stored may negatively impact on their desirable qualities. Again, many practices of fruit handlers along the postharvest value chain, may pose a high risk to the safety of apples. A common example is the different temperatures under which apples are stored and sold on the Ghanaian market; from cold temperature storage to a direct-sun market. Consequently, there is uncertainty regarding the acclaimed quality and nutritive attributes of apples on the Ghanaian market.

Even though lots of agronomic studies have been done on apples, the changes in quality of imported apples along the postharvest value chain have scarcely been reported in literature. As fruit vending in the open-sun on the streets is becoming more lucrative to the Ghanaian trader, there is the need to investigate practices that may alter the rich quality of apples.

1.3 Main Objective

The aim of this study is to investigate the relationship between storage conditions and changes that occur in the quality of imported apples along the post-harvest value chain in Ghana.

Specific Objective(s):

- To measure antioxidant activity of apples at three different stages along the postharvest import chain
- To measure pH, total soluble solids, titratable acidity, loss of mass and percentage moisture of apples, at three different stages along the postharvest import chain
- To observe physical changes that may occur during the storage of apples: discolouration, bitter pit and soft rot

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Apples

The Apple, *Malus pumilia*, is one of the leading important tree fruits of the world. The apple was first cultivated in Greece around 600 BC or earlier; whereas other genetic analysis have revealed a Central Asian origin for cultivated apples (Cornille *et al*, 2014). Apples belong to the Rosaaceae (Rose) Family and the subfamily of Pomoideae along with pear and quince (Kamas *et al*, n.d.). Quoting Janick *et al*, "The apple is the most ubiquitous of temperate fruits and has been cultivated in Europe and Asia from antiquity" (Janick *et al*, 1996). It is a highly nutritive fruit and a rich source of antioxidants, vitamin C, vitamin A, thiamin and other vitamin complexes (Ali *et al*, 2004). A 100 g fresh apple contains, water 84.7 %, fibre 0.8 g, carbohydrates 13.9 g, proteins 0.4 g, lipid 0.3 g, ash 0.3 g, vitamin C 8 mg/100 gm, sodium 0.3 mg/100 g, potassium 145 mg/100 g, calcium 7 mg/100 g, magnesium 6 mg/100 gm, iron 480 µg/100 g, phosphorus 12 mg and iodine 2 µg (Ali *et al*., 2004; Biedrzycka and Amorowicz, 2008). Due to the high nutritional value of the apple fruit, it ranks third in consumption after citrus and banana (Boyer and Liu, 2004b).

Tracing back in history, apples have become the mark of healthiness: "An apple a day keeps the doctor away" is a favourite saying and apple juices and other products have become associated with wellness and good health. Reasons for the popularity of apples are the many ways in which they can be consumed, the convenience in handling and their durability. Its beautiful appearance, crispy flesh, pleasant flavour and sweet taste also attract consumers(King and Henderson, 2012).

2.2 Apple Growth, Production and Export in the World

Apples are temperate fruits, just like plum, peach, strawberry and black berries; all in the Rose family (Janick *et al*., 1996). They are grown in orchards. Apples grow best in the temperate regions of the world (Jan and Rab, 2012). Apples may be imported into a temperate country or location from another temperate country or location. As a result, apples are imported into the tropical zones of the world. The current production of apples worldwide stands at 1.2 million tonnes annually (FAOSTAT, 2014); many of which are exported to various parts of the world including growing areas. Major exporters in the world as at 2014 were United States of America, China, Italy, Chile, France, Poland, New Zealand, South Africa, Netherlands and Belgium (FAOSTAT, 2014). Even though China produces the most apples in the world, it is the United States, with exports worth well over a billion dollars, that allows it to top the list of 10 countries that export the most apples in the world. Ghana imports apples primarily from South Africa. Occasionally purchases are made from China, USA and Poland.

Apples are propagated vegetatively in orchards. In many parts of the world where apples are cultivated, apple fruits are harvested during August and September. After harvesting, different technologies are adopted to prolong the shelf life of apples in storage. Where advanced storage technology is not available, maximum fruit is supplied to neighbouring markets during these two harvest months (Ganai *et al*., 2014).

2.2.1 Apple Production in South Africa

South Africa's agribusinesses and retailers have set themselves up to exploit the opportunity of climate diversity among others on the African continent. Its organizations began expanding their cooperation in the world not long after 1994 when the nation was acknowledged in the worldwide group. South African exports to the rest of the world have dramatically increased from the mid-1990s to 2014. Currently, the production of apples in South Africa stands at 918,085 tonnes annually (FAOSTAT, 2016). In Ghana, fruits and vegetables imported from South Africa include not only apples, but grapes, bananas, mangoes, carrots, cucumbers and root tubers like potatoes. The Golden Delicious cultivar and the Pink Lady cultivar are the two cultivars exported from South Africa to Ghana.

2.3 Apple Cultivars

Throughout the history of cultivation of apples, at least 10,000 apple cultivars were developed, many of which are now lost. This was due in part to the older practice of seed propagation. It is generally assumed that M. *pumila* evolved from chance hybridization among these wild species (Collett, 1945). Several thousands of apple cultivars have been grown from wild to domesticated species (Cornille *et al*., 2014). Over the years, a number of cultivars have been identified as nutritious and healthy for consumption, with high economic value. Commercially there are about 100 cultivars currently being grown, but only 10 of the most popular ones make up over 90% of US production. They include Red Delicious, Golden Delicious, McIntosh, Rome Beauty and Granny Smith (Jha *et al*, 2012); and these account for the major production of apple in the world.

Different types of apple trees, or cultivars, are bred for various purposes, such as to obtain different tastes and textures. These differences allow apples to be used for a variety of purposes, such as to be eaten raw, for juice processing, for processing cider or cooked.

Different cultivars of apples also have different chemical composition and contain different amounts of nutrients including vitamin C (Ali *et al*., 2004).

In Ghana, apples are typically classified according to their skin colour: green or red, even though ripe apples range in colour from green to yellow to red or a mixture of these colours. However, two main apple cultivars are often imported into Ghana. These are the bright green cultivar known as 'Golden Delicious' and the dark red cultivar with green patches known as 'Pink lady'. Either of these two cultivars may be imported under different brand names. While some brand names are related to the exporting farm, others are also related to the local importer's brand. For example, apples with 'Freshmark' labels are solely imported by Shoprite, a multinational grocery with Head office in South Africa. 'Green leaf' labelled apples are also exported exclusively by the Green leaf farm based in South Africa. However, to many direct fruit importers, the final consumers do not know the difference between apples from different countries of origin.

2.3.1 Golden Delicious Cultivar

Golden Delicious is the apple cultivar with a bright green to golden yellow colour, with tiny spots or lenticels on the skin. They range from small to medium and large sizes. They are white-fleshed, firm and crisp, with a sweet aromatic flavour described as 'honeyed'. It is one of the most popular apple cultivars in the United States, China and South Africa. Typically in Ghana, they are identified as the popular green apples sold on the market.

Plate 1: Golden Delicious apples displayed on a tray

2.3.2 Pink Lady Cultivar

The Pink Lady apple cultivar is identified by its bright red to wine colour. Its parentage comes from a cross between 'Golden Delicious' and 'Lady Williams' (Corrigan *et al*, 1997; Kamas *et al*., n.d.). Oblong, green fruit turns yellow at maturity and is overlaid with pink or light red. Thus, this cultivar mostly has green skin areas which may cover about 40% of its total surface area. Anthocyanin in plants account for the red colour pigments in this cultivar of apples.

Plate 2: Pink Lady apple displayed on a tray

2.4 Quality Attributes of Apples

In general, the harvest quality attributes of apples include appearance, taste, texture, microbial safety and nutritional value. These qualities depend on a number of factors such as plant genetics, variety or cultivar, agricultural practices, soil composition, time of harvesting and storage conditions (Jan *et al*, 2012b). These may decline in storage due to continuing respiration, ethylene production and the occurrence of post-harvest diseases.

2.4.1 Physical Quality attributes

Fruit quality can be described as what makes fruit appealing to the various senses: sight, touch, taste and smell (Thedy *et al*, 2005). It is the sum of both the external and internal features of a fruit. Fruits are selected by consumers on the basis of appearance only, since it is not possible to know fruit sweetness or sourness at the point of purchase. The physical attributes of a fruit, such as size, shape and colour, are very important fruit quality characteristics to a consumer. However, during the shelf-life of an apple, it may suffer detrimental defects that can impact largely on its physical quality attributes. For instance fruit softening which occurs as ripening progresses, happens as a result of loss of cell cohesion (Zdunek *et al*, 2007). Changes in pectin composition and pectolytic enzymes are responsible for the reduced adhesion between cells which results in the formation of soft spots.

Bitter Pit is another physical defect in apples. It leads to the formation of brown areas on apple skins and the development of a ‘pit’ on the surface. Affected areas are typically around the calyx end of the fruit and symptoms typically show prior to harvest. This disorder, bitter pit, occurs in soils deficient in calcium. The resulting calcium deficiency in fruits can cause a number of different maladies, including bitter pit, all of which affect the integrity and storage quality of apples.

Ethylene also plays an important role in enhancing apple softening. Thus, the ability to minimize ethylene action could be a means of reducing rapid softening. On the other hand, ethylene is also essential for stimulating other ripening processes other than softening, such as aroma and flavour development, which are important attributes of fruit quality.

2.4.2 Chemical attributes

pH, Titratable Acidity and Total Soluble Solids are some key indicators of the freshness or deterioration of apples. They also impact on other sensory properties of the apple fruit including its taste. These may fall rapidly after harvesting due to biological processes such as respiration. As fruits and vegetables respire, they convert starch and other sugars, as well as oxygen into carbon dioxide, water and heat. Understanding the biochemical composition of harvested fruit could be used as a tool for planning postharvest management to maintain quality and reduce the loss of fresh fruit.

2.4.2.1 Total Soluble Solids (Brix)

Total Soluble Solids (TSS) of a fruit refer to all the dissolved solutes in the fruit. It is also known as Soluble Solids Content (SSC) and can be measured in a small sample of fruit juice using a handheld refractometer. Sugars are the main soluble solids in juices from fruits. TSS is a key characteristic in determining the taste, texture and feel.

2.4.2.2 Titratable Acidity

Acidity is a major contributor to fruit quality. It impacts on taste. There are several organic acids present in apples, but predominant among them is Malic acid (Ali *et al*., 2004; Ganai *et al*., 2014). Titratable acidity (TA) of apples can be measured by titrating a known volume of juice with 0.1N NaOH till all acids present are completely neutralised. A pink coloured end point is obtained as indicated by phenolphthalein indicator. The volume of NaOH needed for the complete neutralisation of the acid, is used to calculate Titratable Acidity.

2.4.3 Nutritional value

Predominant among the rich nutrients in apples are Antioxidants and Vitamin C. Apples are a rich source of phytochemicals and epidemiological studies have linked the consumption of apples with reduced risk of some cancers, cardiovascular disease, asthma and diabetes. In vitro studies have also revealed that apples have a very strong antioxidant activity, inhibit cancer cell proliferation, decrease lipid oxidation and lower cholesterol (Boyer and Liu, 2004a).

2.4.3.1 Major Antioxidants in Apples

Several substances exhibiting antioxidant property have been found in the apple fruit. Dominant among these are polyphenolic compounds such as quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-rhamnoside, catechin, epicatechin, procyanidin, cyanidin-3-galactoside, coumaric acid, chlorogenic acid, gallic acid, phloridzin, anthocyanins, flavonoids and cyanidine-3-glucoside, all of which are strong antioxidants (Boyer and Liu, 2004b). The total phenolics in apple fruits are concentrated in the peels of apples. In a study by Wolfe *et al* (2003), the peels of apples were found to be high in phenolics and flavonoids contents as compared with the measure in its flesh and peel.

These antioxidants and phenolic compounds have been studied to have abilities in preventing diseases such as cardiovascular diseases and cancer (Zardo *et al*, 2015). Phytochemicals including phenolics, flavonoids and carotenoids from fruits and vegetables may play a key role in reducing chronic disease risk (Biedrzycka and Amorowicz, 2008).

2.4.3.2 Vitamin C

Vitamins C, including ascorbic acid and dehydroascorbic acid, is one of the most important nutritional quality factors in many horticultural crops and has many biological activities in the human body. The content of vitamin C in fruits and vegetables can be influenced by various factors such as genotypic differences, preharvest climatic conditions and cultural practices, maturity and harvesting methods and postharvest handling procedures. The higher the intensity of light during the growing season, the greater is the vitamin C content in plant tissues. Nitrogen fertilizers at high rates tend to decrease vitamin C content in many fruits and vegetables (El-ramady *et al*, 2015). Vitamin C content of many crops can be increased with less frequent irrigation.

