

POSTHARVEST STORAGE STUDIES IN POMEGRANATE AND KALE

by

HARWINDER SINGH SIDHU

(Under the Direction of Juan Carlos Díaz-Pérez)

ABSTRACT

Research completed from 2010 to 2012 evaluated the role of storage conditions in preserving produce quality for fresh market. Pomegranate (*Punica granatum* L.) fruit from Ponder Farm (PF) in 2010, 2011 and Alma Farm (AF) in 2011 were stored in CA (Controlled Atmosphere storage; 5% CO₂ + 3% O₂; 5°C, 90%-95% RH) and RA (Regular Air storage; 5°C, 90%-95% RH) for three months. Fruit had better quality and lesser deterioration (disease, injury and storage disorders) when stored in CA compared to RA based on physical and physiochemical quality attributes. Kale (*Brassica oleracea* var. *acephala*) leaves of two cultivars (Red Russian and Konavale 2) were studied for effect of storage temperature (5°C and 18°C) and bagging (bagging or no bagging) on leaf quality. Leaf were evaluated for weight loss, change in chlorophyll index and leaf yellowing. Kale leaves stored at low temperature (5°C) in bags maintained best quality for longest period of time.

INDEX WORDS: Pomegranate, controlled air (CA) storage, regular air (RA) storage, juice content, anthocyanins, total soluble solids (TSS), titratable acidity (TA), kale leaves, storage temperature, relative humidity, bagging.

POSTHARVEST STORAGE STUDIES IN POMEGRANATE AND KALE

by

HARWINDER SINGH SIDHU

B.S., Punjab Agricultural University, India, 2010

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment
of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2013

© 2013

HARWINDER SINGH SIDHU

All Rights Reserved

POSTHARVEST STORAGE STUDIES IN POMEGRANATE AND KALE

by

HARWINDER SINGH SIDHU

Major Professor: Juan Carlos Díaz-Pérez

Committee: Anish Malladi
Smiljana Goreta-Ban

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2013

DEDICATION

I dedicate this work to my family for always being supportive. I would particularly thank my father Karam Singh and mother Kanwaljit Kaur for keeping me motivated all along. I thank my wife Rattandeep who helped, guided and encouraged me to give my best at all times.

ACKNOWLEDGEMENTS

Dr. Juan Carlos Díaz-Pérez, I am lucky to have you as advisor. You are a great mentor and guide with a lot of patience who always cooperated the best way possible. Thank you for your knowledge and insight which helped me to get this work completed.

Dr. Daniel (Dan) MacLean, thank you for the amazing opportunity at UGA you provided me. It was a great experience working with you and I learnt a lot.

Dr. Anish Malladi, thank you for your expertise and suggestions in the thesis. Your seminar class helped me many times for presentations and organizing this thesis.

Dr. Smiljana Goreta-Ban thank you for your insights in pomegranate studies and it was good to know your work while I was in Croatia.

Mr. Anthony Bateman, thank you for the technical help you provided at innumerable times. Finally I would like to thank my fellow graduate students in horticulture for being such nice friends and colleagues.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	v
LIST OF TABLES	viii
LIST OF FIGURES	xiii
CHAPTER	
1 INTRODUCTION	1
Pomegranate.....	1
Kale	2
References	3
2 LITERATURE REVIEW	4
Pomegranate.....	4
Kale	7
References	9
3 POMEGRANATE FRUIT QUALITY IS AFFECTED BY CONTROLLED AIR STORAGE	13
Abstract	14
Introduction.....	15
Objectives	22
Materials and Methods.....	22
Results.....	26

Discussion	33
Conclusion	37
References	38
4 CULTIVAR, STORAGE TEMPERATURE AND BAGGING AFFECT KALE	
WATER LOSS, QUALITY AND SHELF LIFE	81
Abstract	82
Introduction	83
Objective	85
Materials and Methods	85
Results	87
Discussion	90
Conclusion	92
References	93
5 CONCLUSION	113
Pomegranate	113
Kale	113
Summary	114

LIST OF TABLES

	Page
Table 3.1: Physical attributes of pomegranate fruit harvested from Ponder Farm in 2010	
immediately after storage [(cold storage in either controlled atmosphere storage (CA; 5% CO ₂ and 3% O ₂ , 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)] or after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) following storage.	49
Table 3.2: Physical attributes of pomegranate fruit harvested from Ponder Farm in 2011	
immediately after storage [(cold storage in either controlled atmosphere storage (CA; 5% CO ₂ and 3% O ₂ , 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)]	52
Table 3.3: Physical attributes of pomegranate fruit harvested from Alma Farm in 2011	
immediately after storage [(cold storage in either controlled atmosphere storage (CA; 5% CO ₂ and 3% O ₂ , 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)] or after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) following storage	54
Table 3.4: Effect of cultivar and storage [controlled air (CA; 5% CO ₂ and 3% O ₂ , 5°C and 90%-95% RH) or regular air (RA; 5°C and 90%-95% RH)] interaction immediately after storage and after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) on various attributes of pomegranate fruit from Ponder Farm, Ty Ty, GA, 2010.....	56

Table 3.5: Effect of cultivar and storage [controlled air (CA; 5% CO ₂ and 3% O ₂ , 5°C and 90%-95% RH) or regular air (RA; 5°C and 90%-95% RH)] interaction immediately after storage and after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) on various attributes of pomegranate fruit from Alma Farm, Alma, GA, 2011	58
Table 3.6: Effect of cultivar maturity interaction on skin smoothness of pomegranate fruit from Ponder Farm, Ty Ty, GA, 2010	60
Table 3.7: Effect of cultivar and storage [controlled air (CA; 5% CO ₂ and 3% O ₂ , 5°C and 90%-95% RH); regular air (RA; 5°C and 90%-95% RH)] on various fruit attributes in fruit from Ponder farm 2010	61
Table 3.8: Effect of fruit maturity (early and late) and storage [controlled air (CA; 5% CO ₂ and 3% O ₂ , 5°C and 90%-95% RH) or regular air (RA; 5°C and 90%-95% RH)] interaction for attributes immediately after storage and after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) in pomegranate fruit harvested from Ponder farm, 2010	62
Table 3.9: Effect of cultivar and storage [(cold storage in either controlled atmosphere storage (CA; 5% CO ₂ and 3% O ₂ , 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)] interaction immediately after storage or after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) following storage on various attributes in pomegranate fruit harvested from Alma farm, 2011	63
Table 3.10: Juice attributes of pomegranate fruit harvested from Ponder Farm in 2010 immediately after storage [(cold storage in either controlled atmosphere storage (CA; 5% CO ₂ and 3% O ₂ , 5°C and 90%-95% RH) or regular air storage (RA; 5°C and	

90%-95% RH)] or after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D)	
following storage	65
Table 3.11: Juice attributes of pomegranate fruit harvested from Ponder Farm in 2011	
immediately after storage [(cold storage in either controlled atmosphere storage (CA;	
5% CO ₂ and 3% O ₂ , 5°C and 90%-95% RH) or regular air storage (RA; 5°C and	
90%-95% RH)]	68
Table 3.12: Juice attributes of pomegranate fruit harvested from Alma Farm in 2011 immediately	
after storage [(cold storage in either controlled atmosphere storage (CA; 5% CO ₂ and	
3% O ₂ , 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)] or	
after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) following storage	69
Table 3.13: Effect of cultivar and storage [(cold storage in either controlled atmosphere storage	
(CA; 5% CO ₂ and 3% O ₂ , 5°C and 90%-95% RH) or regular air storage (RA; 5°C and	
90%-95% RH)] interaction immediately after storage or after 7 days at 25°C and	
45%-50% RH (CA+7D & RA+7D) following storage on juice attributes in	
pomegranate fruit harvested from Alma farm, 2011	70
Table 3.14: Effect of cultivar and storage [(cold storage in either controlled atmosphere storage	
(CA; 5% CO ₂ and 3% O ₂ , 5°C and 90%-95% RH) or regular air storage (RA; 5°C and	
90%-95% RH)] interaction on total soluble solids (TSS) of pomegranate fruit	
immediately after storage harvested from Ponder farm in 2011	71
Table 3.15: Anthocyanins content of pomegranate fruit harvested from Ponder farm 2010	
immediately after storage [(cold storage in either controlled atmosphere storage (CA;	
5% CO ₂ and 3% O ₂ , 5°C and 90%-95% RH) or regular air storage (RA; 5°C and	

90%-95% RH)] or after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D)	
following storage.	72
Table 3.16: Anthocyanins content of pomegranate fruit harvested from Ponder farm 2011	
immediately after storage [(cold storage in either controlled atmosphere storage (CA;	
5% CO ₂ and 3% O ₂ , 5°C and 90%-95% RH) or regular air storage (RA; 5°C and	
90%-95% RH)].	74
Table 3.17: Anthocyanins content of pomegranate fruit harvested from Alma Farm 2011	
immediately after storage [(cold storage in either controlled atmosphere storage (CA;	
5% CO ₂ and 3% O ₂ , 5°C and 90%-95% RH) or regular air storage (RA; 5°C and	
90%-95% RH)] or after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D)	
following storage	76
Table 3.18: Fruit husk color attributes of fruit from Ponder Farm 2010 immediately after storage	
[(cold storage in either controlled atmosphere storage (CA; 5% CO ₂ and 3% O ₂ , 5°C	
and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)] or after 7	
days at 25°C and 45%-50% RH (CA+7D & RA+7D) following storage.....	77
Table 3.19: Fruit husk color attributes of fruit from Alma Farm 2011 immediately after storage	
[(cold storage in either controlled atmosphere storage (CA; 5% CO ₂ and 3% O ₂ , 5°C	
and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)] or after 7	
days at 25°C and 45%-50% RH (CA+7D & RA+7D) following storage.....	78
Table 4.1: Fresh weight, dry weight and leaf area of kale cultivars Konavale 2 and Red Russian	
in a two year study in Tifton, GA	96

Table 4.2: Weight loss (% of initial weight) of field grown kale leaves of cultivars Konavale 2 and Red Russian stored bagged or non-bagged (open) at either 5°C or 18°C in a two year study in Tifton, GA	97
Table 4.3: Chlorophyll index (CI) in field grown kale leaves of cultivars Konavale 2 and Red Russian stored bagged or non-bagged (open) at either 5°C or 18°C in a two year study in Tifton, GA.....	98
Table 4.4: Yellowing ^z of field grown kale leaves of cultivars Konavale 2 and Red Russian stored bagged and non-bagged (open) at either 5°C or 18°C in year 2012.....	99
Table 4.5: Lightness (L*) ^z of kale leaves from cultivars Konavale 2 and Red Russian stored bagged or non-bagged (open) at either 5°C or 18°C in a two year study.....	100
Table 4.6: Hue angle ^z of kale leaves from cultivars Konavale 2 and Red Russian stored bagged or non-bagged (open) in either 5°C or 18°C in a two year study	101
Table 4.7: Chroma ^z of kale leaves from cultivars Konavale 2 and Red Russian stored bagged or non-bagged (open) in either 5°C or 18°C in two year study	102

LIST OF FIGURES

	Page
Figure 3.1: Anthocyanins of pomegranate juice identified at 520nm in HPLC	79
Figure 3.2: Various colors represented as an angle called hue angle	80
Figure 4.1: Cumulative weight loss (% of initial weight) of field grown kale leaves from cultivars Konavale 2 and Red Russian bagged or non-bagged stored at either 5°C or 18°C in a two year study in Tifton, GA	103
Figure 4.2: Chlorophyll index of kale leaves from cultivars Konavale 2 and Red Russian bagged or non-bagged stored at either 5°C or 18°C	105
Figure 4.3: Yellowing of kale leaves stored with or without bag at either 5°C or 18°C up to 22 days in year 2012.	108
Figure 4.4: Correlation between decrease in the relative decrease in chlorophyll index (% of initial value) and yellowing of kale leaves in 2012	110
Figure 4.5: Chlorophyll index (CI) was recorded on the encircled portions of leaves generally	111
Figure 4.6: Various colors represented as an angle called hue angle	112

CHAPTER 1

INTRODUCTION

Pomegranate

Pomegranate (*Punica granatum* L.) is a fruit plant native to the region extending from Iran to the Himalayas. It is believed to be among the first fruit cultivated by humans in around 4000BC in the Middle East. Within past few thousand years it has spread all over the world, from Asia to Europe & to North America. Presently cultivated in the tropical and subtropical regions, it thrives in different climate and soil conditions and can tolerate drought and salt stress. Pomegranate fruit are consumed fresh or used for the production of wine and syrup. Pomegranate production has been increasing worldwide in response to increased popularity due to its health benefits (Basu and Penugonda, 2009).

Pomegranate fruit is spherical in shape and has a persistent calyx. Pomegranates are covered with a leathery pericarp (rind). The color of the pericarp can range from yellow-green to dark red. It develops from a flower with a hypogenous ovary. The fruit are derived from the hermaphrodite flowers. Aril is the edible portion of the fruit. It is a juicy pulp around the seeds formed from the ovules present in ovary of the fertilized fruit (Shulman et al., 1984).

Quality of pomegranate is determined by both internal and external attributes of fruit. Fruit size is determined at an early stage of fruit development and proper crop management practices are required to achieve desired fruit size (Wetzstein et al., 2011). Appearance of fruit plays an important role in marketability and it is determined by skin qualities such as skin

smoothness, shriveling and appearance. Skin appearance is affected by presence of sunscald damage, disease spots/damage to fruit and injuries, bruises and cracking on skin.

Kale

Vegetables are an excellent source of health beneficial compounds as antioxidants. Kale (*Brassica oleracea*, var. *acephala*) is one of the richest sources of antioxidants among 22 commonly used vegetables which include some other *Brassica* species, carrot and potato (Cao et al., 1996). Market of fresh vegetables as whole or minimally processed as chopped has been increasing in recent years. Leafy vegetables have been utilized as salads in many cuisines.

Kale (*Brassica oleracea*) is a crucifer but, unlike cabbage and broccoli which have edible heads and flowers, kale has large flat leaves in many cultivars but demand for curly leaves has grown over the years in market (Salunkhe and Kadam, 1998). Kale has its origin in Europe, specifically around Mediterranean and Southern Europe. Kale is a very cold tolerant plant. Various *B. oleracea* species are grown around the world under different weather conditions although being a temperate wild species from origin (Vaughan and Geissler, 2009). Kale is grown in eastern Africa, Asia, Europe and Latin America while being referred as poor man's vegetable in Africa (Mwithiga and Olwal, 2005).

References

- Basu A., Penugonda K. (2009) Pomegranate juice: a heart-healthy fruit juice. *Nutrition Reviews* 67:49-56.
- Cao G., Sofic E., Ronald L. (1996) Antioxidant capacity of tea and common vegetables. *Journal of Agricultural and Food Chemistry* 44:3426-3431.
- Mwithiga G., Olwal J.O. (2005) The drying kinetics of kale (*Brassica oleracea*) in a convective hot air dryer. *Journal of Food Engineering* 71:373-378.
- Salunkhe D.K., Kadam S. (1998) Handbook of vegetable science and technology. Production, composition, storage, and processing Marcel Dekker Inc pp 365-366.
- Shulman Y., Fainberstein L., Lavee S. (1984) Pomegranate fruit-development and maturation. *Journal of Horticultural Science* 59:265-274.
- Vaughan J., Geissler C. (2009) The new Oxford book of food plants OUP Oxford. pp 179-206.
- Wetzstein H.Y., Zhang Z., Ravid N., Wetzstein M.E. (2011) Characterization of attributes related to fruit size in pomegranate. *Hortscience* 46:908-912.

CHAPTER 2

LITERATURE REVIEW

Pomegranate

Flowers of pomegranate have been used in Unani medicines for treating diabetes by significantly reducing blood glucose levels in animals (Wang et al., 2010). Pomegranate juice has been reported to have antioxidant and antitumor activities (Singh et al., 2002). Various alkaloids, flavonoids, polyphenolic compounds and hydrolyzable tannins, such as punicalin, pedunculagin, punicalagin and ellagic acid esters of glucose, which possess strong antioxidant properties, are present in the juice of the whole fruit. The juice has shown potential anti-atherogenic properties *in vivo* (Fuhrman et al., 2005).

High temperature during fruit development can cause sunburn leading to decrease in concentration of phenolic compounds along with loss of fruit visual quality. Fruit affected by high temperature are subjected to oxidative stress which eventually develops as sunscald (Weerakkody et al., 2010). Fruit cracking influences fruit quality greatly and can cause significant economic losses in fruit production. As the fruit grow there is an increase in stress on fruit pericarp (Considine and Brown, 1981). Cracking is a problem in mature pomegranate fruit. Cracking in tomato is also influenced by day and night temperature differential, higher the difference more is the tendency of a fruit to crack (Kamimura et al., 1972).

Disease spots on pomegranate in Georgia are predominantly from *Cercospora punicae*. Black circular spots due to the fungal spores affect the fruit quality. Other fungal diseases like *Botryospheria* and *Alternaria* also influence fruit quality rendering fruit unmarketable.

Juice Properties

Pomegranate juice flavor is determined by the ratio of its sweetness to acidity. The Total Soluble Solids (TSS) content of the juice is a measure of sweetness of the juice. The TSS content of juice increases with fruit development and eventually stabilizes at maturity. Glucose and fructose are major contributors to fruit sweetness (Lee et al., 1974). Citric acid is the biggest contributor to the titratable acidity of pomegranate juice. Other organic acids as malic, fumaric, acetic, tartaric and lactic acids are also found in the fruit (Kulkarni and Aradhya. 2005).

Six anthocyanins have been reported in the pomegranate; 3-glucosides and 3, 5 diglucosides of delphinidin, cyanidin, and pelargonidin (Du et al., 1975; Gil et al., 1995; Artes et al., 1996). Color of juice is due to presence of anthocyanadins and flavan-3-ols and their concentration is dependent on stage of fruit maturity. Cyanidin 3-glucoside is found in higher concentration as compared to other anthocyanins whereas Pelargonidin 3-glucoside is usually present in scarce amount (D'Aquino et al., 2010).

Alkaloids and organic acids are the other compounds that are present in the pomegranate juice. Important alkaloids present in the juice are serotonin, melatonin and tryptamine (Badria, 2002). The organic acids present are straight chain fatty acids such as malic acid and citric acid (Neuhofer, 1990). The resulting antioxidant activity of the pomegranate juice is due to the contribution of all the potential compounds present in it.

Harvest Maturity

Maturity stage of pomegranate fruit at harvest plays an important role in fruit quality, especially its juice properties such as flavor, TSS, TA and concentration of phenolic compounds (Lopez-Rubira et al., 2005). Many irreversible physiological, biochemical changes take place in a fruit during ripening, all of which are driven genetically. Degradation of chlorophyll, increased

respiration rate, production of ethylene for ripening and biosynthesis of health beneficial compounds as anthocyanins are some of the changes that occur in a fruit during ripening (Brady, 1987). Generally there is an increase in fruit sweetness due to gluconeogenesis, breakdown of polysaccharides such as starch and a decrease in fruit acidity (Gray et al., 1992). Changes in physical appearance of fruit occur due to changes in cell wall and intercellular spaces (Tucker and Grierson, 1987). With all the changes occurring during fruit development maturity is thus an important factor in deciding harvest time of the fruit.

Postharvest Storage

Controlled atmosphere storage extends storage life of agricultural produce. It has been used successfully for maintaining quality of many tropical and sub-tropical fruit (Kader, 2001; Yahia, 2006). Storage of fruit under controlled atmosphere and cold temperature reduces fruit respiration rate, ethylene production, suppresses or delays senescence processes and eventually increasing postharvest shelf life (Beaudry, 1999; Ding et al., 1998; Smock, 1979). Increased CO₂ concentration in storage atmosphere reduces rate of respiration and CO₂ production (Hertog et al., 2003). Controlled atmosphere storage affects both host fruit and any pathogens on it. With better physiological health of fruit due to reduced senescence in CA storage, fruit can resist attack of pathogens more effectively. In a 2-month storage study in apple, CA storage reduced fruit decay as compared to regular air storage (Conway et al., 2007; Lidster et al., 1983). CA storage conditions suppress pathogen growth and spreading (Yackel et al., 1971). Storing pomegranates adequately is an important factor in maintaining regular availability of good quality fruit in market. Pomegranate fruit quality is influenced by water loss and presence of disease, injury and decay. Due to water loss shriveling symptoms appear on fruit surface along with desiccation and loss of crispness and fruit firmness. Decrease of 5% in fruit weight due to

water loss leads to visible shriveling symptoms (Palou et al., 2007). For sweet pomegranate minimum safe storage temperature is 10°C yet it does not reduce incidence of fungal decay. Decay due to various pathogens as *Botrytis cynerea*, *Alternaria spp.*, and *Aspergillus spp.* causes significant loss during storage (Mitra, 1997).

Pomegranate is a newly introduced fruit crop in the southeastern USA. Local cultivars being used for cultivation have not been studied for fruit physical and physiochemical attributes in detail. Postharvest storage of the fruit from these local cultivars is an important aspect that needed to be studied in detail.

Kale

Kale has large water content (about 85% by weight) thus it loses water quickly, wilts and perishes under room temperature (21-25°C) conditions (Imungi, 1992). Other leafy vegetables follow similar trend and perish very quickly after harvest (Paull, 1992). Water loss is a function of evaporative demand which is determined by temperature and humidity. High relative humidity during storage reduces moisture loss from the produce. Modified atmosphere packaging results in increased relative humidity of microenvironment and reduces water loss by evaporation. Enclosing leaves in polyethylene bags reduces water loss and extends the shelf life (Porat et al., 2004). Storing produce in plastic film is a vastly used method of storage for fresh market.

Storage conditions (temperature and bagging) play a very important role in postharvest shelf life of produce. It is important aspect to minimize losses of produce quality from time of harvest to produce consumption. Reducing losses during storage increases available quantity of produce for consumption, decreases pressure on production chain and thus conserving various natural resources. Visual appearance of fresh produce is an important attribute a consumer considers while buying and so do wholesalers and retailers. In kale, appearance of leaves can be

affected by damage due to insect or pest, disease, and loss of quality during postharvest handling and storage. Color is an important representative of quality and should be preserved as much as possible. Color change seems to be due to senescence which is limited by reducing storage temperature due to suppression of metabolic activities (Able et al., 2003). In many other horticultural crops color change is observed to be one of the first symptoms of senescence (Kader, 2000). Color of leaves starts turning yellow with degradation of chlorophyll. Degradation is escalated by unfavorable storage conditions such as high temperature and low relative humidity during storage. In other *Brassica* species carbohydrates have been observed to be utilized extensively due to respiration during storage (Finger et al., 1999).

Very limited literature had been available about quality changes in kale leaves during storage. Kale cultivars from Croatia (Konavale 2) and local Georgia cultivar (Red Russian) needed to be studied in detail for leaf quality changes, leaf weight loss and chlorophyll loss during storage.

References

- Able A.J., Wong L., Prasad A., O'Hare T. (2003) The effects of 1-methylcyclopropene on the shelf life of minimally processed leafy asian vegetables. *Postharvest Biology and Technology* 27:157-161.
- Artés F., Marín J.G., Martínez J.A., (1996) Controlled atmosphere storage of pomegranate. *Zeitschrift für Lebensmittel-Untersuchung und Forschung* 203:33-37.
- Badria F.A. (2002) Melatonin, serotonin, and tryptamine in some egyptian food and medicinal plants. *Journal of Medicinal Food* 5:153-157.
- Beaudry R.M. (1999) Effect of O₂ and CO₂ partial pressure on selected phenomena affecting fruit and vegetable quality (vol 15, pg 293, 1999). *Postharvest Biology and Technology* 16:199-199.
- Brady C.J. (1987) Fruit ripening. *Annual review of plant physiology* 38:155-178.
- Considine J., Brown K. (1981) Physical aspects of fruit growth theoretical analysis of distribution of surface growth forces in fruit in relation to cracking and splitting. *Plant physiology* 68:371-376.
- Conway W.S., Janisiewicz W.J., Leverentz B., Saftner R.A., Camp M.J. (2007) Control of blue mold of apple by combining controlled atmosphere, an antagonist mixture, and sodium bicarbonate. *Postharvest Biology and Technology* 45:326-332.
- D'Aquino S., Palma A., Schirra M., Continella A., Tribulato E., La Malfa S. (2010) Influence of film wrapping and fludioxonil application on quality of pomegranate fruit. *Postharvest Biology and Technology* 55:121-128.

