Institute of Specialty Crops and Crop Physiology
University of Hohenheim
Fruit Sciences
Prof. Dr. J. N. Wünsche

Physiological Responses of 'Jonagold' Apple (*Malus domestica* Borkh.) following Postharvest 1-Methylcyclopropene (1-MCP) Application

Dissertation

Submitted in fulfilment of the requirements for the degree
"Doktor der Agrarwissenschaften"
(Dr. sc. agr. / Ph.D. in Agricultural Sciences)

to the

Faculty of Agricultural Sciences

presented by

Claudia Susanne Heyn
from Kassel
2009

This thesis was accepted as a doctoral dissertation in fulfilment of the requirements for the degree “Doktor der Agrarwissenschaften” by the Faculty of Agricultural Sciences at University of Hohenheim on 20. April 2009.

Date of oral examination: 7. October 2009.

Examination Committee

Supervisor and Review	Prof. Dr. J. N. Wünsche
Co-Reviewer	Prof. Dr. Dr. h.c. R. Carle
Additional examiners	Prof. Dr. J. Müller
Vice-Dean and Head of the Committee	Prof. Dr. W. Bessei

ACKNOWLEDGEMENTS

I extend my sincere gratitude to Prof. Dr. Jens Wünsche. His kindness, support, confidence and encouragement during the supervision of this research project is highly appreciated. Thanks for all the good advices and considerable patience in proof reading this thesis.

My thanks go to Prof. Dr. Dr. h.c. Reinhold Carle and Prof. Dr. Joachim Müller for co-reviewing this doctoral thesis and for serving on my examination committee.

The financial support of AgroFresh, Inc. for this extensive, but very interesting research project is highly acknowledged. My special thanks are due to André Vink, AgroFresh, Inc., for his friendly, straight-forward support, advice and interest.

Thanks are due to Dr. Josef Streif for instructions and supervision during my stay in Ravensburg. I am grateful with Thomas Kininger, Uwe Spenninger and Michael Zoth for their work in the orchards and for maintaining and controlling the storage facilities in Ravensburg. Thanks to Marlene Stark, Renate Wirsing, Rosi Slodczyck and Sabine Sonnentag for kindly helping me with the considerable quality determinations.

I am grateful to farmer Joseph Bentele, Ravensburg, for kindly providing the apple fruit in 2005.

My thanks are due to Marlene Stark, Dominikus Kittemann, Roy McCormick and Dr. Daniel Neuwald for assistance in consumer taste panels in 2007.

I thank Christiane Beierle and Dr. G. Bufler, Institute of Specialty Crops and Crop Physiology, Vegetable Sciences, University Hohenheim, for instructions in determination of ATP and ADP concentrations.

Acknowledgements are due to Dr. P. Esquivel, Dr. D.R. Kammerer and Dr. E. Sadilova, Institute for Food Science and Biotechnology, Section Plant Foodstuff Technology, University Hohenheim, for kindly providing protocols for determination of phenolic compounds and total antioxidant capacity. Thanks for valuable comments on the results and helpful, stimulating discussion.

Furthermore thanks to my friends and fellow students for interest, encouragement, understanding and from time to time pleasant changes during that time-consuming project.

Finally, I thank my parents and my family for their unconditional support.

TABLE OF CONTENTS

LIST OF FIGURES

1. General Introduction

2. Effect of 1-MCP on Apple Fruit Quality and Consumer Acceptability

3. Effect of 1-MCP on Climacteric Characteristics of Apple Fruit

4. Effect of 1-MCP on Antioxidant Capacity of Apple Fruit

Appendix

LIST OF TABLES

2. Effect of 1-MCP on Apple Fruit Quality and Consumer Acceptability

3. Effect of 1-MCP on Climacteric Characteristics of Apple Fruit

4. Effect of 1-MCP on Antioxidant Capacity of Apple Fruit

Appendix

ABBREVIATIONS

1-MCP	1-Methylcyclopropene
AAPH	2,2‘-azobis(2-amidinopropane)dihydrochloride
ABTS	2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic-acid)
ACC	1-aminocyclopropane-1-carboxylic acid
ACC-O	1-aminocyclopropane-1-carboxylic acid oxidase
ACC-S	1-aminocyclopropane-1-carboxylic acid synthase
ADP	adenosine diphosphate
ANOVA	analysis of variance
ATP	adenosine triphosphate
BGC	background colour
C_2H_4	ethylene
C_4H_6	1-Methylcyclopropene
CA	controlled atmosphere
CAT	catalase
CO_2	carbon dioxide
DAH	days after harvest
DF	degree of freedom
DHA	dehydroascorbic acid
DNA	deoxyribonucleic acid
DPPH	1-diphenyl-2-picrylhydrazyl
DW	dry weight
EDTA	ethylenediamine tetraacetic acid
FCR	Folin-Ciocalteu’s reagent
Fe^{2+}	iron
FF	flesh firmness
FW	fresh weight
g	relative centrifuge force
H°	hue angle
H_2O_2	hydrogen peroxide

HPLC	high pressure liquid chromatography
HPO_3	meta-phosphoric acid
KH_2PO_4	potassium dihydrogen phosphate
$KI\text{-}I_2$	potassium iodide-iodine
L-AA	L-ascorbic acid; ascorbate
LSD	least significant difference
M.9	Malling rootstock No. 9
mM	millimolar
mPa	millipascal
Na_2CO_3	sodium carbonate
Na_2HPO_4	disodium hydrogen phosphate
NaOH	sodium hydroxide
ns	not significant
O_2	oxygen
O_2^-	superoxide
OH	hydroxyl radical
P	probability
PAL	phenylalanine ammonia-lyase
POX	peroxidase
ppb	parts per billion
RH	relative humidity
ROS	reactive oxygen species
SAM	S-adenosyl-methionine
SOD	superoxide dismutase
SSC	soluble solids concentration
TA	titratable acidity
TCA	tricarboxylic acid
TE	Trolox® equivalent
TEAC	Trolox® equivalent antioxidant capacity
TOSC	total oxidant scavenging capacity
ULO	ultra low oxygen
VCEAC	vitamin C equivalent antioxidant capacity
w/v	weight per volume

SUMMARY

Storage technologies, such as controlled atmosphere (CA) storage and recently 1-Methylcyclopropene (1-MCP) treatments have led to an all-year-round global supply of high qualitative apple fruit. As a consequence, pressure of competition between several apple growing areas is increasing and in the same way consumers demands and expectations for apple fruit quality. However, throughout storage fruit quality is generally preserved at a high level whereas conditions at several points throughout the distribution chain are not adequate for fresh commodities. It is critically important to maintain consistently high fruit quality throughout the marketing period to the final consumer and that fruit quality at the point of sale meets consumer requirements. Although decision for purchasing apple fruit is mainly due to appearance (size, shape and colour) and firmness, consumer are increasingly concerned about nutritional quality and health-protecting compounds in foods. In addition, fruit with high antioxidant capacities may have improved fruit quality, storage characteristics and shelf-life.

The plant hormone ethylene influences many of the ripening processes in climacteric fruit such as e.g. apple, pear, banana and kiwifruit. Climacteric fruit exhibit a distinct upsurge in respiration rates and ethylene production rates ('climacteric') during maturation and ripening. To induce the many biochemical changes associated with ripening (colour change from green to yellow, aroma development, softening, increased respiration etc.) and to stimulate further ethylene biosynthesis (positive feedback regulation) ethylene needs to bind to specific ethylene binding sites (receptors). After switching the receptors off, certain signal transduction pathways are activated and the different ripening processes are initiated. Since harvested fruit are still living biological systems, the respiration rate is an important indicator of metabolic activity. The main substrates involved in aerobic respiration of harvested fruit are sugars and acids. The subsequent depletion in these compounds leads to a loss of fruit taste and quality, storability and shelf-life. Therefore, the rate of respiration is generally inversely related to storability of fruit. The main aim of postharvest technologies is to reduce metabolism (ethylene production, respiration, transpiration) of harvested produce. The ripening process of climacteric fruit could be retarded by means of reduced temperatures, high relative humidity and

supplementary controlled atmospheres (reduced oxygen- and elevated carbon dioxide concentrations) and recently 1-MCP treatments. 1-MCP is an effective tool for maintaining fruit quality during storage and post-storage handling. 1-MCP, a synthetic unsaturated cyclic olefin, is thought to act as a competitive substance to ethylene, occupying the ethylene receptor site so that ethylene can not bind to trigger its action, i.e. the autocatalytic ethylene production (system II ethylene) and subsequently the initiation of ripening is prevented. In general, 1-MCP is able to counteract ripening effects triggered by ethylene during and after storage by blocking its action in fruit rather than inhibiting its production. However, once ripening commenced and autocatalytic ethylene biosynthesis started, 1-MCP can not stop the ripening process.

The present research project consists of three studies. The aim of the first study was to determine the effect of 1-MCP treatment, storage condition and –duration on apple fruit quality and consumer acceptability. The second part of the study focused on the effect of 1-MCP treatment, storage condition and –duration on climacteric characteristics of apple fruit. The effect of 1-MCP treatment, storage condition and –duration on antioxidant capacity of apple fruit was studied in the third part of the present research.

'Jonagold' apple fruit (*Malus domestica* Borkh.) were picked at commercial maturity in 2004, 2005 and 2006. Fruit were treated with 1-MCP on the day of harvest in 2004 (0 days after harvest, 0 DAH) and 7 DAH in 2005 and 2006 and stored the following day either in cold storage, CA- (0.8 % CO_2, 3 % O_2) or ULO-storage (3 % CO_2, 1 % O_2). Fruit were held in cold storage prior to commencement of storage in 2005 and 2006. After 2, 4 and 6 months in 2004/05, 3, 6 and 9 months in 2005/06 and 3 and 5 months in 2006/07 fruit samples from each storage atmosphere ± 1-MCP were removed. Fruit quality parameters (flesh firmness, soluble solid concentration, titratable acidity and background colour) were assessed after harvest, commencement of storage and after each sample removal in 2004/05, 2005/06 and 2006/07 following 10 days shelf-life at 20°C. Consumer preference mapping was performed after 3 and 5 months of cold- and ULO-storage in 2006/07. Shelf-life respiration rate and fruit ethylene production was measured after harvest, commencement of storage and after each sample removal in 2004/05 and 2005/06, respectively. In 2005/06 ATP and ADP concentration was additionally determined. Nutritional quality and health-protecting compounds were examined by means of ascorbic acid concentration (L-AA), phenolic compounds and total

non-enzymatic antioxidant capacity in 2005/06 following 10 days shelf-life after harvest, commencement of storage and after each sample removal.

The results of the first part of this study showed that fruit quality generally decreased during storage and shelf-life depending on 1-MCP treatment, storage condition and -duration. However, 1-MCP delayed ripening more and maintained fruit quality better than CA- or even ULO-storage alone. The greatest impact of 1-MCP was seen in a clear firmness retention in all samples, regardless of storage condition and –duration. Since not all ripening and quality parameters are ethylene-dependent, not all of them will be regulated and influenced by 1-MCP in the same intensity. This might have an impact on the overall quality of the fruit and the consumer acceptance of 1-MCP treated apples. However, most consumers, regardless of age or gender, preferred the 1-MCP treated fruit from ULO-storage, particularly when stored longer. Therefore, it is concluded that firmness and tartness ('freshness') were the most important drivers of consumer preference. In the present study all analytical measurements were in good agreement with corresponding sensory evaluations from consumer panels. Though sensory evaluation studies are time-consuming and there might be some flaws and difficulties to generate representative results from consumer taste panels, they are a useful tool to assess food quality and consumer preference.

The results of the second part of the study proved that 1-MCP is a potent antagonist in terms of reducing and delaying ethylene production and respiratory rise. Although CA- and ULO-storage reduced ethylene production significantly in 'Jonagold' apples, 1-MCP treatment inhibited ethylene biosynthesis and respiration rate more than CA- and ULO-storage alone. In general, the effect of 1-MCP on shelf-life ethylene production diminished with storage time in both years. Furthermore, the magnitude of respiration and ethylene production rates was higher in 2005/06 when compared with 2004/05, which might be due to later 1-MCP treatment and commencement of storage after harvest as well as lower treatment temperatures. The present study (Chapter 3) clearly shows that immediate 1-MCP treatment and appropriate storage management after harvest is critical for a maximum reduction of climacteric characteristics such as ethylene production and respiration rate as well as maintenance of postharvest and post-storage apple fruit quality.

L-AA concentration significantly decreased during storage, irrespective of storage condition and 1-MCP treatment. Though L-AA concentration was significantly higher in 1-MCP treated fruit than in untreated fruit at commencement of storage, however, following 9 months of storage L-AA concentration was significantly lower in all 1-MCP treated fruit when compared with untreated fruit. Vitamin C equivalent phenolic concentration decreased after 6 months of storage and gradually increased again after 9 months of storage. Neither storage condition nor 1-MCP treatment had a significant effect on phenolic compounds in apple fruit. In the same way vitamin C equivalent antioxidant capacity (VCEAC) decreased after 6 months of storage and increased again after 9 months of storage. 1-MCP treatment had no effect on VCEAC. The results of the third part of the study showed that the nutritional value of apple fruit was not influenced by 1-MCP. Moreover, storage conditions had little effect on phenolic compounds and total antioxidant capacity. Only L-AA concentration was affected by different storage conditions and slightly influenced by 1-MCP. Since L-AA contributes only to a small extend to the antioxidant capacity of apple fruit, this does not affect the total nutritional value of apple fruit.

In conclusion, this research proved that 1-MCP, alone and especially in combination with controlled atmosphere storage, is an effective tool for maintaining fruit quality during storage and post-storage handling. However, in the literature it is found that 1-MCP treatment and even CA/ULO-storage might impair the development of aroma and flavour compounds of apple fruit due to the reduction of ethylene production. Therefore, the present study will be continued with measurements of aroma volatile profiles and determinations of precursors (fatty acids) of 'Jonagold' apples following 1-MCP treatment and storage in different storage conditions (cold storage, CA- and ULO-storage). Moreover, further research is necessary regarding the enzymatic antioxidant capacity, namely the enzymes superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX), of 1-MCP treated 'Jonagold' apple fruit under the above mentioned experimental conditions. Since no effect of 1-MCP on phenolic compounds and total non-enzymatic antioxidant capacity (VCEAC) was found in the present study, it would be of interest whether antioxidant enzymes would be affected by 1-MCP treatment and/or storage conditions and –durations. The improved fruit quality and storage characteristics of 1-MCP treated apple fruit in the present study might be due to higher contents of antioxidant enzymes.

ZUSAMMENFASSUNG

Lagertechnologien wie die Lagerung bei veränderter Gaszusammensetzung der Lageratmosphäre (CA- und ULO-Lagerung; CA = 'controlled atmosphere'; ULO = Lagerung bei sehr niedriger Sauerstoffkonzentration, 'ultra low oxygen') und seit einiger Zeit die Behandlung mit dem Ethyleninhibitor 1-Methylcyclopropen (1-MCP) haben weltweit zu einem ganzjährigen Angebot an qualitativ hochwertigen Äpfeln geführt. Daraus folgt ein erhöhter Konkurrenzdruck zwischen den einzelnen Apfelanbaugebieten und in gleicher Weise steigen auch die Ansprüche und Erwartungen der Konsumenten an die Qualität der Äpfel. Obwohl die Fruchtqualität während der Lagerung auf einem hohen Level erhalten werden kann, sind die Bedingungen in der weiteren Vermarktungskette nicht immer optimal für frische pflanzliche Erzeugnisse. Es ist besonders wichtig, dass die hohe Fruchtqualität auf dem gesamten Weg der Vermarktung ununterbrochen bis zum Endkonsumenten erhalten wird und dass die Fruchtqualität am Verkaufsort den Ansprüchen und Wünschen der Konsumenten entspricht. Obwohl die Kaufentscheidung bei Äpfeln hauptsächlich vom Aussehen (Größe, Form und Farbe) sowie der Festigkeit beeinflusst wird, sind die Konsumenten zunehmend interessiert am gesundheits- und ernährungsphysiologischen Wert der Nahrungsmittel. Früchte mit hoher antioxidativer Kapazität haben vermutlich eine verbesserte Fruchtqualität, Lagereigenschaften sowie Haltbarkeit.

Das Pflanzenhormon Ethylen initiiert und beeinflusst viele Reifeprozesse in klimakterischen Früchten, wie z. B. bei Apfel, Birne, Banane und Kiwi. Bei klimakterischen Früchten zeigt sich zu Beginn der Reife ein deutlicher Anstieg in der Atmung sowie in der Ethylenproduktion. Um die vielen einzelnen, mit der Reife verbundenen biochemischen Veränderungen (Farbwechsel von grün nach gelb, Aromaentwicklung, Weichwerden, erhöhte Atmung, etc.) einzuleiten und um die weitere Ethylenproduktion (autokatalytische Ethylenproduktion) zu gewährleisten, muss sich Ethylen mit spezifischen Ethylenrezeptoren verbinden. Nach dem Andocken von Ethylen an den Rezeptoren werden bestimmte Signale weitergeleitet und die einzelnen verschiedenen Reifevorgänge eingeleitet. Da geerntete Früchte nach wie vor lebende biologische Systeme sind, stellt die Atmungsrate einen wichtigen Indikator der Stoffwech-

selaktivität dar. Die wichtigsten Substrate der aeroben Atmung in geernteten Früchten sind Zucker und Säuren. Eine daraus folgende Verarmung dieser Bestandteile führt zu einem Verlust an Frucht- und Geschmacksqualität, Lagerfähigkeit und Haltbarkeit. Aus diesem Grund verhalten sich die Atmungsrate der Früchte und ihre Lagerfähigkeit grundsätzlich gegensätzlich. Das Hauptziel verschiedener Nacherntetechnologien ist, den Reifeprozess der geernteten Früchte zu verlangsamen, d. h. die Atmung, Transpiration und Ethylenproduktion zu reduzieren. Bei klimakterischen Früchten lässt sich das durch die Lagerung bei niedrigen Temperaturen, hoher relativer Luftfeuchtigkeit und zusätzlich einer veränderten Gaszusammensetzung der Lageratmosphäre (reduzierte Sauerstoff- und erhöhte Kohlendioxid-Konzentrationen), sowie seit einiger Zeit mit Hilfe von 1-MCP-Behandlungen erreichen. 1-Methylcyclopropen (1-MCP) ist ein effektives Mittel zur Erhaltung der Fruchtqualität während der Lagerung sowie während der nachfolgenden Vermarktung. 1-MCP, ein synthetisches, ungesättigtes, zyklisches Olefin, ist eine mit Ethylen konkurrierende Substanz, die die Rezeptoren besetzt, so dass Ethylen nicht andocken kann. Auf diese Weise wird die autokatalytische Ethylenproduktion (System II Ethylen) sowie die reifestimulierende Wirkung von Ethylen verhindert. Grundsätzlich ist die Fähigkeit von 1-MCP, dem Reifeprozess entgegenzuwirken, mehr einer verhinderten Wirkung, als einer reduzierten Produktion von Ethylen zuzuschreiben. Sobald jedoch die Reifevorgänge initiiert sind und die autokatalytische Ethylenbiosynthese begonnen hat, kann 1-MCP den Reifeprozess nicht mehr stoppen.

Das vorliegende Forschungsprojekt besteht aus drei einzelnen Studien. Das Ziel der ersten Studie war es, den Effekt einer 1-MCP-Behandlung und verschiedener Lagerungsbedingungen (Lageratmosphäre und -dauer) auf die Apfelfruchtqualität sowie die Akzeptanz dieser Früchte beim Konsumenten zu untersuchen. Der zweite Teil der Studie befasste sich mit den Auswirkungen einer 1-MCP-Behandlung und verschiedenen Lagerungsbedingungen (Lageratmosphäre und -dauer) auf die typischen klimakterischen Merkmale bei Apfel (Atmungsrate, Ethylenproduktion, Energiestoffwechsel). Im dritten Teil des vorliegenden Projektes wurde der Einfluss einer 1-MCP-Behandlung in Kombination mit verschiedenen Lagerungsbedingungen (Lageratmosphäre und –dauer) auf den gesundheits- und ernährungsphysiologischen Wert, d. h. die antioxidative Kapazität von Äpfeln untersucht.

'Jonagold' Äpfel (*Malus domestica* Borkh.) wurden in den Jahren 2004, 2005 und 2006 in einem für Langzeitlagerung üblichen Reifezustand geerntet. Nach der Behandlung mit 1-Methylcyclopropen (1-MCP) noch am Tag der Ernte in 2004 und 7 Tage nach der Ernte in den Jahren 2005 und 2006 wurden die Früchte am folgenden Tag im Kühllager, CA- (0.8 % CO_2, 3 % O_2) beziehungsweise ULO-Lager (3 % CO_2, 1 % O_2) eingelagert. Bis zur Einlagerung in 2005 und 2006 wurden die Früchte im Kühllager aufbewahrt. Nach 2, 4 und 6 Monaten in der Saison 2004/05 und nach 3, 6 und 9 Monaten in 2005/06 sowie nach 3 und 5 Monaten in 2006/07 wurden aus jedem Lager ± 1-MCP Früchte entnommen. Fruchtqualitätsfaktoren (Fruchtfleischfestigkeit, lösliche Trockensubstanz, titrierbare Säure und Hintergrundfarbe) wurden nach der Ernte, nach der Einlagerung sowie nach jeder Probenahme jeweils nach 10 Tagen Nachlagerung (Shelf-life) bei 20°C in allen drei Versuchsjahren analysiert. Zusätzliche Verkostungen wurden nach 3 und 5 Monaten Kühl- beziehungsweise ULO-Lagerung in der Saison 2006/07 durchgeführt. Die Atmungsrate sowie die Ethylenproduktion der Früchte wurde während der Nachlagerung nach der Ernte, nach der Einlagerung sowie nach jeder Probenahme in 2004/05 und 2005/06 gemessen. In 2005/06 wurden zusätzlich die ATP und ADP-Konzentrationen bestimmt. Zur Bestimmung der gesundheits- und ernährungsphysiologisch wertvollen Bestandteile wurden in 2005/06 die Vitamin C-Konzentration, die Gesamtphenole sowie die gesamte nichtenzymatische antioxidative Kapazität der Äpfel nach 10 Tagen Nachlagerung (20°C) im Anschluss an die Ernte, die Einlagerung sowie nach jeder Auslagerung analysiert.

Die Ergebnisse der ersten Studie zeigten, dass die Fruchtqualität grundsätzlich nach Lagerung und Shelf-life in Abhängigkeit von 1-MCP-Behandlung und Lagerungsbedingungen (Atmosphäre und Dauer) abnimmt. 1-MCP verzögerte die Reife deutlicher und erhielt die Fruchtqualität besser als die Lagerung unter kontrollierter Atmosphäre (Standard-CA- und ULO-Lagerung) allein. Unabhängig von Lageratmosphäre und Lagerdauer war bei allen Proben die deutlichste Auswirkung von 1-MCP in einer einheitlich besseren Erhaltung der Fruchtfleischfestigkeit zu sehen. Da aber nicht alle Reife- und Qualitätsparameter von Ethylen abhängig sind, werden auch nicht alle von 1-MCP in gleicher Intensität reguliert und beeinflusst. Das kann einen Einfluss auf die Gesamtqualität der Früchte sowie die Akzeptanz 1-MCP behandelter Äpfel beim Konsumenten haben. Dennoch bevorzugten die meisten Konsumenten, unabhängig von Alter und Geschlecht, die mit 1-MCP behandelten und im ULO-Lager gela-

gerten Früchten. Dieser Effekt war besonders deutlich nach längerer Lagerung festzustellen. Daraus lässt sich schließen, dass für die Konsumenten hauptsächlich die Qualitätsfaktoren Festigkeit und Sauerkeit ('Frische') von Bedeutung waren. In der vorliegenden Studie entsprachen die analytischen Ergebnisse der einzelnen Qualitätsparameter in allen Fällen den Aussagen der parallel durchgeführten sensorischen Konsumententests. Sensorische Untersuchungen sind sehr zeitaufwändig und es kann schwierig sein, bei Konsumentenbefragungen und –tests repräsentative Ergebnisse zu erhalten. Dennoch sind Verkostungen ein hilfreiches Mittel, um Qualität und geschmacklichen Wert der Früchte sowie die Ansprüche, Wünsche und Zufriedenheit der Konsumenten zu ermitteln.

In der zweiten Studie konnte festgestellt werden, dass 1-MCP die Ethylenproduktion sowie den damit verbundenen Atmungsanstieg bei Apfel deutlich reduziert und verzögert. Obwohl die Ethylenproduktion von 'Jonagold' Äpfeln auch durch CA- und ULO-Lagerung signifikant reduziert wurde, verminderte eine Behandlung mit 1-MCP die Ethylenbiosynthese sowie den damit verbundenen Atmungsanstieg stärker als CA- und ULO-Lagerung allein. In beiden Versuchsjahren wurde jedoch mit fortschreitender Lagerdauer der Einfluss von 1-MCP auf die Ethylenproduktion während der Nachlagerung schwächer. Darüberhinaus waren Atmungs- und Ethylenproduktionsrate in 2005/06 höher als in 2004/05, was an der späteren 1-MCP-Behandlung und Einlagerung nach der Ernte sowie niedrigeren Behandlungstemperaturen liegen kann. Die vorliegenden Ergebnisse zeigen deutlich, dass es besonders wichtig ist, die Äpfel möglichst bald nach der Ernte mit 1-MCP zu behandeln und einzulagern. Nur so kann ein maximaler Effekt auf die klimakterischen Parameter sowie die Erhaltung der Fruchtqualität während und nach der Lagerung erzielt werden.

Unabhängig von Lageratmosphäre und 1-MCP-Behandlung war während der Lagerung von 'Jonagold' Äpfeln eine signifikante Reduktion der Vitamin C –Gehalte festzustellen. Obwohl die Vitamin-C-Konzentrationen in den mit 1-MCP-behandelten Früchten bei der Einlagerung signifikant höher waren als in den unbehandelten Kontrollfrüchten, waren nach 9 Monaten Lagerung im Vergleich zu den Kontrollfrüchten in allen mit 1-MCP behandelten Früchten signifikant niedrigere Vitamin C-Konzentrationen festzustellen. Die Gesamtphenolkonzentration verringerte sich während der ersten 6 Lagermonate, nahm dann aber nach 9 Monaten Lagerung wieder zu. Die Gesamtphenolkonzentration wurde weder durch die Lageratmosphäre

(Kühllager, CA- bzw. ULO-Lager), noch durch eine 1-MCP-Behandlung signifikant beeinflusst. Auch die Vitamin-C äquivalente antioxidative Kapazität (VCEAC) verringerte sich während der ersten 6 Lagermonate, um nach 9 Monaten wieder anzusteigen. Die Behandlung mit 1-MCP hatte keinen signifikanten Effekt auf die untersuchte antioxidative Kapazität. Die Ergebnisse der dritten Studie zeigten, dass der gesundheits- und ernährungsphysiologische Wert von Äpfeln nicht von 1-MCP beeinflusst wird. Außerdem war auch nur ein leichter Effekt der verschiedenen Lagerbedingungen auf die Gesamtphenole sowie die antioxidative Kapazität festzustellen. Lediglich bei der Vitamin-C-Konzentration konnte sowohl ein Einfluss der verschiedenen Lagerungsbedingungen als auch der Behandlung mit 1-MCP gefunden werden. Da aber Vitamin C nur einen sehr kleinen Teil der antioxidativen Kapazität von Apfel ausmacht, zeigte sich, dass der allgemeine gesundheits- und ernährungsphysiologische Wert von Apfel dadurch nicht beeinflusst wird.

Zusammenfassend lässt sich festhalten, dass 1-MCP alleine oder in Kombination mit CA-Lagerung, ein effektives Mittel zur Erhaltung der Fruchtqualität während und nach der Lagerung ist. In der Literatur ist allerdings zu lesen, dass 1-MCP-Behandlungen und sogar eine CA-/ULO-Lagerung die Entwicklung von typischem Geschmack und Aroma bei Apfel beeinträchtigen können. Vermutlich liegt das in der deutlich reduzierten Ethylenbiosynthese begründet. Aus diesem Grund wird die vorliegende Studie mit Messungen der flüchtigen Aromastoffe sowie einer Bestimmung der Aromavorstufen (Fettsäuren) bei ‘Jonagold’ Äpfeln nach 1-MCP-Behandlung und Kühl-, CA- bzw. ULO-Lagerung fortgesetzt. Außerdem besteht weiterer Untersuchungsbedarf bezüglich der enzymatischen antioxidativen Kapazität, d. h. mögliche Veränderungen der Enzyme Superoxid-Dismutase (SOD), Catalase (CAT), und Peroxidase (POX) bei mit 1-MCP behandelten ‘Jonagold’ Äpfeln unter den oben genannten Versuchsbedingungen sollten untersucht werden. Da in der vorliegenden Studie weder die Gesamtphenole noch die gesamte nicht-enzymatische antioxidative Kapazität (VCEAC) von 1-MCP beeinflusst wurden, wäre es interessant zu sehen, ob die antioxidativ wirkenden Enzyme durch 1-MCP und/oder verschiedenen Lagerungsbedingungen beeinflusst werden. Die deutlich höhere Fruchtqualität sowie bessere Lagereigenschaften und Haltbarkeit der mit 1-MCP behandelten Äpfel könnten in höheren Gehalten an antioxidativ wirkenden Enzymen begründet liegen.

1.

General Introduction

Apples are by far the most common fruit crop consumed in Germany. In general, fruits are an important part in the human diet in that they provide many essential nutrients, such as vitamins, minerals, complex carbohydrates, dietary fibre and antioxidants (Lee et al., 1995; Salunkhe and Kadam, 1995). Regular consumption of fruit is associated with reduced risk of cancer, cardiovascular disease, stroke and other chronic diseases (Salunkhe and Kadam, 1995; Kader, 2002). The positive impact of fruit consumption on human health and welfare is mainly due to their antioxidant activity (Scalzo et al., 2005). However, purchase decision is mainly influenced by external appearance (size, shape, colour and freshness) of fruit. Hidden attributes such as minerals, vitamins and phenolic compounds also affect consumer perception and are increasingly considered by purchase decision. It is suggested that postharvest life based on flavour and nutritional quality is shorter than postharvest life based on firmness and appearance of most fresh fruit (Kader, 2003).

Fruit quality is affected by genetic make up and environmental, cultural and developmental preharvest factors. One of the most important factors determining at-harvest fruit quality and storability is the stage of maturity. Harvested apples are still living biological systems with an active metabolism and since they are removed from the supplies of water, photosynthates and minerals form the tree, they are depending on their stored water and carbohydrate reserves. Between harvest and consumption apples undergo many biochemical changes. Some of these changes are desirable for consumption, such as development of flavour, conversion of starch to sugars and reduction in organic acid contents, while others (mainly transpiration and respiration) may lead to inevitable postharvest losses (physical loss and loss of fruit quality) (Lee et al., 1995; Giovannoni, 2001; Wills et al., 2007). However, the plant hormone ethylene influences many of the ripening processes in apple fruits. Therefore, the main aim of postharvest technologies is to reduce metabolism, such as respiration, transpiration and ethylene production of harvested produce by means of low temperatures, high relative humidity and sup-

plementary controlled atmospheres (reduced oxygen and elevated carbon dioxide concentrations). However, senescence and deterioration of harvested fruit can not be stopped, but delayed and slowed down by appropriate postharvest management. As a rule, the fruit quality offered to the consumer is determined by the level of quality achieved at harvest and is only maintained, never improved by postharvest handling (Wertheim, 2005; Hewett, 2006). Fruit quality usually decreases during storage. It is the challenge of a good storage management to minimize that decline (Tromp, 2005). Responses of apple fruit to storage conditions (storage duration, temperature, relative humidity and atmosphere) are depending on cultivar, season, growing conditions (environmental and cultural factors) and maturity at harvest. The relationship between several preharvest factors and postharvest fruit quality is complex and influenced by many interactions (Shewfelt and Prussia, 1993). However, throughout storage fruit quality is generally preserved at a high level whereas conditions at several points throughout the distribution chain are not adequate for fresh commodities (de Jager, 1994; Paull, 1999; Johnston et al., 2002). It is critically important to maintain consistently high fruit quality throughout the marketing period to the final consumer.

1-Methylcyclopropene (1-MCP), an inhibitor of ethylene action (Sisler et al., 1996) is an effective tool for maintaining fruit quality during storage and post-storage handling. The reduced rate of ethylene production and respiration leads to an improved and extended storage life. However, 1-MCP is not recommended as an alternative to conventional storage technologies such as controlled atmosphere (CA) storage. Especially for long-term storage 1-MCP application can be used as an excellent supplement to CA-storage (Watkins and Nock, 2004).

1.1 Preharvest factors affecting fruit quality

Genetic factors. In general, the storage potential of harvested apple fruit is genetically determined (Johnson, 2000). The pre-defined characteristics of each cultivar influence postharvest behaviour and storability (Lee et al., 1995). New varieties are expected having a higher crop yield, better fruit quality and some of them are even resistant to various diseases and pests (Salunkhe and Kadam, 1995). However, since most pome fruit trees consist of a fruiting wood (scion) grafted onto a genetically different rootstock, genetic factors affecting the final composition and quality of fruit involve cultivar, rootstock and eventually in-

terstocks. Dwarfing rootstocks which lead to optimized productivity and fruit quality (Hewett, 2006) are commonly used in commercial apple production. Rootstocks can exert several effects on the storage behaviour (e.g. respiration rate and ethylene production) and factors of fruit quality such as soluble solids and acidity (Beverley et al., 1993). In addition to a general vigour control, adaptation to adverse soil and climatic conditions, water and nutrient uptake as well as translocation within the tree and the cropping behaviour in general are influenced by the rootstocks (Lal Kaushal and Sharma, 1995). However, the basic genetic background of apple fruit quality at harvest may be modified by environmental and cultural factors in the orchard.

Environmental factors. Depending on the stage of development temperature and light (intensity) are the most important environmental factors determining fruit growth and final fruit quality. Final fruit size is mainly controlled by temperature during the first weeks after bloom, i.e. during the period of cell division (Lurie, 2002; Tromp and Wertheim, 2005). It is described that cold spring temperatures lead to an extended period of cell division and presumably larger fruit sizes (Lurie, 2002). During the entire fruit growing season temperatures influence the uptake of water and nutrients by the trees (Kader, 2002). High temperatures close to harvest may accelerate maturation (Beverly et al., 1993) and lead to reduced accumulation of anthocyanins (Lurie, 2002). In contrast, it is known that cold nights and especially large differences between night and day temperatures in that period favour skin colouring. Appropriate light conditions are generally needed for sufficient production of photosynthates for both fruit and tree growth (Wünsche et al., 2005). However, excessive sunlight may result in sunburn or sunscald (Sams, 1999), whereas insufficient light leads to smaller fruit with unsatisfactory quality (Kays, 1999). Other factors such as available soil moisture, nutrient availability in addition to adverse weather effects such as frost, hail and wind also influence fruit development and final quality. In general, environmental factors are virtually unchangeable under field conditions (Beverly et al., 1993). Adaptation to given conditions requires an appropriate choice of suitable cultivar and rootstock as well as several cultural practices.

Cultural factors. Pruning and thinning are mainly used to determine the crop load and by that fruit size and quality. Pruning enhances the light penetration into the canopy (Beverly et al., 1993), whereas thinning increases the leaf to fruit ratio which leads to higher quality of

individual fruit and has an impact on tree physiology and return bloom as well (Tromp, 2005; Wünsche et al., 2005). Fruit growth is generally dependent on the photosynthetic capacity of the tree (Wertheim, 2005). Fruit from light cropping trees have a better flesh firmness, higher percentage of soluble solids and a larger fruit size when compared with high-cropping trees (Wünsche et al., 2005). Moreover, light-cropping advances fruit maturity. However, excessive thinning and pruning practices which would shift the balance between vegetative and generative development should be avoided (Tromp, 2005). Even moderate water-stress might increase quality parameters such as soluble solids, acidity and ascorbic acid concentration (Kader, 2002) without major negative effects on fruit size and yield. Nutrient deficiencies which may lead to reduced fruit quality and storability can be avoided by timely application of fertilizers. Calcium nutrition is of particular importance since deficiency causes numerous physiological disorders such as e.g. bitter-pit in apples. Plant growth regulators may be used to regulate growth and physiology of trees as well as development and uniformity of maturity of fruits (Beverly et al., 1993; Shewfelt and Prussia, 1993). Pests and diseases can be limited to acceptable levels by the use of several pesticides or alternative methods such as use of beneficial insects.

Harvest date. Maturity at harvest has a very important influence on storage life and the compositional quality of apple fruit. However, the 'optimum' harvest date is dependent on the cultivar, the planed use of the fruit, the given storage technology and the marketing strategy (Tromp, 2005; Wertheim, 2005). The date of apple harvest is always a compromise between storability and fruit quality (Figure 1.1). In early harvested apples quality is low but storability is high, whereas late picked apples have a high sensory quality but reduced storability (Tromp, 2005). In most apple varieties ethylene production increases markedly during the harvest period. The plant hormone ethylene influences many of the ripening processes and the increased production leads to improved quality (flavour, aroma) but decreased storability (softening).

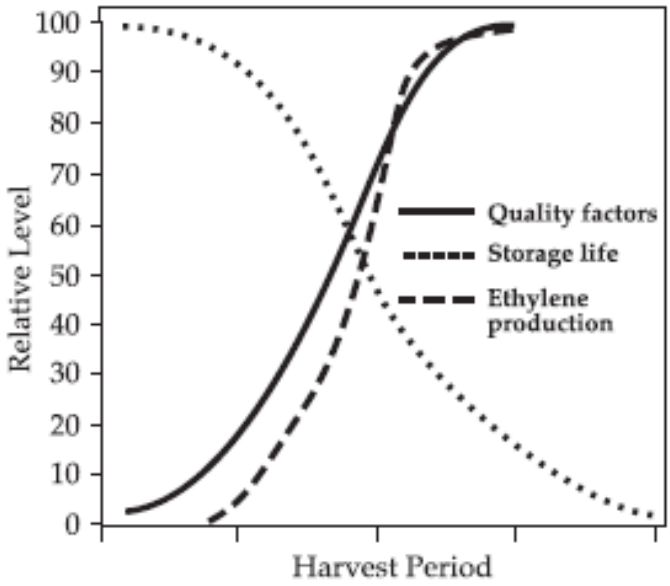


Figure 1.1: Compromise between increasing fruit quality and decreasing storability of apple fruit. Ethylene production increases markedly during the harvest period in most apple varieties (Watkins and Nock, 2000).