Regardless of all the growth conditions that affect Vitamin C content in harvested farm produce, temperature management after harvest is the most important factor to maintain vitamin C levels in fruits and vegetables; losses are accelerated at higher temperatures and with longer storage durations (Ali *et al*., 2004). However, some chilling sensitive crops show more losses in vitamin C at lower temperatures.

2.5 The Postharvest Chain of Apples

Not much growth history of imported apples is available with individual shipped containers. There is little to no information on harvesting and postharvest treatment, specific to delivered apples. However, apples are known to be harvested at full maturity (Arah *et al*, 2015) and taken through a number of treatments prior to storage (Li and Li, 2008). These treatments remove detrimental elements and improve product appearance, as well as ensure that the product conforms to recognized quality standards for fresh produce.

Typically, the postharvest chain of apples begins with harvesting and includes precooling and pre-storage, chemical treatment, classification, washing and waxing, packaging, transportation and storage (El-ramady *et al*., 2015; Li and Li, 2008).

2.5.1 Harvesting

There are different harvesting times for different apple cultivars. Typically, early maturing cultivars are harvested about 100 days after florescence, approximately 100 to 140 days for mid-maturing cultivars and almost 140 to 175 days for late-maturing cultivars (Li and Li, 2008). Apple which will be stored for any length of time should be harvested in advance, usually 7 to 10 days before its regular harvesting period. Being a climacteric fruit, the apple can be harvested at physiological maturity, stored and ripening induced for a high market value.

Generally, apple fruit harvested before maturity has poor colour and flavour development and is prone to defects such as bitter pit and soft rot (Jan *et al*, 2012a; Johnston *et al*, 2002). On the contrary, over-mature fruit are likely to be soft and easily injured after harvest. Thus poor harvesting time increases the susceptibility of apples to diseases and physiological disorders as well as postharvest quality deterioration (Arah *et al*., 2015; Ganai *et al*., 2014; Jan and Rab, 2012).

2.5.2 Postharvest Treatment

2.5.2.1 Precooling and Pre-storage

In many apple producing countries, apples are harvested from September to October (Li and Li, 2008). Due to the comparatively high temperature around this period, the crop must be precooled by mechanical refrigeration as quickly as possible. In the absence of large cooling equipment, apples may also be precooled by the normal cold weather at night in most areas.

2.5.2.2 Chemical treatment

The effect of various chemical treatments on the quality of apples during storage is essential for precooled apples. It helps reduce the occurrence of disease and improve the storage performance of apples. Precooled apples are often cleaned by immersion in chemical solutions before the preservation process. Chemical solutions used for immersion-cleaning include calcium chloride solution (3%-6%), which enhances firmness of apples and retards aging or ripening; ethoxyquin solution (0.25%-0.35%) and thiabendazole solution (1000-2500mg/kg) (Li and Li, 2008).

2.5.2.3 Classification

Classification or grading of apples is done manually by hands or automatically before storing or later after storing. Several classification indices have been established, with the predominant factors being colour, size, mechanical injury, diseases and pests (Li and Li, 2008). In many apple producing and exporting nations, apples are classified in line with these four indices.

2.5.2.4 Washing and waxing

Prior to storage and transportation, apples are washed for cleaning and sterilization. The chemical solutions used for washing apples are hydrochloric acid (1%) or 200-500 mg/kg of potassium permanganate solution or 200 mg/kg bleaching powder. Treatment with food grade waxes help to replace natural waxes removed during harvesting and sorting. It also prevents water and weight loss of apples during transportation and storage; it can also increase the brightness of the fruit surface. Edible coatings used, offer a possible method of extending postharvest storage life by providing a semipermeable barrier to gases and water vapour, thus reducing respiration and water loss.

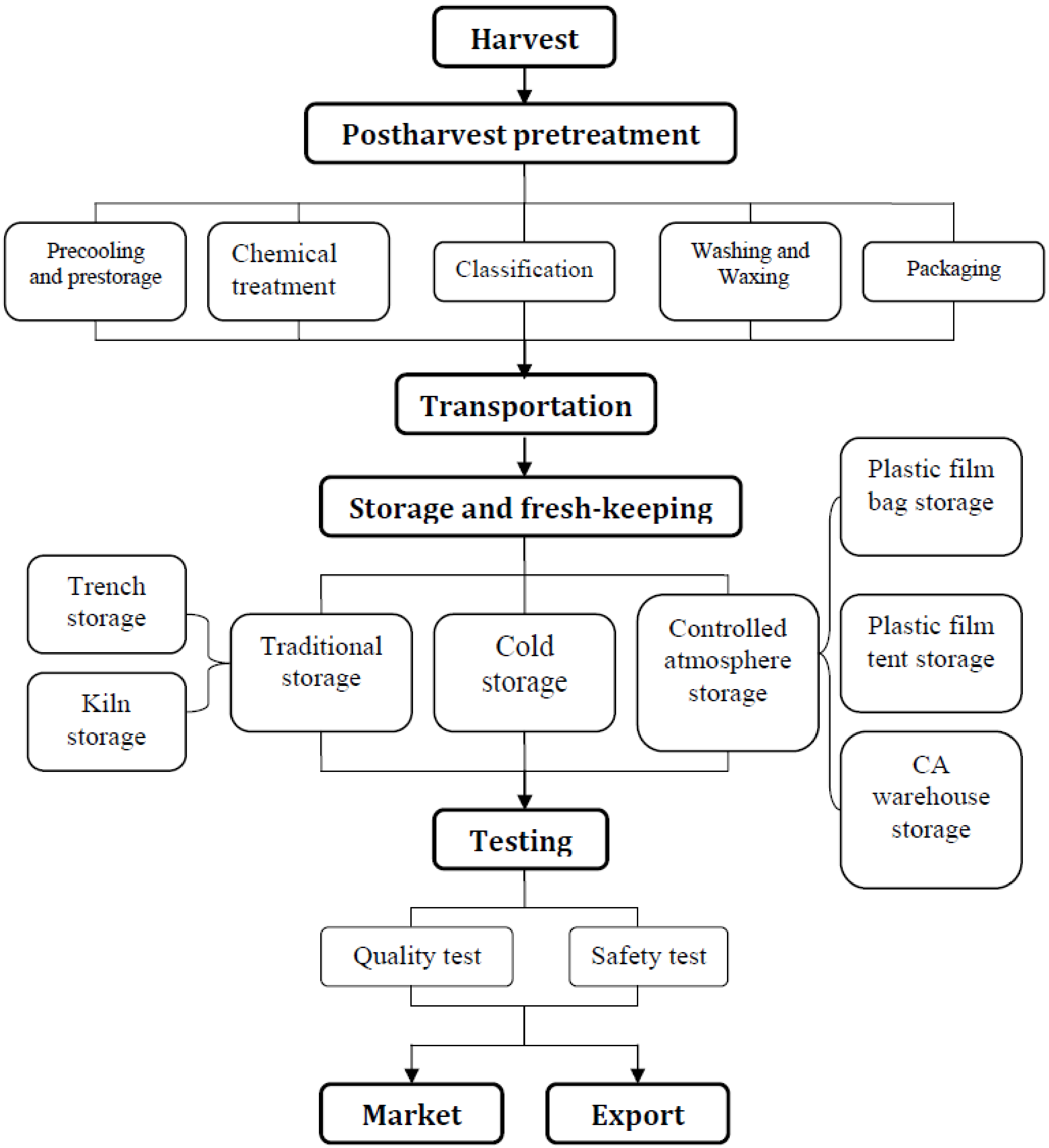
2.5.2.5 Packaging

Wrapping papers containing diphenylamine or ethoxyquin are widely used for the inner lining of packaging for apples (Li and Li, 2008). As for the outer packaging, cartons are the main choice. Generally, cartons made of high-strength corrugated paperboard are used for export packaging in most countries.

2.5.2.6 Transportation

During the transportation of apples for storage or distribution in the market, mechanical damage must be avoided and respiration minimized as much as possible.

Figure 1: A typical Postharvest Value Chain for apples in China (Li and Li, 2008).



2.5.3 Storage

Storage of apples both in the long term and short term, involve physical and chemical changes that can negatively modify its quality. Quality cannot be improved, but it can be largely maintained during storage. Therefore, an effective storage method should prevent all of the above quality changes. In Ghana, the most common method for storage of apples is cold storage or the use of cold rooms. It is a method widely used for preserving apples, even among leading producers like China (Li and Li, 2008). Other common methods of apple storage are Controlled Atmosphere storage, which is used for the preservation of top quality cultivars of apples such as Red Delicious apples (Steele and Vera-filho, 1970); and the Traditional method of storage.

2.5.3.1 Cold storage

Cold storage is the primary way to preserve apples in southern China because of the natural high air temperatures. Usually, apples are stored in refrigerated (-1 to -3°C) warehouses within 1-2 days after harvest and the temperature of the apples is lowered to -1 to 5°C after 3-5 days. The relative humidity in refrigerated warehouses should be controlled at 90-95% during storage. This method of storage can keep apples fresh for more than 6 months.

2.5.3.2 Controlled atmosphere storage

Controlled atmosphere (CA) storage is a method used for storing apples for local and export markets across the globe. It is established either as simple CA storage (also called spontaneous regulating CA storage) or film packaging storage. It is a very common but effective method and includes plastic film bag storage and plastic tent storage. It involves the reduction of oxygen concentration in the air surrounding a fruit to less than 10% (usually between 1-3%), which consequently reduces the rate of respiration in the fruit (Dixon and Hewett, 2000).

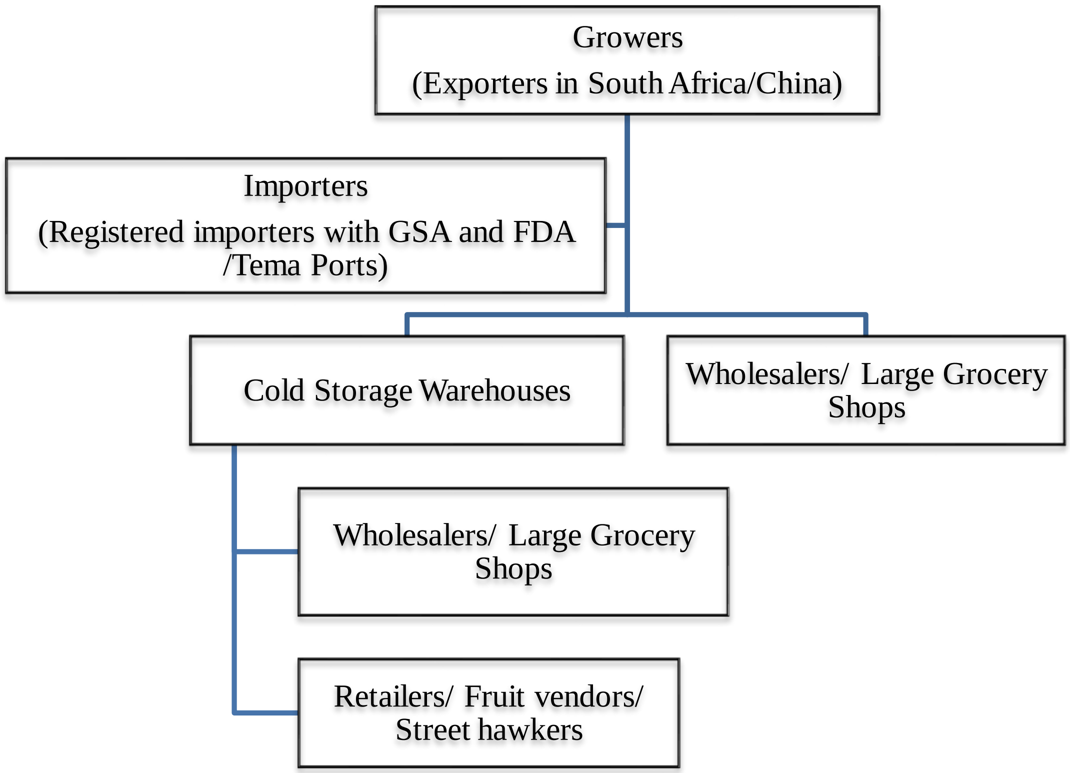
CA or modified atmosphere storage is usually supplemented by refrigeration to extend storage life and maintain quality. CA warehouses, due to its high cost, accounts for a relatively small proportion and is mainly used for high-grade export apples.