- Ding C.K., Chachin K., Hamauzu Y., Ueda Y., Imahori Y. (1998) Effects of storage temperatures on physiology and quality of loquat fruit. *Postharvest Biology and Technology* 14:309-315.
- Du C., Wang P., Francis F. (1975) Anthocyanins of pomegranate, *Punica granatum*. *Journal of Food Science* 40:417-418.
- Finger F.L., Endres L., Mosquim P.R., Puiatti M. (1999) Physiological changes during postharvest senescence of broccoli. *Pesquisa Agropecuária Brasileira* 34:1565-1569.
- Fuhrman B., Volkova N., Aviram M. (2005) Pomegranate juice inhibits oxidized LDL uptake and cholesterol biosynthesis in macrophages. *Journal of Nutritional Biochemistry* 16:570-576.
- Gil M.I., Garcaviguera C., Artes F., Tomasbarberan F.A. (1995) Changes in pomegranate juice pigmentation during ripening. *Journal of the Science of Food and Agriculture* 68:77-81.
- Gray J., Picton S., Shabbeer J., Schuch W., Grierson D. (1992) Molecular biology of fruit ripening and its manipulation with antisense genes. *Plant Molecular Biology* 19:69-87.
- Hertog M.L.A.T.M., Nicholson S.E., Whitmore K. (2003) The effect of modified atmospheres on the rate of quality change in 'Hass' avocado. *Postharvest Biology and Technology* 29:41-53.
- Imungi J. (1992) Postharvest handling of perishable crops, *Proceedings of the Postharvest Management of Food Crops Seminar Held in Serena Hotel. Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.*
- Kader A. (2000) Quality assurance of harvested horticultural perishables, *IV International Conference on Postharvest Science* 553. pp. 51-56.

- Kader A.A. (2001) A summary of ca requirements and recommendations for fruits other than apples and pears. pp. 737-740.
- Kamimura H., Hammock B.D., Yamamoto I., Casida J.E. (1972) Potent juvenile hormone mimic, 1-(4'-ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene, labeled with tritium in either the ethylphenyl- or geranyl-derived moiety. *Journal of Agricultural and Food Chemistry* 20:439-442.
- Kulkarni A.P., Aradhya S.M. (2005) Chemical changes and antioxidant activity in pomegranate arils during fruit development. *Food Chemistry* 93:319-324.
- Lee S., Kim K., Kim S. (1974) Studies on changes in the composition of the pomegranate fruit during maturation. I. Changes in sugars, organic acids, amino acids, and the respiration rate. *Journal of the Korean Society for Horticultural Science* 15:57-68.
- Lidster P., Lightfoot H., McRae K. (1983) Fruit quality and respiration of 'McIntosh' apples in response to ethylene, very low oxygen and carbon dioxide storage atmospheres. *Scientia Horticulturae* 20:71-83.
- Lopez-Rubira V., Conesa A., Allende A., Artes F. (2005) Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. *Postharvest Biology and Technology* 37:174-185.
- Mitra S.K. (1997) Postharvest physiology and storage of tropical and subtropical fruits *Cab International* 18:365-373.
- Neuhöfer H. (1990) The existence of pelletierine derivatives in *Punica granatum*. *World Phytomed* 5:604.
- Palou L., Crisosto C.H., Garner D. (2007) Combination of postharvest antifungal chemical treatments and controlled atmosphere storage to control gray mold and improve

- storability of 'Wonderful' pomegranates. *Postharvest Biology and Technology* 43:133-142.
- Paul R. (1992) Postharvest senescence and physiology of leafy vegetables. *Postharvest News and Information* 3:11N-20N.
- Porat R., Weiss B., Cohen L., Daus A., Aharoni N. (2004) Reduction of postharvest rind disorders in citrus fruit by modified atmosphere packaging. *Postharvest Biology and Technology* 33:35-43.
- Singh R.P., Murthy K.N.C., Jayaprakasha G.K. (2002) Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models. *Journal of Agricultural and Food Chemistry* 50:81-86.
- Smock R.M. (1979) Controlled atmosphere storage of fruits. *Horticultural reviews*:301-336
- Tucker G., Grierson D. (1987) Fruit ripening. *The biochemistry of plants: A comprehensive treatise* 12:265-318.
- Yackel W.C., Nelson A., Wei L., Steinberg M. (1971) Effect of controlled atmosphere on growth of mold on synthetic media and fruit. *Applied microbiology* 22:513-516.
- Yahia E.M. (2006) Modified and controlled atmospheres for tropical fruits. *Stewart Postharvest Review* 2:1-10.

CHAPTER 3
POMEGRANATE FRUIT QUALITY IS AFFECTED BY CONTROLLED AIR
STORAGE¹

¹ Sidhu, H.S., J.C. Díaz-Pérez, D.D. MacLean, and S. Goreta-Ban. To be submitted to *HortScience*.

Abstract

Pomegranate fruit are consumed fresh as whole fruit or arils and used for production of wine and syrup. Pomegranate production has been increasing worldwide in response to increased popularity due to pomegranate health benefits. Pomegranate is a newly introduced fruit crop in the southeastern USA. Storage conditions determine fruit quality. Low temperature and high relative humidity in combination with controlled atmosphere storage have been found beneficial in maintaining produce quality. The objectives were to determine the effects of controlled atmosphere storage and fruit maturity at harvest on physical and physiochemical properties of pomegranate fruit grown under Georgia conditions. Pomegranate fruit from Ponder Farm (PF), Ty Ty, GA in 2010, 2011 and Alma Farm (AF), Alma, GA in 2011 were stored in controlled air (CA) storage (5% CO₂ + 3% O₂; 5 °C, 90%-95% RH) and regular air (RA) storage (5°C, 90%-95% RH) for three months. Pomegranate whole fruit and juice were evaluated for various physical and physiochemical attributes at end of storage. Skin shriveling, fruit cracking, husk scald, chilling injury, and disease severity caused by *Cercospora* and other fungal pathogens were lower in fruit under CA than in RA storage. Fruit husk color and juice TSS was better preserved in fruit under CA than in RA. Fruit quality deteriorated rapidly during shelf period due to high temperature and low relative humidity. In conclusion, controlled atmosphere storage was more effective in maintaining the quality of pomegranate fruit compared to regular air storage.

Introduction

Pomegranate (*Punica granatum* L.), a species of *Punicaceae* family, is a fruit plant native to the region extending from Iran to the Himalayas. It is believed to be among the first fruit tree cultivated by humans in around 4000BC in the Middle East. Within past few thousand years it has spread all over the world, from Asia to Europe & to America. Presently cultivated in the tropical and subtropical regions, it thrives in different climate and soil conditions and can tolerate drought and salt stress. Pomegranate fruit are consumed fresh and used for production of wine and syrup. Pomegranate production has been increasing worldwide in response to increased popularity due to its health benefits (Basu and Penugonda, 2009).

Pomegranate fruit is spherical in shape and has a persistent calyx. Fruit are covered with a leathery pericarp (rind), the color of which can range from yellow-green to dark red. Fruit develops from a flower with a hypogenous ovary. Pomegranate tree bears two kinds of flowers, hermaphrodite and male while fruit are derived only from hermaphrodite flowers while male flowers do not form fruit. Aril is the edible portion of fruit. It is a juicy pulp around the seeds formed from ovules present in ovary of the fertilized fruit (Shulman et al., 1984).

Flowers of pomegranate have been used in Unani medicines for treating diabetes by significantly reducing blood glucose levels in animals (Wang et al., 2010). Gallic acid present in the methanol extract of the flowers is believed to be responsible for improved sensitivity of the insulin receptor, which will in turn lower blood glucose level (Li et al., 2005). Pomegranate juice has been reported to have antioxidant and antitumor activities (Singh et al., 2002). Various alkaloids, flavonoids, polyphenolic compounds and hydrolyzable tannins, such as punicalin, pedunculagin, punicalagin and ellagic acid esters of glucose, which possess strong antioxidant properties, are present in pomegranate's juice. Juice has shown potential anti-atherogenic

properties *in vivo* (Fuhrman et al., 2005). The inhibitory effect of pomegranate juice on lipid peroxidation in plasma contributes to its anti-atherogenic properties (Aviram et al., 2000). The antioxidant activity of the juices varies by cultivar (Borochoy-Neori et al., 2009). Pomegranate juice and pericarp extracts have been shown to contain compounds that can induce apoptosis, programmed cell death, in human prostate cancer cells *in vitro* (Albrecht et al., 2004). The pericarp extracts from the fruit have been demonstrated to inhibit the cancerous cells selectively, while affecting the surrounding normal cells minimally (Kawaii and Lansky, 2004).

Pomegranate fruit quality is affected by both physical and physiochemical attributes. Fruit size is determined at an early stage of fruit development and proper crop management practices are required to achieve potential fruit size (Wetzstein et al., 2011). Appearance of fruit is affected by skin smoothness, shriveling and presence of fruit disorders, such as, sunscald, skin fungal decay, internal fruit decay, injuries, bruises and cracking.

High temperature during fruit development can cause sunburn leading to decrease in concentration of phenolic compounds and loss of fruit visual quality. Fruit affected by high temperature are subjected to oxidative stress which eventually develops as sunscald. Age of fruit bearing tree influences the extent of sunscald damage to a fruit. Fruit on younger trees tend to be affected more by high temperature during fruit development leading to sunscald, possibly due to reduced canopy development in young trees causing exposure of the fruit to direct sunlight for longer periods of time (Weerakkody et al., 2010).

Fruit cracking influences fruit quality greatly and can cause significant economic losses in fruit production. As the fruit grow there is an increase in stress on fruit pericarp (Considine and Brown, 1981). Fruit crack open on sudden rainfall or irrigation after a long stress period. In tomato, incidences of cracking increase after irrigation. Irrigation was shown to reduce strength

of fruit skin and led to appearance of small cracks (Kamimura et al., 1972). Cracking in tomato is also influenced by day and night temperature differential, higher the difference more is the tendency of a fruit to crack. Cracking is a disorder in mature pomegranate fruit.

Disease spots on pomegranate in Georgia are predominantly caused *Cercospora punicae*. Black circular spots due to the fungal spores develop on fruit skin affecting the visual quality. Other fungi like *Botryospheria* and *Alternaria* cause problems as internal fruit decay influence fruit quality rendering fruit unmarketable.

Juice Properties

Total soluble solids (TSS) content of the juice is a measure of sweetness of the juice. TSS content increases with fruit development and eventually stabilizes at maturity (Kulkarni and Aradhya. 2005). Glucose and fructose are major contributors to pomegranate fruit sweetness (Lee et al., 1974). Citric acid is the biggest contributor to the titratable acidity (TA) of pomegranate juice. Other organic acids such as malic, fumaric, acetic, tartaric and lactic acids are also found in the fruit (Kulkarni and Aradhya. 2005).

The primary antioxidants found in the juice are phenolic compounds with hydroxyl groups and double bonds. There is a strong correlation between the antioxidant potential of juices and the amount of phenolic compounds present in them (Schubert et al., 1999; Sun et al., 2007). Polyphenols present in pomegranate juice can help in reducing systolic blood pressure (Aviram et al., 2004). Pomegranate juice is an important source of flavonoids, specifically anthocyanins. Flavonoids play an important role of scavenging free radicals and have a stronger affinity for free radicals compared to vitamins like C and E. There was a significant decrease in the amount of free radicals in the livers, hearts and kidneys of rats when they were administered solely on the flavonoids derived from pomegranate. There was also an increase in the activity of

various endogenous antioxidant enzymes, such as catalases, in the rats' organs (Kar and Parmar, 2007; Sudheesh and Vijayalakshmi, 2005).

Six anthocyanins have been reported in pomegranate; 3-glucosides and 3, 5 diglucosides of delphinidin, cyanidin, and pelargonidin (Du et al., 1975; Gil et al., 1995; Artes et al., 1996). Anthocyanadins are the sugar free counterparts of anthocyanins. Color of juice is due to presence of anthocyanadins and flavan-3-ols and concentration of anthocyanadins is dependent on fruit maturity. Anthocyanins strengthen blood capillaries, reduce platelet aggregation and assist in vasodilation of arteries (Heinonen et al., 1998). They have been used to treat diabetic retinopathy, fibrocystic disease and disorders related to eyesight (Scharrer and Ober, 1981.) Anthocyanins can be utilized to provide potential radiation protection for chemotherapy patients. They can also help reduce the lipoperoxidation risks induced by carbon tetrachloride (Wang et al., 1997). Cyanidin 3-glucoside is found in higher concentration as compared to other anthocyanins whereas Pelargonidin 3-glucoside is usually present in scarce amount (D'Aquino et al., 2010).

Hydrolysable tannins as ellagitannins and gallotannins are the most prevalent compounds present in pomegranate. Ellagic acid and its derivatives, ellagitannins, such as punicalagins and punicalins and ellagic acid, play an important role in antioxidant potential of pomegranate juice (Gil et al., 2000; Sestili et al., 2007). Commercial juices showed three to four times more antioxidant activity as compared to green tea and red wine and juice from arils because commercial juice contains tannins extracted from pericarp (Gil et al., 2000). Ellagic acid has a chelating affinity and can prevent lipid peroxidation in mitochondria and microsomes (Osawa et al., 1987). Other parts of pomegranate tree, including bark, leaves, and pericarp are a rich source of ellagitannins and gallotannins (Tanaka et al., 1985; Nawwar et al., 1994b). Punicalagin is the

major compound in juice responsible for antioxidant activity but the compound is not efficiently absorbed into blood circulation, which could be due to effect of the metabolism of intestinal flora (Aradhya et al., 2004). Various apigenin and luteolin glycosides have been identified in pomegranate leaves whereas hydrolyzable tannins, such as punicalagin and punicalin, were identified in pomegranate pericarp (Mayer et al., 1977; Tanaka et al., 1986a).

Alkaloids and organic acids are the other compounds that are present in the pomegranate juice. Important alkaloids present in the juice are serotonin, melatonin and tryptamine (Badria, 2002). The organic acids present are straight chain fatty acids such as malic acid and citric acid (Neuhofer, 1990). Unsaturated fatty acids present in the juice also play an additional role in the antioxidant activity (Melgarejo et al., 1995). One g/L citric acid and 7 mg/L ascorbic acid are present in the juice as well (El-Nemr et al., 1990). The resulting antioxidant activity of the pomegranate juice is due to the contribution of all the potential compounds present in it.

Harvest Maturity

Generally, there is an increase in fruit sweetness, breakdown of polysaccharides such as starch and a decrease in fruit acidity as fruit mature (Gray et al., 1992). Changes in physical appearance of fruit occur due to changes in cell wall and intercellular spaces (Tucker and Grierson, 1987). Maturity stage of pomegranate fruit at harvest plays an important role in fruit quality, especially juice properties such as flavor, TSS, TA and concentration of phenolic compounds (Lopez-Rubira et al., 2005). Many irreversible physiological, biochemical changes take place in a fruit during ripening, all of which are driven genetically. Degradation of chlorophyll, increased respiration rate, production of ethylene for ripening and biosynthesis of health beneficial compounds as anthocyanins are some of the changes that occur in a fruit during ripening (Brady, 1987).

Over-ripe pomegranate fruit develop browning of arils and have decreased TSS and TA in juice (Prabhu Desai, 1989). Juice TSS increases with fruit development and stabilizes at fruit maturity. In a study on cultivar Ganesh TSS increased from 13% to 15.3% from day 40 to 140 of fruit development (Kulkarni and Aradhya, 2005). Increase in TSS can be a result of breakdown of starch into sugars, as in mango and banana (Biale 1961; Bashir and Abu-Goukh, 2003). TA decreases in fruit with maturity and coincides with TSS increase, delivering a characteristic flavor to fruit (Kulkarni and Aradhya, 2005). Total phenolic content of fruit decreases during maturity resulting in decreased total antioxidant activity of the juice. With maturity fruit lose astringency which is caused by one of the polyphenols, gallic acid. Decrease in astringency is also important for flavor quality pomegranate in as a fresh fruit (Ozawa et al., 1986). With all the changes occurring during fruit development maturity is thus an important factor in deciding harvest time of the fruit.

Postharvest Storage

Controlled atmosphere (CA) storage extends storage life of agricultural produce. It has been used successfully for maintaining quality of many tropical and sub-tropical fruit (Kader, 2001; Yahia, 2006). Economic value of fruit is limited by their short ripening periods and short postharvest shelf life. It becomes important to manage post-harvest factors to maintain fruit quality. Storage of fruit in CA and low temperature reduces fruit respiration rate, ethylene production, suppresses or delays senescence processes and eventually increasing postharvest shelf life (Beaudry, 1999; Ding et al., 1998; Smock, 1979). Increased CO₂ concentration in storage atmosphere reduces rate of respiration and CO₂ production (Hertog et al., 2003). Ethylene production in fruit, responsible for its senescence and decay, is suppressed under low O₂ and high CO₂ conditions. Such suppression of ethylene production during CA storage was

observed in celery (Gómez and Artés, 2004). Controlled atmosphere storage affects both host fruit and any pathogens on it. With better physiological health of fruit due to reduced senescence in CA storage, fruit can resist attack of pathogens more effectively. In a 2-month storage study in apple, CA storage reduced fruit decay as compared to regular air storage (Conway et al., 2007; Lidster et al., 1983). CA storage conditions suppress pathogen growth and spreading (Yackel et al., 1971). Fruit decay in stored loquat (*Eriobotrya japonica*) fruit was controlled better by CA storage than modified packaging (Ding et al., 2006).

Storing pomegranates adequately is an important factor in maintaining regular availability of good quality fruit in market. Harvest season of pomegranate varies depending on the region and cultivar. In Spain it ranges from mid-September to mid-November.. Moreover, pomegranate fruit quality is influenced by fruit water loss and disease, injury and decay. Due to water loss shriveling symptoms appear on pomegranate fruit surface along with desiccation and loss of crispness and fruit firmness. Decrease of 5% in fruit weight due to water loss leads to visible shriveling symptoms (Palou et al., 2007). For sweet pomegranate minimum safe storage temperature is 10°C yet it does not reduce incidence of fungal decay. Decay due to various pathogens as *Botrytis cynerea*, *Alternaria spp.*, and *Aspergillus spp.* causes significant loss during storage (Mitra, 1997).

Storing fruit below 5°C under standard refrigerated conditions for more than two months can lead to chilling injury. Chilling injury is the development of browning in pericarp, arils and seeds. Chilled fruit becomes sensitive to fungal decays (Kader et al; 1984). Chilling injury can be inhibited at 2%-4% O₂ concentration and 2 to 6°C (Ben-Arie and Or, 1986). In another study, decline in water loss, chilling injury and fungal decay was observed when fruit were kept in CA storage. Additionally, vitamin C and fruit sugars content decreased during CA storage (Artes et

al, 1996). Chilling injury symptoms are observed in other agricultural produce stored in low temperature conditions for long periods of time.

Objectives

The objectives were to determine the effects of controlled atmosphere storage and fruit maturity at harvest on physical and physiochemical properties of pomegranate fruit grown under Georgia conditions.

Materials and Methods

Pomegranate fruit were harvested from Ponder Farm (PF), Ty-Ty, GA in 2010 ('PF2010') and 2011 ('PF2011') and Alma Farm ('AF2011'), Alma, GA in 2011, both farms being part of the University of Georgia. Seven cultivars ('Afganski', 'Crab', 'Cranberry', 'Entek-habi-saveh', 'Kaj-acik-anor', 'Nikitski-ranni', 'Salavatski') were harvested from PF in 2010 and 6 cultivars (same as 2010 except 'Entek-habi-saveh') in 2011 while five cultivars ('Al-sirin-nar', 'Bala Miursal', 'Eversweet', 'Nikitski-ranni' and 'Sweet') were harvested from AF in 2011. In 2010 fruit from PF were harvested on 2nd week Sept. (early) and 1st week Oct. (late).. In 2011 fruit from AF were harvested on 14th Sept. and on 5th Oct. from PF. At early maturity fruit were unripe and had not reached marketable quality.

Fruit were brought to Vidalia Onion Research Laboratory (VORL) at the University of Georgia, Tifton Campus. Fruit free from physical damage and decay were randomly divided into two groups and stored either in regular air (RA; 5°C, 90%-95% RH) or controlled air (CA; 3% O₂; 5% CO₂; 90%-95% RH) for three months. After removal from storage fruit were divided into two groups. First group was examined immediately after storage (day 1; CA, RA) and other was kept at room temperature for 7 days (shelf storage; CA+7D & RA+7D, 20-25°C and RH 45%50%). Fruit were evaluated for various physical quality attributes such as weight, skin

qualities, disease incidence and physiochemical attributes as total soluble solids, titratable acidity, maturity index, anthocyanins content.

Physical Evaluation

Four fruit per treatment were weighed and graded visually for the following fruit attributes: a) Skin smoothness (5= high skin smoothness; 4= moderately high; 3= moderate, skin with rashes or patches; 2= moderately low, rough skin with major portion of fruit covered with rashes; 1= low skin smoothness, very rough skin); b) skin shriveling (1= no shriveling; 2= mild shriveling, fruit is marketable; 3= moderate shriveling, fruit is not marketable; 4= severe shriveling, fruit is not marketable); c) Fruit cracking (1= no cracking; 2= mild cracking with very thin cracks, possibly superficial, fruit marketable; 3= moderate cracking, small but wide cracks, arils visible, fruit unmarketable; 4= severe cracking, long wide cracks, fruit unmarketable); d) Sunscald (1= no sunscald; 2= mild sunscald, sunscald damage on less than 25% fruit area, easily visible sunscald damage; 3= moderate sunscald, 25%-50% fruit area with sunscald; 4= severe sunscald, more than 50% fruit surface area with sunscald damage, unmarketable fruit, sunburn); e) Husk scald (1= no husk scald, 2= mild husk scald, small size browning, fruit marketable, 3= moderate husk scald, more than quarter of fruit skin damaged, fruit unmarketable, 4= severe husk scald, about half fruit skin damaged by skin scald); f) Chilling injury (CI) (1= no chilling injury, 2: mild chilling injury, less than 10% fruit arils affected, fruit marketable; 3: moderate chilling injury, 10%-50% fruit arils show chilling injury , fruit unsuitable for marketing 4: severe chilling injury, more than 50% of arils affected, fruit unmarketable). Sunscald results from injury to fruit surface during fruit growth by exposure to high temperature and sun radiation. It leads to loss of fruit visual quality of fruit.

Fruit diseases caused by *Cercospora punicae* and other fungi (*Botryospheria spp.* and *Alternaria spp.*) were recorded on individual fruit on a 1-4 scale. *Cercospora* severity on fruit was graded on 1-4 scale (1= negligible, 0-2 spots; 2= mild, 5-10 spots scattered on fruit surface, fruit marketable; 3= moderate, more than 10 spots, unmarketable fruit, 4= high, more than 1/4th fruit skin covered with spots, unmarketable fruit). *Botryospheria spp.* and *Alternaria spp.* caused internal decay which was observed after cutting fruit open. It was graded from 1-4 scale (1= no disease damage, fruit marketable, 2= mild disease damage, less than 25% of fruit affected, disease not visible outside from the skin, 3= moderate disease damage, 25%-50% of fruit damaged, disease symptoms visible from outside and fruit unmarketable, 4= high disease damage, more than 50% fruit damaged, unmarketable fruit). *Cercospora* severity was recorded during both years of study. Internal fruit decay was recorded only in year 2011 of the study.

Juice Properties

TSS/TA ratio determines fruit flavor. For physicochemical analysis 50 arils were separated from fruit and weighed (g/50 arils). Arils were squeezed in a cheese cloth and juice was weighed (g/50 arils) to calculate percentage juice content (Juice/weight ratio) of arils. Total soluble solids (TSS) were measured using a Brix-stix digital handheld refractometer (Livermore, CA) which was calibrated with distilled water. Titratable acidity (TA) was measured using an automatic titrator DL-15 (Mettler Toledo, Switzerland) by using 500 µl of juice diluted with 25 ml of water. Juice sample was titrated to pH 8.2 using 0.1M NaOH after recording the initial pH. Titratable acidity was expressed as a percentage of malic acid present in juice.

