



Università degli Studi della Tuscia di Viterbo

Dipartimento per l'innovazione nei sistemi biologici, agroalimentari e forestali –DIBAF

Corso di Dottorato di Ricerca in

Scienze, tecnologie e biotecnologie per la sostenibilità XXXII ciclo

**Effects of different storage technologies and temperatures on the quality and
nutritional value of yellow-fleshed kiwifruit**

(s.s.d. AGR/15)

Tesi di dottorato di:

Dott. Valentina Goffi

Coordinatore del corso

Prof. M. Moresi and A. Vannini

Tutore

Prof. Rinaldo Botondi

Co-tutore

Dott.ssa Milena Petriccione

A.A. 2016/2019 (A.Y. 2016/2019)

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SHORT ABSTRACT

The PhD project set out to study and optimise storage yellow-flesh kiwifruit ‘Soreli’. It compared two storage methods: air storage and gaseous ozone treatment during cold storage. Different temperatures and gaseous ozone concentrations were used. The efficacy of ozone is affected by extrinsic and intrinsic factors, which get difficult the prediction of ozone behaviour in presence of specific compounds and environmental factors such as medium pH, temperature and humidity.

Initially the yellow-flesh kiwifruit ‘Soreli’ was studied in comparison with the green-flesh fruit ‘Hayward’ to determine the characteristics of ‘Soreli’ and set the limit of effectiveness of the gaseous ozone and used temperatures on both cultivars. During the first trial fruit were stored at 0 °C and at gaseous ozone concentration of 150 ppb in continuous flow for 42 days; and during the second trial fruits underwent at 4 °C and 300 ppb storage conditions for 60 days, comparing the treated fruits with the fruits stored at air cold storage.

Subsequently, the main objective was to understand the behaviour of the studied yellow-flesh kiwifruit when it stored at different storage conditions for 60 days. The chosen gaseous ozone concentration was 300 ppb, according to some regarding researches, and the temperatures were 2 °C and 4 °C. To evaluate the performance of ‘Soreli’, the response of antioxidant system to ozone postharvest treatment and cold temperature and physico-chemical, microbiological and qualitative traits were studied. Moreover, due to the allergenicity emerging problem, the detection of the allergens in kiwifruit-containing food products was considered.

KEYWORDS Yellow-flesh kiwifruit, cold storage, gaseous ozone, postharvest quality, antioxidant system, allergens, traceability.

ATTACHEMENT 1

Brief description of the carried-out activities

During the I year of PhD the aim was to evaluate the effects of the synergic relation between 0 °C and the low concentration of gaseous ozone (150 - 180 ppb) during 42 days of cold storage of kiwifruit and their behaviour at the shelf-life conditions. Two cultivars were studied: green flesh kiwifruit 'Hayward' and yellow flesh kiwifruit 'Soreli'. 'Hayward' kiwifruit has been taken as reference due to its worldwide importance in the kiwifruit industry. The storage at 0 °C was the first temperature considered because it is the recommended temperature to prolong post-harvest storability of 'Hayward' kiwifruit. Whereas the applied ozone concentration during cold storage was lower compared to the concentration used in other studies in order to study the effectiveness of a minimum level of gaseous ozone.

During the II year the focus of the PhD activity was to optimize ozone processing conditions, regarding the management of ozone concentration and the temperature of the storage. The level of ozone was increased twofold compared to that used in the I trial (300 ppb) and the temperature was increased up to 4 °C. The storage run 6 weeks (42 days), followed by shelf-life at 18 °C for 10 days. The studied cultivars were the same cultivars of the first year. The analyses carried out had been increased to examine in depth the behaviour of the kiwifruit under ozone treatment combined with low temperature. Therefore, the effects of gaseous ozone and cold storage on qualitative and antioxidant-related properties of 'Hayward' and 'Soreli' kiwifruit were studied. The analyses of bioactive compounds and enzyme activities took place at Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia agraria (CREA), Centro di ricerca per Olivicoltura, Frutticoltura e Agrumicoltura, Caserta, Italy.

During the same year, an international exchange took place at Massey University, Centre for Postharvest and Refrigeration Research, New Zealand, during which I actively participated in the main activities assayed by the Postharvest group.

During the III year of PhD, a new manipulation of temperature combined with gaseous ozone concentration has been conducted. The aim was to maximise the postharvest performance of 'Soreli', studying its behaviour when stored at 2 °C and 4 °C under continuous flow of gaseous ozone at 300 ppb and under air storage – untreated samples – for 60 days. The used temperatures were higher than 0 °C due to Soreli susceptibility to low temperatures. Moreover, due to the allergenicity emerging problem, the detection of the allergens in kiwifruit-containing food products has been studied. The analysis of bioactive compounds,

186 enzyme activities and expression of genes and allergens took place at Consiglio per la Ricerca
187 in Agricoltura e l'Analisi dell'Economia agraria (CREA), Centro di ricerca per Olivicoltura,
188 Frutticoltura e Agrumicoltura, Caserta, Italy.

ATTACHEMENT 2

Technological transfer of the carried-out activities

The current research aims to investigate parameters management and a cultivar-specific response of kiwifruit to specific storage conditions, in order to maximise the performance of commercial handling and storability of kiwifruit in the worldwide market.

The findings from the trials underlight that it is important to tailor the synergic combination among gaseous ozone concentration, temperature and cultivar of stored kiwifruit. More information has been got about the performance of yellow-flesh kiwifruit ‘Soreli’ and the storage management. Nowadays, the kiwifruit market lives a transition phase from domination by one only species *Actinidia chinensis* var. *deliciosa* to increasing species diversity. Different fruit cultivars show a large disparity among cold storage.

Therefore, the present study might be helpful for stakeholders, growers and academics.

Storage temperature influences the post-harvest life of ‘Soreli’. Storage at 2 °C and under 300 ppb of continuous flow of gaseous ozone improved the yellow-fleshed kiwifruit storage. Whereas storage at 4 °C under 300 ppb of continuous flow of gaseous ozone did not show advantages in preserving the fruit quality neither of ‘Soreli’ nor of ‘Hayward’.

In kiwifruit industry there are huge expenses in establishment of chambers, management of gaseous composition related to postharvest technologies such as the curing. Hence, with this study we try to define the best practice of management on postharvest storage field.

Moreover, the applied ozone treatment improved the efficiency of antioxidative system when ‘Soreli’ kiwifruit were stored at 2 °C and under 300 ppb of continuous flow of gaseous ozone. So, the study contributes to clarify the role of ozonation on antioxidant system and enzymology, neglected sometimes.

Kiwifruit have allergenicity potential. Thus, the investigation of the allergens contained in ‘Soreli’ kiwifruit and their traceability in kiwifruit-containing food products has consequences on human health. It is useful know how the expressed allergen genes may be involved in the expression of their corresponding proteins in kiwifruit during cold storage and under gaseous ozone treatment.

Therefore, the current research provides additional information that could be helpful for the management of post-harvest treatments on fruit with allergenic impact, to obtain fruit with low allergenic impact.

ATTACHMENT 3

Elenco ed indirizzo dei 2 docenti esterni e supplemente

List and address of 2 external professors proof-readers and substitute

disponibili per la revisione della tesi

(art. 8- comma 6 del DM 8 febbraio 2013, n. 45).

1. Prof.ssa Di Matteo Marisa, Università degli Studi di Salerno, Campus di Fisciano, UNISA.

mdimatteo@unisa.it

Docente di *Food Preservation, Gestione della qualità in ortofloro-frutticoltura e processi delle tecnologie alimentari.*

2. Prof.re Pietro Tonutti, Scuola Superiore Sant'Anna Pisa.

pietro.tonutti@santannapisa.it

Coordinatore del laboratorio di fisiologia postraccolta e qualità dei prodotti frutticoli.

3. Prof.re Emanuele Boselli, Facoltà di Scienze e Tecnologie, Free University of Bozen.

emanuele.boselli@unibz.it

Docente di agro-industry, Innovation and authenticity of winery products, sensory analysis approaches for mountain wines and wine production processes and plants.

247

ATTACHMENT 4

248

Elenco degli insegnamenti frequentati e/o sostenuti

249

List of courses attended and/or supported

DIDATTICA OBBLIGATORIA	CFU	Frequentato	Sostenuto	Certificazione
Sicurezza nelle strutture di ricerca	2	X	27/04/2017	X
Statistica Applicata	7	X	05/04/2017	X
Soft Skills	2	X	21/03/2017	X
Basi di programmazione	4	X	21/02/2017	X
Fundamentals of Intellectual Property and Academic Technology Transfer	2	X	09/05/2018	X
Bioeconomia	4	X	27/04/2017	X
Inglese	6	X	15/09/2017	X
Subtotale Didattica obbligatoria	27			
DIDATTICA SPECIALISTICA	(CFU)			
Oral communication at IX International Symposium on kiwifruit, Porto, Portugal	4	X	06/09/2017 09/09/2017	X
Inglese B2	6	X	05/07/2018	X
HACCP analisi dei pericoli e punti di controllo critici		X	07/02/2018	X
Health and Safety Port Induction Certificate (Port of Tauranga, New Zealand)		X	28/06/2018	X
Research Experience Massey University New Zealand		X	16/06/2018- 16/09/2018	X
Elsevier on Campus @University of Campania 'Luigi Vanvitelli'		X	27/02/2019	X
Giornata S.O.I, Gruppo di Lavoro ACTINIDIA 2019: L'actinidia al		X	19/03/2019	

tempo del cambiamento climatico; Latina, Italy.				
Oral communication at IX National Chestnut Symposium, Pergine Valsugana, TN, Italy	4	X	11-14/06/2019	X
Workshop: Trasferimento d'innovazione alla filiera del Castagno: risultati del progetto Castarray, CREA, Caserta, Italy		X	10/07/2019	
Oral communication at XXIV Workshop on the developments in the Italian PhD on Food Science, Technology and Biotechnology, Firenze, Italy	5	X	11-13/09/2019	X
Certificate of Workshop attendance and Successful Learning Outcomes	2	X	11-13/09/2019	X
Lead Auditor 9001	8	X	13-14-19-20- 26/07/2019	X
IFS – BRC	3	X	13-14-20- 21/09/2019	X
Subtotale Didattica specialistica	32			
Totale Attività didattiche	59			

250

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252

**CORSO DI DOTTORATO DI RICERCA in
SCIENZE, TECNOLOGIE E BIOTECNOLOGIE PER LA SOSTENIBILITÀ**

Alla cortese attenzione del Coordinatore del Corso di DR, Prof. Mauro Moresi

Si certifica che la dottoranda Valentina Goffi, iscritta al XXXII Ciclo di questo corso di Dottorato di Ricerca, ha superato in data 21/02/2017 con **esito positivo** il colloquio in **Basi di Programmazione**.

Il Docente del Corso
Prof. Dario Papale



**CORSO DI DOTTORATO DI RICERCA in
SCIENZE, TECNOLOGIE E BIOTECNOLOGIE PER LA SOSTENIBILITÀ**

Alla cortese attenzione del Coordinatore del Corso di DR, Prof. Mauro Moresi

Si certifica che la dottoranda **Valentina Goffi**, iscritta al XXXII Ciclo di questo corso di Dottorato di Ricerca, ha superato in data 04/05/2017 con **esito positivo** il colloquio in **Statistica Applicata**.

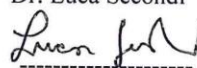
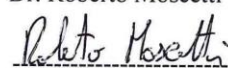
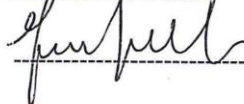
I Docenti del Corso

Prof.ssa Ines Delfino

Prof. Marcello Fidaleo

Dr. Roberto Moschetti

Dr. Luca Secondi





DIBAF

DIPARTIMENTO PER LA INNOVAZIONE NEI SISTEMI
BIOLOGICI AGROALIMENTARI E FORESTALI

**CORSO DI DOTTORATO DI RICERCA in
SCIENZE, TECNOLOGIE E BIOTECNOLOGIE PER LA SOSTENIBILITÀ**

Alla cortese attenzione del Coordinatore del Corso di DR, Prof. Mauro Moresi

Si certifica che il/la dottorando/a GOFFI VALENTINA, iscritto al XXXII Ciclo di questo corso di Dottorato di Ricerca, ha superato in data 21 Marzo 2017 con **esito positivo (very good)** la prova finale in

Soft Skills

Il Docente del Corso

Dott. Stefano Bonometti

**CORSO DI DOTTORATO DI RICERCA IN
SCIENZE, TECNOLOGIE E BIOTECNOLOGIE PER LA SOSTENIBILITÀ**

Alla cortese attenzione del Coordinatore del Corso di DR, Prof. Mauro Moresi

Si certifica che il dottorando Valentina Goffi iscritto al XXXII Ciclo di questo corso di Dottorato di Ricerca, ha superato in data 16.9.2024 con **esito positivo** la prova prevista per il corso "Fundamental of Intellectual Property and Academic Technology Transfer" (2 CFU)

Il Docente del Corso

Prof. Antoine Harfouche



**CORSO DI DOTTORATO DI RICERCA in
SCIENZE, TECNOLOGIE E BIOTECNOLOGIE PER LA SOSTENIBILITÀ**

Alla cortese attenzione del Coordinatore del Corso di DR, Prof. Mauro Moresi

Si certifica che il/la dottorando/a VALENTINA GOFFI, iscritto/a al
XXXII o **XXXI** Ciclo di questo corso di Dottorato di Ricerca, ha superato in data 27 Aprile 2017
con **esito positivo** il colloquio in **Bioeconomia**.

I Docenti del Corso
Prof. Franco Silvio
Dr. Emanuele Blasi

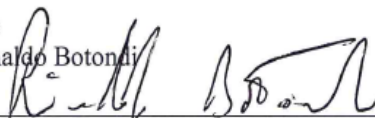
**CORSO DI DOTTORATO DI RICERCA in
SCIENZE, TECNOLOGIE E BIOTECNOLOGIE PER LA SOSTENIBILITÀ**

Alla cortese attenzione del Coordinatore del Corso di DR, Prof. Mauro Moresi

Si certifica che il dottorando Valentina Goffi, iscritto al XXXII Ciclo di questo corso di Dottorato di Ricerca, ha partecipato al IX INTERNATIONAL SYMPOSIUM ON KIWIFRUIT sul kiwi, tenutosi a Porto (Portogallo) a settembre, 6-9 dell'anno 2017, presentando una comunicazione orale, la cui preparazione ha comportato un impegno di 100 h, equivalenti all'acquisizione di 4 CFU. Si allega il programma del IX international symposium on kiwifruit.

Il Tutore

Prof. Rinaldo Botondi





UNIVERSITÀ DEGLI STUDI DELLA TUSCIA
Dipartimento di Studi linguistico-letterari,
storico-filosofici e giuridici

ATTESTATO

1

Si attesta che la studentessa PHD G'OFFI VALENTINA, ha frequentato con profitto, nell'anno 2016/2017, il corso di Lingua Inglese della durata di 50 ore in modalità blended organizzato dall'Unità Servizi Linguistici DISTU superando la verifica finale ed attestandosi al livello B1.

This is to certify that the PHD student G'OFFI VALENTINA, in 2016/2017, has successfully attended a 50 hours (Blended mode) English language course organized, by the Language Services Unit and passing the final test, reaching to level B1.

Viterbo, 15/09/2017



**CORSO DI DOTTORATO DI RICERCA in
SCIENZE, TECNOLOGIE E BIOTECNOLOGIE PER LA SOSTENIBILITÀ**

Alla cortese attenzione del Coordinatore del Corso di DR, Prof. Mauro Moresi

Si certifica che la dottoranda Valentina Goffi, iscritta al XXXII Ciclo di questo corso di Dottorato di Ricerca, ha superato in data 21/02/2017 con **esito positivo** il colloquio in **Basi di Programmazione**.

Il/Docenti del Corso
Prof. Dario Papale





CERTIFICATE OF PARTICIPATION

This is to certify that

Valentina Goffi

Attended the IX INTERNATIONAL SYMPOSIUM ON KIWIFRUIT (KIWI-2017), held in Porto,
Portugal.