2.6 The Postharvest Import Chain of Apples.

In Ghana, imported apples agro foods are mostly transported in refrigerated containers (reefer container). These refrigerated containers are temperature regulated containers or vessels used in shipping products from an exporting country into an importing destination. To enable an importer ship fruits into Ghana, fruit importers will typically register with the Agro division of the Ghana Food and Drugs Authority (FDA) and have their import food product registered with them. Required documents for the importation of fruits and other agro-foods include a phytosanitary certificate from the exporting party or the country of origin. This is a document or statement of declaration stating that the import products are free of pests and other harmful chemical residue.

After port processes for the clearance of produce, the containing vessels are then transported on trailers to their destinations, typically cold storage warehouses for apples. The imported apples are graded before storage and subsequently sold out to large grocery stores for retailing on cold shelves, or to other retailers and street hawkers who sell under the direct sun or ambient conditions.

Figure 2: A typical Postharvest Import Chain for apples in Ghana (*Source: Researcher*)



2.6.1 Poor Handling Activity along the Apple Import Chain

At the point of offloading in the cold storage warehouses, it is difficult to maintain fruits at their optimum temperatures. Additionally, fruits are also exposed to non-optimal temperatures during grading, packing, distribution, vehicle loading and unloading, transporting and in retail outlets while on display; which may consequently impact on the internal and external quality of apples.

Many imported apples are retailed by the unorganised sector, including street vendors and traffic hawkers. The rest of the apples imported into the country are sold wholesale from cold storage rooms, or retailed on cold shelves in large grocery stores.

2.7 Factors affecting the Postharvest Quality of Apples

Postharvest physiology of apple fruits has received much attention over the last decade. After a fruit has been harvested and denied nourishment, it inevitably faces senescence. However, it remains a biological system and responds to internal and external stimuli such as hormones, metabolites, pH, temperature and atmospheric condition (El-ramady *et al*., 2015). Thus postharvest activities and handling can be engineered to prolong the life of a fruit, for as long as possible before senescence.

It is essential to maintain not only high apple fruit quality, but also to ensure their physiological and microbiological safety during storage. The key features of quality maintenance during storage are effective control of skin background colour and weight loss, high retention of flesh firmness and the retention of soluble solids and acid to give the desired sugar to acid ratio (Arah *et al*., 2015; Ganai *et al*., 2014; Khan *et al*., 2017; Schrader *et al*., 2009).

2.7.1 Temperature

It has been estimated that, 5 to 25% of fruits and vegetables harvested from farms are never consumed (El-ramady *et al*., 2015). Among several contributing factors to this loss are packaging and temperature management.

In spite of 0 - 3 °C being the ideal postharvest temperature for slowing down the loss of desirable quality attributes of apples, it is difficult to maintain fruits at these optimum temperatures through the whole postharvest import or handling chain (Johnston *et al*, 2002) especially in Tropical countries like Ghana. Fruits are often exposed to non-optimal temperatures during grading, packing, distribution, ship loading and unloading and in retail outlets while on display; which may consequently impact on the internal and external quality of apples. In order to satisfy the need of consumers to get high quality fresh fruits and to preserve their quality during storage, it is necessary to ensure that the optimum storage conditions are closely met. When this is achieved, not only are fruit quality attributes greatly preserved, but storage losses are reduced as much as possible and also the life span of fruits usage is extended (Kvikliene *et al*, 2006).

Temperature is the single most essential factor in maintaining the quality of apples during storage. Low temperature (0 – 5 °C) storage can reduce postharvest respiration and consequently quality deterioration and nutrient reduction. Cold storage can also create a high humidity (85 - 95%) environment, which prevents weight loss due to transpiration. The low temperature delays the ripening process, but does not halt it. Usually during long term cold storage, apples lose firmness and have their appearance altered significantly due to evaporation and other chemical interaction within the fruit cell walls (Zdunek *et al*., 2007).

2.7.2 Moisture Loss

Postharvest water loss has a great effect on fruit quality and is a major cause of deterioration. Transpiration is the process by which fresh produce lose moisture (Becker and Fricke, n.d.). This process involves the movement of moisture through the skin of the foods, the evaporation of this moisture from the food surface and the convective mass transport of the moisture to the surroundings. Significant moisture loss may lead to a substantial loss of product weight, causing a drop in market value if product is traded by weight. Slight moisture loss can equally cause subtle quality changes in the colour and texture of fruits; and when moisture is lost beyond the threshold, negative changes in firmness, aroma or flavour and even nutritional value may occur.

2.7.3 Storage Duration

Storage duration is a key decisive factor for changes in apple quality. The longer fruits stay in storage, the more prone they are to defects and deterioration. Several studies have shown a negative correlation between storage duration and the quality parameters of fruits. Storage duration influences the reduction of flesh firmness for Golden Delicious apples according to Jan *et al*, (2012b). Thus, carefully controlling storage conditions can extend the shelf life of produce.

It must however be noted that, various apple cultivars vary significantly in their storage performance.

2.8 Role of Storage in Ensuring Quality, Safety And Nutrition

Postharvest fruits and vegetables remain physiologically active after harvesting. Respiration, which comprises the primary metabolism, can affect and constrain the life span of fruit and vegetable products, as well as quality and nutrition changes during storage. Continuing respiration after harvest of fruits and vegetables results in quality deterioration and nutrient reduction. Most fruits and vegetables are high in water content (between 65% and 96%), so they lose weight due to transpiration of water during storage. This leads to a deterioration in the quality of fruits and vegetables and may even result in loss of their commercial value. Postharvest fruits and vegetables also produce ethylene during maturation. Ethylene, a ripening agent, accelerates fruit and vegetable aging and weakens antiviral and antimicrobial abilities. Postharvest fruits and vegetables are also prone to decay because of spoilage and pathogenic microorganism infestation during storage. All of these result in a decline in the quality, nutrition and safety of postharvest fruits and vegetables (Khan *et al*., 2017).

Generally speaking, effective storage can create environments with low temperature and high humidity or low oxygen and high carbon dioxide, or low ethylene and asepsis, which are beneficial for fruit and vegetable preservation. Low temperature and high humidity inhibit enzyme activities that are necessary for respiration and the growth of spoilage and pathogenic microorganisms which make fruits and vegetables decay; it can also prevent water loss. Low oxygen and high carbon dioxide prevent fruits and vegetables from maturing by inhibiting respiration, while low ethylene and asepsis decrease the rate of maturation and spoilage by microorganisms (Li and Li, 2008; Thedy *et al*., 2005).

Thus effective storage can postpone fruit and vegetable ripening and senescence, inhibit respiration and transpiration, reduce the formation of ethylene and increase their antiviral and antimicrobial abilities to maintain their quality, nutrition and safety, for as long as possible.

2.9 The DPPH Antioxidant Assay

The scavenging activity of 2, 2-diphenyl-1-picrylhidrazyl (DPPH) radical is the basis of the popular DPPH antioxidant assay. In other words, the ability of food substances to scavenge the free radicals of DPPH is the basis for this antioxidant activity assay. In this assay, the blue-violet colour of DPPH solution changes gradually to green and yellow (absorption maximum at 405 nm) and a decrease in absorbance at 517 nm is monitored during the reaction in neutral medium (Stratil *et al*, 2006).

The DPPH assay is a rapid and low cost method frequently used for the assessment of the anti-oxidative ability of numerous natural foods (Elbadrawy and Sello, 2016). The rate of DPPH destruction after the addition of a sample containing phenolic compounds is proportional to the concentration of added antioxidant; thus, the classical calibration procedure based on Trolox as a standard can be used for quantification and 1 mmol/L of Trolox corresponds to the antioxidant activity of 1 mmol/L of phenolic compounds.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Reagents and Instruments

Ethanol, Methanol, DPPH, pH meter, Spectrophotometer, weighing scale, Drying Oven, Refractometer

3.2 Sample Collection

Two apple cultivars, Golden Delicious (green apple) and Pink lady (red apple) were collected directly from a refrigerated container imported from South Africa, for a fruit warehouse at Nungua in the Greater Accra region of Ghana. The container was accompanied by a temperature logger unit which recorded an average temperature of 2 ^{o}C within the forty-eight day shipping and clearing period. The shipment was also accompanied by a phytosanitary certificate declaring the products to be pests and disease-free. Healthy looking apples with no defects at the time of offloading were randomly selected as samples for this experiment.

The samples of each cultivar were separated into four groups with fifty apples in Group A and eighty apples in each of the remaining groups; each group representing a major stage in the postharvest value chain of imported apples in Ghana.

Group A: Fresh samples (from the refrigerated container)

Group B: Samples in a local cold storage warehouse (0 – 4 ^{o}C)

Group C: Samples stored on the cold shelf of a grocery shop (10 – 18 ^{o}C)

Group D: Samples stored at ambient temperature as for street vending (25 – 38 ^{o}C)

Samples in Group A were placed in an ice chest with icepacks to minimize temperature loss. They were then analysed in the laboratory for Antioxidant activity, pH, Total Soluble solids, Titratable acidity, weight and percentage moisture.

Samples in the remaining groups were stored for 0 to 90 days and sub samples were selected after every 15 days to measure the following parameters: Antioxidant activity, pH, Total Soluble solids, Titratable acidity, weight loss and percentage moisture. Each of the sub samples was also observed for physical changes such as discolouration, rot, dark patches and soft spots.

3.3 Antioxidant Activity Assay

3.3.1 Sample preparation

To prepare samples for the determination of antioxidant activity, apples were washed in the laboratory, wiped dry and cut manually with a knife into small pieces (flesh and peel, except seeds).

3.3.2 Extraction

According to the procedure described by Wolfe *et al.* (2003), 50 g of cut apple samples with the peel were blended with 200 g of chilled 80% methanol solution in a blender for 5 min. The sample was then homogenized for 3 min using a laboratory homogenizer. The slurry was filtered through Whatman No. 1 filter paper in a Buchner funnel. The solids were scraped into 150 g of 80% methanol and homogenized again for 3 min before re-filtering. The filtrate was recovered by evaporating the solvent using a stirred water bath at 45 °C until less than 10% of the initial volume remained. The extract was made up to 50 mL with distilled water and frozen at -4 °C until analysed. All extracts were made in triplicate (Wolfe *et al*., 2003).

3.3.3 The DPPH Free Radical Scavenging Assay

The percentage of antioxidant activity (AA%) of each sample extract was assessed by DPPH free radical assay. This method of determination was described by Brand-William *et al* (1995). The juice extract from the samples was reacted with the stable DPPH radical in an ethanol solution; the reaction mixture consisting of, 0.5mL of sample, 3 mL of absolute ethanol and 0.3 mL of DPPH radical solution 0.5 mM in ethanol (Brand-Williams *et al*, 1995). When DPPH reacts with any antioxidant compounds present, which can donate hydrogen, it is reduced. This reduction will result in a change in colour (from deep violet to light yellow) and absorbance read at 517 nm after 100 min of reaction using a spectrophotometer. A mixture of ethanol (3.3 mL) and sample (0.5 mL) was used as blank. A control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution (0.3 mL). The scavenging activity percentage (AA%) was determined according to the formula below (Garcia *et al*., 2012):

$$\left[AA\% = 100 - \frac{(Abs\ sample - Abs\ blank)\ x\ 100}{Abs\ control}\right]$$

3.4 Percentage Moisture

Five grams of sample (flesh and peel) was obtained by size reduction and weighed into an empty pre-dried tray. The sample was then dried in an oven pre-set at 105 ^{o}C, for three hours. The difference in weight was calculated as a percentage on wet basis (AOAC, 2000).

$$\left[\%\ Moisture = \frac{(Weight\ before\ drying - Weight\ after\ drying)\ x\ 100}{Weight\ before\ Drying}\right]$$

3.5 Weight Loss

All samples retained for storage were identified with labeled tags on their stalk. The weight of each whole apple was measured and recorded on an electronic mass balance (Mettler Toledo) with an accuracy of 0.01g. Weight loss was calculated as a difference between the recorded weights of samples within the duration of storage. The results were expressed as a percentage.

3.6 Other Physico-chemical Analysis

Sample preparation

To prepare samples for the determination of chemical properties, juice extract was obtained from the apple samples. Apples were washed, cut manually with a knife into small pieces and crushed using a kitchen-type blender. The mixture was then squeezed in a four-layered muslin cloth to obtain a clear filtrate.

3.6.1 Total Soluble Solids

The total dissolved solids of the samples were determined from the liquid filtrate using a Density Meter Analyzer. It was measured as degree Brix at 20 ^oC.

3.6.2 pH

The pH of liquid filtrate was measured using a pH meter (Hanna Instrument, Hanna) at 20 ^oC.