Harvest maturity indices (e.g. 'Streif-index') which use a combination of different ripening and quality characteristics such as flesh firmness, starch index and soluble solids concentration, can be calculated for determining the optimum harvest date with respect to cultivar, storage regime and duration (Wertheim, 2005; Hewett, 2006). Several cultivars such as 'Jonagold' and 'Elstar' have a wide within-tree maturity variability which is due to the extended period of flowering and fruit set as well as differences in fruit position within the canopy. In order to harvest all fruits at the right time, i.e. at the optimum stage of maturity, sequential picking is recommended (Kingston, 1992; de Jager, 1994). Harvesting of apple fruit is generally carried out by hand. Physical damage due to mechanical injuries such as bruising, surface abrasions and cuts (Lee and Kader, 2000) lead to a loss of quality and storability and should be minimized by proper harvest management. Moreover, postharvest fruit quality is influenced by harvesting method, transport and handling conditions, storage conditions and conditions in retail.

1.2 Fruit quality criteria at- and post-harvest

Development of fruit. The development of fruits can be divided into the three major physiological stages growth, maturation and senescence (Wills et al., 2007). However, the individual stages of development can overlap (Figure 1.2).

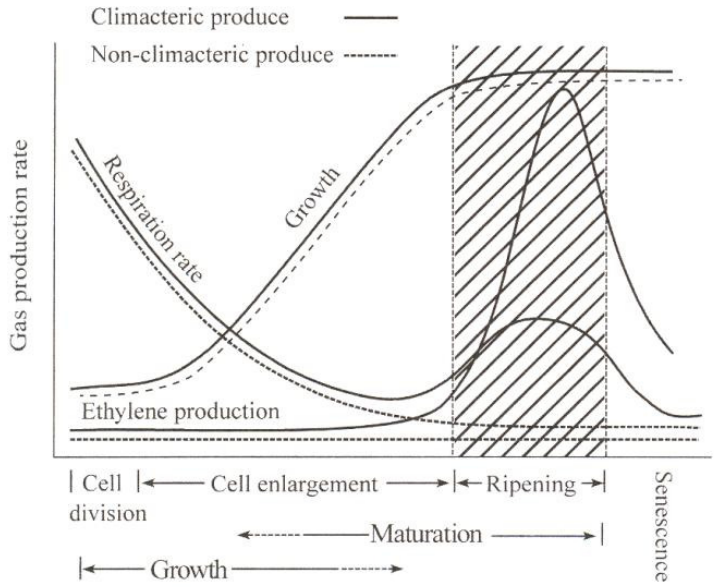


Figure 1.2: Growth, respiration and ethylene production patterns of climacteric and non-climacteric plant organs (Wills et al., 2007).

Growth, the first physiological stage, involves cell division and subsequent cell enlargement and leads to a generally irreversible physical increase of the developing plant or fruit. Maturation describes the time between the stages growth and senescence. During maturation the fruit develops from an immature stage to the attainment of maturity (Watada et al., 1984). Many physical and chemical changes occur during maturation. However, in the mature stage, the fruit is in most cases still uneatable but it has reached the ability to ripen (Tromp, 2005). Therefore, the stage of maturity at harvest of climacteric fruit is an important factor affecting the development of the final eating quality during ripening and postharvest handling (Shewfelt, 1993). The two stages growth and maturation are mainly completed while fruit is attached to the tree, whereas senescence may proceed on or off the plant (Wills et al., 2007).

Ripening is the process by which the physiologically mature but inedible fruit attains its characteristic appearance and/or food quality (Watada et al., 1984; Kader, 2002; Wills et al., 2007). The term is restricted to changes within the fruit and is considered to commence during the final stages of maturation until the first stages of senescence. Changes during ripening include loss of chlorophyll, synthesis of carotenoids and anthocyanins, loss of acidity, increase in sweetness, tissue softening and formation of flavour volatiles (Lee et al., 1995). Senescence, the final physiological stage, is characterized by degradative processes which lead to deterioration and subsequently death of the plant tissue (Watada et al., 1984). The process of senescence can not be stopped but delayed and slowed down with effective postharvest storage regimes and proper handling. Hence, storability of fruit might be prolonged with delayed senescence.

Fruit can be classified into two groups according to their pattern of respiration and ethylene production rates (Figure 1.2). Climacteric fruit such as e. g. apple, pear, banana, kiwifruit and apricot are distinguished from non-climacteric fruits (berry, cherry, citrus, grape, etc.) by the incidence of a so-called climacteric (Abeles et al., 1992), i.e. climacteric fruit exhibit a distinct upsurge in respiration rates and ethylene production rates during ripening (Abeles et al., 1992; Giovannoni, 2001; Wills et al., 2007). They generally reach fully ripe stage after the respiratory climacteric (Wills et al., 2007). While most climacteric fruit can be harvested mature and can ripen on or off the plant, non-climacteric fruit are not able to ripen after harvest (Kader, 2002). Since ethylene is not required for ripening of non-climacteric fruit, fruit produce very small quantities of ethylene (Abeles et al., 1992; Giovannoni, 2001). In contrast, ethylene is necessary for the completion of ripening in climacteric fruit. Exogenously applied ethylene leads to faster and more uniform ripening (Kader, 2002).

Parameters of maturity and fruit quality.

Flesh firmness. In general, flesh firmness decreases with successive harvests, i.e. with advanced maturity. Moreover, a distinct decline in flesh firmness is found during postharvest ripening depending on cultivar, storage regime (temperature, relative humidity, atmosphere) and duration. It is known that an inverse relationship between fruit size and flesh firmness exists (Kingston, 1992). Smaller fruit are generally firmer than larger fruit and this might be

due to having more cell wall material per unit volume (Johnston et al., 2002). Apple softening is largely due to disruption of the cementing material between cells (middle-lamella) which leads to a loss of cell to cell contact (Kingston, 1992; Johnston et al., 2002; Tromp, 2005). Softening, which occurs in almost all fruits during ripening is considered being an undesirable process in apples. Many reports describe firmness as a crucial factor for consumer acceptance of apple fruit (Daillant-Spinnler et al., 1996; Jaeger et al., 1998; Harker et al., 2002). Hence, firmness is very important from a commercial viewpoint, because softening limits the post-harvest life of fruits by enhancing physical damage during handling and increasing the fruit susceptibility to diseases (Brady, 1987). Therefore, flesh firmness is an indicator of maturity and also an important parameter for judging quality throughout the distribution chain (Tromp, 2005). Measurements of flesh firmness are easily done by using a penetrometer which records the force needed to insert a probe of known head diameter to a defined depth into the peeled fruit flesh.

Soluble solids. With advanced maturity and during postharvest apple ripening fruit generally become sweeter and more acceptable. The increase in sweetness is mainly due to the conversion of starch to sugars. Sugars are primarily found in the cell vacuole (Kader, 2002) and are usually a major component of soluble solids in the cell sap (Wills et al., 2007). Therefore, it is easier and generally accepted to measure the percentage of soluble solids (% Brix) by using a refractometer, rather than directly measuring the sugar content by chemical means (Kingston, 1992; Wills et al., 2007).

Titratable acidity. The predominant acid in apple fruit is malic acid. Organic acids generally decline during maturation and apple ripening. Since organic acids can be considered as a source of energy (Wills et al., 2007), their gradual decline during ripening might be explained by their utilization during postharvest respiration (Kader, 2002). The degree of decline in organic acids is dependent on cultivar, preharvest environmental and cultural factors as well as on postharvest storage- and handling conditions. Since acidity in interaction with sweetness mainly contributes to fruit flavour, it is considered to be an important quality factor. Acidity is usually determined by titration.

Starch content. The conversion of starch into sugars in maturing and ripening apples generally starts in the core area and gradually progresses outwards (Kingston, 1992; Tromp, 2005). The degree of starch conversion can be subjectively estimated by comparing iodine

stained fruit surfaces with pictures on a chart. Most cultivars have a characteristic pattern of disappearance, therefore starch charts might be cultivar specific and the starch index value at the 'optimum' harvest date can vary between cultivars (Kingston, 1992). Although starch pattern index is a good tool for determination of maturity, it is described that starch disappearance is not closely related to sensory changes (Wills et al. 2007).

Skin colour. Colour changes are the most obvious signal for fruit ripening. During ripening apple fruit generally show a rapid loss of green colour, which results from the degradation of chlorophyll structures (Tromp, 2005; Wills et al., 2007). The yellow to red colour of apple fruit, which is due to anthocyanins and carotenoids in the peel, becomes visible with chlorophyll decline (Kingston, 1992; Tromp, 2005; Wills et al., 2007). However, synthesis of carotenoids and development of anthocyanins might also occur *de novo* during ripening (Knee, 1988; McLean et al. 2006). Since the degradation of chlorophyll is a good indicator of maturity, background colour (BGC) is visually assessed by comparison with colour charts or objectively measured by using 'chromameters' (Tromp, 2005). The degree of red colour can not be used as a good indicator of maturity, since colour development is influenced by many preharvest factors.

Respiration rate and rate of ethylene production. Apple fruit exhibit a distinct upsurge in respiration rates and ethylene production rates during maturation and ripening (Abeles et al., 1992; Giovannoni, 2001; Wills et al., 2007). The extent of the rise in ethylene production is much more pronounced than the respiratory peak (Figure 1.2), however, both respiration rate and ethylene production rate (magnitude and timing) can vary greatly among varieties and seasons (Tromp, 2005). In general, early season varieties have high respiration and ethylene production rates and subsequently show short storability. Late season varieties with low ethylene rates and low respiration ripen slowly and can be stored long periods (Wertheim, 2005). Although the physiological stage of apple fruit can be accurately determined by measurements of respiration and/or ethylene production, these determinations are generally not used in praxis for harvest decisions.

Aroma volatiles. The development of volatiles (low-molecular weight compounds) during ripening is generally responsible for the characteristic aroma and optimal sensory quality of fruits (Kader, 2002). The volatile compounds in apple fruit are mainly esters, alcohols, acids, ketones and aldehydes (Kader, 2002; Tromp, 2005; Wills et al., 2007). Although a large

number of volatiles has been identified in ripe fruit, the characteristic aroma is mostly due to the presence of one or two so-called 'character impact' compounds, i.e. ethyl 2-methylbutyrate in apples (Shewfelt, 1993; Tromp, 2005; Wills et al., 2007). However, the major volatile in apple fruit is ethylene though it does not contribute to the typical fruit aroma (Kader, 2002).

Nutritional value. Nutritional quality and degree of healthful constituents of fruits are related to contents of vitamins, minerals, dietary fibre and phytochemicals with antioxidant properties, such as phenolic compounds (Kader, 2002; Awad and de Jager, 2003; Sánchez-Moreno et al., 2006). Nutritional value of apple fruit is generally not a major criterion affecting purchase decision for most consumer. The nutrient composition varies depending on cultivar, preharvest environmental and cultural factors, stage of maturity at harvest and postharvest regime and duration. However, consumers are increasingly concerned about nutritional quality and health-protecting compounds in foods (Larrigaudière et al., 2004; Vilaplana et al., 2006; Kevers et al., 2007).

1.3 Physiological changes during fruit ripening

Respiration metabolism in ripening apples. Since harvested fruit are still living biological systems, the respiration rate is an important indicator of metabolic activity (Lee et al., 1995). Fruit respiration is mainly needed to supply energy for catabolic processes during ripening (Abeles et al., 1992) and to maintain basic metabolism. The remainder energy is lost as heat. Climacteric fruit such as apples is generally characterised by a typical respiratory peak during ripening. Depending on the availability of oxygen, respiration can occur under aerobic or anaerobic conditions (Lee et al., 1995). In postharvest fruit aerobic respiration, which mainly consists of the processes glycolysis, tricarboxylic acid (TCA) cycle (which involves CO_2 release) and the electron transport chain (which involves O_2 uptake) (Lee et al., 1995; Wills et al., 2007) is desirable since anaerobic respiration might produce off-flavours (Saltveit, 2003; Wills et al., 2007) and might lead to tissue damage (Lee et al., 1995). The main substrates involved in aerobic respiration of harvested fruit are sugars and acids. The subsequent depletion in these compounds leads to a loss of fruit taste and quality, storability and shelf-life (de Baerdemaeker et al., 1994; Wertheim, 2005). Therefore, the rate of respiration is

generally inversely related to storability of fruit (Lee et al., 1995; Wertheim, 2005). Apples for long term storage should be harvested pre-climacteric when respiration rates are still low (Wertheim, 2005). However, the increase in respiration during ripening of climacteric fruit seems to be a consequence of the increase in ethylene production (Brady, 1987; Tromp, 2005).

Biosynthesis of ethylene in ripening apples. The plant hormone ethylene regulates many physiological aspects during plant growth, development and fruit ripening (Abeles et al., 1992; Saltveit, 1999; Blankenship, 2001). Sensitivity of fruit to ethylene varies with stage of development and maturity, cultivar and postharvest storage conditions (temperature and storage atmosphere) (Lee et al., 1995).

McMurchie et al. (1972) described a generally accepted model of ethylene biosynthesis by differentiating between system I and system II ethylene production. System I ethylene is found in all vegetative tissues, non-climacteric fruit and immature (i.e. pre-climacteric) climacteric fruits until ripening commences (Brady, 1987; Lee et al., 1995). The rate of system I ethylene is generally low as the tissue sensitivity to ethylene is also low. However, climacteric fruit become more sensitive to ethylene during development and maturation (Abeles et al., 1992; Watkins, 2002). After a special ethylene level is reached in fruit during maturation and the fruit reached the competency to ripen (McMurchie et al., 1972; Bufler, 1986), there is a shift to system II ethylene production. System II ethylene production is responsible for the rise in ethylene at the beginning of ripening in climacteric fruit (Golding et al., 1998).

It has been shown that ethylene regulates its own biosynthesis (Yang and Hoffman, 1984). System I ethylene production is under negative feedback regulation and ethylene inhibits its own synthesis (Yang and Hoffman, 1984; Nakatsuka et al., 1998; de Wild et al., 2003). However, system II ethylene production during the climacteric stage of ripening is under positive feedback regulation, i.e. system II ethylene production is an autocatalytic process (Tromp, 2005). Continuous perception of ethylene is needed to initiate the autocatalytic system II ethylene production and to commence the ripening process (Bufler, 1984; Sisler et al., 1996). The increased autocatalytic ethylene production (system II) and the accompanied rise in respiration, is characteristic of ripening climacteric fruits (Burg and Burg, 1967; Zarembinski and Theologis, 1994; Nakatsuka et al., 1998). In contrast, non-climacteric fruits do not show autocatalytic ethylene biosynthesis (Tromp, 2005).

The pathway of ethylene biosynthesis was elucidated and described in detail by Adams and Yang (1979) and Yang and Hoffman (1984) (Figure 1.3). The amino acid methionine is the overall precursor of ethylene. Methionine and ATP are converted by the enzyme S-adenosyl-methionine synthase (SAM synthase) to form the intermediate S-adenosylmethionine (SAM) (Yang and Hoffman, 1984; Abeles et al., 1992; Zarembinski and Theologis, 1994). The enzymes involved in the subsequent pathway of ethylene synthesis are 1-amino-cyclopropane-1-carboxylic acid (ACC) synthase (ACC-S) and ACC oxidase (ACC-O). The conversion of SAM to ACC by the enzyme ACC-S is the rate-limiting step in the ethylene biosynthesis (Adams and Yang, 1979; Yang, 1980). The conversion of ACC to ethylene requires oxygen (Adams and Yang, 1979; Zarembinski and Theologis, 1994) and Dong et al. (1992) describe that ACC-O is activated by CO_2, requiring both ascorbate and Fe^{2+} as co-factors. The auto-catalytic ethylene production (system II) is due to increased abundance of ACC-S and ACC-O (Abeles et al., 1992; Rupasinghe et al., 2000).

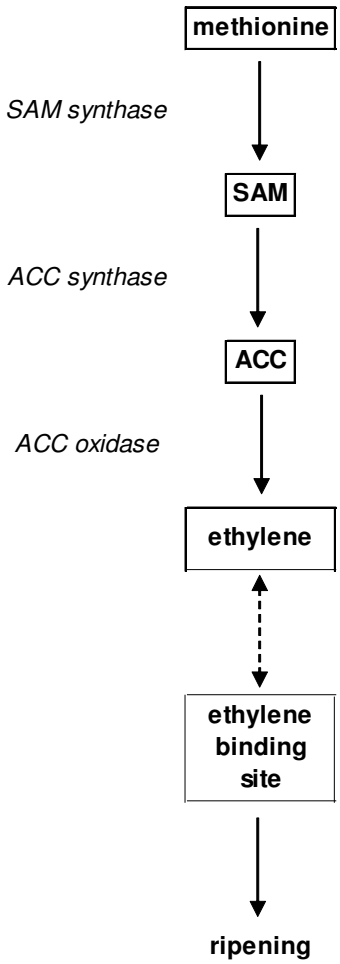


Figure 1.3: Pathway of ethylene biosynthesis and action (adapted and simplified from Adams and Yang, 1979; Yang and Hoffman, 1984; Wills et al., 2007).

To induce the many biochemical changes associated with ripening (colour change from green to yellow, aroma development, softening, increased respiration etc.) and to stimulate further ethylene biosynthesis (positive feedback regulation) (Gorny and Kader, 1996) ethylene needs to bind to specific ethylene binding sites (receptors) (Watkins and Nock, 2000; Wills et al., 2007) (Figure 1.3 and 1.4). After switching the receptors off, certain signal transduction pathways are activated (Tromp, 2005) and the different ripening processes are initiated. However, not all parameters of fruit ripening are controlled by ethylene (ethylene-dependent). The binding of ethylene to the receptors is reversible (Lieberman, 1979; Wills et al., 2007).

Several different postharvest strategies (low temperature, controlled atmospheres, 1-MCP treatment etc.) are known to control ethylene production and/or perception and by that retarding the ripening process of climacteric fruit (Lee et al., 1995; Watkins, 2002).

1.4 Control factors for maintaining postharvest fruit quality

Temperature management. It is generally accepted and often described that ethylene plays a critical role in apple ripening (Abeles et al., 1992; Gorny and Kader, 1996; Mathooko, 1996). Therefore, suppression of ethylene biosynthesis and action is the main concern of postharvest strategies. The most simple and effective means of controlling ethylene production and reducing the overall metabolism is achieved by storage at low temperatures (Lurie, 2002; Watkins, 2002; Kader, 2003). Low temperatures considerably reduce respiration (Lee et al., 1995; Wills et al., 2007) and it is described that both enzymes involved in the ethylene biosynthetic pathway, ACC-S and ACC-O are sensitive to low temperatures (Larrigaudière et al., 1997). Moreover, tissue sensitivity and the rate of ethylene biosynthesis are dependent on temperature (Lee et al., 1995; Wills et al., 2007). At lower temperatures fruit are less sensitive to ethylene and at a given ethylene concentration it takes longer until response, i.e. ripening is initiated. Moreover, it has been described that loss of vitamin C is generally reduced at lower temperatures (Paull, 1999) and low temperatures also reduce fungal infection and decay during apple storage (Lee et al., 1995; Lurie, 2002). The fact that respiration generally releases heat is another important reason for storage at low temperatures (Lurie, 2002; Wills et al., 2007). In general, low temperatures significantly delay the initiation of ripening and clearly decrease all metabolic activities and biochemical reactions during ripening in harvested fruit

(Kingston, 1992; Lee et al., 1995). Apples are ideally stored at temperatures just above the freezing point, depending on the variety and the sensitivity to 'chilling injury' (Johnston et al., 2002; Wertheim, 2005; Wills et al., 2007). Too high temperatures generally result in faster ripening and in turn in shorter storage life whereas too low temperatures might induce physiological disorders and 'chilling injury' (Lee et al., 1995). It is important to rapidly cool apples after harvest. The quicker the optimum storage temperature is achieved, the better the maintenance of fruit quality and the longer the storage life (Paull, 1999; Wertheim, 2005; Wills et al., 2007).

Therefore, an appropriate temperature management after harvest is the most important factor in maintaining fruit quality, delaying ripening of climacteric fruit and extending and improving storage- and shelf-life (Lee et al., 1995; Lee and Kader, 2000; Lurie, 2002). Other postharvest technologies such as controlled atmosphere (CA) storage or 1-MCP application can be used as supplementary improvements, not as a substitute (Kader, 2003; Watkins and Nock, 2004). Moreover, to maximize longevity and to maintain fruit quality until consumption, low temperatures should also be maintained through the entire postharvest handling chain, i.e. during grading, packing and transport until retail display (Johnston et al., 2002; Kader, 2003; Wertheim, 2005; Wills et al., 2007).

Relative humidity. Since harvested fruit are still living biological systems with an active metabolism they respire. During respiration fruit release water from the intracellular space to the environment which is a normal and necessary process (Lee et al., 1995; Wertheim, 2005). In this case, water loss is always dependent on the rate of respiration. Respiration is in turn affected by temperature and any method which reduces respiration might lead to decreased water loss (Lee et al., 1995). However, harvested fruit mainly loose intercellular water by transpiration, i.e. water loss controlled by the water vapour difference between fruit and surrounding air (Lee et al., 1995; Lurie, 2002; Wertheim, 2005). The rate of fruit transpiration is influenced by the surface area to volume ratio and by the structure and composition of surface tissues (Paull, 1999; Wills et al., 2007). A waxy cuticle on the surface of some apple varieties is advantageous in restricting water loss by transpiration (Wills et al., 2007). Wrapping fruit boxes with plastic sheets is a further simple method to increase relative humidity of the air surrounding the fruit and by that decreasing water and weight loss of the

fruit. In general, the weight loss during storage of apples is primarily due to water loss rather than to loss of respirable substrates such as sugars and acids (Johnson, 2000).

Water loss is a significant cause of fruit deterioration during storage (Nunes et al., 1998) and, moreover, a quantitative loss in fresh weight results in financial loss. To avoid shrivelling and by that changes in appearance, texture and flavour (Lee et al., 1995; Wertheim, 2005) during postharvest storage, it is important to maintain a high relative humidity in combination with reduced temperatures (Ezell and Wilcox, 1959; Nunes et al., 1998; Lurie 2002). Apples are ideally stored at relative humidity between 90 – 95 % in combination with temperatures just above the freezing point as previously described. However, condensation of water on the surface of apples needs to be avoided because this might lead to increased fungal and bacterial growth (Wills et al., 2007).

Controlled atmosphere storage. It is now common commercial practice to store apples at controlled atmospheres with low O_2- and/or elevated CO_2-concentrations. The controlled atmosphere is usually combined with reduced temperature which is, as previously described, the most effective tool in postharvest storage techniques for maintaining the quality of apples and by that increasing storability (Lurie, 2002; Wills et al., 2007) and extending marketing season. Controlled atmosphere storage considerably increases the positive effects brought about by low temperatures (Wertheim, 2005). The beneficial impact of these practices on quality maintenance are due to reduced rates of fruit respiration and suppression of fruit ethylene production and action (Mir and Beaudry, 2002; Watkins, 2002; Mattheis, 2004).

Since O_2 is a critical substrate in the respiratory process, respiration can be reduced by restricted availability of O_2. Elevated concentrations of CO_2 lead to a lesser extent to reduced respiration rates (Mir and Beaudry, 2002) and can be seen as an additive effect to low O_2 effects. Moreover, during normal aerobic respiration reactive oxygen species (ROS) are continuously formed in excess (Masia, 2003; Wood et al., 2006) which can lead to oxidative stress and accelerate ripening and senescence (Bartosz, 1997). Therefore, reduced respiration rates produce less ROS and cause a delay in ripening which consequently results in a better maintenance of fruit quality and storability. However, CA-storage might also be considered as a stress factor and inappropriate conditions lead to damage. Adequate quantities of enzymatic and non-enzymatic antioxidants are needed to counteract any kind of stress (Bartosz, 1997; Wertheim, 2005).

Suppression of O_2-concentration in the atmosphere is limited. Excessively reduced O_2–availability might lead to a shortage of energy needed for maintenance. Therefore aroma volatile development might be reduced in long-term storage at controlled atmospheres (Beaudry, 1999; Wertheim, 2005). Exceedingly low respiration rates could not provide adequate levels of energy, i.e. adenosine triphosphate (ATP), which is needed for the synthesis of fatty acids (Mir and Beaudry, 2002; Wertheim, 2005). Fatty acids are the predominant precursors of aroma volatiles (Saquet et al., 2003). Moreover, anaerobic respiration induces fermentation (Wertheim, 2005; Wills et al., 2007). Recommended O_2 levels for many apple varieties are ≤ 3 % and storage at O_2 levels ≤ 1.5 % is called ultra low oxygen (ULO) CA-storage (Wertheim, 2005). In the same way, excessive high CO_2 levels during storage should be avoided, because it might also result in anaerobic respiration, which leads to fermentation, the development of off-flavours and the induction of tissue injuries (Lee et al., 1995; Beaudry, 1999; Wertheim, 2005). Apples are normally stored at CO_2 concentrations up to 3 %, however, CO_2-sensitive varieties such as 'Braeburn', 'Granny Smith' and 'Fuji' are stored at CO_2-concentrations below 1 %.

Besides that, controlled atmosphere storage by means of low O_2- and elevated CO_2-concentrations has an impact on ethylene biosynthesis and action (Mir and Beaudry, 2002; Watkins, 2002). Mir and Beaudry (2002) and Tromp (2005) describe that for climacteric fruit in which ripening is initiated by ethylene, the impact of controlled atmosphere storage on suppression of ethylene synthesis and action is more important than the reduced respiration brought about by low O_2 concentrations.

Burg and Burg (1967) suggested that low O_2-concentrations inhibit ethylene biosynthesis by preventing the binding of ethylene (system I) to the receptors responsible for the initiation of autocatalytic ethylene production (system II). Hence low O_2-levels inhibit the positive feedback regulation of ethylene biosynthesis (Gorny and Kader, 1996). Moreover, the conversion of ACC to ethylene by the enzyme ACC-O requires O_2 (Adams and Yang, 1979; Abeles et al., 1992; Zarembinski and Theologis, 1994) and it is described that low O_2-concentrations directly reduce ethylene biosynthesis (Tromp, 2005; Wertheim, 2005) by reduction of enzyme activity (Gorny and Kader, 1996).

Previously, Burg and Burg (1967) proposed that inhibition of ethylene action by elevated CO_2 concentrations is due to competition with ethylene for active receptor sites. Moreover, it is

described that CO_2-enriched atmospheres reduce ethylene production by inhibiting synthesis and action of ACC-S and ACC-O (Li et al., 1983; Bufler, 1984; Gorny and Kader, 1997). However, in most cases inhibition of ACC-S activity is the major site at which elevated CO_2 atmospheres inhibit ethylene biosynthesis (Gorny and Kader, 1996, 1997; Mathooko, 1996).

In general, the effect of CA-storage on maintenance of fruit quality and storability is dependent on cultivar, stage of maturity, the concentrations of O_2 and CO_2, the temperature and the duration of storage (Jobling and McGlasson, 1995; Lee et al., 1995). Once autocatalytic ethylene biosynthesis (system II) starts, the effectiveness of CA-storage is reduced and ethylene biosynthesis can not be diminished to preclimacteric production levels (Jobling and McGlasson, 1995; Gorny and Kader, 1997).

However, since no postharvest technology has the ability to improve produce quality, initial fruit quality at harvest and all conditions at- and post-harvest need to be optimal.

1.5 Use of 1-MCP and effects on ripening of apple

1-Methylcyclopropene (1-MCP) is thought to act as a competitive substance to ethylene, occupying the ethylene receptor site so that ethylene can not bind to trigger its action, i.e. the autocatalytic ethylene production (system II ethylene) and subsequently the initiation of ripening is prevented (Figure 1.4) (Watkins and Nock, 2000; Agrofresh, 2003; Blankenship and Dole, 2003). Because 1-MCP protects apples from both endogenous and exogenous ethylene (Blankenship and Dole, 2003), it seems to be a promising tool in postharvest technology (Watkins, 2006). In general, 1-MCP is able to counteract ripening effects triggered by ethylene during and after storage by blocking its action in fruit rather than inhibiting its production. However, once ripening commenced and autocatalytic ethylene biosynthesis started, 1-MCP can not stop the ripening process.

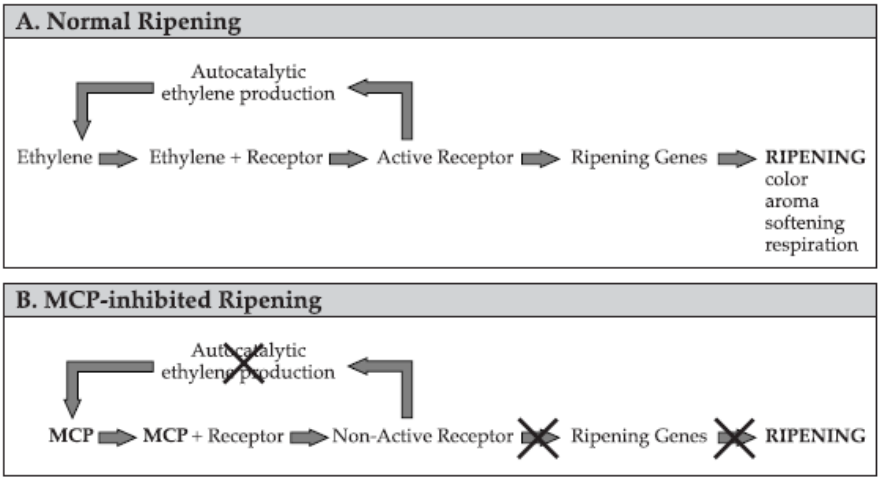


Figure 1.4: Comparison of normal ripening processes and effects of fruit treated with 1-MCP (Watkins and Nock, 2000).

1-MCP, a synthetic unsaturated cyclic olefin, which is structurally related to ethylene (C_4H_6 vs. C_2H_4) is considered a safe product for farmers, workers in the packhouse, consumers and the environment (Blankenship, 2001; Regiroli, 2004; Watkins, 2006). It is sold as a vapour release formulation under the trade name SmartFresh™ (Agrofresh, Inc.). 1-MCP has a non-toxic mode of action and is usually applied one-time following harvest at a very low dose level that leaves no detectable residue in treated fruit (Blankenship, 2001; Agrofresh, 2003). Application of 1-MCP requires gas tight coolroom facilities (Bates and Warner, 2001).

1-MCP reduces respiration rates (Fan et al., 1999; Fan and Mattheis, 1999) and clearly inhibits or reduces and delays ethylene production in different apple varieties (Fan et al., 1999; Fan and Mattheis, 1999; Rupasinghe et al., 2000). Therefore, application of 1-MCP leads to a better retention of apple fruit quality during storage (Fan and Mattheis, 1999; Watkins et al., 2000; DeEll et al., 2002) and also post-storage (Watkins et al., 2000; Watkins, 2006). Consequently, storage- and shelf-life of climacteric fruit can be significantly increased. Firmness is generally best maintained in apple fruit treated with 1-MCP (Fan et al., 1999; Watkins et al., 2000; Tatsuki et al., 2007). Effects of 1-MCP on other quality parameters such as soluble solids concentration, titratable acidity and retention of green background colour are not consistently described in the literature. 1-MCP can, however, impair the development of typical aroma volatiles in apples which is due to the suppression of the climacteric, i.e. the inhibition of the upsurge in respiration and the rise in autocatalytic ethylene production (Golding et al.,

1998; Fan and Mattheis, 1999; Watkins and Nock, 2000). The inconsistent effect of 1-MCP on several quality parameters is likely due to cultivar specific responses to 1-MCP (Watkins et al., 2000; DeEll, 2002; Watkins, 2006) and that not all quality parameters are ethylene-dependent. The efficacy of 1-MCP refers just to retention of ethylene-dependent quality and ripening parameters (De Castro et al., 2003; Saftner et al., 2003).

Moreover, it is described that 1-MCP delayed ripening more than CA- or even ULO-storage (Mir et al., 2001; Saftner et al., 2003). A combination of CA/ULO-storage and 1-MCP application seems to be more effective in postponing the climacteric than either alone. Both technologies may complement one another, but 1-MCP can not replace long-term CA/ULO-storage (Watkins and Nock, 2004).

In contrast to ethylene, the binding of 1-MCP is irreversible to the receptors present at the time of treatment (Sisler et al., 1996; Blankenship and Dole, 2003). It has much higher affinity to receptors (Blankenship and Dole, 2003) and binds ethylene receptor sites more strongly than ethylene (Tatsuki et al., 2007). However, the inhibition of ethylene action may be overcome and ethylene binds to receptors that were produced after 1-MCP application (Watkins et al., 2000; Tatsuki et al., 2007). The gradual production of new receptors as well as the affinity of 1-MCP might be dependent on the ethylene production rate at the time of and after 1-MCP treatment (Tatsuki et al., 2007). Moreover, the efficacy of 1-MCP is depending on various factors such as treatment temperature (Mir et al., 2001; DeEll et al., 2002), storage atmosphere and duration (DeEll et al., 2002; Watkins et al., 2002; Johnson, 2003), cultivar (Watkins et al., 2000; DeEll et al., 2002; Blankenship and Dole, 2003), stage of maturity (Watkins et al., 2000) and the time between harvest and 1-MCP treatment (Blankenship and Dole, 2003; Tatsuki et al., 2007). The longer the time from harvest to 1-MCP treatment, the less the effect of 1-MCP on retention of quality parameters (Tatsuki et al., 2007). Once autocatalytic ethylene biosynthesis begun it can neither be controlled by reduced O_2- and elevated CO_2-concentrations (Gorny and Kader, 1997), nor by 1-MCP application to reduce ethylene production rates to preclimacteric levels (Golding et al., 1998; Bates and Warner, 2001; Watkins and Nock, 2004).

1.6 Research objectives

'Jonagold' apple fruit (*Malus domestica* Borkh.) were picked at commercial maturity in 2004, 2005 and 2006. Fruit were treated with 1-MCP on the day of harvest in 2004 (0 days after harvest, 0 DAH) and 7 DAH in 2005 and 2006 and stored the following day either in cold storage, CA- (0.8 % CO_2, 3 % O_2) or ULO-storage (3 % CO_2, 1 % O_2). After 2, 4 and 6 months in 2004/05, 3, 6 and 9 months in 2005/06 and 3 and 5 months in 2006/07 fruit samples from each storage atmosphere ± 1-MCP were removed. Fruit quality parameters were assessed after harvest, commencement of storage and after each sample removal in 2004/05, 2005/06 and 2006/07 following 10 days shelf-life at 20°C. Consumer preference mapping was performed after 3 and 5 months of cold- and ULO-storage in 2006/07. Shelf-life respiration and ethylene production was measured after harvest, commencement of storage and after each sample removal in 2004/05 and 2005/06, respectively. ATP and ADP concentration was additionally determined in 2005/06. In 2005/06 ascorbic acid concentration, phenolic compounds and total non-enzymatic antioxidant capacity were examined following 10 days shelf-life after harvest, commencement of storage and after each sample removal.

The research objectives were:

- To determine the impact of 1-MCP treatment, storage condition and –duration on maintenance of commonly measured quality parameters of 'Jonagold' apple fruit. Particular focus was given to sensory evaluation of 1-MCP treated 'Jonagold' apples by consumer preference mapping. Which quality parameters are driving consumer liking and preference? Is there a correlation between instrumental values of common quality parameters of apples and corresponding consumer scores?

- To examine the effect of 1-MCP treatment, storage condition and –duration on ethylene production and shelf-life respiration rate as well as ATP concentration of 'Jonagold' apples. Are there differences in the efficacy of 1-MCP on climacteric characteristics due to time between harvest and 1-MCP treatment, treatment temperature, commencement of storage, storage condition and –duration?

- To study the influence of 1-MCP treatment, storage condition and –duration on ascorbic acid concentration, phenolic compounds and total non-enzymatic antioxidant capacity. Is the nutritional and health-protecting value of apple fruit affected by different storage conditions? Does 1-MCP influence nutritional values of apple fruit following storage and shelf-life?

The working hypotheses of this study were:

- Controlled atmosphere storage is commercial practice with significant effects on reduction of climacteric characteristics, such as fruit respiration and ethylene production and action and in turn on maintenance of postharvest fruit quality. 1-MCP is an effective inhibitor of ethylene synthesis and action in climacteric fruit. It is suggested that the combined effect of CA-storage and 1-MCP on reduction of climacteric characteristics and maintenance of fruit quality is stronger than either factor alone.

- One of the main factors influencing apple purchase decision is flesh firmness. Since flesh firmness is consistently described being best maintained in apple fruit treated with 1-MCP, it seems likely that consumer would prefer 1-MCP treated fruit. However, not all quality factors are ethylene-dependent and will be affected by 1-MCP to the same extent. It is suggested that consumer would give their preference to 1-MCP treated fruit from ULO-storage rather than to 1-MCP treated fruit from cold storage or even untreated fruit.

- It is described that 1-MCP likely competes with ethylene for binding sites. The longer the time between harvest and 1-MCP treatment, the more ethylene may already be produced and bound to receptor sites. Since apples were treated 0 DAH in 2004 and 7 DAH in 2005 and 2006, it is suggested that the efficacy of 1-MCP on reduction of climacteric characteristics and on maintenance of fruit quality might be reduced in the latter cases.

- Several articles in the literature report little to no effects of CA-storage on phenolic compounds and total antioxidant capacity of climacteric fruit. The nutritional value of apple fruit seems to be not ethylene-dependent. Phenolic compounds mainly contribute to the total antioxidant capacity of apple fruit, whereas ascorbic acid concentration of apple con-

tributes to a small extent to the total antioxidant capacity. Since the overall synthesis and accumulation of phenolic compounds is completed in the early stages of fruit development, it seems likely that the overall nutritional value of apple fruit is not affected by postharvest 1-MCP application.

1.7 References

Abeles, F.B., P.W. Morgan and M.E. Saltveit. (1992) Ethylene in plant biology. 2nd Edition, Academic Press, Inc.

Adams, D.O. and S.F. Yang. (1979) Ethylene biosynthesis: Identification of 1-amino-cyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. *Proc. Natl. Acad. Sci. USA*, 76(1), 170-174.

AgroFresh Inc., a Rohm and Haas Company (July 2003) *SmartFresh™ The Smart Choice for Apples. A Breakthrough in Fruit Storage Technology for Maintaining "fresh-picked" Apple Quality.* Apple Tech. Bul.

Awad, M.A. and A. de Jager. (2003) Influences of air and controlled atmosphere storage on the concentration of potentially healthful phenolics in apples and other fruits. *Postharvest Biol. Technol.* 27, 53-58.

Bartosz, G. (1997) Oxidative stress in plants. *Acta Physiol. Plant.* 19(1), 47-64.

Bates, B.R. and H. Warner. (2001) 1-MCP and fruit quality. *Perish. Handl. Quart.* 108, 10-12.

Beaudry, R.M. (1999) Effect of O_2 and CO_2 partial pressure on selected phenomena affecting fruit and vegetable quality. *Postharvest Biol. Technol.* 15, 293-303.

Beverly, R.B., J.G. Latimer and D.A. Smittle. (1993) Preharvest physiological and cultural effects on postharvest quality. In: Shewfelt, R.L. and S.E. Prussia (Eds.), *Postharvest handling: a systems approach.* Academic Press, Inc., 73-98.

Blankenship, S. (2001) Ethylene effects and the benefits of 1-MCP. *Perish. Handl. Quart.*, 108, 2-4.

Blankenship, S.M. and J.M. Dole. (2003) 1-Methylcyclopropene: a review. *Postharvest Biol. Technol.* 28, 1-25.