Convener

Maria Dulce Antunes

Convener

Pedro Pablo Gallego



Associação
Portuguesa de
Horticultura



UAlg

UNIVERSIDADE DO ALGARVE

Universidade de Vigo

ISHS

International Society for Horticultural Science





UNIVERSITÀ DEGLI STUDI DELLA TUSCIA
Dipartimento di Studi linguistico-letterari,
storico-filosofici e giuridici

ATTESTATO



Si attesta che la studentessa PHD VALENTINA GOFFI, nata a Senigallia (AN) il 26/01/1989, ha frequentato con profitto nell'anno 2017/2018 il corso di Lingua Inglese della durata di 50 ore in modalità blended organizzato dall'Unità Servizi Linguistici del Dipartimento DISTU dell'Università degli Studi della Tuscia attestandosi al livello B2 del Quadro di Riferimento Europeo delle Lingue.

This is to certify that the PHD student VALENTINA GOFFI, born in Senigallia (AN) on 26/01/1989, in 2017/2018 has successfully attended a 50 hours (Blended mode) English language course organized by the Language Services Unit in Distu Department of the University of Tuscia reaching to level B2 of the Common European Framework of Languages.

Viterbo, 05 luglio 2018



F.to UNITÀ SERVIZI LINGUISTICI



ATTESTATO DI PARTECIPAZIONE

SI CERTIFICA CHE

GOFFI VALENTINA

HA PARTECIPATO AL CORSO DI FORMAZIONE

***“HACCP (HAZARD ANALYSIS AND CRITICAL CONTROL POINTS”
ANALISI DEI PERICOLI E PUNTI DI CONTROLLO CRITICI***

PRESSO DIBAF - UNIVERSITÀ DEGLI STUDI DELLA TUSCIA
VIA SAN CAMILLO DE LELLIS, SNC - 01100 VITERBO

MERCOLEDÌ, 07 FEBBRAIO 2018 (09:00 – 16:00)

IL PRESIDENTE DELL'ORDINE DEI TECNOLOGI ALIMENTARI CAMPANIA E LAZIO

SALVATORE VELOTTI



ORDINE DEI
TECNOLOGI ALIMENTARI
CAMPANIA E LAZIO
www.otacl.it



Health and Safety Port Induction Certificate

This certificate is to certify that

valentina goffi

from

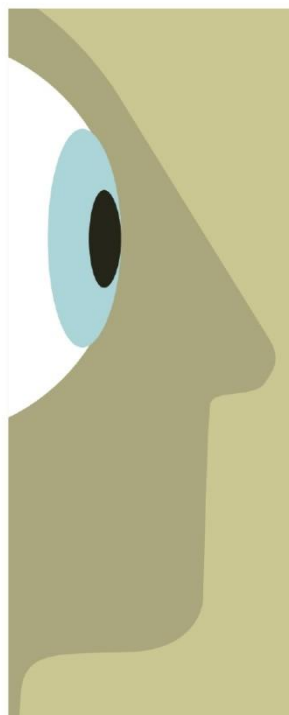
ZESPRI

has completed the
Port of Tauranga Health and Safety Induction
on 28/06/2018

Your Port Induction will expire 28/06/2020

Safety starts with me





Researcher Academy On Campus
Certificate of Attendance



This certifies that

valentina goffi

has attended the following

Elsevier on Campus @ University of Campania "Luigi Vanvitelli"

at University of Campania "Luigi Vanvitelli",
on Wednesday 27 February, 2019

Presented by Massimiliano Bearzot, Customer Consultant, Luigi Rucco, Customer Consultant

Suzanne BeDell
Managing Director, Education
Reference & Continuity Books

Philippe Terheggen
Managing Director
Science, Technology & Medical Journals





VII CONVEGNO NAZIONALE SUL CASTAGNO
Pergine Valsugana (TN), 11-14 giugno 2019

Si attesta che

VALENTINA GOFFI

ha partecipato al

VII Convegno Nazionale sul Castagno
Castanea 2019

11-14 giugno—Pergine Valsugana (TN)



Dr. Luisa Palmieri
Convenor



FONDAZIONE
EDMUND
MACH



SOI

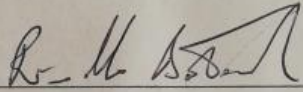
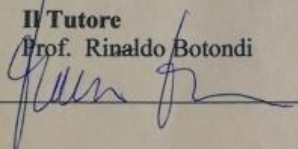
**CORSO DI DOTTORATO DI RICERCA in
SCIENZE, TECNOLOGIE E BIOTECNOLOGIE PER LA SOSTENIBILITÀ**

Alla cortese attenzione del Coordinatore del Corso di DR, Prof. Mauro Moresi

Si certifica che la dottoranda Valentina Goffi, iscritta al XXXII Ciclo di questo corso di Dottorato di Ricerca, ha partecipato al Convegno su "VII Convegno Nazionale sul Castagno, *Castanea 2019*", tenutosi a Pergine Valsugana (Trento) nel giugno, 11 – 14 dell'anno 2019, presentando una comunicazione orale, la cui preparazione ha comportato un impegno di 100/75 h, equivalenti all'acquisizione di 4 CFU. Si allega il programma del Convegno.

Il Tutore

Prof. Rinaldo Botondi



con il patrocinio di



COMUNE
DI LATINA



COMUNE
DI CISTERNA
DI LATINA



SOI

GIORNATA S.O.I, GRUPPO DI LAVORO ACTINIDIA 2019

L'ACTINIDIA AL TEMPO DEL CAMBIAMENTO CLIMATICO

COME ADATTARSI, MANTENENDO BUONA QUANTITA'
ED ELEVATA QUALITA'

19

MARZO 2019

LATINA

Palazzo della Cultura
viale Umberto I, 43

Dalle ore 9,00 alle ore 18,00

Scienziati e Tecnici del settore affronteranno, in una
tavola rotonda, problemi, risorse e prospettive future
della coltura dell'actinidia

NUOVE VARIETA' IN MOSTRA, A CURA DEI CLUB



*Temi
all'ordine
del giorno:*

*Coperture, Nutrizione, Irrigazione,
Fitopatie emergenti, Nuove varietà, Post Raccolta*

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l'Europa investe
nelle zone rurali



TRASFERIMENTO D'INNOVAZIONE ALLA FILIERA DEL CASTAGNO: RISULTATI DEL PROGETTO CASTARRAY

Misura 16.1.1 azione 1

WORKSHOP

10 Luglio 2019 ore 9:00

CREA - Centro di ricerca per Olivicoltura, Frutticoltura e Agrumicoltura
Via Torino, 2 - Caserta
SALA CONVEGNI

ore 9:00 Iscrizioni

ore 9:30 Saluti istituzionali e apertura dei lavori

Milena Petriccione, Responsabile del CREA-OFA di Caserta

Emidio de Francischi di Casanova, Funzionario UOD 06 Responsabile di Misura 16.1
PSR 2014-2020 Regione Campania

Moderatore: Mimmo Pelagalli, giornalista Agronotizie

ore 9:50 "Etnobotanica del castagno: origine e distribuzione delle principali specie del
genere Castanea"

Relatrice: Milena Petriccione, Responsabile del CREA-OFA di Caserta, Capofila
CASTARRAY

ore 10:10 "Lo stato della castanicoltura campana dopo oltre 10 anni dall'introduzione del
cinipide"

Relatrice: Marina Maura Calandrelli, CNR-IRET di Napoli, Partner CASTARRAY

ore 10:30 "Profili del DNA, relazioni genetiche e identificazione di castagni dell'areale di
Roccamonfina"

Relatore: Luigi De Masi, CNR-IBBR di Portici (Napoli), RTS CASTARRAY

ore 10:50 Pausa caffè

ore 11:20 "La fattibilità tecnica del trasferimento di nuovi strumenti, semplici ed economici,
per il riconoscimento genetico varietale"

Relatrice: Angelina Nunziata, CREA-OFA Caserta, Capofila CASTARRAY

ore 11:40 "L'interesse del settore castanicolo per il riconoscimento genetico varietale"

Relatore: Mario Conti, Perito Agrario Azienda Agricola "Franco Di Pippo", partner
CASTARRAY

ore 12:00 Tavola rotonda aperta al pubblico tra ricercatori, agronomi e castanicoltori

ore 13:00 Discussione e conclusione

La partecipazione dei professionisti iscritti comporterà
l'attribuzione di 0,5 Crediti Formativi Professionali, ex art.
4, comma 1, del Regolamento CONAF n. 3/2013 per la
formazione professionale continua.



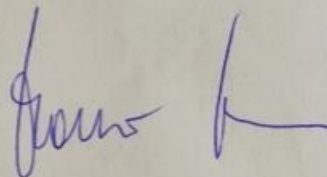
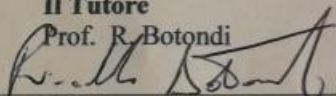
**CORSO DI DOTTORATO DI RICERCA in
SCIENZE, TECNOLOGIE E BIOTECNOLOGIE PER LA SOSTENIBILITÀ**

Alla cortese attenzione del Coordinatore del Corso di DR, Prof. Mauro Moresi

Si certifica che la dottoranda Valentina Goffi, iscritta al XXXII Ciclo di questo corso di Dottorato di Ricerca, ha partecipato al corso per Lead Auditor di Sistemi di Gestione per la Qualità tenutosi ad Ancona nel luglio (12-13, 19-20, 26) dell'anno 2019, la cui partecipazione ha comportato un impegno di 40 h, equivalenti all'acquisizione di 8 CFU. Si allega attestato di partecipazione e programma del corso di abilitazione

Il Tutore

Prof. R. Botondi



Attestato N° 2376/VSQ

Con il presente si attesta che
This is to Certify that

GOFFI VALENTINA

**ha frequentato con successo e superato l'esame finale del corso
per la qualifica di**
has successfully completed the course and passed the final examination of

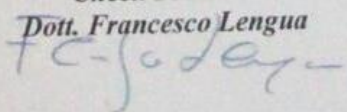
**Auditor/Lead Auditor di Sistemi
di Gestione per la Qualità**
in base alla norma UNI EN ISO 9001:2015
QMS Auditor/Lead Auditor UNI EN ISO 9001:2015

svoltosi ad Ancona nei giorni 12-13-19-20-26 Luglio 2019

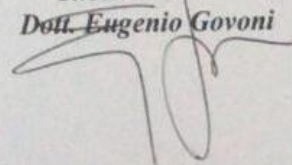
Corso iscritto al n. Q019 del Registro dei corsi Qualificati KHC



Area Formazione – Gestione Corsi
Check Fruit srl
Dott. Francesco Lengua



Il Presidente
Check Fruit srl
Dott. Eugenio Govoni



Bologna, 26 Luglio 2019

FD143 Rev. 03 del 21/06/2018



XXIV Workshop on the Developments
in the Italian PhD Research
on Food Science, Technology and Biotechnology



UNIVERSITÀ
DEGLI STUDI
FIRENZE
DAGRI
DEPARTMENT OF
AGRICULTURE, FOOD
ENVIRONMENT AND FORESTRY



Florence, 11-13 September 2019

Certificate of Successful Learning Outcomes – 3rd year PhD Candidate

The Coordinators of the XXIV Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology have reviewed the academic work presented by

Valentina Goffi

that accounts for a total of **125 hours of work** that ensure that the learning outcomes have been met.

The 125 hours have been completed throughout the preparation in English of an extended contribution for the Proceedings and of an oral presentation and its discussion and they account for a total of **5 CFU**.

Florence, 13 September 2019

The XXIV Workshop Coordinators

Prof. Bruno Zanoni

Prof. Erminio Monteleone

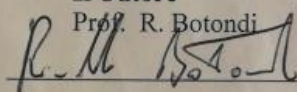
**CORSO DI DOTTORATO DI RICERCA in
SCIENZE, TECNOLOGIE E BIOTECNOLOGIE PER LA SOSTENIBILITÀ**

Alla cortese attenzione del Coordinatore del Corso di DR, Prof. Mauro Moresi

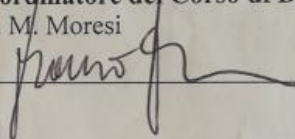
Si certifica che la dottoranda Valentina Goffi, iscritta al XXXII Ciclo di questo corso di Dottorato di Ricerca, ha partecipato al XXIV Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology, tenutosi a Firenze nel settembre, 11 – 13 dell'anno 2019, presentando una comunicazione orale, la cui preparazione ha comportato un impegno di 125 h, equivalenti all'acquisizione di 5 CFU. Si allega il certificato di partecipazione.

Il Tutore

Prof. R. Botondi

**Il coordinatore del Corso di DR**

Prof. M. Moresi





Attestato N° 881/IAIS

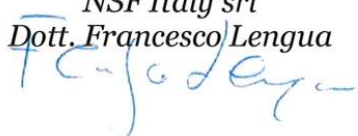
Con il presente si Certifica che
This is to Certify that

GOFFI VALENTINA

ha frequentato e superato con successo l'esame finale del corso
per la qualifica di
has successfully completed the course and passed the final examination of

**Auditor Interno per Standard
Internazionali delle catene
distributive
Analisi degli Standard BRC e IFS**

svoltosi ad Ancona nei giorni 13-14-20-21 Settembre 2019
per un totale di 24 ore

Area Formazione – Gestione Corsi
NSF Italy srl
Dott. Francesco Lengua


Il Presidente
NSF Italy srl
Dott. Eugenio Govoni


Bologna, 21 Settembre 2019

Rev. 01 del 15/03/2018

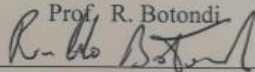
**CORSO DI DOTTORATO DI RICERCA in
SCIENZE, TECNOLOGIE E BIOTECNOLOGIE PER LA SOSTENIBILITÀ**

Alla cortese attenzione del Coordinatore del Corso di DR, Prof. Mauro Moresi

Si certifica che la dottoranda Valentina Goffi, iscritta al XXXII Ciclo di questo corso di Dottorato di Ricerca, ha partecipato al corso per auditor interno per standard internazionale (BRC e IFS) delle catene distributive tenutosi ad Ancona nel settembre (13-14, 20-21) dell'anno 2019, la cui partecipazione ha comportato un impegno di 24 h, equivalenti all'acquisizione di **3** CFU. Si allega attestato di partecipazione e programma del corso di abilitazione

Il Tutore

Prof. R. Botondi

**Il Coordinatore del Corso di DR**

Prof. M. Moresi



ATTACHMENT 5

Stage esterno e periodo di svolgimento

Foreign Experience

Ente esterno	Tutore esterno	Periodo	Attività svolte
Massey University, Centre for Postharvest and Refrigeration Research. Palmerston North, New Zealand	Professor Andrew East	16/06/2018 - 21/09/2018	Report added

REPORT

VISITOR'S EXCHANGE AT MASSEY UNIVERSITY

During the visiting period at Massey University, Centre for Postharvest and Refrigeration Research, located in Palmerston North, I have had a pleasure to take part at the activities of the Postharvest group.

After the first introducing week, where I gained confidence with the Laboratory and with the international post-harvest group (Professors, Technicians, Post-Doc and PhD students), I actively participated in the post-harvest work.

Main Activities unrolled:

- ✓ *Lab induction.*
- ✓ *Meeting of Presentation of the “Università degli Studi della Tuscia” and my PhD activity to the post-harvest group.*
- ✓ *Tauranga Workfield on packaging experiment on containers.*

We carried out the initial stage of an experiment on kiwifruit packaging set at one of the factories belonged to Zespri group. The purpose was modelling a system to measure gas exchange and flow during the international trade.
- ✓ *Meeting of presentation the “Università degli Studi della Tuscia” and my PhD activity to Zespri company at Tauranga, North Island.*

✓ *International kiwifruit trade at Tauranga Port.*

This activity consisted of first completing the port of Tauranga Health and Safety Primary Induction (port of Tauranga Primary Induction Certificate) and then checking the equipment and supply chain activities before the ship could leave the port of Tauranga for the international trade.

✓ *Writing week Retreat*

During the writing retreat that set at Castelpoint Station for one-week, North Island, I have started the writing of my thesis: Index, Chapter I: Literature review and chapter II: Experimental Section. After that, we decided to carry on this activity; so, we scheduled the writing session for two hours every two Friday morning.