3.6.3 Titratable Acidity

Acidity of the samples was determined by titration. Five (5g) of apple juice was diluted with distilled water (25 ml) and titrated against 0.1N NaOH solution using phenolphthalein (3-5 drops) as an indicator. The titre value was noted at the formation of a pink colour and calculated as Malic acid equivalent using following the formula below (Ganai *et al*., 2014):

$$\text{Titratable acidity} = \frac{Titre\ x\ Normality\ of\ alkali\ x\ Vol.make\ up\ x\ Equivalent\ weight\ of\ acid}{\text{Vol of sample taken x Weight or Volume of sample}}\ x\ 100$$

3.7 Physical Analysis:

The incidence of physical defects was observed visually in each group after every 15 days during the retention period. Defects were noted as discolouration, rot, dark patches and soft spots and recorded per affected fruit.

Bitter pit (%): Percentage bitter pit incidence was observed visually in each storage condition by calculating the surface area of each fruit covered with the symptoms of bitter pit on Day 0 through to Day 90 in storage.

Soft rot (%): Percentage soft rot in each storage condition was examined visually and counted during 90 days storage and the disease percentage of fruits was calculated by formula as:

$$\text{Disease Incidence (\%)} = \frac{\text{Number of diseased fruits}}{\text{Total number of fruits}} x100$$

3.8 Statistical Analysis:

The results obtained were analysed using the one-way ANOVA to determine whether these are significant differences in the results from samples at different stages of the postharvest import chain. The *T-test* was then used to determine the first incidence of this significant change during storage.

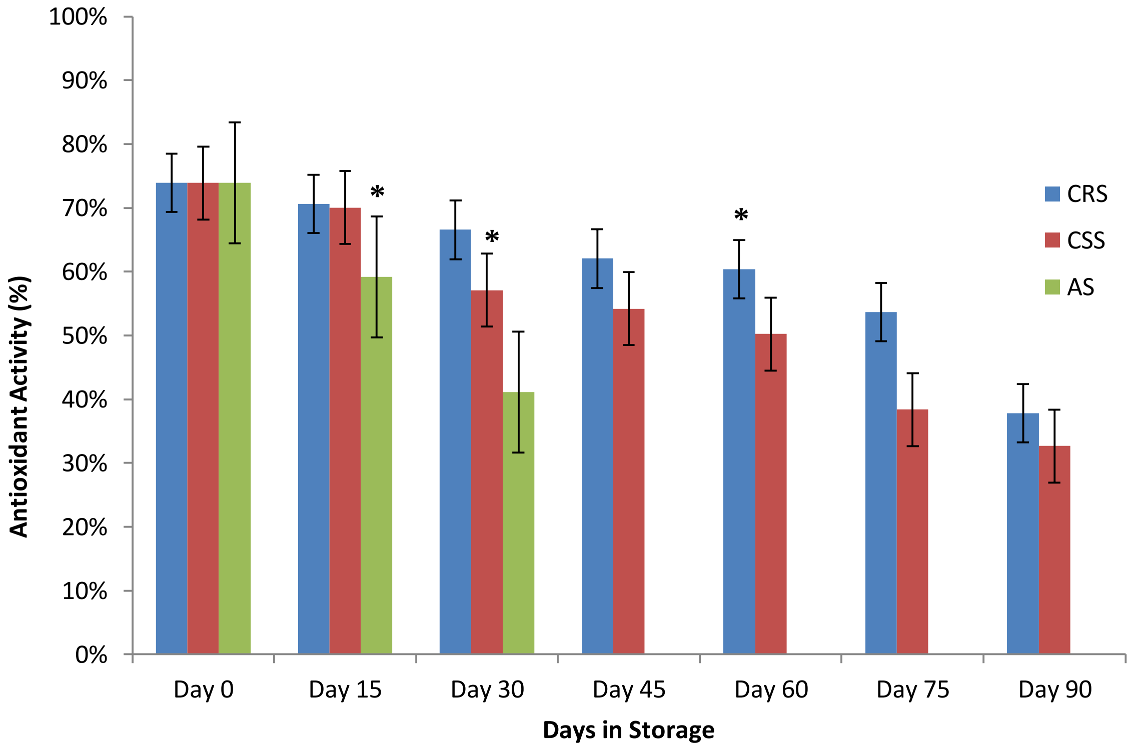
CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Changes in Antioxidant Activity (AA)

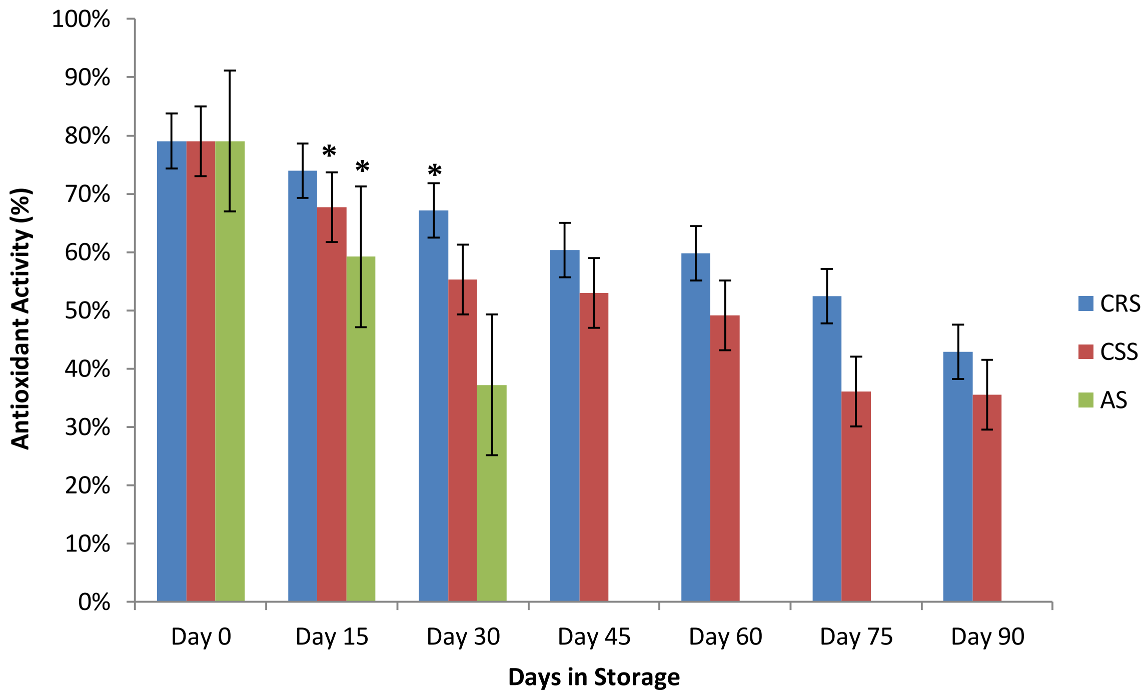
The effect of storage duration and temperature on Antioxidant activity (AA) was determined. AA% measured in fresh samples of Golden Delicious was 73.90% (Figure 3) and 79.05% (Figure 4) for Pink lady cultivar. This corresponded with results obtained by Wolfe *et al* (2003) in a similar study on harvested apples in cold storage for 7 days.

Figure 3: Antioxidant Activity (%) of Golden Delicious samples



Key: CRS= Cold Room Storage, CSS= Cold Shelf Storage, AS: Ambient Storage.
Error bars represent standard error
Columns with '*' show the first significant difference in antioxidant activity during the 90-day storage

Figure 4: Antioxidant Activity (%) of Pink Lady samples



Key: CRS= Cold Room Storage, CSS= Cold Shelf Storage, AS: Ambient Storage.
Error bars represent standard error
Columns with '*' show the first significant difference in antioxidant activity during the 90-day storage

The higher AA of the Pink Lady samples can be attributed to the presence of higher anthocyanin in this red coloured cultivar. Studies have revealed a relation between the red colour in the skin of fruits and phenolic content, particularly with anthocyanins (Zardo *et al*., 2015). The higher the intensity of the red colour, the higher its total phenolic content and consequently its AA.

AA varied significantly ($p < 0.05$) in both cultivars over the storage period for each condition observed. For the Golden Delicious samples, the first significant drop in antioxidant activity was recorded on day 15 for ambient storage, compared with day 30 and day 60 for cold shelf storage and cold room storage respectively.

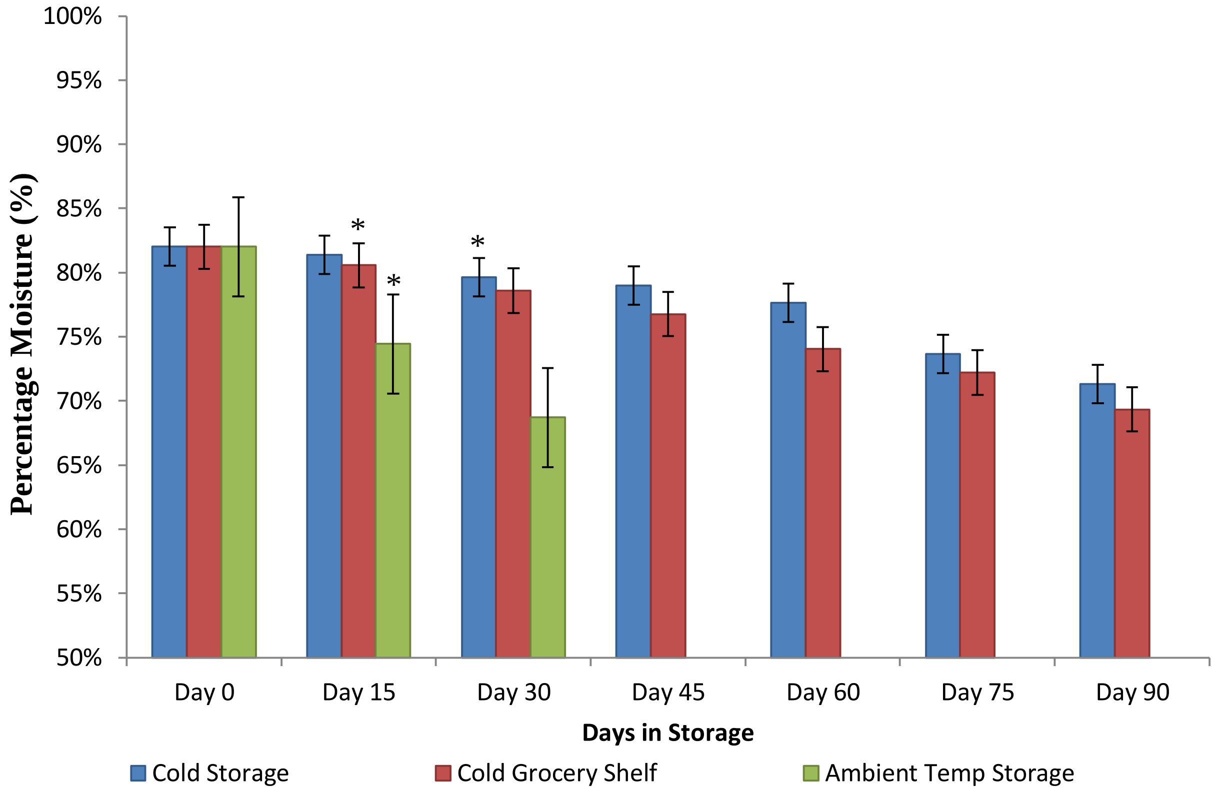
Cold room storage provided a longer retention of antioxidant activity. A similar pattern was observed in the Pink lady samples, where the first significant drop in AA was seen on day 15 for both ambient storage and cold shelf storage; and on day 30 for the cold room samples. Results from *Figure 3 and 4* imply that antioxidants and by inference, other heat sensitive nutrients in apples are retained optimally under cold storage conditions between 0 and 4 ^{o}C up to a maximum of 60 days. Beyond 60 days under cold storage, significant changes in nutritional value could occur. The Golden Delicious cultivar again performed better on the cold grocery shelf than the Pink Lady cultivar.

4.2 Percentage Moisture

Golden Delicious apples had a moisture content of 82.01 ± 0.33%, whereas fresh Pink Lady apple cultivar had 81.75 ± 1.00% moisture; falling within the moisture content range for similar apple cultivars in previous studies (Arah *et al*., 2015; Thedy *et al*., 2005). The percentage moisture results were used as reference for all changes in percentage moisture that were recorded over the storage duration under the various storage temperatures or conditions. Summary of moisture results obtained for Golden Delicious and Pink Lady cultivar are presented in Figure 5 and Figure 6 respectively.

The highest moisture content was measured in samples kept in cold storage for both Golden Delicious and Pink Lady cultivars; followed by the samples stored on cold grocery shelves. The lowest moisture value was however observed on day 30 for apple samples stored under ambient condition. All samples stored under ambient condition were rotten after day 30. In all conditions and for both cultivars, a significant difference was observed in the percentage moisture recorded over the storage duration.