Anthocyanins

Anthocyanins in pomegranate juice were separated with HPLC (Agilent technologies 1200 series, model g1316, Santa Clara, LA) equipped with a XDB-C18 (Zorbax eclipse, Agilent

technologies) 3.5 micron column. Juice samples were centrifuged (Allegra 25R centrifuge, Beckman coulter, Atlanta, GA) at 25155 g for 30 min at 4° C. Supernatant of 0.75mL was added to the HPLC tubes. Flow rate was maintained at 0.4 mL/min, with solution A as 5% formic acid (FA) and Solution B as 95% acetonitrile. The gradient used was starting from 5% A and 95% B at 2 min; 15% A, 85% B at 19 min; 20% A, 80% B at 20 min, 100% A at 21 min, 100% A at 24 min, 5% A, 95% B at 24.9 min; 5% A, 95% B at 28 min. The run time was 28 min per sample. Five anthocyanins were observed at spectral scan of 520nm (Fig 1). Anthocyanin concentrations were determined using a standard curve and results were expressed as Cyanidin, 3-glucoside ($\mu\text{g/g}$ in arils) equivalent.

Color

A CR-400 (8mm aperture, D65 illuminant) handheld colorimeter (Konica Minolta, Ramsey, NJ) was used to record fruit skin color. Five color readings were taken per fruit. Color was measured as L^* , a^* , b^* . The value of L^* describes the degree of darkness or lightness with $L=0$ being black and $L=100$ being white. The b^* value refers to the colors in the range yellow to blue. The a^* refers to colors ranging from red-purple to blue-green. Chroma and hue angle were calculated from the L^* , a^* and b^* values using Spectramagic NX software. Chroma represents richness of color on a 0-60 scale with 0 being gray and 60 being the true color. Hue angle represents dominant color wavelength (Fig.3.2).

Statistical Design and Analysis

In 2010, the experimental design was completely randomized with 2 replications and 64 treatments [8 cultivars x 4 storage (CA, CA+7D, RA & RA+7D) x 2 fruit maturity (early and late)]. Four fruit per treatment were used to record physical and physiochemical attributes. In 2011, for both PF and AF four fruit per treatment were used for physical and physiochemical

attributes. Color data transformations for analysis were done with Spectramagic NX software.

The experiment was a completely randomized design. ANOVA procedure from the SAS Enterprise (SAS Institute, Cary, SC) was used to carry out the statistical analysis for all response variables. Whenever interactions between main factors were significant, effects of main factors were not discussed separately.

Results

Fruit Weight

Fruit weight in PF2010 and AF2011 varied significantly by cultivar ($p < 0.01$). ‘Cranberry’ (297g), ‘Kaj-acik-anor’ (319 g), ‘Nikitski-ranni’ (286 g) and ‘Salavatski’ (297 g) had highest fruit weight in PF2010. In AF2011 ‘Al-sirin-nar’ (276 g) had highest fruit weight while fruit weight for ‘Bala Miursal’ was (215g), ‘Eversweet’ (205g), ‘Nikitski-ranni’ (230g) and ‘Sweet’ (202g). Average fruit weight for ‘Afganski’ (256g), ‘Crab’ (236g), ‘Cranberry’ (216g), ‘Kaj-acik-anor’ (260g), ‘Nikitski-ranni’ (238g), and ‘Salavatski’ (241g) was not significantly different in PF2010.

Skin Smoothness

Fruit in general had better skin smoothness when stored in CA than under RA. Cultivar-storage interaction for skin smoothness was observed in PF2010 and AF2011 fruit while cultivar and storage significantly affected skin smoothness in PF2011 fruit (Table 3.1, 3.2 & 3.3). Skin smoothness of PF2010 fruit did neither differ due to modification of air (CA, RA) nor decrease during shelf period. Among fruit stored in CA conditions ‘Salavatski’ had highest skin smoothness (Table 3.4). PF2011 fruit of ‘Salavatski’ stored in CA had higher skin smoothness compared to those in RA (Table 3.2). AF2011 fruit cultivars Bala Miursal, Eversweet and Nikitski-ranni had higher skin smoothness under CA than RA (Table 3.5). In PF 2010 fruit late

harvested cultivars Kaj-acik-anor and Salavatski had lower skin smoothness than early harvested fruit (Tables 3.1 & 3.6).

Fruit Cracking

Fruit cracking was significantly affected by cultivar and storage in PF2010 and AF2011 fruit while cultivar-storage interaction was observed in PF2011 (Table 3.1, 3.2 & 3.3). Fruit from cultivars Afganski and Crab had highest cracking damage in PF2010 (Table 3.1). Fruit cracking did not worsen during shelf period. In PF2011 cultivar Afganski showed higher fruit cracking in RA compared to CA storage (Table 3.7). In RA storage ‘Afganski’ and ‘Cranberry’ had highest fruit cracking damage. Fruit cracking increased during maturity in PF2010 fruit (Table 3.1). AF2011 fruit from treatment RA+7D had highest cracking damage (Table 3.3).

Sunscald

Fruit sunscald for all cultivars in PF2010 (Mean= 2.08) and PF2011 (Mean= 2.59) did not differ significantly due to storage conditions (Table 3.7). Sunscald damage tended to worsen during shelf storage although a significant difference was observed only in cultivars Al-sirin-nar and Nikitski-ranni from AF2011 (Table 3.5). There was cultivar-maturity-storage interaction for sunscald in PF2010 and cultivar-storage interaction in PF2011 and AF2011 (Tables 3.1, 3.2 & 3.3).

Skin Shriveling

Fruit skin shriveling in general was reduced in CA compared to RA stored fruit (Tables 3.1-3.3). There was cultivar-storage interaction in PF2010 (Table 3.4) and AF2011 (Table 3.5). In AF2011 all cultivars except ‘Sweet’ had lower skin shriveling under CA compared to other treatments. During shelf period, skin shriveling increased significantly in fruit from CA storage.

Maturity affected skin shriveling in PF2010 fruit with late harvested fruit having increased shriveling (Table 3.1).

Husk Scald (Skin scald)

Fruit husk scald was generally reduced in CA compared to other treatments (Table 3.1-3.3). Fruit from RA+7D had highest husk scald damage among all treatments (Table 3.1).

Cultivar-storage interaction was observed in AF2011 fruit with lowest husk scald in CA stored fruit of ‘Al-sirin-nar’, ‘Bala Miursal’ and ‘Nikitski-ranni’ (Table 3.5).

Decay Caused by Cercospora

Cercospora severity was mostly reduced in fruit stored under CA conditions (Table 3.1-3.3). PF 2010 fruit showed maturity-storage interaction although no significant differences were found among treatments (Table 3.8). CA stored ‘Afganski’, ‘Crab’ and ‘Cranberry’ fruit from PF2011 had lesser cercospora severity than those in RA (Table 3.7). Fruit of ‘Bala Miursal’, ‘Eversweet’ and ‘Nikitski-ranni’ from AF2011 had lower cercospora severity under CA compared to RA+7D treatment.

Fruit skin smoothness was influenced by skin shriveling (-0.896 ; $P < 0.0001$) and cercospora severity (-0.442 ; $p=0.02$) on fruit surface. As cercospora severity was higher in fruit in RA compared to those in CA storage ($p < 0.05$) it resulted in decreased skin smoothness in fruit in RA storage in fruit from PF2011 and AF2011.

Decay Caused by Other Fungi

CA stored fruit normally had lesser damage due to other fungal species as those caused by *Botryospheria spp.* and *Alternaria spp.* (Table 3.2 & 3.3). ‘Crab’ and ‘Cranberry’ fruit from PF2011 had the greatest disease damage from other fungal species. Cultivar-storage interaction

in AF2011 fruit was observed where ‘Bala Miursal’ fruit from RA+7D had higher disease damage compared to CA and CA+7D (Table 3.9).

Chilling Injury

Cultivar-storage interaction was significant for chilling injury and majority of fruit under CA storage had reduced or no damage (Table 3.2 & 3.3). All cultivars except ‘Cranberry’ and ‘Kaj-acik-anor’ exhibited significant chilling injury in RA storage (Table 3.7). Fruit of ‘Al-sirin-nar’ and ‘Sweet’ from AF2011 showed significant chilling injury under RA compared to CA treatment (Table 3.9). RA stored fruit from all cultivars except ‘Sweet’ showed significant increase in chilling injury symptoms during shelf period.

Aril Weight

Aril weight (weight of 50 arils) was affected by cultivar in the three trials (Tables 3.10-3.12). ‘Cranberry’ (PF2010) and ‘Crab’ and ‘Nikitski-ranni’ (PF2011) were among the cultivars with the highest aril weight. ‘Entek-habi-saveh’ (13.96 g) and ‘Salavatski’ (15.9 g) in PF2011 and ‘Salavatski’ (15.3 g) in PF2011 ($P < 0.0001$) had the lowest aril weight. ‘Al-sirin-nar’ (24.1 g) had the highest aril weight in AF2011. Aril weight was not affected by fruit maturity or storage conditions.

Juice Weight

Juice weight was affected by cultivar in PF2010 (Table 3.10) and PF2011 (Table 3.11), but not in AF2011 (Table 3.12). As for aril weight, ‘Cranberry’ (PF2010) and ‘Crab’ and ‘Nikitski-ranni’ (PF2011) were among the cultivars with the highest juice weight. Late harvested fruit had increased juice/weight ratio (Table 3.10). Storage had no consistent effect on juice weight.

Juice/Weight Ratio

Juice/weight ratio (juice content) was affected by cultivar in PF2010, with ‘Crab’, ‘Kaj-acik-anor’, ‘Cranberry’, ‘Nikitsk-ranni’ and ‘Salavatski’ having among the highest juice content. Juice/weight ratio ranged from 72% to 80% of total aril weight. Early harvested PF2010 fruit from CA treatment had higher juice/weight ratio compared to RA treatment (Table 3.8). Fruit stored in CA had 79.2% juice in arils whereas RA stored fruit had 73.5% juice content. Cultivar Eversweet fruit stored in CA from AF11 had a significant decrease in juice/weight ratio during shelf storage (Table 3.13).

Total Soluble Solids, Titratable Acidity, and pH

Total soluble solids (TSS) were affected by cultivar in the three trials (Tables 3.10-3.12). TSS was lower under RA stored fruit compared to CA for only a few cultivars. There was cultivar-maturity-storage interaction in PF2010 fruit (Table 3.10) and cultivar-storage interaction in PF2011 (Table 3.11) and AF2011 (Table 12). Late harvested ‘Kaj-acik-anor’ fruit had higher juice TSS under CA storage (16.15%) compared to those in RA storage (15.53%). Among PF2011 cultivars only ‘Kaj-acik-anor’ fruit had higher TSS under CA storage than RA storage (Table 3.14). ‘Eversweet’ from AF2011 had higher TSS in RA stored fruit compared to CA stored fruit (Table 3.13). Late harvested ‘Crab’ fruit from PF2010 had higher TSS (mean = 15.56%) content than early harvested fruit (mean = 14.29%).

Titrateable acidity was affected by cultivar, fruit maturity and storage (Tables 3.10-3.12). There were, however cultivar x maturity x storage interactions in PF2010 (Table 3.10) and cultivar x storage interactions in AF2011 (Table 3.12). Late harvested ‘Afganski’, ‘Cranberry’ and ‘Salavatski’ fruit in CA storage had higher TA compared to RA storage (data not shown). Similarly, late harvested CA stored ‘Cranberry’ fruit (4.08%) had higher TA than early harvested

RA stored fruit (1.44%). Fruit from CA+7D treatment than higher TA compared to those from RA and RA+7D treatments for early harvested ‘Entek-habi-saveh’ (CA+7D=5.07%, RA= 2.50% and RA+7D=2.45%) and ‘Kaj-acik-anor’ (CA+7D=4.67%, RA=2.35%, RA+7D=2.52%). PF2011 (Table 3.11) and AF2011 (Table 3.12) fruit under CA storage had lower TA compared to those under RA storage. PF 2011 ‘Afganski’ and ‘Kaj-acik-anor’ fruit had the highest TA among all cultivars. AF2011 ‘Bala Miursal’ fruit had the highest TA among other cultivars. Generally TA of AF2011 fruit was higher than other trials possibly because fruit were harvested very early in maturity and were not at optimum maturity for harvest.

Anthocyanins

Delphinidin 3, 5-diglucoside content of fruit juice was stable during storage. PF2010 fruit (Table 3.15) showed cultivar x maturity x storage interaction for delphinidin 3, 5-diglucoside with early harvested ‘Crab’ fruit stored in RA having reduced concentration of delphinidin 3, 5-diglucoside (70.7 µg/g). PF2011 fruit did not show an effect of storage on its concentration (Table 3.16). Cultivar-storage interaction was observed in AF2011 fruit (Table 3.17) and only ‘Bala Miursal’ fruit under RA storage (112µg/g) had higher delphinidin 3, 5-diglucoside content compared to CA storage (43µg/g).

Cyanidin 3, 5-diglucoside content was also stable during storage. Cultivar x maturity x storage interaction in PF2010 (Table 3.15) and cultivar x storage interaction in PF2011 (Table 3.16) were observed although no significant differences between storage conditions were found. CA+7D fruit of ‘Cranberry’ had higher concentration when harvested late (776µg/g) as compared to early harvested (299µg/g).

Cyanidin, 3-glucoside changed little during storage. There was cultivar x maturity x storage interaction in PF2010 fruit (Table 3.15) where late harvested fruit had higher

concentration than early harvested fruit of ‘Afganski’ in CA+7D (early= 170 μ g/g and late = 571 μ g/g) treatment, ‘Crab’ in RA treatment (early = 59 μ g/g & late = 418 μ g/g) and ‘Kaj-acik-anor’ in CA treatment (early = 211 μ g/g and late = 949 μ g/g).

Pelargonidin-3 glucoside and Petunidin 3-glucoside were affected by cultivar in PF2010 and PF2011 but not in AF2011 (Tables 3.15-3.17). Pelargonidin-3 glucoside and Petunidin 3-glucoside had higher concentrations in late harvested than in early harvested fruit. Pelargonidin-3 glucoside and Petunidin 3-glucoside concentrations were unaffected by storage conditions.

Total anthocyanins was affected by cultivar in the three trials (Tables 3.15-3.17), by maturity in PF2010 (Table 3.15) and by storage in PF2010 and AF2011 (Table 3.17). Cultivar-maturity-storage interaction in PF2010 was observed in which late harvested fruit of ‘Crab’ under RA treatment (late = 2063 μ g/g and early = 298 μ g/g) and ‘Kaj-acik-anor’ under CA treatment (late = 2175 μ g/g and early = 1053 μ g/g) had higher total anthocyanins concentration compared to early harvested fruit. Fruit of ‘Bala Miursal’ in CA+7D treatment (79 μ g/g) had lower total anthocyanins than RA treatment fruit (222 μ g/g).

Fruit Skin Color

Lightness (L^*) of fruit skin was in general affected by cultivar, maturity and storage (Tables 3.18-3.19). There were, however, several interactions among cultivar, maturity and storage treatments. Lightness did not differ between CA and RA stored fruit from PF2010 (Table 3.18) and AF2011 (Table 3.19) while it decreased in some cultivars during shelf period. Decrease in L^* during shelf period was observed in early harvested RA stored fruit of ‘Afganski’ (RA=46.4 & RA+7D=39.4), late harvested fruit of ‘Crab’ (RA=40.3 & RA+7D=34.4) and ‘Entek-habi-saveh’ (RA=50.4 & RA+7D=44.1) under RA storage and ‘Kaj-acik-anor’ (CA=50.0 & CA+7D= 39.9) under CA storage. Early harvested fruit from treatment CA+7D had lower skin

L* compared to RA treatment in 'Afganski' (CA+7D=35.7 & RA=46.4), 'Entek-habi-saveh' (CA+7D=46.8 & RA=54.3) and 'Salavatski' (CA+7D=47.8 & RA=56.1). In AF 2011 fruit L* decreased in 'Al-sirin-nar' (CA=49.7 & CA+7D=43.6) and 'Nikitski-ranni' (CA=46.5 & CA+7D=41.2) during shelf period.

Hue angle was affected by cultivar (Tables 3.18-3.19). There were, however, several interactions among cultivar x storage treatments (Tables 3.18 and 3.19). CA stored fruit had higher hue angle than RA stored fruit in PF2010 'Cranberry' (CA = 37.1 and RA = 30.8) and AF2011 'Eversweet' (CA = 57.0 and RA = 54.9). Late harvested fruit had lower hue angle than early harvested fruit in 'Cranberry' (Early = 36.3 and Late = 32.2), 'Entek-habi-saveh' (Early = 59.1 and Late = 52.2), 'Kaj-acik-anor' (Early = 58.3 and Late=52.4) and 'Salavatski' (Early = 60.1 and Late = 53.2).

Chroma value of fruit skin was in general affected by cultivar, maturity and storage (Tables 3.18-3.19). There were, however, several interactions among cultivar, maturity and storage treatments. Chroma did not differ between CA or RA stored fruit. A few cultivars showed a decreased chroma during shelf period, as in early harvested fruit of PF2010 cultivars Kaj-acik-anor (CA = 41.9 and CA+7D = 35.9), Nikitski-rani (CA = 46.0 and CA+7D = 40.2), Afganski (RA = 42.8 and RA+7D = 36.7) and Crab (RA = 43.0 and RA+7D = 40.3). AF2011 fruit similarly showed decreased chroma during shelf period in cultivars Al-sirin-nar (CA = 42.8 and CA+7D = 30.4), Bala Miursal (CA = 45.8 and CA+7D = 35.8) and Nikitski-ranni (CA = 42.5 and CA+7D = 34.1).

Discussion

Fruit response to cold storage conditions was strongly influenced by cultivar. CA storage had a positive effect on most of the fruit attributes. After storage, during shelf period, fruit

quality deteriorated quickly. Water loss represents a major portion of fruit weight loss (75%-90%; Kader et al., 1984). Increase in vapor pressure deficit (VPD) during shelf period due to higher temperature and lower relative humidity must have increased rate of fruit water loss. Skin of pomegranate is very porous and allows rapid loss of moisture (Kader et al., 1984). Water loss from fruit reduced juice content of fruit arils. Increased water loss during shelf period possibly resulted in increased shriveling (Küpper et al., 1994). Increase in shriveling along with increase in fruit cracking during shelf period lead to decreased skin smoothness. This resulted in lower visual skin quality as excessive water loss and shriveling gave fruit skin a leathery appearance. Shriveling in pomegranate fruit is clearly visible on more than 5% weight loss (Kader et al., 1984).

Our finding that husk scald was reduced in CA storage is in accordance with one study in which pomegranates under CA storage had lesser incidence of husk scald as compared to those under RA storage (Defilippi et al., 2006). Husk scald during storage can result from the enzyme mediated oxidation of *o*-dihydroxyphenols present in the skin, although its amount in pomegranate peel is very low; pointing to possibility of different biochemical basis of scald. Enzyme mediated denaturation of skin tannins was reported to be the basis of fruit browning in another study (Zhang and Zhang, 2008). Rise in temperature during shelf period might be responsible for increase in scald symptoms, possibly due to increased enzymatic activity to oxidize skin components (Defilippi et al., 2006).

Reduced fungal damage (caused by *Cercospora* spp., *Botrytisphaeria* spp. and *Alternaria* spp.) in CA stored fruit was possibly because elevated CO₂ and lowered O₂ concentration in storage might have inhibited fungal infection and growth on fruit. Moreover, during RA storage due to enzymatic activity causing weakening of cell wall and increase in chilling injury

symptoms other fruit like avocado, papaya and carambola have been observed to become more susceptible to disease damage (Wang, 1993; Yon, 1994). Elevated CO₂ level has profound effect on fungal growth by suppressing respiration. Fungal growth is further suppressed with decrease in storage temperature. High CO₂ and low O₂ in storage atmosphere inhibits spore germination and mycelium growth of fungi like *Botrytis cinerea*, *Penicillium spp* (Conway et al., 2007; Lidster et al., 1983). CA storage conditions suppress pathogen growth and spreading (Yackel et al., 1971).

Elevated level of CO₂ during cold storage reduces chilling injury in fruit (Artes et al., 1996). Fruit under modified storage packaging are subjected as in CA to conditions of elevated CO₂ and low O₂ concentration where a decrease in chilling injury during storage has been observed. Modified atmosphere due to packaging showed lowered chilling injury in carambola (*Averrhoa carambola* L. cv. B10) fruit with possible suppression of enzymatic activity (Ali et al., 2004; Wang, 1993). High temperature during shelf period may trigger polyphenol oxidase (PPO) activity leading to increase in chilling injury (Ben-Arie and Or, 1986). Weight loss from fruit has been reported to positively influence chilling injury symptoms by decreasing fruit physiological health (Miller and McDonald, 1997; Mitra, 1997).

CA showed a positive effect on fruit TSS and TA. Sugars and organic acids are substrates for respiration in fruit. Increased CO₂ and decreased O₂ in CA storage reduce respiration rate and thus TSS and TA (Ahumada et al., 1996; Klieber et al., 1996; YongHua et al., 2000). Higher TSS in fruit from 'Eversweet' in AF2011 might be a sampling error as there is a lot of variation in fruit properties even from the same tree. Our data showed reduced TA in CA stored fruit in PF2011 and AF2011 fruit possibly because of increased air CO₂ concentration. Similar results were found in strawberries under CA storage where increased CO₂ concentration (and decreased

O₂) led to decreased acidity of fruit (Almenar et al., 2005; Gil et al., 1997). CA stored fruit had lower titratable acidity (TA) than RA stored fruit in 2011, while in 2010 CA stored fruit had higher TA than RA stored fruit. This difference in observation of fruit titratable acidity during storage can be due to difference in fruit maturity at harvest. Fruit for study were taken from a farm at a different location. Moreover in 2011 there was a drought which might have affected the fruit acidity during storage.

Flavonoids are not substrates for polyphenol oxidase activity thus their concentration does not decrease during storage and shelf period (Baruah and Swain, 1959; Roberts, 1960). Various anthocyanins and total anthocyanins content were almost stable during storage and did not differ between CA and RA. Anthocyanins in apple have also been observed to be fairly stable during storage (Lin et al., 1989; Reay, 1998). Higher anthocyanins in 'Bala Miursal' under RA than CA storage might have resulted due to anthocyanin biosynthesis in fruit during RA conditions. As anthocyanin biosynthesis has been observed to occur in fruit even during storage (Gil et al., 1995), elevated CO₂ levels in storage atmosphere might have suppressed it in CA storage. Similar response was reported in pomegranate arils in which modified atmospheric packaging led to suppression of anthocyanin biosynthesis whereas it occurred normally in control unpackaged fruit (Artés et al., 2000; Holcroft et al., 1998).

Decrease in L* and Chroma during shelf storage shows that fruit skin becomes dull and decreased color richness. This might be due to degradation of some coloring pigments in fruit skin during shelf storage due to increased temperature during shelf. L* did not differ between CA & RA stored fruit are similar to earlier findings where fruit skin color characteristics did not change significantly during storage (Artés et al., 1998). High CO₂ and O₂ in storage atmosphere were shown to dampen the decrease in hue value in other fruit. This might have caused the richer

red color (lower hue angle) in RA stored fruit of some cultivars compared to CA stored. It is caused by a delay in synthesis of coloring pigments under controlled atmospheres (Ali et al., 2010; Buescher, 1979; Holcroft et al., 1998).

Maturity at harvest influenced fruit quality attributes. Changes in fruit attributes as increased fruit cracking, decreased skin smoothness and increased TSS were observed during fruit maturation. TSS increases during maturity due to increase in soluble sugars from starch hydrolysis. Many fruit like pomegranate and kiwifruit show increased TSS with maturation (Kulkarni and Aradhya, 2005). Anthocyanins content in fruit increases during maturity in apple and pomegranate (Ju et al., 1996; Kulkarni and Aradhya, 2005).