✓ *Weekly meeting*

During this section we scheduled the weekly work, the laboratory activities, we checked the work done and the equipments involved in the trials (machineries, cold rooms, sensors) and followed-up meeting of some cases: sharing of skills and knowledge of statistic programmes, of activities and papers e.g. Zespri supply chain.

✓ *Student laboratory work on Horticulture quality*

The undergrad student's laboratory consisted of gain experience with some methodologies of measuring quality and physiology of horticultural products. One of that was the measure of physiological status of fresh produce by measuring the interaction of the product with its environment through gas exchange: ETD-300 ethylene analysis equipment and CO₂/O₂ gas analyser; visual quality assessment measures and textural assessment measures with destructive and non-destructive methods (Avocado M., Handihit, acoustic, hand penetrometer, electric penetrometer, compression, juiciness, snap) followed-up sensory analysis and, at the end, taste assessment measures; this last lab looked at the means in which two main properties (sourness and sweetness) can be assessed and the interpretation of the results obtained; and once characterizing the taste, quantitative methods assessment carried out (digital refractometer, visual refractometer and SCiO molecular sensor and titratable acidity with automatic titrator and dry matter).

✓ *Support lab activity during trial of post-harvest group.*



MASSEY UNIVERSITY
COLLEGE OF SCIENCES
TE WAIHANGA PUTAIAO

Monday, 5 October 2018

RE: Period of research experience of Valentina Goffi at Massey University, New Zealand.

To Whom It May Concern:

This letter is to confirm that Ms Valentina Goffi travelled to New Zealand from June to September 2018, and was hosted by myself, Prof. Andrew East, at Massey University, Palmerston North, New Zealand for 12 weeks of research experience. Valentina approached Massey University for work at a different research location as part of her studies, and I was happy to offer her an opportunity given that her area of study (postharvest physiology of kiwifruit) is a key expertise of our research group. As a visitor to Massey University, Valentina was not formally enrolled as a student.

I found that Valentina was a pleasant and capable student. She took advantage of her time in New Zealand by engaging in the research work being conducted by the group and interacting with industry representative. It was a pleasure for us to host her, and I hope that her experience with us will be of value to her in the future.

Please contact me directly on +64 6 336 9099 extn 83257 or +64 210552610 or email on a.r.east@massey.ac.nz if you have any further questions regarding Valentina's period of visit to Massey University.

Regards

Prof. Andrew East
Professor of Postharvest Engineer
Massey AgriTech Partnership
Massey University

Te Kōwhiri
Iwi Pūwhiri

Massey AgriTech Partnership
Private Bag 11222, Palmerston North 4442, New Zealand T 06 650 7576 <http://www.massey.ac.nz>

ATTACHMENT 6

Elenco dei lavori scientifici pubblicati

List of publications

Goffi V., Modesti M., Forniti R. and Botondi R. (2018). Quality of green (*Actinidia chinensis* var. *deliciosa* 'Hayward') and yellow (*A. chinensis* var. *chinensis* 'Soreli') kiwifruit during cold storage at 0°C in normal atmosphere and with gaseous ozone. *Acta Hort.* 1218, 473-480. <https://doi.org/10.17660/ActaHortic.2018.1218.65>

Goffi, V., Zampella, L., Forniti, R., Petriccione M. and Botondi R. (2019). Effects of ozone postharvest treatment on physicochemical and qualitative traits of *Actinidia chinensis* 'Soreli' during cold storage. *Journal of Science and Food Agriculture*. doi:10.1002/jsfa.9823

Vettraino AM., Bianchini L., Caradonna V., Forniti R., Goffi V., Zambelli M., Antonino T., Vinciguerra V. and Botondi R. (2019). Ozone gas as a storage treatment to control *Gnomoniopsis castanea* preserving chestnut quality. *Journal of the Science of Food and Agriculture*. <https://doi.org/10.1002/jsfa.9883>

Goffi V., Magri A., Botondi R., Petriccione M. (2020). Response of antioxidant system to ozone postharvest treatment in 'Soreli' kiwifruit. *Journal of Science and Food Agriculture*. 100:961-968. <https://doi:10.1002/jsfa.10055>

Vettraino AM., Vinciguerra V., Pacini G., Forniti R., Goffi V. and Botondi R. (2020). Gaseous ozone as a suitable solution for postharvest chestnut storage: evaluation of quality parameter trends. *Food and Bioprocess Technology*.

Carboni C., Goffi V., Petriccione M. and Botondi R. (2019). The effect of gaseous ozone treatment and different storage temperatures on the quality of yellow-flesh kiwifruit cv. 'Soreli'. *Proceedings of IOA World Congress & Exhibition, Nice, France, 20-25 October 2019*.

STATE OF THE ART

Understanding the physiological and environmental factors associated with postharvest decay of fruits and vegetables is one of the challenges of postharvest research. Fruit ripening and senescence directly affect the sensorial and nutritional quality during postharvest life. It has been clarified that reactive oxygen species and oxidative damage are responsible for fruit senescence. Some kiwifruit cultivars with yellow flesh can be stored for a short period compared to green fleshed kiwifruit. The postharvest performance is affected by the physiological state of the fruit at harvest, associated with the applied postharvest management. Among several applications in postharvest, ozone treatment is considered as a cost effective and eco-friendly food processing technology to preserve the fruit quality during cold storage (Carletti et al., 2013; Miller et al., 2013; Botondi et al., 2016; Goffi et al., 2019).

Ozone is a strong oxidant and its spontaneous decomposition into oxygen without any residue makes its attractive as an alternative agent for food applications. In 2001, it was approved by the US Food and Drug Administration as an antimicrobial additive for direct contact with foods. Ozone treatment may utilize ozone in aqueous or gaseous phase in the food processing industry (Brodowska et al., 2017). Ozone treatment system have few basic components: the gas (air or pure oxygen), an ozone generator, an electric power source, a contactor (if ozone is in the water phase), a reactor, a surplus gas elimination unit, and an ozone analyzer. Several reports have shown the benefits and disadvantages of gaseous ozone as storage postharvest treatment (Glowacz et al., 2015). Ozone can be used as antimicrobial agent that causes the progressive oxidation of some cellular components (Barboni et al., 2010).

Ozone is efficient in promoting higher firmness retention; it is effective at suppressing ripening and senescence process, and therefore can extend kiwifruit post-harvest life during cold storage (at 0 °C under 95% of relative humidity) with a gaseous ozone atmosphere (0.3 ppm) for up to 5 months (Minas et al., 2012). Minas and coworkers (2012) demonstrated a key postharvest function of ozone that could be the main aetiological reason for the markedly retarded ripening process. Minas and coworkers (2014) demonstrated that ethylene biosynthesis and cell walls turnover were specifically targeted by ozone (0.3 μ L L⁻¹ at 0 °C for 2 or 4 months) in ‘Hayward’; generating the reduction of flesh softening and cell wall disassembly. Moreover, kiwifruit are characterized by a time lag between the increase in

respiration rate and the induction of ethylene production, as numerous observations confirmed.

Respiration rate is enhanced by ozone treatment after short or intermediate cold storage and subsequent transfer to 20 °C; so, kiwifruit experience physiological injury as a result of the oxidative stress caused by ozone exposure. The respiration rate is reduced in ozone-treated kiwifruit following extended cold storage compared with control fruit. Ozone acts as an antioxidant and anti-radical elicitor in kiwifruit during ripening. Minas and coworkers (2012) showed that the exposure of kiwifruit to ozone stimulated their antioxidant activity, presumably because of its action as a signal molecule for the activation of antioxidant-stress responses in kiwifruit, which possibly experienced an oxidative stress during the cold storage period. Ozone can induce oxidative stress involving enzymatic and non-enzymatic antioxidant system responsible to steady-state ROS level (Xia et al., 2016). The reaction between ozone and antioxidants results in the formation of reactive oxygen species (ROS) and thereby protect cell structures from oxidative damage (Brodowska et al., 2017).

Moreover, ozone exposure stimulates sucrose degradation and reduces the emission of volatile esters and modulates protein and enzymatic profiles (Minas et al., 2012; Barboni et al., 2010). Kiwifruit soften rapidly after transfer from low-temperature storage conditions (0 °C) to ripening temperature (20 °C).

In conclusion, ozone is considered as a valid postharvest treatment, cost-effective and eco-friendly, to delay ripening process, prolonging the shelf-life of produce and preserving fruit quality during cold storage (Goffi et al., 2018).

Kiwifruit are nutrient and healthy fruit due to the high concentration of vitamins C, A, E, K, polyphenols, carotenoids, flavonoids, folate, carbohydrates, minerals, aminoacids and dietary fibers. Nevertheless, some researchers report some adverse effects of kiwifruit on human health concerning the kiwifruit allergy (Meena et al., 2018).

Allergy to kiwifruit was first described in 1981; this allergy shows a wide range of symptoms from localized oral allergy syndrome (OAS) to life-threatening anaphylaxis (Lucas et al., 2003). Kiwifruit allergy has been associated with allergy to pollen and in latex-fruit syndrome (Maddumage et al., 2013) and other fruits and foods, including apple, avocado, banana, carrot, celery, hazelnut and potato (Ciardiello et al., 2009). Food allergies are typically IgE-mediated, but certain reactions result from different immunologic mechanisms (Shahali Y. and Dadar M., 2018).

Kiwifruit contain detectable number of specific allergens (Moller et al., 1997). Pastorello and coworkers (1998) provided the evidence that actinidin is the major allergen of the fruit *Actinidia chinensis*; others IgE-binding proteins have been identified in kiwifruit such as the above-mentioned actinidin, a thaumatin-like protein (TLP, Act d 2), 40kDa protein (Act d 3), kiwellin (Act d 5), a phytocystain and a chitinase (www.allergome.org). Actinidin (Act c 1) and kiwellin (Act c 5) have also been found in gold kiwifruit. Studies have shown that environmental cultivation conditions, harvest maturity and storage conditions have an important role on the fruit allergen content (Wang et al., 2019). During the storage of fruits, many studies found that a considerable change in the level of allergens depending on environmental stress and/or storage conditions (Sánchez-Monge et al 1999). For instance, the rate of overall reduction of some allergens is greatest when fruit were stored at controlled atmosphere conditions (Bolhaar et al., 2005) and, in contrast, an allergen increased during cold storage at 4 °C. Another study conducted by Minas and coworkers (2018) revealed that O₃ modulated some proteins. Ozone probably acts as a protein repressor since most proteins are exclusively affected by O₃ are down-accumulated. Thus, it is important understand the effect of the ozone, as postharvest treatment, on the allergen-related gene expression of yellow flesh kiwifruit ‘Soreli’ as kiwifruit’s availability and consumption have increased recently, the incidence of kiwifruit allergy is greater, too.

CHAPTER 1: LITERATURE REVIEW

1.1 ECONOMIC ASPECTS OF KIWIFRUIT CROP

1.1.1. Worldwide kiwifruit industry

Actinidia has become an important fruit species in several countries. According to FAO data (www.fao.org/faostat/en) global kiwifruit production amounts to about 4.02 million metric tons from 2000 to 2018, an increase from 3.89 million metric tons in the previous years (Figure 1.1).

Production and marketing situation: New Zealand domesticated the species and remained very important into international markets. At the end of last century, Italy became the first producing country in the Northern hemisphere, followed by France, Iberian Peninsula and Greece while in the Southern hemisphere, New Zealand and Chile were the most important countries. The biggest change over the last ten years has been the increasing importance of China, which is now producing more than half the world's total production of kiwifruit. Also, Turkey and Iran consistently increased their acreage (Costa et al., 2018). According to FAO data during 2018 China produced 2,035,158 t, Italy 562,188 t, New Zealand 414,261 t, Iran 266,319 t, Greece 265,280 t, Chile 230,267 t, Turkey 61,920 t, France 53,201 t, United State of America 34,290 t and Portugal 34,057 (Figure 1.2). Thus, the market will probably change since export of kiwifruit from China and other countries might create an impact on the actual situation (Figure 1.3).

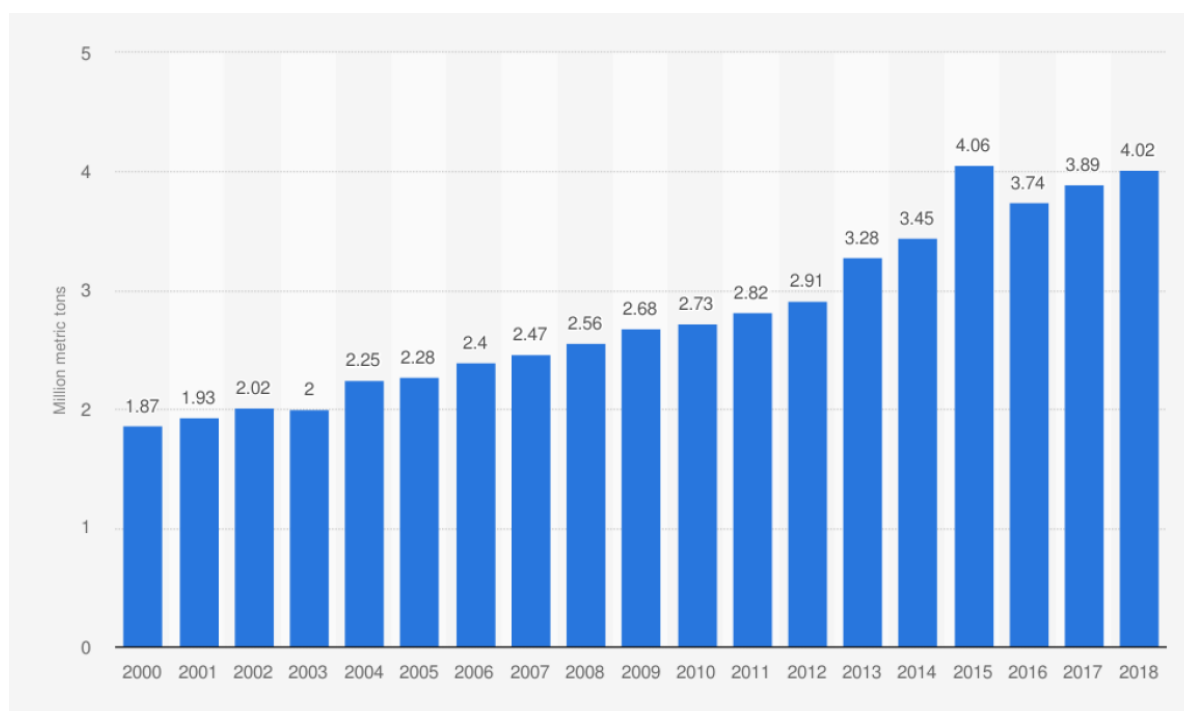


Fig.1.1. The worldwide production of kiwifruit from 2000 to 2018 in million metric tons (<http://www.fao.org/faostat/en>).

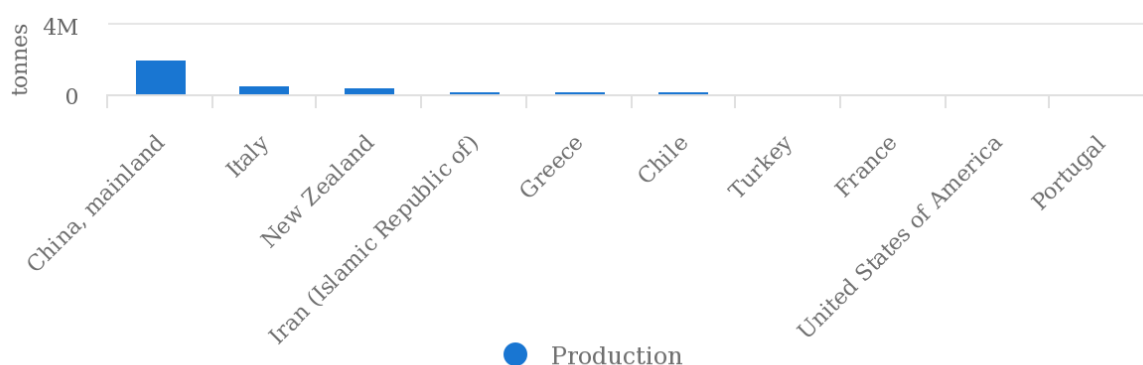


Fig.1.2. Top ten producers of kiwifruit during 2018 (<http://www.fao.org/faostat/en>).