Figure 5: Percentage moisture of Golden Delicious samples stored for 90 days under various conditions.

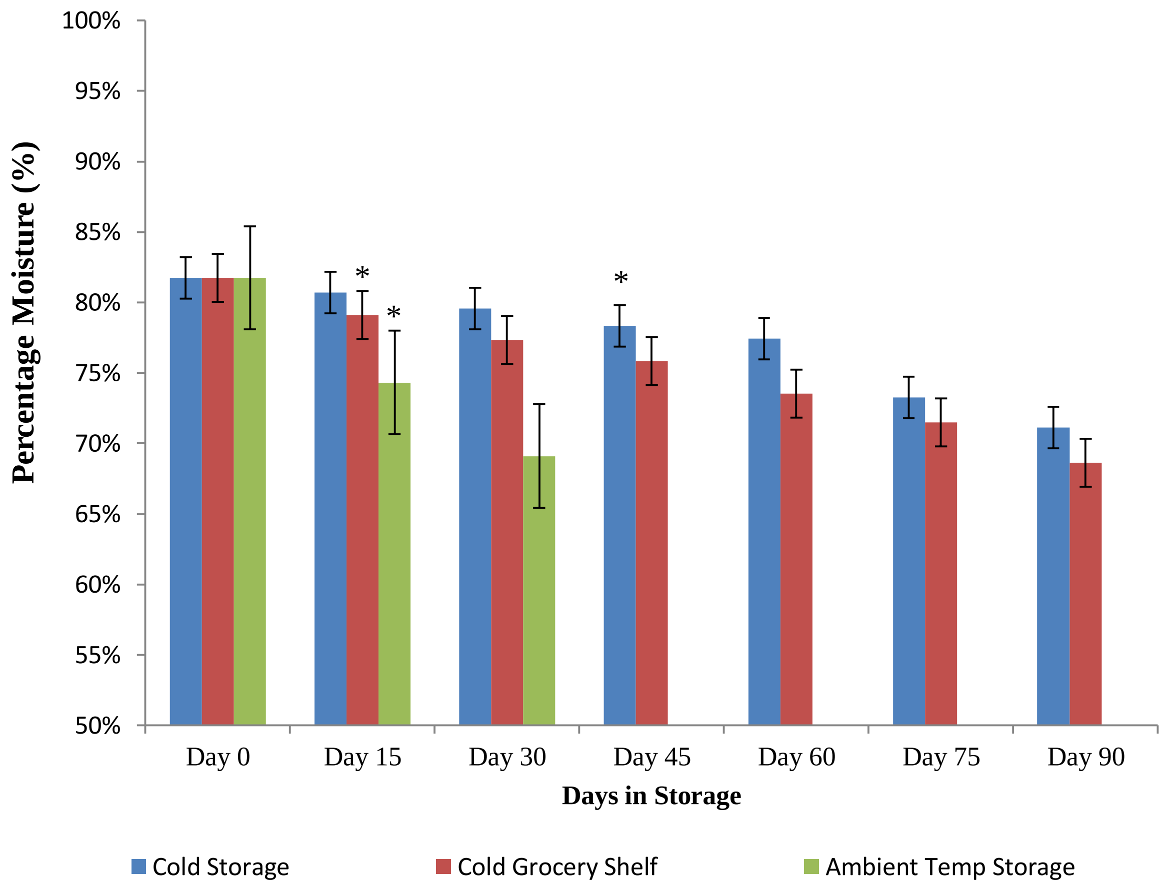


Bars with '*' shows the first incidence of significant difference
Error bars represent standard error

The significant difference recorded in the percentage moisture results shows that the incidence of lose varied across the storage duration under the three storage conditions. Under the Cold Room storage, this difference was recorded first on Day 30 and Day 45 for Golden Delicious and Pink Lady cultivars, respectively.

However, in cold shelf storage and under ambient storage, a significant difference was recorded in both cultivars on Day 15. Thus to retain the most moisture, apples stored on cold shelf must be consumed or used before 15 days.

Figure 6: Percentage moisture of Pink Lady samples stored for 90 days under different conditions



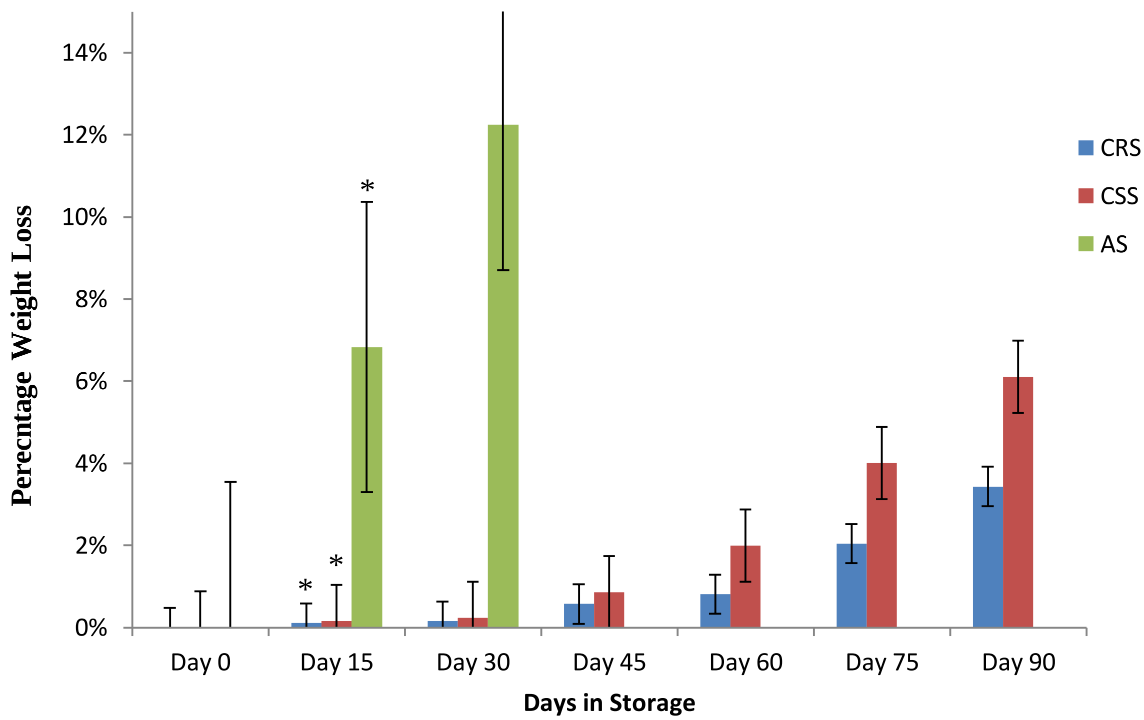
Bars with '*' shows the first incidence of significant difference.
Error bars represent standard error

4.3 Percentage Weight Loss

All through the 90-day storage period, weight loss occurred regardless of apple cultivar and storage temperature. The lowest weight loss was recorded in the cold storage samples, followed by the samples stored on cold shelf. On the 90th day in storage, the weight lost in the cold shelf samples was over 70% higher than the cold room samples, in both cultivars.

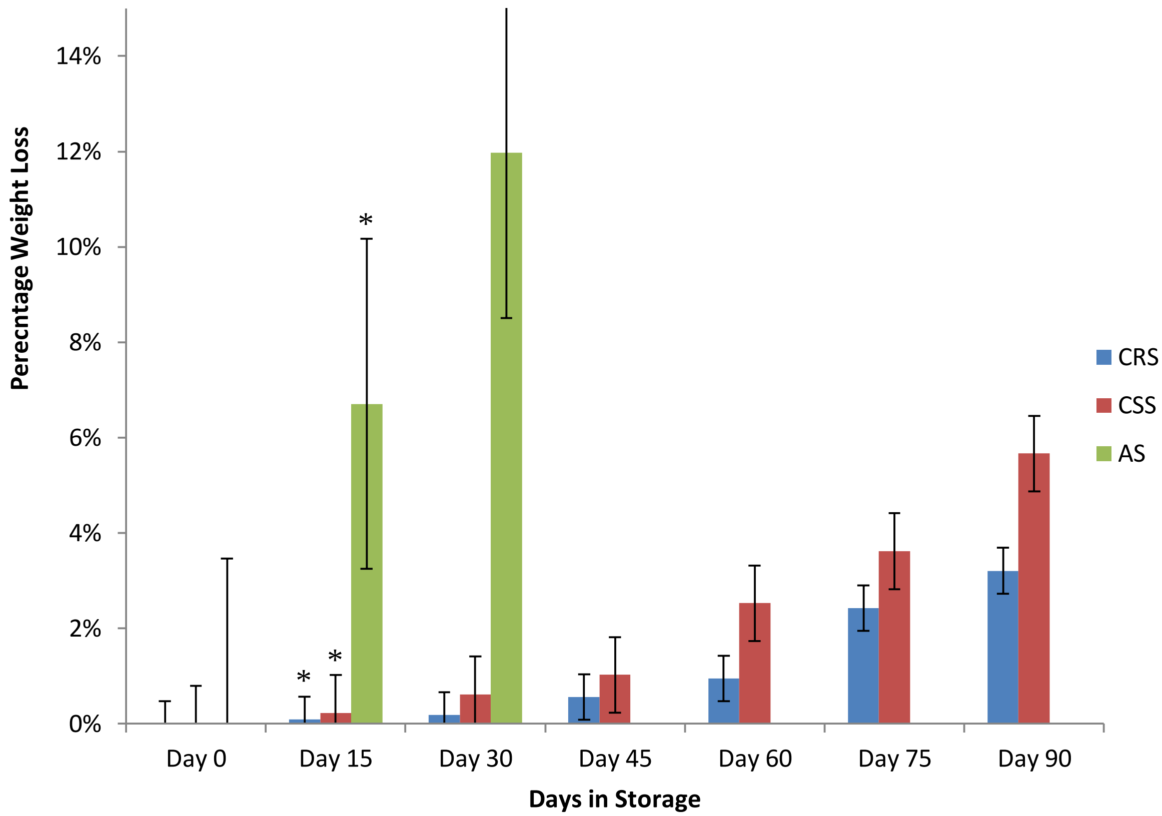
The maximum weight loss in the study was recorded on day 30 under ambient temperature. All samples stored under ambient temperature were rotten after day 30.

Figure 7: Percentage Weight loss in Golden Delicious apple samples stored over 90 days under different temperature conditions



Key: CRS= Cold Room Storage, CSS= Cold Shelf Storage, AS: Ambient Storage
Columns with '*' show the first significant weight loss during the 90-day storage
Error bars represent standard error

Figure 8: Percentage Weight loss in Pink Lady apple cultivars stored over 90 days under different temperature conditions

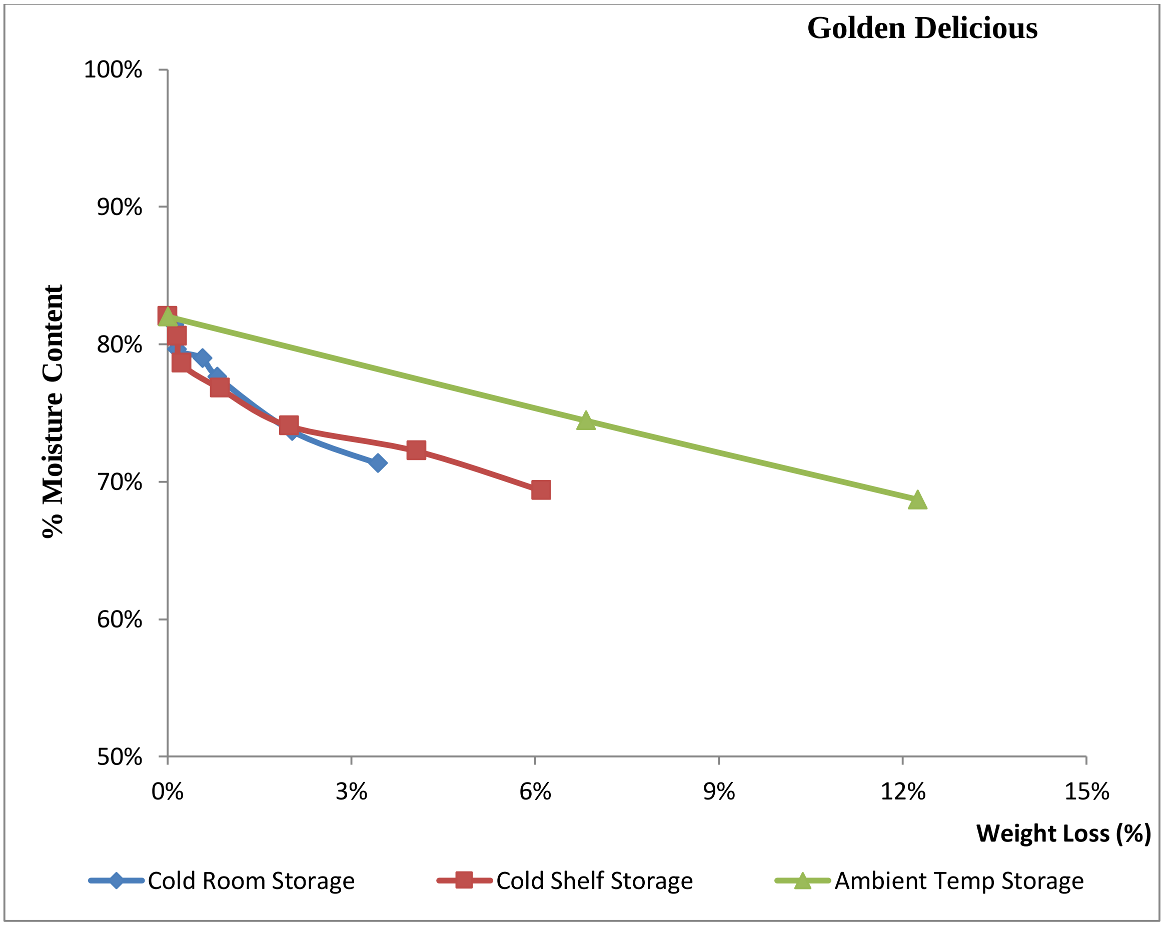


Key: CRS= Cold Room Storage, CSS= Cold Shelf Storage, AS: Ambient Storage
Columns with '*' show the first significant weight loss during the 90-day storage
Error bars represent standard error