Conclusion

Pomegranate fruit being a non-climacteric fruit needs to be harvested at right maturity status. Changes in TSS and TA during maturity play very important role determining the final flavor of fruit. Changes in fruit weight and juice/weight content influence marketability of fruit. CA stored fruit had better fruit quality with smoother skin, less skin shriveling, lower fruit cracking, lesser husk scald and lower chilling injury compared to RA stored fruit. Decay caused by *Cercospora* and other fungal pathogens was lesser in fruit stored under CA compared to RA. Fruit color and juice TSS were better preserved in fruit under CA storage than RA. Fruit quality deteriorated rapidly during shelf storage due to higher temperature (20-25°C) and low relative humidity (45%-50%). Thus it is evident that controlled atmosphere storage is definitely a better option for storing pomegranates.

References

- Ahumada M.H., Mitcham E.J., Moore D.G. (1996) Postharvest quality of thompson seedless' grapes after insecticidal controlled-atmosphere treatments. *HortScience* 31:833-836.
- Albrecht M., Jiang W., Kumi-Diaka J., Lansky E.P., Gommersall L.M., Patel A., Mansel R.E., Neeman I., Geldof A.A., Campbell M.J. (2004) Pomegranate extracts potently suppress proliferation, xenograft growth, and invasion of human prostate cancer cells. *Journal of Medicinal Food* 7:274-283. DOI: doi:10.1089/jmf.2004.7.274.
- Ali A., Maqbool M., Ramachandran S., Alderson P.G. (2010) Gum arabic as a novel edible coating for enhancing shelf-life and improving postharvest quality of tomato (*Solanum lycopersicum* L.) fruit. *Postharvest Biology and Technology* 58:42-47.
- Ali Z.M., Chin L.-H., Marimuthu M., Lazan H. (2004) Low temperature storage and modified atmosphere packaging of carambola fruit and their effects on ripening related texture changes, wall modification and chilling injury symptoms. *Postharvest Biology and Technology* 33:181-192.
- Almenar E., Hernández-Muñoz P., Lagarón J.M., Catalá R., Gavara R. (2005) Controlled atmosphere storage of wild strawberry fruit (*Fragaria vesca* L.). *Journal of Agricultural and Food Chemistry* 54:86-91.
- Aradhya S.M., Kulkarni A.P., Divakar S. (2004) Isolation and identification of a radical scavenging antioxidant - punicalagin from pith and carpellary membrane of pomegranate fruit. *Food Chemistry* 87:551-557.
- Artés F., Marín J.G., Martínez J.A., (1996) Controlled atmosphere storage of pomegranate. *Zeitschrift für Lebensmittel-Untersuchung und Forschung* 203:33-37.

- Artés F., Tudela J.A., Gil M.I. (1998) Improving the keeping quality of pomegranate fruit by intermittent warming. *Zeitschrift für Lebensmitteluntersuchung und -Forschung A* 207:316-321.
- Artés F., Villaescusa R., Tudela J.A. (2000) Modified atmosphere packaging of pomegranate. *Journal of Food Science* 65:1112-1116.
- Aviram M., Dornfeld L., Rosenblat M., Volkova N., Kaplan M., Colemann R., Hayek T., Presser D., Fuhrman B. (2000) Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. *American Journal of Clinical Nutrition* 71:1062-1076.
- Aviram M., Rosenblat M., Gaitini D., Nitecki S., Hoffman A., Dornfeld L., Volkova N., Presser D., Attias J., Liker H., Hayek T. (2004) Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation. *Clinical Nutrition* 23:423-433.
- Badria F.A. (2002) Melatonin, serotonin, and tryptamine in some egyptian food and medicinal plants. *Journal of Medicinal Food* 5:153-157.
- Baruah P., Swain T. (1959) The action of potato phenolase on flavonoid compounds. *Journal of the Science of Food and Agriculture* 10:125-129.
- Bashir H.A., Abu-Goukh A.-B.A. (2003) Compositional changes during guava fruit ripening. *Food Chemistry* 80:557-563.
- Basu A., Penugonda K. (2009) Pomegranate juice: a heart-healthy fruit juice. *Nutrition Reviews* 67:49-56.

- Beaudry R.M. (1999) Effect of O₂ and CO₂ partial pressure on selected phenomena affecting fruit and vegetable quality (vol 15, pg 293, 1999). *Postharvest Biology and Technology* 16:199-199.
- Ben-Arie R., Or E. (1986) The development and control of husk scald on «Wonderful» pomegranate fruit during storage. *Journal of the American Society for Horticultural Science* 111:395-399.
- Biale J.B. (1961) The postharvest biochemistry of tropical and subtropical fruits. *Advances in food research* 10:293-354
- Borochoy-Neori H., Judeinstein S., Tripler E., Harari M., Greenberg A., Shomer I., Holland D. (2009) Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (*Punica granatum* L.) fruit. *Journal of Food Composition and Analysis* 22:189-195.
- Brady C.J. (1987) Fruit ripening. *Annual review of plant physiology* 38:155-178.
- Buescher R. (1979) Influence of carbon dioxide on postharvest ripening and deterioration of tomatoes. *Journal American Society for Horticultural Science* 104:545-547.
- Considine J., Brown K. (1981) Physical aspects of fruit growth theoretical analysis of distribution of surface growth forces in fruit in relation to cracking and splitting. *Plant physiology* 68:371-376.
- Conway W.S., Janisiewicz W.J., Leverentz B., Saftner R.A., Camp M.J. (2007) Control of blue mold of apple by combining controlled atmosphere, an antagonist mixture, and sodium bicarbonate. *Postharvest Biology and Technology* 45:326-332.
- D'Aquino S., Palma A., Schirra M., Continella A., Tribulato E., La Malfa S. (2010) Influence of film wrapping and fludioxonil application on quality of pomegranate fruit. *Postharvest Biology and Technology* 55:121-128.

- Defilippi B.G., Whitaker B.D., Hess-Pierce B.M., Kader A.A. (2006) Development and control of scald on wonderful pomegranates during long-term storage. *Postharvest Biology and Technology* 41:234-243.
- Ding C.K., Chachin K., Hamauzu Y., Ueda Y., Imahori Y. (1998) Effects of storage temperatures on physiology and quality of loquat fruit. *Postharvest Biology and Technology* 14:309-315.
- Ding Z., Tian S., Wang Y., Li B., Chan Z., Han J., Xu Y. (2006) Physiological response of loquat fruit to different storage conditions and its storability. *Postharvest Biology and Technology* 41:143-150.
- Du C., Wang P., Francis F. (1975) Anthocyanins of pomegranate, *Punica granatum*. *Journal of Food Science* 40:417-418.
- El-Nemr S., Ismail I., Ragab M. (1990) Chemical composition of juice and seeds of pomegranate fruit. *Food/Nahrung* 34:601-606
- Fuhrman B., Volkova N., Aviram M. (2005) Pomegranate juice inhibits oxidized LDL uptake and cholesterol biosynthesis in macrophages. *Journal of Nutritional Biochemistry* 16:570-576.
- Gil M.I., Garcavioguera C., Artes F., Tomasbarberan F.A. (1995) Changes in pomegranate juice pigmentation during ripening. *Journal of the Science of Food and Agriculture* 68:77-81.
- Gil M.I., Holcroft D.M., Kader A.A. (1997) Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. *Journal of Agricultural and Food Chemistry* 45:1662-1667.

- Gil M.I., Tomas-Barberan F.A., Hess-Pierce B., Holcroft D.M., Kader A.A. (2000) Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry* 48:4581-4589.
- Gómez P.A., Artés F. (2004) Controlled atmospheres enhance postharvest green celery quality. *Postharvest Biology and Technology* 34:203-209.
- Gray J., Picton S., Shabbeer J., Schuch W., Grierson D. (1992) Molecular biology of fruit ripening and its manipulation with antisense genes. *Plant Molecular Biology* 19:69-87.
- Heinonen I.M., Meyer A.S., Frankel E.N. (1998) Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *Journal of Agricultural and Food Chemistry* 46:4107-4112.
- Hertog M.L.A.T.M., Nicholson S.E., Whitmore K. (2003) The effect of modified atmospheres on the rate of quality change in 'Hass' avocado. *Postharvest Biology and Technology* 29:41-53.
- Holcroft D.M., Gil M.I., Kader A.A. (1998) Effect of carbon dioxide on anthocyanins, phenylalanine ammonia lyase and glucosyltransferase in the arils of stored pomegranates. *Journal of the American Society for Horticultural Science* 123:136-140.
- Holcroft D.M., Kader A.A. (1999) Carbon dioxide-induced changes in color and anthocyanin synthesis of stored strawberry fruit. *Hortscience* 34:1244-1248.
- Horak P., Zilmer M., Saks L., Ots I., Karu U., Zilmer K. (2006) Antioxidant protection, carotenoids and the costs of immune challenge in greenfinches. *Journal of Experimental Biology* 209:4329-4338.
- Kader A.A., Chordas A., Elyatem S. (1984) Responses of pomegranates to ethylene treatment and storage temperature. *California Agriculture* 38:14-15.

- Kader A.A. (2001) A summary of ca requirements and recommendations for fruits other than apples and pears. pp. 737-740.
- Kader A.A. (2006) 14 Postharvest Biology and Technology of Pomegranates. In *Pomegranates. Ancient roots to modern medicine*; Seeram, N.P., Schulman, R.N., Heber, D., Eds.; CRC Press-Taylor and Francis: Boca Raton, FL; pp 211-218.
- Kamimura H., Hammock B.D., Yamamoto I., Casida J.E. (1972) Potent juvenile hormone mimic, 1-(4'-ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene, labeled with tritium in either the ethylphenyl- or geranyl-derived moiety. *Journal of Agricultural and Food Chemistry* 20:439-442.
- Kar A., Parmar H.S. (2007) Protective role of *Citrus sinensis*, *Musa paradisiaca*, and *Punica granatum* peels against diet-induced atherosclerosis and thyroid dysfunctions in rats. *Nutrition Research* 27:710-718.
- Kawaii S., Lansky E.P. (2004) Differentiation-promoting activity of pomegranate (*Punica granatum*) fruit extracts in HL-60 human promyelocytic leukemia cells. *Journal of Medicinal Food* 7:13-18.
- Klieber A., Ratanachinakorn B., Simons D. (1996) Effects of low oxygen and high carbon dioxide on tomato cultivar 'Bermuda' fruit physiology and composition. *Scientia Horticulturae* 65:251-261.
- Kulkarni A.P., Aradhya S.M. (2005) Chemical changes and antioxidant activity in pomegranate arils during fruit development. *Food Chemistry* 93:319-324.
- Küpper W., Pekmezci M., Henze J. (1994) Studies on CA-storage of pomegranate (*Punica granatum* L., cv. Hicaz). *Postharvest Physiology of Fruits* 398:101-108.

- Lee S., Kim K., Kim S. (1974) Studies on changes in the composition of the pomegranate fruit during maturation. In. Changes in sugars, organic acids, amino acids, and the respiration rate. Journal of the Korean Society for Horticultural Science 15:57-68.
- Li Y.H., Huang T.H.W., Peng G., Kota B.P., Li G.Q., Yamahara J., Roufogalis B.D. (2005) Anti-diabetic action of *Punica granatum* flower extract: Activation of PPAR-gamma and identification of an active component. Toxicology and Applied Pharmacology 207:160-169.
- Lidster P., Lightfoot H., McRae K. (1983) Fruit quality and respiration of 'McIntosh' apples in response to ethylene, very low oxygen and carbon dioxide storage atmospheres. Scientia Horticulturae 20:71-83.
- Lin T., Koehler P., Shewfelt R. (1989) Stability of anthocyanins in the skin of starkrimson apples stored unpackaged, under heat shrinkable wrap and in-package modified atmosphere. Journal of Food Science 54:405-407.
- Lopez-Rubira V., Conesa A., Allende A., Artes F. (2005) Shelf life and overall quality of minimally processed pomegranate arils modified atmosphere packaged and treated with UV-C. Postharvest Biology and Technology 37:174-185.
- Mayer W., Gorner A., Andra K. (1977) Punicalagin und punicalin, zwei gerbstoffe aus den schalen der granatapfel Liebigs. Ann. Chem. 1976-1986.
- Melgarejo P., Salazar D.M., Amorós A., Artés F. (1995) Total lipids content and fatty acid composition of seed oils from six pomegranate cultivars. Journal of the Science of Food and Agriculture 69:253-256.
- Miller W., McDonald R. (1997) Carambola quality after ethylene and cold treatments and storage. Hortscience 32:897-899.

- Mitra S.K. (1997) Postharvest physiology and storage of tropical and subtropical fruits *Cab International* 18:365-373.
- Nawwar, M.A.M., Hussein, S.A.M., Merfort, I. (1994) Leaf phenolics of *Punica granatum*. *Phytochemistry*, 37:1175-1177.
- Neuhöfer H. (1990) The existence of pelletierine derivatives in *Punica granatum*. *World Phytomed* 5:604
- Osawa T., Ide A., Su J.D., Namiki M. (1987) Inhibition of in vitro lipid peroxidation by ellagic acid. *Journal of Agricultural and Food Chemistry* 35:808-812.
- Ozawa, T.H. Lillas, E. Haslam (1987) Polyphenol interactions: astringency and the loss of astringency in ripening fruit. *Phytochemistry*, 26:2937–2942
- Palou L., Crisosto C.H., Garner D. (2007) Combination of postharvest antifungal chemical treatments and controlled atmosphere storage to control gray mold and improve storability of ‘Wonderful’ pomegranates. *Postharvest Biology and Technology* 43:133-142.
- Prabhu Desai, V. G. (1989). Investigation on internal breakdown of pomegranate fruit, Ph.D. Thesis, MPWV, Rahuri
- Reay P.F. (1998) The effects of maturity on colour and concentration of pigments in the blush and shaded sides of Gala 'apple fruit during cool storage. *Journal of Horticultural Science and Biotechnology* 73: 841-845.
- Roberts E. (1960) Effect of glycosylation on the enzymic oxidation and translocation of flavonoids. *Nature* 185:536-537.
- Scharrer A., Ober M. (1981) Anthocyanosides in the treatment of retinopathies. *Klinische Monatsblätter Für Augenheilkunde* 178:386-389.

- Schubert S.Y., Lansky E.P., Neeman I. (1999) Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. *Journal of Ethnopharmacology* 66:11-17.
- Sestili P., Martinelli C., Ricci D., Fraternale D., Bucchini A., Giamperi L., Curcio R., Piccoli G., Stocchi V. (2007) Cytoprotective effect of preparations from various parts of *Punica granatum* L. fruit in oxidatively injured mammalian cells in comparison with their antioxidant capacity in cell free systems. *Pharmacological Research* 56:18-26.
- Shulman Y., Fainberstein L., Lavee S. (1984) Pomegranate fruit-development and maturation. *Journal of Horticultural Science* 59:265-274.
- Singh R.P., Murthy K.N.C., Jayaprakasha G.K. (2002) Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models. *Journal of Agricultural and Food Chemistry* 50:81-86.
- Smock R.M. (1979) Controlled atmosphere storage of fruits. *Horticultural reviews*:301-336.
- Sudheesh S., Vijayalakshmi N.R. (2005) Flavonoids from *Punica granatum* - potential antiperoxidative agents. *Fitoterapia* 76:181-186.
- Sun M., Surveswaran S., Cai Y.Z., Corke H. (2007) Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. *Food Chemistry* 102:938-953.
- Tanaka, T.; Nonaka, G.I.; Nishioka, I. (1985) Punicafolin, and ellagitannin from the leaves of *Punica granatum*. *Phytochemistry*, 24:2075-2078.
- Tanaka T., Nonaka G. I., Nishioka I. (1986) Tannins and related compounds. XLI. Isolation and characterization of novel ellagitannins, punicacorteins A, B, C and D and punigluconin from the bark of *Punica granatum* L., *Chem. Pharm. Bull.* 34:656-663.

- Tucker G., Grierson D. (1987) Fruit ripening. *The biochemistry of plants: A comprehensive treatise* 12:265-318.
- Wang C.Y. (1993) Approaches to reduce chilling injury of fruits and vegetables. *Hort. Rev* 15:63-95.
- Wang H., Cao G.H., Prior R.L. (1997) Oxygen radical absorbing capacity of anthocyanins. *Journal of Agricultural and Food Chemistry* 45:304-309.
- Wang R., Ding Y., Liu R., Xiang L., Du L. (2010) Pomegranate: Constituents, Bioactivities and Pharmacokinetics. *FVCSB* 4:77–87.
- Weerakkody P., Jobling J., Infante M.M.V., Rogers G. (2010) The effect of maturity, sunburn and the application of sunscreens on the internal and external qualities of pomegranate fruit grown in Australia. *Scientia Horticulturae* 124:57-61.
- Wetzstein H.Y., Zhang Z., Ravid N., Wetzstein M.E. (2011) Characterization of attributes related to fruit size in pomegranate. *Hortscience* 46:908-912.
- Yackel W.C., Nelson A., Wei L., Steinberg M. (1971) Effect of controlled atmosphere on growth of mold on synthetic media and fruit. *Applied microbiology* 22:513-516.
- Yahia E.M. (2006) Modified and controlled atmospheres for tropical fruits. *Stewart Postharvest Review* 2:1-10.
- Yon R.M. (1994) Papaya: fruit development, postharvest physiology, handling and marketing in ASEAN. ASEAN Food Handling Bureau.
- YongHua Z., XinGuo S., QianJiu L., SanYu L., YuFang X. (2000) Effect of high oxygen on respiration rate, polyphenol oxidase activity and quality in postharvest loquat fruits. *Plant Physiology Communications* 36:318-320.

Zhang Y., Zhang R. (2008) Study on the Mechanism of Browning of Pomegranate (*Punica granatum*. Ganesh) Peel in Different Storage Conditions. Agricultural Sciences in China 7:65-73.

Table 3.1. Physical attributes of pomegranate fruit harvested from Ponder Farm in 2010

immediately after storage [(cold storage in either controlled atmosphere storage (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)] or after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) following storage.

	Skin smoothness ^z	Fruit Cracking ^y	Sunscald ^x	Skin Shriveling ^w	Husk scald ^v	Cercospora severity ^u
Cultivar						
Afganski	2.1	1.4 ab ^t	2.2	2.9	1.2 a	3.1 a
Crab	3.0	1.5 a	2.1	2.4	1.3 a	2.1 c
Cranberry	3.1	1.2 cd	2.3	1.9	1.1 b	2.3 b
Entek-habi-saveh	2.8	1.0 d	1.9	1.8	1.0 b	2.9 a
Kaj-acik-anor	3.2	1.2 bc	2.0	1.5	1.0 b	2.9 a
Nikitski-ranni	3.5	1.1 cd	2.0	1.7	1.2 a	2.1 c
Salavatski	3.8	1.0 d	2.0	1.3	1.0 b	2.3 b
Maturity ^s						
Early	3.3	1.1 b	2.0	1.8 b	1.1	2.5
Late	2.8	1.3 a	2.2	2.1 a	1.1	2.7
Storage						
CA	3.1	1.1 b	2.0	1.8	1.1 b	2.5
CA+7D	3.2	1.2 ab	2.1	2.0	1.1 b	2.5
RA	3.0	1.2 ab	2.1	2.0	1.0 b	2.6
RA+7D	2.9	1.3 a	2.1	2.1	1.2 a	2.6
Source						
Cultivar (C)	<.0001	<.0001	0.0032	<.0001	<.0001	<.0001
Maturity (M)	<.0001	0.0002	0.0026	<.0001	0.7689	0.0098
Storage (S)	0.0112	0.0446	0.3035	0.0106	0.0011	0.0697
C x M	0.0043	0.3451	0.8785	0.0626	0.9436	0.0703
C x S	0.0019	0.6697	0.301	0.0004	0.2334	0.4114
M x S	0.1156	0.2771	0.7287	0.2485	0.3891	0.0031
C x M x S	0.1379	0.7036	0.0019	0.0806	0.9977	0.146

^zSkin smoothness was graded on 1-5 scale (1= low, 5= high)

^yFruit cracking on 1-4 scale (1= no cracking; 4= severe cracking; fruit split open on one side)

^xSunscald on a scale of 1-4 (1= no sunscald, 4= severe sunscald)

^wSkin shriveling on 1-4 scale (1= no shriveling; 4= severe shriveling, unmarketable)

^vHusk scald on 1-4 scale (1= no husk scald, 4= severe; more than half of fruit husk damaged)

^uCercospora severity on 1-4 scale (1= negligible , 4= high, unmarketable fruit)

^tValues followed by the same letter within a column are not significantly different at $P < 0.05$

level, according to Duncan multiple range test.

^sFruit harvested in 2nd week September (Early) and 1st week October (Late).

Table 3.2. Physical attributes of pomegranate fruit harvested from Ponder Farm in 2011 immediately after storage [(cold storage in either controlled atmosphere storage (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)].

	Skin smoothness ^z	Fruit cracking ^y	Sunscald ^x	Skin shriveling ^w	Husk Scald ^v	Cercospora severity ^u	Other fungal diseases ^t	Chilling injury ^s
Cultivar								
Afganski	2.8 c ^r	2.5 a	2.6	2.5 ab	2.8 a	2.5 a	1.6 bc	2.0 a
Crab	4.1 a	1.3 c	2.1	1.9 b	2.5 ab	2.0 b	2.3 ab	1.5 bc
Cranberry	3.3 b	2.0 ab	2.5	2.5 ab	2.0 ab	2.5 a	2.7 a	1.2 c
Kaj-acik-anor	3.5 b	1.1 c	2.4	2.3 ab	1.1 c	2.0 b	1.3 c	1.1 c
Nikitski-ranni	3.1 bc	1.3 c	2.5	2.6 a	2.4 ab	2.1 b	1.6 bc	1.6 ab
Salavatski	4.1 a	1.8 bc	2.4	1.9 b	1.9 b	2.3 ab	1.1 c	1.6 ab
Storage								
CA	3.9 a	1.5 a	2.2 b	2.0 b	1.6 b	1.9 b	1.3 b	1.0 b
RA	3.1 b	1.8 a	2.7 a	2.5 a	2.6 a	2.6 a	2.1 a	2.0 a
Source								
Cultivar (C)	<0.0001	0.0007	0.395	0.0291	0.0011	0.0052	0.0139	0.0008
Storage (S)	<0.0001	0.0975	0.0016	0.0117	<0.0001	<0.0001	0.0021	<0.0001
C x S	0.9345	0.0141	0.0083	0.9379	0.1556	0.0003	0.7011	0.0008

^zSkin smoothness was graded on 1-5 scale (1= low, 5= high)

^yFruit cracking on 1-4 scale (1= no cracking; 4= severe cracking; fruit split open on one side)

^xSunscald on a scale of 1-4 (1= no sunscald, 4= severe sunscald)

^wSkin shriveling on 1-4 scale (1= no shriveling; 4= severe shriveling, unmarketable)

^vHusk scald on 1-4 scale (1= no husk scald, 4= severe; more than half of fruit husk damaged)

^uCercospora severity on 1-4 scale (1= negligible , 4= high, unmarketable fruit)

^t Severity of other fungal diseases (*Alternaria* and *Botryospheria*) on 1-4 scale (1= no disease, 4= high, unmarketable fruit)

^sChilling injury on 1-4 scale (1= no chilling injury, 4= severe, more than half of arils affected, fruit unmarketable).

^rValues followed by the same letter within a column are not significantly different at $P < 0.05$ level, according to Duncan multiple range test.

Table 3.3. Physical attributes of pomegranate fruit harvested from Alma Farm in 2011 immediately after storage [(cold storage in either controlled atmosphere storage (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)] or after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) following storage.