Brady, C.J. (1987) Fruit ripening. *Annu. Rev. Plant. Physiol.* 38, 155-178.

Bufler, G. (1984) Ethylene-enhanced 1-aminocyclopropane-1-carboxylic acid synthase activity in ripening. *Plant Physiol.* 75, 192-195.

Bufler, G. (1986) Ethylene-promoted conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene in peel of apple at various stages of fruit development. *Plant Physiol.* 80, 539-543.

Burg, S.P. and E.A. Burg. (1967) Molecular requirements for the biological activity of ethylene. *Plant Physiol.* 42, 144-152.

Daillant-Spinnler, B., H.J.H. MacFie, P.K. Beyts and D. Hedderley. (1996) Relationships between perceived sensory properties and major preference directions of 12 varieties of apples from the southern hemisphere. *Food Qual. Pref.* 7(2), 113-126.

De Baerdemaeker, J., E. Verstreken and X. Vanderwalle. (1994) Quality sensing and maintenance in post harvest and storage systems. *The post-harvest treatment of fruit and vegetables – current status and future prospects*; Proceedings of the sixth international symposium of the European concerted action program COST 94, 19-22 Oct. 1994, Oosterbeek, The Netherlands, 447-456.

De Castro, E., W.V. Biasi and E. J. Mitcham. (2007) Quality of Pink Lady apples in relation to maturity at harvest, prestorage treatments and controlled atmosphere during storage. *HortScience* 42(3), 605-610.

De Jager, A. (1994) Review of the workshop on determination and prediction of optimum harvest date of apples and pears. *The post-harvest treatment of fruit and vegetables – current status and future prospects*; Proceedings of the sixth international symposium of the European concerted action program COST 94, 19-22 Oct. 1994, Oosterbeek, The Netherlands, 267-279.

De Wild, H.P.J., E.C. Otma and H.W. Peppelenbos. (2003) Carbon dioxide action on ethylene biosynthesis of preclimacteric and climacteric pear fruit. *J. Exp. Bot.*, 54, 387, 1537-1544.

DeEll, J.R., D.P. Murr, M.D. Porteous and H.P.V. Rupasinghe. (2002) Influence of temperature and duration of 1-Methylcyclopropene (1-MCP) treatment on apple quality. *Postharvest Biol. Technol.* 24, 349-353.

Dong, J.G., J.C. Fernández-Maculet and S.F. Yang. (1992) Purification and characterization of 1-aminoyclopropane-1-carboxylate oxidase from apple fruit. *Proc. Natl. Acad. Sci. USA,* 89, 9789-9793.

Ezell, B.D. and M.S. Wilcox. (1959) Loss of vitamin C in fresh vegetables as related to wilting and temperature. *Agric. Food Chem.* 7(7), 507-509.

Fan, X. and J.P. Mattheis. (1999) Development of apple superficial scald, soft scald, core flush, and greasiness is reduced by MCP. *J. Agric. Food Chem.* 47, 3063-3068.

Fan, X., S.M. Blankenship and J.P. Mattheis. (1999) 1-Methylcyclopropene inhibits apple ripening. *J. Amer. Soc. Hort. Sci.* 124(6), 690-695.

Giovannoni, J. (2001) Molecular biology of fruit maturation and ripening. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52, 725-749.

Golding, J.B., D. Shearer, S.G. Wyllie and W.B. McGlasson. (1998) Application of 1-MCP and propylene to identify ethylene-dependent ripening processes in mature banana fruit. *Postharvest Biol. Technol.* 14, 87-98.

Gorny, J.R. and A.A. Kader. (1996) Controlled-atmosphere suppression of ACC synthase and ACC oxidase in 'Golden Delicious' apples during long-term cold storage. *J. Amer. Soc. Hort. Sci.* 121(4), 751-755.

Gorny, J.R. and A.A. Kader. (1997) Low oxygen and elevated carbon dioxide atmospheres inhibit ethylene biosynthesis in preclimacteric and climacteric apple fruit. *J. Amer. Soc. Hort. Sci.* 122(4), 542-546.

Harker, F.R., F.A. Gunson, P.L. Brookfield and A. White. (2002) An apple a day: the influence of memory on consumer judgment of quality. *Food Qual. Pref.* 13, 173-179.

Hewett, E.W. (2006) An overview of preharvest factors influencing postharvest quality of horticultural products. *Int. J. Postharvest Technol. Inn.* 1(1), 4-15.

Jaeger, S.R., Z. Andani, I.N. Wakeling and H.J.H. MacFie. (1998) Consumer preferences for fresh and aged apples: a cross-cultural comparison. *Food Qual. Pref.* 9(5), 355-366.

Jobling, J.J. and W.B. McGlasson. (1995) A comparison of ethylene production, maturity and controlled atmosphere on storage life of 'Gala', 'Fuji' and 'Lady Williams' apples. *Postharvest Biol. Technol.* 6, 209-218.

Johnson, D.S. (2000) Mineral composition, harvest maturity and storage quality of 'Red Pippin', 'Gala' and 'Jonagold' apples. *J. Hort. Sci. Biotechnol.* 75(6), 697-704.

Johnson, D.S. (2003) Improvement in the storage quality of apples in the UK by use of 1-MCP (SmartFresh^{TM}). *Acta Hort.* 599, 39-45.

Johnston, J.W., E.W. Hewett and M.L.A.T.M. Hertog. (2002) Postharvest softening of apple (*Malus domestica*) fruit: a review. *N.Z. J. Crop Hort. Sci.* 30, 145-160.

Kader, A.A. (2002) Fruits in the global market. In: Knee, M. (Ed.), *Fruit quality and its biological basis.* Sheffield Academic Press Ltd; 1-16.

Kader, A.A. (2003) A perspective on postharvest horticulture (1978-2003). *HortScience* 38(5), 1004-1008.

Kays, S.J. (1999) Preharvest factors affecting appearance. *Postharvest Biol. Technol.* 15, 233-247.

Kevers, C., M. Falkowski, J. Tabart, J.-O. Defraigne, J. Dommes and J. Pincemail. (2007) Evolution of antioxidant capacity during storage of selected fruits and vegetables. *J. Agric. Food Chem.* 55, 8596-8603.

Kingston, C.M. (1992) Maturity indices for apple and pear. *Hort. Rev.* 13(10), 407-432.

Knee, M. (1988) Carotenol esters in developing apple fruits. *Phytochemistry* 27(4), 1005-1009.

Lal Kaushal, B.B. and P.C. Sharma. (1995) Apple. In: Salunkhe, D.K. and S.S. Kadam (Eds.), *Handbook of Fruit Science and Technology – Production, composition, storage, and processing.* Marcel Dekker, Inc., 95.

Larrigaudière, C., J. Graell, J. Salas and M. Vendrell. (1997) Cultivar differences in the influence of a short period of cold storage on ethylene biosynthesis in apples. *Postharvest Biol. Technol.* 10, 21-27.

Larrigaudière, C., R. Vilaplana, Y. Soria and I. Recasens. (2004) Oxidative behaviour of Blanquilla pears treated with 1-Methylcyclopropene during cold storage. *J. Sci. Food Agric.* 84, 1871-1877.

Lee, L., J. Arul, R. Lencki and F. Castaigne. (1995) A review on modified atmosphere packaging and preservation of fresh fruits and vegetables: physiological basis and practical aspects. Part I. *Packaging Technol. Sci.* 8, 315-331.

Lee, S.K. and A.A. Kader. (2000) Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biol. Technol.* 20, 207-220.

Li, Z.-G., Y. Liu, J.G. Dong, R.J. Xu and M.Z. Zhu. (1983) Effect of low oxygen and high carbon dioxide on the levels of ethylene and 1-aminocyclopropane-1-carboxylic acid in ripening apple fruits; *J. Plant Growth Regul.* 2, 81-87.

Lieberman, M. (1979) Biosynthesis and action of ethylene. *Ann. Rev. Plant Physiol.* 30, 533-591.

Lurie, S. (2002) Temperature management. In: Knee, M. (Ed.), *Fruit Quality and its biological basis*. Sheffield Academic Press Ltd; 107-121.

MacLean, D.D., D.P. Murr, J.R. DeEll and C.R. Horvath. (2006) Postharvest variation in apple (Malus x domestica Borkh.) flavonoids following harvest, storage, and 1-MCP treatment. *J. Agric. Food Chem.* 54, 870-878.

Masia, A. (2003) Physiological effects of oxidative stress in relation to ethylene in postharvest produce. In: Hodges, D.M. (Ed.), *Postharvest oxidative stress in horticultural crops.* The Haworth Press, Inc., 165-197.

Mathooko, F.M. (1996) Review. Regulation of ethylene biosynthesis in higher plants by carbon dioxide. *Postharvest Biol. Technol.* 7, 1-26.

Mattheis, J.P. (2004) 1-MCP on apples: From physiology to application. *Obstbau/Weinbau* 10, 299.

McMurchie, E.J., W.B. McGlasson and I.L. Eaks. (1972) Treatment of fruit with propylene gives information about the biogenesis of ethylene. *Nature*, 237, 235-236.

Mir, N. and R. Beaudry. (2002) Atmosphere control using oxygen and carbon dioxide. In: Knee, M. (Ed.), *Fruit Quality and its biological basis*. Sheffield Academic Press Ltd; 122-156.

Mir, N.A., E. Curell, N. Khan, M. Whitaker and R.M. Beaudry. (2001) Harvest maturity, storage temperature and 1-MCP application frequency alter firmness retention and chlorophyll fluorescence of 'Redchief Delicious' apples. *J. Amer. Soc. Hort. Sci.* 126(5), 618-624.

Nakatsuka, A., S. Murachi, H. Okunishi, S. Shiomi, R. Nakano, Y. Kubo and A. Inaba. (1998) Differential expression and internal feedback regulation of 1-aminocyclopropane-1-carboxylate synthase, 1-aminocyclopropane-1-carboxylate oxidase, and ethylene receptor genes in tomato fruit during development and ripening. *Plant Physiol.* 118, 1295-1305.

Nunes, M.C.N., J.K. Brecht, A.M.M.B. Morais and S.A. Sargent. (1998) Controlling temperature and water loss to maintain ascorbic acid levels in strawberries during postharvest handling. *J. Food Sci.* 63(6), 1033-1036.

Paull, R.E. (1999) Effect of temperature and relative humidity on fresh commodity quality. *Postharvest Biol. Technol.* 15, 263-277.

Regiroli, G. (2004) Introducing SmartFresh™ (1-Methylcyclopropene). *Obstbau/Weinbau* 10, 297.

Rupasinghe, H.P.C., D.P. Murr, G. Paliyath and L. Skog. (2000) Inhibitory effect of 1-MCP on ripening and superficial scald development in 'McIntosh' and 'Delicious' apples. *J. Hort. Sci. Biotechnol.* 75(3), 271-276.

Saftner, R.A., J.A. Abbott, W.S. Conway and C.L. Barden. (2003) Effects of 1-Methylcyclopropene and heat treatments on ripening and postharvest decay in 'Golden Delicious' apples. *J. Amer. Soc. Hort. Sci.* 128(1), 120-127.

Saltveit, M.E. (1999) Effect of ethylene on quality of fresh fruits and vegetables. *Postharvest Biol. Technol.* 15, 279-292.

Saltveit, M.E. (2003) Is it possible to find an optimal controlled atmosphere? *Postharvest Biol. Technol.* 27, 3-13.

Salunkhe, D.K. and S.S. Kadam. (1995) Introduction. In: Salunkhe, D.K. and S.S. Kadam (Eds.), *Handbook of Fruit Science and Technology – Production, composition, storage, and processing.* Marcel Dekker, Inc., 1-6.

Sams, C.E. (1999) Preharvest factors affecting postharvest texture. *Postharvest Biol. Technol.* 15, 249-254.

Sánchez-Moreno, C., S. de Pascual-Teresa, B. de Ancos and M.P. Cano. (2006) Nutritional values of fruits. In: Hui, Y.H. (Ed.), *Handbook of fruits and fruit processing*. Blackwell Publishing Professional, 29-43.

Saquet, A.A., J. Streif and F. Bangerth. (2003) Impaired aroma production of CA-stored 'Jonagold' apples as affected by adenine and pyridine nucleotide levels and fatty acid concentrations. *J. Hort. Sci. Biotechnol.* 78(5), 695-705.

Scalzo, J., A. Politi, N. Pellegrini, B. Mezzetti and M. Battino. (2005) Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition* 21, 207-213.

Shewfelt, R.L. (1993) Measuring quality and maturity. In: Shewfelt, R.L. and S.E. Prussia (Eds.), *Postharvest Handling: a systems approach*. Academic Press, Inc., 99-124.

Shewfelt, R.L. and S.E. Prussia. (1993) Challenges in handling fresh fruits and vegetables. In: Shewfelt, R.L. and S.E. Prussia (Eds.) *Postharvest Handling: a systems approach*. Academic Press, Inc., 27-41.

Sisler, E.C., E. Dupille and M. Serek. (1996) Effect of 1-Methylcyclopropene and methylenecyclopropane on ethylene binding and ethylene action on cut carnations. *Plant Growth Regul.* 18, 79-86.

Tatsuki, M., A. Endo and H. Ohkawa. (2007) Influence of time from harvest to 1-MCP treatment on apple fruit quality and expression of genes for ethylene biosynthesis enzymes and ethylene receptors. *Postharvest Biol. Technol.* 43, 28-35.

Tromp, J. (2005) Fruit ripening and quality. In: Tromp, J., A.D. Webster and S.J. Wertheim (Eds.), *Fundamentals of temperate zone tree fruit production.* Backhuys Publishers, Leiden, The Netherlands; 295-310.

Tromp, J. and S.J. Wertheim (2005) Fruit growth and development. In: Tromp, J., A.D. Webster and S.J. Wertheim (Eds.), *Fundamentals of temperate zone tree fruit production*. Backhuys Publishers, Leiden, The Netherlands; 240-266.

Vilapana, R., M.C. Valentines, P. Toivonen and C. Larrigaudière. (2006) Antioxidant potential and peroxidative state of 'Golden Smoothee' apples treated with 1-methylcylcopropene. *J. Amer. Soc. Hort. Sci.* 131(1), 104-109.

Watada, A.E., R.C. Herner, A.A. Kader, R.J. Romani and G.L. Staby. (1984) Terminology for the description of developmental stages of horticultural crops. *HortScience* 19(1), 20-21.

Watkins, C.B. (2002) Ethylene synthesis, mode of action, consequences and control. In: Knee, M. (Ed.), *Fruit quality and its biological basis*. Sheffield Academic Press Ltd; 180-224.

Watkins, C.B. (2006) 1-Methylcyclopropene (1-MCP) based technologies for storage and shelf life extension. *Int. J. Postharvest Technol. Inn.* 1(1), 62-68.

Watkins, C.B. and J. Nock. (2004) SmartFresh™ (1-MCP) – an update; *Cornell Fruit Handling and Storage Newsletter,* 2-12.

Watkins, C.B. and J.F. Nock. (2000) MCP: Facts, Speculation, and How could it affect the New York apple industry? *N.Y. Fruit Quart.* 8(3), 5-9.

Watkins, C.B., J.F. Nock and B.D. Whitaker. (2000) Responses of early, mid and late season apple cultivars to postharvest application of 1-Methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. *Postharvest Biol. Technol.* 19, 17-32.

Wertheim, S.J. (2005) Fruit storage. In: Tromp, J., A.D. Webster and S.J. Wertheim (Eds.), *Fundamentals of temperate zone tree fruit production*. Backhuys Publishers, Leiden, The Netherlands; 311-324.

Wills, R.B.H., W.B. McGlasson, D. Graham and D.C. Joyce. (2007) *Postharvest: an introduction to the physiology and handling of fruit, vegetables and ornamentals.* 5th Edition; CABI.

Wood, L.G., P.G. Gibson and M.L. Garg. (2006) A review of the methodology for assessing *in vivo* antioxidant capacity. *J. Sci. Food Agric.* 86, 2057-2066.

Wünsche, J.N., D.H. Greer, W.A. Laing and J.W. Palmer. (2005) Physiological and biochemical leaf and tree responses to crop load in apple. *Tree Physiol.* 25, 1253-1263.

Yang, S.F. (1980) Regulation of ethylene biosynthesis. *HortScience*, 15(3), 238-243.

Yang, S.F. and N.E. Hoffman. (1984) Ethylene biosynthesis and its regulation in higher plants. *Ann. Rev. Plant Physiol.* 35, 155-189.

Zarembinski, T.I. and A. Theologis. (1994) Ethylene biosynthesis and action: a case of conservation. *Plant Mol. Biol.* 26, 1579-1597.

2.

Effect of 1-MCP on Apple Fruit Quality and Consumer Acceptability

Abstract

'Jonagold' apple fruit (*Malus domestica* Borkh.) were picked at commercial maturity in 2004, 2005 and 2006. Following 1-MCP treatment fruit were stored in cold storage, CA- (0.8 % CO_2, 3 % O_2) and ULO-storage (3 % CO_2, 1 % O_2). After 2, 4 and 6 months (2004/05) and 3, 6 and 9 months (2005/06) fruit samples from each storage atmosphere ± 1-MCP were removed to assess fruit quality parameters (flesh firmness FF, soluble solids SSC, titratable acidity TA, background colour BGC) following 10 days shelf-life (20°C). In 2006/07 fruit samples were removed from storage (cold storage and ULO-storage) after 3 and 5 months to perform consumer preference mapping. Additionally fruit quality (FF, SSC, TA and BGC) was evaluated by instrumental measurements. Which quality parameters are driving consumer liking and preference? Is there a correlation between instrumental values of common quality parameters of apples and corresponding consumer scores? In general, fruit quality decreased during storage and shelf-life depending on 1-MCP treatment, storage condition and -duration. However, 1-MCP delayed ripening more and maintained fruit quality better than CA- or even ULO-storage. Since not all ripening and quality parameters are ethylene-dependent, not all of them will be regulated and influenced by 1-MCP in the same intensity. This might have an impact on the overall quality of the fruit and the consumer acceptance of 1-MCP treated apples. However, most consumers, regardless of age or gender, preferred the 1-MCP treated fruit in ULO-storage, particularly after 5 months. The preference of 1-MCP treated apples held in cold storage declined with storage time. Therefore it is concluded that firmness and tartness ('freshness') were the most important drivers of consumer preference. In our study all analytical measurements were in good agreement with corresponding sensory evaluations from consumer panels. Overall, the scores of firmness, sweetness, tartness and background colour followed the trend for instrumental values of FF, SSC, TA and BGC, respectively. Though sensory evaluation studies are time-consuming and there might be some flaws and

difficulties to generate representative results from consumer taste panels, they are a useful tool to assess food quality and consumer preference.

2.1 Introduction

'Jonagold' is currently the most popular apple cultivar in Germany. In 2005 private households bought 21.9 kg apples, 17.7 % of that was 'Jonagold' (Ellinger, 2006).

Because of a wide variation of maturity within-trees, 2 - 3 select picks are recommended in order to harvest all fruit at the right stage of maturity (de Jager, 1994; Girard and Lau, 1995) depending on the intended storage condition and -duration. All fruit have to be picked at the stage of maturity which assures for consumers best eating quality ex-store (de Jager, 1994; Hurndall et al., 1994; Tromp, 2005). While eating quality of the apples will improve with maturity, storability decreases (Tromp, 2005). Independent changes of both, sugars and acids, during ripening have an impact on the overall flavour (Paull, 1999). Texture (Shewfelt, 1999; Harker et al., 2002) and appearance (size and shape) (Francis, 1995; Lange et al., 2000) are important quality attributes for consumer. EU quality specifications for apple, however, define just appearance (Jack et al., 1997). Unfortunately flavour and sensory quality are not particularly considered.

During maturation and ripening, several quality parameters are not constant. Maturation is the time between the stages of growth and ripening (Tromp, 2005). During maturation the fruit develop from an immature stage to maturity (Watada et al., 1984; Tromp, 2005). However, at mature stage, the fruit is still uneatable but it has reached the ability to ripen (Tromp, 2005). Ripening is the process by which the physiologically mature but inedible fruit develops its characteristic appearance and eating quality (Watada et al., 1984; Kader, 2002; Wills et al., 2007). Maturation and ripening may overlap. Growth and maturation are completed while fruit is attached to the plant, whereas ripening may proceed on or off the plant (Wills et al., 2007) and may be considered as the first part of the senescence process (Watada et al., 1984; Tromp, 2005). Changes occur to different extents while fruit are still attached to the tree as well as after harvest, during storage and post-storage. 'Jonagold' apples show a relatively rapid loss of firmness and titratable acids during ripening. The concomitant increase in the concentration of soluble solids is due to the conversion of starch (Watkins et al., 2000;

Tromp, 2005). Because of the degradation of chlorophyll and also synthesis of carotenoids the background colour changes from green to yellow. All these quality parameters are commonly used for determining the optimum harvest date and to control fruit quality during and after storage (Watada et al., 1980).

Storage and post-storage apple quality is primarily affected by harvest maturity of the fruit and storage condition and duration (Hurndall et al., 1994; Girard and Lau, 1995). 'Jonagold' can be stored for up to 10 months under controlled atmosphere (CA) conditions (Stow, 1987; Lau, 1988), i.e. under reduced O_2- and elevated CO_2-concentrations. These conditions decrease the loss of firmness and titratable acidity (TA) as well as the degradation of chlorophyll (Johnson, 2000). In general, storage under controlled atmosphere leads to a better maintenance of apple quality and a longer storage life (Echeverría et al., 2002; Wills et al., 2007).

Throughout storage, apple quality is preserved at a high level, whereas conditions at several points throughout the distribution chain are often not adequate for fresh commodities (de Jager, 1994; Paull, 1999; Johnston et al., 2002). Fruit quality retention from harvest to the point of sale and consumption requires continuous optimum conditions, especially with regard to temperature and relative humidity (Tijskens et al., 1994; Paull, 1999). It is critically important that fruit quality meets consumer requirements (Cardello, 1995; Lawless, 1995).

Ethylene, the so-called 'ripening hormone' is produced naturally during fruit ripening and regulates many aspects associated with ripening. Fruit quality parameters are either ethylene-dependent or ethylene-independent (Jeffrey et al., 1984; Mir et al., 2001). 1-Methylcyclopropene (1-MCP), an inhibitor of ethylene (Sisler et al., 1996) leads to a better fruit quality retention during storage (Fan and Mattheis, 1999; Watkins et al., 2000; DeEll et al., 2002) and also ex-store (Watkins et al., 2000; Watkins, 2006). 1-MCP maintains firmness (Fan and Mattheis, 1999; Watkins et al., 2000; Mir et al, 2001; Johnson, 2003) and reduces the loss of acidity (Fan and Mattheis, 1999; Watkins et al., 2000; Johnson, 2003; Saftner et al., 2003; Moya-Leon et al., 2007). 1-MCP treated fruits have a fresh and crunchy texture (AgroFresh Inc., 2003).

Although a lot of reports describe the impact of harvest maturity, storage atmosphere and -duration (e.g. Girard and Lau, 1995; Plotto et al., 1997; Echeverría et al., 2002) and 1-MCP treatment (e.g. Watkins et al., 2000; Mir et al., 2001; Johnson, 2003; de Castro et al., 2007) on

the retention of fruit quality, reports on consumer acceptability of 1-MCP treated apple fruit are lacking.

The research objectives of this study were to determine the impact of 1-MCP treatment, storage condition (cold storage, CA- and ULO-storage) and -duration (ex-store after 2 – 9 months) on maintenance of commonly measured quality attributes (flesh firmness, soluble solids concentration, titratable acidity, background colour) of 'Jonagold' apples grown in Southwest Germany. Particular focus was given to sensory evaluation of 1-MCP treated 'Jonagold' apples stored for 3 and 5 months in cold-storage and ULO-storage by consumer preference mapping. Which quality parameters are driving consumer liking and preference? Is there a correlation between instrumental values of common quality parameters of apples and corresponding consumer scores?

2.2 Materials and methods

2.2.1 Plant material and harvest management

The experiments were carried out at 'Kompetenzzentrum Obstbau – Bodensee', Ravensburg, Germany, using the apple cultivar 'Jonagold' (*Malus domestica* Borkh.) in the 2004, 2005 and 2006 growing season. In 2004 and 2006 'Jonagold' fruit were picked at the experimental site, in 2005 fruit were harvested at a commercial orchard nearby. All trees were grown on rootstock M.9 and trained as slender spindle. Three harvests were taken throughout the commercial harvest period for long-term CA-storage of 'Jonagold' apples in 2004 and 2005. In 2006 fruit were harvested from representative trees in two harvests (Table 2.1).

2.2.2 1-MCP treatments

Immediately after each harvest fruit were graded for uniformity by hand. Fruit were divided at random in 12 kg plastic boxes, according to number of storage conditions, sample removals, treatments (± 1-MCP) and replications.

In 2004 immediately after grading half of the boxes with fruit were placed in gas-tight storage containers (volume 0.560 m^3, regular air, 1°C, 92 % relative humidity (RH)) and treated with 625 ppb 1-Methyl-cyclopropene (1-MCP) for 24 hours (0 days after harvest, 0 DAH). The

temperature of the container was at 1°C (± 0.5). Control fruit were held at the same conditions but without 1-MCP.

To simulate commercial practice fruits were held in cold storage (1°C, 92 % RH) for 6 days prior to MCP–treatments in 2005 and 2006, respectively (Table 2.1). 1-MCP treatments on day 7 after harvest (7 DAH) were performed as described above.

All boxes with 1-MCP-treated and untreated fruit were divided at random into three equal groups. These groups were distributed to containers with different storage atmospheres.

2.2.3 Fruit storage and sampling procedure

Fruit were stored for up to 6 months in 2004/05, 9 months in 2005/06 and 5 months in 2006/07 (Table 2.1). Storage atmospheres were as follows: cold storage, CA- (0.8 % CO_2, 3 % O_2) and ULO-storage (3 % CO_2, 1 % O_2). Temperature was about 1°C (± 0.5) and RH at 92 % (± 2 %) in each storage atmosphere. For each harvest one independent storage container was used for CA- and ULO-storage, respectively. Fruit boxes for cold storage were covered lightly with plastic sheets to minimize water loss and were placed in a cold storage room. In 2006/07 no fruit were stored in CA-storage.

After 2, 4 and 6 months (2004/05), 3, 6 and 9 months (2005/06), 3 and 5 months (2006/07) fruit samples from each storage atmosphere (cold storage, CA- and ULO-storage ± 1-MCP) were removed to assess fruit quality parameters and to perform consumer preference mapping only in 2006/07 (Table 2.1).

Table 2.1: Picking dates, time of 1-MCP treatment and storage durations in 2004, 2005 and 2006.

	2004/05	2005/06	2006/07
Picking date			
1st harvest	29. Sept.	27. Sept.	06. Oct.
2nd harvest	07. Oct.	07. Oct.	16. Oct.
3rd harvest	14. Oct	17. Oct.	-
1-MCP treatment	0 DAH	7 DAH	7 DAH
Commencement of storage (cold storage, CA- and ULO-storage)	1 DAH	8 DAH	8 DAH
Sample removals			
Ex-store 1	2 months	3 months	3 months
Ex-store 2	4 months	6 months	5 months
Ex-store 3	6 months	9 months	-

2.2.4 Fruit quality evaluations by instrumental measurements

In 2004/05 and 2005/06 instrumental measurements of quality parameters were conducted following 10 days of shelf-life at 20°C after each harvest and after each sample removal from storages. After 6 and 9 months in 2005/06 fruit quality evaluations of the untreated 3rd harvest from cold store were not possible due to a lack of fruit. In 2006/07 fruit samples were taken from storage (cold- and ULO-storage) after 3 and 5 months to assess fruit quality parameters and to perform sensory panels. Shelf-life period at 20°C was 5 days after 3 months and 6 days after 5 months of storage. Each of the 3 replicates consisted of 8 (2004/05 and 2006/07) and 9 fruits (2005/06) at harvest dates and at sample removal of 6 fruits (2004/05 and 2006/07) and 5 fruits (2005/06), respectively. Each time all fruit of the replicates were analysed.

Starch degradation. Starch degradation was analysed at harvest (KI-I_2 staining pattern). Fruit were cut horizontally at the equatorial region and the cut surfaces were stained with an iodine solution (10 g potassium iodide (KI) and 3 g iodine (I_2) per 1 litre). Starch degradation was estimated visually on a rating scale from 1 (full starch, unripe) to 10 (no starch, overripe). All assessments were made by the same person in order to eliminate variations between operators.

Flesh firmness (FF). Flesh firmness was determined between exposed and shaded site of each fruit. After removing the peel measurements were made using a penetrometer (Chatillon (2004/05), GÜSS, Fruit Texture Analyser Type GS 14 (2005/06, 2006/07)) with a 11 mm diameter probe (penetration depth 8 mm) and expressed in N.

Background colour (BGC). Background colour was measured on the greenest region of each fruit using a chromameter (CR 300, Minolta). Hue angle was calculated from a* and b* values (H° = arctan (b*/a*)). The higher the value (H°) the greener and less ripe the fruit.

Soluble solids concentration (SSC) and titratable acidity (TA) were measured by using freshly prepared juice from all fruit of each replicate. Fruit were cut horizontally at the equatorial region and the lower half was mixed in a blender. Juice was filtered through paper tissue and used for determination of SSC and TA. Soluble solids concentration (% SSC) was assessed with a refractometer (Leica Digital Refractometer AR 200 (2004/05), Atago Digital Refractometer, PR 1 (2005/06, 2006/07)). Titratable acidity (TA) was measured by titrating 10 ml freshly prepared juice with 0.1 N NaOH to an endpoint pH of 8.1 using manual pH-meter in 2004/05 and an automatic titration system (Metrohm, Type 702 SM Titrino with 815 Robotic USB Sample Processor KL) in 2005/06 and 2006/07. Titratable acidity was expressed as g malic acid per litre.

2.2.5 Fruit quality evaluations by consumer preference mapping

In 2004 a survey was conducted with 118 consumers of several households in Southwest Germany. Consumer were asked if they would also purchase apples which have additionally been treated with a fruit ripening regulator to preserve their quality and freshness.

In 2007 consumer preference mapping was performed to assess correlation between measured standard quality parameters and consumer acceptance of 1-MCP treated ‘Jonagold’ apple (Table 2.1). Instrumental quality assessments were made after fruit were held for 5 and 6 days at 20°C after 3 and 5 months of storage, respectively. Sensory evaluations by consumer taste panels were performed two days after instrumental quality measurements.

Consumer preference mapping took place at the farmer’s market in Ravensburg, Germany. Participants were not selected and participated voluntarily. Table 2.2 provides an overview of the number of participating consumers in various age groups.

Table 2.2: Participating consumer at consumer taste panels in 2007.

	1st harvest		2nd harvest	
	3 months	5 months	3 months	5 months
< 20 years	16	20	7	21
20 - 39 years	20	30	17	19
40 - 60 years	38	32	20	40
> 60 years	23	26	8	28
Total	97	108	52	108

Based on the total number of 365 participants, 17.5 % were younger than 20 years, 23.6 % were between 20 and 39 years, 35.6 % were between 40 and 60 and 23.3 % were older than 60 years. Fifty seven percent of the participants were female, 43 % male.

Consumer were asked to score the intensity of the quality parameters firmness, sweetness, tartness and background colour on a scale from 'much too less', 'too less', 'just right', 'too great', 'much too great'. In the end consumers were asked for their overall preference among the four treatments.

For scoring the background colour, apples were presented to consumers on a table with a white sheet. In addition, unpeeled fresh fruit segments were given to consumers to assess the above mentioned eating quality parameters. On cold days in January fruits were held in poly-styrene boxes to guarantee good eating temperature. Consumer could taste fruit from each treatment as often as they desired.

2.2.6 Statistical analysis

Data of instrumental measurements were statistically analysed by analysis of variance (ANOVA) using GenStat 7.2 (Rothamsted, UK) to determine the effects of 1-MCP treatment, harvest date, storage condition and -duration on fruit quality parameters. Results of consumer preference mapping were not statistically analysed.

2.3 Results

2.3.1 Fruit quality

Even though 3 harvests were taken throughout the commercial harvest period for long-term CA-storage of 'Jonagold' apples in both years, no consistent trends were found due to significant interactions between harvest dates, 1-MCP treatment, storage condition and -duration. Shown data present the means averaged values for all 3 harvests in season 2004/05 and 2005/06, respectively. Fruit quality of 'Jonagold' apples at harvest in 2004 and 2005 is shown in Table 2.3. In general, fruit maturity was more advanced at harvest in 2004 than in 2005. Since starch scores, FF and SSC in 2004 were 7.7, 64.7 N and 13.3 %, respectively, values in 2005 were 7.0, 70.6 N and 14.0 %. Moreover, TA in 2004 was lower and BGC higher (6.8 g L^{-1} and 109.1 H°) when compared with 2005 values (7.1 g L^{-1} and 101.8 H°).

Table 2.3: Average fruit quality of 'Jonagold' apples from 3 picks in 2004 and 2005, respectively.

	2004			2005		
		P	$LSD_{0.05}$		*P*	$LSD_{0.05}$
Size (mm)	81.6	**	2.30	85.2	**	2.70
Starch (score)	7.7	***	0.57	7.0	***	0.62
FF (N)	64.7	ns		70.6	ns	
SSC (%)	13.3	ns		14.0	ns	
TA (g L^{-1})	6.8	**	0.74	7.1	*	0.99
BGC (H°)	109.1	ns		101.8	*	8.09

ns, ***, **, *: not significant or significant at *P*-value ≤ 0.001, ≤ 0.01 and ≤ 0.05, respectively; least significant difference LSD (*P* ≤ 0.05).

Figure 2.1 and 2.2 show data from measurements of standard quality parameters (FF, SSC, TA, BGC) of 'Jonagold' apples ± 1-MCP after 2, 4 and 6 months of storage in different storage conditions + shelf-life (20°C) in 2004/05 and 3, 6 and 9 months in 2005/06, respectively.

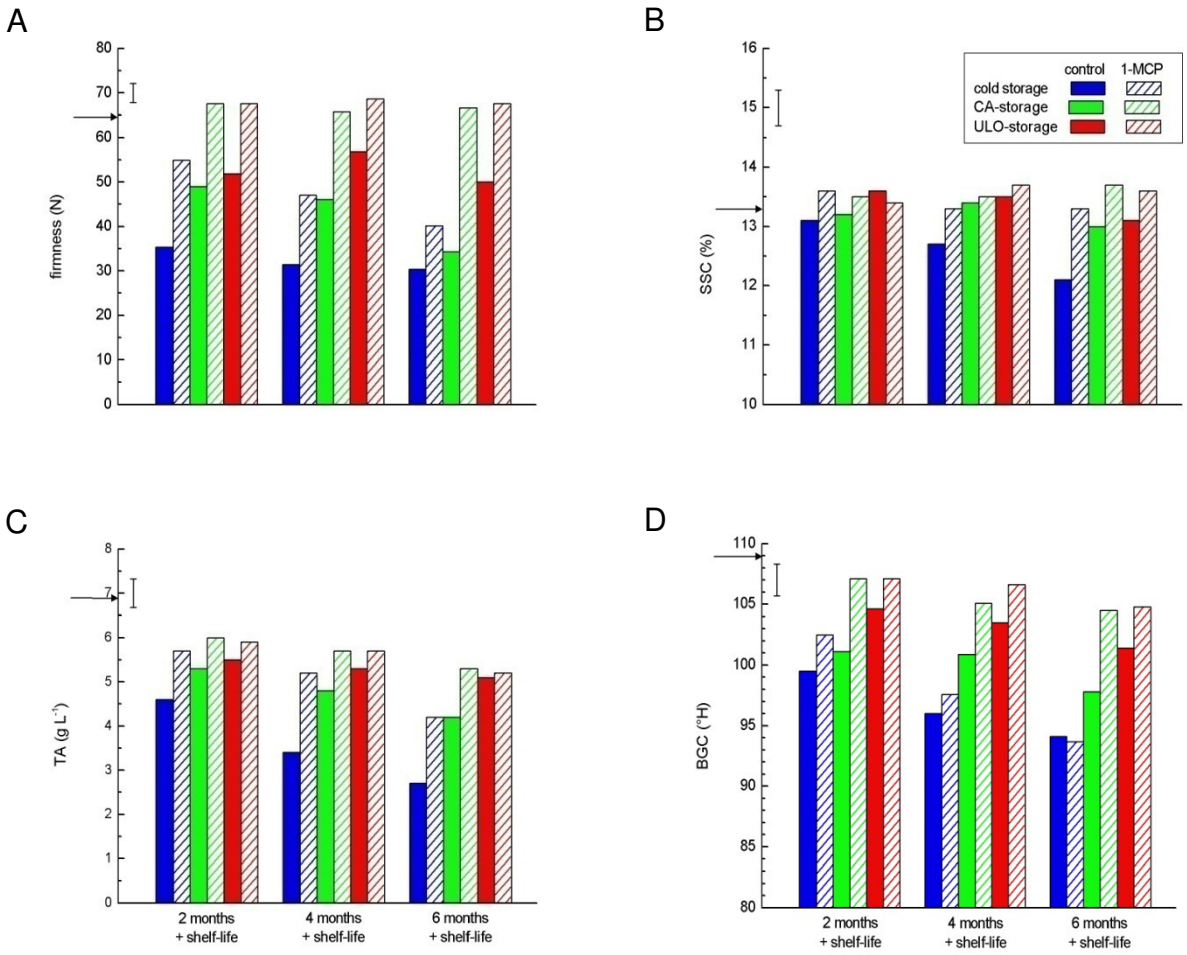


Figure 2.1: Firmness FF (N) (A), soluble solid concentration SSC (%) (B), titratable acidity TA (g L^{-1} (C) and background colour BGC (H°) (D) of 'Jonagold' apples ± 1-MCP after 2, 4 and 6 months of storage in different storage conditions (cold storage, CA- and ULO-storage) plus 10 days shelf-life (20°C) in 2004/05. Bars show the $LSD_{0.05}$ for all treatments at each sampling time. Arrow at the y-axis represents harvest value.