4.4 Percentage Moisture versus Weight Loss

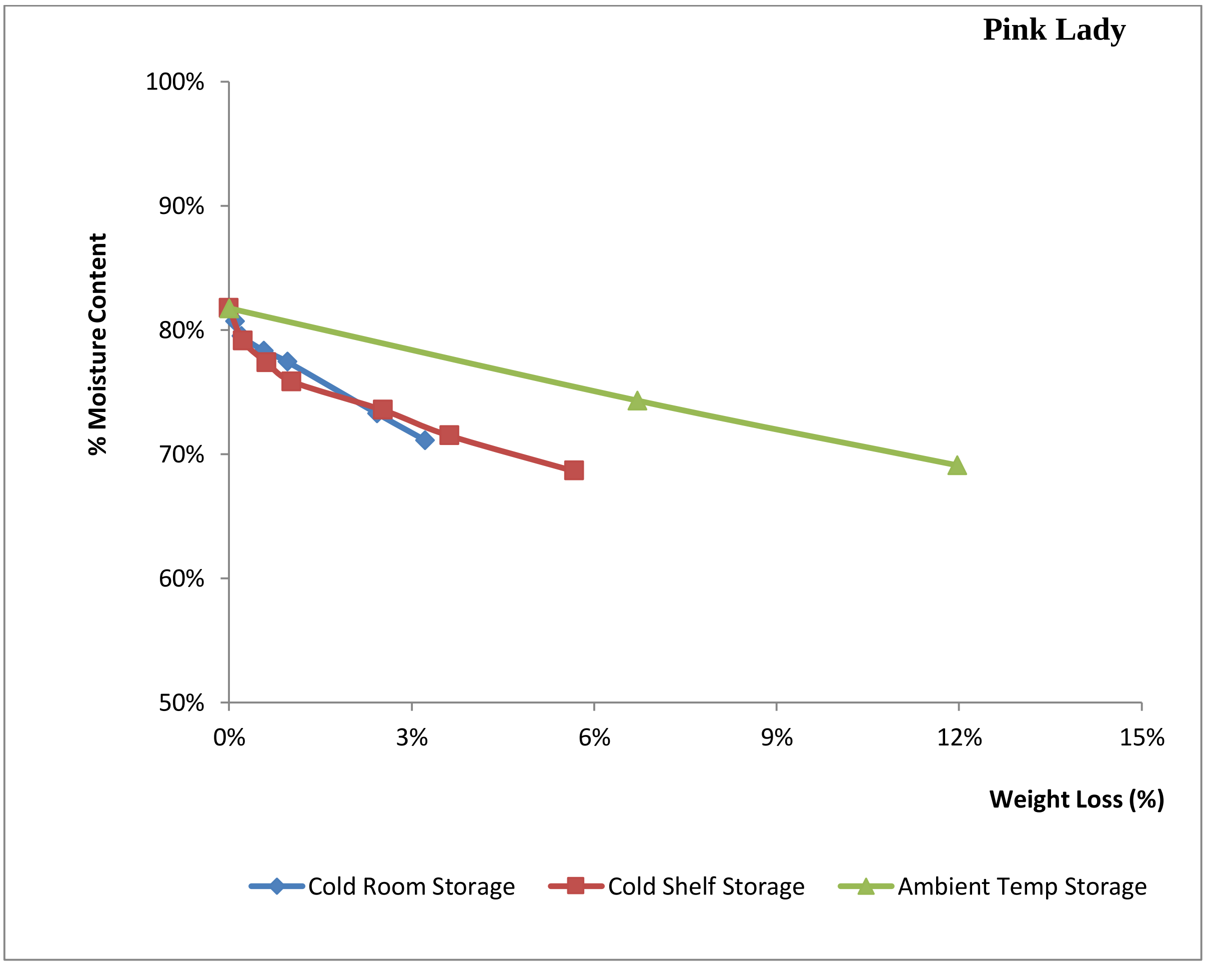
Several studies have revealed a direct relationship between moisture content and weight loss in harvested crops (Holcroft, 2015; Nunes and Emond, 2007). This corresponded with the findings of this study. Weight loss in both cultivars was highest in samples under ambient storage where moisture loss was highest; and conversely low in samples where moisture loss was lowest. In other words, weight loss increases as storage temperature increases.

Figure 9: Percentage Moisture against Weight loss in Golden Delicious apple cultivar



Transpiration and respiration in fruits are two metabolic processes that may account for this relationship in harvested foods. In transpiration, the rate at which moisture is lost from a fruit is a function of the water vapour in the air and the diffusion of water vapour through the permeable skin of the fruit, also termed as the diffusion coefficient (Becker and Fricke, n.d.). Temperature directly impacts on the amount of water vapour diffused through the fruit surface and into its surrounding. Respiration also occurs when sugars are converted into water, CO_2 and heat (Jan *et al*, 2012a). The heat generated as a result of respiration tends to increase the temperature of a food crop. This, in turn increases the water vapour pressure just below the surface of a food crop, leading to increased transpiration (Nunes and Emond, 2007). Thus, it can be said that respiration can cause transpiration to occur in apples, which results in moisture reduction and consequently loss of weight.

Figure 10: Percentage Moisture against Weight loss in Pink Lady apple cultivar



4.5 Chemical Changes

Table 1: pH, Total Soluble Solids and Titratable Acidity of 'Golden Delicious' and 'Pink Lady' samples stored under different conditions over 90 day storage duration.

Parameter	Storage Duration (Days)	Cold Storage (0 - 4°C)		Cold Grocery Shelf (10 - 18°C)		Ambient Storage (25 - 38°C)	
		Golden Delicious	Pink Lady	Golden Delicious	Pink Lady	Golden Delicious	Pink Lady
pH	0	3.24±0.00	3.22±0.00	3.24±0.00	3.22±0.00	3.24±0.00	3.22±0.00
	15	3.46±0.02	3.43±0.00	3.62±0.04	3.55±0.01	3.81±0.02	3.81±0.00
	30	3.62±0.04	3.58±0.01	3.88±0.04	3.88±0.01	4.45±0.03	4.42±0.00
	45	3.82±0.02	3.82±0.00	4.21±0.02	4.17±0.00	Samples Rotten	Samples Rotten
	60	4.19±0.02	4.19±0.01	4.58±0.03	4.53±0.01		
	75	5.22±0.10	4.86±0.04	5.54±0.05	5.02±0.00		
	90	5.62±0.06	5.32±0.03	5.63±0.04	5.22±0.04		
Total Soluble Solids (TSS) (°Brix)	0	12.25±0.02	12.26±0.01	12.25±0.02	12.26±0.01	12.25±0.02	12.26±0.01
	15	12.50±0.03	12.43±0.00	12.55±0.03	12.52±0.01	12.91±0.05	12.93±0.01
	30	13.10±0.03	13.11±0.00	13.54±0.06	13.49±0.00	13.89±0.03	13.93±0.00
	45	13.81±0.06	13.82±0.00	14.07±0.13	14.13±0.04	Samples Rotten	Samples Rotten
	60	14.09±0.06	14.01±0.00	14.28±0.04	14.22±0.00		
	75	14.22±0.03	14.17±0.03	14.35±0.02	14.38±0.00		
	90	14.33±0.03	14.27±0.02	14.41±0.02	14.46±0.02		
Titratable Acidity (TA) (g/l)	0	0.36±0.00	0.36±0.00	0.36±0.00	0.36±0.00	0.36±0.00%	0.36±0.00%
	15	0.35±0.00	0.36±0.00	0.33±0.00	0.34±0.00	0.29±0.01%	0.28±0.00%
	30	0.34±0.00	0.34±0.00	0.32±0.00	0.32±0.00	0.26±0.00%	0.26±0.00%
	45	0.32±0.00	0.32±0.00	0.29±0.01	0.28±0.00	Samples Rotten	Samples Rotten
	60	0.31±0.01	0.30±0.00	0.27±0.00	0.27±0.00		
	75	0.30±0.00	0.29±0.00	0.26±0.00	0.26±0.00		
	90	0.27±0.00	0.29±0.00	0.24±0.00	0.25±0.00		

Values are averages of triplicate determinations
Data is represented as Mean ± Standard Deviation

The lowest pH recorded in this study was 3.22 in Pink Lady cultivar and 3.24 in Golden Delicious cultivar. Throughout the study, the highest pH measured was 5.63, which occurred on day 90 for Golden Delicious cultivar. All measured pH values increased as storage duration increased. Other studies have revealed the 'market-acceptable' pH range of apples as 2.80 and 5.80, regardless of all other factors such as age after harvesting (Jan *et al*, 2012b; Kvikliene *et al*., 2006). This indicates that for both Golden Delicious and Pink Lady apple cultivars, 90 days of storage in Cold room conditions and Cold Shelf conditions does not alter the pH acceptable for marketable apples.

Total Soluble Solids (TSS) is a major characteristic of fruit quality and marketplace value. TSS was generally higher with increasing storage duration and in storage conditions with higher temperatures. The lowest TSS recorded in the study was 12.25 ^{o}Brix on day 0 for Golden Delicious apples, as compared with 12.26 ^{o}Brix for Pink Lady apples on day 0. The highest TSS was measured on day 90 for each cultivar; 14.46 ^{o}Brix for Pink Lady apples and 14.41 ^{o}Brix in Golden Delicious apples. However, all recorded values fell within the 'market-acceptable' range of 11.50 to 14.50 (Arah *et al*., 2015; Jha *et al*., 2012; Schrader *et al*., 2009). As explained by Jha *et al*., (2012) the increase in TSS during storage is due to the conversion of starch present into simple sugars such as glucose, fructose and sucrose. Thus, for the purpose of extraction in juice manufacturing where higher sweetness may be desired, late stored apples may be a preferable and more economical option.

Titratable acidity (TA) of the test samples measured over the storage duration, decreased with longer storage duration, but gave results within the acceptable range for consumer acceptability (Jan *et al*, 2012a). Fresh samples with the lowest pH values recorded the highest acidity. Acidity decreased for both cultivars as storage duration increased, as pH increased and TSS also increased.

Many apple cultivars are very acidic on harvesting, but improve in flavour with storage as a result of the drop in titratable acidity levels (Corrigan *et al*., 1997). The titratable acidity of a fruit is influenced by its rate of metabolism particularly respiration which uses up organic acids and consequently decrease acidity (Schrader *et al*., 2009). The fruit being living tissue respire even after harvesting from the tree and during storage which consume the organic acids and hence decrease the titratable acidity of the fruit.

4.6 Physical Changes

Soft rot, Bitter pit incidence and discolouration were the physical defects that were monitored in this study. In all of these defects observed, apple samples of both cultivars kept in cold storage showed better performance (Figure 11). The first incidence of soft rot was seen in 10% (Figure 11) of the samples for both cultivars, on the 60th day in Cold Room storage. This increased to 45% for Golden Delicious and slightly to 15% in the Pink Lady cultivar by Day 90 (Figure 11). Bitter pit incidence was also first observed on Day 75 in 10% of the Golden Delicious samples in Cold Room storage; and on the 90th day in 5% of the Pink lady samples (Figure 11).