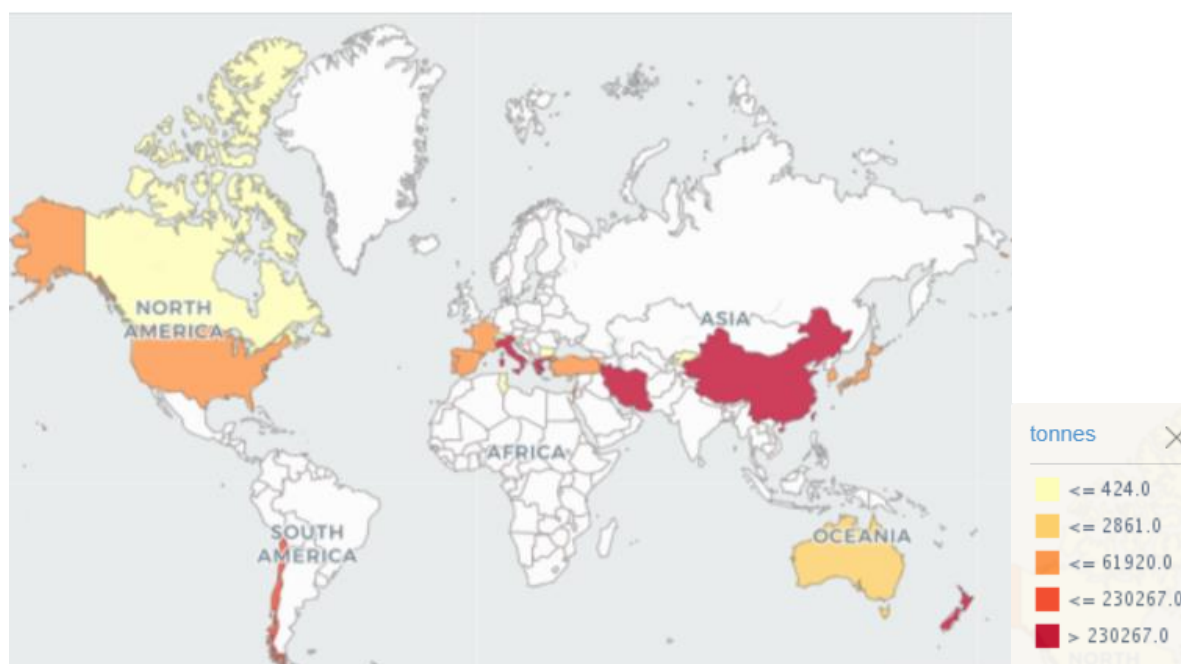


Fig.1.3. Production of kiwifruit by country during 2018 (<http://www.fao.org/faostat/en>).

Kiwifruit industry changes. Thirty years ago, the kiwifruit industry was based on a cultivar ‘Hayward’ belong to a single *Actinidia* specie (*Actinida chinensis* var. *deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson) but now other yellow- and some red-flesh cultivars have been introduced. Consequently, most of the pre- and post-harvest management techniques and parameters have been defined for Hayward cv. grown under New Zealand conditions and may not be suitable for other cultivars, recently introduced too. How concern diseases, PSA is a real issue and new cultivars seem susceptible (Costa et al., 2018). The goal of future worldwide research and kiwifruit industry is replacement of the existing cultivar with tolerant or resistant to PSA.

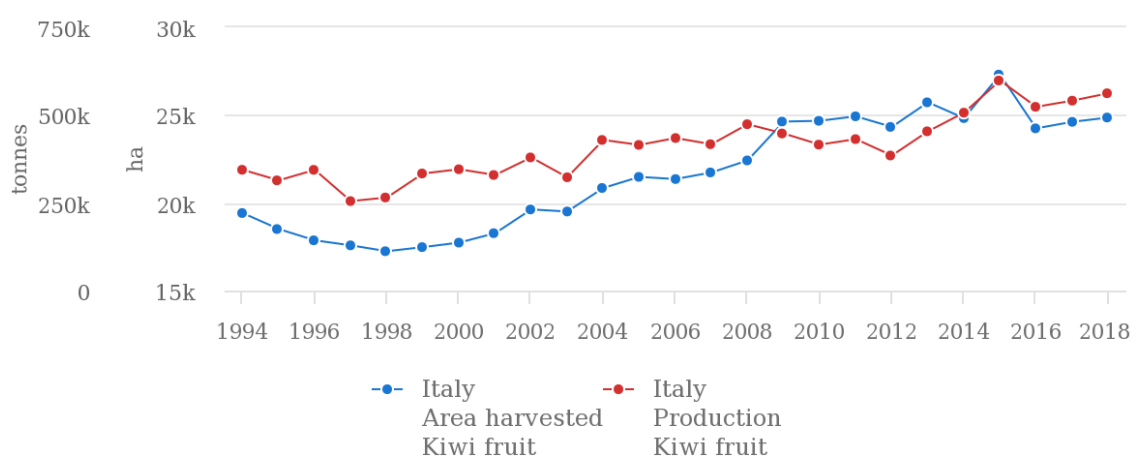
1.1.2. The current state of Italian kiwifruit industry compared to the kiwifruit industries of other countries, especially New Zealand.

The Italian kiwifruit industry is important both nationally and internationally. It makes up only a small part of Italian horticulture in general, but it is nevertheless profitable and expanding. In the context of the total Italian fruit production, and in terms of ‘marketable gross production’ (crop value) kiwifruit are the sixth most valuable fruit crop after citrus, apples, table grapes, peaches/nectarines and pears excluding grapes grown for wine production and olives, that belong to an other important category of fruit crops. The first kiwifruit orchards were established in Italy only 40 years ago. One of the first attempts at commercial-scale

kiwifruit plantings in Latina in 1971, probably using plants from New Zealand imported through France, failed because the plants died from heat stress due to the misapprehension that kiwifruit were tropical plants. Elsewhere, establishment of kiwifruit plantings depend to a large extent on the relative profitability of competing crops. For example, in Latina (Lazio region) kiwifruit tended to replace table grapes, because during the 1970s and 1980s the returns for grapes were low and kiwifruit appealed as an alternative potentially more profitable crop (Testolin et al., 2009).

According to FAO data the Italian production of kiwifruit increased from 1994 to 2018 (Figure 1.4)

Italy produced 562,188 tons during 2018 according to FAO data (Figure 1.5) (<http://www.fao.org/faostat/en>).



Source: FAOSTAT (Nov 14, 2020)

Fig.1.4. Production/Yield quantities of kiwifruit in Italy from 1994 to 2018 (<http://www.fao.org/faostat/en>).

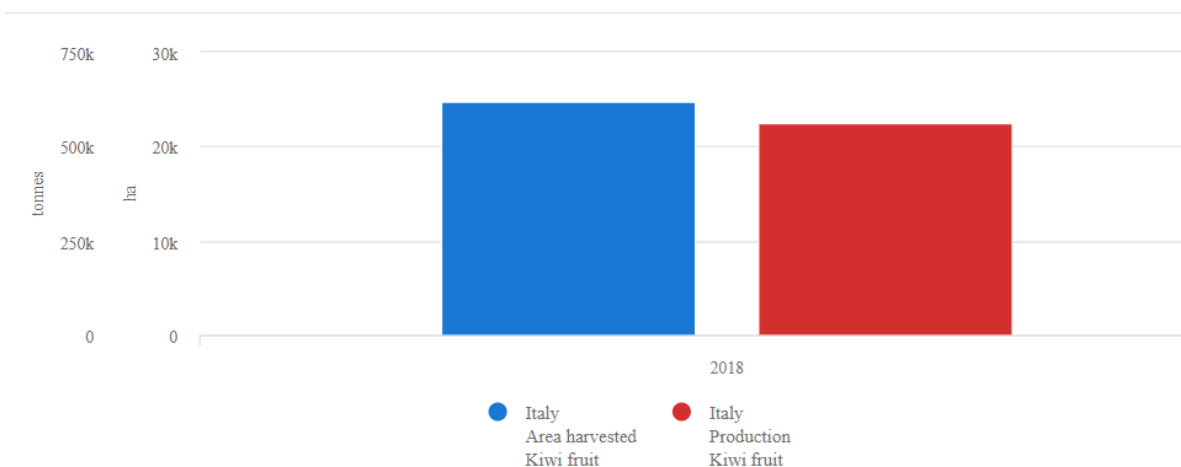


Fig.1.5. Production/Yield quantities of kiwifruit in Italy during 2018 (<http://www.fao.org/faostat/en>).

Most kiwifruit plantings in Italy are small: 71% are 1 ha or less, 86 % 2 ha or less, 95 % less than 5 ha (<http://rica.crea.gov.it>). Kiwifruit plantings are therefore much less consolidated than they are in New Zealand where only 31% of plantings are 2 ha or less (NZKGI, 2016). This is typical of Italian horticulture in that orchards, like Italian farms, are generally small or average only 5 ha.

The cultivation methods used in Italy are like those in New Zealand but with regional differences. According to regional differences in vine phenology, kiwifruit orchards extend almost the whole of the great length of the Italian peninsula between latitudes 46 and 39 °N: the most northern orchards are on the northern shore of Lago di Garda, in Trento or in the high Friuli plain of north-eastern Italy. The most southern around Lamezia in Calabria. Consequently, kiwifruit grow under varying climatic conditions; and there is not a significative indicator of their climatic adaptability. The most suitable region for kiwifruit cultivation, as determined by length of growing season, annual solar radiation and satisfaction of chilling requirements, appears to be central Italy, especially the west coast, along the Tyrrhenian Sea, an area that corresponds to the Lazio region. There, agricultural land is more readily available, the growing season is long (8-9 months), insolation is high, and winters are mild but cold enough to meet winter chilling requirements without application of dormancy-breaking chemicals (Costa et al., 2018).

Most kiwifruit produced in Italy are eaten fresh, although some fruits sold fresh undergo subsequent industry processing. Most of Italian kiwifruit belong to ‘Hayward’ cultivar. Nowadays, there are yellow-fleshed kiwifruit including into the market. The Italian food industry has followed New Zealand firms in devising procedures for production of clear juices, fortified drinks, purées, kiwifruit preserved in syrup, candies, frozen, dehydrated, lyophilised products, kiwifruit leathers, distilled spirits etc. (Cassano et al., 2007).

Green kiwifruit are not easily processed because the chlorophyll which makes them so distinctive visually usually becomes a muddy brown on processing (Torreggiani et al., 1994). The introduction of yellow-fleshed kiwifruit presents new opportunities to food processors. The first small-scale trials on Jintao fruit showed that the yellow colour survives well in processed kiwifruit products (Costa et al., 2018). The high prices currently fetched by yellow-fleshed kiwifruit reduce the need to consider processing options, but this could change in the future.

Italy and New Zealand are the world’s two biggest kiwifruit exporters. Nearly all kiwifruit from New Zealand are exported whereas most Italian kiwifruit are consumed within Italy or in other countries of the European Union (Costa et al., 2018).

Chile, Italy and New Zealand are responsible for nearly 80 % of international kiwifruit exports. New Zealand and Chile are both dependent on exports. In theory, supplies of kiwifruit from the two hemispheres should be complementary not competitive. Italian kiwifruit export starts early in September and peak in the December through April period (Belrose, 2007). Kiwifruit from New Zealand are mainly exported May-November whereas exports from Chile would arrive in Europe a little earlier. There can be an overlap of kiwifruit supplies in Europe, especially, at the beginning of the Italian harvest and this has led to conclusions that such overlap leads eventually to a lowering of returns, during the European winter when most kiwifruit are supplied by Italy. Growing cultivars that are harvested earlier in the season would increase the potential overlap; likewise, increased exports of fruit as production expands in different countries may result in further competition and its subsequent effects on fruit returns.

The biggest difference with New Zealand is in the marketing of the crop. The New Zealand industry has placed great emphasis on quality standards and a proprietary brand (ZESPRI) to distinguish New Zealand kiwifruit in the hope of ensuring a price premium. About 90 % of the New Zealand production comes from a single region within New Zealand, kiwifruit growers usually produce no other fruits and all exports, apart from those to Australia, are

under the control of one marketing organization, ZESPRI International Ltd. In contrast, Italian kiwifruit industry is fragmented, even if coordination is better than other horticultural industries (Testolin, 2017). In Italy distinctions are also being made for marketing purposes between kiwifruit produced in different regions. For example, Protected Geographic Indication (IGP) status for kiwifruit from Lazio – Kiwi Latina – was granted in 2004 (Cacioppo, 2015) and this means that kiwifruit from Latina are being promoted for their claimed superiority, especially in flavour, not as Italian kiwifruit in general. About 80 % of Italian kiwifruit exportations are destined to other European Union countries, so, a long sea voyage is not required, and no tariffs need be paid; it is relatively easy to export within the European Union and this encourage fragmentation at all levels of the industry. The promotion is limited and usually by individual exporters or grower cooperatives. The diversity within the Italian kiwifruit industry induces to take advantage of new opportunities. For instance, in Italy has seen the adoption of new cultivar such as ‘Jintao’ and the Summerkiwi cultivars.

The challenges for the Italian kiwifruit industry are to improve organisational coordination, to improve and monitor more stringently quality standards and to develop new markets to absorb rapidly increasing production. The industry of the future is to become even more important in terms of international trade.

1.2 *ACTINIDIA CHINENSIS*

1.2.1 Botanical description

Kiwifruit belong to *Actinidia*, a genus comprising more than 50 species found mainly in southern China. All members of the genus are climbing plants and are functionally dioecious. Kiwifruit is a berry with seed embedded in a fleshy mesocarp. The commercial kiwifruit are large-fruited selections of two varieties of the species, *A. chinensis* Planc.: *A. chinensis* var. *chinensis* and *A. chinensis* var. *deliciosa* (A. Chev.) A. Chev., formerly known as *A. deliciosa* (A. Chev.) C.F. Liang et A.R Ferguson. Other *Actinidia* species are grown on a small scale or have limited commercial potential: *A. arguta* (Sieb. et Zucc.) Planch. ex Miq., *A. eriantha* Benth. and *A. kolomikta* (Maxim. et Rupr.) Maxim. (Testolin et al., 2016).

The name kiwifruit, or regrettably ‘kiwi’, is often used to refer to any member the genus *Actinidia* Lindl. The closest Chinese equivalent is ‘mihoutao’ (monkey peach), widely used

for any *Actinidia* species but particularly for fruit of *A. chinensis* Planch. var. *chinensis* and *A. chinensis* var. *deliciosa*. Some small-fruited *Actinidia* species, such as *A. arguta*, are distinguished by the common names: ‘hardy kiwifruit’, ‘baby kiwifruit’ or ‘kiwiberry’, the last of which is becoming the most widely used (Testolin et al., 2016).

Morphologically, *Actinidia* fruit are fleshy and produced from a single flower containing a single ovary; the entire ovary wall ripens into an edible mesocarp with the seed embedded in the fleshy interior of the ovary (Figure 1.6). So, they are different from many of the well-known fruits as berryfruits e.g. strawberries, raspberries or mulberries, which are aggregate fruits. There is a great variation between and within *Actinidia* species in fruit characteristics such as: size, shape, skin hardness, colour internal and external, flesh texture, flesh flavour, flesh composition of health-promoting, time of maturity, time of ripening and responses to prolonged storage (Figure 1.7) (Testolin et al., 2016). Therefore, this variation is important horticulturally and for the handling, storage and marketing of the fruit.

Edible parts are mesocarp and placenta. Fruit size is usually 20-70 g, maximum up to 200 g or even larger. Fruit shapes are quite different for various species and varieties, but mostly oval, oblong, flat-round, cylindrical, oval, peel thin, green, brown, orange, red, purple and flesh color is green, yellow, and red. Ripe fruit have high soluble solids content (SSC), soft texture, special flavor, moderately sweet or sour taste.

The seeds are very small. In the most species, grain weight is about 1.2-1.6 g of mass 1000 seeds, the minimum only about 0.2 g. Seeds are oblong shape, fresh seeds are mostly brown or dark brown color, dry seeds brown with moire on surface. The seeds have high oil content of 22-24 %, even up to 36.5%. Seed also contain 15-16 % proteins.