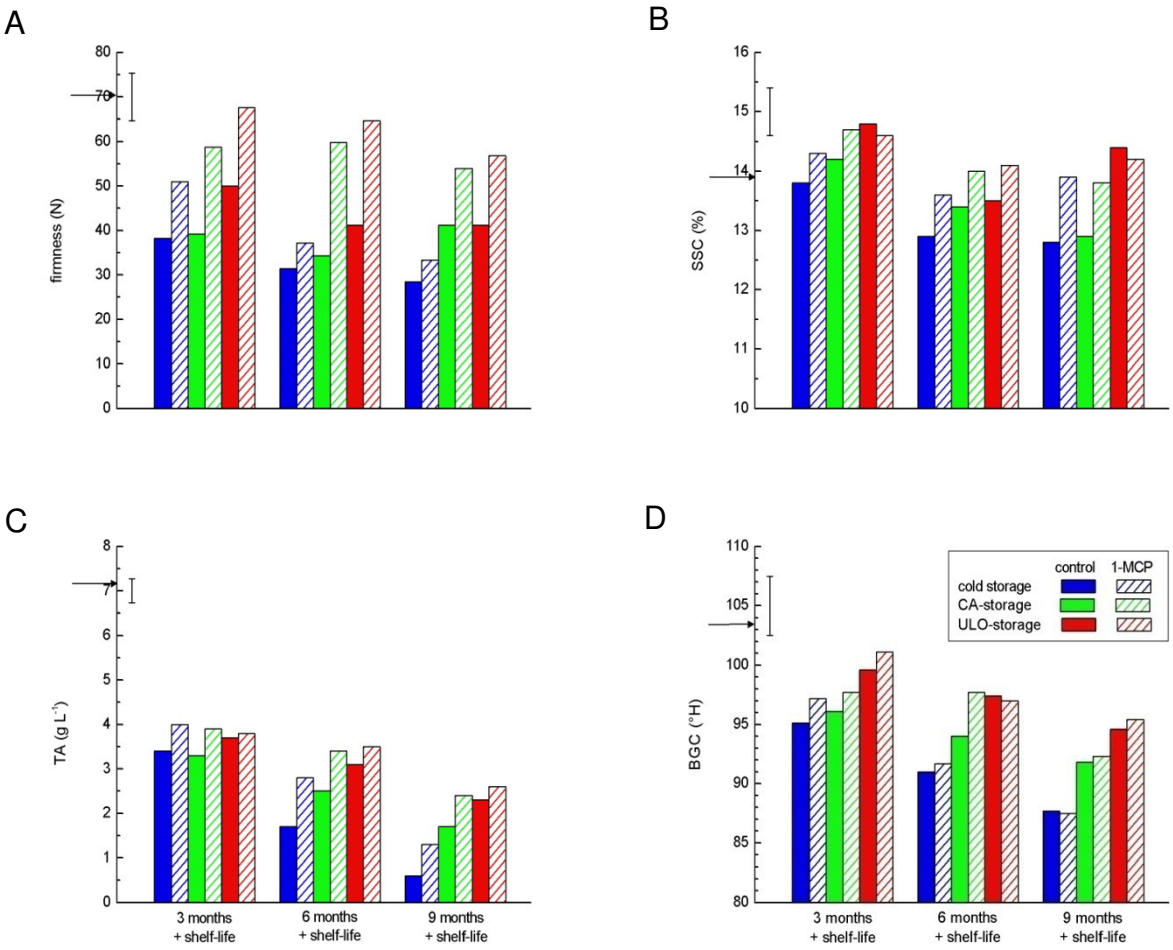


Figure 2.2: Firmness FF (N) (A), soluble solid concentration SSC (%) (B), titratable acidity TA (g L^{-1}) (C) and background colour BGC (H°) (D) of 'Jonagold' apples ± 1-MCP after 3, 6 and 9 months of storage in different storage conditions (cold storage, CA- and ULO-storage) plus 10 days shelf-life (20°C) in 2005/06. Bars show the $LSD_{0.05}$ for all treatments at each sampling time. Arrow at the y-axis represents harvest value.

After each storage removal + shelf-life in both years firmness was best maintained in 1-MCP treated fruit from ULO-storage. However, after 2 and 6 months of storage in 2004/05 1-MCP treated CA stored fruit reached the same value like 1-MCP treated fruit from ULO-storage. Lowest firmness values after 2, 4 and 6 months in 2004/05 and after 3, 6 and 9 months in 2005/06, respectively, were found for untreated cold stored fruit. In both years firmness of cold stored fruit decreased continuously during the entire storage duration, irrespective of 1-MCP treatment.

Again, following 2, 4 and 6 months storage in 2004/05 and 3, 6 and 9 months in 2005/06, respectively, lowest SSC were found for untreated fruit from cold storage. SSC in untreated cold stored fruit declined continuously during the entire storage duration. Highest SSC in 2004/05 with 13.7 % was found in 1-MCP treated fruit from ULO-storage after 4 months and in 1-MCP treated fruit from CA-storage after 6 months. In contrast, highest SSC in 2005/06 (14.8 %) was found in untreated ULO stored fruit after 3 months.

In both years TA declined in all cases during the entire storage duration, irrespective of 1-MCP treatment and storage condition. In the end of the entire storage duration in 2004/05 (after 6 months) TA in untreated fruit from cold storage was reduced to 2.6 g L^{-1} whereas TA in untreated fruit after 9 months of cold storage in 2005/06 was drastically reduced to 0.8 g L^{-1}. After each sample removal in 2004/05 TA was best maintained in 1-MCP treated CA stored fruit, followed by 1-MCP treated fruit from the ULO-storage. In contrast, highest TA values after 6 and 9 months of storage were found for 1-MCP treated fruit from the ULO-storage. After 3 months storage in 2005/06 TA was best maintained in 1-MCP treated fruit from CA-storage (4.0 g L^{-1}) and TA in 1-MCP treated fruit from cold- and ULO-storage was slightly lower (3.9 and 3.8 g L^{-1}). In all cases 1-MCP treated fruit had higher TA values when compared with untreated fruit.

The effect of 1-MCP on maintenance of green BGC in cold stored fruit declined with storage duration in 2004/05. Since untreated fruit after 2 and 4 months of cold storage had BGC of 99.5 and 96.0 H°, BGC of 1-MCP treated fruit was 102.5 and 97.6 H°, respectively. After 6 months of cold storage untreated and 1-MCP treated fruit had nearly the same BGC value. In 2005/06 BGC of untreated and 1-MCP treated cold stored fruit was nearly the same after each sample removal. BGC was best maintained in 1-MCP treated ULO stored fruit after each sample removal in both years. However, after 2 months of storage in 2004/05 highest BGC was found for both, 1-MCP treated fruit from CA- and ULO-storage (107.1 H°) and in 2005/06 highest BGC after 6 months was found in 1-MCP treated fruit from CA-storage (97.8 H°).

Table 2.4 presents the effects of 1-MCP, storage condition and -duration on FF, SSC, TA and BGC of ‘Jonagold’ apples in 2004/05 and 2005/06, respectively. In 2004/05 highly significant main effects were found for all quality parameters. Moreover, highly significant interactions between all main effects were found except for SSC. In 2005/06 main effects were also sig-

nificant for all quality parameters but again significant interactions between most of the main effects were found.

Table 2.4: Effects of 1-MCP, storage condition (cold storage, CA- and ULO-storage) and storage duration (2, 4, 6 months in 2004/05 and 3, 6, 9 months in 2005/06) on FF (N), SSC (%), TA (g L^{-1}) and BGC (H°) of 'Jonagold' apples in 2004/05 (A) and 2005/06 (B), respectively.

			FF		SSC		TA		BGC	
Treatment		DF	*P*	$LSD_{0.05}$	*P*	$LSD_{0.05}$	*P*	$LSD_{0.05}$	*P*	$LSD_{0.05}$
Main effects										
± 1-MCP	A	1	***	0.49	***	0.07	***	0.08	***	0.31
	B		***	1.27	***	0.09	***	0.06	*	0.58
Storage condition (SC)	A	2	***	0.59	***	0.09	***	0.09	***	0.38
	B		***	1.57	***	0.12	***	0.08	***	0.71
Storage duration (SD)	A	5	***	0.88	***	0.12	***	0.13	***	0.54
	B		***	2.16	***	0.16	***	0.11	***	1.01
Interactions										
SC*SD	A	10	***	1.57	***	0.21	***	0.23	***	0.93
	B		ns		***	0.28	***	0.19	***	1.75
SD*± 1-MCP	A	5	***	1.27	***	0.17	***	0.18	***	0.76
	B		**	3.04	*	0.23	*	0.16	ns	
SC*± 1-MCP	A	2	***	0.88	***	0.12	***	0.13	***	0.54
	B		***	2.16	***	0.16	***	0.11	***	1.01
SC*SD*± 1-MCP	A	10	***	2.16	ns		***	0.32	***	1.32
	B		***	5.29	*	0.40	*	0.27	ns	

ns, ***, **, *: not significant or significant at *P*-value ≤ 0.001, ≤ 0.01 and ≤ 0.05, respectively; least significant difference LSD (*P* ≤ 0.05).

2.3.2 Sensory evaluation by consumer panels

In general, fruit of the 2nd harvest were more mature than fruit of the 1st harvest in 2006 (Table 2.5). Fruit starch was significantly higher at the 2nd harvest date (9.1) when compared with the 1st harvest (7.6). Fruit from the 2nd harvest had also significantly lower TA- and BGC-values in comparison with fruit from the 1st harvest (6.2 vs. 6.4 g L^{-1} and 108.8 vs. 104.9 H°). Surprisingly no differences in FF and SSC between the two harvest dates were found.

Table 2.5: Fruit quality of 'Jonagold' apples at harvests in 2006.

	1st harvest	2nd harvest	*P*	$LSD_{0.05}$
Starch (score)	7.6	9.1	***	0.54
Firmness (N)	68.6	67.6	ns	
SSC (%)	14.5	14.4	ns	
TA (g L^{-1})	6.4	6.2	*	0.20
BGC (H°)	108.8	104.9	*	1.78

ns, ***, **, *: not significant or significant at *P*-value ≤ 0.001, ≤ 0.01 and ≤ 0.05, respectively; least significant difference LSD (*P* ≤ 0.05).

Figure 2.3 compares data from instrumental measurements (FF, SSC, TA and BGC) of the 2nd harvest after 5 months storage + shelf-life with corresponding results obtained from consumer panels. Firmness of untreated fruit from cold storage was the lowest with 34.3 N and highest in 1-MCP treated fruit from ULO-storage (60.8 N). A good correlation between measured values and sensory scores by consumer preference panels was found for FF. In general, consumer found untreated fruit stored in cold storage too soft and fruit stored in ULO + 1-MCP slightly too firm. Untreated fruit stored in ULO were with 53.9 N 'just right' for most consumers.

Untreated fruit kept in cold storage had significant lower SSC (12.9 %) when compared to the other storage regimes which had around 13.8 % SSC. All treatments were scored closely around 'just right' level by the consumers. Sweetness tended to be less appreciated under ULO-storage condition than in cold storage, but in both cases was even less liked when fruit was treated with 1-MCP.

TA increased significantly from cold storage to ULO-storage, but under both storage conditions TA was significantly higher in 1-MCP treated fruit than in untreated fruit. TA in untreated fruit stored in cold storage was at only 2.2 g malic acid per litre after 5 months storage. TA in 1-MCP treated fruit stored in cold storage was higher at 3.6 g malic acid per litre. In contrast, untreated and treated fruit stored in ULO-storage had significantly higher TA levels at 4.6 and 5.1 g malic acid per litre, respectively. There was also a good correlation between measured values and sensory scores for acidity. While 1-MCP treated fruit stored in ULO were scored slightly above 'just right' level, untreated ULO fruit were scored slightly below

the ‘just right’ level. Both cold storage treatments were scored below ‘just right’ level, with untreated fruit having the lowest score. A significant increase in BGC for fruit stored in cold storage compared to fruit stored in ULO-storage indicates that the latter fruit was less ripe. Within each storage condition a significant difference between treated and untreated fruit could not be observed.

Background colour scores for cold storage fruit were above ‘just right’ level whereas scores for ULO-storage were below the ‘just right’ level. This was in agreement with instrumental measurements and indicates that fruit from ULO-storage were greener than those in cold storage.

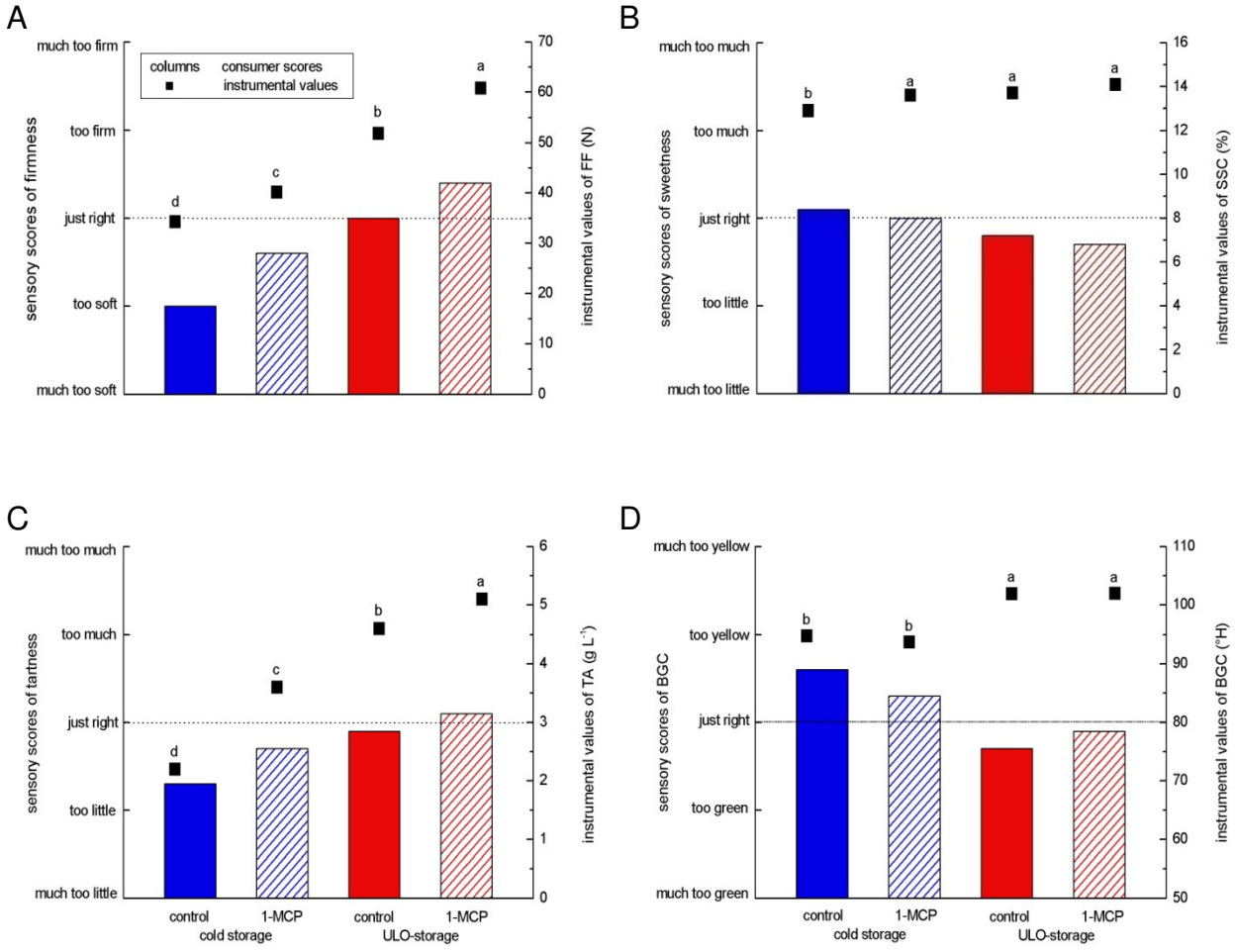


Figure 2.3: Instrumental measurements vs. consumer scores of FF (N) (A), SSC (%)/sweetness (B), TA (g L^{-1})/tartness (C) and BGC (H°) (D); 2nd harvest after 5 months of storage + shelf-life (20°C); different letters indicate significant differences at $P \leq 0.05$.

The overall consumer preference, considering all four consumer panels is shown in Figure 2.4. Results are presented for 4 different age groups, < 20 years, 20 - 39 years, 40 - 60 years and > 60 years. In general, 1-MCP treated fruit and ULO stored fruit were in preference to untreated and cold stored fruit, respectively. However, consumer > 60 years found 1-MCP treated fruit from the cold store more likeable rather than untreated fruit from ULO-storage. In contrast, 52 % of < 20 years consumer gave their preference to 1-MCP and ULO-treated fruit while only 8 % of this age group preferred untreated cold stored fruit.

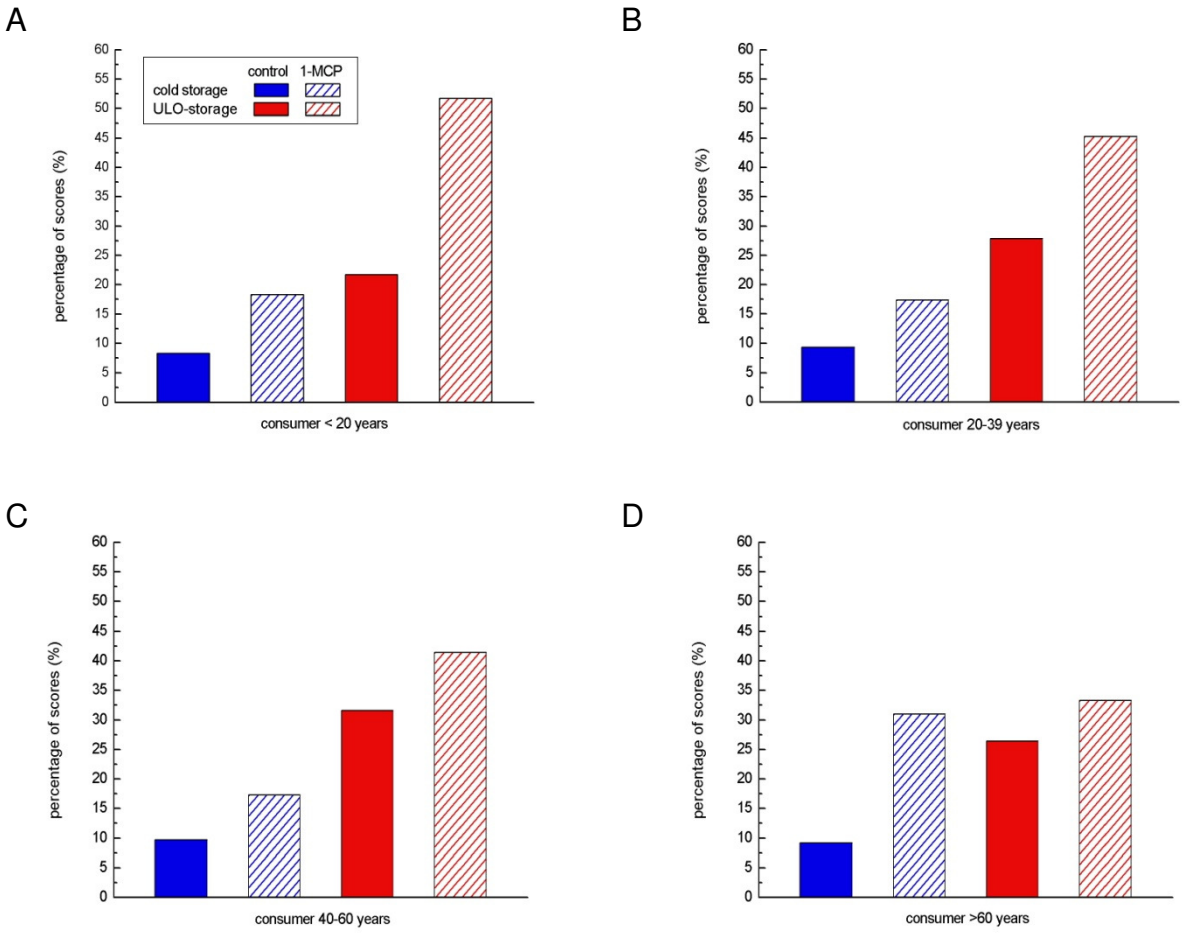


Figure 2.4: Overall preference of consumers among the 4 treatments cold storage ± 1-MCP and ULO-storage ± 1-MCP in 2007.

Table 2.6 presents results on the overall consumer preference for fruit stored at 4 storage conditions either 3 or 5 months. Fruit treated with 1-MCP and stored in ULO-storage was most

preferred from consumers. The preference for fruit decreased with time in cold storage, while increased from 65.4 to 73.2 % in ULO-storage. Consumer also preferred more 1-MCP treated fruit when stored longer.

Table 2.6: Effect of 1-MCP treatment and storage condition on consumer preference (%).

	3 months	5 months
cold storage, control	11.8	7.5
cold storage, 1-MCP	22.9	19.2
ULO-storage, control	28.1	27.7
ULO-storage, 1-MCP	37.3	45.5
control	39.9	35.2
1-MCP	60.1	64.8
cold storage	34.6	26.8
ULO-storage	65.4	73.2
# consumer	153	213

In 2004 a survey was conducted by asking 118 consumers 'Would you also purchase apples which have additionally been treated with a fruit ripening regulator to preserve their quality and freshness?'. The older the respondent, the more answered with 'No' (< 20 years: 32 %, 20 - 39 years: 42 %, 40 - 60 years: 49 %, > 60 years: 56 %). However, in the group of consumer between 20 - 39 years the number of pro and con answers was the same (Figure 2.5).

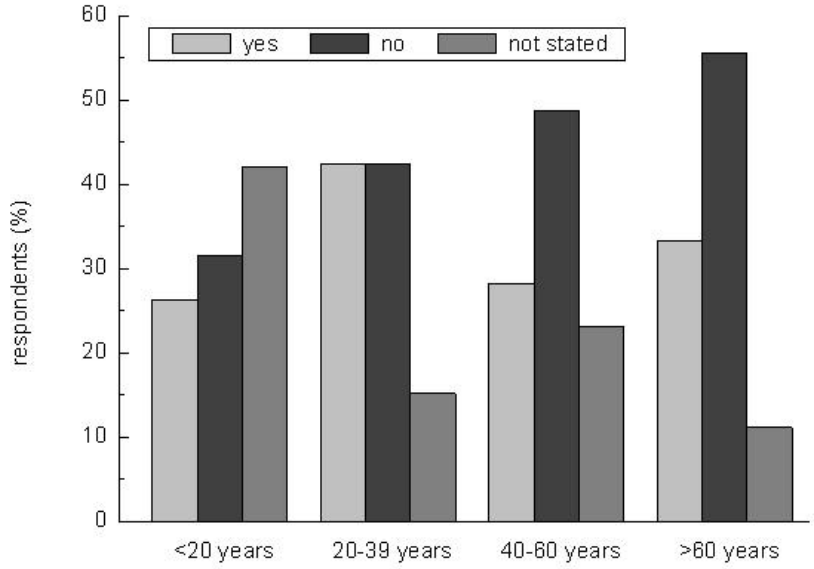


Figure 2.5: Consumers response (%) of various age groups to the question: 'Would you also purchase apples which have additionally been treated with a fruit ripening regulator to preserve their quality and freshness?'

2.4 Discussion

In general, fruit quality decreased during storage and shelf-life depending on storage duration, storage condition and 1-MCP treatment. This effect was found in all cases, regardless of harvest date or storage condition but was more pronounced in cold storage.

Fruit quality is made in the orchard during the growing season, and at best, can be maintained but never be improved by any postharvest technology (Hewett, 2006). Harvested apples are still living biological systems with an active metabolism and they deteriorate sooner or later.

1-MCP, an effective ethylene inhibitor (Sisler et al., 1996; Fan and Mattheis, 1999; Tatsuki et al., 2007) has the ability to inhibit or reduce the autocatalytic production of ethylene, hence to prevent or delay ethylene-dependent quality loss in climacteric fruit. In contrast to ethylene, the binding of 1-MCP to the receptors present at the time of treatment is irreversible (Sisler et al., 1996; Blankenship and Dole, 2003*)*. This is why 1-MCP is not only effective in maintaining fruit quality during storage but also during shelf-life of climacteric fruit (Fan et al., 1999; Lafer, 2003). In agreement with Saftner et al. (2003), 1-MCP delayed ripening more than CA-

or even ULO-storage. However, ethylene binds to receptors that were produced after 1-MCP treatment (Watkins et al., 2000; Tatsuki et al., 2007). This is why ripening after 1-MCP treatment was not entirely inhibited but reduced or delayed (Figure 1, 2). Compared to fruit firmness at harvest of 70.6 N, 1-MCP treated fruit stored for 3, 6 and 9 months in ULO-storage followed by 10 d shelf-life had a decline in firmness to 67.6, 64.7 and 56.8 N, respectively. The reduction in firmness was even more pronounced for fruit stored in cold storage + 1-MCP. After 3, 6 and 9 months of storage fruit firmness declined to 43.1, 38.2 and 33.3 N, respectively. In general, retention of firmness was better in ULO-storage than in CA and cold storage, but a combination of ULO with 1-MCP produced even firmer fruit ex-store. Similar findings were reported by Watkins et al., (2000).

The greatest impact of 1-MCP was seen in a clear firmness retention in all samples, regardless of storage condition and duration. Several other reports on the effect of 1-MCP on maintenance of flesh firmness during storage are consistent with these findings (Fan et al., 1999; Watkins et al., 2000; Lafer, 2003; Moya-Leon et al., 2007 and Tatsuki et al., 2007).

Effects of 1-MCP on other quality parameters such as soluble solids concentration (SSC), titratable acidity (TA) and retention of green background colour (BGC) are not consistently described in other reports. While Mir et al. (2001) found no effect of 1-MCP on TA of 'Redchief Delicious', many other authors like Watkins et al. (2000), DeEll et al. (2002), Saftner et al. (2003) and Johnson (2003) reported a loss of acidity of different apple cultivars during 1-MCP-storage.

Similar unequivocal 1-MCP effects were found on SSC; 1-MCP treated fruit can have similar (Fan et al., 1999) or higher (Fan et al., 1999; Fan and Mattheis, 1999) sugar concentrations than untreated controls. It is unclear whether SSC accumulation is necessarily depending on ethylene (Fan et al., 1999). The retention of TA and SSC by 1-MCP may result from the lower respiration rate of 1-MCP treated fruit (Fan and Mattheis, 1999).

Chlorophyll degradation was prevented or at least delayed after 1-MCP treatment in the experiments conducted by Fan and Mattheis (1999), Johnson (2003) and Saftner et al. (2003). In general, BGC values declined during the entire storage duration, regardless of storage condition and 1-MCP treatment. Treatments, storage conditions and –durations showed significant effects but were influenced by several interactions. However, green BGC was best maintained in ULO-storage, followed by CA-storage and cold storage. During ripening most of climacteric fruits show a rapid loss of green colour, which is due to degradation of chlorophyll struc-

tures (Wills et al., 2007). The yellow to red colour of apple fruit, which is due to anthocyanins and carotenoids in the peel, becomes visible with chlorophyll decline (Kays, 1999; Niemann, 2005; Wills et al., 2007). Colour development is promoted by light (Chalmers and Faragher, 1977), temperature (Blankenship, 1987) and ethylene (Chalmers and Faragher, 1977; Saks et al., 1990) during fruit development and maturation. Carotenoid synthesis also occurs *de novo* during ripening and Knee (1988) suggested that the synthesis of carotenoids in ripening apples might be an ethylene-dependent process. Our results support this hypothesis. Since all fruit were grown under same conditions the differences in BGC after storage must be due to varying ethylene synthesis rates. Ethylene production significantly decreased from cold storage to CA- and ULO-storage (data not shown; Heyn et al., 2009, submitted). However, it is unclear why application of 1-MCP, an ethylene inhibitor, did not influence BGC significantly. Consistent trends of single quality parameters for each harvest are difficult to realize due to many significant interactions. The inconsistent effect of 1-MCP on several quality parameters is likely due to cultivar specific responses to 1-MCP (Watkins et al., 2000; DeEll et al., 2002; Watkins, 2006) and that not all quality parameters are ethylene-dependent. The efficacy of 1-MCP refers just to retention of ethylene-dependent quality and ripening parameters (De Castro et al., 2003; Saftner et al., 2003).

Johnson (2003) suggests that 1-MCP treated fruit can be stored in air 30 days longer than untreated apples. Moreover, elevated temperatures in apple storage might be possible after 1-MCP treatment (Mir et al., 2001). However, to achieve the best efficacy of 1-MCP fruit need to be picked at the appropriate stage of maturity for long-term storage (Johnson, 2003). The production of new receptors as well as the affinity of 1-MCP might be dependent on the ethylene production rate at the time of and after 1-MCP treatment (Tatsuki et al., 2007). Moreover, the efficacy of 1-MCP is depending on various factors such as treatment temperature (Mir et al., 2001; DeEll et al., 2002), storage atmosphere and -duration (Watkins et al., 2000; DeEll et al., 2002; Johnson, 2003), cultivar (Watkins et al., 2000; DeEll et al., 2002; Blankenship and Dole, 2003) and time between harvest and 1-MCP treatment (Blankenship and Dole, 2003; Tatsuki et al., 2007). The longer the time from harvest to 1-MCP the less the effect of 1-MCP on retention of quality parameters (Tatsuki et al., 2007).

It is noticeable that the greatest difference of firmness between untreated and 1-MCP treated fruit was after 6 months of storage (48.6 %). Difference of firmness after 3 and 9 months was 36.4 and 36.1 %, respectively. This suggests that the efficacy of 1-MCP declines greatly after

6 months of storage. Johnson (2003) reported diminished beneficial effects of 1-MCP when fruit was harvested late and stored long. While reduced effects of 1-MCP in long-term storage (> 6 months) were also found in this study, 1-MCP effects on several quality attributes were rather inconsistent when fruit harvest was delayed. Both, CA-storage and 1-MCP treatment delayed ripening of 'Jonagold' apples as indicated by better maintenance of flesh firmness, titratable acidity, less loss of green background colour, reduced respiration and ethylene production rates (data not shown). The instrumental quality measurements suggest that the 1-MCP effects on apple quality retention of cold stored fruit are similar to those of CA-storage.

Since not all ripening and quality parameters are ethylene-dependent, not all of them will be regulated and influenced by 1-MCP in the same intensity. That means that some parameters such as flesh firmness are well maintained following 1-MCP treatment, while others may be considerably changing during storage and especially shelf-life. This might have an impact on the overall quality of the fruit and the consumer acceptance of 1-MCP treated apples.

Consumer preference profiling in 2007 was conducted to investigate the effect of 1-MCP on fruit quality of 'Jonagold' apples. Fruit quality was evaluated by instrumental measurements and correlated with sensory outputs from consumer panels. It is important to perform consumer tests with products subjected to new technologies, such as 1-MCP treatment on apple storage, in order to realize consumers' opinion. Dissatisfied consumer who had unpleasant experience might stop buying this variety, apples from this origin or in the worst case change to competitive products like banana, oranges (Harker et al., 2003) or even manufactured snacks. Especially when fruits have to compete with other fruit types or other snacks, product quality is of particular importance (Jack et al., 1997). 1-MCP treatments might impair the development of typical aroma and flavour compounds due to the reduction of ethylene production (Saftner et al., 2003; Fan and Mattheis, 1999).

Although a correlation coefficient between instrumental values and sensory scores obtained by consumer preference mappings was not calculated, Figure 2.3 clearly indicates that all analytical measurements are in good agreement with corresponding sensory evaluations. Overall, the scores of firmness, sweetness, tartness and background colour followed the trend for instrumental values of firmness, SSC, TA and BGC, respectively. In general it seems that consumers were satisfied with the fruit quality of all given treatments. For example following 5

months cold storage untreated fruit had the lowest firmness (34.3 N) and was still scored 'too soft'. Results were similar for tartness and TA. Untreated and cold stored fruit with just 2.2 g malic acid per litre did not even reach the score 'too little'. Acceptability of apples by consumers seems to depend on varying interacting factors. However it is interesting to understand which fruit attributes mainly drive consumer preference in order to adapt apple quality with the help of postharvest technologies.

Most consumers, regardless of age or gender, preferred the 1-MCP treated fruit in ULO-storage and particular when fruit was stored longer (Figure 2.4, Table 2.6). In contrast the preference of 1-MCP treated apples held in cold storage declined with storage time. We therefore conclude that firmness and tartness ('freshness') were the most important drivers of consumer preference. Firmness, or in the broader sense texture, is also described in many other reports as a crucial factor for consumers acceptance (Daillant-Spinnler et al., 1996; Jaeger et al., 1998; Shewfelt, 1999; Harker et al., 2002; Höhn et al., 2003). Harker et al. (2002) stated that improving firmness is likely to increase sales and our results support this hypothesis. Daillant-Spinnler et al. (1996), Jaeger et al. (1998), Höhn et al. (2003) and López et al. (2007) describe also sweetness as an important determinant of consumer preference. However, SSC was not different between the treatments cold storage + 1-MCP and ULO-storage ± 1-MCP, hence sweetness was not an important factor for consumer preference in our study.

MacFie and Hedderely (1993) refer to some flaws and difficulties in relating sensory data to instrumental measurements. To generate a representative result from consumer test panels it is important to include a sufficient number of participants in the questionnaire (MacFie and Hedderely, 1993; Cliff et al., 1998). The number of participating consumer for fruit from the first harvest were 97 after 3 months storage and 108 after 5 months storage (Table 2.2). Number of participating consumers was just 52 for evaluating fruit from second harvest after 3 months storage, presumably due to the unsuitably weather conditions. After 5 months storage, 108 consumer were participating in mapping acceptability of fruit from the second harvest. Cliff et al. (1998) recommended that panels should consist of at least 100 consumer, hence most of our results were representative estimates of customer preferences.

Consumer tests are time-consuming and laborious to obtain meaningful data (Hampson et al. 2000). Consequently, most of the postharvest fruit quality assessment is based on instrumental measurements without knowing consumer preferences. There are inconsistent results in the

literature regarding the use and relevance of instrumental measurements for predicting consumer preferences. Moreover, there are varying definitions of quality (Moskowitz, 1995) depending on gender, age, ethnic groups and consumers often do not agree in their preferences. Preference and perceived quality might also be influenced by the intended use of the produce (Oude Ophuis and Van Trijp, 1995; Tromp, 2005). Some of the participating consumer explained they would have chosen fruit stored in cold storage for baking apple cake while they would have taken fruit stored in ULO as a fresh snack. These statements were mostly based on the appearance of the different treatments. Appearance is the first (Francis, 1995; Kays, 1999; Lange et al., 2000) and in most cases the only quality parameter consumer can judge at the point of sale. If the appearance is unacceptable for an individual consumer, he or she would not buy that apple, even if other quality factors would be satisfying. Interactions between different quality attributes are obvious, for example it is known that there is a clear effect of colour on the perception of sweetness (Francis, 1995). Consequently it is not clear whether the acceptance or rating of the fruit was entirely due to sensory parameters or also related to consumers expectations after viewing the fruit. Similar situation was described by Daillant-Spinnler et al. (1996). In this context it is important to distinguish between expected, perceived and actual quality. Consumers' expectations of fruit quality are due to product information and the memory of previous eating experiences (Harker et al., 2002, 2003; Höhn et al., 2003), which might be influenced by exaggerations of good and bad points in the course of time (Schutz, 1999; Harker et al., 2003). Availability of product information is a very important point for the consumer. Consumers need to be informed about the origin of the food, the production pathway and possible treatments to decide whether to buy and consume the fruit or not. The results of our 2004 survey might be affected by the lack of information we provided. The question if consumers 'Would purchase apples which have additionally been treated with a fruit ripening regulator to preserve their quality and freshness' did not provide any specific product information. The consumer was not informed about the substance, its mode of action, the application rate and duration and the impact on consumer and the environment. When this type of information would have been made available, the results might have been different and 45.8 % of overall consumers may not have answered 'no'. However, consumers are increasingly concerned about food safety (Moskowitz, 1995; Paull, 1999), pesticides (Harker et al., 2002) and chemical use in food production systems. Cardello (1995) stated that not the actual measurable quality (or safety) is important for consumer, but the

perceived quality (or safety). Consumers perception of food is not constant and changes with people, time and situation (Lawless, 1995; Cardello, 1995; Harker et al., 2002). Perception of food is also influenced by experience and knowledge, hence age is important. The perceived quality is determined by many extrinsic and intrinsic factors (Oude Ophuis and Van Trijp, 1995; Lange et al., 2000; Hewett, 2006) and interactions with consumers' expectations. While intrinsic quality attributes like size, colour, sweetness or firmness are physical characteristics of the fruit and easy to estimate, extrinsic factors, such as production method and technology, chemical treatments and special storage techniques (Hewett, 2006) are mostly unknown to the consumer. Extrinsic factors are very useful for producers and throughout the distribution chain, because changes are not (immediately) recognized by the consumer (Oude Ophuis and Van Trijp, 1995) and might provide opportunities to reduce costs. Therefore the extrinsic factors are important additional information for consumers and they might have an impact on purchasing decision. The results of our survey in 2004 are indicative of this.
Moreover, the results of consumer preference mapping might be influenced by the presentation of the sample, the environment and the way how the moderator of the experiment interacts with the panelists (Schutz, 1999). Consumer tests should ensure that questions can be easily understood and answered and the questionnaire should not take much time.

A limitation of consumer data is the so-called halo-effect (Lawless, 1995; Schutz, 1999), which describes 'a tendency for an estimate of judgment to be influenced by an irrelevant or only loosely associated factor, impression, etc.' (Colliers's Dictionary, 1994). Due to the halo-effect judgments are not objective. A slight tendency of a halo-effect might be seen in some of the results of our preference mapping sessions. While no significant differences in the analytical measurements of SSC were found between the treatments cold storage + 1-MCP and ULO-storage ± 1-MCP, consumers scores for ULO stored apples had the tendency to have little sweetness, while fruit stored in cold storage were 'just right'. Assessments of single fruit characters also seem to be affected by other characters. For example, it is noticeable that sweetness was scored low when titratable acidity was found high and fruit were green.

Nevertheless sensory evaluation studies are a useful tool to assess food quality and consumer preference (Cardello, 1995; Lawless, 1995; Daillant-Spinnler et al., 1996; Schutz, 1999). Although consumers might be less sensitive to quality issues than a trained panel they do represent product preference behaviour and market signals.

2.5 References

AgroFresh Inc., a Rohm and Haas Company (July 2003) *SmartFresh™ The Smart Choice for Apples. A Breakthrough in Fruit Storage Technology for Maintaining "fresh-picked" Apple Quality*. Apple Tech. Bul.

Blankenship, S.M. (1987) Night-temperature effects on rate of apple fruit maturation and fruit quality. *Scientia Hort.* 33, 205-212.

Blankenship, S.M. and J.M. Dole. (2003) 1-Methylcyclopropene: a review. *Postharvest Biol. Technol.* 28, 1-25.

Cardello, A.V. (1995) Food quality: relativity, context and consumer expectations. *Food Qual. Pref.* 6, 163-170.

Castro, de, E., W.V. Biasi and E. J. Mitcham. (2007) Quality of Pink Lady apples in relation to maturity at harvest, prestorage treatments, and controlled atmosphere during storage. *HortScience* 42(3), 605-610.

Chalmers, D.J. and J.D. Faragher. (1977) Regulation of anthocyanin synthesis in apple skin. II. Involvement of ethylene. *Aust. J. Plant Physiol.* 4, 123-131.

Cliff, M.A., M.C. King and C. Hampson. (1998) Comparison of mean scores and R-indices for consumer preferences of apple cultivars. *HortScience* 33(7), 1239-1240.

Colliers's Dictionary, (1994).