	Skin smoothness ^z	Fruit cracking ^y	Sunscald ^x	Shriveling ^w	Husk scald ^v	Cercospora severity ^u	Other fungal diseases ^t	Chilling injury ^s
Cultivar								
Al-sirin-nar	2.4	1.6 a ^r	2.3	3.3	2.9	1.9	1.5	1.8
Bala Miursal	2.8	1.9 a	2.1	3.0	2.8	1.9	2.0	1.4
Eversweet	3.1	1.4 ab	2.1	2.6	2.3	1.6	2.3	2.6
Nikitski-ranni	2.5	1.6 a	2.1	3.3	2.6	1.9	1.8	1.6
Sweet	3.8	1.1 b	1.6	1.6	1.2	1.3	1.6	3.1
Storage								
CA	4.5	1.1 b	1.3	1.5	1.2	1.1	1.3	1.5
CA+7D	2.4	1.4 b	2.5	3.1	2.6	1.7	1.5	1.5
RA	3.0	1.5 b	1.8	2.9	2.5	1.7	2.1	2.0
RA+7D	1.9	2.1 a	2.6	3.5	3.0	2.4	2.5	3.4
Source								
Cultivar (C)	<.0001	0.0109	0.0004	<.0001	<.0001	<.0001	0.1722	<.0001
Storage (S)	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001
C x S	<.0001	0.0523	0.0085	0.0245	<.0001	0.0033	0.0012	<.0001

^zSkin smoothness was graded on 1-5 scale (1= low, 5= high)

^yFruit cracking on 1-4 scale (1= no cracking; 4= severe cracking; fruit split open on one side)

^xSunscald on a scale of 1-4 (1= no sunscald, 4= severe sunscald)

^wSkin shriveling on 1-4 scale (1= no shriveling; 4= severe shriveling, unmarketable)

^vHusk scald on 1-4 scale (1= no husk scald, 4= severe; more than half of fruit husk damaged)

^uCercospora severity on 1-4 scale (1= negligible , 4= high, unmarketable fruit)

^t Severity of other fungal diseases (*Alternaria* and *Botryospheria*) on 1-4 scale (1= no disease, 4= high, unmarketable fruit)

^sChilling injury on 1-4 scale (1= no chilling injury, 4= severe, more than half of arils affected, fruit unmarketable).

^rValues followed by the same letter within a column are not significantly different at $P < 0.05$ level, according to Duncan multiple range test.

Table 3.4. Effect of cultivar and storage [controlled air (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH) or regular air (RA; 5°C and 90%-95% RH)] interaction immediately after storage and after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) on various attributes of pomegranate fruit from Ponder Farm, Ty Ty, GA, 2010.

	Skin smoothness ^z				Skin shriveling ^y			
	CA	CA+7D	RA	RA+7D	CA	CA+7D	RA	RA+7D
Cultivar								
Afganski	1.8 c ^x	2.0 b	2.4 c	2.2 b	3.1 Aab	3.4 A ^w	2.7 AB	2.6 B
Crab	2.8 b	3.3 a	3.2 ab	2.8 ab	2.3 b	2.4	2.4	2.6
Cranberry	3.0 b	3.5 a	3.1 ab	2.7 ab	1.9 bc	1.9	1.9	2.1
Entek-habi-saveh	3.1 b	2.8 ab	2.6 bc	3.0 ab	1.8 bd	1.9	1.8	1.8
Kaj-acik-anor	3.4 bc	3.4 a	2.8 bc	3.1 ab	1.2 cd	1.4	1.8	1.8
Nikitski-ranni	3.8 b	3.9 a	3.8 ab	2.6 ab	1.4 b	1.9	1.4	2.3
Salavatski	4.2 a	4.0 a	3.6 ab	3.6 a	1.1 d	1.3	1.5	1.4
<i>P</i>	0.0019				0.0004			
	pH ^v							
	CA	CA+7D	RA	RA+7D				
Cultivar								
Afganski	2.9	2.79	3.09	2.76				
Crab	3.08 A	2.76 B	3.08 AB	2.92 AB				
Cranberry	2.90 B	2.98 AB	3.23 A	2.88 AB				
Entek-habi-saveh	3.05 AB	2.82 B	3.23 A	2.92 AB				
Kaj-acik-anor	2.87	2.75	3.04	2.69				
Nikitski-ranni	3.04	2.87	3.36	3.09				
Salavatski	3.03 B	2.83 B	3.35 A	2.61 B				
<i>P</i>	0.0121							

^zSkin smoothness was graded on 1-5 scale (1= low, 5= high)

^ySkin shriveling on 1-4 scale (1= no shriveling and 4= severe shriveling; unmarketable)

^xValues followed by the same lower case letter within a column are not significantly different at $P < 0.05$ level according to Tukey Lsmeans comparison.

^wValues followed by the same upper case letter within a row are not significantly different at $P < 0.05$ level according to Tukey Lsmeans comparison.

^vJuice pH

Table 3.5. Effect of cultivar and storage [controlled air (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH) or regular air (RA; 5°C and 90%-95% RH)] interaction immediately after storage and after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) on various attributes of pomegranate fruit from Alma Farm, Alma, GA, 2011.

	Skin smoothness ^z				Sunscauld ^y			
	CA	CA+7D	RA	RA+7D	CA	CA+7D	RA	RA+7D
Cultivar								
Al-sirin-nar	4.3 A ^x	1.0 B	3.0 A	1.5 B	1.3 B	2.5 AC	2.0 BC	3.3 A
Bala Miursal	4.8 A	2.8 B	2.5 C	1.0 D	1.5	2.5	2	2.5
Eversweet	4.5 A	3.3 AB	3.0 BC	1.8 C	1.5	2	2	2.8
Nikitski-ranni	4.3 A	1.5 B	2.5 B	1.8 B	1.3 B	3.3 A	1.8 B	2.3 AB
Sweet	4.8 A	3.3 B	3.8 AB	3.3 B	1	2	1.3	2
<i>P</i>	<.0001				0.0085			
	Skin Shriveling ^w				Husk scald ^v			
	CA	CA+7D	RA	RA+7D	CA	CA+7D	RA	RA+7D
Cultivar								
Al-sirin-nar	1.8 B	4.0 A	3.3 A	4.0 A	1.5 B	3.5 A	3.3 A	3.3 A
Bala Miursal	1.5 B	3.3 A	3.3 A	4.0 A	1.0 B	3.3 A	2.8 A	4.0 A
Eversweet	1.3 B	2.5 A	3.0 A	3.5 A	1.5	2	2.5	3.3
Nikitski-ranni	2.0 B	3.8 A	3.5 A	3.8 A	1.0 B	3.0 A	3.3 A	3.0 A
Sweet	1	2	1.5	2	1	1.3	1	1.5
<i>P</i>	0.0245				<.0001			

^zSkin smoothness was graded on 1-5 scale (1= low, 5= high)

^ySunscald on a scale of 1-4 (1= no sunscald, 4= severe sunscald)

^xValues followed by the same upper case letter within a row are not significantly different at $P < 0.05$ level, according to Tukey Lsmeans comparison

^wSkin shriveling on 1-4 scale (1= no shriveling and 4= severe shriveling, unmarketable fruit)

^vHusk scald (1= no husk scald, 4= severe; more than half of fruit skin damaged by scald)

Table 3.6. Effect of cultivar maturity interaction on skin smoothness of pomegranate fruit from Ponder Farm, Ty Ty, GA, 2010.

Cultivar	Skin smoothness ^z	
	Early ^y	Late
Afganski	2.3 d ^x	1.9 c
Crab	3.0 c	3.0 ab
Cranberry	3.4 bc	2.8 b
Entek-habi-saveh	2.9 c	2.8 b
Kaj-acik-anor	3.7 A ^w ab	2.7 Bb
Nikitski-ranni	3.5 bc	.
Salavatski	4.2 Aa	3.5 Ba
<i>P</i>	0.0043	

^zSkin shriveling on 1-4 scale (1= no shriveling and 4= severe shriveling, unmarketable fruit)

^yFruit harvested in 2nd week September (Early) and 1st week October (Late).

^xValues followed by the same lower case letter within a column are not significantly different at $P < 0.05$ level, according to Tukey Lsmeans comparison.

^wValues followed by the same upper case letter within a row are not significantly different at $P < 0.05$ level, according to Tukey Lsmeans comparison.

Table 3.7. Effect of cultivar and storage [controlled air (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH); regular air (RA; 5°C and 90%-95% RH)] on various fruit attributes in fruit from Ponder farm 2010.

Cultivar	Fruit Cracking ^z		Sunscauld ^y		Cercospora severity ^x		Chilling injury ^w	
	CA	RA	CA	RA	CA	RA	CA	RA
Afganski	1.8 A ^u	3.3 B*a	2.3	3.0	2.0 B	3.0 Aa	1.0 B	3.0 Aa
Crab	1.0	1.5 b ^v	1.8	2.5	1.3 B	2.8 Aab	1.0 B	2.0 Abc
Cranberry	1.7	2.3 ab	2.3	2.7	2.0 B	3.0 Aa	1.0	1.3 c
Kaj-acik-anor	1.0	1.3 b	2.0	2.8	2.0	2.0 b	1.0	1.3 c
Nikitski-ranni	1.3	1.3 b	2.0	3.0	2.0	2.3 ab	1.0 B	2.3 Aab
Salavatski	2.3	1.3 b	2.8	2.0	2.0	2.5 ab	1.0 B	2.3 Aab
<i>P</i>	0.0141		0.0083		0.0003		0.0008	

^zFruit cracking on 1-4 scale (1= no cracking; 4= severe cracking; fruit split open on one side)

^ySunscauld on a scale of 1-4 (1= no sunscauld, 4= severe sunscauld)

^xCercospora severity on 1-4 scale (1= negligible, 4= high, unmarketable fruit)

^wChilling injury on 1-4 scale (1= no chilling injury, 4= severe, more than half of arils affected, fruit unmarketable).

^vValues followed by the same lower case letter within a column are not significantly different at $P < 0.05$ level, according to Tukey Lsmeans comparison.

^uValues followed by the same upper case letter within a row are not significantly different at $P < 0.05$ level, according to Tukey Lsmeans comparison.

Table 3.8. Effect of fruit maturity (early and late) and storage [controlled air (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH) or regular air (RA; 5°C and 90%-95% RH)] interaction for attributes immediately after storage and after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) in pomegranate fruit harvested from Ponder farm, 2010.

	Cercospora severity ^z		Juice/weight ratio ^y	
	Early ^v	Late	Early	Late
Storage				
CA	2.5	2.5	0.79 a ^w	0.80
CA+7D	2.5	2.6	0.81 a	0.80
RA	2.4	2.9	0.71 b	0.76
RA+7D	2.5	2.7	0.81 a	0.81
<i>P</i>	0.0031		0.0308	

^zCercospora severity on 1-4 scale (1= negligible, 4= severe, unmarketable fruit)

^wValues followed by the same lower case letter within a column are not significantly different at *P* <0.05 level, according to Tukey Lsmeans comparison.

^vFruit harvested in 2nd week September (Early) and 1st week October (Late).

Table 3.9. Effect of cultivar and storage [(cold storage in either controlled atmosphere storage (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)] interaction immediately after storage or after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) following storage on various attributes in pomegranate fruit harvested from Alma farm, 2011.

	Cercospora severity ^z				Other fungal diseases ^y			
	CA	CA+7D	RA	RA+7D	CA	CA+7D	RA	RA+7D
Cultivar								
Al-sirin-nar	1.3	2.0	2.0	2.3	1.0	1.0	2.7	1.8
Bala Miursal	1.0 B ^w	1.8 B	2.0 AB	3.0 A	1.0 B	1.0 B	2.3 AB	3.8 A
Eversweet	1.0 B	1.0 B	2.0 AB	2.3 A	1.8	1.8	2.5	3.0
Nikitski-ranni	1.3 B	2.3 AB	1.5 B	2.8 A	1.0	1.0	2.3	3.0
Sweet	1.0	1.3	1.0	1.8	1.8	2.5	1.0	1.0
<i>P</i>	0.0033				0.0012			
Chilling injury ^x								
	CA	CA+7D	RA	RA+7D				
Cultivar								
Al-sirin-nar	1.0 C	1.0 C	2.0 B	3.3 A				
Bala Miursal	1.0 B	1.0 B	1.0 B	2.8 A				
Eversweet	2.3 B	2.5 B	2.0 B	3.5 A				
Nikitski-ranni	1.0 B	1.0 B	1.0 B	3.5 A				
Sweet	2.3 B	2.0 B	4.0 A	4.0 A				
<i>P</i>	<.0001							

^zCercospora severity on 1-4 scale (1= negligible, 4= high, unmarketable fruit)

^ySeverity of other fungal diseases (*Alternaria*, *Botryospheria* etc.) on 1-4 scale (1= no disease, 4= high, unmarketable fruit)

^xChilling injury on 1-4 scale (1= no chilling injury, 4= severe; more than half of arils turned brown due to chilling injury, fruit unmarketable).

^wValues followed by the same upper case letter within a row are not significantly different at $P < 0.05$ level, according to Tukey Lsmeans comparison.

Table 3.10. Juice attributes of pomegranate fruit harvested from Ponder Farm in 2010

immediately after storage [(cold storage in either controlled atmosphere storage (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)] or after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) following storage.

	Aril weight (g/50 arils)	Juice weight (g/50 arils)	Juice/weight ratio	TSS (%) ^z	TA (%) ^y	TSS/TA ratio
Cultivar						
Afganski	17.11 b ^x	13.18 cd	0.77 bc	14.1	3.1	5.4
Crab	17.98 b	14.54 b	0.81 a	14.9	2.5	7.0
Cranberry	22.43 a	17.77 a	0.79 ab	14.5	2.5	7.0
Entek-habi-saveh	13.96 d	10.62 e	0.76 c	15.4	3.4	5.2
Kaj-acik-anor	17.12 b	13.67 c	0.80 ab	15.5	3.0	6.0
Nikitski-ranni	21.83 a	17.08 a	0.78 abc	14.3	2.3	6.9
Salavatski	15.88 c	12.54 d	0.79 ab	15.1	2.9	6.5
Maturity ^w						
Early	17.74 a	13.83 a	0.78	14.6	2.8	6.2
Late	17.77 a	14.13 a	0.79	15.2	2.9	6.3
Storage						
CA	17.63 b	14.00 b	0.79	15.1	3.0	6.2
CA+7D	18.39 a	14.80 a	0.80	14.9	3.6	4.4
RA	17.30 b	12.75 c	0.74	14.7	2.1	8.0
RA+7D	17.70 ab	14.38 ab	0.81	14.8	2.5	6.2
Source						
Cultivar (C)	<.0001	<.0001	0.0029	<.0001	<.0001	0.0005
Maturity (M)	0.0074	0.0007	0.0349	<.0001	0.9619	0.6478
Storage (S)	0.042	<.0001	<.0001	0.0038	<.0001	<.0001
C x M	0.3875	0.5016	0.7965	0.002	0.2977	0.0438
C x S	0.1475	0.1474	0.1276	0.0503	0.0034	<.0001
M x S	0.32	0.3186	0.0308	0.0049	<.0001	<.0001
C x M x S	0.244	0.1249	0.6846	0.0005	<.0001	<.0001

^zTSS (Total soluble solids).

^yTA (Titratable acidity) measured with an automatic titrator, expressed as % malic acid.

^xValues followed by the same letter within a column are not significantly different at $P < 0.05$ level, according to Duncan multiple range test.

^wFruit harvested in 2nd week September (Early) and 1st week October (Late).

Table 3.11. Juice attributes of pomegranate fruit harvested from Ponder Farm in 2011 immediately after storage [(cold storage in either controlled atmosphere storage (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)].

	Aril weight (g/50 arils)	Juice weight (g/50 arils)	Juice/weight ratio	TSS (%) ^z	TA (%) ^y	TSS/TA ratio
Cultivar						
Afganski	20.19 b ^x	15.51 b	0.77	14.6 c	1.65 a	9.02 c
Crab	21.24 ab	16.01 ab	0.75	16.4 a	1.04 b	15.97 a
Cranberry	23.63 a	17.93 a	0.77	14.3 c	0.96 b	15.20 a
Kaj-acik-anor	20.10 b	15.72 ab	0.78	15.6 ab	1.73 a	9.34 c
Nikitski-ranni	21.13 ab	16.4 ab	0.78	14.3 a	1.13 b	12.73 b
Salavatski	15.28 c	11.61 c	0.76	15.0 bc	1.19 b	12.76 b
Storage						
CA	19.90 a	15.15 a	0.76 a	15.2 a	1.23 b	13.07 a
RA	19.67 a	15.23 a	0.77 a	15.0 a	1.41 a	11.35 b
Source						
Cultivar (C)	<0.0001	<0.0001	NS	<0.0001	<0.0001	<0.0001
Storage (S)	0.8931	0.5364	NS	0.1874	0.0149	0.0001
C x S	0.5302	0.4543	NS	0.0317	0.7113	0.2191

^zTSS (Total soluble solids).

^yTA (Titratable acidity) measured with an automatic titrator, expressed as % malic acid.

^xValues followed by the same letter within a column are not significantly different at $P < 0.05$ level, according to Duncan multiple range test.

Table 3.12. Juice attributes of pomegranate fruit harvested from Alma Farm in 2011 immediately after storage [(cold storage in either controlled atmosphere storage (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)] or after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) following storage.

	Aril weight (g/50 arils)	Juice weight (g/50 arils)	Juice/weight ratio	TSS (%) ^z	TA (%) ^y	TSS/TA ratio
Cultivar						
Al-sirin-nar	24.14 a ^x	17.8	0.74	10.3	0.89 b	12.6
Bala Miursal	20.75 b	15.6	0.75	11.0	1.28 a	9.3
Eversweet	20.29 b	15.6	0.76	12.6	0.49 c	27.9
Nikitski-ranni	21.42 b	15.4	0.71	10.3	1.00 b	11.0
Sweet	18.57 b	13.5	0.73	12.3	0.27 c	49.8
Storage						
CA	21.39	16.8	0.78	11.2	0.71 b	24.0
CA+7D	20.69	13.9	0.67	11.1	0.71 b	21.8
RA	21.94	16.5	0.75	11.6	1.19 a	12.0
RA+7D	21.19	15.6	0.73	11.0	0.88 b	14.6
Source						
Cultivar (C)	0.0082	0.1038	0.1782	<.0001	<.0001	<.0001
Storage (S)	0.7575	0.0707	<.0001	0.0101	0.005	0.0016
C x S	0.1943	0.0447	0.0109	0.0005	0.245	0.0202

^zTSS (Total soluble solids).

^yTA (Titratable acidity) measured with an automatic titrator, expressed as % malic acid.

^xValues followed by the same letter within a column are not significantly different at $P < 0.05$ level, according to Duncan multiple range test.

Table 3.13. Effect of cultivar and storage [(cold storage in either controlled atmosphere storage (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)] interaction immediately after storage or after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) following storage on juice attributes in pomegranate fruit harvested from Alma farm, 2011.

	Juice weight (g/50 arils)				Juice/weight ratio			
	CA	CA+7D	RA	RA+7D	CA	CA+7D	RA	RA+7D
Cultivar								
Al-sirin-nar	19.2	16.1	17.1	17.6	0.76	0.70	0.74	0.73
Bala Miursal	15.2	15.9	15.7	15.2	0.76	0.74	0.75	0.72
Eversweet	20.60 A ^y	10.23 B	15.63 AB	15.90 AB	0.87 A	0.57 B	0.78 A	0.83 A
Nikitski-ranni	15.3	15.2	18.8	12.5	0.75	0.63	0.74	0.65
Sweet	13.7	13.2	.	.	0.78	0.67	.	.
<i>P</i>	0.0447				0.0109			
	TSS ^x (%)				TSS/TA ratio			
	CA	CA+7D	RA	RA+7D	CA	CA+7D	RA	RA+7D
Cultivar								
Al-sirin-nar	10.75	9.90	10.00	10.33	12.1	16.3	8.8	14.5
Bala Miursal	11.70	10.28	11.28	10.20	12.0	9.0	7.0	9.5
Eversweet	11.73 B	11.60 B	13.90 A	13.25 AB	39.97 A	24.63 B	22.34 B	22.92 B
Nikitski-ranni	9.95	10.40	11.00	10.10	11.4	12.9	11.3	9.0
Sweet	11.97	12.63	.	.	56.9	42.8	.	.
<i>P</i>	0.0005				0.0202			

^zTSS (Total soluble solids).

^yValues followed by the same upper case letter within a row are not significantly different at $P < 0.05$ level, according to Tukey

Lsmeans comparison.

Table 3.14. Effect of cultivar and storage [(cold storage in either controlled atmosphere storage (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)] interaction on total soluble solids (TSS) of pomegranate fruit immediately after storage harvested from Ponder farm in 2011.

	TSS (%)	
	CA	RA
Cultivar		
Afganski	14.47 ac ^z	14.68
Crab	16.63 b	16.28
Cranberry	14.65 ab	13.70
Kaj-acik-anor	16.38 Abc	14.57 B ^y
Nikitski-ranni	13.85 a	14.87
Salavatski	15.10 ab	14.95
<i>P</i>	0.0317	

^zValues followed by the same letter within a column are not significantly different at $P < 0.05$ level.

^yValues followed by the same upper case letter within a row are not significantly different at $P < 0.05$ level, according to Tukey Lsmeans comparison.

Table 3.15. Anthocyanins content of pomegranate fruit harvested from Ponder farm 2010

immediately after storage [(cold storage in either controlled atmosphere storage (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)] or after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) following storage. Fruit were harvested at two maturity stages (Early and Late).

	Anthocyanins content (µg/g aril fresh weight)					
	Del-dig ^z	Cya-dig ^y	Cya-glu ^x	Pet-glu ^w	Pel-glu ^v	Total ^u
Cultivar						
Afganski	109	265	339	90.5 b ^t	26	829
Crab	338	726	229	60.7 c	21	1373
Cranberry	285	557	147	47.2 c	12	1042
Entek-habi-saveh	221	408	200	60.9 c	10	888
Kaj-acik-anor	293	565	510	132.6 a	27	1527
Nikitski-ranni	190	385	59	19.3 d	5	655
Salavatski	83	314	176	25.6 d	10	605
Maturity ^s						
Early	214	383	155	53.4 a	10	811
Late	230	594	395	85.1 b	27	1326
Storage						
CA	216	475	261	64	16	1024
CA+7D	234	460	243	73	20	1024
RA	199	473	267	63	23	1017
RA+7D	238	463	228	66	12	1005
Source						
Cultivar (C)	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Maturity (M)	0.1869	<.0001	<.0001	<.0001	<.0001	<.0001
Storage (S)	0.137	0.0153	0.0011	0.1141	0.2159	0.0146
C x M	0.0024	<.0001	0.0002	0.0858	<.0001	0.0009
C x S	0.5093	0.1873	0.2801	0.9618	0.7453	0.5186
M x S	0.0144	0.3545	0.0588	0.3296	0.4537	0.7615
C x M x S	0.0155	0.0003	0.0147	0.0957	0.4992	0.0078

^zDel-dig= Delphinidin 3, 5-diglucoside

^yCya-dig= Cyanidin 3, 5-diglucoside

^xCya-glu= Cyanidin 3-glucoside

^wPet-glu= Petunidin 3- glucoside

^vPel-glu= Pelargonidin 3-glucoside

^uTotal=Total anthocyanins

^tValues followed by the same letter within a column are not significantly different at $P < 0.05$ level, according to Duncan multiple range test.

^sFruit harvested in 2nd week Sept. (Early) and 1st week Oct. (Late).

Table 3.16. Anthocyanins content of pomegranate fruit harvested from Ponder farm 2011 immediately after storage [(cold storage in either controlled atmosphere storage (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)].