Only some skins are palatable. The skins could be separated into three types:

- 1) Soft, hairless skin of species such as *A. arguta*. The skin is edible if somewhat chewy.
- 2) Hairless but rough and ‘warty’ skin of species such as *A. Indochinensis*.
- 3) Rough, hair skin as in *A. chinensis* var. *chinensis*, *A. chinensis* var. *deliciosa* and *A. eriantha*, varying in hairiness.

The skin of most *Actinidia* species are unpalatable and fruit are normally eaten with a spoon or after peeling using a knife.

Externally, most *Actinidia* fruit are brownish green to yellowish brown if any hairs are removed.

Internally the core of immature actinidia fruit is white, cream or pale green and the mesocarp is almost always green. The mesocarp of most actinidia taxa remains green as the fruit ripen;

in many genotypes of *A. chinensis* var. *chinensis* the green is lost and the mesocarp appears pale green, lime-green, yellow-green, or golden-yellow. The mesocarp of ripe fruit of *A. arguta* (some genotypes only) and *A. melanandra* can be brick-red to purplish-red, of *Actinidia kolomikta*, pale yellow to pale orange, of *A. polygama* apricot-yellow, and of *A. macrosperma*, *A. tetramera* and *A. valvata*, bright, intense orange. Some genotypes, especially of *A. chinensis* var. *chinensis* and *A. chinensis* var. *deliciosa* also have red pigmentation, mainly in the mesocarp around the core.

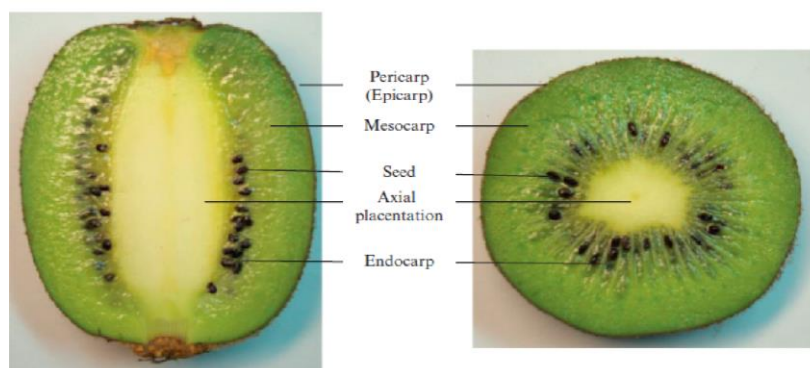


Fig 1.6. Cross section of fruit *A. chinensis* var. *deliciosa* (Huang H., 2016a).

Species	Fruit shape	Fruit wt. (g)	Skin color	Skin hairs	Flesh color	Fruit core	Seed size and amount	Juiciness	Flavor
<i>A. arguta</i>	Ovoid-globose, flat globose, oblong, near globose	4-20	Green, light red, purple	Glabrous, smooth	Green, jade green, purple	Large	Small and numerous	Juicy	Sweet, slightly acid
<i>A. callose</i>	Nearly globose, short cylindrical	8-9	Green	Glabrous	Jade green	Small		Juicy	Optimum sweet acid
<i>A. chengkouensis</i>	Nearly globose, cylindrical	10-35	Brown	Brown tomentum					
<i>A. chinensis</i> var. <i>chinensis</i>	Ellipsoidal	20-120	Brown	Short brown tomentum but readily shed	Green, yellow	Small	Med-large, numerous	Juicy	Sweet acid
<i>A. chinensis</i> var. <i>deliciosa</i>	Long cylindrical	30-200	Brown-green	Dense coarse yellow-brown hairs	Green	Large	Med-large, numerous	Juicy	Sweet acid
<i>A. chinensis</i> var. <i>setosa</i>	Nearly globose, ovoid	20-35	Brown	Dense with long brown strigose hairs					
<i>A. chrysantha</i>	Short cylindric, ovoid, long globose	10-30	Chestnut brown, brown-green	Glabrous	Green, light green	Medium	Large and numerous	Juicy	Sweet acid
<i>A. cylindrica</i>	Long cylindrical	0.5-1	Dark green	Glabrous	Light jade green	Small	Small-medium		
<i>A. eriantha</i>	Long cylindrical	10-40	Green	Dense white villous hairs	Jade green	Small	Less	Juicy	Acid
<i>A. farinosa</i>	Ovoid-cylindrical	1-2	Light green	Short tomentum but readily shed	Green	Medium	Less	Less juicy	Acid sweet
<i>A. fasciculoides</i>	Long cylindrical		Dark green		Dark green		Small and numerous		
<i>A. fulvicoma</i> var. <i>cinnamensis</i>	Ovoid-cylindrical	5-10					Small		
<i>A. fortunatii</i>	Long cylindrical	1-2	Dark green	Short yellow tomentum	Green		Small and numerous		Acid
<i>A. fulvicoma</i>	Nearly cylindrical	3-4	Dark green	Long yellow tomentum					
<i>A. glauco-allosa</i>	Flat globose	10-15	Green, red-brown	Glabrous					
<i>A. grandiflora</i>	Cylindrical, ellipsoidal	20-60	Brown	Grayish white or yellow-brown downy hairs	Green	Medium	Small and numerous	Juicy	Very acid
<i>A. hemsleyana</i>	Cylindrical	16-30	Brown	Dense grayish to yellowish-brown setose hairs	Yellow-green or green		Small and numerous	Juicy	Acid sweet and very fragrant
<i>A. henryi</i>	Nearly cylindrical, long cone	2-8	Green	Glabrous but distinctly spotted	Green		Numerous		Slightly acid and mouth-numbing
<i>A. hubeiensis</i>	Ovoid-cone	5-9	Dark green	Glabrous but distinctly spotted	Dark green	Medium	Few	Less juicy	Sweet
<i>A. indochinensis</i>	Short ellipsoidal	6-8	Brown	Glabrous but distinctly spotted	Green, dark green		Numerous	Juicy	Acid sweet
<i>A. kolomikta</i>	Long-cylindrical or long-globose	2-10	Green and yellow-green	Glabrous, smooth	Dark green	Small	Small	Juicy	Sweet acid and fragrant
<i>A. lanceolata</i>	Long cylindrical, globose ovoid	1	Light brown	Glabrous but distinctly spotted	Dark green	Small	Few	Juicy	
<i>A. latifolia</i>	Cylindrical	2-4	Brown-green	Glabrous but distinctly spotted	Jade green	Small	Large and numerous	Juicy	

Fig 1.7. Variation in fruit characters of genus *Actinidia* (Huang H., 2016a).

1.2.2. *A. chinensis* var. *deliciosa*, Hayward

Hayward is one or two generations derived from plants raised from kiwifruit seed introduced through Yichang, Hubei province, China to New Zealand in 1904. Originally the name was ‘Wright’s Large Oval’ but it was later renamed Hayward in honor of the nurseryman (Hayward Wright), who raised several seedlings and selected a superior plant in the early 1930s. When other countries started growing kiwifruit, Hayward became the main cultivar, becoming the only kiwifruit in international trade.

The fruit are large, mean weight 80-110 g, with rounded shoulders and flat at the stylar end, distinctly broad ovoid, slightly flattened laterally so that they are elliptical in cross section. The skin is greenish-brown, covered with dense, bristly, ginger-brown hairs, not easily removed. The flesh is light green; the large, elliptical, often fluted, core is white or greenish white. The flesh is juicy with a good acid-sugar balance.

The soluble solids content (SSC) is 12-18 %, the total sugars 9.8 %, acids 1.0-1.6 %, proteins 1 %, aminoacids 0.5 %, and vitamin C 50-80 mg/100 g FW. The flavour is slightly sour. The fruit can be stored for long periods. The storage can last 49 days under ambient temperature and 6-8 months in cold storage (0 °C), even longer under modified atmosphere (Huang H., 2016b).

1.2.3 *A. chinensis* var. *chinensis*, Soreli

Soreli is yellow-fleshed; it is a selection of tetraploid *Actinidia chinensis* originated from the controlled cross A137 x A134.41, carried out in 1997, and developed by R. Testolin and G. Cipriani, breeders at the Università di Udine, Italy.

A 137 is a female *A. chinensis* selection of unknown origin introduced in Italy from China in the early 1990s; A134.41 is a male obtained from open-pollination of the Chinese selection 79-05 ‘Ganmi No 1’ (syn. ‘Zaoxian’, ‘FT-79-05’) (Dicho et al., 2015). Soreli is protected by IPR (Huang H., 2016a).

Soreli adapts readily to different climates and soils. Bud fertility is rather similar at the different locations with little differences between years. Soreli appears to have a lower chilling requirement, much lower (600h-300h less) than that of Hayward cv., which is reported to require more than 900h (chilling units, CU, that is hours below 7.2 °C) (Dicho et al., 2015). Budburst occurs 4-17 days before Hayward, with the earlier bud burst recorded in the south. Soreli almost always flowers two weeks before Hayward, but over a range of dates of up to three weeks in the different seasons and locations.

The second year after grafting individual vines carried 12-16 Kg fruit and the third year after grafting 33-68 Kg.

Mean fruit weight at harvest ranges from 85 to 147 g, according to the location and the season, with the lowest values recorded when artificial pollination is absent.

The fruit is ovoid-long and regular in shape, the skin is brilliant brown and the flesh is intense yellow. The green flesh of the unripe fruit turns yellow as soon as ripening starts.

Harvest date, as determined by the fruit reaching 8.0 °Brix, varies by up to 25 days, with the extreme dates of 23 September and 18 October.

Soluble solid content averages from 12.5° to 15.7 °Brix, varying according to the location and the season.

According to some sensory tests, key good points of Soreli are the higher flesh colour intensity if compared with ‘Jintao’, the lower flesh fibrosity, the excellent sugar/acid balance, the lower intensity of the grassy taste typical of green kiwifruit, the marked citrus and tropical fruit flavours (Dicho et al., 2015).

Fruit can be harvested within a period of 20-30 days with an SSC between 8° - 12 °Brix.

The storage under normal atmosphere is limited to 90-120 days. Controlled atmosphere storage can prolong the storage life up to 6 months (Huang H., 2016b).

1.3 STORAGE LIFE

1.3.1. Storage

Storage life is the time for a stored fruit to remain marketable. Storage potential can be considered as storage life. Nowadays, the supply chain of kiwifruit wants to ensure the continuous supply of premium fruit. Supply chain refers to a management of tasks, which initiates the process of sources, production and product delivery to the market to satisfy the needs of a customer (Jabbar A., 2014). Therefore, successful kiwifruit storage and efficient supply chain management has fundamental importance in the industry. For this reason, postharvest handling and technology play an important role.

A challenge for postharvest technology is to be environmentally sustainable from an economical and a community perception perspective. One objective is to reduce the produce deterioration as much as possible during the period between harvest and the use, ensuring eating quality and maximum market value for the produce. Quality management starts in the field and continues until produce reaches the end user. The use of molecular biology is of

increasing interest to researchers to try better understanding specific postharvest problems. While several genetically modified (GM) fruits and vegetables have been developed, commercialisation has been almost non-existent as many consumers remain resistant to accepting GM foods (Wills RB, 2016).

Postharvest management of fruits and vegetables requires a thorough knowledge of their nature, physiology and responses to the surrounding environment, such as temperature and relative humidity, composition of the atmosphere and metabolic products to which they are subjected from harvest to consumption (Yahia E.M., 2018).

Fruits and vegetables are living structures; even after harvest, the produce is still living as it continues to perform most of the metabolic reactions. The life of fruits and vegetables can be conveniently divided into three major physiological stages following initiation or germination. These are: growth, maturation, and ripening/senescence. Growth involves cell division and cell enlargement. Maturation is the final stage of fruit growth, where fruit reach maximum size, volume and weight. Senescence is defined as the period when anabolic biochemical processes give way to catabolic (degradative) processes, leading to ageing and finally death of the tissue (Wills RB, 2016). In some fruit species it may be an overlapping between late ripening and early senescence, that are two distinct developmental processes.

Ripening is a developmental stage where complex physiological processes take place inducing several changes characterizing the early stages of senescence. The associated physiological changes during ripening are: increase of respiration rate, ethylene production, loss of chlorophyll, synthesis of pigments such for instance carotenoids or flavonoids in climacteric fruits. Senescence is the final stage of the biological development that leads to cellular breakdown and death. (Yahia E.M., 2018).

There are many factors which influence the storage quality and storage potential. The postharvest performance of kiwifruit is dependent on the physiological state of the fruit at harvest in conjunction with the management of the pre and post-harvest environment.

Harvest date has a marked influence on fruit quality as well as storage performance. Fruit maturity at the time of harvest affects storage quality. Kiwifruit with higher SSC at the time of harvest can store well and have flavour and more sweetness at the ripe stage (Jabbar A., 2014). Fruit should be above 9 °Brix for SSC at harvest if the fruit must be stored for longer period. A delay in harvest can increase the risk of damage from frost or winter storms. Late harvests result in softer fruit, that are more prone to softening disorders than fruit harvested early. For these reasons, it is important to define an index, able to predict tendency of fruit to soften and develop storage disorders, and consequently, the storage life without forgetting

the variability related to regional/national environmental characteristics. Indeed, in Japan the optimum harvest time for yellow-fleshed kiwifruit is at 9-10 % of SSC (Huang H., 2016d); while in New Zealand when the fruit show 18-21 % of dry matter, 10% of SSC and hue angle at or below 103°. In addition, kiwifruit can be quite susceptible to post-harvest handling operations. Any damage during handling can lead to premature softening, can result in defects, permitting invasion by disease-causing microorganisms. Therefore, kiwifruit are harvested at the optimal maturity and immediately transported to the packhouse to complete the following steps of the supply chain.

After harvest, an efficient practice in terms of preserving quality in storage is the curing. Curing is a post-harvest approach well-established in the kiwifruit industry because it can reduce rot incidence (*Botrytis*). It consists of keeping fruit at ambient temperature for 48 or 72 hours, during which is important monitoring temperature and humidity levels. Temperature management in the first two weeks after the harvest has been associated with the development of storage breakdown disorder and softening after long-term storage (Zhao JM, 2017).

At-harvest fruit temperature represents the ambient air temperature, absorbed heat from sunlight and respiratory heat (Zhao et al., 2015). Therefore, it is vital to minimize and remove the field heat from the fresh produce by post-harvest practices to reach the optimal storage temperature for the produce. If the fruit are kept at high temperatures for long time, fruit quality, nutritional content and flavour might decrease; while fruit aging could accelerate (Huang H., 2016c).

Postharvest practices that result in rapid cooling are recommended for most fresh produce. Often refrigerated transport spaces (trucks and shipping containers) are not designed to remove heat or have adequate cool air circulation; but they are designed to maintain precooled produce at the selected transport temperature (Wills RB, 2016). Most of the kiwifruit are usually warehoused for up to 4–6 months at 0 °C, with relative humidity 90-95 % and no ethylene during the storage. Kiwifruit are very susceptible to small concentrations of ethylene in the atmosphere. Losses occur if fruit soften prematurely in storage. Kiwifruit slowly deteriorate over time even in cool store with softening being the major limiting factor of storage life. Hayward is stored at temperatures around 0 °C and yellow-flesh kiwifruit around 1 °C. When kiwifruit are stored at 2 °C, a reduction of 1-2 months in storage life can be expected, compared with the storage at the optimum temperature of 0 °C. In general, an increase from 0 to 5 °C in fruit temperature leads to a doubling of the respiration rate, and

approximately halves the storage life. A storage below 0.5 °C should be avoided for kiwifruit, to maintain an adequate margin of safety above the freezing point. The highest temperature at which freezing of kiwifruit has been observed is -1.5 °C, but a typical value is -1.7 °C. The freezing temperatures of fruit stored for 6 months at 0 °C were measured between -1.8 °C and -2.1 °C, this lower freezing temperature probably being due to the higher sugar content, which increase during storage (Beever et al., 1990). Lowering the temperature reduce ethylene production but also the rate of response of the tissue to ethylene (Wills RB, 2016). Rot incidence and low temperature breakdown are other issues for kiwifruit storage; thus, for successful management the application of strict regimes of temperature and humidity in an ethylene free environment is necessary to ensure fruit long storage.

New techniques for reducing undesired microbial contamination, spoilage and decay, as well as maintaining product's visual, textural and nutritional quality are required in all steps of the production and distribution chain.