Daillant-Spinnler, B., H.J.H. MacFie, P.K. Beyts and D. Hedderley. (1996) Relationships between perceived sensory properties and major preference directions of 12 varieties of apples from the southern hemisphere. *Food Qual. Pref.* 7(2), 113-126.

DeEll, J.R., D.P. Murr, M.D. Porteous and H.P.V. Rupasinghe. (2002) Influence of temperature and duration of 1-Methylcyclopropene (1-MCP) treatment on apple quality. *Postharvest Biol. Technol.* 24, 349-353.

Echeverría, G., J. Graell and M.L. López. (2002) Effect of harvest date and storage conditions on quality and aroma production of 'Fuji' apples. *Food Sci. Tech. Int.* 8(6), 351-360.

Ellinger, W. (2006) *ZMP-Marktbilanz Obst 2006*. Deutschland, EU, Weltmarkt, 32.

Fan, X. and J.P. Mattheis. (1999) Development of apple superficial scald, soft scald, core flush, and greasiness is reduced by MCP. *J. Agric. Food Chem.* 47, 3063-3068.

Fan, X., S.M. Blankenship and J.P. Mattheis. (1999) 1-Methylcyclopropene inhibits apple ripening. *J. Amer. Soc. Hort. Sci.* 124(6), 690-695.

Francis, F.J. (1995) Quality as influenced by color. *Food Qual. Pref.* 6, 149-155.

Girard, B. and O.L. Lau. (1995) Effect of maturity and storage on quality and volatile production of 'Jonagold' apples. *Food Res. Int.* 28(5), 465-471.

Hampson, C.R., H.A. Quamme, J.W. Hall, R.A. MacDonald, M.C. King and M.A. Cliff. (2000) Sensory evaluation as a selection tool in apple breeding. *Euphytica* 111, 79-90.

Harker, F.R, F.A. Gunson and S.R. Jaeger. (2003) The case for fruit quality: an interpretive review of consumer attitudes, and preferences for apples. *Postharvest Biol. Technol.* 28, 333-347.

Harker, F.R., F.A. Gunson, P.L. Brookfield and A. White. (2002) An apple a day: the influence of memory on consumer judgment of quality. *Food Qual. Pref.* 13, 173-179.

Hewett, E.W. (2006) An overview of preharvest factors influencing postharvest quality of horticultural products. *Int. J. Postharvest Technol. Inn.* 1(1), 4-15.

Höhn, E., F. Grasser, B. Guggenbühl and U. Künsch. (2003) Efficacy of instrumental measurements for determination of minimum requirements of firmness, soluble solids, and acidity of several apple varieties in comparison to consumer expectations. *Postharvest Biol. Technol.* 27, 27-37.

Hurndall, R.F., M.C. Dodd, R. Bester and P. de Vries. (1994) Management of fruit quality in relation to harvest maturity and storage potential of apples. *The post-harvest treatment of fruit and vegetables – current status and future prospects*; Proceedings of the sixth international symposium of the European concerted action program COST 94, 19-22 Oct. 1994, Oosterbeek, The Netherlands; 289-294.

Jack, F.R., J. O'Neill, M.G. Piacentini and M.J.A. Schröder. (1997) Perception of fruit as a snack: a comparison with manufactured snack foods. *Food Qual. Pref.* 8(3), 175-182.

Jaeger, S.R., Z. Andani, I.N. Wakeling and H.J.H. MacFie. (1998) Consumer preferences for fresh and aged apples: a cross-cultural comparison. *Food Qual. Pref.* 9(5), 355-366.

Jager de, A. (1994) Review of the workshop on determination and prediction of optimum harvest date of apples and pears. *The post-harvest treatment of fruit and vegetables – current status and future prospects*; Proceedings of the sixth international symposium of the European concerted action program COST 94, 19-22 Oct. 1994, Oosterbeek, The Netherlands; 267-279.

Jeffrey, D., C. Smith, P. Goodenough, I. Prosser and D. Grierson. (1984) Ethylene-independent and ethylene-dependent biochemical changes in ripening tomatoes. *Plant Physiol.* 74, 32-38.

Johnson, D.S. (2000) Mineral composition, harvest maturity and storage quality of 'Red Pippin', 'Gala' and 'Jonagold' apples. *J. Hort. Sci. Biotechnol.* 75(6), 697-704.

Johnson, D.S. (2003) Improvement in the storage quality of apples in the UK by use of 1-MCP (SmartFresh^{TM}). *Acta Hort.* 599, 39-45.

Johnston, J.W., E.W. Hewett and M.L.A.T.M. Hertog. (2002) Postharvest softening of apple (*Malus domestica*) fruit: a review. *N.Z. J. Crop Hort. Sci.* 30, 145-160.

Kader, A.A. (2002) Fruits in the global market. In: Knee, M. (Ed.), *Fruit Quality and its biological basis*. Sheffield Academic Press Ltd.; 1-16.

Kays, S.J. (1999) Preharvest factors affecting appearance. *Postharvest Biol. Technol.* 15, 233-247.

Knee, M. (1988) Carotenol esters in developing apple fruits. *Phytochemistry* 27(4), 1005-1009.

Lafer, G. (2003) Effects of 1-MCP treatments on fruit quality and storability of different apple varieties. *Acta Hort.* 599, 65-69.

Lange, C., S. Issanchou and P. Combris. (2000) Expected versus experienced quality: trade-off with price. *Food Qual. Pref.* 11, 289-297.

Lau, O.L. (1986) Dessert quality and storability of Jonagold apples. 30. Oct. 2007, http://postharvest.tfrec.wsu.edu.

Lau, O.L. (1988) Harvest indices, dessert quality, and storability of 'Jonagold' apples in air and controlled atmosphere storage. *J. Amer. Soc. Hort. Sci.* 113(4), 564-569.

Lawless, H. (1995) Dimensions of sensory quality: a critique. *Food Qual. Pref.* 6, 191-199.

López, M.L., C. Villatoro, T. Fuentes, J. Graell, I. Lara and G. Echeverría. (2007) Volatile compounds, quality parameters and consumer acceptance of 'Pink Lady®' apples stored in different conditions. *Postharvest Biol. Technol.* 43, 55-66.

MacFie, H.J.H. and D. Hedderley. (1993) Current practice in relating sensory perception to instrumental measurements. *Food Qual. Pref.* 4, 41-49.

Mir, N.A., E. Curell, N. Khan, M. Whitaker and R.M. Beaudry. (2001) Harvest maturity, storage temperature, and 1-MCP application frequency alter firmness retention and chlorophyll fluorescence of 'Redchief Delicious' apples. *J. Amer. Soc. Hort. Sci.* 126(5), 618-624.

Moskowitz, H.R. (1995) Food quality: conceptual and sensory aspects. *Food Qual. Pref.* 6, 157-162.

Moya-Leon, M.A., M. Vergara, C. Bravo, M. Pereira and C. Moggia. (2007) Development of aroma compounds and sensory quality of 'Royal Gala' apples during storage. *J. Hort. Sci. Biotechnol.* 82(3), 403-413.

Niemann, N. (2005) *The effect of 1-MCP, controlled atmosphere and heat treatment on Santa tomatoes.* PhD Thesis, University of Johannesburg.

Oude Ophuis, P.A.M. and H.C.M. Van Trijp. (1995) Perceived quality: a market driven and consumer oriented approach. *Food Qual. Pref.* 6, 177-183.

Paull, R.E. (1999) Effect of temperature and relative humidity on fresh commodity quality. *Postharvest Biol. Technol.* 15, 263-277.

Plotto, A., A. N. Azarenko, M.R. McDaniel, P.W. Crockett and J.P. Mattheis. (1997) Eating quality of 'Gala' and 'Fuji' apples from multiple harvests and storage durations. *HortScience* 32(5), 903-908.

Saftner, R.A., J.A. Abbott, W.S. Conway and C.L. Barden. (2003) Effects of 1-Methylcyclopropene and heat treatments on ripening and postharvest decay in 'Golden Delicious' apples. *J. Amer. Soc. Hort. Sci.* 128(1), 120-127.

Saks, Y., L. Sonego and R. Ben-Arie. (1990) Artificial light enhances red pigmentation, but not ripening, of harvested 'Anna' apples. *HortScience* 25(5), 547-549.

Schutz, H.G. (1999) Consumer data – sense and nonsense. *Food Qual. Pref.* 10, 245-251.

Shewfelt, R.L. (1999) What is quality? *Postharvest Biol. Technol.* 15, 197-200.

Silbereisen, R. (1982) Die Apfelsorte 'Jonagold' im Anbau- und Qualitätsvergleich mit anderen Sorten. *Erwerbsobstbau* 24, 76-81, 96-103.

Sisler, E.C., E. Dupille and M. Serek. (1996) Effect of 1-Methylcyclopropene and methylenecyclopropane on ethylene binding and ethylene action on cut carnations. *Plant Growth Regul.* 18, 79-86.

Stow, J. (1987) Storage of 'Jonagold' apples. *Scientia Hort.* 31, 245-251.

Tatsuki, M., A. Endo and H. Ohkawa. (2007) Influence of time from harvest to 1-MCP treatment on apple fruit quality and expression of genes for ethylene biosynthesis enzymes and ethylene receptors. *Postharvest Biol. Technol.* 43, 28-35.

Tijskens, L.M.M., M. Sloof and E.C. Wilkinson. (1994) Quality of perishable produce. A philosophical approach. *The post-harvest treatment of fruit and vegetables – current status and future prospects*; Proceedings of the sixth international symposium of the European concerted action program COST 94, 19-22 Oct. 1994, Oosterbeek, The Netherlands; 493-502.

Tromp, J. (2005) Fruit ripening and quality. In: Tromp, J., A.D. Webster and S.J. Wertheim (Eds.), *Fundamentals of Temperate Zone Tree Fruit Production*. Backhuys Publishers, Leiden, The Netherlands; 295-310.

Watada, A.E., J.A. Abbott and R.E. Hardenburg. (1980) Sensory characteristics of apple fruit. *J. Amer. Soc. Hort. Sci.* 105(3), 371-375.

Watada, A.E., R.C. Herner, A.A. Kader, R.J. Romani and G.L. Staby. (1984) Terminology for the description of developmental stages of horticultural crops. *HortScience* 19(1), 20-21.

Watkins, C.B. (2006) 1-Methylcyclopropene (1-MCP) based technologies for storage and shelf life extension. *Int. J. Postharvest Technol. Inn.* 1(1), 62-68.

Watkins, C.B., J.F. Nock and B.D. Whitaker. (2000) Responses of early, mid and late season apple cultivars to postharvest application of 1-Methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. *Postharvest Biol. Technol.* 19, 17-32.

Wills, R.B.H., W.B. McGlasson, D. Graham and D.C. Joyce. (2007) *Postharvest: an introduction to the physiology and handling of fruit, vegetables and ornamentals*. 5th Edition. CABI.

3.

Effect of 1-MCP on Climacteric Characteristics of Apple Fruit

Abstract

'Jonagold' apple fruit (*Malus domestica* Borkh.) were picked at commercial maturity in 2004 and 2005. Fruit were treated with 1-MCP on the day of harvest in 2004 and 7 days after harvest in 2005 and stored the following day either in cold storage, controlled atmosphere- (CA) or ultra low oxygen-(ULO) storage. Fruit samples from each treatment were removed after 2, 4 and 6 months (2004/05) and 3, 6 and 9 months (2005/06) to examine the effect of 1-MCP, storage condition and –duration on ethylene production and respiration rate of 'Jonagold' apples. ATP and ADP concentration was additionally determined in 2005/06. Fruit respiration rate was measured daily during 10 days shelf-life (at 20°C) in terms of CO_2-production. Measurements of ethylene production were performed every third day during 10 days at 20°C in 2004/05 and 11 days in 2005/06, respectively. Concentrations of ATP and ADP were determined by bioluminescence technique and ATP detection kit (luciferin-luciferase test kit) after harvest, commencement of storage and after each storage removal plus 10 days ripening. 1-MCP treatment had highly significant effects on fruit ethylene production and shelf-life respiration in both years. Although fruit ethylene production and respiration rate were significantly reduced in CA and ULO-storage, both processes were even greater inhibited by 1-MCP when compared to untreated control fruit. The magnitude of 'Jonagold' respiration and ethylene production rates was higher in 2005/06 than in 2004/05 likely due to late 1-MCP application and commencement of storage. However, the 1-MCP effect on fruit ethylene production during shelf-life diminished with storage duration in both years. Efficacy of 1-MCP is primarily influenced by storage condition and -duration, treatment temperature, time from harvest to treatment and commencement of storage.

3.1 Introduction

The plant hormone ethylene regulates, in interaction with the other plant hormones, many aspects of plant growth, development and the initiation of fruit ripening (Lieberman, 1979; Yang and Hoffman, 1984; Abeles et al., 1992; Dong et al., 1992). Ethylene biosynthesis is regulated by developmental and environmental factors (Yang, 1980; Yang and Hoffman, 1984; Mathooko, 1996) and the production rate is generally low in climacteric fruit close to the beginning of ripening (<0.005 µL/L (Wills et al., 2007).

Ethylene plays a critical role in ripening of climacteric fruit like apple. Ripening is the plant process by which the physiologically mature but inedible fruit obtains its characteristic appearance and eating quality (Kader, 2002; Watkins, 2002; Wills et al., 2007). Most of the climacteric fruit like apple, pear, apricot, banana, peach and kiwifruit, can be harvested at mature stage and ripen then off the plant (Kader, 2002). Climacteric fruit, in contrast to non-climacteric fruit exhibit a distinct upsurge in respiration and ethylene biosynthesis rates at the beginning of ripening (Abeles et al., 1992; Giovannoni, 2001; Wills et al., 2007). They generally reach the fully ripe stage after the respiratory climacteric (Wills et al., 2007). Non-climacteric fruits, such as cherry, citrus, strawberry and grape, may respond dose-dependent to exogenous applied ethylene with a temporary increase in respiration (Wills et al., 2007). However, ethylene is not required for ripening of non-climacteric fruit (Abeles et al., 1992; Giovannoni, 2001; Fleancu, 2007).

Normal fruit ripening of climacteric fruit requires energy (Tromp, 2005). Therefore respiration has to increase to provide the energy used in the catabolic processes during ripening (Abeles et al., 1992). Accompanied with the respiratory rise at the beginning of ripening, adenosine triphosphate (ATP) level increases (Liebermann, 1979; Brady, 1987; Tan, 1999) which is described by Mir and Beaudry (2002) as a kind of ‘currency’ for metabolic processes in plant tissues. Tan (1999) found that the production of ATP is directly related with the respiration rate and an inhibition of respiration results in decreased ATP levels (Solomos and Laties, 1976; de Wild, et al., 1999).

McMurchie et al. (1972) described a model of ethylene biosynthesis by separating between system I and system II ethylene. System I ethylene is found in all vegetative tissues, non-climacteric fruit and immature climacteric fruits (Brady, 1987). Generally, fruit become more

sensitive to ethylene during development and maturation (Abeles et al., 1992; Wills et al., 2007). After a certain ethylene level is reached in fruit during maturation and the fruit reached the competency to ripen (McMurchie et al., 1972; Bufler, 1986), the respiratory climacteric is initiated. Accompanied with the respiratory rise the autocatalytic system II ethylene biosynthesis is induced.

It has been shown that ethylene regulates its own biosynthesis (Yang and Hoffman, 1984). In vegetative tissues and during the pre-climacteric phase (system I) of climacteric fruit there is a negative feedback regulation and ethylene inhibits its own synthesis (Yang and Hoffman, 1984; Nakatsuka et al., 1997, 1998; de Wild et al., 2003). In the climacteric stage, which is introduced by an upsurge in absolute ethylene levels, apples and climacteric fruit in general, are under positive feedback regulation. Continuous availability of ethylene is needed to induce the autocatalytic system II ethylene production and to commence the ripening process (Bufler, 1984, 1986; Sisler et al., 1996). The autocatalytic ethylene production (system II) and the accompanied rise in respiration, is characteristic of ripening climacteric fruits (Burg and Burg, 1967; Zarembinski and Theologis, 1994; Nakatsuka et al., 1998; Wills et al., 2007).

The pathway of ethylene biosynthesis was elucidated and described in detail by Adams and Yang (1979) and Yang and Hoffman (1984). The amino acid methionine, the overall precursor of ethylene is converted by the enzyme S-adenosyl-methionine synthase (SAM synthase) to S-adenosyl-methionine (SAM) (Kende, 1993; Zarembinski and Theologis, 1994). For the production of SAM, ATP is required (Lieberman, 1979; Yang, 1980; Kende, 1993). The enzymes involved in the pathway of ethylene synthesis are ACC synthase (ACC-S) and ACC oxidase (ACC-O). ACC-S converts SAM to the intermediate ACC, which is in turn catalyzed by ACC-O to ethylene. The conversion of SAM to ACC by the enzyme ACC-S is the rate-limiting step in the ethylene biosynthesis (Adams and Yang, 1979; Yang, 1980; Hoffman and Yang, 1980; Yang and Hoffman, 1984). The conversion of ACC to ethylene requires oxygen (Adams and Yang, 1979; Yang, 1980; Zarembinski and Theologis, 1994; Mathooko, 1996) and Dong et al. (1992) state that ACC-O also requires CO_2 for its activity. The autocatalytic ethylene production (system II) is due to increased abundance of ACC-S and ACC-O (Abeles et al., 1992; Rupasinghe et al, 2000).

Ethylene needs to bind to specific ethylene binding sites (receptors) to induce the many biochemical changes associated with ripening (e.g. colour change from green to yellow, aroma

development, softening, increased respiration) (Watkins and Nock, 2000; Wills et al., 2007) and to stimulate further ethylene biosynthesis (positive feedback regulation) (Gorny and Kader, 1996a). The binding of ethylene to the receptors is reversible (Lieberman, 1979; Wills et al., 2007).

The main concern of postharvest technology is to minimize the exposure of harvested climacteric fruit to ethylene and in general to slow down the overall metabolism. It is commercial practice to store apples at controlled atmospheres with low O_2-concentrations and/or elevated CO_2-concentrations in combination with low temperatures which reduce ethylene biosynthesis, ethylene sensitivity and responses (Li et al., 1983; Abeles et al., 1992; Mir and Beaudry, 2002; Wills et al., 2007) during storage.

1-Methylcyclopropene (1-MCP) is an effective inhibitor of ethylene synthesis and action which is acting at the receptor binding sites (Sisler et al., 1996; Blankenship and Dole, 2003). Because 1-MCP protects apples from both endogenous and exogenous ethylene (Blankenship and Dole, 2003), it seems to be a promising tool in postharvest technology (Watkins, 2002).

The research objective of this study was to examine the effect of 1-MCP, storage condition and –duration on ethylene production and accompanied respiration rate as well as ATP concentration of 'Jonagold' apples. Since 1-MCP inhibits ethylene action we suggested that both respiration rate and ATP concentration would be reduced after 1-MCP application. Are there differences in the efficacy of 1-MCP on climacteric characteristics due to time between harvest and 1-MCP treatment, treatment temperature, commencement of storage, storage condition and -duration?

3.2 Materials and methods

3.2.1 Plant material and harvest management

The experiments were carried out at the 'Kompetenzzentrum Obstbau – Bodensee' (KOB), Ravensburg, Germany, using the apple cultivar 'Jonagold' (*Malus domestica* Borkh.) in the 2004/05 and 2005/06 growing season. 'Jonagold' fruit were picked at commercial maturity at the research orchard in 2004 and at a commercial orchard in 2005 (Table 3.1). All trees were grown on rootstock M.9 and trained as slender spindle.

3.2.2 1-MCP treatments

Immediately after harvest fruit were colour- and size-graded by hand. Fruit were divided at random in 12 kg plastic boxes, according to number of storage conditions, storage duration, 1-MCP vs. control and replications.

In 2004 fruit were 1-MCP treated immediately after harvest (0 DAH). Half of the boxes with fruit were placed in gas–tight storage containers (volume 0.560 m³) and treated with 625 ppb 1-Methylcyclopropene (1-MCP) for 24 hours. The temperature of the container was at 1°C (± 0.5), the actual temperature of the apples during treatment with 1-MCP was not recorded. Control fruit were held at the same conditions but without 1-MCP.

To simulate commercial conditions fruit were held in cold storage (1°C, 92 % relative humidity (RH)) for 6 days prior to MCP–treatments in 2005 (Table 3.1). 1-MCP treatment on day 7 after harvest (7 DAH) was performed as described above.

Following 1-MCP treatment, all boxes with treated and untreated fruit were divided at random into three equal groups and each placed in a different storage atmosphere.

3.2.3 Fruit storage and sample collection

Fruit were stored for up to 6 month in 2004/05 and 9 month in 2005/06 (Table 3.1) in: cold storage, CA (0.8 % CO_2, 3 % O_2) and ULO (3 % CO_2, 1 % O_2). Temperature was about 1°C (± 0.5) and RH at 92 % (± 2 %) in each storage atmosphere. Fruit boxes for cold storage were covered lightly with plastic sheets to minimize water loss.

After 2, 4 and 6 months (2004/05) and 3, 6 and 9 months (2005/06) representative samples of 4 fruit were removed from each storage (cold storage, CA, ULO, ± 1-MCP) to measure respiration and ethylene production rates during 10 days at 20°C (shelf-life).

In 2005/06 ATP and ADP concentration in fruit samples was also determined. Samples were taken at harvest plus 10 days at 20°C and following each storage removal plus 10 days ripening at 20°C and ~85 % RH.

Table 3.1: Picking dates, time of 1-MCP treatments and sample removals in the 2004/05 and 2005/06 season.

	2004/05	2005/06
Picking date	14. Oct.	27. Sept.
1-MCP treatment	0 DAH	7 DAH
Commencement of storage (cold storage, CA- and ULO-storage)	1 DAH	8 DAH
Sample removals		
Ex-store 1	2 months	3 months
Ex-store 2	4 months	6 months
Ex-store 3	6 months	9 months

3.2.4 Respiration and ethylene measurements

Respiration and ethylene measurements were made after each harvest date, following commencement of storage and after each sample removal date. For each treatment four apple fruit were placed in a glass jar (volume 3 L) of a respiration measuring system and continuously flushed with air. Each jar of 4 fruit was considered a replicate. With the help of an infrared CO_2 analyser (URAS-2, Mannesmann, Germany) fruit respiration was measured in terms of CO_2 production and expressed as (mL $(kg*h)^{-1}$). Measurements were performed daily for respiration and for ethylene production every third day during 10 days at 20°C and ~85 % RH. In 2005/06 ethylene production was measured during 11 days shelf-life.

Ethylene samples (10 mL) were withdrawn with a syringe from the headspace of the jars and 1 mL of it was analyzed by GC (Carlo Erba, series 2150, Italy) equipped with activated alumina 60 mesh column (0.9 m*1.8 inch). Ethylene values were expressed as µL C_2H_4 $(kg*h)^{-1}$.

3.2.5 Determination of ATP and ADP concentrations

Concentrations of ATP and ADP were determined by a bioluminescence technique and ATP detection kit (luciferin-luciferase test kit) from Bio-Orbit Oy (Turku, Finland) in 2005/06.

Eight fruit of each treatment were cut horizontally. A thin layer of the equatorial region was immediately frozen in liquid nitrogen. Fruit were not peeled, but apple core was removed. Prior to determination of ATP and ADP concentrations frozen samples were lyophilized and powdered in liquid nitrogen in an analytical mill (IKA, Staufen, Germany). Lyophilized and powdered samples were stored at -28°C until further analysis.

0.5 g of lyophilized and powdered sample was dissolved in 5 mL cold solution of 5 % (w/v) trichloracetic acid/EDTA (2 mM) and incubated on ice for 30 min. Thereafter samples were centrifuged at 40,000 *g* for 15 min at 4°C. An aliquot (1 mL) of the clear supernatant was filled in vials, immediately frozen in liquid nitrogen and stored at -28°C until measurement. Samples were diluted with Tris-EDTA-buffer (pH 7.75) prior to assessment with a luminometer (model 1250, LKB-Wallac, Turku, Finland) at 25°C. ATP and ADP determinations were carried out as described by Tan (1999). For measurement of ADP, ATP samples were incubated with pyruvate kinase at 25°C for 30 min. During that time ADP was converted to ATP. ATP and ADP values were expressed in nmol g^{-1} dry weight (DW).

3.2.6 Statistical analysis

Data were statistically analysed by analysis of variance (ANOVA) using GenStat 7.2 (Rothamsted, UK.) to determine the effects of 1-MCP treatment, storage condition and -duration on climacteric characteristics of 'Jonagold' apple.

3.3 Results

3.3.1 Fruit respiration rate

Respiration rate of untreated 'Jonagold' fruit was 9.0 mL$(kg*h)^{-1}$ during 10 days (20°C) after harvest in 2004 and shelf-life respiration rate in 1-MCP treated fruit (0 DAH) was significantly reduced to 3.6 mL$(kg*h)^{-1}$. In 2005 respiration rate during 10 days shelf-life after harvest was 7.7 mL$(kg*h)^{-1}$). At commencement of storage following 6 days cold storage and 1 day of 1-MCP treatment (7 DAH) a significant reduction of shelf-life respiration rate was found in 1-MCP treated fruit compared to untreated control fruit. Throughout 10 days ripening at 20°C respiration rate in untreated fruit was more than twofold higher than in 1-MCP treated fruit (10.2 vs. 4.7 mL$(kg*h)^{-1}$).

In both years 1-MCP treatment, storage condition and an interaction between 1-MCP treatment and storage condition had highly significant effects on shelf-life respiration rate. Storage duration had no effect on shelf-life respiration rate neither in 2004/05 nor in 2005/06 (Table 3.2). Shelf-life respiration rate decreased significantly from cold storage to CA- and ULO-storage after 2, 4 and 6 months in 2004/05 in 1-MCP treated and untreated fruit (Figure 3.1 A). In contrast, after 3, 6 and 9 months storage in 2005/06 highest shelf-life respiration rate was found in untreated fruit from CA-storage, followed by untreated fruit from cold- and ULO-storage (Figure 3.1 B). However, shelf-life respiration rate of untreated fruit stored in ULO-storage for 3 and 9 months (7.5 $mL(kg*h)^{-1}$) was identical with respiration rate of 1-MCP treated fruit from cold storage at the same time. The accumulated effect of controlled atmosphere storage plus 1-MCP on reduction of shelf-life respiration rate was considerably given in both years.

Figure 3.2 A compares average respiration rate of 'Jonagold' apples ± 1-MCP (0 DAH in 2004 and 7 DAH in 2005) during shelf-life following 6 months of storage in 2004/05 and 2005/06, respectively. Shelf-life respiration rate after 6 months of cold storage was higher in 2004/05 when compared with fruit from 2005/06. In contrast, 1-MCP treated fruit from cold storage and fruit ± 1-MCP from CA- and ULO-storage had considerably higher shelf-life respiration rate in 2005/06 in comparison with 2004/05. Shelf-life respiration rate of 1-MCP treated fruit after 6 months was about 90 % higher in cold storage, 43 % in CA-storage and 27 % in ULO-storage as opposed to 2004/05.

Table 3.2: Effects of 1-MCP treatment, storage condition (cold storage, CA- and ULO-storage) and storage duration (2, 4, 6 months in 2004/05 and 3, 6, 9 months in 2005/06) on average respiration rate (mL(kg*h)$^{-1}$) and ethylene production rate (µL(kg*h)$^{-1}$) of 'Jonagold' apples during 10 days shelf-life (20°C) in 2004/05 (A) and 2005/06 (B), respectively (exception: ethylene production in 2005/06 was measured during 11 days shelf-life). 1-MCP treatment 0 days after harvest (DAH) in 2004 and 7 DAH in 2005.

			respiration rate during shelf-life at 20°C		ethylene production during shelf-life at 20°C	
Treatment		DF	*P*	$LSD_{0.05}$	*P*	$LSD_{0.05}$
Main effects						
± 1-MCP	A	1	***	0.34	***	5.99
	B		***	0.37	***	7.49
Storage condition (SC)	A	2	***	0.42	***	7.33
	B		***	0.46	***	9.17
Storage duration (SD)	A	2	ns		***	7.33
	B		ns		***	9.17
Interactions						
SC*SD	A	4	ns		**	12.70
	B		ns		***	15.89
SD*± 1-MCP	A	2	ns		***	10.37
	B		ns		***	12.97
SC*± 1-MCP	A	2	***	0.59	***	10.37
	B		***	0.65	***	12.97
SC*SD*± 1-MCP	A	4	ns		***	17.96
	B		ns		***	22.47

ns, ***, **, *: not significant or significant at *P*-value ≤ 0.001, ≤ 0.01 and ≤ 0.05, respectively; least significant difference LSD (*P* ≤ 0.05).

3.3.2 Fruit ethylene rate

Ethylene production during 10 days shelf-life after harvest of 'Jonagold' fruit in 2004 was 55.5 µL(kg*h)$^{-1}$. After 1-MCP treatment at the harvest day (0 DAH) shelf-life ethylene production was significantly reduced to 1.7 µL(kg*h)$^{-1}$. In 2005 shelf-life ethylene production after harvest was 44.1 µL(kg*h)$^{-1}$. Following 6 days cold storage and 1 day 1-MCP treatment shelf-life ethylene production in untreated fruit after commencement of storage was 84.3 µL(kg*h)$^{-1}$ and significantly reduced to 3.5 µL(kg*h)$^{-1}$ in 1-MCP treated fruit.

In both years 1-MCP treatment, storage condition and –duration had highly significant effects on ethylene production during 10 (2004/05) and 11 (2005/06) days shelf-life (20°C). But all interactions between these main factors were also highly significant in 2004/05 and 2005/06, respectively (Table 3.2).

Shelf-life ethylene production rate in 2004/05 was significantly reduced from cold storage to CA- and ULO-storage. This effect was found in both, untreated and 1-MCP treated apple fruit in 2004/05. Highest shelf-life ethylene production of untreated fruit from cold store, CA- and ULO-storage in 2004/05 was found after 4 months (184.9, 100 and 79.8 $\mu L(kg*h)^{-1}$). Following 6 months of storage in 2004/05 shelf-life ethylene production of untreated fruit with all storage conditions decreased again (Figure 3.1 C). In contrast, ethylene production during shelf-life after sample removal of 1-MCP treated fruit from cold storage, CA- and ULO-storage continuously increased during the entire storage period 2004/05.

In 2005/06 shelf-life ethylene production consistently increased during the entire storage period, irrespective of 1-MCP treatment and storage condition (Figure 3.1 D). As an exception, ethylene production in untreated fruit from cold storage was lowest after the 2nd sample removal following 6 months of storage. Since shelf-life ethylene production of 1-MCP treated fruit after the last sample removal decreased from cold storage to CA- and ULO-store, shelf-life ethylene production of untreated CA-stored fruit was significantly higher (325.4 $\mu L(kg*h)^{-1}$) than ethylene production of untreated fruit following 9 months of cold store (305.5 $\mu L(kg*h)^{-1}$) and ULO-storage (205.4 $\mu L(kg*h)^{-1}$), respectively.

1-MCP reduced shelf-life ethylene production with all storage conditions and –durations when compared with untreated fruit. Magnitude of shelf-life ethylene production after each sample removal was significantly lower in 1-MCP treated fruit than in untreated fruit in both years. However, in both years effect of 1-MCP on shelf-life ethylene production diminished with storage time and ethylene production of fruit increased with storage time (Figure 3.1 C, D). Following 6 and 9 months of storage in 2005/06 shelf-life ethylene production of 1-MCP treated fruit from cold store was nearly identical with ethylene production of untreated fruit from ULO-storage.

After 6 months of storage in different storage conditions shelf-life ethylene production in 2005/06 was considerably higher when compared to 2004/05 (Figure 3.2 B). In both seasons,

2004/05 and 2005/06 shelf-life ethylene production after 6 months of storage was significantly lower in 1-MCP treated fruit than in untreated fruit, regardless of storage condition. Moreover, reduced ethylene production due to controlled atmosphere (CA and ULO) was observed in both years, irrespective of 1-MCP treatment. Average ethylene production during shelf-life following 6 months of storage was in descending order from cold storage, to CA- and ULO-storage.

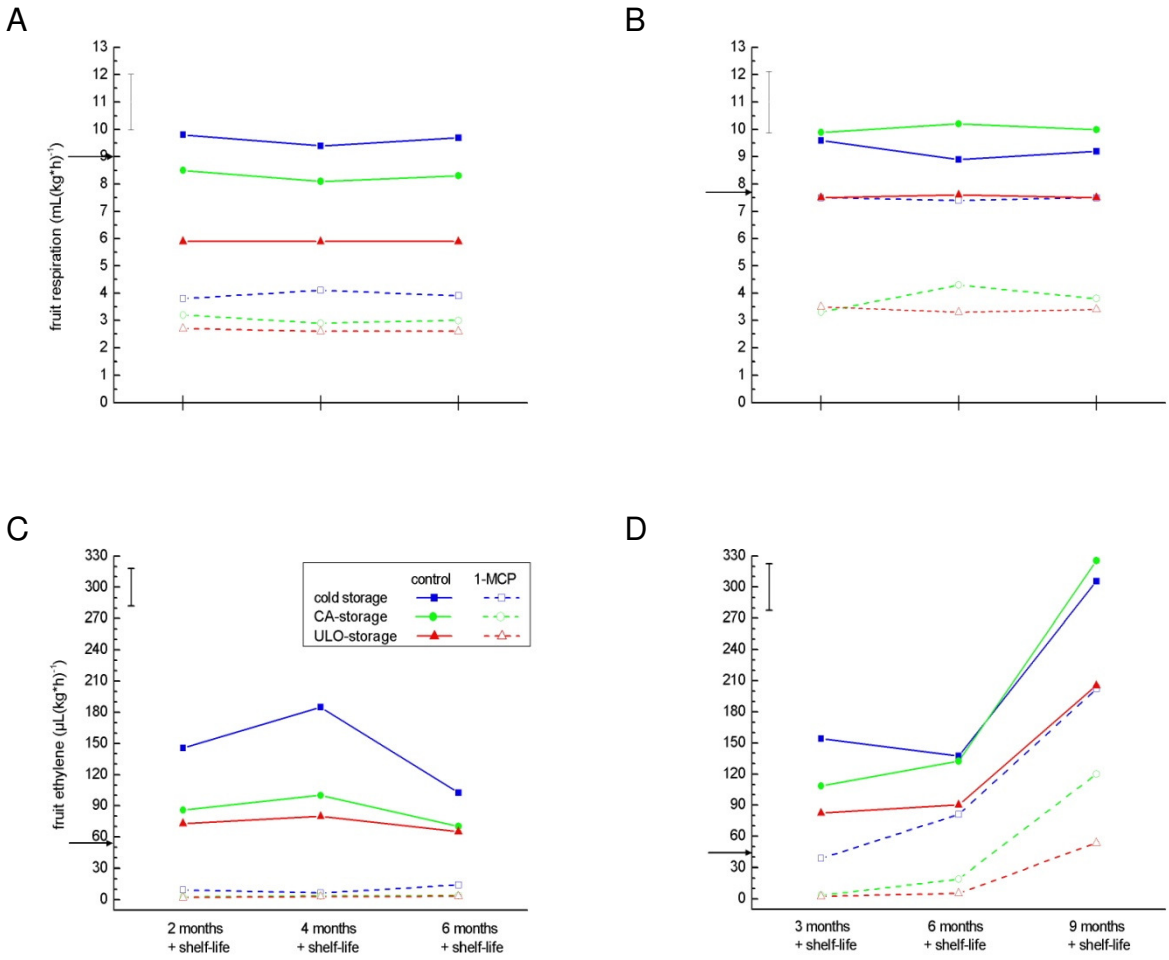
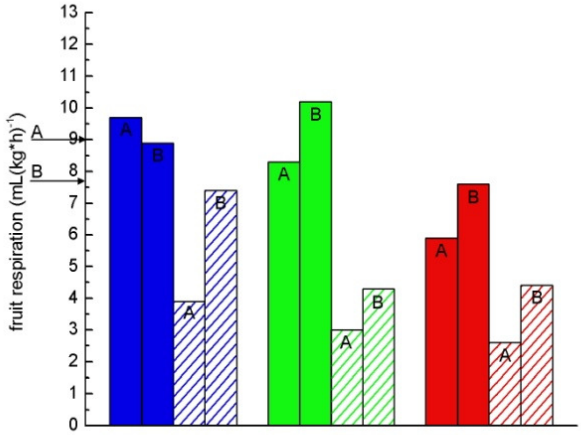


Figure 3.1: Average respiration rate (mL(kg*h)$^{-1}$) (A, B) and ethylene production rate (µL(kg*h)$^{-1}$) (C, D) of 'Jonagold' apples ± 1-MCP during 10 days shelf-life (20 °C) (exception: ethylene production in 2005/06 was measured during 11 days shelf-life) after 2, 4, 6 and 3, 6, 9 months of cold storage, CA- and ULO-storage in 2004/05 (A, C) and 2005/06 (B, D), respectively. 1-MCP treatment was 0 DAH in 2004 and 7 DAH in 2005. Bars show $LSD_{0.05}$ for all treatments at each sampling time. Arrow at y-axis represents harvest value.

A



B

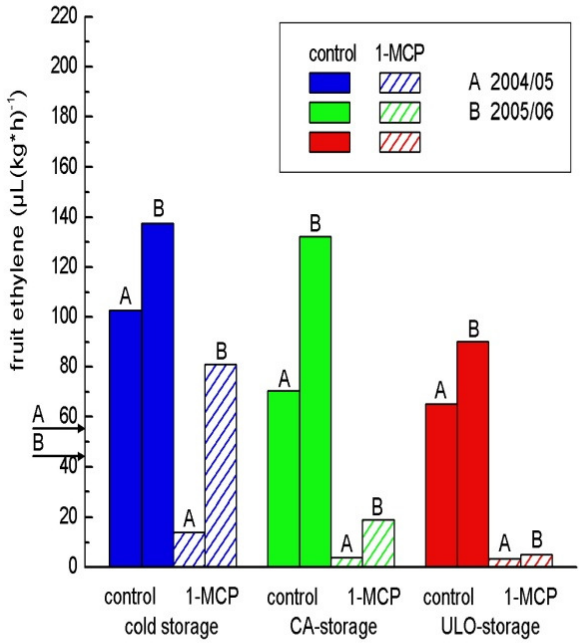


Figure 3.2: Average respiration rate (mL(kg*h)$^{-1}$) (A) and ethylene production rate (µL(kg*h)$^{-1}$) (B) of 'Jonagold' apples ± 1-MCP during 10 days shelf-life at 20°C (exception: ethylene production in 2005/06 was measured during 11 days) following 6 months of storage in different storage conditions (cold storage, CA- and ULO-storage) in 2004/05 and 2005/06. 1-MCP treatment was 0 DAH in 2004 and 7 DAH in 2005. Arrows at y-axis represent harvest values.