No discolouration was observed in the Golden Delicious samples until day 75 and on day 90 in the Pink Lady samples. The late observation of discolouration in the Pink lady samples can be attributed to its red skin colour. Unlike the Golden Delicious samples where the slightest change of the bright green colour to yellow is easily noticeable, the red colour of the Pink lady samples has the tendency to mask slight colour changes that may occur.

Bitter pit is a deficiency of calcium within a fruit as it develops on a tree. The sensitivity to bitter pit depends on genetic factors as well as growth conditions and maturity at harvest (Jan *et al*, 2012b), thus significant variations may be observed in different apple cultivars. Bitter pit is very common in apples and its development can be accelerated by the storage environment of the apples.

It was observed in this study that, in cold storage, Golden Delicious cultivar showed more susceptibility to bitter pit incidence than Pink Lady cultivar, even though this occurred late in storage. In the Cold Shelf Storage samples, incidence of bitter pit was at Day 75 for both Golden Delicious and Pink Lady cultivar at 25% incidence. No further incidence was observed in Pink Lady cultivar at day 90 in storage. An increase from 25% to 40% was however seen in Golden Delicious cultivar (Figure 12).

Figure 11: Percentage of physical defects in Cold room storage samples

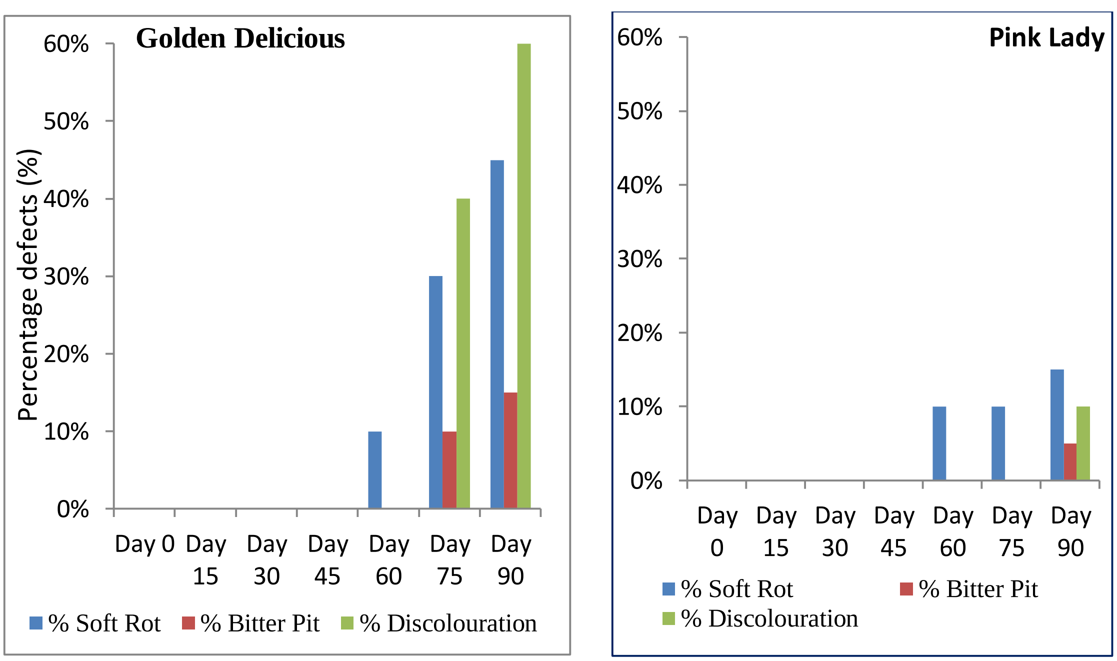
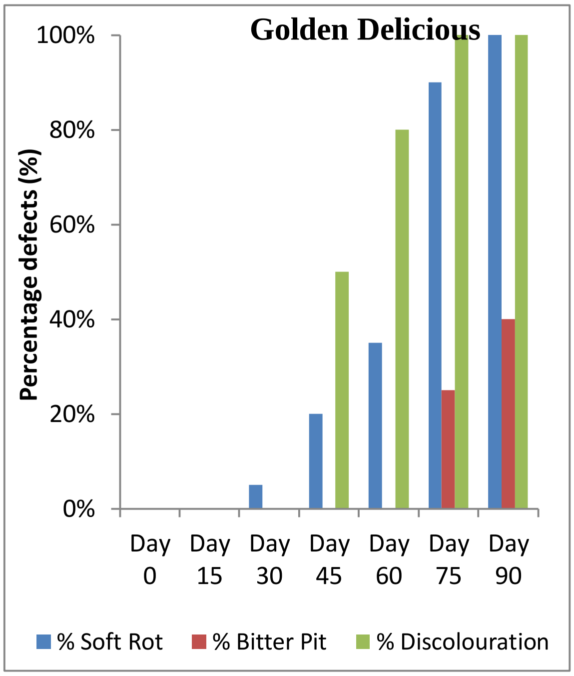


Figure 12: Percentage of physical defects in Cold shelf storage samples



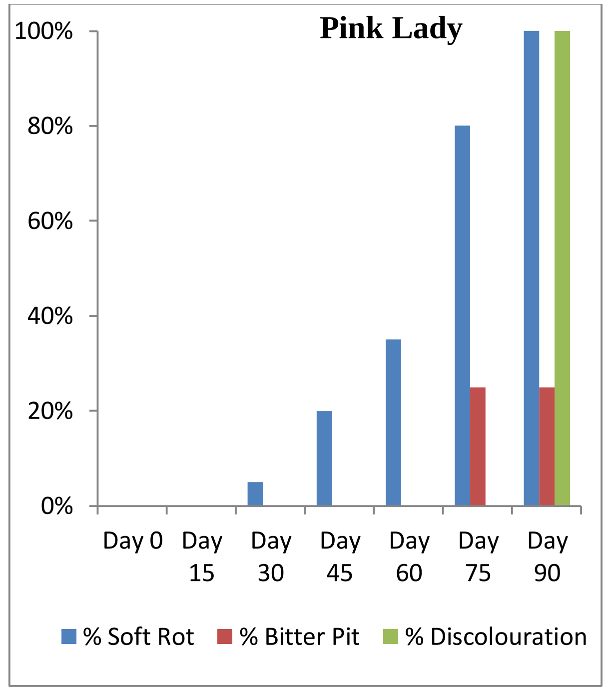
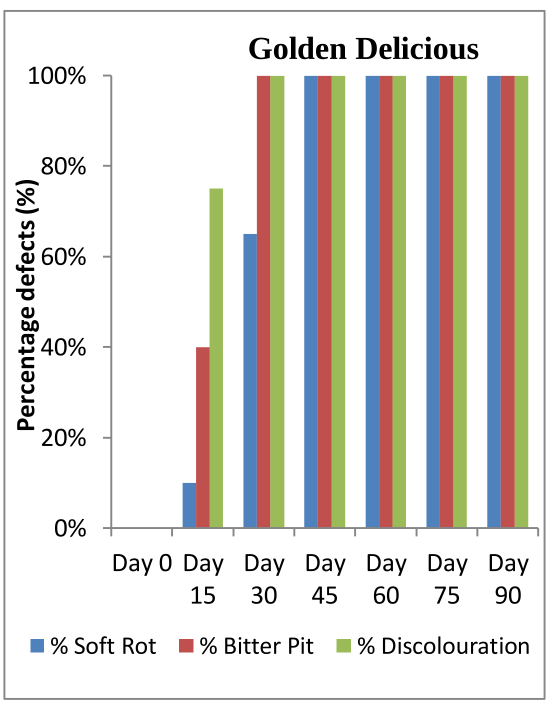
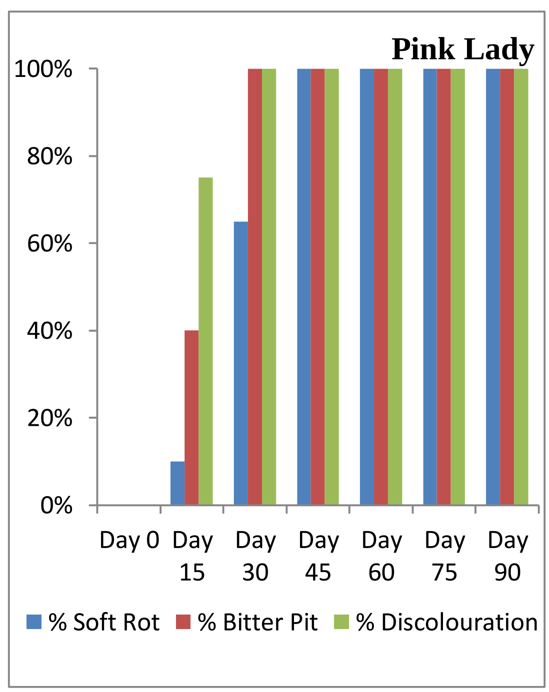


Figure 13: Percentage of physical defects in Ambient storage samples





For samples stored under ambient conditions, a different trend was observed for the incidence of defects. On day 15 in storage, 10% of the samples of both cultivars had developed soft rot (Figure 13). This increased to 65% by day 30 and 100% by day 30. Samples were visibly rotten after day 30 (Figure 13). Again on day 15, 40% of samples from both cultivars had bitter pit formation and 75% of them had discoloured (Figure 13). Incidence of bitter pit and discolouration had progressed to 100% by Day 30. All samples were visibly rotten by after day 30.

Weight loss correlates with the visual quality of fruits (Nunes and Emond, 2007). It was therefore expected that samples in ambient storage, which had recorded the highest loss of moisture and weight, would record more visual defects than samples stored in the other storage conditions (cold room and cold shelf storage).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The measured quality parameters of the freshly imported apples samples gave results as follows. For Golden Delicious cultivar: 73% antioxidant activity, 80% moisture, pH of 3.34, total soluble solids of 11.45 ^{o}Brix and 3.45 g/L acidity. For Pink Lady cultivar: 79% antioxidant activity, 79% moisture, pH of 3.46, total soluble solids of 11.48 ^{o}Brix and acidity of 3.42 g/L. No physical defects were observed on the fresh samples selected.

Cold room storage of apples for both Golden Delicious and Pink Lady cultivar was found to retain most of the quality characteristics measured on import, within a 90 day storage period as compared with storage on a Cold Grocery shelf and storage under ambient conditions. Cold room storage samples in both cultivars retained the highest levels of antioxidant activity as storage duration progressed. Samples stored in cold rooms were again able to retain the highest amount of moisture and maintain the least weight loss while in storage.

The changes in pH, total soluble solids and titratable acidity, which occurred in the samples from different storage conditions, were found to be within the range suitable for consumer acceptance; regardless of the duration and temperature in storage. Samples under ambient storage were however rotten after day 30.

In terms of physical defects observed, incidence of bitter pit, soft rot and discolouration were observed later in samples from cold room storage than in all other storage conditions. Apples stored under ambient condition were noticed to develop bitter pit, soft rot and discolouration making it unacceptable for market sale after day 15. Further to that, all samples under ambient storage were completely rotten by the 30th day in storage.

5.2 Recommendation

Improved ways of retailing apples to optimize quality could be investigated, as street hawking of apples have become very popular in Ghana. The technology of irradiation in delaying quality deterioration of imported apples could also be studied.