1.3.2 Ozone

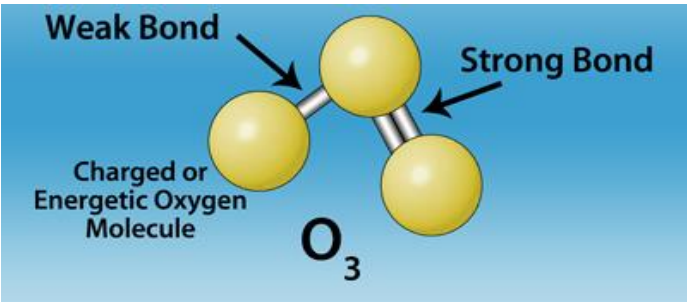
Among several applications in postharvest, one option could be the use of ozone (Glowacz, 2014). Ozone treatment is considered as a cost effective and eco-friendly food processing technology to preserve the fruit quality during cold storage (Carletti et al., 2013; Miller et al., 2013; Botondi et al., 2016; Goffi et al., 2019). Ozone is a strong oxidant; its high oxidizing potential is + 2.07 V (Table 1.1) (Guzel-Seydim et al., 2004), which conveys bactericidal and viricidal properties (Kim et al., 1999; Kim and Yousef, 2000).

Its spontaneous decomposition into oxygen without any residue makes its attractive as an alternative agent for food applications. Indeed, it is an unstable gas at room temperature; it readily degrades but has a longer half-life in the gaseous state than in aqueous solution (Colm O'Donnell et al., 2012). It is (O₃) a triatomic form of oxygen and an oxygen allotrope (Figure 1.8). Its major physical properties are given in Table 1.2. The strong reactivity of ozone is due to the structure of the molecule: it has three oxygen atoms; in the valence shell of each oxygen atom are two unpaired electrons, each one occupying one 2p orbital. This mean that during its formation, three oxygen atoms are combined, with the central oxygen rearranged in a plane sp² from the 2s and two 2p atomic orbitals of the valence band. With this rearrangement the three new sp² hybrid orbitals form a triangle with an oxygen nucleus at its centre; that is, with an angle of 116°49', as shown in Figure 1.9.

815 Table 1.1. Oxidizing agents and their oxidation potential (Guzel-Seydim et al., 2004).

Oxidizing agent	Oxidation potential (mV)
Fluorine	3.06
Ozone	2.07
Permanganate	1.67
Chloride dioxide	1.50
Hypochlorous acid	1.49
Chlorine gas	1.36

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818 Fig.1.8. Ozone structure (www.prozoneint.com)

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820 Table 1.2. Major physical properties of pure ozone (Guzel-Seydim et al., 2004).

Property	Value
Boiling point	-111.9 ± 0.3 °C
Melting point	-192.5 ± 0.4 °C
Critical temperature	-12.1 °C
Critical pressure	54.6 atm

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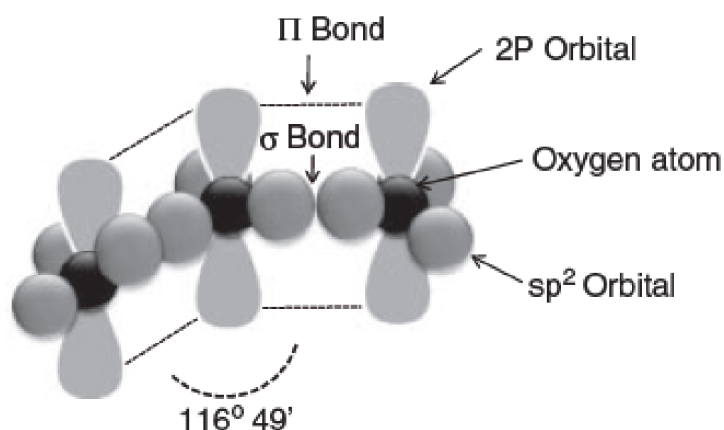


Fig.1.9. The molecular structure of ozone (Greene et al., in Colm O'Donnell et al., 2012).

Ozone results from the rearrangement of atoms when oxygen molecule (O₂) are subjected to high-voltage electric discharge. It is formed in the stratosphere, in photochemical smog and by UV sterilization lamps, high voltage electric arcs, and gamma radiation plants (Mustafa MG, 1990). In 2001, it was approved by the US Food and Drug Administration as an antimicrobial additive for direct contact with foods. Its toxicity is dependent on concentration and length of symptoms include headaches, nosebleeds, eye irritation, dry throat and respiratory irritation. At higher exposure levels (1-100 ppm), symptoms become more severe and include asthma-like symptoms, tiredness and loss of appetite.

In the food processing industry (Figure 1.10) ozone treatment can be used in aqueous or gaseous phase (Brodowska et al., 2017). Gaseous ozone is slightly denser (2.14 g/L) compared to air (1.28 g/L) at 0 °C and atmospheric pressure.

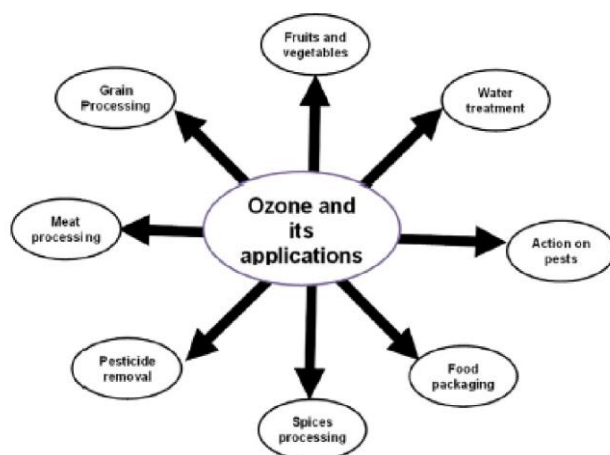


Figure 1.10. Application areas of ozone in food processing.

Ozone treatment is characterized by few basic components: the gas (air or pure oxygen), an ozone generator, an electric power source, a contactor (if ozone is in the water phase), a reactor, a surplus gas elimination unit, and an ozone analyzer. Several reports have shown the benefits and disadvantages of gaseous ozone as storage postharvest treatment (Glowacz et al., 2015). Ozone can be used as antimicrobial agent that cause the progressive oxidation of some cellular components (Barboni et al., 2010). Fruits and vegetables contaminations are mostly associated with bacteria such as *Salmonella*, *E. coli*, *Listeria monocytogenes*, *Shigella*, *Campylobacter*, *Clostridium botulinum* and *Bacillus cereus*, some viruses like Norwalk and hepatitis A, and some fungi such as *Aspergillus* and *Penicillium*. Ozone can destroy microorganisms by progressive oxidation of the phospholipid and protein molecules in the cell membrane, which are vital for functioning membrane. This increases the permeability of cell membranes, allowing for the leakage of cellular content and eventually leading to the death of microorganisms (Han et al., 2017). The germicid effect is explained by the mechanism of ozonolysis studied by Criegee in 1975. Therefore, ozone reacts with the carbon-carbon double bonds of unsaturated fatty acids, according to the mechanism described by Criegee (1975), giving rise to the formation of chemical species, such as ozonides and peroxides that are responsables for the germicidal action, as well as the properties of stimulating tissue repair and regeneration (De Almeida et al., 2012).

Ozone is also effective in removing ethylene from the atmosphere, extending the shelf-life of many ethylene-sensitive produce during storage (Skog and Chu, 2001). Minas and co-workers (2014) showed that ozone treatment along with cold storage inhibited AdACS1 and AdACO1 gene expression; these ethylene biosynthetic genes encode ACC synthase and ACC oxidase, respectively, which are closely related with climacteric ethylene production. So, ozone treatment could modulate responses to stress events. The physiological action of ozone is interconnected with ethylene, salicylic acid (SA), abscisic acid (ABA) and nitric oxide (NO) during stress responses. Thus, apart ethylene biosynthesis, ozone may regulate the kiwifruit ripening through alterations of the balance, interaction and constant recalibration of the different plant hormones (Minas et al., 2014).

Moreover, ozone can reduce or eliminate odour when added to the storage atmosphere of fruits and vegetables (Aguayo et al., 2006). Ozone can be added continuously or intermittently to the storage room atmosphere throughout the storage period to prevent or delay fruit decay (Chu and Skog, 2001; Palou et al., 2003; Cayuela et al., 2009).

Ozone is efficient in promoting higher firmness retention; it is effective at suppressing ripening and senescence process, and therefore can extend kiwifruit post-harvest life during

cold storage (at 0 °C under 95 % RH) with a gaseous ozone atmosphere (0.3 ppm) for up to 5 months (Minas et al., 2012).

Minas and coworkers (2012) demonstrated a key postharvest function of ozone that could be the main aetiological reason for the markedly retarded ripening process. Minas and coworkers (2014) demonstrated that ethylene biosynthesis and cell walls turnover were specifically targeted by ozone (0.3 μ L L⁻¹ at 0 °C for 2 or 4 months) in ‘Hayward’ generating the reduction of flesh softening and cell wall disassembly. Moreover, kiwifruit are characterized by a time lag between the increase in respiration rate and the induction of ethylene production, as numerous observations confirmed.

Respiration rate is enhanced by ozone treatment after short cold storage and subsequent transfer to 20 °C; so, kiwifruit experience physiological injury due to the oxidative stress caused by ozone exposure.

Ozone acts as an antioxidant and anti-radical elicitor in kiwifruit during ripening. Minas and coworkers (2012) showed that the exposure of kiwifruit to ozone stimulated their antioxidant activity, presumably because of its action as a signal molecule for the activation of antioxidant-stress responses in kiwifruit, which possibly experienced an oxidative stress during the cold storage period. Ozone can induce oxidative stress involving enzymatic and non-enzymatic antioxidant system responsible to steady-state ROS level (Xia et al., 2016). The reaction between ozone and antioxidants results in the formation of reactive oxygen species (ROS) and thereby protect cell structures from oxidative damage (Brodowska et al., 2017). Moreover, ozone exposure stimulates sucrose degradation and reduces the emission of volatile esters and modulates protein and enzymatic profiles (Minas et al., 2012; Barboni et al., 2010).

The efficacy of ozone is influenced by extrinsic and intrinsic factors. It is not always predictable its behaviour in the presence of specific compounds and environmental factors such as pH, temperature, and humidity. The instability of ozone-consuming materials affects the level of residual ozone available in the medium. Therefore, it is important to distinguish between the concentration of applied ozone and that of residual ozone necessary for effective disinfection. Higher ozone concentration results in inactivation, shorter time, and smaller D value. However, high ozone concentrations may cause the oxidation of health-promoting compounds. An increase in temperature renders ozone less soluble and less stable and so, the decomposition rate increases. The ability of ozone to inactivate bacteria decreases with decreasing temperature (7-22 °C). The simultaneous effect of these two factors

(solubility/stability and reactivity) on ozone efficacy can vary with experimental conditions, making it difficult to predict the influence of temperature in an application.

Some studies indicate that a decrease in pH increases ozone efficacy. It is reported that the kinetics of inactivation of *E. coli* are much faster in an acidic medium than in a basic one. The presence of organic substances with high ozone demand in a medium may compete with microorganisms for ozone. The presence of high level of organic solids provides protection for microorganisms.

Kiwifruit morphology provides information clarifying the interaction between ozone and kiwifruit, since the elements play a central role in the quality of the fruit after harvest (Celano et al., 2009).

Kiwifruit are berries with an internal structure consisting of a central fleshy columella with multiple locules containing multiple seeds, a fleshy mesocarp and the surface skin (Ferguson A. R., 1984). Fruit skin is complex and is composed of surface structures such as hairs and epicuticular waxes, with several layers below the surface (Celano et al., 2009). Surface skin presents lenticels, where gaseous exchanges take place. They have circular shape with a pore in the centre (Hayward P., 1974). Therefore, fruit skin directly interacts with the surrounding environment, modulating gas and moisture transfer, protecting against physical, chemical and biological attack (Lara et al., 2014).

Therefore, ozone enters the cell through the lenticels, the wall and plasma membrane, where there are some sites for primary oxidation events: olefinic compounds, phenolics groups and proteins and lipids (some of that are unsaturated).

The primary site of ozone interaction with plant cells is the extracellular matrix where ozone challenges the antioxidant protection of the cells (Baier et al., 2005). A continuous exposure to low concentrations (0.1-0.3 $\mu\text{L/L}$) during storage can oxidize ethylene and can elicit the accumulation of antioxidants. The effect of ozone and its primary decomposition metabolites are associated with multiple reactions, including the inactivation of enzymes, the alteration of nucleic acids, and the oxidation of membrane lipids (Rodoni et al., 2010). When lipid peroxidation occurs due to be the target of attack by ozone, the malondialdehyde concentrations (MDA) increases. Ozone can directly react with different cellular constituent, such as membrane lipids, causing membrane dysfunction or even cell lysis, enzymes, altering metabolic homeostasis, and/or nucleic acids, disrupting gene expression and cell division (Rodoni et al., 2010). When ozone reacts with unsaturated fatty acids, it produces H_2O_2 , aldehydes, organic radicals; so, the priority reaction between ozone and any of that compounds results in production of active oxygen species. Once formed free radicals or other active

oxygen species, they will be capable to initiate subsequent non-catalysed oxidation events (Eva J. Pell, 1997). Oxidant agents can abstract electrons from essential organic molecules and disturb cellular functions. Ozone can reduce cell wall disassembly during cold storage because it interacts with polygalacturonase (PG) and endo-1-4-glucanase activity (enzymes involved in pectin and hemicellulose degradation), slackening their activity and reducing the solubilisation of pectin; for chain-effect the water moves into voids, that are left empty in the cellulose-hemicellulose matrix by the solubilised pectin. Therefore, the swelling of cell wall is reduced, and in addition, ozone can elicit antioxidant activity, induce cell repair or cell protection if the concentration is not very high, otherwise it accelerates the senescence (Pell et al., 1997). For instance, Booker and Miller (1998) found that ozone treatments induce phenylalanine ammonia lyase (PAL), a key regulatory enzyme in the biosynthesis of phenolic compounds. The correlation between phenolic compounds and reduced fruit damage might favor hypotheses related to the involvement of fruit defense responses in preventing the spread of fungal pathogens and to the contribution to reinforce the cell walls. Accordingly, ozone sensitivity is generally correlated with the ascorbate status of the apoplast, which is an important signal initiation point. Ascorbic acid, which gives an additional value to the fruits for its nutritional aspect, is commonly used as indicator for deciding the suitability of a treatment in terms of nutritional value of the product; that's because it is prone to degradation reactions during processing and storage of agricultural products due to its vulnerable structure. The ascorbic acid content usually has decreasing trend during storage. Some researchers observed that at the beginning of the ozone treatment on black mulberries (5.14 mg m^{-3}) a slight increase of ascorbic acid content occurs, followed by a decrease in the rest of storage (Tabakoglu and Karaca, 2018); similar behaviour was also observed in strawberries (Pérez et al., 1999) and citrus plants (Iglesias et al., 2006). These increases occur most likely for the stimulation of the natural defense mechanisms of the plants in response to ozone treatment. These mechanisms trigger the synthesis/accumulation of antioxidant compounds such as ascorbic acid in the cell to resist oxidative stress (Figure 1.11).