3.3.3 ATP and ADP concentrations

ATP and ADP concentration at harvest + shelf-life in 2005 was 112.8 and 30.2 nmol g^{-1} DW, respectively. After 10 days shelf-life following commencement of storage (8 DAH) ATP concentration decreased when compared with harvest date (85.3 nmol g^{-1}). 1-MCP had no effect on ATP concentration after commencement of storage + shelf-life. In contrast, ADP concentration after commencement of storage was influenced by 1-MCP application. When compared with harvest value ADP concentration in untreated fruit was reduced to 26.2 nmol g^{-1} DW, whereas ADP concentration in 1-MCP treated fruit was increased to 31.6 nmol g^{-1} DW. 1-MCP had no significant effect on ATP:ADP-ratio, which was reduced from 3.8 to 3.0 after commencement of storage + shelf-life.

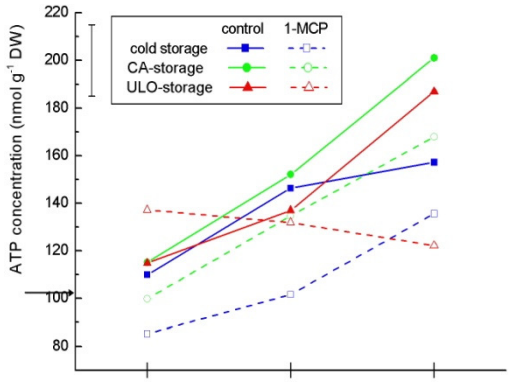
Table 3.3: Effects of 1-MCP treatment, storage condition (cold storage, CA- and ULO-storage) and storage duration (3, 6, 9 months) on ATP and ADP concentrations (nmol g^{-1} DW) and ATP:ADP-ratio of 'Jonagold' apples following 10 days shelf-life (20 °C) in 2005/06.

		ATP nmol g^{-1} DW		ADP nmol g^{-1} DW		ATP:ADP ratio	
Treatment	DF	*P*	LSD	*P*	LSD	*P*	LSD
Main effects							
± MCP	1	***	4.98	ns		**	0.35
Storage condition (SC)	2	***	6.1	**	3.42	*	0.43
Storage duration (SD)	2	***	6.1	*	3.42	***	0.43
Interactions							
SC*SD	4	***	10.57	***	5.93	**	0.74
SD*1-MCP	2	***	8.63	ns		ns	
SC*1-MCP	2	ns		*	4.84	ns	
SC*SD*1-MCP	4	***	14.94	ns		**	1.05

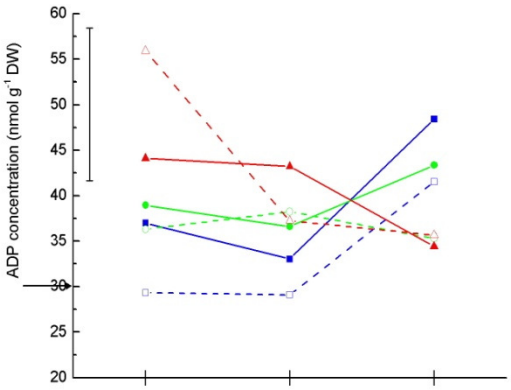
ns, ***, **, *: not significant or significant at *P*-value ≤ 0.001, ≤ 0.01 and ≤ 0.05, respectively; least significant difference LSD (*P* ≤ 0.05).

ATP concentration was highly significantly influenced by all main factors, 1-MCP treatment, storage condition and –duration and also by several interactions between these main factors (Table 3.3). ATP concentration of 1-MCP treated and untreated apple fruit increased consistently during cold- and CA-storage and coincided with the increasing tendency of ethylene production. However, 1-MCP treated fruit from ULO-storage showed an inverse trend and ATP concentration decreased with increasing storage duration. 1-MCP application reduced ATP concentration significantly in all storage conditions when compared with untreated fruit, except in ULO-storage after 3 months of storage 1-MCP treated fruit had higher ATP concentration than untreated fruit (Figure 3.3 A). In contrast, ADP concentration was not influenced by 1-MCP treatment during storage. Because of significant effects of storage condition and -duration and also significant interactions, no consistent trends of ADP concentration could be found during 9 months of storage (Figure 3.3 B). However, ATP:ADP-ratio was affected by 1-MCP treatment and by interactions between storage condition, -duration and 1-MCP treatment (Figure 3.3 C).

A



B



C

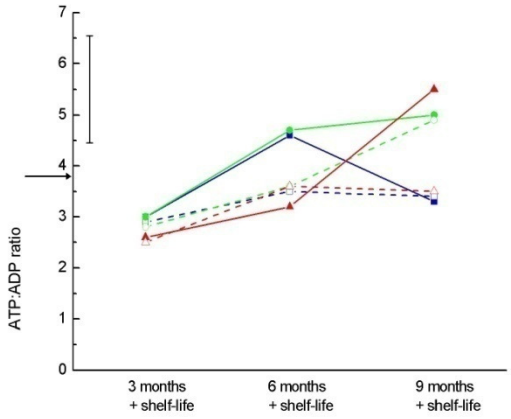


Figure 3.3: ATP (A), ADP (B) concentration (nmol g^{-1}) and ATP:ADP-ratio (C) of 'Jonagold' apples ± 1-MCP (7 DAH) after 3, 6 and 9 months of storage following 10 days shelf-life (20 °C) in 2005/06. Bars show $LSD_{0.05}$ for all treatments at each sampling time. Arrow at y-axis represents harvest value.

3.4 Discussion

It is generally accepted that ethylene plays a critical role in apple ripening (Abeles et al., 1992; Gorny and Kader, 1996a; Mathooko, 1996). Suppression of ethylene biosynthesis and action is the main concern of postharvest technologies and the simplest and most effective method for inhibiting ethylene production of climacteric fruit is achieved by low temperatures (Lurie, 2002; Watkins, 2002). Enzymes involved in the ethylene biosynthetic pathway, ACC-S and ACC-O, are sensitive to low temperatures (Larrigaudière et al., 1997) (Figure 3.3); however, tissue sensitivity and the rate of ethylene biosynthesis of climacteric fruit are also dependent on temperature (Lee et al., 1995; Wills et al., 2007). Therefore, an appropriate temperature management after harvest is the most important factor in maintaining fruit quality, delaying ripening of climacteric fruit, thus extending and improving storage- as well as shelf-life (Lee et al., 1995; Lee and Kader, 2000; Lurie, 2002). Other postharvest technologies such as CA-storage or 1-MCP application can be used in addition but not as a substitute for cold storage (Kader, 2003; Watkins and Nock, 2004). Nevertheless, CA-storage considerably increases the positive effects brought about by low temperatures (Wertheim, 2005). Moreover, ethylene production and respiration rate were consistently lower in ULO than in CA in 2004/05 (Figure 3.1 C).

The beneficial effect of CA-storage on maintaining fruit quality is due to both, reduced rates of fruit respiration and suppression of fruit ethylene production (Mir and Beaudry, 2002; Watkins, 2002). However, Mir and Beaudry (2002) and Tromp (2005) describe that for climacteric fruit in which ripening is initiated by ethylene, the impact of CA-storage on suppression of ethylene synthesis and resulting responses is more important than the reduced respiration.

Burg and Burg (1967) suggested that low O_2-concentrations inhibit ethylene biosynthesis by preventing the binding of ethylene (system I) to the receptors responsible for the initiation of autocatalytic ethylene production (system II). Hence low O_2-levels inhibit the positive feedback regulation of ethylene biosynthesis (Gorny and Kader, 1996a), i.e. the autocatalytic ethylene production which is needed to commence the normal ripening process in climacteric fruit (Bufler, 1984, 1986; Sisler et al., 1996; Golding et al., 1998), is prevented by low O_2-levels. Since the conversion of ACC to ethylene requires O_2 (Adams and Yang, 1979; Abeles et al., 1992; Zarembinski and Theologis, 1994) it seems likely that this is the point were low

O_2-levels directly reduce ethylene biosynthesis (Adams and Yang, 1979; Li et al., 1983; Gorny and Kader, 1996 b; Mir and Beaudry, 2002) (Figure 3.3).

Previously, Burg and Burg (1967) proposed that inhibition of ethylene biosynthesis by elevated CO_2-concentrations is due to competition of CO_2 with ethylene for receptor sites. Thereafter many other articles reported that CO_2-enriched atmospheres reduce ethylene production by inhibiting synthesis and action of ACC-S and ACC-O (Li et al., 1983; Bufler, 1984; Chaves and Tomás, 1984; Gorny and Kader, 1997). However, it is described that in most cases inhibition of ACC-S activity is the major site at which elevated CO_2 atmospheres inhibit ethylene biosynthesis (Gorny and Kader, 1996a, 1997; Mathooko, 1996) (Figure 3.3). Since CO_2 is essential for ACC-O activity (Dong et al., 1992) a direct inhibiting effect of elevated CO_2-levels on ACC-O is questionable or might be dependent on the concentration.

The inhibitory effect of CA-storage on ripening of climacteric fruit might further be due to its effect on respiration. Since O_2 is a critical substrate in the respiratory process, respiration can be reduced predominantly by restricted availability of O_2. Elevated concentrations of CO_2 lead to a lesser extent to reduced respiration rates (Mir and Beaudry, 2002) and can be seen as an additive to low O_2 effects.

It is described that the inhibition of respiration by elevated CO_2 and reduced O_2 leads to reduced ATP production (Solomos and Laties, 1976; Gorny and Kader, 1996a; de Wild et al, 1999; Tan, 1999). Since ATP is required in the methionine-cycle for conversion of methionine to SAM (Murr and Yang, 1975; Adams and Yang, 1977) and may also be needed for the conversion of ACC to ethylene (Yu et al., 1980; Apelbaum et al., 1981), reduced respiration would consequently reduce ethylene biosynthesis (Figure 3.3). However, in our study respiration of 1-MCP treated and untreated apple fruit in all storage conditions was not influenced by storage duration, neither in 2004/05 nor in 2005/06. This is surprising, since ethylene production was significantly affected by storage duration in both years. An increase in respiration during ripening of climacteric fruit is thought to be a consequence of the increase in ethylene production (Brady, 1987; Tromp, 2005) (Figure 3.3).

However, none of the above describes an ethylene inhibition via the receptor site as postulated by Burg and Burg (1967). In our study the ethylene inhibitory effect of 1-MCP was greater in CA-storage than in cold storage for ‘Jonagold’ apple fruit, suggesting that the CA-atmosphere

reduced ethylene production not at the receptor level since 1-MCP is an effective inhibitor of ethylene production and its responses at the receptor sites (Sisler et al., 1996; Blankenship and Dole, 2003). Control fruit from cold store, CA- and ULO-storage had a shelf-life ethylene production of 102.7, 70.3 and 65.1 µL(kg*h)$^{-1}$ after 6 months, respectively, in 2004/05, whereas 1-MCP treated fruit from cold store, CA- and ULO-storage produced 14, 3.8 and 3.3 µL(kg*h)$^{-1}$, respectively, at the same time (Figure 3.1 C). Similar results with 1-MCP treated pears were obtained and discussed by de Wild et al. (1999).

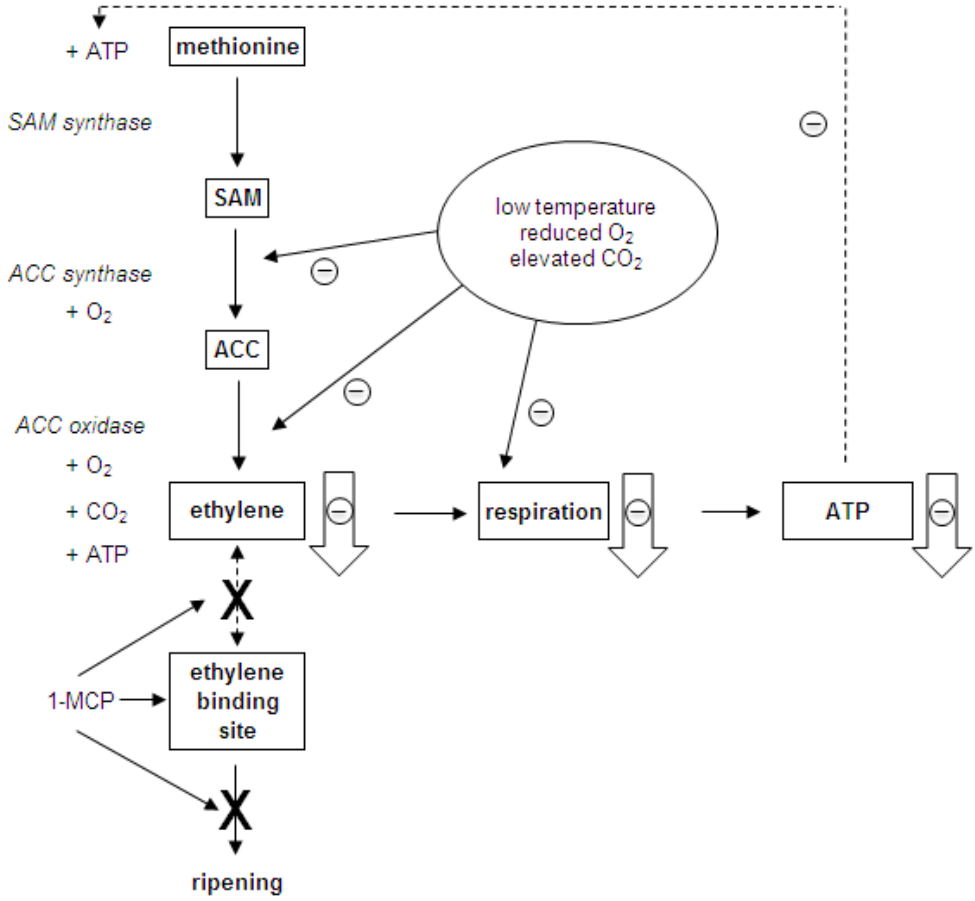


Figure 3.4: Suggested effects of controlled atmosphere storage (low O_2- and elevated CO_2-concentrations in combination with reduced temperatures) and 1-MCP treatment on ethylene biosynthesis, respiration and ATP-concentration.

The ethylene antagonist 1-MCP considerably blocked ethylene production (Figure 3.1 C, D), concomitantly respiration rate (Figure 3.1 A, B) and other ethylene-dependent ripening processes such as softening, yellowing and loss of titratable acidity (data not shown) in 'Jonagold' apples (Heyn et al., 2009, submitted). It is described that 1-MCP irreversibly blocks the autocatalytic ethylene production (system II ethylene) (Sisler et al., 1996; Watkins, 2002), i.e. the normal positive feedback regulation during climacteric as found for example for tomato (Nakatsuka et al., 1997, 1998) and banana (Golding et al., 1998). While untreated fruit stored for 3 months in CA and ULO-storage had average ethylene production rates of 108.5 and 82.3 $\mu L(kg*h)^{-1}$ during shelf-life at 20°C, respectively, 1-MCP treated fruit showed much reduced ethylene production rates of 3.3 and 2.2 $\mu L(kg*h)^{-1}$ following same storage conditions and durations. However, after 9 months of CA- and ULO-storage ethylene production was considerably higher in both, untreated control fruit (325.4 and 205.4 $\mu L(kg*h)^{-1}$) and 1-MCP treated fruit (120 and 53.6 $\mu L(kg*h)^{-1}$). This indicates that irrespective of storage condition and -duration ethylene production was reduced but not entirely suppressed in 1-MCP treated fruit, likely due to some receptor sites not being blocked by 1-MCP (Figure 3.1 C, D). Similar findings were published by Mir et al. (2001) and Saftner et al. (2003) for 'Redchief Delicious' and 'Golden Delicious' apples. A combination of CA- or ULO-storage and 1-MCP seems to be more effective in postponing the climacteric than either factor alone (Figure 3.1). Watkins and Nock (2004) state that both technologies may complement one another; however, 1-MCP can not replace long-term CA/ULO-storage. The results from 2004/05 support this statement (Figure 3.1 A, C). However, in 2005/06 ethylene production of 1-MCP treated and cold stored fruit was identical with ethylene production of untreated fruit stored in ULO after 6 and 9 months (Figure 3.1 D). The beneficial combined effect of CA- or ULO-storage and 1-MCP treatment was also not consistently given in ATP concentrations (Figure 3.3). Moreover, shelf-life respiration was nearly identical for 1-MCP treated cold-stored fruit and untreated fruit from ULO-storage at each removal date in 2005/06 (Figure 3.1 B).

There might be different explanations for increasing ethylene production rates in 1-MCP treated fruit with storage duration.

In our experiment it seems that the efficacy of 1-MCP was, at least partly, influenced by the time between harvest and 1-MCP treatment. Fruit ethylene rates at the commencement of storage were 55.5 in untreated and 1.7 $\mu L(kg*h)^{-1}$ in 1-MCP treated fruit in 2004 (1-MCP

treatment at 0 DAH), whereas 84.3 and 3.5 µL(kg*h)$^{-1}$, respectively, in 2005 (1-MCP treatment at 7 DAH). This suggests that by the time of 1-MCP treatment in 2005 some ethylene was presumably already bound to the receptor sites. This might also explain why ethylene production was not absolutely inhibited after 1-MCP treatment. It is assumed that the efficacy of 1-MCP might be reduced by high ethylene production at the time of (Watkins et al., 2000; Tatsuki et al., 2007) and after 1-MCP treatment (Tatsuki et al., 2007).

Moreover, Rupasinghe et al. (2000) suggested that a gradual recovery of ethylene production during storage of 1-MCP treated apple fruit might be due to partial release of the bound 1-MCP from the receptor sites, hence they might become active again and regain ethylene sensitivity. To achieve continuous insensitivity to ethylene and thus retarding climacteric in apples, re-treatment with 1-MCP might be promising (Mir et al., 2001).

The last and most plausible explanation for increased ethylene production in 1-MCP treated fruit with prolonged storage is the synthesis of new receptor binding sites (Sisler et al., 1996; Jiang et al., 1999; Blankenship and Dole, 2003). This suggests that ethylene can bind to receptors which were produced after 1-MCP treatment. A higher number of ethylene-bound receptors would result in increasing ethylene-sensitivity (Tatsuki et al., 2007) and initiate positive feedback regulation (system II ethylene). After the initiation of ripening the amount of active receptors would increase rapidly (Yen et al., 1995; Golding et al., 1998) because normal ripening is dependent on sufficient and functioning ethylene receptors (Golding et al., 1998).

It is also likely that interactions of the three possible mechanisms as described above are responsible for the gradual increase of ethylene production in 1-MCP treated apple fruit during and after storage.

Therefore, the present study clearly shows that immediate 1-MCP treatment and appropriate storage management after harvest is critical for a maximum reduction of climacteric characteristics such as ethylene production and respiration rate as well as maintenance of postharvest and post-storage apple fruit quality. Similar findings were reported for different apple varieties by Watkins and Nock (2004) and Tatsuki et al. (2007) and for banana by Golding et al. (1998). In ‘Orin’ apple fruit, the later 1-MCP was applied after harvest, the less was the suppression of ethylene production (Tatsuki et al., 2007), thus very late applications of 1-MCP after harvest should be avoided.

Another reason for higher ethylene production rates in 2005 might be due to the temperature of fruit at the time of 1-MCP application. Whereas 1-MCP was applied to relatively warm fruit at the day of harvest in 2004, fruit was held in cold storage for 1 week before being treated with 1-MCP in 2005. It seems to be likely that affinity and sensitivity of the binding sites for 1-MCP might decline with lower temperatures (Mir et al., 2001; DeEll et al., 2002; Blankenship and Dole, 2003). Watkins and Miller (2003) suggested that a reduced effect of 1-MCP at low temperatures might be, at least partially, due to non-specific binding of 1-MCP molecules in plant tissues. If 1-MCP binding to the receptor is reduced at lower temperatures, Mir et al. (2001) concluded that it might be useful to increase the 1-MCP concentration in order to achieve a greater amount of receptors saturated with 1-MCP. Efficacy of 1-MCP can also be affected by treatment duration (DeEll et al., 2003; Blankenship and Dole, 2003) and with lower temperatures extended treatment duration might be needed to achieve a maximum effect. However, since shelf-life respiration rate and ethylene production was higher in untreated control and 1-MCP treated fruit in 2005/06 than in 2004/05, this effect could not exclusively be due to treatment temperature.

Our results confirmed that the efficacy of 1-MCP is besides other factors, greatly influenced by storage condition and -duration, treatment temperature, time from harvest to 1-MCP treatment and commencement of storage (Watkins et al., 2000; DeEll et al., 2002; Blankenship and Dole, 2003; Tatsuki et al., 2007). The present study clearly shows that apple fruit shall be exposed as soon as possible to 1-MCP treatment and appropriate storage conditions after harvest for achieving a maximum effect on reduction of climacteric characteristics and maintenance of postharvest and post-storage apple fruit quality.

3.5 References

Abeles, F.B., P.W. Morgan and M.E. Saltveit. (1992) *Ethylene in plant biology*. 2nd Edition, Academic Press, INC.

Adams, D.O. and S.F. Yang. (1977) Methionine metabolism in apple tissue. *Plant Physiol.* 60, 892-896.

Adams, D.O. and S.F. Yang. (1979) Ethylene biosynthesis: Identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. *Proc. Natl. Acad. Sci. USA*, 76(1), 170-174.

Apelbaum, A., S.Y. Wang, A.C. Burgoon, J.E. Baker and M. Lieberman. (1981) Inhibition of the conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene by structural analogs, inhibitors of electron transfer, uncouplers of oxidative phosphorylation, and free radical scavengers. *Plant Physiol.* 67, 74-79.

Blankenship, S.M. and J.M. Dole. (2003) 1-Methylcyclopropene: a review. *Postharvest Biol. Technol.* 28, 1-25.

Brady, C.J. (1987) Fruit ripening. *Annu. Rev. Plant. Physiol.* 38, 155-178.

Bufler, G. (1984) Ethylene-enhanced 1-aminocyclopropane-1-carboxylic acid synthase activity in ripening. *Plant Physiol.* 75, 192-195.

Bufler, G. (1986) Ethylene-promoted conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene in peel of apple at various stages of fruit development. *Plant Physiol.* 80, 539-543.

Burg, S.P. and E.A. Burg. (1967) Molecular requirements for the biological activity of ethylene. *Plant Physiol.* 42, 144-152.

Chaves, A.R. and J.O. Tomás. (1984) Effect of a brief CO_2 exposure on ethylene production. *Plant Physiol.* 76, 88-91.

De Wild, H.P.J., E.C. Otma and H.W. Peppelenbos. (2003) Carbon dioxide action on ethylene biosynthesis of preclimacteric and climacteric pear fruit. *J. Exp. Bot.* 54, 387, 1537-1544.

De Wild, H.P.J., E.J. Woltering and H.W. Peppelenbos. (1999) Carbon dioxide and 1-MCP inhibit ethylene production and respiration of pear fruit by different mechanisms. *J. Exp. Bot.* 50, 335, 837-844.

DeEll, J.R., D.P. Murr, M.D. Porteous and H.P.V. Rupasinghe. (2002) Influence of temperature and duration of 1-Methylcyclopropene (1-MCP) treatment on apple quality. *Postharvest Biol. Technol.* 24, 349-353.

Dong, J.G., J.C. Fernández-Maculet and S.F. Yang. (1992) Purification and characterization of 1-aminoyclopropane-1-carboxylate oxidase from apple fruit. *Proc. Natl. Acad. Sci. USA,* 89, 9789-9793.

Fleancu, M. (2007) Correlations among some physiological processes in apple fruit during growing and maturation processes. *Int. J. Agri. Biol.*, 9(4), 613-616.

Giovannoni, J. (2001) Molecular biology of fruit maturation and ripening. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52, 725-749.

Golding, J.B., D. Shearer, S.G. Wyllie and W.B. McGlasson. (1998) Application of 1-MCP and propylene to identify ethylene-dependent ripening processes in mature banana fruit. *Postharvest Biol. Technol.* 14, 87-98.

Gorny, J.R. and A.A. Kader. (1996a) Controlled-atmosphere suppression of ACC synthase and ACC oxidase in 'Golden Delicious' apples during long-term cold storage. *J. Amer. Soc. Hort. Sci.* 121(4), 751-755.

Gorny, J.R. and A.A. Kader. (1996b) Regulation of ethylene biosynthesis in climacteric apple fruit by elevated CO_2 and reduced O_2 atmospheres. *Postharvest Biol. Technol.* 9, 311-323.

Gorny, J.R. and A.A. Kader. (1997) Low oxygen and elevated carbon dioxide atmospheres inhibit ethylene biosynthesis in preclimacteric and climacteric apple fruit. *J. Amer. Soc. Hort. Sci.* 122(4), 542-546.

Hoffman, N.E. and S.F. Yang. (1980) Changes of 1-Aminocyclopropane-1-carboxylic acid content in ripening fruits in relation to their ethylene production rates. *J. Amer. Soc. Hort. Sci.* 105(4), 492-495.

Jiang, Y., D.C. Joyce and A.J. Macnish. (1999) Extension of the shelf life of banana fruit by 1-MCP in combination with polyethylene bags. *Postharvest Biol. Technol.* 16, 187-193.

Kader, A.A. (2002) Fruits in the global market. In: Knee, M. (Ed.), *Fruit Quality and its biological basis*. Sheffield Academic Press Ltd; 1-16.

Kader, A.A. (2003) A perspective on postharvest horticulture (1978-2003). *HortScience* 38(5), 1004-1008.

Kende, H. (1993) Ethylene biosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 44, 283-307.

Larrigaudière, C., J. Graell, J. Salas and M. Vendrell. (1997) Cultivar differences in the influence of a short period of cold storage on ethylene biosynthesis in apples. *Postharvest Biol. Technol.* 10, 21-27.

Lee, L., J. Arul, R. Lencki and F. Castaigne. (1995) A review on modified atmosphere packaging and preservation of fresh fruits and vegetables: physiological basis and practical aspects. Part I. *Packaging Technol. Sci.* 8, 315-331.

Lee, S.K. and A.A. Kader. (2000) Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biol. Technol.* 20, 207-220.

Li, Z.-G., Y. Liu, J.G. Dong, R.J. Xu and M.Z. Zhu. (1983) Effect of low oxygen and high carbon dioxide on the levels of ethylene and 1-aminocyclopropane-1-carboxylic acid in ripening apple fruits. *J. Plant Growth Regul.* 2, 81-87.

Lieberman, M. (1979) Biosynthesis and action of ethylene. *Ann. Rev. Plant Physiol.* 30, 533-591.

Lurie, S. (2002) Temperature management. In: Knee, M. (Ed.), *Fruit Quality and its biological basis*. Sheffield Academic Press Ltd; 107-121.

Mathooko, F.M. (1996) Review. Regulation of ethylene biosynthesis in higher plants by carbon dioxide. *Postharvest Biol. Technol.* 7, 1-26.

McMurchie, E.J., W.B. McGlasson and I.L. Eaks. (1972) Treatment of fruit with propylene gives information about the biogenesis of ethylene. *Nature,* 237, 235-236.

Mir, N. and R. Beaudry. (2002) Atmosphere control using oxygen and carbon dioxide. In: Knee, M. (Ed.), *Fruit Quality and its biological basis*. Sheffield Academic Press Ltd; 122-156.

Mir, N.A., E. Curell, N. Khan, M. Whitaker and R.M. Beaudry. (2001) Harvest maturity, storage temperature, and 1-MCP application frequency alter firmness retention and chlorophyll fluorescence of 'Redchief Delicious' apples. *J. Amer. Soc. Hort. Sci.* 126(5), 618-624.

Murr, D.P. and S.F. Yang. (1975) Inhibition of *in vivo* conversion of methionine to ethylene by L-canaline and 2,4-dinitrophenol. *Plant Physiol.* 55, 79-82.

Nakatsuka, A., S. Murachi, H. Okunishi, S. Shiomi, R. Nakano, Y. Kubo and A. Inaba. (1998) Differential expression and internal feedback regulation of 1-Amino-cyclopropane-1-Carboxylate Synthase, 1-Aminocyclopropane-1-Carboxylate Oxidase, and ethylene receptor genes in tomato fruit during development and ripening. *Plant Physiol.* 118, 1295-1305.

Nakatsuka, A., S. Shiomi, Y. Kubo and A. Inaba. (1997) Expression and internal feedback regulation of ACC synthase and ACC oxidase genes in ripening tomato fruit. *Plant Cell Physiol.* 38(10), 1103-1110.

Rupasinghe, H.P.C., D.P. Murr, G. Paliyath and L. Skog. (2000) Inhibitory effect of 1-MCP on ripening and superficial scald development in 'McIntosh' and 'Delicious' apples. *J. Hort. Sci. Biotechnol.* 75(3), 271-276.

Saftner, R.A., J.A. Abbott, W.S. Conway and C.L. Barden. (2003) Effects of 1-Methylcyclopropene and heat treatments on ripening and postharvest decay in 'Golden Delicious' apples. *J. Amer. Soc. Hort. Sci.* 128(1), 120-127.

Sisler, E.C., E. Dupille and M. Serek. (1996) Effect of 1-Methylcyclopropene and methylenecyclopropane on ethylene binding and ethylene action on cut carnations. *Plant Growth Regul.* 18, 79-86.

Solomos, T. and G.G. Laties. (1976) Effects of cyanide and ethylene on the respiration of cyanide-sensitive and cyanide-resistant plant tissues. *Plant Physiol*. 58, 47-50.

Tan, T. (1999) *Stoffwechselphysiologische Untersuchungen zur Ursache der verminderten Aromabildung von früh geernteten Apfel- und Erdbeerfrüchten sowie von unter ULO (ultra low oxygen)-Bedingungen langzeitgelagerten Apfelfrüchten*. Dissertation, Universität Hohenheim.

Tatsuki, M., A. Endo and H. Ohkawa. (2007) Influence of time from harvest to 1-MCP treatment on apple fruit quality and expression of genes for ethylene biosynthesis enzymes and ethylene receptors. *Postharvest Biol. Technol.* 43, 28-35.

Tromp, J. (2005) Fruit ripening and quality. In: Tromp, J., A.D. Webster and S.J. Wertheim (Eds.), *Fundamentals of temperate zone tree fruit production*. Backhuys Publishers, Leiden, The Netherlands; 295-310.

Watkins, C.B. (2002) Ethylene synthesis, mode of action, consequences and control. In: Knee, M. (Ed.), *Fruit Quality and its biological basis*. Sheffield Academic Press Ltd; 180-224.

Watkins, C.B. and J. Nock. (2004) SmartFresh™ (1-MCP) – an update. *Cornell Fruit Handl. Storage Newsletter*, 2-12.

Watkins, C.B. and J.F. Nock. (2000) MCP: Facts, Speculation, and How could it affect the New York apple industry? *N.Y. Fruit Quart*. 8(3), 5-9.

Watkins, C.B. and W.B. Miller. (2003) Implications of 1-Methylcyclopropene registration for use on horticultural products. In: Vendrell, M., H. Klee, J.C. Pech and F. Romojaro (Eds.) *Proceedings of the NATO Advanced Research Workshop on Biology and Biotechnology of the Plant Hormone Ethylene III*; IOS Press, 385-290.

Watkins, C.B., J.F. Nock and B.D. Whitaker. (2000) Responses of early, mid and late season apple cultivars to postharvest application of 1-Methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. *Postharvest Biol. Technol.* 19, 17-32.

Wertheim, S.J. (2005) Fruit storage. In: Tromp, J., A.D. Webster and S.J. Wertheim (Eds.), *Fundamentals of temperate zone tree fruit production*. Backhuys Publishers, Leiden, The Netherlands; 311-324.

Wills, R.B.H., W.B. McGlasson, D. Graham and D.C. Joyce. (2007) *Postharvest: an introduction to the physiology and handling of fruit, vegetables and ornamentals*. 5th Edition. CABI.

Yang, S.F. (1980) Regulation of ethylene biosynthesis. *HortScience*, 15(3), 238-243.

Yang, S.F. and N.E. Hoffman. (1984) Ethylene biosynthesis and its regulation in higher plants. *Ann. Rev. Plant Physiol.* 35, 155-189.

Yen, H.-C., S. Lee, S.D. Tinsley, M.B. Manahan, H.J. Kale and J.J. Giovannoni. (1995) The tomato *never-ripe* locus regulates ethylene inducible gene expression and is linked to a homolog of the *Arabidopsis* ETR 1 gene. *Plant Physiol.* 107, 1343-1353.

Yu, Y.-B., D.O. Adams and S.F. Yang. (1980) Inhibition of ethylene production by 2,4-dinitrophenol and high temperature. *Plant Physiol.* 66, 286-290.

Zarembinski, T.I. and A. Theologis. (1994) Ethylene biosynthesis and action: a case of conservation. *Plant Mol. Biol.* 26, 1579-1597.

4.

Effect of 1-MCP on Antioxidant Capacity of Apple Fruit

Abstract

'Jonagold' apple fruit (*Malus domestica* Borkh.) were picked at commercial maturity in 2005. Following 1-MCP treatment 7 days after harvest fruit were stored in cold storage, CA- (0.8 % CO_2, 3 % O_2) and ULO-storage (3 % CO_2, 1 % O_2). After 3, 6 and 9 months fruit samples from each storage atmosphere ± 1-MCP were removed. Is the nutritional value of apple fruit affected by different storage conditions? Does 1-MCP influence nutritional values of apple fruit? Following 10 days shelf-life (20°C) after harvest, commencement of storage and each storage removal ascorbic acid (L-AA) concentration, phenolic compounds and total antioxidant capacity were examined. L-AA concentrations were analysed by HPLC and phenolic compounds by Folin-Ciocalteu's reagent (FCR). A modified 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic-acid) (ABTS) decoloration method was used for determination of total antioxidant capacity. In both, Folin-Ciocalteu and ABTS determinations (vitamin C equivalent antioxidant capacity VCEAC) ascorbic acid was used as standard. L-AA concentration significantly decreased during storage, irrespective of storage condition and 1-MCP treatment. Though L-AA concentration was significantly higher in 1-MCP treated fruit than in untreated fruit at commencement of storage, however, following 9 months of storage L-AA concentration was significantly lower in all 1-MCP treated fruit when compared with untreated fruit. Vitamin C equivalent phenolic concentration decreased after 6 months of storage and gradually increased again after 9 months of storage. Neither storage condition nor 1-MCP treatment had a significant effect on phenolic compounds in apple fruit. In the same way VCEAC decreased after 6 months of storage and increased again after 9 months of storage. 1-MCP treatment had no effect on VCEAC. In general, the nutritional value of apple fruit was not influenced by 1-MCP. Moreover, storage conditions had little effect on phenolic compounds and total antioxidant capacity. Only L-AA concentration was affected by different storage conditions and slightly influenced by 1-MCP. However, since L-AA contributes to a small

extend to the antioxidant capacity of apple fruit, this does not affect the total nutritional value of apple fruit.

4.1 Introduction

Oxidative stress is an unavoidable consequence of life in an oxygen environment (Bartosz, 1997; Kalt, 2005). Oxygen has two contrasting sides. On the one hand it is a molecule essential for aerobic forms of life; on the other hand it is also a destructive, toxic agent for living tissues (Larson, 1988; Bartosz, 1997). Reactive oxygen species (ROS) are continuously formed by oxidative processes such as respiration, photosynthesis and oxidative phosphorylation (Masia, 2003; Wood et al., 2006). Indeed, ROS are by-products of normal oxygen metabolism (Wang et al., 1996; Bartosz, 1997; Awad and de Jager, 2003; Wood et al., 2006) and can be enhanced by unfavourable environmental conditions (Hancock and Viola, 2005b). ROS include compounds such as superoxide (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical (OH) (Noctor and Foyer, 1998; Davey et al., 2000; Lurie, 2003). ROS can cause protein damage, lipid peroxidation, DNA damage and finally cell death (Wang et al., 1996; Davey, 2000; Masia, 2003; Chun et al, 2005; Kalt, 2005).

All oxygen-consuming organisms have crucial enzymatic and non-enzymatic antioxidant defence systems to protect against the deleterious effects of ROS (Wang et al., 1996; Noctor and Foyer, 1998; Lurie, 2003; Wood et al., 2006). An imbalance between ROS and antioxidants in favour of ROS leads to oxidation and damage (oxidative stress) (Bartosz, 1997). An antioxidant is described as a 'substance that inhibits the destructive effects of oxidation' (Bloomsbury English Dictionary) without undergoing conversion to a deleterious radical (Noctor and Foyer, 1998; Lurie, 2003; Masia, 2003). Antioxidant enzymes of plants, such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) (Shewfelt and del Rosario, 2000; Lurie, 2003; Masia, 2003) are acting concomitantly with non-enzymatic antioxidants. The non-enzymatic antioxidants, which are mostly scavengers of free radicals (i.e. ROS) (Bartosz, 1997; Awad and de Jager, 2003; Hancock and Viola, 2005a), can be divided by their solubility in water or lipids (water- and lipid-soluble; hydrophilic and lipophilic) (Klein and Kurilich, 2000; Lurie, 2003). The major water-soluble antioxidants in fruits and vegetables are

ascorbate (L-AA) and glutathione (Bartosz, 1997; Noctor and Foyer, 1998; Arnao et al., 2001; Davey et al., 2004) and the majority of phenolic compounds (Ju and Bramlage, 1999). Tocopherols, carotenoids and xanthophylls are important lipid-soluble antioxidants (Arnao, et al., 2001; Huang et al., 2002).

Although decision for purchasing fruit is mainly due to appearance (size, shape and colour) (Francis, 1995; Kays, 1999; Kevers et al., 2007), consumer are increasingly concerned about nutritional quality and health-protecting compounds in foods (Larrigaudière et al., 2004; Vilaplana et al., 2006; Kevers et al., 2007). Nutritional quality and healthful constituents of fruits and vegetables are related to contents of vitamins, minerals, dietary fibre and phytochemicals with antioxidant properties, such as phenolic compounds (Kader, 2002; Awad and de Jager, 2003; Sánchez-Moreno et al., 2006).

Regular consumption of fruit and vegetables is associated with reduced risk of cancer, cardiovascular disease and other chronic diseases which have their origin in oxidative stress (Robards et al., 1999; Sun et al., 2002; Wolfe et al., 2003; Boyer and Liu, 2003-04; Scalzo et al., 2005; Sánchez-Moreno et al., 2006). The beneficial effects of fruits and vegetables on human health and welfare are mainly attributed to their total antioxidant concentration (Scalzo et al., 2005) in general and their balanced mixture of several antioxidants (Wang et al., 1996) and synergism among them (Klein and Kurilich, 2000; Wood et al., 2006). L-AA, which is synthesized from the precursor D-glucose (Hancock and Viola, 2005b), is the most effective and least toxic antioxidant (Davey et al., 2000; Sánchez-Moreno et al., 2006). In addition to its role as an antioxidant L-AA is involved in many metabolic processes in plants (Davey et al., 2000; Lurie, 2003). Regular L-AA intake is essential for humans, since they are not able to synthesize L-AA in their body (Davey et al., 2000; Hancock and Viola, 2005a). Phenolic compounds, which are derived from the shikimate pathway and phenylpropanoid metabolism (Awad and de Jager, 2000) are highly diverse and extensively distributed in all fruits (Robards et al., 1999). Flavonoids are the most common phenolic compounds in fruits (Podsędek et al., 2000; Chun et al., 2005) and they are divided in several subgroups (flavonols, flavanols, anthocyanins, etc.). Phenolic compounds not only play an important role in antioxidant defense but they contribute to the ‘inner’ as well as ‘outer’ quality of apple fruit (Treutter, 2001). Phenolic compounds influence flavour and taste, astringency and colour of apple (Klein and Kurilich, 2000; Golding et al., 2001).