	Anthocyanins content (µg/g aril weight)					
	Del-dig ^z	Cya-dig ^y	Cya-glu ^x	Pet-glu ^w	Pel-glu ^v	Total ^u
Cultivar						
Afganski	52.8 b ^t	188.2 b	178.4 b	52.5 b	20	492.2 b
Crab	234.5 a	600.8 a	133.3 bc	61.4 b	16	1045.6 a
Cranberry	65.0 b	319.2 b	35.7 c	13.1 c	.	433.0 b
Kaj-acik-anor	228.4 a	488.2 a	454.9 a	133.2 a	37	1341.3 a
Nikitski-ranni	138.8 ab	271.5 b	59.8 bc	25.1 bc	8	475.2 b
Salavatski	28.7 b	140.5 b	42.0 c	11.2 c	9	212.5 b
Storage						
CA	134.0 a	361.0 a	167.0 a	56.5 a	25	701.2 a
RA	138.9 a	300.2 a	152.0 a	51.7 a	18	655.5 a
Source						
Cultivar (C)	0.0008	<.0001	<0.0001	<0.0001	NS	<0.0001
Storage (S)	0.3115	0.286	0.7515	0.9403	NS	0.7958
C x S	0.1058	0.0128	0.7694	0.5928	NS	0.0711

^zDel-dig= Delphinidin 3, 5-diglucoside

^yCya-dig= Cyanidin 3, 5-diglucoside

^xCya-glu= Cyanidin 3-glucoside

^wPet-glu= Petunidin 3- glucoside

^vPel-glu= Pelargonidin 3-glucoside

^uTotal=Total anthocyanins

^tValues followed by the same letter within a column are not significantly different at $P < 0.05$

level, according to Duncan multiple range test.

Table 3.17. Anthocyanins content of pomegranate fruit harvested from Alma Farm 2011

immediately after storage [(cold storage in either controlled atmosphere storage (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)] or after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) following storage.

	Anthocyanins content (µg/g aril weight)				
	Del-dig ^z	Cya-dig ^y	Cya-glu ^x	Pet-glu ^w	Total ^v
Cultivar					
Al-sirin-nar	24	32.9 b ^u	4	.	41
Bala Miursal	58	82.6 a	8	5	144
Eversweet	33	40.4 b	10	9	55
Nikitski-ranni	23	17.0 b	3	3	18
Sweet	.	24.4 b	5	.	29
Storage					
CA	40	47	4	6	58
CA+7D	21	34	4	7	44
RA	65	60	6	9	118
RA+7D	21	28	.	4	34
Source					
Cultivar (C)	0.0138	0.0003	NS	0.3061	<.0001
Storage (S)	0.0122	0.1149	NS	0.5958	0.0053
C x S	0.0299	0.0604	NS	.	0.0394

^zDel-dig= Delphinidin 3, 5-diglucoside

^yCya-dig= Cyanidin 3, 5-diglucoside

^xCya-glu= Cyanidin 3-glucoside

^wPet-glu= Petunidin 3- glucoside

^vTotal=Total anthocyanins

^uValues followed by the same letter within a column are not significantly different at $P < 0.05$

level, according to Duncan multiple range test.

Table 3.18. Fruit husk color attributes of fruit from Ponder Farm 2010 immediately after storage [(cold storage in either controlled atmosphere storage (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)] or after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) following storage. Fruit were harvested at two maturity stages (Early and Late).

	L* ^z	Hue ^y	Chroma ^x
Cultivar			
Afganski	38.3	35.4	35.7
Crab	40.2	30.6	36.8
Cranberry	43.1	34.3	39.1
Entek-habi-saveh	48.3	55.6	32.4
Kaj-acik-anor	48.6	55.6	35.8
Nikitski-ranni	45.8	34.3	42.6
Salavatski	49.7	56.6	37.1
Maturity ^w			
Early	47.1	45.1	38.9
Late	42.1	42.5	34.0
Storage			
CA	46.3	44.7	38.1
CA+7D	41.8	43.9	34.6
RA	47.3	43.5	38.4
RA+7D	43.8	43.5	35.5
Source			
Cultivar (C)	<.0001	<.0001	<.0001
Maturity (M)	<.0001	<.0001	<.0001
Storage (S)	<.0001	0.1559	<.0001
C x M	<.0001	<.0001	<.0001
C x S	0.0002	<.0001	0.016
M x S	0.0002	0.3226	0.0116
C x M x S	0.0261	0.1505	0.0244

^zL* describes the degree of darkness or lightness with L=0 being black and L=100 is white.

^yHue angle represents the dominant color wavelength.

^xChroma describes deviation of color from gray (0=gray and 60= true color).

^wFruit harvested in 2nd week Sept. (Early) and 1st week Oct. (Late).

Table 3.19. Fruit husk color attributes of fruit from Alma Farm 2011 immediately after storage [(cold storage in either controlled atmosphere storage (CA; 5% CO₂ and 3% O₂, 5°C and 90%-95% RH) or regular air storage (RA; 5°C and 90%-95% RH)] or after 7 days at 25°C and 45%-50% RH (CA+7D & RA+7D) following storage.

	L* ^z	Hue ^y	Chroma ^x
Cultivar			
Al-sirin-nar	46.9	40.0	39.0
Bala Miursal	46.7	33.4	41.0
Eversweet	61.8	59.9	40.7
Nikitski-ranni	43.8	33.5	39.5
Sweet	66.5	75.0	45.6
Storage			
CA	54.4	46.8	43.6
CA+7D	51.3	49.6	36.6
RA	54.1	49.2	43.5
Source			
Cultivar (C)	<.0001	<.0001	<.0001
Storage (S)	<.0001	0.0495	<.0001
C x S	0.0381	<.0001	<.0001

^zL* describes the degree of darkness or lightness with L=0 being black and L=100 is white.

^yHue angle represents the dominant color wavelength.

^xChroma describes deviation of color from gray (0=gray and 60= true color)

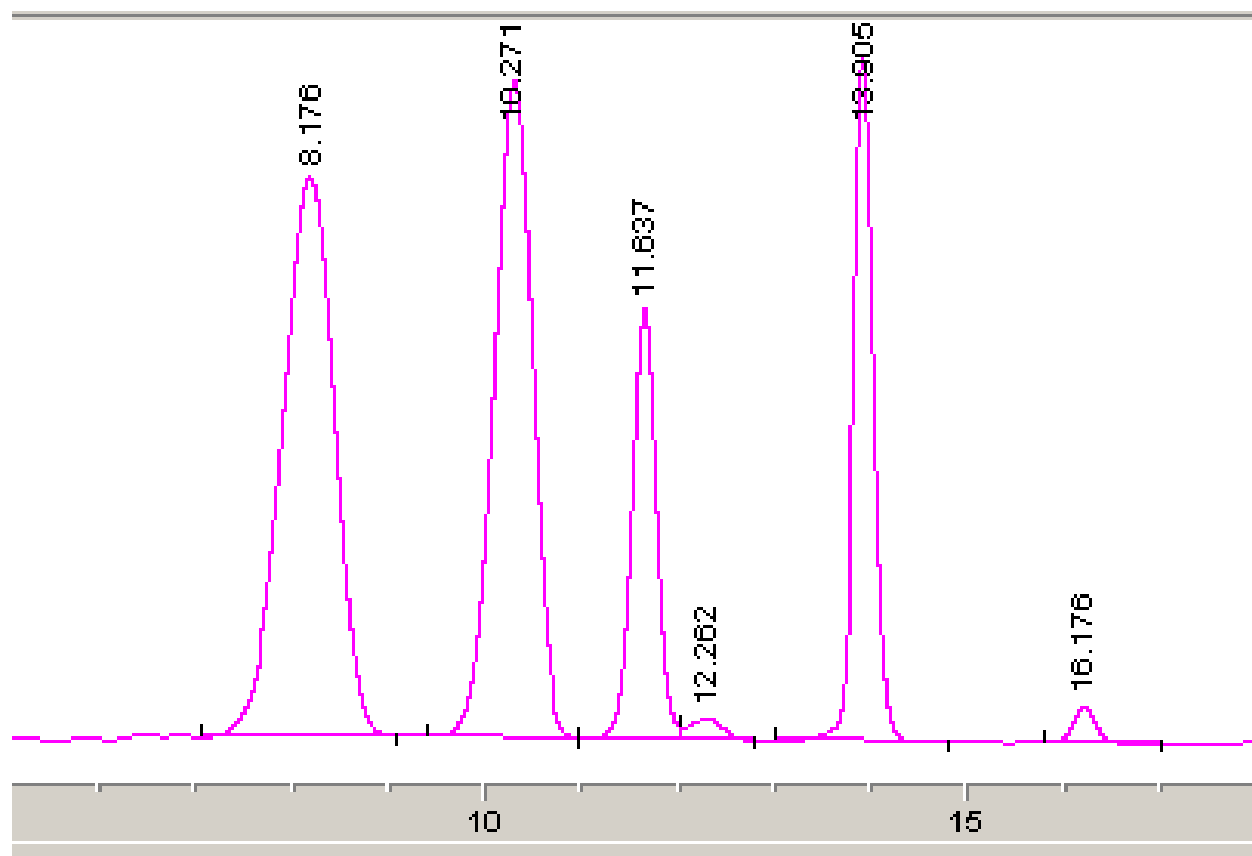


Figure 3.1. Anthocyanins of pomegranate juice identified at 520nm in HPLC.

8.176 min= Delphinidin 3, 5-diglucoside

10.271 min= Cyanidin 3, 5-diglucoside

11.637 min= Petunidin 3- glucoside

13.905 min= Cyanidin 3-glucoside

16.176 min= Pelargonidin 3-glucoside

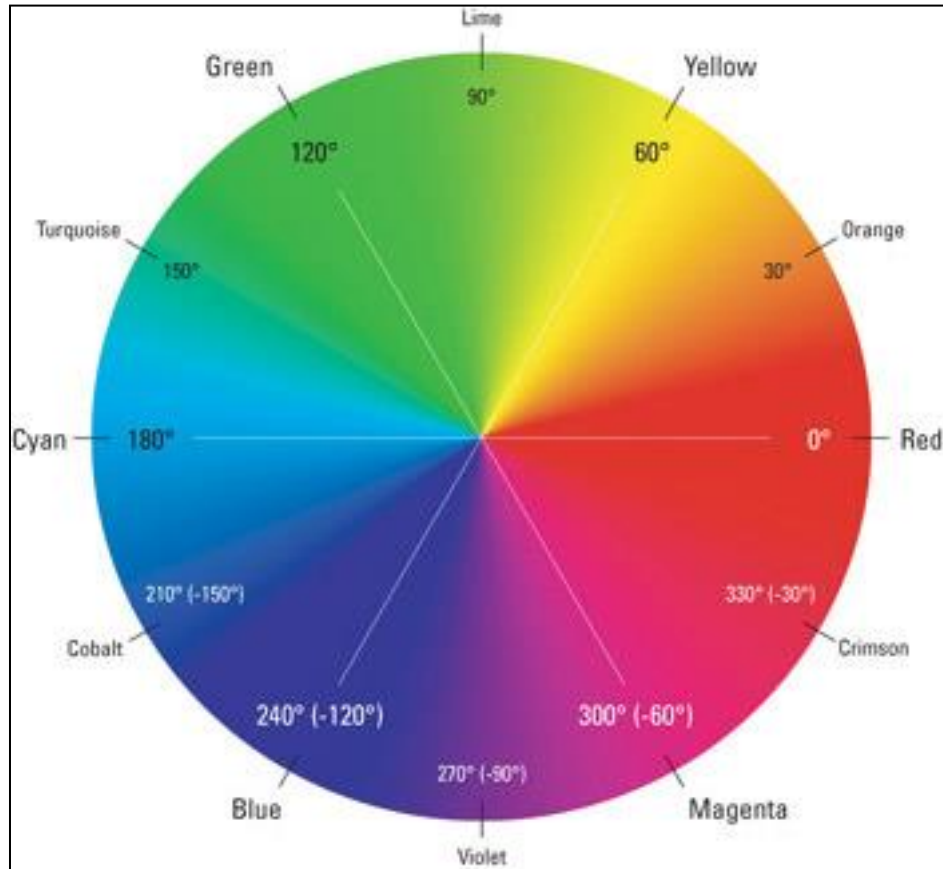


Figure 3.2. Various colors represented as hue angle. Red is between 330° - 30° .

<http://flylib.com/books/en/2.816.1.70/1/>

CHAPTER 4
CULTIVAR, STORAGE TEMPERATURE AND BAGGING AFFECT KALE WATER
LOSS, QUALITY AND SHELF LIFE²

² Sidhu, H.S., J.C. Díaz-Pérez, G. Dumičić, D.D. MacLean, and S. Goreta-Ban. To be submitted to *HortScience*.

Abstract

Kale (*Brassica oleracea*, var. *acephala*) is a green leafy vegetable with about 80%-90% water by weight, which is lost quickly after harvest along with chlorophyll degradation and yellowing thus loss of quality. The objective of this study was to determine the effects of cultivar (Konavale 2 and Red Russian), storage temperature (5°C or 18°C) and bagging (bagged or non-bagged) on leaf weight loss, quality and shelf life of fully developed kale leaves. Leaf weight loss was lesser in leaves of 'Konavale 2' than 'Red Russian', lesser at 5°C than 18°C and lesser in bagged leaves than those without bags. Decrease in CI and yellowing were lesser in leaves of 'Konavale 2' than 'Red Russian' and lesser in leaves stored at 5°C than 18°C. Bagging reduced yellowing of leaves when stored at 18°C.

Introduction

Vegetables are an excellent source of health beneficial compounds as antioxidants. Kale (*Brassica oleracea*, var. *acephala*) is one of the richest sources of antioxidants among 22 commonly used vegetables which include some other *Brassica* species, carrot and potato (Cao et al., 1996). Market of fresh vegetables as whole or minimally processed as chopped has been increasing in recent years. Leafy vegetables have been utilized as salads in many cuisines.

Kale is a leafy vegetable unlike cabbage and broccoli which have edible heads and flowers. Kale has large flat leaves in many cultivars but demand for curly leaves has grown over the years in market (Salunkhe and Kadam, 1998). *Brassica oleracea* has its origin in Europe, specifically around Mediterranean and Southern Europe. Kale is a very cold tolerant plant. Various *B. oleracea* species are grown around the world under different weather conditions although being a temperate wild species from origin (Vaughan and Geissler, 2009). Kale is grown in eastern Africa, Asia, Europe and Latin America while being referred as poor man's vegetable in Africa (Mwithiga and Olwal, 2005).

Kale has a large water content (about 85% by weight). Kale loses water quickly, wilts and perishes under room temperature (21-25°C) conditions (Imungi, 1992). Other leafy vegetables follow similar trend and perish very quickly after harvest (Paull, 1992). Water loss is a function of evaporative demand which is determined by temperature and humidity. High relative humidity during storage reduces moisture loss from the produce. Modified atmosphere packaging results in increased relative humidity of microenvironment and reduces water loss by evaporation. Enclosing leaves in polyethylene bags reduces water loss and extends the shelf life (Porat et al., 2004). Storing produce in plastic film is a vastly used method of storage for fresh market.

Storage conditions (temperature and bagging) play a very important role in postharvest shelf life of produce. It is important aspect to minimize losses of produce quality from time of harvest to produce consumption. Reducing losses during storage increases available quantity of produce for consumption, decreases pressure on production chain and thus conserving various natural resources. Postharvest losses are higher (range from 1%-50% of the produce) in developing nations as compared to developed countries (2%-23%) due to lack of storage facilities and infrastructure equipped with new technologies (Kader, 2003).

Visual appearance of fresh produce is an important attribute a consumer considers while buying and so do wholesalers and retailers. In kale, appearance of leaves can be affected by damage due to insect or pest, disease, and loss of quality during postharvest handling and storage. Color is an important representative of quality and should be preserved as much as possible. Detaching produce from a plant renders it devoid of various nutrients and thus senescence escalates (Noodén et al., 2006). Color change seems to be due to senescence which is limited by reducing storage temperature due to suppression of metabolic activities (Able et al., 2003). In many other horticultural crops color change is observed to be one of the first symptoms of senescence (Kader, 2000). Color of leaves starts turning yellow with degradation of chlorophyll. Degradation is escalated by unfavorable storage conditions such as high temperature and low relative humidity during storage. Exposure of produce to light during storage also affects its quality attributes. During storage light enhances degradation of carotenoids in leafy vegetables. This causes breakdown of chlorophyll molecules and leads to loss of green color (Biswal, 1995). In other *Brassica* species carbohydrates have been observed to be utilized extensively due to respiration during storage (Finger et al., 1999).

Ethylene plays an important role in senescence of produce during storage (Kasai et al., 1996; King and Morris, 1994; Tian et al., 1994). Increase in ethylene levels in storage causes rapid yellowing of green vegetables (broccoli, lettuce etc.) and reduces their shelf life. Any injury due to insects, pests and during postharvest handling increases ethylene production causing rapid postharvest decay (Able et al., 2003).

Objective

The objective was to determine the effects of cultivar, temperature, and bagging on water loss, postharvest quality and shelf life of kale leaves.

Materials and Methods

Plants for study were planted on 13 Mar. 2011 and 7 Dec. 2011 at the Horticulture Farm, The University of Georgia Tifton, GA, USA. Fully developed leaves (leaves after first 5 new leaves) of kale cultivars Red Russian and Konavale 2 (IAC, Split, Croatia) were harvested early morning of 1st Jul. 2011 (110 days after plantings) and 16th Apr. 2012 (131 days after planting), placed in polythene bags and brought to Vidalia Onion Research Lab (VORL), Tifton, GA in an ice box. Leaves were cleaned to remove dirt and checked for insect or disease damage. Leaves were divided equally for storage at 18°C or 5°C temperature (90%-95% RH). At both storage temperatures leaves were kept either inside a polyethylene bag ('bagged') or unbagged. . Four replications of 4 leaves/treatment (treatment= Cultivar-temperature-bagging) were set up. Leaf attributes as weight loss, chlorophyll index and yellowing were recorded on 0, 1, 3, 5, 7, 10, 17 & 21 days after harvest (DAH) in 2011 and 0, 1, 2, 4, 7, 10, 17 & 20 DAH in 2012. Leaves were green, healthy and without any yellowing at day 0.

Leaf Weight Loss and Leaf Area

Leaf weight was recorded to understand the kinetics of water loss and its effect on leaf quality. Initial leaf weight was measured as soon as leaves were brought from field for storage. Percentage weight loss (WL) of leaves was calculated based on initial leaf weight (W_0).

$$\text{Percentage weight loss (WL)} = \frac{\text{Final leaf weight} - \text{Initial leaf weight}}{\text{Initial leaf weight}} * 100$$

After the last weighing of leaves, leaf area (cm^2) was measured with a leaf area meter (LI-3000C, LICOR, Lincoln, NE) at end of storage study. Leaves were then dried at 80°C for 24-36 h for dry weight determination.

Leaf Yellowing

During storage leaf yellowing was graded on a 1-6 scale (1= no yellowing, 2= yellowing on less than 10% leaf area, 3= yellowing on 10%-25% leaf area, 4= 25%-50% of leaf with yellowing, 5= 50%-75% leaf with yellowing and 6= completely yellow leaf). Leaves with score 3 or above were considered unmarketable.

Chlorophyll Index

Handheld chlorophyll meter (SPAD 502, Konica Minolta, Minolta Corp, Ramsey, N.J.) was used to record chlorophyll index of leaves. Observations at two points on each leaf were recorded (Figure 4.5). Percent change in chlorophyll index was calculated on the basis of initial leaf chlorophyll index reading taken immediately after harvest.

$$\text{Chlorophyll index change} = \frac{\text{Initial CI} - \text{Final CI}}{\text{Initial CI}} * 100$$

Leaf Color

Handheld colorimeter (CR-400, Konica Minolta, Minolta Corp., Ramsey, N.J.) was used to measure leaf color. Color was recorded as L^* , a^* , b^* and from these values hue and chroma

were calculated with Spectramagic NX software. Color readings were taken on same position on leaf where chlorophyll index was measured. Color attribute 'L*' (0=black & 100= white) represents lightness of leaf surface. Hue angle represents the dominant color wavelength expressing the color of leaf. It is represented in as an angle (0° - 360°) and 45° - 90° is yellow, 90° - 135° is green (Fig.4.2). Chroma represents the trueness of color or its deviation from gray (0=gray and 60= true color).

Statistical Analysis

Statistical software SAS (SAS version 9.3; SAS Institute Inc., Cary, NC.) was used to analyze the data. ANOVA procedure was used to determine the effect of cultivar, storage temperature, and bagging on different kale leaf weight loss and quality attributes. Duncan multiple range test was used for means separation and tukey test for comparison of lsmeans in interactions. Significant differences at $P < 0.05$ were reported.

Results

Kale leaf fresh weight, dry weight and leaf area in 2011 and 2012 are shown in table 4.1. Leaf fresh weight and dry weight of cultivar Konavale 2 were greater than those of 'Red Russian'. Leaves during 2012 had more than twice the weight than those in 2011, probably due to older plants in 2012 (131 days) than in 2011 (110 days). In 2012 leaves of 'Konavale 2' had slightly larger leaf area than those of 'Red Russian'. Leaves stored at 18°C (Bagged and not bagged) were discarded at day 8 following their deterioration in quality and considered unmarketable. Reason of their unmarketable quality was either weight loss or chlorophyll degradation individually or both of them together.

Leaf Weight Loss

Reduced temperature and bagging decreased the leaf weight loss 7 days after harvest (DAH; Table 4.2). Temperature-bagging interaction was observed in 2011 and cultivar-temperature-storage interaction in 2012. In 2011, non-bagged leaves at 18°C (28.9%) had greatest leaf weight loss compared to nonbagged-5°C (21.4%), bagged-18°C (7.2%) and bagged-5°C (3.1%). In 2012 non-bagged leaves of ‘Red Russian’ had greater weight loss (34.6% at 18°C and 32.4% at 5°C) than those of ‘Konavale 2’ (23.3% at 18°C and 13.7% at 5°C). Bagged leaves did not differ in weight loss due to cultivar or temperature.

Non-bagged leaves had greater weight loss than bagged ones. This difference was detectable as early as 1 DAH. Leaf weight loss by 21 DAH (at 5°C) was significantly lowered due to bagging (Table 4.2). By 21 DAH, bagged leaves had lost <10% of initial their weight, while non-bagged leaves lost >30% of their weight. In 2011 bagged leaves had lesser weight loss (‘Konavale 2’=6.3% & ‘Red Russian’=6.7%) compared to non-bagged leaves (‘Konavale 2’=34.6% & ‘Red Russian’=31.8%). In 2012 non-bagged leaves of ‘Red Russian’ (40.3%) had greater weight loss than those of ‘Konavale 2’ (27.1%), while bagged leaves had reduced weight loss. (‘Konavale 2’=4.1% and ‘Red Russian’=5.4%).

Chlorophyll Index

Leaves of ‘Konavale 2’ had lesser decrease in CI during storage 7DAH compared to ‘Red Russian’ (Table 4.3). Cultivar-temperature interaction was observed for decrease in CI 7DAH. In 2011 leaves of ‘Konavale 2’ (33.9%) had lesser decrease in CI than ‘Red Russian’ (53.9%) stored at 18°C while cultivars did not differ in decrease of CI when stored at 5°C (‘Konavale 2’=10.9% & ‘Red Russian’=9.1%). In 2012 leaves of ‘Konavale 2’ (70.0%) had

lesser decrease in CI than 'Red Russian' (94.8%) stored at 18°C while cultivars did not differ in decrease of CI when stored at 5°C ('Konavale 2'=9.1% & 'Red Russian'=7.5%).

Leaves at 5°C storage 21DAH lost lesser CI when stored without bags (Table 4.3).

Leaves of 'Konavale 2' maintained CI better compared to 'Red Russian'. Cultivar-bagging interaction was observed in 2012 and 'Red Russian' leaves stored in bags (82.0%) lost maximum CI compared to those not in bags (49.0%) whereas 'Konavale 2' leaves had equal decrease in CI (55.0%) irrespective of storage with or without bags.

Leaves of 'Red Russian' had lowest CI at end of storage period (Fig.4.2). CI started decreasing in leaves at 18°C storage between 2-4DAH whereas it started decreasing around 5-8DAH when stored at 5°C.

Yellowing

Leaves stored at 18 °C had considerable greater yellowing than those at 5 °C (Fig. 4.3). Yellowing was detectable earlier at 18 °C (2 DAH) than at 5 °C (> 7 DAH). By 7 DAH leaves at 18°C had the highest score of yellowing ('Konavale 2'=5.0 and 'Red Russian'=6.0) whereas leaves at 5°C had no yellowing (Fig.4.3). By 7 DAH leaves at 18°C were severely deteriorated and were discarded. Yellowing ratings were higher in 'Red Russian' than in 'Konavale 2' and bagging reduced the degree of leaf yellowing in both cultivars. At 18 °C, however, bagging was more beneficial in reducing yellowing in 'Red Russian' than in 'Konavale 2'. Leaves stored at 18°C were unmarketable due to yellowing by 3-6 DAH depending on cultivar and bagging. Bagged leaves of 'Konavale 2' maintained marketability for longest time (about 5-6 DAH).