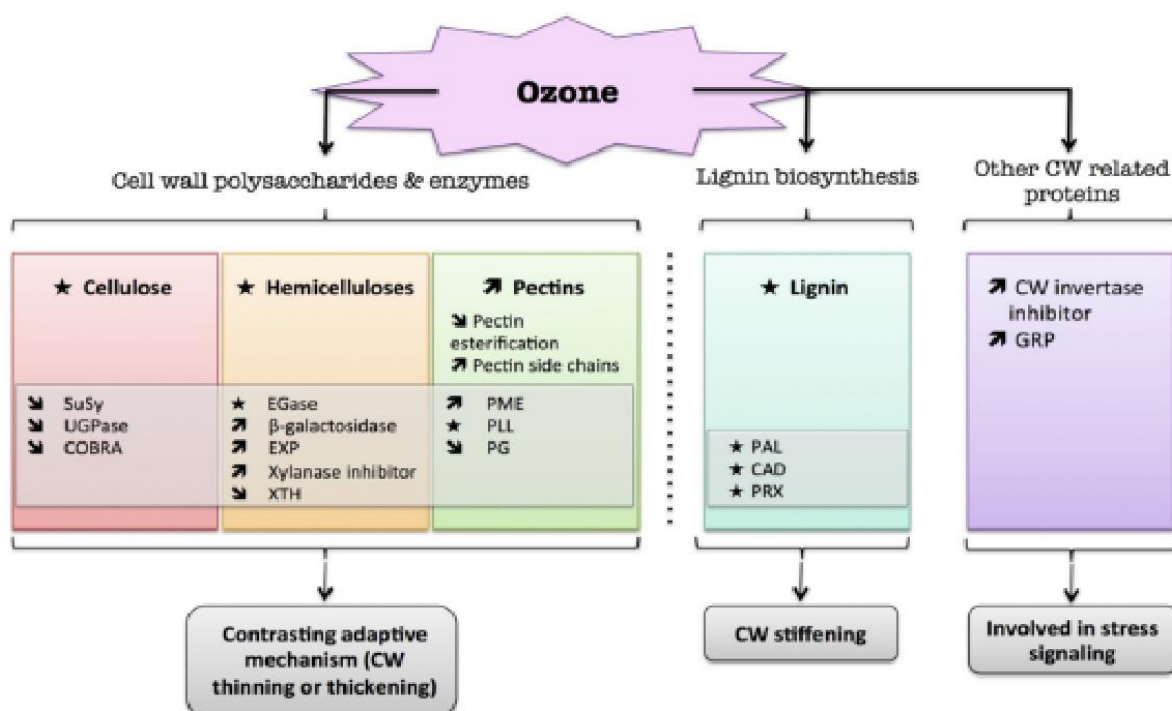


Figure 1.11. Diagram summarizing the plant cell wall response to ozone. Arrow (↑) means increased abundance and arrow (↓) means decreased abundance of the molecules. Star means contrasting data on the gene/protein or the molecule studied according to the literature cited in the review. Cell wall (CW); xyloglucan endo-β-transglucosylases/hydrolases (XET/XTH); expansin (EXP); sucrose synthase (SuSy); UDP-glucose pyrophosphorylases (UGPase); endoglucanase (EGase); pectin methylesterase (PME); pectin/pectate lyase-lyke (PLL); polygalacturonase (PG); phenylalanine ammonia-lyase (PAL); hydroxycinnamyl alcohol dehydrogenase (CAD); glycine-rich protein (GRP); cell wall peroxidases (PRX) (Le Gall et al., 2015).

Han and coworkers (2017) showed, using scanning electron microscopy (SEM) observation, that after a storage period, control samples of black mulberry presented deformed stomata. Thinned guard cells, and external projections on the surface, indicating the loss of moisture control functions in the epidermal cells. In addition, also appeared on the surface of control fruit, which cracked the skin and destroyed the epidermal tissue. Conversely, the stomata were regular on the fruit surface of samples treated (with precooling and ozone), morphology of guard cells complete, the closed status could be effectively suppressed water loss. The ozone treatment matched with the precooling suppressed the degradation of epidermal tissue of fruit, allowing for maintenance of their morphological features. Ozone can reduce the size of pores on fruit skin, allowing for the simultaneous inhibition of bacterial infection and water

transpiration. These observations suggest that ozone can inhibit the decomposition of fruit skin and suppress fruit softening (Han et al., 2017). In general, fruits are quite susceptible to water loss. Respiration, transpiration and electrolyte leakage are the possible causes of water loss and consequent weight loss.

Although ozone inhibits ripening, the mechanism has not been clearly defined. The critical question raised by some researches is whether the mode of action of ozone in kiwifruit ripening stems from permanent oxidative damage or from potentially reversible biochemical inhibition (Minas et al., 2018).

1.4 HEALTH ATTRIBUTES OF KIWIFRUIT

1.4.1 Nutraceutical quality and antioxidant system

Kiwifruit are highly appreciated among consumers for high bioactive compounds content with health-beneficial effects. Indeed, it is a rich basket of phytonutrient and bioactive compounds, including carbohydrates, vitamin A, C, E and K, folate, and polyphenols (Iwasawa et al., 2011) (Figure 1.12).

Extensive researches over the last decade on the health benefits of kiwifruit has linked their regular consumption to improvements not only in nutritional status, but also benefits to digestive, immune and metabolic health; and, also, they have ascertained that fruit senescence is greatly related to reactive oxygen scavenge (ROS), whose accumulation accelerates the quality decay of the fruit.

The contribution of kiwifruit to digestive health is attracting attention owing to a growing body of evidence from human intervention studies. There are several plausible mechanisms of action that are likely to act together including the fibre content and type, the presence of actinidin, a proteolytic enzyme unique to kiwifruit which breaks down protein and facilitates gastric and ileal digestion, and other phytochemicals which may stimulate motility (Richardson et al., 2017).

Nutrient	Units/100 g	Green Kiwifruit	Gold Kiwifruit
Proximates			
Water	g	83.1	82.4
Energy	kcal	61	63
Energy	kJ	255	262
Protein	g	1.14	1.02
Total lipid (fat)	g	0.52	0.28
Ash	g	0.61	0.47
Carbohydrate, by difference	g	14.7	15.8
Fiber, total dietary	g	3	1.4
Sugars, total	g	9.0	12.3
Minerals			
Calcium, Ca	mg	34	17.0
Iron, Fe	mg	0.31	0.21
Magnesium, Mg	mg	17	12.0
Phosphorus, P	mg	34	25
Potassium, K	mg	312	315
Sodium, Na	mg	3	3
Zinc, Zn	mg	0.14	0.08
Copper, Cu	mg	0.13	0.103
Manganese, Mn	mg	0.098	0.05
Selenium, Se	µg	0.2	0.44
Vitamins			
Vitamin C, total ascorbic acid	mg	92.7	161.3
Vitamin B1-Thiamin	mg	0.027	<0.01
Vitamin B2-Riboflavin	mg	0.025	0.074
Vitamin B3-Niacin	mg	0.341	0.231
Vitamin B5-Pantothenic acid	mg	0.183	0.12
Vitamin B6-Pyridoxine	mg	0.063	0.079
Vitamin B9-Folate	µg, DFE	25	31.0
Choline	mg	7.8	1.9
Vitamin B-12	µg	0	0.08
Vitamin A, RAE	µg _RAE	4	1
Vitamin A	IU	87	23
Vitamin E (α-tocopherol)	mg	1.46	1.51
Vitamin K	µg	40.3	6.1
Others			
Carotene, beta	µg	52	14
Lutein + zeaxanthin	µg	122	24
Scientific Name:		<i>Actinidia deliciosa</i>	<i>Actinidia chinensis</i>
Cultivar		<i>Hayward</i>	<i>SunGold</i>
USDA NDB No		09148	09520

Figure 1.12. Nutritional composition of kiwifruit green and gold raw kiwifruit per 100g (Richardson et al., 2017).

Gold kiwifruit do not contain large amounts of actinidin. Kiwellin is the dominant protein, with probably less of the proteolysis product KiTH because there is no amount of actinidin to produce it. Proteins are often referred in the literature by their allergen numbers (Table 1.3).

Table 1.3. Common forms of nomenclature for the major kiwifruit proteins.

Common name	Allergen name	Genetic identifier	Protein database number	Enzyme number
Actinidin	Act d 1	Act 1A	P84527	EC 3.4.22.14
Kiwellin	Act d 5		A5HII1	
Thaumatococcus-like protein	Act d 2		P81370	
Kirola	Act d 11		P85524	

Typically, kiwifruit (*A. chinensis* var. *deliciosa* and *chinensis*) are eaten with the skin removed. Recently, several consumers choose to eat gold kiwifruit with the skin, since it is smoother, thinner, and hairless; so, its consumption increases the fibre, vitamin E and folate contents by 50, 32 and 34 %, respectively.

Vitamin C is the most distinctive nutritional attribute of kiwifruit. Hayward contains generally 80 and 120 mg per 100 g FW. Yellow flesh kiwifruit (branded Zespri) contain 161.3 mg vitamin C per 100 g, almost three times the amount found in orange (Figure 1.13).

This different amount of vitamin C in kiwifruit depends to some factors: growing conditions, use of fertilisers, maturity at harvest, time of harvest, storage and ripening conditions. The role of vitamin C in human diet is essential as humans have not the terminal enzyme L-gulonolactone oxidase in the ascorbate biosynthetic pathway. It is a cofactor of metallo-enzymes necessary for the biosynthesis of collagen, L-carnitine, catecholamine, neurotransmitters, and peptide hormones such as oxytocin; and it is involved in the regulation of transcription factors. It protects biomolecules such as lipids and DNA against oxidative damage. Its role is in the functioning of the immune system (Richardson et al., 2018).

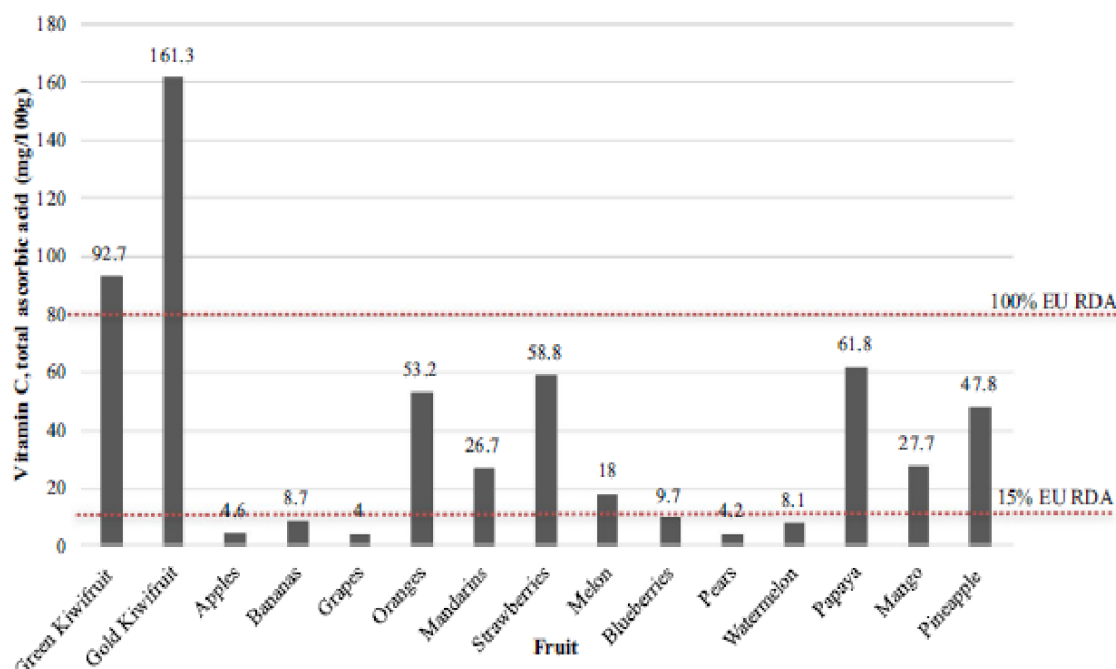


Figure 1.13. Comparison of the vitamin C content in kiwifruit with other commonly consumed fruits (Richardson et al., 2018).

It has been also identified a new form of vitamin E in kiwifruit: δ -tocomonoenol; its radical scavenging and antioxidant capacity contributes to the total antioxidant activity of kiwifruit. Yellow flesh kiwifruit contains 1.40 mg per 100 g while green kiwifruit 1.46 mg per 100 g of Vitamin E, main form α -tocopherol present in the flesh (Richardson et al., 2018).

Folate content (31 μ g per 100 g) in gold kiwifruit meets the criteria of EU Regulation to make a 'source' claim as it exceeds the 15% of the reference intake of 200 μ g/day. As folate is extremely labile and its presence in green leafy vegetables is easily destroyed by cooking, fresh kiwifruit can contribute to the diet uptake.

Green and gold kiwifruit are good sources of potassium; they contain typically 301-315 mg per 100 g; these amounts are enough to meet the criteria of EU Regulation (EC) no. 1924/2006.

The dietary fibre of kiwifruit comes from the plant cell walls: polysaccharides that form the mayor structural components of these walls. The soluble fibre fraction contains almost exclusively pectin polysaccharides, whereas the insoluble fibre is mostly cellulose and hemicellulose. The high swelling and water retention of kiwifruit fibre in comparison with other forms of dietary fibre such as wheat bran, commercial preparations of sugar beet fibre and apple fibre, accentuate the value of consuming kiwifruit as a natural whole product that has had minimal processing (Richardson et al., 2018).

The predominant sugars present in Actinidia are glucose and fructose.

Kiwifruit contain a complex network of minor compounds that can also be associated with beneficial physiological functions. This network includes carotenoids, lutein, zeaxanthin and β -carotene, chlorophyll, quinic acid, caffeic acid glucosyl derivatives, β -chlorogenic acid, phenolics, including flavones and flavonones. The total antioxidant capacity of kiwifruit is higher than apple, grapefruit and pear, but less than raspberry, strawberry, orange and plum. There is significant variation in the types and levels of antioxidant activity between Actinidia species. Although there are no dietary intake recommendations for antioxidants in general, the scientific data suggest that eating kiwifruit has the potential to inhibit oxidative and inflammatory processes (Richardson et al., 2018).

In relation to the importance of phytochemicals and antioxidant power for functional aspects of kiwifruit some changes happen during maturation and ripening (Ferguson et al., 2003). Kiwifruit are harvested firm and may be in cold stored for three or more months before eating. During long-term cool storage, they undergo several chemical and physical changes. A loss of eating quality can occur (e.g., a decrease in flavour, a loss of chlorophyll or change in the acid/sugar ratio) (Ferguson et al., 2003).

It is known that reactive oxygen species (ROS) and oxidative damage take part to fruit decay. Antioxidative system controls the steady-state level of reactive oxygen species (ROS) under normal conditions. Under abiotic stress conditions, like low or high temperature and water deficit stress, antioxidant system may be overwhelmed, and ROS accumulation leads to increased organelle and cell damage (Xia et al., 2016). In response to stresses, certain mechanisms may activate antioxidative systems by a program of gene expression to control the ROS level.

Post-harvest oxidative stress is involved in potential storability and susceptibility to development of physiological disorders during storage of several fruits. Indeed, postharvest industry applied several treatments to slow down ripening process and to delay the onset of senescence (Xia et al., 2016). For instance, the post-harvest application of ozone, oxalic acid, controlled atmosphere storage and gradual cooling treatment.

Several studies have demonstrated that postharvest treatments in kiwifruit can improve the antioxidant enzymes system. (Xu et al., 2000; Yang et al., 2013; Xia et al., 2016; Goffi et al., 2020). Xu and co-workers (2000) demonstrated that lysozyme and 1-methylcyclopropene (1-MCP) treatments prolong the shelf-life of 'Hayward' kiwifruit, improve ascorbate peroxidase (APX), superoxide dismutase (SOD) and catalase (CAT) activities, showing higher values in treated kiwifruit than untreated. Gradual postharvest cooling, from 15 °C to 0 °C, in

‘Hongyang’ kiwifruit results in higher SOD, CAT, APX, and glutathione peroxidase (GPX) activities compared to fruit treated by direct cooling during storage (Yang et al., 2013). Furthermore, nitric oxide postharvest treatment reduces peroxidase (POD) activity but increases the CAT and SOD activity in ‘Xuxiang’ kiwifruit during storage at 4 °C for 80 days (Zhu et al., 2008).

Ozone treatment induces an increase in SOD, CAT, and APX activities in papaya and pear fruits (Pasquariello et al.2013; Ong et al., 2014). Modesti and co-workers (2018) showed that the postharvest treatments by methyl-jasmonates and ozone in wine grape induce an initial increase of SOD, APX, CAT, GPX. Ozone at low concentration can active the antioxidant system, reduces intracellular ROS content and protects the membrane lipid peroxidation (Zhang et al., 2011; Adiletta et al., 2018). Ozone treatment (4 ppm) inhibites the decrease of POD and CAT activity and reduces membrane oxidative damage in strawberries during 20 days of cold storage (Villa-Rodriguez et al., 2015).