Many pre- and postharvest factors such as growing conditions, cultural practices, maturity at harvest, harvesting method and storage conditions and –duration can influence nutritional composition and total antioxidant levels of fruits and vegetables (Davey et al., 2000; Lee and Kader, 2000; Boyer and Liu, 2003-04; Kalt, 2005). Genetic variation within several fruit species and even among different varieties within species can be substantial (Kalt, 2005). However, the nutritional and health-protecting value of various fruit and vegetables depends not only on the concentrations but also on the amounts of such produce consumed daily (Lee et al., 2003; Davey et al., 2004; Chun et al., 2005; Wills et al., 2007). Apples are a good source of antioxidants (Boyer and Liu, 2003-04; Chun et al., 2005) and they are one of the most frequently consumed fruits. In Germany, apples were by far the most common (27.5 %) type of fruit consumed in 2004/05, followed by banana (16.6 %) and oranges (10.9 %) (ZMP, 2006).

Ripening of fruits generally involve oxidative stress (Rabinovitch and Sklan, 1981). During fruit ripening and senescence prooxidant ROS are produced in excess and can outperform the antioxidant defense mechanism of the host organism (Bartosz, 1997; Noctor and Foyer, 1998; Davey et al., 2000; Wood et al., 2006). Additive stress conditions result from various factors of harvesting and different strategies of postharvest handling (Lurie, 2003; Toivonen, 2003). Postharvest oxidative stress leads to accelerated senescence (Toivonen, 2003) and loss of fruit quality, consumer acceptability (Shewfelt and del Rosario, 2000) and storability. Several storage conditions, such as reduced storage temperatures and controlled storage atmospheres with low O_2- and elevated CO_2-concentrations are known methods to minimize ethylene biosynthesis, ethylene sensitivity and responses (Abeles et al., 1992; Mir and Beaudry, 2002; Wills et al., 2007) of harvested climacteric fruit and by that to slow metabolic changes during ripening. Because harvested fruit are removed from its source of carbohydrates, water and nutrient supply, there is no possibility for further quality improvement (Hewett, 2006) and maintenance of vitamins and other bioactive compounds during storage and post-storage handling should gain increasing consideration. Since harvested fruit are still living biological systems with an active metabolism phytochemical profiles are subject to continual changes (Davey et al., 2000; Lee and Kader, 2000; Kalt, 2005). Fruits with high antioxidant capacities may have improved fruit quality, nutritional values, storage characteristics (Davey et al., 2000, 2004, 2007) and shelf-life.

1-Methylcyclopropene (1-MCP) is an effective inhibitor of ethylene action and synthesis (Sisler et al., 1996; Fan and Mattheis, 1999; Blankenship and Dole, 2003). Ripening is the process by which the physiologically mature but inedible fruit attains its characteristic appearance and eating quality (Kader, 2002; Watkins, 2002; Wills et al., 2007). Most of the climacteric fruits such as apple can be harvested mature and ripening may proceed off the plant (Kader, 2002; Wills et al., 2007). Ripening may be considered as the first part of senescence (Tromp, 2005). Ethylene plays a critical role in ripening of climacteric fruit. 1-MCP reduces and/or delays respiration rates (Fan et al., 1999; Fan and Mattheis, 1999) and clearly inhibits or reduces and delays ethylene production in different apple varieties (Fan et al., 1999; Fan and Mattheis, 1999; Rupasinghe et al., 2000). Therefore, application with 1-MCP leads to a better retention of apple fruit quality during storage (Fan and Mattheis, 1999; Watkins et al., 2000; DeEll et al., 2002) and also post-storage (Watkins et al., 2000; Watkins, 2006). 1-MCP maintains firmness (Fan and Mattheis, 1999; Watkins et al., 2000; Mir et al., 2001) and reduces the loss of acidity (Fan and Mattheis, 1999; Watkins et al., 2000). Similar effects of 1-MCP on apple fruit quality (cv. 'Jonagold') and on climacteric characteristics were found in two related studies by Heyn et al. (2009, submitted).

However, little is known about the effect of 1-MCP on total antioxidant capacity in general, L-AA concentrations and phenolic compounds in particular. Does 1-MCP influence nutritional values of apple fruit?

In this study 'Jonagold' apples were harvested at commercial maturity, treated with 1-MCP and stored in cold storage, CA- and ULO-storage, respectively. After 3, 6 and 9 months fruit samples were removed from the storages. L-AA concentrations were analysed by HPLC and phenolic compounds by Folin-Ciocalteu's reagent (FCR). A modified 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic-acid) (ABTS) decoloration method was used for determination of total antioxidant capacity. With this method, both hydrophilic and lipophilic antioxidants can be determined simultaneously in the same sample (van den Berg et al., 1999; Arnao et al., 2001). Ascorbic acid rather than Trolox® was used as standard in Folin-Ciocalteu and ABTS determinations. As opposed to Trolox®, an unfamiliar artificial chemical (Chun et al., 2005), ascorbic acid is a known naturally occurring substance with antioxidant activity in fruits and vegetables (Kim et al., 2002).

4.2 Materials and methods

4.2.1 Plant material and harvest management

The experiments were carried out at 'Kompetenzzentrum Obstbau – Bodensee', Ravensburg, Germany, using the apple cultivar 'Jonagold' (*Malus domestica* Borkh.) in the 2005/06 growing season. 'Jonagold' fruit were harvested at a commercial orchard. All trees were grown on rootstock M.9 and trained as slender spindle. Three harvests were taken over the commercial harvest period for long-term CA-storage of 'Jonagold' apples ('Streif'-index 0.11, 0.09 and 0.06). However, no consistent trends were found due to significant interactions between 1-MCP treatment, harvest dates, storage condition and -duration. Shown data present the average mean values of all three harvests.

4.2.2 1-MCP treatments

Immediately after each harvest fruit were colour- and size-graded by hand. Fruit were divided at random in 12 kg plastic boxes, according to number of storage conditions, -durations, 1-MCP treatment and replications. To simulate commercial conditions fruit were held in cold storage (1°C, 92 % relative humidity (RH)) for 6 days prior to 1-MCP treatments.

Half of the boxes with fruit were placed in gas-tight storage containers (volume 0.560 m^3) and treated with 625 ppb 1-Methylcyclopropene (1-MCP) for 24 hours. The temperature of the container was at 1°C (± 0.5); the actual temperature of the apples during treatment with 1-MCP was not measured. Control fruit were held at the same conditions but without 1-MCP.

Following each 1-MCP treatment, all boxes with treated and untreated fruit, were divided at random into three equal groups. Each group was then placed in containers with different storage atmospheres.

4.2.3 Fruit storage and sampling procedure

Fruit was exposed to the storage atmospheres as follows: cold storage, CA- (0.8 % CO_2, 3 % O_2) and ULO-storage (3 % CO_2, 1 % O_2). Temperature was about 1°C (± 0.5) and RH at 92 % (± 2 %) in each storage atmosphere. For each harvest one independent storage container

was used for CA- and ULO-storage, respectively. Fruit boxes for cold storage were covered lightly with plastic sheets to minimize water loss and were placed in a cold storage room.

After 3, 6 and 9 months fruit samples from each storage atmosphere (cold storage, CA- and ULO-storage ± 1-MCP) were removed. Following 10 days of shelf-life at 20°C after harvest, commencement of storage and each storage removal 8 fruit of each replicate were cut horizontally, respectively. A thin layer of the equatorial region was immediately frozen in liquid nitrogen. Fruit were not peeled, but apple core was removed. Frozen samples were held at -28°C until analysis. A fraction of each sample was lyophilized and powdered in liquid nitrogen using an analytical mill (IKA, Staufen, Germany) for determination of phenolic compounds and total antioxidant capacity. Vitamin C concentration, phenolic compounds and total antioxidant capacity were always determined after 10 days shelf-life at 20°C. All samples were analysed in triplicates.

4.2.4 Extraction and quantification of vitamin C

Vitamin C (L-ascorbate; L-AA; ascorbic acid) content was determined using HPLC (LC-CaDI 22-14, Bischoff, Germany) equipped with Prontosil 60-5-C 18-H (5.0 µm, 4.0 x 125 mm) column. Prior to assessment of vitamin C content frozen samples were ground in an analytical mill (IKA, Staufen, Germany) under addition of liquid nitrogen. Approximately 8 g powder was added to 15 ml of 3 % HPO_3 solution and homogenized for 1 min.

After centrifugation at 25,000 *g* for 15 min. at 4°C the supernatant was filtered (0.45 µm) into small vials; vials were closed and held in a fridge until analysis. 50 mL of the supernatant was injected to HPLC with the following conditions: eluent: tetra-n-butyl ammoniumhydrogensulfate (2.5 g) + methanol (55 mL) in 1 L H_2O; 800 mL min^{-1} flow; 10.6 MPa pressure; 30°C; 20 mL sample volume. Samples were detected at 254 nm wavelength and L-AA concentrations were calculated from ascorbic acid standard curves. Vitamin C concentration was expressed on a fresh weight (FW) basis (mg 100 g^{-1}).

4.2.5 Extraction of phenolic compounds and total antioxidant capacity

500 mg lyophilized sample were dissolved in 80 % aqueous methanol (30 mL), covered and extracted while shaking for 30 min. Samples were centrifuged at 40,000 *g* at 4°C for 15 min. The clear supernatant was decanted into small vials and kept in a cold and dark place until determination of phenolics and total antioxidant capacity.

4.2.6 Quantification of phenolic compounds

Quantification of phenolic compounds was performed by using Folin-Ciocalteu's reagent (Sigma). Determination of phenols was conducted with an UV/VIS spectrometer (PU 8700, Philips) and absorption was read at wavelength 720 nm. Ascorbic acid (VWR) was used for the calibration and results were expressed on a dry weight (DW) basis (mg g^{-1}).

Sample extracts (59 µL) were diluted 1:10 with deionised water and 100 µL Folin-Ciocalteu reagent was added and solution was mixed using vortex. After 3 min., 800 µL sodium carbonate solution (7.5 %) were added and solution was mixed on vortex and placed in a dark place at room temperature. Absorption at 720 nm was read after 60 min.

4.2.7 Quantification of total antioxidant capacity

Total antioxidant capacity was analysed by a modified TEAC (Trolox equivalent antioxidant capacity) assay as described by van den Berg et al. (1999). This TEAC assay is based on scavenging of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic-acid) (ABTS) radical anions by both water- and lipid-soluble compounds with antioxidant capacity (hydrogen- or electron-donating antioxidants) in the sample (van den Berg et al., 1999). Briefly, ABTS stock solution was prepared by solving 20 mmol ABTS in the crystallized diammoniumsalt form in 1 mL phosphate buffer (0.066 mol L^{-1} Na_2HPO_4*2 H_2O and 0.066 mol L^{-1} KH_2PO_4; pH 7.4) and 2,2'-azobis(2-amidinopropane)-dihydrochloride (AAPH) stock solution was made by solving 2.5 mmol AAPH in 100 mL phosphate buffer (pH 7.4). 0.5 mL of ABTS stock solution was mixed with 100 mL of AAPH stock solution, covered with aluminium foil and heated for 15 min at 60°C until colour was fully developed. ABTS/AAPH stock solution was kept covered at room temperature. Extinction of the blue-green ABTS/AAPH stock solution

was checked to have an absorbance between 0.35 and 0.40. ABTS/AAPH stock solution was freshly prepared every day. Because absorption of ABTS/AAPH stock solution is not stable, blanks were measured regularly in between the samples. Prior to measurement of total antioxidant capacity sample extracts were diluted 1:10 with aqueous methanol (80 %). 1960 µL of the ABTS/AAPH stock solution were mixed with 40 µL sample extract and deionised water (blank) or ascorbic acid (calibration), respectively. For the determination of total antioxidant capacity an UV/VIS spectrometer (PU 8700, Philips) was used. The decrease in absorption was read at wavelength 734 nm exactly 6 min. after addition of the sample extract. Ascorbic acid was used for the calibration and results were expressed as mg g^{-1} DW vitamin C equivalent antioxidant capacity (VCEAC).

Ascorbic acid, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic-acid) (ABTS) and 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH) were obtained from VWR. All other chemicals used for the assays were of analytical grade.

4.2.8 Statistical analysis

Data were statistically analysed by analysis of variance (ANOVA) using GenStat 7.2 (Rothamsted, UK) to determine the effects of 1-MCP treatment, harvest date, storage condition and -duration on vitamin C concentration, phenolic compounds and antioxidant capacity of 'Jonagold' apple.

4.3 Results

4.3.1 Vitamin C concentrations

Fruit vitamin C concentration decreased continuously during the entire storage time (Figure 4.1 A), but the greatest loss of L-AA was during the first 17 days after harvest (Table 4.1). Fruit vitamin C concentration at harvest in 2005 was 7.3 mg $100g^{-1}$ FW. Following 6 days of cold storage, 1 day of 1-MCP treatment and 10 days shelf-life, vitamin C concentration of control fruit was considerably reduced to 2.5 mg $100g^{-1}$ FW (Table 4.1). Application of 1-MCP, however, maintained fruit vitamin C levels significantly ($P \leq 0.001$) better when compared to non-treated fruit (5.1 vs. 2.5 mg 100 g^{-1} FW).

Table 4.1: L-AA concentrations (mg 100 g^{-1} FW), phenolic compounds (mg g^{-1} DW) and antioxidant capacity (VCEAC) (mg g^{-1} DW) of 'Jonagold' apples ± 1-MCP at harvest and commencement of storage + 10 days shelf-life (20°C) in 2005.

		L-AA (mg 100g^{-1} FW)	phenolics (mg g^{-1} DW)	VCEAC (mg g^{-1} DW)
harvest time + 10 d		7.3	11.3	1.9
Commencement of storage + 10 d	control	2.5	11.2	2.0
	1-MCP	5.1	11.7	1.9
P-value		***	ns	ns
$LSD_{0.05}$		0.51		

ns, ***, **, *: not significant or significant at *P*-value ≤ 0.001, ≤ 0.01 and ≤ 0.05, respectively; least significant difference LSD (*P* ≤ 0.05).

Effect of 1-MCP treatment (± 1-MCP), storage condition (cold storage, CA- and ULO-storage) and -duration (3, 6 and 9 months) on L-AA concentration (mg 100 g^{-1} FW) of 'Jonagold' apples after 10 days shelf-life (20°C) are shown in Table 4.2 and Figure 4.1 A.

In general, vitamin C concentration significantly decreased during storage, irrespective of storage condition and 1-MCP treatment. Loss of vitamin C was influenced by storage duration ($P \leq 0.001$), storage condition ($P = 0.028$) and 1-MCP treatment ($P = 0.038$) and several interactions between these three main factors (Table 4.2).

After 3 and 6 months L-AA content was best maintained in 1-MCP treated fruit stored in CA-storage. However, following 6 months of CA-storage untreated fruit had the same L-AA concentration as 1-MCP treated fruit (3.1 mg 100 g^{-1} FW). With all storage conditions L-AA concentration after 3 months of storage was higher in 1-MCP treated fruit when compared to untreated fruit after 3 months of storage. After 9 months of storage highest L-AA concentration was found for untreated fruit from ULO-storage. In general, following 9 months of storage L-AA concentration was lower in all 1-MCP treated fruit when compared with untreated fruit (Figure 4.1 A).

4.3.2 Concentration of phenolic compounds

Vitamin C equivalent phenol concentration of 'Jonagold' apples was 11.3 mg g^{-1} DW at harvest in 2005 and at commencement of the storage period 11.2 in untreated and 11.7 mg g^{-1}

DW in 1-MCP treated fruit, respectively. Significant differences between 1-MCP treatments were not found at commencement of storage (Table 4.1).

In general, concentrations of phenolic compounds fluctuated over 9 months of storage plus shelf-life (20°C). Phenolic compounds decreased after 6 months of storage and reached the lowest value of 10.8 mg g^{-1} DW (= 95.6 %). After 9 months of storage phenolic compounds gradually increased again and values were similar when compared with harvest (11.3 mg g^{-1} DW; = 100 %) (Figure 4.1 B).

Phenolic compounds were influenced by storage duration (3, 6 and 9 months). Neither storage condition (cold storage, CA- and ULO-storage) nor 1-MCP treatment had a significant effect on phenolic compounds. In the same way, no significant interactions were found for phenolic compounds (Table 4.2).

Table 4.2: Effects of 1-MCP treatment (7 days after harvest (DAH)), storage condition (cold storage, CA- and ULO-storage) and storage duration (3, 6, 9 months) on L-AA concentration (mg 100 g^{-1} FW), phenolic compounds (mg g^{-1} DW) and vitamin C equivalent antioxidant capacity (VCEAC) (mg g^{-1} DW) of 'Jonagold' apples after 10 days shelf-life (20°C) in 2005/06.

		L-AA		phenolics		VCEAC	
Treatment	DF	*P*	$LSD_{0.05}$	*P*	$LSD_{0.05}$	*P*	$LSD_{0.05}$
Main effects							
± 1-MCP	1	*	0.17	ns		ns	
Storage condition (SC)	2	*	0.20	ns		**	0.04
Storage duration (SD)	2	**	0.20	***	0.30	***	0.04
Interactions							
SC*SD	4	ns		ns		*	0.08
SD*± 1-MCP	2	***	0.29	ns		ns	
SC*± 1-MCP	2	*	0.29	ns		ns	
SC*SD*± 1-MCP	4	**	0.50	ns		ns	

ns, ***,**, *: not significant or significant at *P*-value ≤ 0.001, ≤ 0.01 and ≤ 0.05, respectively; least significant difference LSD (*P* ≤ 0.05).

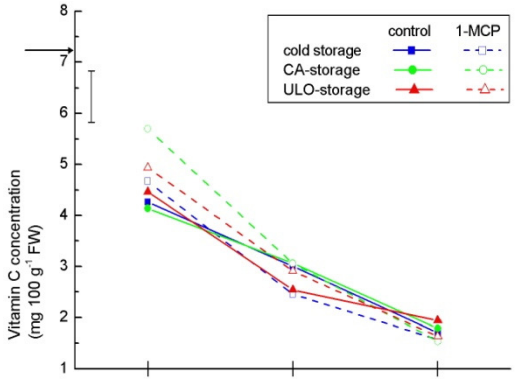
4.3.3 Concentration of total antioxidant capacity

Vitamin C equivalent antioxidant capacity (VCEAC) of 'Jonagold' apples at harvest in 2005 was 1.9 mg g^{-1} DW. At commencement of storage antioxidant capacity was at 1.9 and 2.0 mg g^{-1} DW without significant differences between 1-MCP treated and untreated fruit ($P = 0.052$) (Table 4.1).

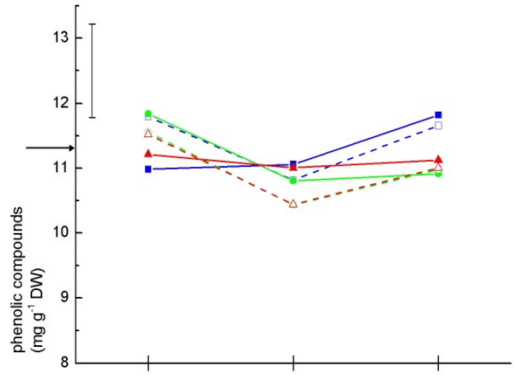
VCEAC decreased after 6 months of storage and increased again after 9 months of storage, irrespective of storage condition and 1-MCP treatment. Lowest VCEAC values were found after 6 months of storage in all cases (Figure 4.1 C).

In general, VCEAC was influenced by storage duration ($P \leq 0.001$), storage condition ($P = 0.007$) and an interaction between both main factors ($P = 0.035$). Values of VCEAC were influenced neither by 1-MCP treatment nor by any other interaction (Table 4.2).

A



B



C

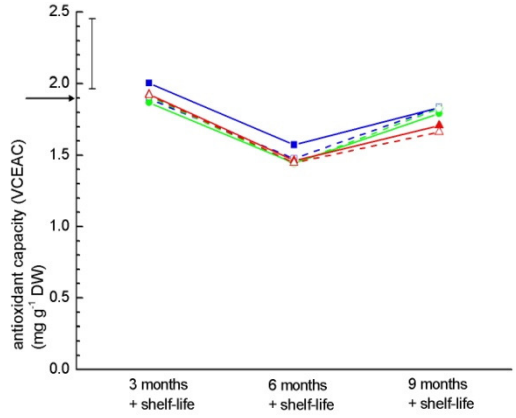


Figure 4.1: Vitamin C (L-AA) concentration (mg 100 g^{-1} FW) (A), phenolic compounds (mg g^{-1} DW) (B) and antioxidant capacity (VCEAC) (mg g^{-1} DW) (C) of 'Jonagold' apples ± 1-MCP (7 DAH) after 3, 6 and 9 months of storage in different storage conditions plus 10 days shelf-life (20°C) in 2005/06. Bars show the $LSD_{0.05}$ for all treatments at each sampling time. Arrow at the y-axis represents harvest value.

4.4 Discussion

The principal biologically active form of vitamin C is L-ascorbic acid (L-AA) (Ball, 2006). L-AA is easily but reversibly oxidized to dehydroascorbic acid (DHA) which is also biologically active (Wills et al., 1984; Lee and Kader, 2000; Hancock and Viola, 2005b). Therefore, to give the total vitamin C content of fruit it is recommended to measure both L-AA and DHA concentrations (Klein, 1987; Nunes et al., 1998; Ball, 2006). The main enzyme responsible for enzymatic degradation of L-AA is ascorbate oxidase (Lee and Kader, 2000; Ball, 2006), but L-AA can also be oxidized by reacting with ROS (Hancock and Viola, 2005b). In the absence of oxygen (Ball, 2006) DHA can irreversibly be converted to form 2,3-diketo-L-gulonic acid, which constitutes a loss of total vitamin C content (Klein, 1987; Hancock and Viola, 2005b). It is assumed that initial DHA concentrations of fruit and vegetables at harvest are low. During storage and shelf-life DHA levels generally increase to different extents while L-AA levels continuously decrease (Wills et al., 1984). However, it is described that there is a loss of vitamin C concentration during storage and poststorage, since not all losses of L-AA are compensated by increased DHA levels (Agar et al., 1997). Therefore, since we did not measure DHA concentrations we suggest that our results underestimate the total vitamin C contents particular at longer storage durations.

In general, freshly harvested fruit contain more vitamin C than fruit held in storage. Since 'Jonagold' apples had 7.3 mg 100 g^{-1} FW L-AA at harvest, L-AA concentrations after 3, 6 and 9 months of storage plus 10 days shelf-life at 20°C dropped down to 4.7, 2.8 and 1.7 mg 100 g^{-1} FW, respectively. Similar results were found in other unpublished own studies with apple cultivars 'Braeburn', 'Royal Gala' and 'Jonagold' and are also described by Davey et al. (2004).

L-AA is very susceptible to chemical and enzymatic oxidation during postharvest handling and storage (Lee and Kader, 2000; Ball, 2006). Reduction of L-AA concentrations during storage can be substantial and is affected by a number of factors, such as extended storage duration, adverse storage temperatures, low relative humidity (Lee and Kader, 2000). However, stability of L-AA is dependent on pH-value of fruit tissue (optimum 3.0 - 4.5) (Davey et al., 2000; Ball, 2006), intracellular compartmentation (Klein, 1987; Kalt et al., 1999) and the protective effect of phenolic antioxidants (Miller and Rice-Evans, 1997). In intact plant tissues ascorbate and degrading enzymes, mainly ascorbate oxidase, are separated by cellular

compartmentation, however, they come in contact after cellular disruption due to bruising, wilting and senescence after harvest (Klein, 1987; Ball, 2006). The vitamin C-sparing activity of phenolics is also due to intracellular compartmentation. Phenolics are localized in the cell vacuole and since they are antioxidants enzyme catalyzed degradation of ascorbate in the vacuole is prevented. Losses of ascorbate may be due to degradation of extravacuolar vitamin C which is not protected by phenolics and the low pH environment of the vacuole (Kalt et al., 1999). It is described that storage conditions and postharvest handling procedures affect vitamin C concentrations more than preharvest conditions (Bangerth, 1977; Lee and Kader, 2000).

In general, it is assumed that postharvest conditions that preserve sensory and eating quality, mainly by slowing down produce metabolism, also maintain the nutritional value of fruit and vegetables (Klein, 1987; Wills et al., 2007). However, it is obvious that there is a progressive loss of L-AA with time (Davey et al., 2000). The most important factor to maintain L-AA concentrations is an appropriate temperature management (Klein, 1987; Lee and Kader, 2000; Wills et al., 2007). Nunes et al. (1998) reported significant L-AA decreases in strawberries with increasing temperatures. While strawberries at 1°C lost 20 – 30 % of the initial L-AA content during 8 days, fruit at 10°C and 20°C lost 30 – 50 % and 55 – 70 %, respectively. Ezell and Wilcox (1959) and Nunes et al. (1998) stated that maintenance of high relative humidity in combination with reduced temperatures is important for preservation of high fruit quality. Water loss is a significant cause of fruit deterioration during storage (Nunes et al., 1998) and leads to a rapid and considerable loss of the water-soluble vitamin C (Ezell and Wilcox, 1959; Nunes et al., 1998; Lee and Kader, 2000). Wrapping strawberry fruit with plastic sheets to reduce water loss leads to better retention of L-AA concentrations and had a greater effect on L-AA levels than temperature (Nunes et al., 1998). Humidity conditions seem to be more important in produce with active metabolism, high respiration rates and rapid loss of moisture during storage.

Storage of climacteric fruit in controlled atmospheres with reduced oxygen concentrations and elevated carbon dioxide concentrations is a known and commonly used method to slow down respiration and the rate of produce metabolism in general. However, higher CO_2 concentrations tend to accelerate L-AA loss during storage of different types of fruit and vegetables (Bangerth, 1977; Agar et al.,1997). While CO_2 concentrations of 0.5 % did not affect the

L-AA content, it was considerably reduced in storage atmospheres with 5.0 % CO_2 (Bangerth, 1977). Both, L-AA and to a lesser extend DHA levels were found to be reduced by high CO_2 concentrations in the study of Agar et al. (1997). Differences in L-AA retention due to storage atmospheres, i.e. with lower O_2- and higher CO_2- concentrations, were also found in our experiment. While 'Jonagold' fruit stored for 9 months in CA-storage had L-AA concentrations of 3.2 mg 100 g^{-1} FW (43.8 % of harvest level), fruit stored for the same duration in ULO-storage had significantly lower L-AA-concentrations of 3.1 mg 100 g^{-1} FW (42.5 % of harvest level).

It is known that the enzyme ACC oxidase catalyses the final step in ethylene biosynthesis (ACC → C_2H_4). ACC oxidase is activated by CO_2 and both ascorbate and Fe^{2+} are required as co-factors (Dong et al., 1992). According to Dong et al. (1992) even 1mM ascorbate is required to maintain the maximum enzyme activity, whereas the required Fe^{2+} concentration is much less (10 µM). In spite of that it is unlikely that L-AA loss during ripening is directly associated with ethylene production (C.B. Watkins, 2008, personal communication). Regarding the stoichiometry of ACC-Oxidation as described by Dong et al. (1992) it seems that there is rather an alteration between L-AA and DHA levels during ethylene biosynthesis than a real loss in total vitamin C concentration

$$(ACC + L - AA + O_2 \xrightarrow{Fe^{2+},CO_2} C_2H_4 + CO_2 + HCN + DHA + 2\,H_2O)$$

Since L-AA concentrations decreased similar in both 1-MCP treated and untreated fruit during the entire storage and post-storage duration in our experiment, it seems likely that L-AA tissue concentration is not ethylene dependent.

Fruit and vegetables contain many different antioxidant components. Eberhardt et al. (2000) demonstrated that vitamin C contributed less than 0.4 % on total antioxidant activity of apple fruit. Total antioxidant activity of 1 g apple with skin had 83.3 ± 8.9 TOSC (total oxidant scavenging capacity) while only 0.32 TOSC of vitamin C. Many other articles describe similar findings and it is generally assumed that the majority of the antioxidant capacity of fruits in general and apple in particular must be due to phytochemicals (phenolic compounds) (Eberhardt et al., 2000; Lee at al., 2003; Wolfe et al., 2003; Kalt, 2005). The results in our experiment show a similar trend. VCEAC levels at harvest were 1.9 mg g^{-1} DW and L-AA concentrations were 7.3 mg $100g^{-1}$ FW. After 9 months of storage VCEAC levels were still

94.7 % (1.8 mg g^{-1} DW) while L-AA concentrations were just 23.3 % (1.7 mg 100 g^{-1} FW) of the initial levels at harvest (Figure 4.1). Even L-AA concentrations were considerably reduced during the entire storage duration (+ shelf-life) and showed 64.4 % after 3 months, 38.4 % after 6 months and 23.3 % after 9 months this was not reflected at VCEAC levels (1.9, 1.5, 1.8 mg g^{-1} DW).

With a few exceptions, the majority of antioxidants in apple fruit are phenolics. Because quantitative data are linked to specific analytical methods, values might be contradictory and it often seems difficult to compare results from different studies or methods. In our experiment, phenolic compounds of 'Jonagold' apples measured at harvest were 11.3 mg g^{-1} DW, whereas total antioxidant capacity at the same time was found to be 1.9 mg g^{-1} DW. Although total values are much different, they follow a similar trend throughout the storage period. Phenolics and total antioxidant capacity during 9 months is comparable. The various storage conditions influenced the concentrations of phenolic compounds and total antioxidant capacity in the same magnitude. In general, the samples with higher phenolics tended to have higher total antioxidant capacity.

Vinson et al. (2001) have shown that the antioxidant capacity of fruit was much greater due to several phenolic compounds than vitamin antioxidants and pure phenolics. Synergistic effects between different individual antioxidant compounds are also suggested by Eberhardt et al., 2000, van der Sluis (2001) and Chun et al. (2005). In our study phenols were measured colorimetrically using Folin-Ciocalteu's reagent (FCR) with L-AA as the standard. It is known that the FCR assay does not measure total quantity of the phenolics in plant extracts (Kähkönen et al., 1999; Singleton et al., 1999; Vinson et al., 2001). Phenolic compounds in plants are either soluble free or bound. Vinson et al. (2001) describe that most of the tested fruit in their experiment had a high percentage of bound phenolics (31 – 94 %). In apple total phenols were 34.1 ± 4.8 µmol g^{-1} on a dry weight basis and it has been shown that 51.9 % of these phenols were conjugated (Vinson et al., 2001). Imeh and Khokhar (2002) describe that extraction with aqueous methanol, as used in this study, is only used for determination of unconjugated, free phenols. For determination of total phenols additional 1.2 M HCL in the extraction is needed (Vinson et al., 2001; Imeh and Khokhar, 2002). Therefore, since bound phenolics were not measured in our experiment, our results underestimate the total phenol concentrations in 'Jonagold' apple fruit.

Moreover, different phenolic compounds, which are very diverse and extensively distributed in plants, have different responses in FCR (Kähkönen et al., 1999). In general, it is known, that phenols have a high chemical reactivity, which also complicates their analysis (Robards et al., 1999). Singleton et al. (1999) state that the results of FCR can include interfering substances and that FCR might measure all oxidizable substrates under the given reaction conditions, not just phenols. Therefore, it is recommended to subtract the concentration of ascorbic acid, which reacts readily with FCR (Singleton et al., 1999) from results given by FCR (Singleton et al., 1999; Vinson et al., 2001). However, since the contribution of L-AA on total antioxidant capacity was found to be less than 0.4 % in apple fruit (Eberhardt et al., 2000), we suggest that its influence on results from FCR is negligible. L-AA contents were not subtracted neither from the results of FCR nor of VCEAC determinations. After 3 months of storage L-AA concentration was significantly reduced and had 64.4 % of the initial value at harvest (4.7 vs. 7.3 mg 100 g^{-1} FW). In contrast, phenolic compounds after 3 months of storage were even significantly increased and reached 101.8 % of the initial harvest levels (11.5 vs. 11.3 mg g^{-1} DW).

Phenylalanine ammonia-lyase (PAL) is the crucial enzyme in phenylpropanoid metabolism (Saltveit, 1999). The production of *trans*-cinnamic acid from phenylalanine by the enzyme PAL is generally the first step in the biosynthesis of a wide range of phenylpropanoid compounds such as simple phenols, flavonoids and anthocyanins (Assis et al. 2001). PAL activity (Faragher and Chalmers, 1977; Blankenship and Unrath, 1988) and thus phenylpropanoid metabolism is stimulated and enhanced by ethylene (Saltveit, 1999). Moreover, phenylpropanoid metabolism is enhanced by postharvest oxidative stress due to wounding, wilting, adverse temperatures, anaerobic storage atmospheres, advanced stages of senescence (Faragher and Chalmers, 1977; Saltveit, 1999). Therefore, PAL activity might be a potential site for regulation of phenylpropanoid metabolism (Assis et al., 2001) and thus total antioxidant capacity of apple fruit. From all this it can be concluded that different storage atmospheres, -durations and 1-MCP treatment should affect phenolic concentrations during storage. Compared to control fruit 1-MCP significantly reduced ethylene production in ‘Jonagold’ apples during 9 months of storage plus 10 days shelf-life at 20°C (57.5 vs. 172.1 mL(kg*h)$^{-1}$) and should lead to significant lower levels of phenolic compounds. MacLean et al. (2006) investigated the impact of 1-MCP on the synthesis and retention of flavonoid compounds during 120

days cold storage of 'Red Delicious' apples. While total flavonoid concentrations were higher in 1-MCP treated apples, chlorogenic acid levels were lower in their experiment. However, in our experiment phenolic compounds were influenced neither by storage condition nor by 1-MCP treatment. Awad et al. (2001) and Renard et al. (2007) point out that the overall production and accumulation of phenols (flavonoids and chlorogenic acid; (Awad et al., 2001)) in apple skin (Awad et al., 2001) and in apple flesh (Renard et al., 2007) is completed in the early stages of fruit development. Even the main accumulation of anthocyanins occurs during growth and maturation (Awad et al., 2001), however, MacLean et al. (2006) reported *de novo* anthocyanin biosynthesis during storage and ripening. Decreasing concentrations of phenolic compounds during fruit development and maturation (Burda et al., 1990) are mainly due to dilution of the initial values (Renard et al., 2007) by cell enlargement.

Many authors describe that phenolics and total antioxidant capacity in apple are stable during storage irrespective of storage atmospheres (Burda et al., 1990; Awad and de Jager, 2000; Golding et al., 2001; van der Sluis et al., 2001). In our experiment mean phenolic compounds increased during the first 3 months of storage (11.5 vs. 11.3 mg g^{-1} DW). This effect was similar in all three storage atmospheres. After 6 months of storage mean phenolic compounds declined to 10.8 mg g^{-1} DW and increased again after 9 months of storage to reach initial levels (11.3 mg g^{-1} DW). Lattanzio et al. (2001) found similar effects in the skin of 'Golden Delicious' apples. Moreover, they found evidence that increased concentrations of phenolics are a consequence of low storage temperatures of 2°C. Cellular adaptation and response to postharvest oxidative stress leads to an up-regulation in the antioxidant defense system (Bartosz, 1997; Toivonen, 2003; Davey et al., 2004). An appropriate antioxidant system is needed to protect against deleterious postharvest stress (Lurie, 2003). Since concentrations of phenolic compounds showed some fluctuation during 9 months of storage, it seems likely, however, that not all phenolic compounds were affected in the same manner and it is generally suggested that individual phenols have different behaviours during ripening (Awad and de Jager, 2003).

Larrigaudière et al. (2004) reported an increase in the enzymatic antioxidant capacity following 1-MCP treatment of 'Blanquilla' pears and interpreted it as a sign of a general metabolic change which is directly or indirectly influenced by ethylene. In contrast, Shaham et al. (2003) observed lower activities of most antioxidant enzymes in 1-MCP treated 'Granny

Smith' apples. However, lipid-soluble antioxidant activity was found to be higher in 1-MCP treated fruit when compared with untreated control apple fruit. Though, Scalzo et al. (2005) described that the lipophilic contribution on total antioxidant capacity in apple is negligible. Since total TEAC value was found to be 1.60 ± 0.29 µmol TE (Trolox® equivalents) g^{-1} FW in their study, the hydrophilic and lipophilic section was 1.49 ± 0.29 and 0.10 ± 0.01 µmol TE g^{-1} FW, respectively.

No effect of 1-MCP neither on phenolic compounds nor on total antioxidant capacity was found in our experiment. Our results show that phenolic compounds were just affected by storage duration ($P \leq 0.001$) whereas total antioxidant capacity (VCEAC) was affected by storage condition ($P = 0.007$), -duration ($P \leq 0.001$) and an interaction ($P = 0.035$) between these two main factors. Similar to our results Vilaplana et al. (2006) could not find significant differences in total antioxidant activity (DPPH; 1-diphenyl-2-picrylhydrazyl) between 1-MCP treated 'Smoothee' apples and untreated control fruit.

Although standard quality factors such as firmness, titratable acidity and retention of green background colour, which mainly influence purchase decision for consumer are generally positively influenced by 1-MCP (Heyn et al., 2009, submitted), the nutritional value was not influenced by 1-MCP. Moreover, storage conditions had little effect on phenolic compounds and total antioxidant capacity. Only L-AA concentration was affected by different storage conditions and slightly influenced by 1-MCP. However, since L-AA contributes to a small extend to the antioxidant capacity of apple fruit, this does not affect the total nutritional value of apple fruit.

4.5 References

Abeles, F.B., P.W. Morgan and M.E. Saltveit. (1992) *Ethylene in plant biology*. 2nd Edition, Academic Press, INC.

Agar, I.T., J. Streif and F. Bangerth. (1997) Effect of high CO_2 and controlled atmosphere (CA) on the ascorbic and dehydroascorbic acid content of some berry fruits. *Postharvest Biol. Technol.* 11, 47-55.

Arnao, M.B., A. Cano and M. Acosta. (2001) The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem.* 73, 239-244.

Assis, J.S., R. Maldonado, T. Munoz, M.I. Escribano and C. Merodio. (2001) Effect of high carbon dioxide concentration on PAL activity and phenolic contents in ripening cherimoya fruit. *Postharvest Biol. Technol.* 23, 33-39.

Awad, M.A. and A. de Jager. (2000) Flavonoid and chlorogenic acid concentrations in skin of 'Jonagold' and 'Elstar' apples during and after regular and ultra low oxygen storage. *Postharvest Biol. Technol.* 20, 15-24.

Awad, M.A. and A. de Jager. (2003) Influences of air and controlled atmosphere storage on the concentration of potentially healthful phenolics in apples and other fruits. *Postharvest Biol. Technol.* 27, 53-58.