Color

Lightness of kale leaves increased during storage period (Table 4.5). In general, leaves at 5°C had lesser L* than those at 18°C. Non-bagged eaves had greater L* than bagged ones. In

2011 Cultivar-temperature interaction was observed for L^* 7 DAH. Leaves at 5°C did not differ in L^* for cultivar but at 18°C, ‘Red Russian’ (61.4) had higher L^* than ‘Konavale 2’ (53.0).

Hue angle of leaves decreased during storage period (Table 4.6). Hue angle was unaffected by cultivar in both years. Hue angle (7 DAH) was generally lower in leaves at 18°C compared to those at 5°C. Hue angle was also generally higher in bagged leaves compared to non-bagged leaves. Temperature-bagging interaction was observed in 2011. Bagged leaves (129.0) had higher hue angle 7 DAH than non-bagged ones (121.9) at 5°C whereas leaves at 18°C did not differ due to bagging.

Chroma value of leaves increased during storage period (Table 4.7). In 2011, ‘Red Russian’ had higher values of chroma than those of ‘Konavale 2’. Leaves at 5 °C had higher chroma values than those at 18°C. Bagged leaves had higher chroma values than non-bagged leaves. In 2012, the effect of temperature and bagging on chroma was similar as in 2011, and there was no difference in chroma value between cultivars.

Decrease in chlorophyll index (% of initial value) of leaves was correlated to the increase in L^* , chroma and decrease in hue angle of leaf color. Decrease in chlorophyll index of leaves was correlated to leaf color L^* 8D (Pearson correlation coefficient, $r = 0.62$ in 2011 and 0.49 in 2012), hue angle ($r = 0.63$ in 2011 and 0.44 in 2012) and chroma 8D ($r = 0.59$ in 2011 and 0.54 in 2012). Shelf life based on yellowing was strongly correlated to changes in color attributes (L^* 8D = -0.79 , Chroma 8D = -0.76 and hue angle 8D= -0.64) at day 8.

Discussion

Produce quality is highest immediately after harvest. Thus it is important to maintain the freshness and quality of produce until it reaches the consumer. Various techniques are used to maintain produce, especially fruits and vegetables. Bagging fruits and vegetables in polythene or

other films has proved to be fairly successful (Miller et al., 1986). Reducing storage temperature reduces respiration rate and water loss resulting in extended postharvest life of produce (Nunes et al., 1998). Different broccoli products have been shown to maintain better quality under low storage temperatures (Reddy et al., 2010). Bagging has been observed to retain turgidity of vegetables preserving moisture to a better extent than storing the produce without bags (Forney et al., 1989; Miller et al., 1986; Rij and Ross, 1987). Bagging the leaves builds up a microenvironment with high relative humidity, which decreases the vapor pressure deficit and as a result reduces leaf moisture loss.

Greenness (CI) of many vegetables is an important quality attribute that affects produce marketability. Leaf greenness was better maintained at 5 °C than at 18°C. Similar results were observed when broccoli heads stored at 23°C lost almost 95% of original chlorophyll content within 4 days of storage while the loss was minimal when stored at 5°C (Deschene et al., 1990).

Impact of bagging on leaf CI varied by cultivar. Bagging the produce during storage leads to increase in concentration of CO₂ and C₂H₄ (Tulio et al., 2002). High respiration rate will thus lead to quicker increase in concentration of CO₂ and C₂H₄. There might be a difference in rate of respiration activity within the cultivars. Decrease in CI and change in hue angle signify loss of chlorophyll from leaves which affects its visual quality. Broccoli is a vegetable similar to kale in which loss of greenness significantly affects the shelf life and marketability. Decreasing hue angle of kale leaves, representing change of leaf color from green to yellow, was similar to observations by Reddy et al., 2010 on broccoli. Reduced change in hue angle of leaves under 5°C storage compared to 18°C showed preservation of green color (Rij and Ross, 1987). Lowering storage temperature helped maintain the initial color of fresh harvested kale leaves for longer period of time.

Bagged leaves of 'Red Russian' when stored at 18°C showed higher yellowing and decreased CI compared to bagged leaves of 'Konavale 2'. This difference could possibly result from higher ethylene production from 'Red Russian' leaves than 'Konavale 2' leading to increased ethylene concentration in bags. Production of ethylene has been shown to accompany chlorophyll degradation in broccoli and pak choy leaves (Aharoni et al., 1985; Pogson and Morris, 1997). Chlorophyll index of kale leaves might be affected by the ethylene rich microenvironment around them due to bagging that possibly led to enhanced chlorophyll degradation. In another study, jute (*Corchorus olitorius* L.) leaves stored in polythene bags showed an increased ethylene concentration but leaf senescence was observed only at high temperature. Carbon dioxide concentration in bags was also observed to be increased when stored at high temperatures (Tulio Jr et al., 2002).

Leaf yellowing was significantly related to relative decrease in chlorophyll index (% of initial value, $r^2=0.939$). This concludes that quality attribute can be graded very precisely both by yellowing of leaf and chlorophyll index. Higher the decrease in chlorophyll index, higher was the yellowing on a leaf (Fig.4.4).

Shelf life of kale leaves was decided both by weight loss (% of initial weight) and yellowing on leaves. Storing leaves in bags is equally important as low temperature storage. Cultivar Konavale 2's leaves had longest shelf life based on yellowing and weight loss.

Conclusion

Lower storage temperature (5°C) led to decrease in leaf weight loss, lower shriveling and wilting thus preserving the quality of leaves. Leaf greenness and visual quality were better maintained at reduced storage temperature. Bagged leaves during storage further enhanced shelf life by greatly reducing moisture loss.

References

- Able A.J., Wong L., Prasad A., O'Hare T. (2003) The effects of 1-methylcyclopropene on the shelf life of minimally processed leafy asian vegetables. *Postharvest Biology and Technology* 27:157-161.
- Aharoni N., Philosoph-Hadas S., Barkai-Golan R. (1985) Modified atmospheres to delay senescence and decay of broccoli. *In* SM Blankenship, ed, *Controlled atmospheres for storage and transport of perishable agricultural commodities*, Horticulture report 126. Department of Horticultural sciences, Raleigh, NC., pp 169-177.
- Biswal B. (1995) Carotenoid catabolism during leaf senescence and its control by light. *Journal of Photochemistry and Photobiology B: Biology* 30:3-13.
- Cao G., Sofic E., Ronald L. (1996) Antioxidant capacity of tea and common vegetables. *Journal of Agricultural and Food Chemistry* 44:3426-3431.
- Deschene A., Paliyath G., Loughheed E.C., Dumbroff E.B., Thompson J.E. (1991) Membrane deterioration during postharvest senescence of broccoli florets: modulation by temperature and controlled atmosphere storage. *Postharvest Biology and Technology* 1:19-31.
- Finger F.L., Endres L., Mosquim P.R., Puiatti M. (1999) Physiological changes during postharvest senescence of broccoli. *Pesquisa Agropecuária Brasileira* 34:1565-1569.
- Forney C., Rij R., Ross S. (1989) Measurement of broccoli respiration rate in film-wrapped packages. *Hortscience* 24:111-113.
- Imungi J. (1992) Postharvest handling of perishable crops, *Proceedings of the Postharvest Management of Food Crops Seminar Held in Serena Hotel. Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya.*

- Kader A. (2000) Quality assurance of harvested horticultural perishables, IV International Conference on Postharvest Science 553. pp. 51-56.
- Kader A.A. (2003) A perspective on postharvest horticulture (1978-2003). Hortscience 38:1004-1008.
- Kasai Y., Kato M., Hyodo H. (1996) Ethylene biosynthesis and its involvement in senescence of broccoli florets. Journal of the Japanese Society for Horticultural Science 65: 185-191.
- King G.A., Morris S.C. (1994) Physiological changes of broccoli during early postharvest senescence and through the preharvest-postharvest continuum. Journal of the American Society for Horticultural Science 119:270-275.
- Miller W., Risse L., McDonald R. (1986) Deterioration of individually wrapped and non-wrapped bell peppers during long-term storage. Tropical science 26:1-8.
- Mwithiga G., Olwal J.O. (2005) The drying kinetics of kale (*Brassica oleracea*) in a convective hot air dryer. Journal of Food Engineering 71:373-378.
- Noodén L.D., Guiamét J.J., John I. (2006) Senescence mechanisms. Physiologia Plantarum 101:746-753.
- Nunes M., Brecht J., Morais A., Sargent S. (1998) Controlling temperature and water loss to maintain ascorbic acid levels in strawberries during postharvest handling. Journal of Food Science 63:1033-1036.
- Paull R. (1992) Postharvest senescence and physiology of leafy vegetables. Postharvest News and Information 3:11N-20N.
- Pogson B.J., Morris S.C. (1997) Consequences of cool storage of broccoli on physiological and biochemical changes and subsequent senescence at 20 C. Journal of the American Society for Horticultural Science 122:553-558.

- Porat R., Weiss B., Cohen L., Daus A., Aharoni N. (2004) Reduction of postharvest rind disorders in citrus fruit by modified atmosphere packaging. *Postharvest Biology and Technology* 33:35-43.
- Reddy Y.V.R., Marcy J.E., Bratsch A.D., Williams R.C., Waterman K.I.M.M. (2010) Effects of bagging and postharvest treatments on the shelf-life quality of crown-cut broccoli. *Journal of food quality* 33:599-611.
- Rij R., Ross S. (1987) Quality retention of fresh broccoli packaged in plastic films of defined CO₂ transmission rates. *Bagging Technol* 17:22-23.
- Salunkhe D.K., Kadam S. (1998) *Handbook of vegetable science and technology. Production, composition, storage, and processing* Marcel Dekker Inc pp 365-366.
- Tian M., Downs C., Lill R., King G. (1994) A role for ethylene in the yellowing of broccoli after harvest. *Journal of the American Society for Horticultural Science* 119:276-281.
- Tulio Jr A.Z., Ose K., Chachin K., Ueda Y. (2002) Effects of storage temperatures on the postharvest quality of jute leaves (*Corchorus olitorius* L.). *Postharvest Biology and Technology* 26:329-338.
- Vaughan J., Geissler C. (2009) *The new Oxford book of food plants* OUP Oxford. pp 179-206.

Table 4.1. Fresh weight, dry weight and leaf area of kale cultivars Konavale 2 and Red Russian in a two year study in Tifton, GA^z.

Cultivar	2011			2012		
	Fresh weight (g/leaf) ^y	Dry weight (g/leaf) ^x	Leaf area (cm ² /leaf) ^w	Fresh weight (g/leaf)	Dry weight (g/leaf)	Leaf area (cm ² /leaf)
Konavale 2	20.9 ± 0.93	2.77 ± 0.14	181 ± 10	48.3 ± 1.89	5.37 ± 0.29	363 ± 12
Red Russian	17.8 ± 0.63	2.11 ± 0.08	179 ± 8	42.1 ± 1.16	4.12 ± 0.15	324 ± 7

^zValues are the mean (±SE) of 64 leaves.

^yFresh weight (g) immediately after harvest.

^xDry weight (g) of leaves dried for 24 h at 82°C.

^wLeaf area measured with an automatic leaf area meter.

Table 4.2. Weight loss (% of initial weight) of field grown kale leaves of cultivars Konavale 2 and Red Russian stored bagged or non-bagged (open) at either 5°C or 18°C in a two year study in Tifton, GA.

	Leaf weight loss (%)			
	2011 ^z		2012	
	7 DAH ^y	21 DAH	7 DAH	21 DAH
Cultivar				
Konavale 2	15.7	20.5	10.8 b	15.6 b
Red Russian	14.5	19.3	18.7 a	22.8 a
Temperature (°C)				
5	12.2 a	21.8	12.9 b	21.8
18	18.0 b	ND ^x	16.6 a	ND
Bagging				
Bagged	5.1 b	6.5 b	3.5 b	4.8 b
Non-bagged	25.1 a	33.3 a	26.0 a	33.6 a
Source				
Cultivar (C)	0.0715	0.1363	<.0001	<.0001
Temperature (T)	<.0001	ND	<.0001	ND
Bagging (B)	<.0001	<.0001	<.0001	<.0001
C x T	0.3639	ND	0.0355	ND
C x B	0.1584	0.0451	<.0001	<.0001
T x B	0.0074	ND	0.0138	ND
C x T x B	0.6541	ND	0.0323	ND

^zYear of study

^yDAH = days after harvest

^xND = not determined as leaves were already discarded. Leaves were discarded due to excessive weight loss and yellowing.

Table 4.3. Chlorophyll index (CI) in field grown kale leaves of cultivars Konavale 2 and Red Russian stored bagged or non-bagged (open) at either 5°C or 18°C in a two year study in Tifton, GA.

	2011 ^z			2012		
	0 DAH ^y	7 DAH	21 DAH	0 DAH	7 DAH	21 DAH
Cultivar						
Konavale 2	69.3 a	54.7 a	58.1 a ^x	72.9 a	44.7 a	41.7 a
Red Russian	60.7 b	44.7 b	39.5 b	62.6 b	37.4 b	17.2 b
Temperature (°C)						
5	65.9	65.2 a	47.4	68.9	63.1 a	34.8
18	63.7	36 b	ND ^w	66.7	14.6 b	ND
Bagging						
Bagged	64.3	46.3 b	47	68.3	38.4	32.4
Non-bagged	65.1	52.6 a	47.8	67.3	45.3	39.3
Source						
Cultivar (C)	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Temperature (T)	.	<.0001	ND	.	<.0001	ND
Bagging (B)	.	0.011	0.8223	.	0.409	0.1112
C x T	.	0.1472	ND	.	0.0043	ND
C x B	.	0.1973	0.7369	.	0.2467	.
T x B	.	0.0188	ND	.	0.3907	ND
C x T x B	.	0.3457	ND	.	0.3118	ND

^zYear of study

^yDAH=Days after harvest

^xValues followed by the same letter within a column are not significantly different at $P < 0.05$ level.

^wND = not determined as leaves were already discarded. Leaves were discarded due to excessive weight loss and yellowing.

Table 4.4. Yellowing^z of field grown kale leaves of cultivars Konavale 2 and Red Russian stored bagged and non-bagged (open) at either 5°C or 18°C in year 2012.

	Yellowing		
	5DAH ^y	7DAH	21DAH
Cultivar			
Konavale 2	2.0 b	3.0	3.2 b
Red Russian	2.8 a	3.0	5.3 a
Temperature (°C)			
5	1.0 b	1.0 b	3.9
18	3.8 a	5.4 a	ND ^x
Bagging			
Bagged	2.1 b	3.2	3.8
Non-bagged	2.8 a	2.7	4.0
Source			
Cultivar (C)	<.0001	<.0001	<.0001
Temperature (T)	<.0001	<.0001	ND
Bagging (B)	<.0001	0.4363	0.0648
C x T	<.0001	0.0002	ND
C x B	0.004	0.7863	0.6366
T x B	<.0001	0.7863	ND
C x T x B	0.004	0.4363	ND

^zYellowing on a 1-6 scale (1=no yellowing, 6= completely yellow leaf). Leaves with yellowing score ≥ 3 were considered unmarketable.

^yDAH=days after harvest.

^xND = not determined as leaves were already discarded. Leaves were discarded due to excessive weight loss and yellowing.

Table 4.5. Lightness (L^*)^z of kale leaves from cultivars Konavale 2 and Red Russian stored bagged or non-bagged (open) at either 5°C or 18°C in a two year study.

	2011 ^y			2012		
	0 DAH ^x	7 DAH	21 DAH	0 DAH	7 DAH	21 DAH
Cultivar						
Konavale 2	39.0	48.1 b	48.4 b ^w	37.2	50.4	47.9 b
Red Russian	39.9	51.6 a	51.7 a	38.0	50.1	57.2 a
Temperature (°C)						
5	39.5	42.5 b	50.1	36.0	41.1 b	50.5
18	39.4	57.2 a	ND ^v	39.2	62.1 a	ND
Bagging						
Bagged	39.5	47.8 b	45.9 b	37.4	49.4 b	49.5 b
Non-bagged	39.3	52.0 a	54.2 a	37.9	51.3 a	52.2 a
Source						
Cultivar (C)	0.1473	0.0021	0.034	0.057	0.2819	<.0001
Temperature (T)	.	<.0001	ND	.	<.0001	ND
Bagging (B)	.	0.0003	<.0001	.	0.0015	<.0001
C x T	.	<.0001	ND	.	0.1215	ND
C x B	.	0.7601	0.2624	.	0.3992	ND
T x B	.	0.0171	ND	.	0.5383	ND
C x T x B	.	0.9202	ND	.	0.3648	ND

^z L^* lightness of leaf (0= black, 100=white)

^yYear of study

^xDAH=days after harvest

^wValues followed by the same letter within a column are not significantly different at $P < 0.05$ level.

^vND = not determined as leaves were already discarded. Leaves were discarded due to excessive weight loss and yellowing.

Table 4.6. Hue angle^z of kale leaves from cultivars Konavale 2 and Red Russian stored bagged or non-bagged (open) in either 5°C or 18°C in a two year study.

	2011 ^y			2012		
	0 DAH ^x	7 DAH	21 DAH	0 DAH	7 DAH	21 DAH
Cultivar						
Konavale 2	128.1	120.1	119.8	134.6	114.9	117.5 a
Red Russian	128.4	119.5	116.9	136.0	117.4	104.4 b
Temperature (°C)						
5	128.3	125.4 a	118.4	137.9	128.0 a	114.0
18	128.3	114.2 b	ND ^w	132.7	100.6 b	ND
Bagging						
Bagged	128.6	121.7 a	122.4 a ^v	134.2	116.7 a	113.5
Non-bagged	127.9	117.9 b	114.3 b	136.4	115.2 b	114.8
Source						
Cultivar (C)	0.6613	0.5675	0.1669	0.2845	0.5128	<.0001
Temperature (T)	.	<.0001	ND	.	<.0001	ND
Bagging (B)	.	0.0008	0.0004	.	0.021	0.0523
C x T	.	0.1436	ND	.	0.1759	ND
C x B	.	0.9459	0.3464	.	0.6501	ND
T x B	.	0.0035	ND	.	0.5711	ND
C x T x B	.	0.6059	ND	.	0.2057	ND

^zHue angle represents the dominant color wavelength

^yYear of study

^xDAH=days after harvest

^wND = not determined as leaves were already discarded. Leaves were discarded due to excessive weight loss and yellowing.

^vValues followed by the same letter within a column are not significantly different at $P < 0.05$ level.

Table 4.7. Chroma^z of kale leaves from cultivars Konavale 2 and Red Russian stored bagged or non-bagged (open) in either 5°C or 18°C in two year study.

	2011 ^y			2012		
	Chroma			Chroma		
	0 DAH ^x	7 DAH	21 DAH	0 DAH	7 DAH	21 DAH
Cultivar						
Konavale 2	16.7	27.6 b	27.7	12.8	27.9	28.9 b
Red Russian	18.8	31.3 a	31.0	12.6	27.3	42.7 a
Temperature (°C)						
5	17.9	21.9 b	29.3	11.0	17.6 b	32.6
18	17.7	37.1 a	ND ^w	14.3	40.4 a	ND
Bagging						
Bagged	17.9	28.2 b	26.1 b ^v	12.9	27.6	33.4
Non-bagged	17.6	30.8 a	32.6 a	12.4	27.6	31.2
Source						
Cultivar (C)	NS ^u	0.0009	0.0801	0.6721	0.9525	<.0001
Temperature (T)	.	<.0001	ND	.	<.0001	ND
Bagging (B)	.	0.0203	0.0016	.	0.369	0.055
C x T	.	0.0025	ND	.	0.0186	ND
C x B	.	0.1583	0.9947	.	0.273	ND
T x B	.	<.0001	ND	.	0.0034	ND
C x T x B	.	0.6999	ND	.	0.1077	ND

^zChroma represents trueness of color (0=gray, 60=true color)

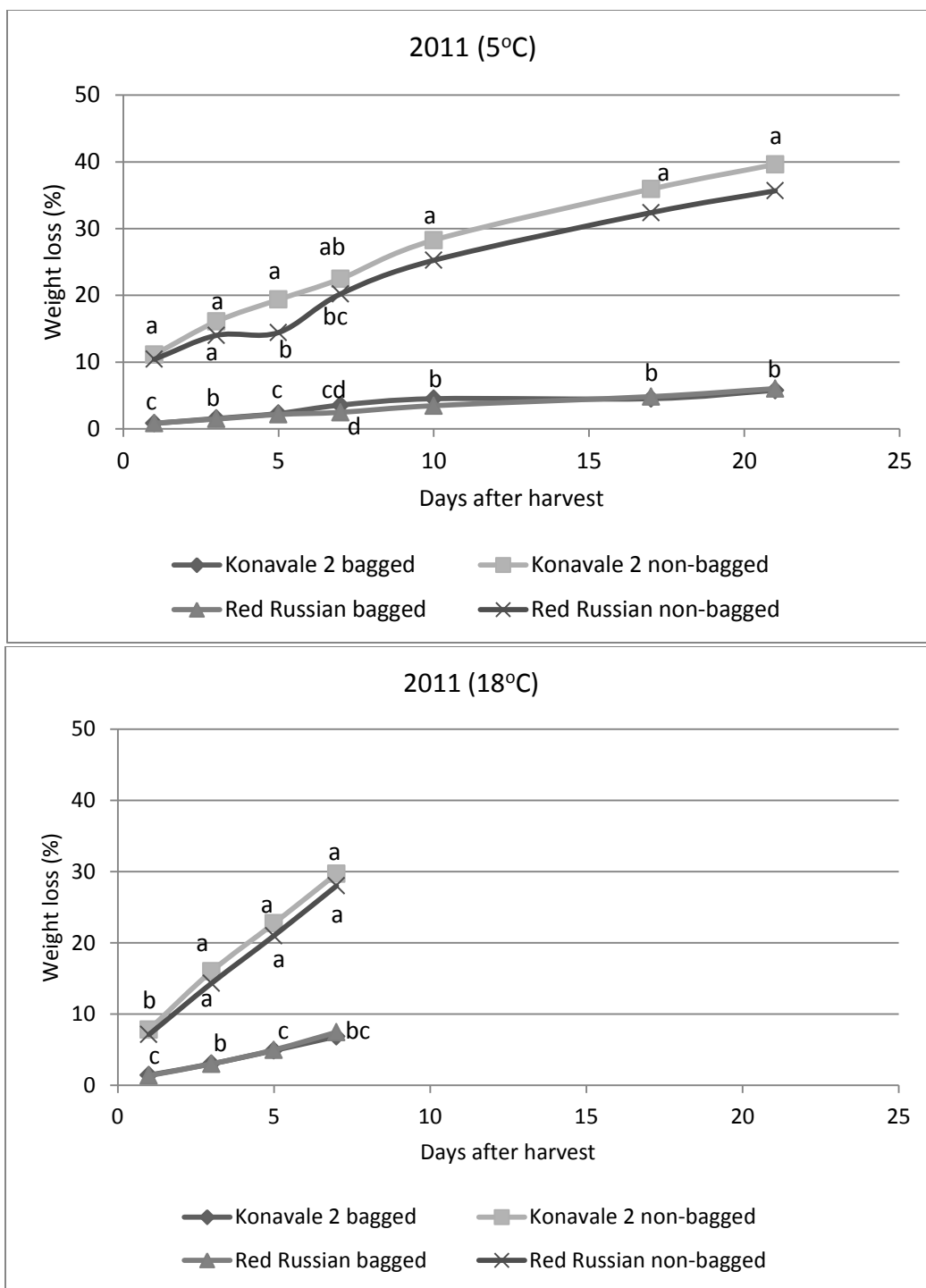
^yYear of study

^xDAH=days after harvest

^wND = not determined as leaves were already discarded. Leaves were discarded due to excessive weight loss and yellowing.