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APPENDIX

1.0 ANTIOXIDANT ACTIVITY

1.1.0 ANTIOXIDANT ACTIVITY: GOLDEN DELICIOUS SAMPLES

1.1.1 ANOVA: Cold Room Storage

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2650.70881	6	441.7848	21.5883351	2.53E-06	2.847726
Within Groups	286.496721	14	20.464052			
Total	2937.20553	20				

1.1.2 t-Test: Two-Sample Assuming Unequal Variances (Cold Room Storage)

	Day 0	*Day 15*
Mean	73.902	70.62
Variance	33.938	26.77
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	0.7289	
P(T<=t) one-tail	0.2532	
t Critical one-tail	2.1318	
P(T<=t) two-tail	0.5065	
t Critical two-tail	2.7764	

	Day 0	*Day 30*
Mean	73.902	66.57
Variance	33.938	26.15
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	1.6379	
P(T<=t) one-tail	0.0884	
t Critical one-tail	2.1318	
P(T<=t) two-tail	0.1768	
t Critical two-tail	2.7764	

	Day 0	*Day 45*
Mean	73.902	62.06
Variance	33.938	20.66
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	2.776	
P(T<=t) one-tail	0.025	
t Critical one-tail	2.1318	
P(T<=t) two-tail	0.05	
t Critical two-tail	2.7764	

	Day 0	*Day 60*
Mean	73.902	60.36
Variance	33.938	21.1
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	3.1608	
P(T<=t) one-tail	0.0171	
t Critical one-tail	2.1318	
P(T<=t) two-tail	0.0342	
t Critical two-tail	2.7764	

1.1.3 ANOVA: Cold Shelf Storage

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4128.21096	6	688.03516	31.6866872	2.271E-07	2.847726
Within Groups	303.991773	14	21.713698			
Total	4432.20273	20				

1.1.4 t-Test: Two-Sample Assuming Unequal Variances (Cold Shelf Storage)

	Day 0	*Day 15*
Mean	73.902	70.62
Variance	33.938	26.77
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	0.7289	
P(T<=t) one-tail	0.2532	
t Critical one-tail	2.1318	
P(T<=t) two-tail	0.5065	
t Critical two-tail	2.7764	

	Day 0	*Day 30*
Mean	73.902	57.08
Variance	33.938	37.78
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	3.4397	
P(T<=t) one-tail	0.0132	
t Critical one-tail	2.1318	
P(T<=t) two-tail	0.0263	
t Critical two-tail	2.7764	

1.1.5 ANOVA: Ambient Storage

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1616.36886	2	808.18443	24.5474921	0.0012916	5.143253
Within Groups	197.539796	6	32.923299			
Total	1813.90865	8				

1.1.6 t-Test: Two-Sample Assuming Unequal Variances (Ambient Storage)

	Day 0	*Day 15*
Mean	73.902393	59.21
Variance	33.937706	31.08
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	3.1551917	
P(T<=t) one-tail	0.0171711	
t Critical one-tail	2.1318468	
P(T<=t) two-tail	0.0343422	
t Critical two-tail	2.7764451	

1.2.0 ANTIOXIDANT ACTIVITY: PINK LADY SAMPLES

1.2.1 ANOVA: Cold Room Storage

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2771.594	6	461.9323	25.78023	8.4E-07	2.847726
Within Groups	250.8531	14	17.91808			
Total	3022.447	20				

1.2.2 t-Test: Two-Sample Assuming Unequal Variances (Cold Room Storage)

	Day 0	*Day 15*
Mean	79	73.959
Variance	6.4	7.1623
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	2.39	
P(T<=t) one-tail	0.04	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.07	
t Critical two-tail	2.78	

	Day 0	Day 30
Mean	79	67.175
Variance	6.4	11.221
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	4.9	
P(T<=t) one-tail	0	
t Critical one-tail	2.13	
P(T<=t) two-tail	0.01	
t Critical two-tail	2.78	

1.2.3 ANOVA: Cold Shelf Storage

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	4512.458	6	752.0764	57.34378	4.69E-09	2.847726
Within Groups	183.6131	14	13.11522			
Total	4696.071	20				

1.2.4 t-Test: Two-Sample Assuming Unequal Variances (Cold Room Storage)

	Day 0	Day 15
Mean	79.0465	67.7219
Variance	6.39774	14.5813
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	4.28246	
P(T<=t) one-tail	0.0117	
t Critical one-tail	2.35336	
P(T<=t) two-tail	0.02339	
t Critical two-tail	3.18245	

1.2.5 ANOVA: Ambient Storage

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2629.696	2	1314.848	87.66458	3.62E-05	5.143253
Within Groups	89.99175	6	14.99862			
Total	2719.688	8				

1.2.6 t-Test: Two-Sample Assuming Unequal Variances (Cold Room Storage)

	Day 0	*Day 15*
Mean	79.0465	59.2425
Variance	6.39774	16.5106
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	7.16668	
P(T<=t) one-tail	0.0028	
t Critical one-tail	2.35336	
P(T<=t) two-tail	0.0056	
t Critical two-tail	3.18245	

2.0 PERCENTAGE MOISTURE

2.1.0 PERCENTAGE MOISTURE: GOLDEN DELICIOUS SAMPLES

2.1.1 ANOVA: Cold Room Storage

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.02832251	6	0.004720418	202.4848239	8.78739E-13	2.847725996
Within Groups	0.000326374	14	2.33125E-05			
Total	0.028648885	20				

2.1.2 t-Test: Two-Sample Assuming Unequal Variances (Cold Room Storage)

	(Day 0)	*(Day 15)*
Mean	0.820136663	0.813902657
Variance	1.06788E-05	1.0453E-05
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	2.348876705	
P(T<=t) one-tail	0.039305879	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.078611757	
t Critical two-tail	2.776445105	

	(Day 0)	(Day 30)
Mean	0.820136663	0.796303556
Variance	1.06788E-05	1.50887E-06
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	11.82446358	
P(T<=t) one-tail	0.000650168	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.001300337	

2.1.3 ANOVA: Cold Shelf Storage

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.037982013	6	0.006330335	120.4001531	3.12486E-11	2.847725996
Within Groups	0.000736085	14	5.25775E-05			
Total	0.038718097	20				

2.1.4 t-Test: Two-Sample Assuming Unequal Variances (Cold Shelf Storage)

	(Day 0)	(Day 15)
Mean	0.820136663	0.805691054
Variance	1.06788E-05	7.12411E-06
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	5.929954393	
P(T<=t) one-tail	0.00202656	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.00405312	
t Critical two-tail	2.776445105	

2.1.5 ANOVA: Ambient Storage

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.01165642	2	0.00582821	39.05662596	0.000362961	5.14325285
Within Groups	0.000895348	6	0.000149225			
Total	0.012551768	8				

2.1.6 t-Test: Two-Sample Assuming Unequal Variances (Ambient Storage)

	(Day 0)	*(Day 15)*
Mean	0.820136663	0.744432
Variance	1.06788E-05	0.000102
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	12.32989426	
P(T<=t) one-tail	0.003256806	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.006513612	
t Critical two-tail	4.30265273	

2.2.0 PERCENTAGE MOISTURE: PINK LADY SAMPLES

2.2.1 ANOVA: Cold Room Storage

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.02741088	6	0.00456848	85.22788994	3.28E-10	2.847726
Within Groups	0.000750444	14	5.36031E-05			
Total	0.028161323	20				

2.2.2 t-Test: Two-Sample Assuming Unequal Variances (Cold Room Storage)

	(Day 0)	*(Day 15)*
Mean	0.817490348	0.80703088
Variance	0.000100382	8.4743E-05
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	1.331489908	
P(T<=t) one-tail	0.126920072	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.253840143	
t Critical two-tail	2.776445105	

	(Day 0)	(Day 30)
Mean	0.817490348	0.79551804
Variance	0.000100382	6.5649E-06
Observations	3	3
Hypothesized Mean Difference	0	
df	2	
t Stat	3.680040061	
P(T<=t) one-tail	0.033276799	
t Critical one-tail	2.91998558	
P(T<=t) two-tail	0.066553598	
t Critical two-tail	4.30265273	

	(Day 0)	(Day 45)
Mean	0.817490348	0.78359152
Variance	0.000100382	2.4447E-05
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	5.255191354	
P(T<=t) one-tail	0.006710782	
t Critical one-tail	2.353363435	
P(T<=t) two-tail	0.013421565	
t Critical two-tail	3.182446305	

2.2.3 ANOVA: Cold Shelf Storage

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.036804313	6	0.006134052	97.81171302	1.29E-10	2.847726
Within Groups	0.00087798	14	6.27129E-05			
Total	0.037682294	20				

2.2.4 t-Test: Two-Sample Assuming Unequal Variances (Cold Shelf Storage)

	(Day 0)	(Day 15)
Mean	0.817490348	0.791157
Variance	0.000100382	9.08E-05
Observations	3	3
Hypothesized Mean Difference	0	
df	4	
t Stat	3.298988017	
P(T<=t) one-tail	0.014980912	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.029961825	
t Critical two-tail	2.776445105	

2.2.5 ANOVA: Ambient Storage

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.033019881	2	0.016509941	286.2384946	1.12E-06	5.143253
Within Groups	0.000346074	6	5.7679E-05			
Total	0.033365955	8				

2.2.6 t-Test: Two-Sample Assuming Unequal Variances (Ambient Storage)

	(Day 0)	*(Day 15)*
Mean	0.81749	0.687054
Variance	0.0001	1.89E-05
Observations	3	3
Hypothesized Mean Difference	0	
df	3	
t Stat	20.68206	
P(T<=t) one-tail	0.000124	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.000247	
t Critical two-tail	3.182446	

3.0 WEIGHT LOSS

3.1.0 WEIGHT LOSS: GOLDEN DELICIOUS SAMPLES

3.1.1 ANOVA: Cold Room Storage

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.00973	6	0.0016212	519.2845	1.195E-51	2.246408
Within Groups	0.0002	63	3.122E-06			
Total	0.00992	69				

3.1.2 t-Test: Two-Sample Assuming Unequal Variances (Cold Room Storage)

	Wt 1 (0)	*Wt (Day 15)*
Mean	0	0.0011173
Variance	0	1.563E-08
Observations	10	10
Hypothesized Mean Difference	0	
df	9	
t Stat	-28.262	
P(T<=t) one-tail	2.1E-10	
t Critical one-tail	1.83311	
P(T<=t) two-tail	4.2E-10	
t Critical two-tail	2.26216	

3.1.3 ANOVA: Cold Shelf Storage

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.03294	6	0.0054907		7.018E-55	2.246408
Within Groups	0.00052	63	8.312E-06	660.6031		
Total	0.03347	69				

3.1.4 t-Test: Two-Sample Assuming Unequal Variances (Cold Shelf Storage)

	Wt 1 (0)	*Wt (Day 15)*
Mean	0	0.0016059
Variance	0	3.514E-08
Observations	10	10
Hypothesized Mean Difference	0	
df	9	
t Stat	-27.09178	
P(T<=t) one-tail	3.081E-10	
t Critical one-tail	1.8331129	
P(T<=t) two-tail	6.161E-10	
t Critical two-tail	2.2621572	

3.1.5 ANOVA: Ambient Storage

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.07522	2	0.0376085	928.68693	1.284E-25	3.3541308
Within Groups	0.00109	27	4.05E-05			
Total	0.07631	29				

3.1.6 t-Test: Two-Sample Assuming Unequal Variances (Ambient storage)

	Wt 1 (0)	*Wt (Day 15)*
Mean	0	0.0682612
Variance	0	2.415E-06
Observations	10	10
Hypothesized Mean Difference	0	
df	9	
t Stat	-138.893	
P(T<=t) one-tail	1.321E-16	
t Critical one-tail	1.8331129	
P(T<=t) two-tail	2.642E-16	
t Critical two-tail	2.2621572	

3.2.0 **WEIGHT LOSS:** PINK LADY SAMPLES

3.2.1 ANOVA: Cold Room Storage

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.00959	6	0.0015981	363.75217	6.679E-47	2.246408
Within Groups	0.00028	63	4.393E-06			
Total	0.00987	69				

3.2.2 t-Test: Two-Sample Assuming Unequal Variances (Cold Room Storage)

	Wt 1 (0)	*Wt (Day 15)*
Mean	0	0.0008545
Variance	0	1.181E-07
Observations	10	10
Hypothesized Mean Difference	0	
df	9	
t Stat	-7.8631	
P(T<=t) one-tail	1E-05	
t Critical one-tail	1.8331	
P(T<=t) two-tail	3E-05	
t Critical two-tail	2.2622	

3.2.3 ANOVA: Cold Shelf Storage

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.02635	6	0.0043922	215.47045	5.137E-40	2.246408
Within Groups	0.00128	63	2.038E-05			
Total	0.02764	69				

3.2.4 t-Test: Two-Sample Assuming Unequal Variances (Cold Shelf Storage)

	Wt 1 (0)	*Wt (Day 15)*
Mean	0	0.0023279
Variance	0	3.228E-08
Observations	10	10
Hypothesized Mean Difference	0	
df	9	
t Stat	-40.97353	
P(T<=t) one-tail	7.653E-12	
t Critical one-tail	1.8331129	
P(T<=t) two-tail	1.531E-11	
t Critical two-tail	2.2621572	

3.2.5 ANOVA: Ambient Storage

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.07204	2	0.036018	227.01443	1.3E-17	3.3541308
Within Groups	0.00428	27	0.0001587			
Total	0.07632	29				

3.2.6 t-Test: Two-Sample Assuming Unequal Variances (Ambient Storage)

	Wt 1 (0)	*Wt (Day 15)*
Mean	0	0.06711
Variance	0	8.3E-05
Observations	10	10
Hypothesized Mean Difference	0	
df	9	
t Stat	-23.31883	
P(T<=t) one-tail	1.168E-09	
t Critical one-tail	1.8331129	
P(T<=t) two-tail	2.335E-09	
t Critical two-tail	2.2621572	