Awad, M.A., A. de Jager, L.H.W. van der Plas and A.R. van der Krol. (2001) Flavonoid and chlorogenic acid changes in skin of 'Elstar' and 'Jonagold' apples during development and ripening. *Scientia Hort.* 90, 69-83.

Ball, G.F.M. (2006) *Vitamins in foods: Analysis, bioavailability, and stability*. CRC Press; Taylor & Francis Group, LLC; 289-308.

Bangerth, F. (1977) Zum Einfluss des Partialdrucks verschiedener Gaskomponenten der Lageratmosphäre auf den Ascorbinsäuregehalt. *Qual. Plant. – Pl. Fds. Hum. Nutr.* XXVII, 2, 125-133.

Bartosz, G. (1997) Oxidative stress in plants. *Acta Physiol. Plant.* 19(1), 47-64.

Blankenship, S.M. and C.R. Unrath. (1988) PAL and ethylene content during maturation of Red and Golden Delicious apples. *Phytochemistry* 27(4), 1001-1003.

Blankenship, S.M. and J.M. Dole. (2003) 1-Methylcyclopropene: a review. *Postharvest Biol. Technol.* 28, 1-25.

Bloomsbury English Dictionary (2004) Bloomsbury Publishing Plc, London.

Boyer, J. and R.H. Liu. (2003-2004) Antioxidants in apples. *N.Y. Fruit Quart.* 11(4), 11-15.

Burda, S., W. Oleszek and C.Y. Lee. (1990) Phenolic compounds and their changes in apples during maturation and cold storage. *J. Agric. Food Chem.* 38, 945-948.

Chun, O.K., D.-O. Kim, N. Smith, D. Schroeder, J.T. Han and C.Y. Lee. (2005) Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *J. Sci. Food Agric.* 85, 1715-1724.

Davey, M.W., A. Auwerkerken and J. Keulemans. (2007) Relationship of apple vitamin C and antioxidant contents to harvest date and postharvest pathogen infection. *J. Sci. Food Agric.* 87, 802-813.

Davey, M.W., C. Franck and J. Keulemans. (2004) Distribution, developmental and stress responses of antioxidant metabolism in *Malus. Plant Cell Environ.* 27, 1309-1320.

Davey, M.W., M. van Montagu, D. Inzé, M. Sanmartin, A. Kanellis, N. Smirnoff, I.J.J. Benzie, J.J. Strain, D. Favell and J. Fletcher. (2000) Plant L-ascorbic acid: chemistry, function metabolism, bioavailability and effects of processing. *J. Sci. Food Agric.* 80, 825-860.

DeEll, J.R., D.P. Murr, M.D. Porteous and H.P.V. Rupasinghe. (2002) Influence of temperature and duration of 1-Methylcyclopropene (1-MCP) treatment on apple quality. *Postharvest Biol. Technol.* 24, 349-353.

Dong, J.G., J.C. Fernández-Maculet and S.F. Yang. (1992) Purification and characterization of 1-aminocyclopropane-1-carboxylate oxidase from apple fruit. *Proc. Natl. Acad. Sci.*, 89, 9789-9793.

Eberhardt, M.V., C.Y. Lee and R.H. Liu. (2000) Antioxidant activity of fresh apples. *Nature* 405, 903-904.

Ezell, B.D. and M.S. Wilcox. (1959) Loss of vitamin C in fresh vegetables as related to wilting and temperature. *Agric. Food Chem.* 7(7), 507-509.

Fan, X. and J.P. Mattheis. (1999) Development of apple superficial scald, soft scald, core flush, and greasiness is reduced by MCP. *J. Agric. Food Chem.* 47, 3063-3068.

Fan, X., S.M. Blankenship and J.P. Mattheis. (1999) 1-Methylcyclopropene inhibits apple ripening. *J. Amer. Soc. Hort. Sci.* 124(6), 690-695.

Faragher, J.D. and D.J. Chalmers. (1977) Regulation of anthocyanin synthesis in apple skin. III. Involvement of phenylalanine ammonia-lyase. *Aust. J. Plant Physiol.* 4, 133-141.

Francis, F.J. (1995) Quality as influenced by color. *Food Qual. Pref.* 6, 149-155.

Golding, J.B., W.B. McGlasson, S.G. Wyllie and D.N. Leach. (2001) Fate of apple peel phenolics during cool storage. *J. Agric. Food Chem.* 49, 2283-2289.

Hancock, R.D. and R. Viola. (2005a) Improving the nutritional value of crops through enhancement of L-ascorbic acid (vitamin C) content: rationale and biotechnological opportunities. *J. Agric. Food Chem.* 53, 5248-2289.

Hancock, R.D. and R. Viola. (2005b) Biosynthesis and catabolism of L-ascorbic acid in plants. *Crit. Rev. Plant Sci.* 24, 167-188.

Hewett, E.W. (2006) An overview of preharvest factors influencing postharvest quality of horticultural products. *Int. J. Postharvest Technol. Inn.* 1(1), 4-15.

Huang, D., B. Ou, M. Hampsch-Woodill, J.A. Flanagan and E.K. Deemer. (2002) Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated β-cyclodextrin as the solubility enhancer. *J. Agric. Food Chem.* 50, 1815-1821.

Imeh, U. and S. Khokhar. (2002) Distribution of conjugated and free phenols in fruits: antioxidant activity and cultivar variations. *J. Agric. Food Chem.* 50, 6301-6306.

Ju, Z. and W.J. Bramlage. (1999) Phenolics and lipid-soluble antioxidants in fruit cuticle of apples and their antioxidant activities in model systems. *Postharvest Biol. Technol.* 16, 107-118.

Kader, A.A. (2002) Fruits in the global market. In: Knee, M. (Ed.), *Fruit quality and its biological basis*. Sheffield Academic Press Ltd; 1-16.

Kähkönen, M.P., A.I. Hopia, H.J. Vuorela, J.-P. Rauha, K. Pihlaja, T.S. Kujala and M. Heinonen. (1999) Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* 47, 3954-3962.

Kalt, W. (2005) Effects of production and processing factors on major fruit and vegetable antioxidants. *J. Food Sci.* 70(1), 11-19.

Kalt, W., C.F. Forney, A. Martin and R.L. Prior. (1999) Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits. *J. Agric. Food Chem.* 47, 4638-4644.

Kays, S.J. (1999) Preharvest factors affecting appearance. *Postharvest Biol. Technol.* 15, 233-247.

Kevers, C., M. Falkowski, J. Tabart, J.-O. Defraigne, J. Dommes and J. Pincemail. (2007) Evolution of antioxidant capacity during storage of selected fruits and vegetables. *J. Agric. Food Chem.* 55, 8596-8603.

Kim, D.-O., K.W. Lee, H.J. Lee and C.Y. Lee. (2002) Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *J. Agric. Food Chem.* 50, 3713-3717.

Klein, B.P. (1987) Nutritional consequences of minimal processing of fruits and vegetables. *J. Food Qual.* 10, 179-193.

Klein, B.P. and A.C. Kurilich. (2000) Processing effects on dietary antioxidants from plant foods. *HortScience* 35(4), 580-588.

Larrigaudière, C., R. Vilaplana, Y. Soria and I. Recasens. (2004) Oxidative behaviour of Blanquilla pears treated with 1-Methylcyclopropene during cold storage. *J. Sci. Food Agric.* 84, 1871-1877.

Larson, R.A. (1988) The antioxidants of higher plants. *Phytochemistry* 27(4), 969-978.

Lattanzio, V., D. Di Venere, V. Linsalata, P. Bertolini, A. Ippolito and M. Salerno. (2001) Low temperature metabolism of apple phenolics and quiescene of *phlyctaena vagabunda*. *J. Agric. Food Chem.* 49, 5817-5821.

Lee, K.W., Y.J. Kim, D.-O. Kim, H.J. Lee and C.Y. Lee. (2003) Major phenolics in apple and their contribution to the total antioxidant capacity. *J. Agric. Food Chem.* 49, 5817-5821.

Lee, S.K. and A.A. Kader. (2000) Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biol. Technol.* 20, 207-220.

Lurie, S. (2003) Antioxidants. In: Hodges, D.M. (Ed.), *Postharvest oxidative stress in horticultural crops*. Food Product Press; 131-150.

MacLean, D.D., D.P. Murr, J.R. DeEll and C.R. Horvath. (2006) Postharvest variation in apple (Malus x domestica Borkh.) flavonoids following harvest, storage, and 1-MCP treatment. *J. Agric. Food Chem.* 54, 870-878.

Masia, A. (2003) Physiological effects of oxidative stress in relation to ethylene in postharvest produce. In: Hodges, D.M. (Ed.), *Postharvest oxidative stress in horticultural crops*. Food Product Press; 165-197.

Miller, N.J. and C.A. Rice-Evans. (1997) The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. *Food Chem.* 60(3), 331-337.

Mir, N. and R. Beaudry. (2002) Atmosphere control using oxygen and carbon dioxide. In: Knee, M. (Ed.), *Fruit Quality and its biological basis*. Sheffield Academic Press Ltd; 122-156.

Mir, N.A., E. Curell, N. Khan, M. Whitaker and R.M. Beaudry. (2001) Harvest maturity, storage temperature, and 1-MCP application frequency alter firmness retention and chlorophyll fluorescence of 'Redchief Delicious' apples. *J. Amer. Soc. Hort. Sci.* 126(5), 618-624.

Noctor, G. and C.H. Foyer. (1998) Ascorbate and glutathione: keeping active oxygen under control. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 49, 249-279.

Nunes, M.C.N., J.K. Brecht, A.M.M.B. Morais and S.A. Sargent. (1998) Controlling temperature and water loss to maintain ascorbic acid levels in strawberries during postharvest handling. *J. Food Sci.* 63(6), 1033-1036.

Podsędek, A., J. Wilska-Jeszka, B. Anders and J. Markowski. (2000) Compositional characterisation of some apple varieties. *Eur. Food Res. Technol.* 210, 268-272.

Rabinovitch, H.D. and Sklan, D. (1981) Superoxide dismutase activity in ripening cucumber and pepper fruit. *Physiol. Plant*, 52, 380-384.

Renard, C.M.G.C., N. Dupont and P. Guillermin. (2007) Concentrations and characteristics of procyanidins and other phenolics in apples during fruit growth. *Phytochemistry* 68, 1128-1138.

Robards, K., P.D. Prenzler, G. Tucker, P. Swatsitang and W. Glover. (1999) Phenolic compounds and their role in oxidative processes in fruits. *Food Chem.* 66, 401-436.

Rupasinghe, H.P.C., D.P. Murr, G. Paliyath and L. Skog. (2000) Inhibitory effect of 1-MCP on ripening and superficial scald development in 'McIntosh' and 'Delicious' apples. *J. Hort. Sci. Biotechnol.* 75(3), 271-276.

Saltveit, M.E. (1999) Effect of ethylene on quality of fresh fruits and vegetables. *Postharvest Biol. Technol.* 15, 279-292.

Sánchez-Moreno, C., S. de Pascual-Teresa, B. de Ancos and M.P. Cano. (2006) Nutritional values of fruits. In: Hui, Y.H. (Ed.), *Handbook of fruits and fruit processing*. Blackwell Publishing Professional; 29-43.

Scalzo, J., A. Politi, N. Pellegrini, B. Mezzetti and M. Battino. (2005) Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition* 21, 207-213.

Shaham, Z., A. Lers and S. Lurie. (2003) Effect of heat or 1-Methylcyclopropene on antioxidative enzyme activities and antioxidants in apples in relation to superficial scald development. *J. Amer. Soc. Hort. Sci.* 128(5), 761-766.

Shewfelt, R.L. and B.A. del Rosario. (2000) The role of lipid peroxidation in storage disorders of fresh fruits and vegetables. *HortScience* 35(4), 575-579.

Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventós. (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol.* 299, 152-178.

Sisler, E.C., E. Dupille and M. Serek. (1996) Effect of 1-Methylcyclopropene and methylenecyclopropane on ethylene binding and ethylene action on cut carnations. *Plant Growth Regul.* 18, 79-86.

Sun, J., Y.-F. Chu, X. Wu and R.H. Liu. (2002) Antioxidant and antiproliferative activities of common fruits. *J. Agric. Food Chem.* 50, 7449-7454.

Toivonen, P.M.A. (2003) Effects of storage conditions and postharvest procedures on oxidative stress in fruits and vegetables. In: Hodges, D.M. (Ed.), *Postharvest oxidative stress in horticultural crops*. Food Product Press; 69-90.

Treutter, D. (2001) Biosynthesis of phenolic compounds and its regulation in apple. *Plant Growth Regul.* 34, 71-89.

Tromp, J. (2005) Fruit ripening and quality. In: Tromp, J., A.D. Webster and S.J. Wertheim (Eds.), *Fundamentals of temperate zone tree fruit production.* Backhuys Publishers, Leiden, The Netherlands; 295-310.

Van den Berg, R., G.R.M.M. Haenen, H. van den Berg and A. Bast. (1999) Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. *Food Chem.* 66, 511-517.

Van der Sluis, A.A., M. Dekker, A. de Jager and W.M.F. Jongen. (2001) Activity and concentration of polyphenolic antioxidants in apple: effect of cultivar, harvest year, and storage conditions. *J. Agric. Food Chem.* 49, 3606-3613.

Vilapana, R., M.C. Valentines, P. Toivonen and C. Larrigaudière. (2006) Antioxidant potential and peroxidative state of 'Golden Smoothee' apples treated with 1-Methylcylcopropene. *J. Amer. Soc. Hort. Sci.* 131(1), 104-109.

Vinson, J.A., X. Su, L. Zubik and P. Bose. (2001) Phenol antioxidant quantity and quality in foods: fruits. *J. Agric. Food Chem.* 49, 5315-5321.

Wang, H., G. Cao and R.L. Prior. (1996) Total antioxidant capacity of fruits. *J. Agric. Food Chem.* 44, 701-705.

Watkins, C.B. (2002) Ethylene synthesis, mode of action, consequences and control. In: Knee, M. (Ed.), *Fruit quality and its biological basis*. Sheffield Academic Press Ltd; 180-224.

Watkins, C.B. (2006) 1-Methylcyclopropene (1-MCP) based technologies for storage and shelf life extension. *Int. J. Postharvest Technol. Inn.*, 1(1), 62-68.

Watkins, C.B., J.F. Nock and B.D. Whitaker. (2000) Responses of early, mid and late season apple cultivars to postharvest application of 1-Methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. *Postharvest Biol. Technol.* 19, 17-32.

Wills, R.B.H., P. Wimalasiri and H. Greenfield. (1984) Dehydroascorbic acid levels in fresh fruit and vegetables in relation to total vitamin C activity. *J. Agric. Food Chem.* 32, 836-838.

Wills, R.B.H., W.B. McGlasson, D. Graham and D.C. Joyce. (2007) *Postharvest: an introduction to the physiology and handling of fruit, vegetables and ornamentals*. 5th Edition. CABI.

Wolfe, K., X. Wu and R.H. Liu. (2003) Antioxidant activity of apple peels. *J. Agric. Food Chem.* 51, 609-614.

Wood, L.G., P.G. Gibson and M.L. Garg. (2006) A review of the methodology for assessing *in vivo* antioxidant capacity. *J. Sci. Food Agric.* 86, 2057-2066.

ZMP (2006) *Marktbilanz Obst*; Deutschland: Versorgung und Verbrauch; 26.

5.

General Conclusion and Outlook

Storage technologies, such as CA-storage and 1-MCP treatments, have led to an all-year-round global supply of high qualitative apple fruit. As a consequence, pressure of competition between several apple growing areas is increasing and in the same way consumers demands and expectations for apple fruit quality. It is critically important that fruit quality at the point of sale meets consumer requirements. The present study (Chapter 2) has shown that postharvest fruit quality is best maintained in 1-MCP treated apple, especially in combination with controlled atmosphere storage (CA and ULO). However, in the literature it is found that 1-MCP treatment and even CA/ULO-storage might impair the development of aroma and flavour compounds due to the reduction of ethylene production. Therefore, the present study will be continued with measurements of aroma volatile profiles and determination of precursors (fatty acids) of 'Jonagold' apple fruit following 1-MCP treatment and storage in different storage conditions (cold storage, CA- and ULO-storage). Nevertheless, the present study (Chapter 2) provides evidence that consumers purchase decision of apple fruit is not necessarily influenced by aroma, if other quality parameters, especially firmness and appearance, are optimal and the sugar:acid-ratio is well-balanced.

Moreover, it would be interesting to determine the enzymatic antioxidant capacity, namely the enzymes superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX), of 1-MCP treated 'Jonagold' apple fruit under the above mentioned experimental conditions. Since no effect of 1-MCP on phenolic compounds and total non-enzymatic antioxidant capacity (VCEAC) was found in the present study (Chapter 4), it would be of interest whether antioxidant enzymes would be affected by 1-MCP treatment and/or storage conditions and -durations. Results presented in the literature are equivocal. The improved fruit quality and storage characteristics of 1-MCP treated apple fruit in the present study might be due to higher contents of antioxidant enzymes.

However, the present study (Chapter 3) clearly shows that immediate 1-MCP treatment and appropriate storage management after harvest is critical for a maximum reduction of climacteric characteristics such as ethylene production and respiration rate as well as maintenance of postharvest and post-storage apple fruit quality.

APPENDIX

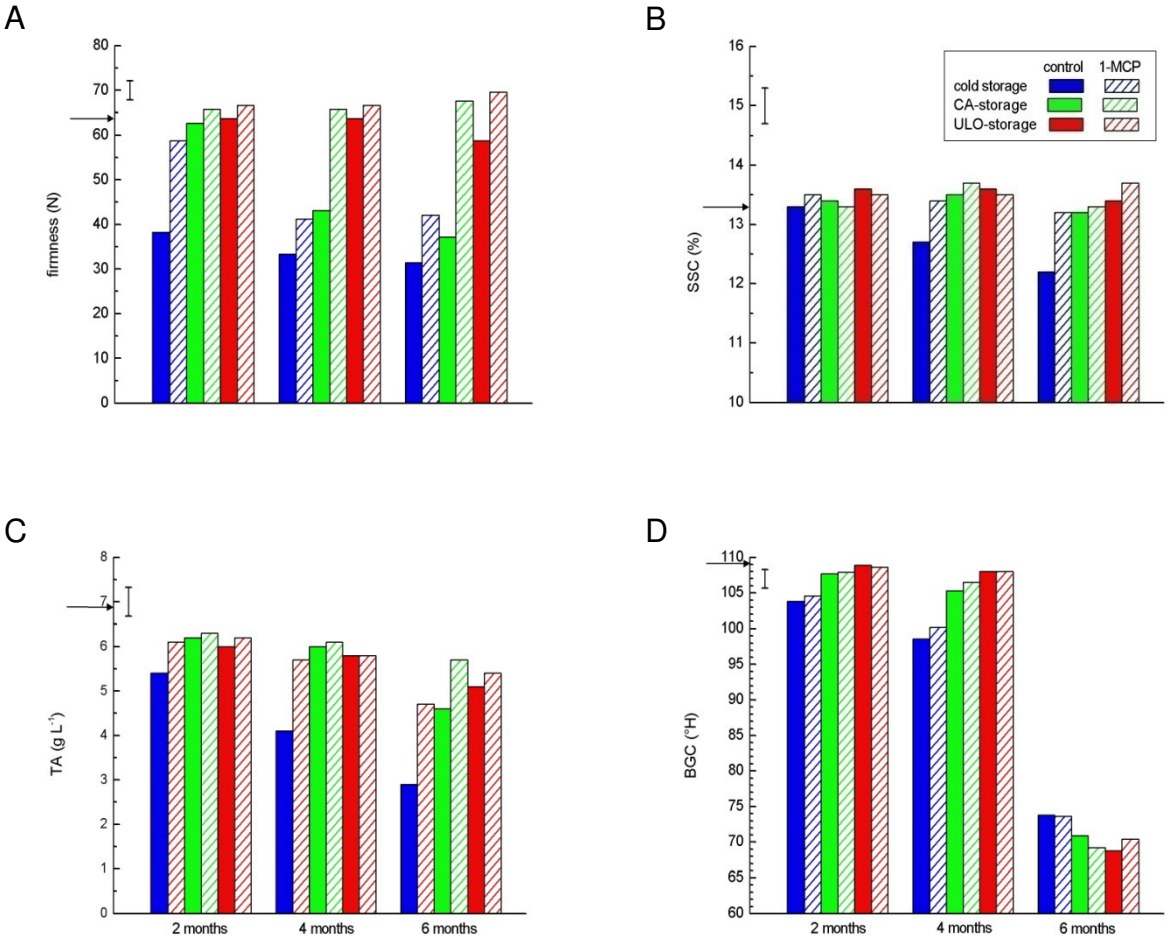


Figure A.1: Firmness FF (N) (A), soluble solid concentration SSC (%) (B), titratable acidity TA (g L^{-1}) (C) and background colour BGC (H°) (D) of 'Jonagold' apples ± 1-MCP after 2, 4 and 6 months of storage in different storage conditions (cold storage, CA- and ULO-storage) in 2004/05. Bars show the $LSD_{0.05}$ for all treatments at each sampling time. Arrow at the y-axis represents harvest value.

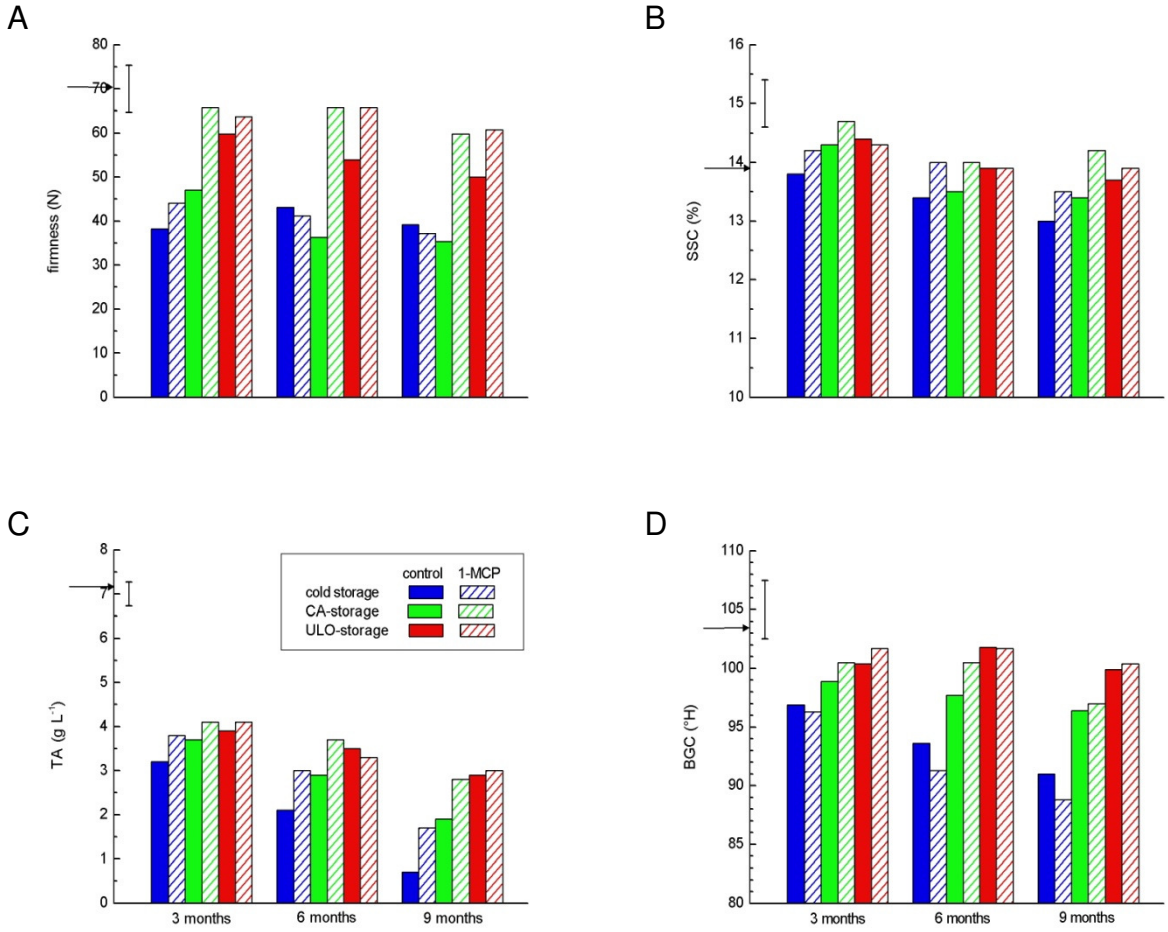


Figure A.2: Firmness FF (N) (A), soluble solid concentration SSC (%) (B), titratable acidity TA (g L^{-1}) (C) and background colour BGC (H°) (D) of 'Jonagold' apples ± 1-MCP after 3, 6 and 9 months of storage in different storage conditions (cold storage, CA- and ULO-storage) in 2005/06. Bars show the $LSD_{0.05}$ for all treatments at each sampling time. Arrow at the y-axis represents harvest value.

Table A.1: Flesh firmness FF (N), soluble solid concentration SSC (%), titratable acidity TA (g L^{-1}) and background colour BGC (H°) of 'Jonagold' apples of the 1st harvest ± 1-MCP after 3 and 5 months of cold- and ULO-storage plus 5 and 6 days shelf-life (20°C) in 2006/07, respectively.

quality parameter	Storage period (months)	cold storage		ULO-storage	
		control	1-MCP	control	1-MCP
FF (N)	3	36.3	39.2	47.0	62.7
	5	39.2	43.1	44.1	63.7
SSC (%)	3	13.3	13.5	14.3	14.6
	5	13.4	14.2	14.1	14.3
TA (g L^{-1})	3	3.6	4.5	5.0	5.6
	5	2.7	4.2	4.2	4.8
BGC (H°)	3	98.6	97.9	106.6	107.7
	5	96.8	95.8	105.4	106.0

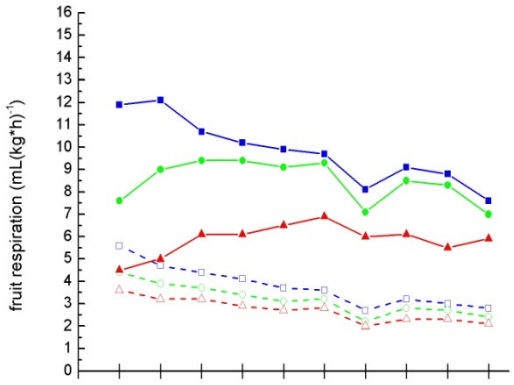
Table A.2: Flesh firmness FF (N), soluble solid concentration SSC (%), titratable acidity TA (g L^{-1}) and background colour BGC (H°) of 'Jonagold' apples of the 2nd harvest ± 1-MCP after 3 and 5 months of cold- and ULO-storage plus 5 and 6 days shelf-life (20°C) in 2006/07, respectively.

quality parameter	Storage period (months)	cold storage		ULO-storage	
		control	1-MCP	control	1-MCP
FF (N)	3	39.2	39.2	50.0	59.8
	5	34.3	40.2	51.9	60.8
SSC (%)	3	13.3	13.4	13.8	14.1
	5	12.9	13.6	13.7	14.1
TA (g L^{-1})	3	3.9	4.5	4.9	5.1
	5	2.2	3.6	4.6	5.1
BGC (H°)	3	95.0	95.2	102.9	103.0
	5	94.8	93.7	101.9	102.0

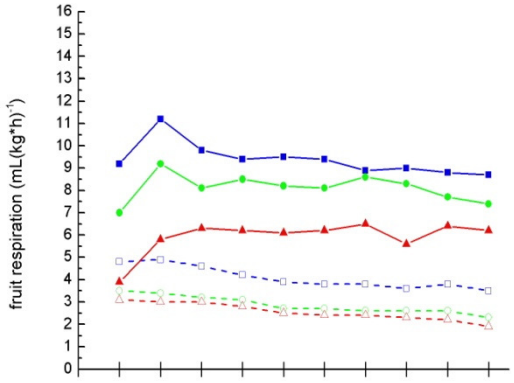
Table A.3: Participating consumer divided by gender at consumer taste panels following 3 and 5 months of cold- and ULO-storage plus 5 and 6 days shelf-life (20°C) of 'Jonagold' apples ± 1-MCP in 2007, respectively.

		1st harvest		2nd harvest	
	gender	3 months	5 months	3 months	5 months
< 20 years	f	9	11	4	14
	m	7	9	3	7
20 - 39 years	f	11	18	12	15
	m	9	12	5	4
40 - 60 years	f	20	16	8	23
	m	18	16	12	17
> 60 years	f	12	18	4	14
	m	11	8	4	14

A



B



C

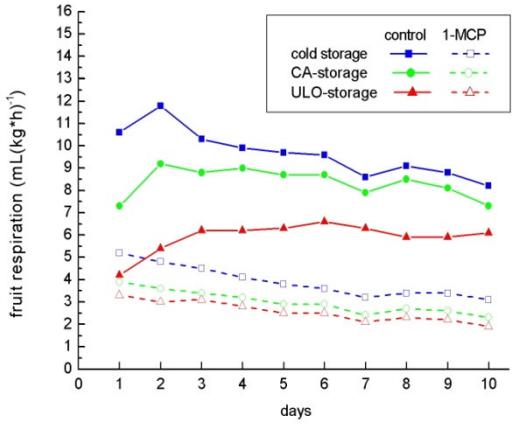
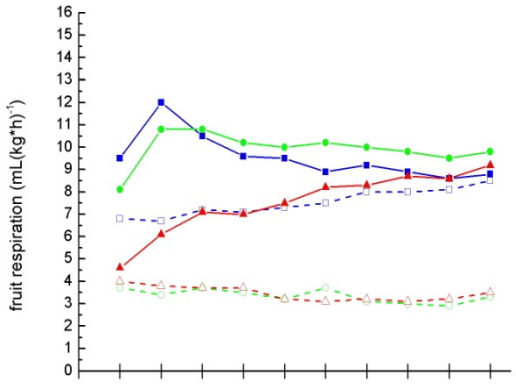
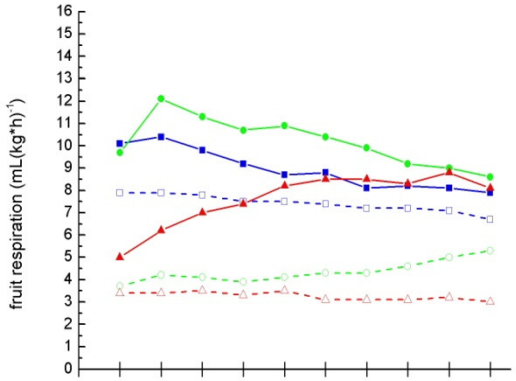


Figure A.3: Daily respiration rate (mL(kg*h)$^{-1}$) of 'Jonagold' apples ± 1-MCP during 10 days shelf-life (20°C) following 2 (A), 4 (B) and 6 (C) months of cold storage, CA- and ULO-storage in 2004/05. 1-MCP treatment was 0 DAH.

A



B



C

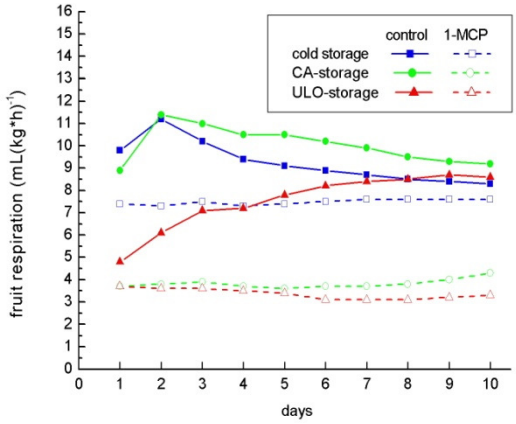
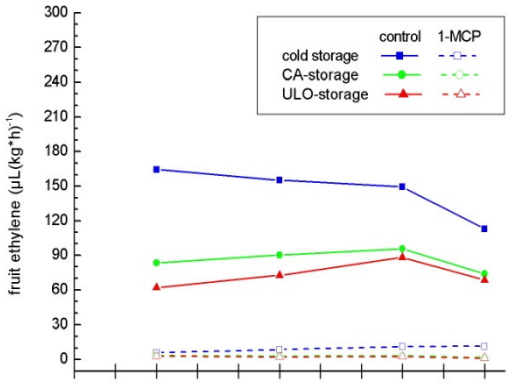
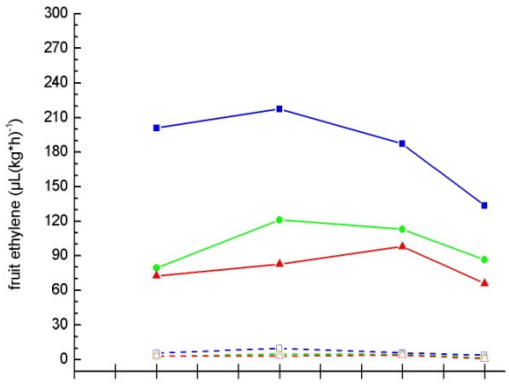


Figure A.4: Daily respiration rate (mL(kg*h)$^{-1}$) of 'Jonagold' apples ± 1-MCP during 10 days shelf-life (20°C) following 3 (A), 6 (B) and 9 (C) months of cold storage, CA- and ULO-storage in 2005/06. 1-MCP treatment was 7 DAH.

A



B



C

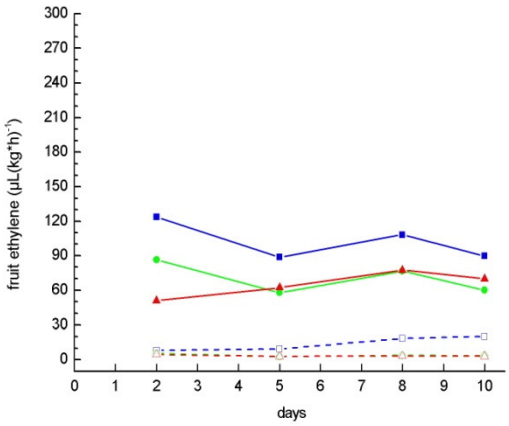
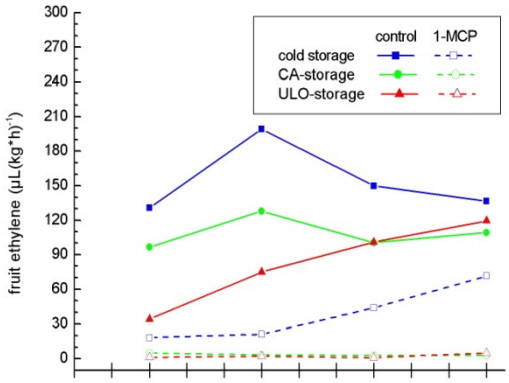
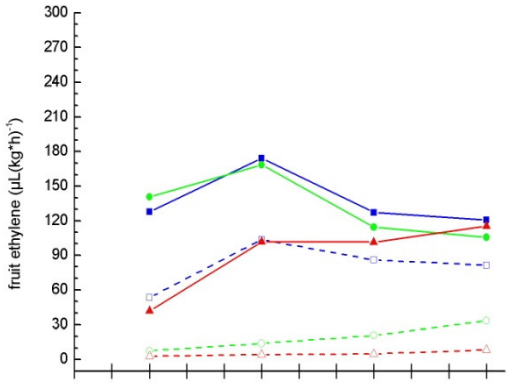


Figure A.5: Ethylene production rate (µL(kg*h)$^{-1}$) of 'Jonagold' apples ± 1-MCP during 10 days shelf-life (20°C) following 2 (A), 4 (B) and 6 (C) months of cold storage, CA- and ULO-storage in 2004/05. 1-MCP treatment was 0 DAH.

A



B



C

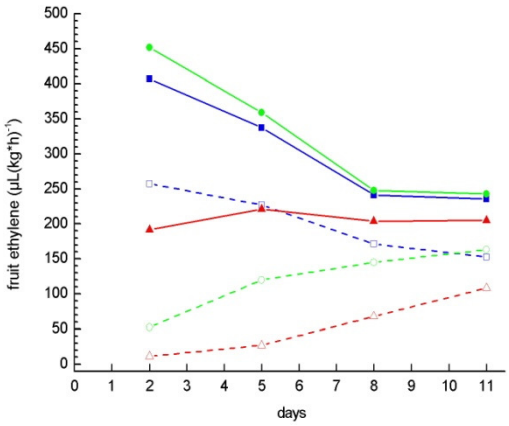
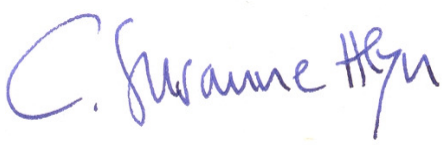


Figure A.6: Ethylene production rate (µL(kg*h)$^{-1}$) of 'Jonagold' apples ± 1-MCP during 11 days shelf-life (20°C) following 3 (A), 6 (B) and 9 (C) months of cold storage, CA- and ULO-storage in 2005/06. 1-MCP treatment was 7 DAH.

DECLARATION OF ORIGINALITY

Hereby I declare that this doctoral thesis is independently written by myself. In addition, I confirm that no other sources than those specified in the thesis have been used. I assure that this thesis, in the current or similar format, has not been submitted to any other institution in order to obtain a Ph.D. or any other academic degree.

Ich erkläre hiermit, dass ich diese Dissertation selbständig angefertigt habe. Es wurden nur die im Literaturverzeichnis aufgeführten Hilfsmittel benutzt und fremdes Gedankengut als solches kenntlich gemacht. Ich versichere, dass ich diese Arbeit in gleicher oder ähnlicher Form noch keiner anderen Institution zur Prüfung vorgelegt habe.



Hohenheim, im Januar 2009 Claudia Susanne Heyn

CURRICULUM VITAE

PERSONAL DATA

Name	Claudia Susanne Heyn
Date and Place of Birth	April 2nd 1977 in Kassel, Germany

UNIVERSITY EDUCATION

Sept. 2004 – to date:	PhD-student at the Faculty of Agricultural Sciences, Institute of Specialty Crops and Crop Physiology, Fruit Sciences, University of Hohenheim, Germany
April – July 2001:	Study abroad, Writtle College, Chelmsford, Essex, UK
Sept. 1997 – July 2003:	Studies in Horticulture University of Applied Sciences Wiesbaden/Geisenheim, Germany
July 2003:	*Diplom-Ingenieur (FH) Gartenbau*

SCHOOL EDUCATION

June 1996:	*Abitur*
1993 – 1996:	Geschwister-Scholl-Schule (gymnasiale Oberstufe), Melsungen, Germany
1989 – 1993:	Gesamtschule (gymnasialer Zweig), Melsungen, Germany

WORKING EXPERIENCE

Nov. 2001 – May 2007:	Research assistant; Kompetenzzentrum Obstbau – Bodensee, Ravensburg, Germany
July – Sept. 2001:	Assistant on the Fruit Farm, Writtle College, Chelmsford, Essex, UK
April – June 2001:	Assistant in Argents Ltd. Nursery, Chelmsford, Essex, UK