^vValues followed by the same letter within a column are not significantly different at $P < 0.05$ level.

^uNS= means not significantly different ($P > 0.05$)



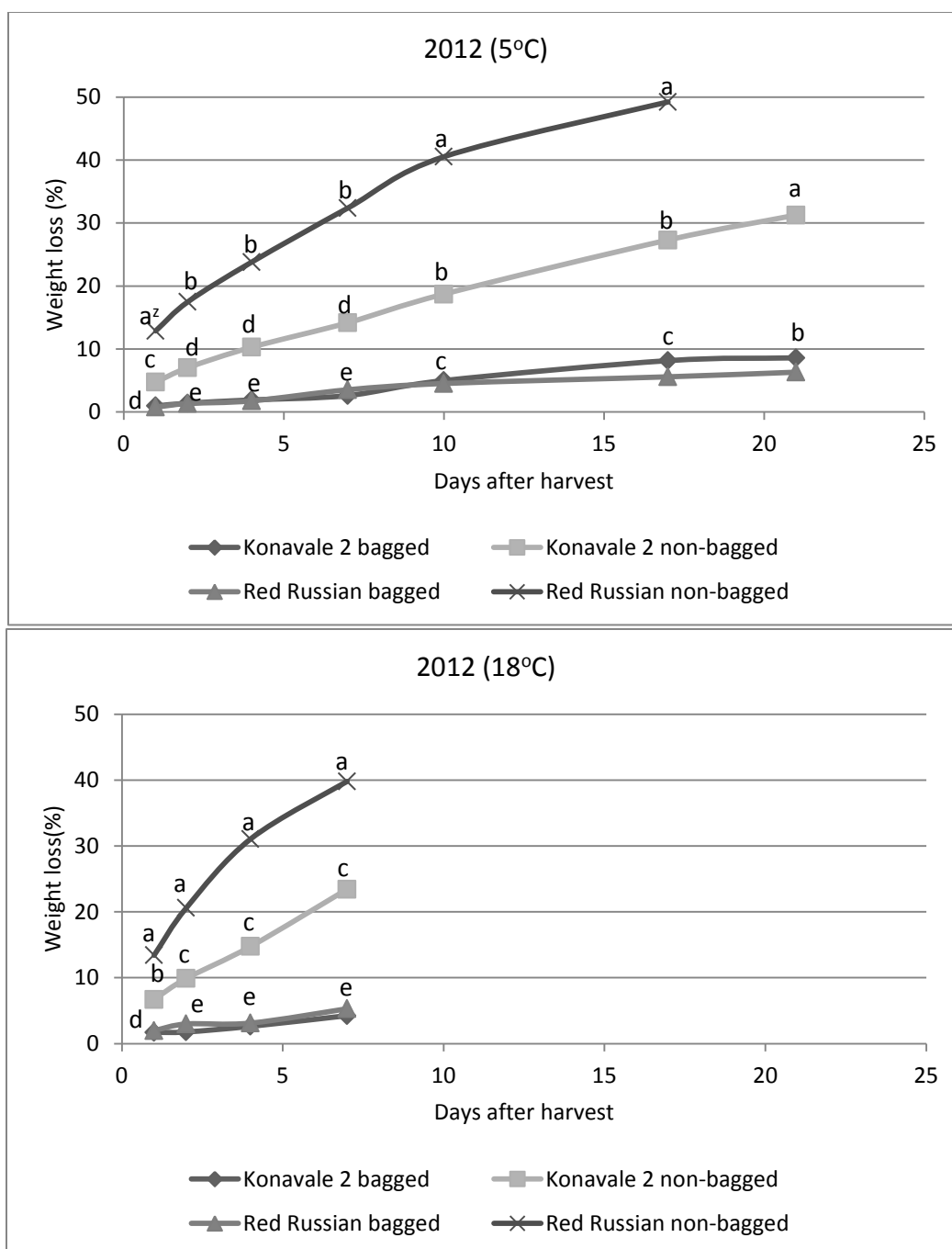
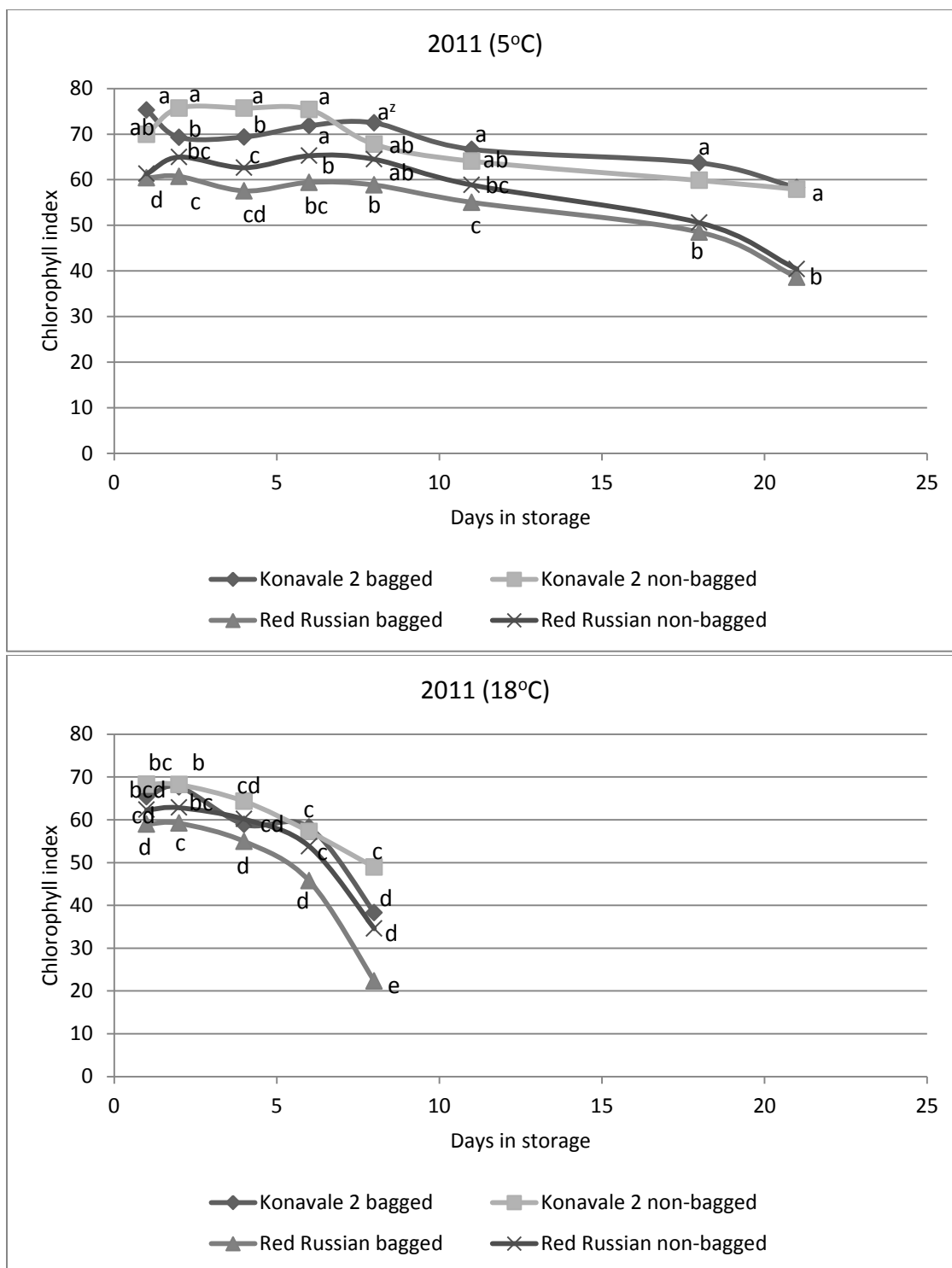


Figure 4.1. Cumulative weight loss (% of initial weight) of field grown kale leaves from cultivars Konavale 2 and Red Russian bagged or non-bagged stored at either 5°C or 18°C in a two year study in Tifton, GA. ²For each year irrespective of the temperature of storage data points followed by the same letter within a day are not significantly different at $P < 0.05$ level.



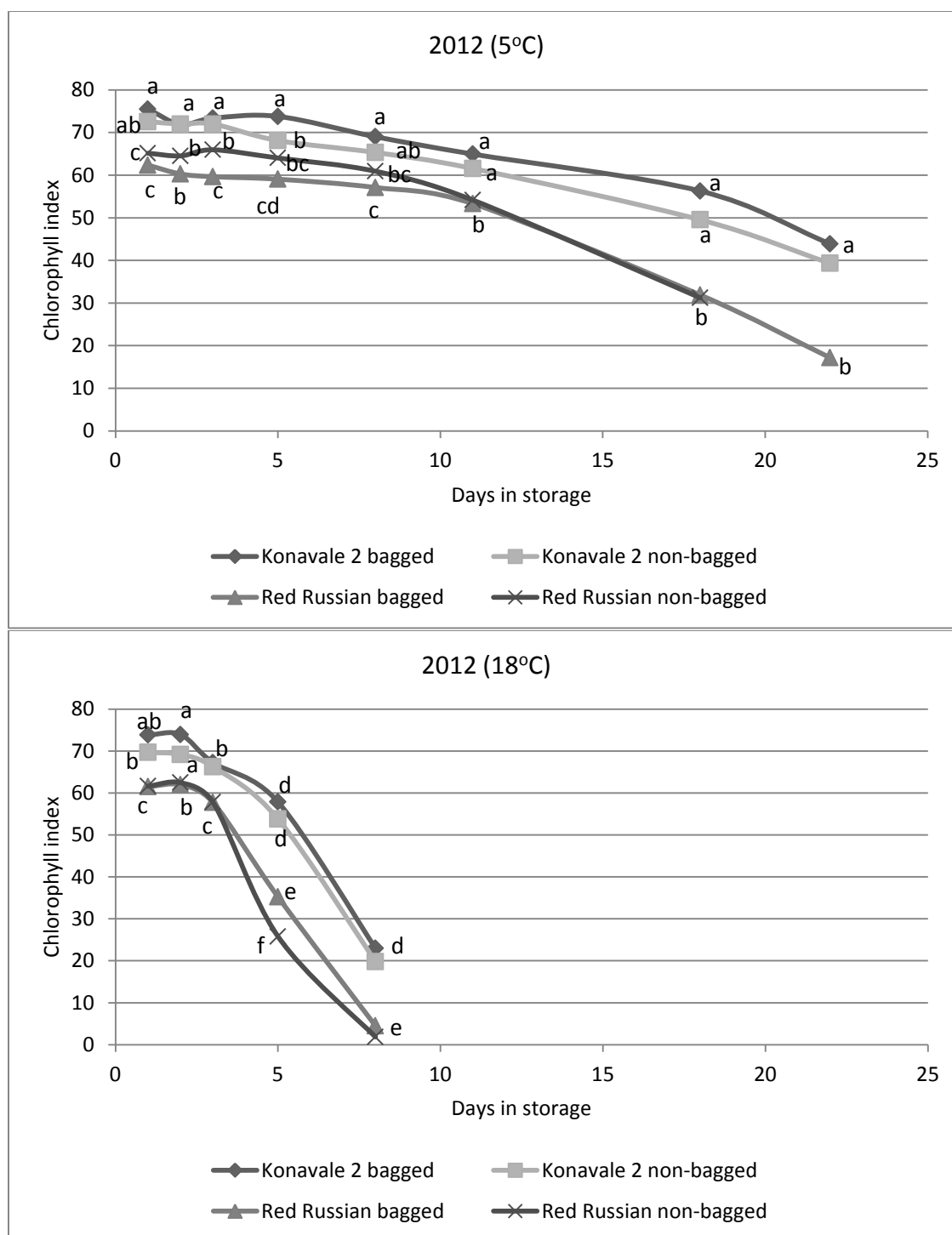


Figure 4.2. Chlorophyll index of kale leaves from cultivars Konavale 2 and Red Russian bagged or non-bagged stored at either 5°C or 18°C.

^zFor each year irrespective of the temperature of storage data points followed by the same letter within a day are not significantly different at $P < 0.05$ level.

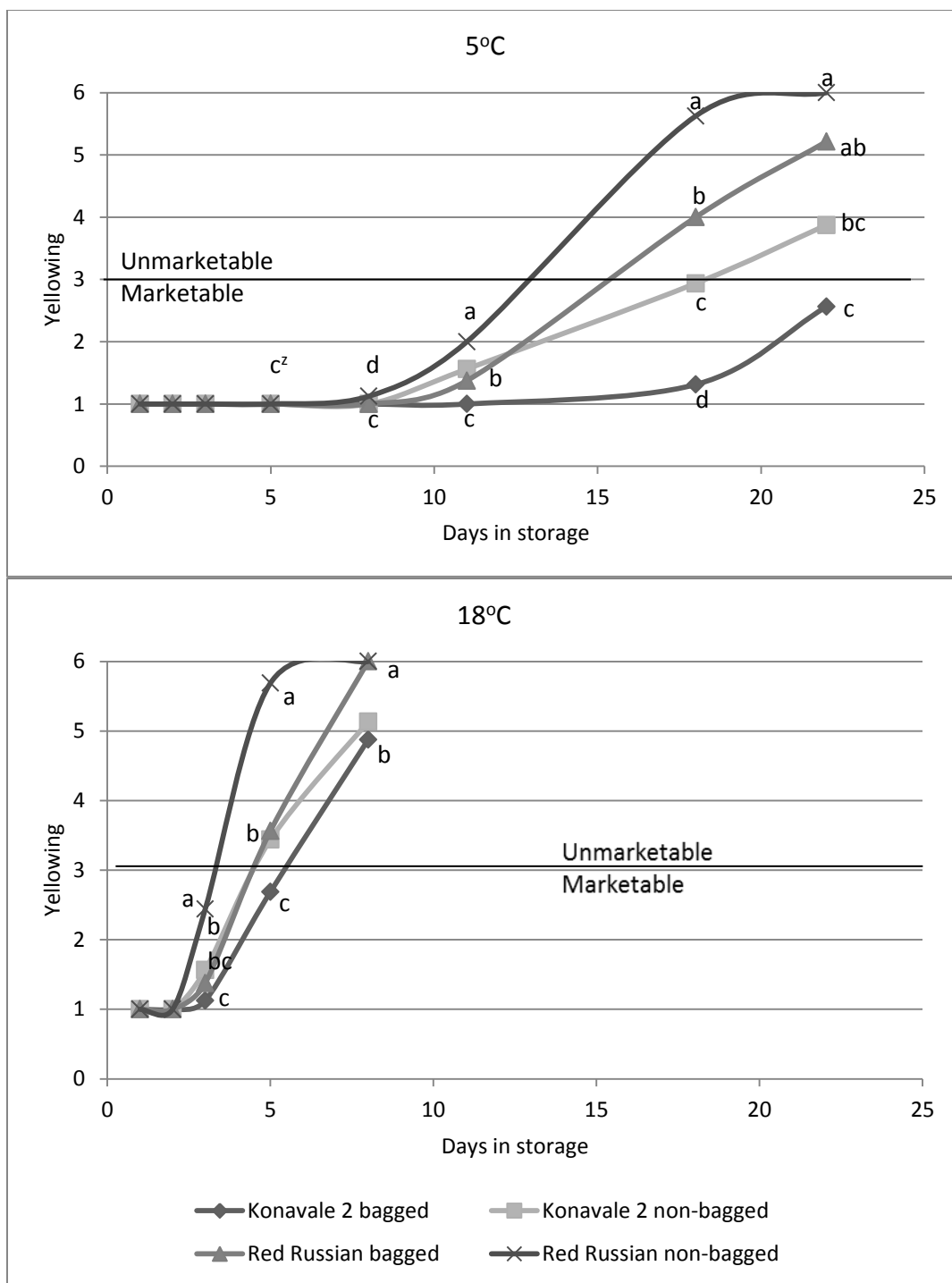


Figure 4.3. Yellowing of kale leaves stored with or without bag at either 5°C or 18°C up to 22 days in year 2012. Leaves were rated in 1-6 scale (1=no yellowing and 6= completely yellow leaves) and leaves with score of 3 or above were considered unmarketable.

^zFor each year irrespective of the temperature of storage data points followed by the same letter within a day are not significantly different at $P < 0.05$ level.

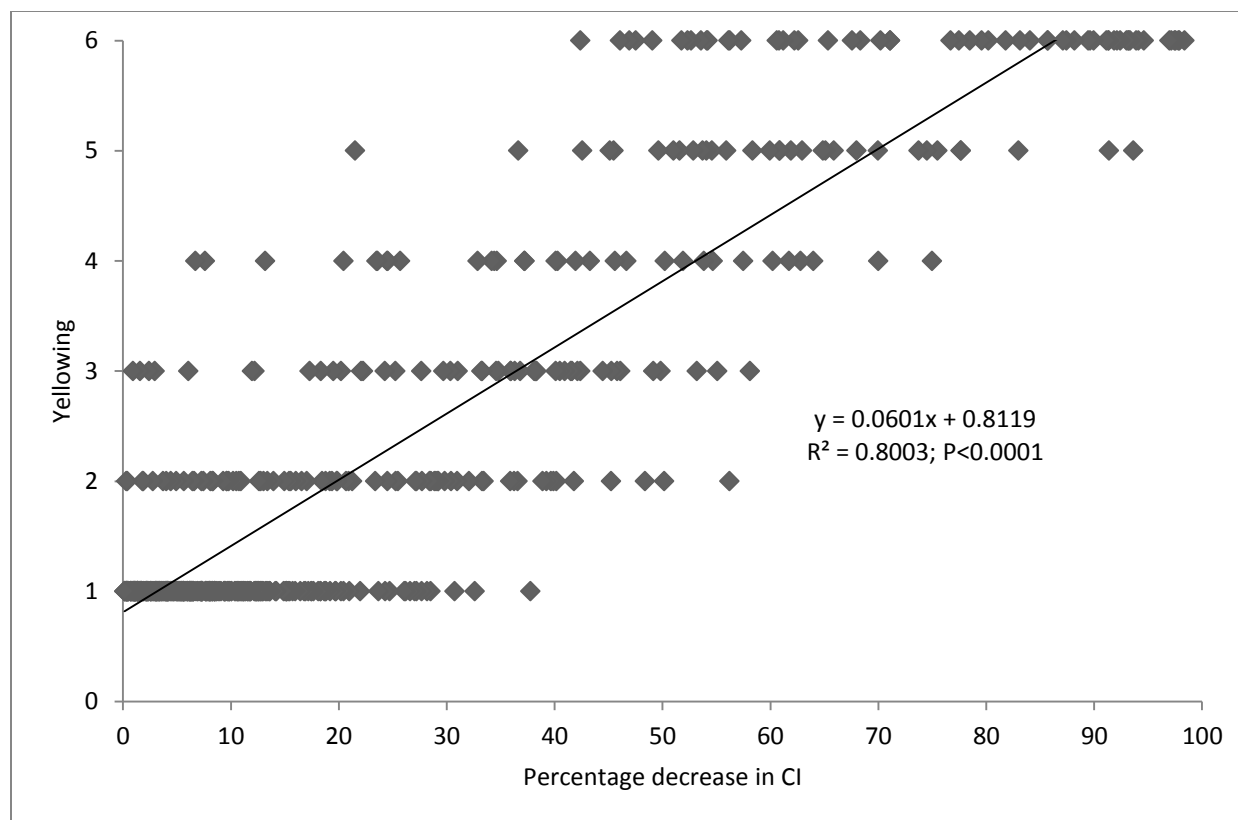
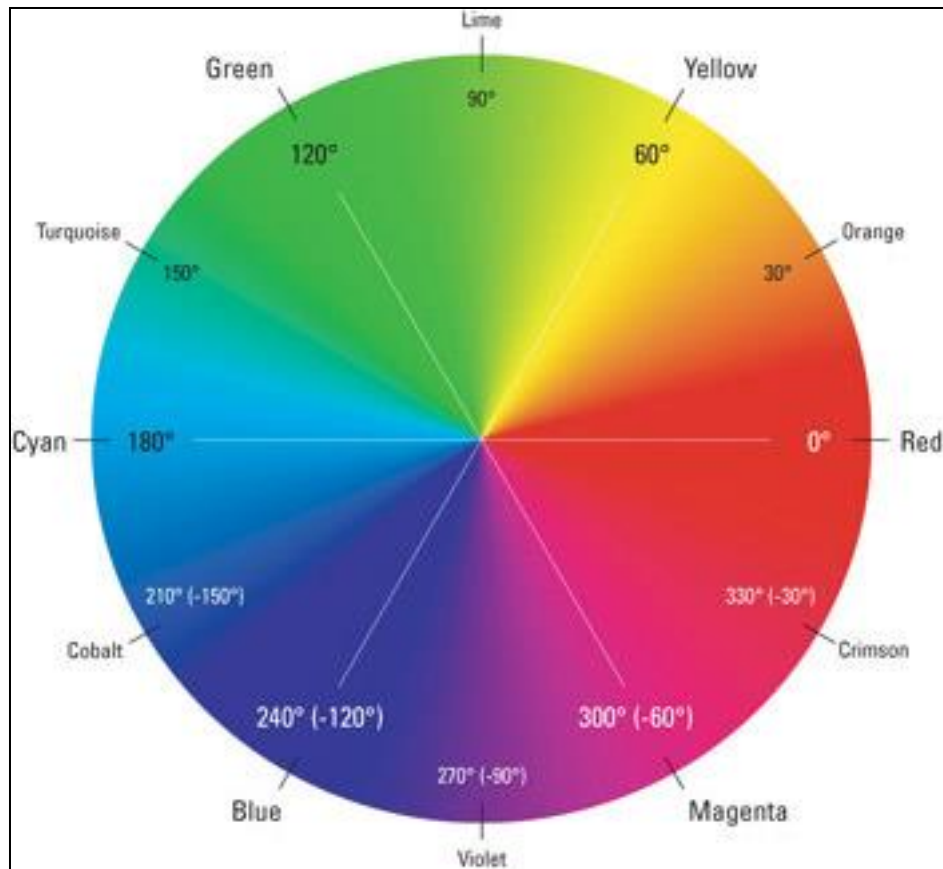


Figure 4.4. Correlation between decrease in the relative decrease in chlorophyll index (% of initial value) and yellowing of kale leaves in 2012.



Figure 4.5. Chlorophyll index (CI) was recorded on the encircled portions of leaves generally.



<http://flylib.com/books/en/2.816.1.70/1/>

Figure 4.6. Various colors represented as an angle called hue angle. Yellow is at 60° and green is at 120° .

CHAPTER 5

CONCLUSION

Pomegranate

Pomegranate fruit being a non-climacteric fruit needs to be harvested at right maturity status. Changes in TSS and TA during maturity play very important role determining the final flavor of fruit. Changes in fruit weight and juice/weight content influence marketability of fruit. CA stored fruit had better fruit quality with smoother skin, less skin shriveling, lower fruit cracking, lesser husk scald and lower chilling injury compared to RA stored fruit. Fruit diseases caused by *Cercospora* spp. and other fungal pathogens were lesser in fruit stored under CA compared to RA. Fruit color and juice TSS were better preserved in fruit under CA storage than RA. Fruit quality deteriorated rapidly during shelf storage due to higher temperature (20-25°C) and low relative humidity (45%-50%). Thus it is evident that controlled atmosphere storage is definitely a better option for storing pomegranates.

Kale

During storage of kale, lower storage temperature (5°C) led to decrease in leaf weight loss, lower shriveling and wilting thus preserving the quality of leaves. Leaf greenness and visual quality were better maintained at reduced storage temperature. Bagged leaves during storage further enhanced shelf life by greatly reducing moisture loss.

Summary

In general, storage conditions greatly affect the quality and shelf life of produce. Temperature and relative humidity during storage affect water loss from the fruit and vegetables thus affecting their quality. Packaging and modifying the storage atmosphere reduce postharvest losses (decay, diseases and injury) of produce. For regular availability of produce in the market it is necessary to maintain its quality by storing it under appropriate conditions.