Several studies have demonstrated that postharvest treatments such as methyl jasmonates and ozone induce a significant decrease of polyphenoloxidase (PPO), that uses polyphenols as substrates in the browning reactions decreasing polyphenol content during storage, and lipoxxygenase (LOX) activity in wine grape (Adiletta et al., 2018). Ozone treatment can induce a rapid response of detoxification system by reducing oxidative stress (Goffi et al., 2020).

1.4.2 Allergens – Possible harm from eating kiwifruit

Some researchers also reported that kiwifruit have some adverse effects on human health (Meena et al., 2018).

Nowadays, along with changes in lifestyle and eating habits and with the increase of fruit and vegetable consumption for health reasons, the prevalence of allergic reactions to plant food has increased (Krebtiz et al., 2003).

In Sweden and Denmark almost half of food allergy individuals reports kiwifruit allergy in self-reported surveys. In Spain about 1.8 % of the total population shows kiwifruit allergy. In France on 182 children 9 % of the population reports hypersensitivity to kiwifruit (Wang et al., 2019).

Fruit allergies are often caused by profilins present in a wide range of fruits and can trigger hypersensitivity because of conflicting immune feedback to dietary antigens which are often difficult to avoid. Accumulation of allergens during development and ripening is identified as an undesirable health attributes in many fruits. The adverse reaction to the consumption of

fruit is caused by an IgE-response and manifests itself through pathophysiological response to food (Yang et al., 2012) (Figure 1.14).

Allergens are the proteins present in plants and many fruits that are responsible of an overreaction of the human immune system (Yang et al., 2012). They have specific antigens with the capacity to induce IgE-mediated reactions.

The allergenic proteins have certain sites, called epitopes with linear or conformational features, which can be combined with the specific antibodies (Wang et al., 2019). After the first consumption of fruit, the specific allergens are recognized by certain antigens present in cells (dendritic cells – DCs) of lamina propria in the intestine and incorporated by DCs cause of receptor-mediated endocytosis process, micropinocytosis and phagocytosis (Wang et al., 2019). Consequently, when the allergy-prone person has a secondary exposure to the same allergen, a hypersensitive reaction is presented with a series of symptoms.

Allergy to kiwifruit has been described in 1981 and some reports present this allergy with a wide range of symptoms from localized oral allergy syndrome (OAS) to life-threatening anaphylaxis (Lucas et al., 2003). Kiwifruit allergy has been associated with allergy to pollen and in latex-fruit syndrome (Maddumage et al., 2013) and other fruits and foods, including apple, avocado, banana, carrot, celery, hazelnut and potatoes (Ciardiello et al., 2009). Pollen allergy is estimated to occur in 20 % to 40 % of the population worldwide. In Europe 40 % of individuals reports hypersensitive to tree and weed pollen (Wang et al., 2019) (Figure 1.15).

The difficulty consists of recruiting large number of patients with IgE sensitization and clinically proven intolerance to a certain food (Pastorello et al., 1998). Food allergies are typically IgE-mediated, but certain reactions result from different immunologic mechanisms (Shahali and Dadar, 2018).

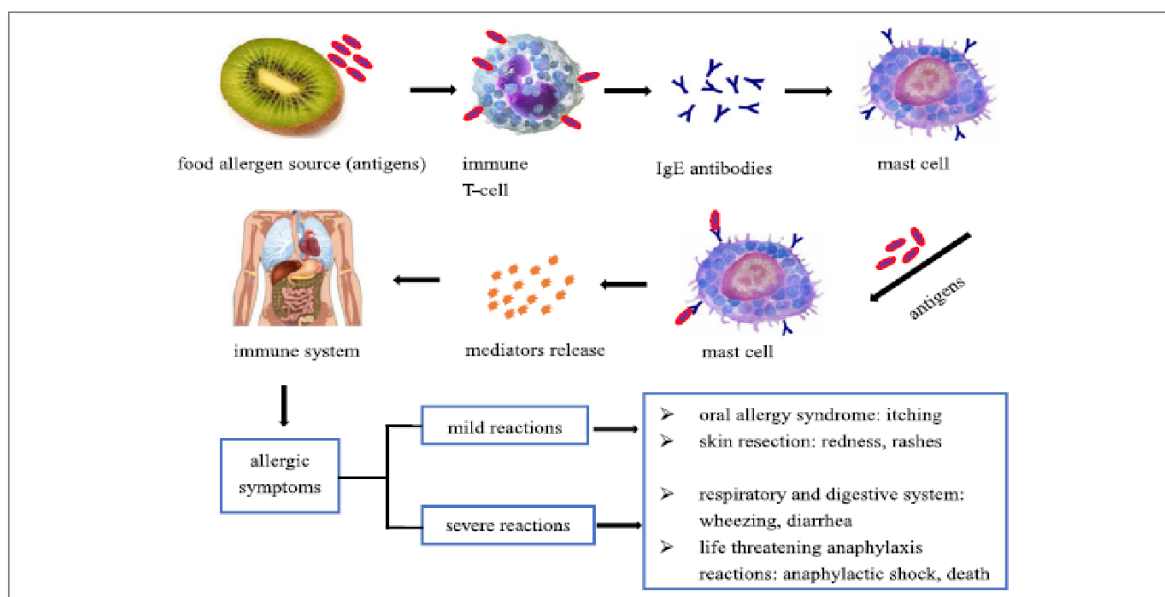


Figure 1.14. Overview of allergic sensitization to kiwifruit and related allergic reactions (Wang et al., 2019).

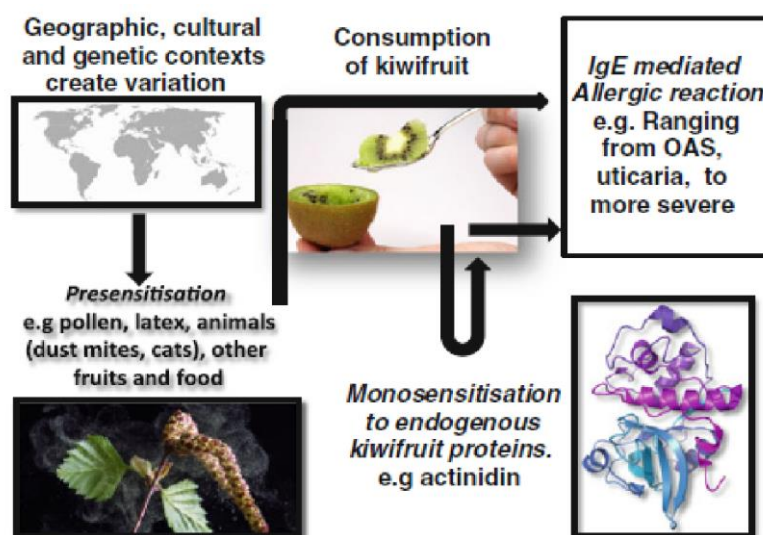


Figure 1.15. The routes towards development of kiwifruit allergy (Bulley SM, 2016).

Kiwifruit contain detectable number of specific allergens (Moller et al., 1997). Pastorello and co-workers (1998) provided the evidence that actinidin is the major allergen of the fruit *Actinidia chinensis*. Indeed, actinidin accounts around 50 % of the soluble proteins of the fruit. There are four main kiwifruit allergen families: PR-10, TLPs, nsLTPs and profilins, that induce allergic reactions. PR-10 (pathogenesis-related) proteins consist of 151-162 amino acids, presented in many fruits, for instance cherry, peach and apple; and they are associated with symptoms of oral allergy syndrome (OAS) reactions (Wang et al., 2019).

LTPs are about 200 aminoacid proteins and are significantly produced when the plants are under stress: viral or bacterial infections. Ns-LTP are found in plant-based food source such as strawberry and peach. Profilins are considered functional proteins to regulate the polymerization of the actin filament. They are the most common allergens of fruits including orange, mango, sweet cherry, strawberry, pineapple and melon (Wang et al., 2019; www.allergome.org).

The World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-Committee have identified 13 key allergenic proteins present in green and gold kiwifruit (*Actinidia chinensis* var. *deliciosa*) and three in gold kiwifruit (*Actinidia chinensis* var. *chinensis*). Among these allergens Act d 1 and Act d 2 are the first two kiwifruit allergens identified by Pastorello and coworkers (1998) (Figure 1.16).

Act d 1 is one the main kiwifruit allergen, with a 30 kDa molecular weight and it belongs to papain-like family of cysteine protease (Bublin et al., 2010).

Act d 2 is a 20 to 26 kDa member of thaumathin-like proteins (TLP) with 200 aminoacid residues (Wang et al., 2019).

Act d 1 and Act d 2 are expressed in fruit tissues when the plants are under stress or when infectious pathogens activate the defense system (Wang et al., 2019).

Act d 3 is a 40 kDa protein, attached to complex glycans (cross-reactive carbohydrate determinants).

Act d 4 is a phytocystain (12 to 16 kDa) and it is considered a cysteine proteinase inhibitor (Bublin et al., 2010).

Act d 5, kiwellin, is a cysteine-rich protein (26 to 28 kDa), which is abundantly present in green and gold kiwifruit (Maddumage et al., 2013). Kiwellin is susceptible to actinidin activity, producing the peptide kissper, and KiTH. Kissper is of interest for human health as it displays a range of beneficial activities, including anti-inflammatory response, reducing oxidative stress at the GI mucosal interface. Kissper is a member of a new class of pore-forming peptides with potential beneficial effects on human health, including a potential effect on gastrointestinal physiology (Richardson et al., 2018).

Act d 6 (18 kDa) is expressed in ripe fruits involving the synthesis of pectin methylesterase (Wang et al. 2019).

Act d 7 belongs to pectin methylesterase (50 kDa) but few consumers show allergy to it.

Act d 8 is pathogenesis related proteins (PR-10), 15-18 kDa, that is found to be homologous to birch pollen.

Act d 9 (profilin) and Act d 10 (nsLTP) are two minor allergens of kiwifruit. Act d 11 is one of the main ripening-related proteins present in kiwifruit (D'Avino et al., 2011). From green kiwifruit seeds, Act d 12 (globulin) and Act d 13 (albumin) were extracted; they show cross reactivity with allergens present in peanuts and tree nuts (Sirvent et al., 2014). The gold kiwifruit show three allergens: Act c 5, Act c 8 and Act c 10. Act c 5 is a kiwellin (28 kDa) and it is the main allergen found in gold kiwifruit, about 30 % of the total protein. Act c 8 belongs to PR-10 family and it is less abundant in gold kiwifruit; it is encoded by open reading frames of 474 nucleotides, corresponding to 158 aminoacid residues (Wang et al., 2019). Act c 10 (10 kDa) belongs to nsLTP, that are involved in the defense mechanism of the plant.

Allergen	Function/biochemical name	Molecular weight (kDa)	Archetypal accession (GenBank)	Experimental sensitisation prevalence (%)
<i>Actinidia chinensis</i> var. <i>deliciosa</i>				
Act d 1	Cysteine protease (actinidin)	30	CAA34486	0–100
Act d 2	Thaumatococin-like protein	24	CAI38795	0–61
Act d 3	Unknown function	40	P85063	33–62
Act d 4	Phytocystatin	11	AAR92223	0–20
Act d 5	Kiwellin	26	P84527	0–18
Act d 6	Pectin methylesterase inhibitor	18	BAC54964	72
Act d 7	Pectin methylesterase	50	P85076	32
Act d 8	Pathogenesis-related protein, PR-10, Bet v 1 family member	17	CAM31909	60–100
Act d 9	Profilin	14	FG438715	20
Act d 10	nsLTP1	10	P86137; P85206	3–56
Act d 11	Major latex protein/ripening-related protein (MLP/RRP), Bet v 1 family member	17	P85524	22–24
Act d 12	Cupin, 11S globulin	50		70–100
Act d 13	2S albumin	11		7–18
<i>Actinidia chinensis</i> var. <i>chinensis</i>				
Act c 5	Kiwellin	28	P85261 (KISSPER cleavage product); AGC39168 (Kiwellin)	
Act c 8	Pathogenesis-related protein, PR-10, Bet v 1 family member	17	CAM31908	54–83
Act c 10	nsLTP1	10	P85204	33

Figure 1.16. Official kiwifruit allergens (www.allergon.org)

Some studies suggest that the number of allergenic proteins in kiwifruit and their clinical relevance is characterized by variation (Ciardiello et al., 2009). Indeed, also the level of

nutrients and phytochemicals varies according to the genotypes of kiwifruit and is affected by different pre and post-harvest factors (Meena et al., 2018).

Studies have shown that maturity related to kiwifruit allergens increases during the storage (Wang et al., 2019). The relationship between allergens and the fruit-ripening process has also been studied in other fruits such as apples, peach and tomato (Yang et al., 2012). For instance, Mal d 1 (PR-proteins) is upregulated by internal and external factors (PPO activity and temperatures, respectively), resulting in its increased synthesis in stored apples; this significant increase is attributed to the increase of respiration rate and ethylene biosynthesis (Wang et al., 2019).

Allergens have an important role as defence-associated proteins in response to biotic and abiotic stresses (Krebitz et al., 2003).

Indeed, studies have shown that environmental cultivation conditions have an impact on the fruit allergen content as well as temperature increase could directly affect photosynthesis, which results in alterations in sugars, organic acids, flavonoids content, firmness and antioxidant activity (Wang et al., 2019). Harvest maturity is another affecting factor. Studies found that the level of allergens present in apples might be influenced by the maturity levels through the regulation of ethylene levels (Sancho et al 2006). Storage conditions are the major factors for preserving quality of fruits after harvest. During the storage of fruits, many studies found that a considerable change in the level of allergens depends on environmental stress and/or storage conditions (Sanchez-Monge et al., 1999). For instance, the rate of overall reduction of some allergens is greatest when fruits are stored at controlled atmosphere conditions, and in contrast an allergen increases during cold storage at 4 °C (Bolhaar et al., 2005).

The three allergenic proteins: actinidin (Act d 1), LTP (Act d 2) and kiwellin (Act d 5) in kiwifruit after commercial harvest (about after 42 days) show higher concentration compared to those picked before commercial harvest (104 days). Thus, there are four ripening-related allergenic proteins synthesized during postharvest ripening: Act d 1, Act d 2, Act d 5 and Act d 11.

Another study conducted by Minas and co-workers (2018) revealed that ozone modulates some proteins; O₃ probably acts as a protein repressor since most proteins are exclusively affected by O₃.

1.5 PROCESSED FOOD KIWIFRUIT-CONTAINED

Interesting is to consider the structural stability of fruit allergens. Most of the fruits are not only consumed fresh but are also used as the ingredients in various related products such as drink, dry powder, jam, yogurt and cereals. Nowadays, minimally processed food with high nutrients content, natural flavour, and taste are increasing in popularity. Studies have demonstrated that the consumption of kiwifruit juice (0.5 L, about 8 kiwifruits) is associated with a decrease of DNA damage in human cells compared to the cells from the water consuming control group (Wang et al., 2019).

In addition, the food industry point of view is focused on the avoidance of food loss, so, kiwifruit are transformed into different products such as juice, dried kiwifruit slices, frozen products, jam and bakery products.

Therefore, a new need is the use of novel technologies to potentially reduce the food allergenicity maintaining the food quality.

The allergenicity of fruits has a relationship to the form in which they are consumed. For instance, cooking can reduce the allergenicity of fresh fruits because it destroys the pollen related fruit allergens through changing their protein secondary and tertiary structures of epitopes (Wang et al., 2019); an example is Act d 11 allergen in kiwifruit. Studies show that conformational epitopes of some protein allergens can be altered by heating or enzymatic hydrolysis because the modification of these secondary and tertiary structures can lead to the loss of their IgE-binding sites in these proteins as IgE-binding is essential for triggering allergic symptoms.

Few allergens have a stable structure in some fruits; for instance, the kiwifruit allergen Act d 2 has the heat stable secondary structures (Asero et al., 2003).

Nowadays, it is mandatory to declare allergenic ingredients on food labelling because allergen proteins, even if they are present in traces, can cause anaphylaxis in hypersensitive consumers. Thus, it is important an accurate allergenic food detection method able to quantify trace amounts of allergens.

CHAPTER 2: EXPERIMENTAL DESIGN

2.1 EXPERIMENTAL DESIGN

Figure 2.1. Mindmap of the trials during the PhD years.

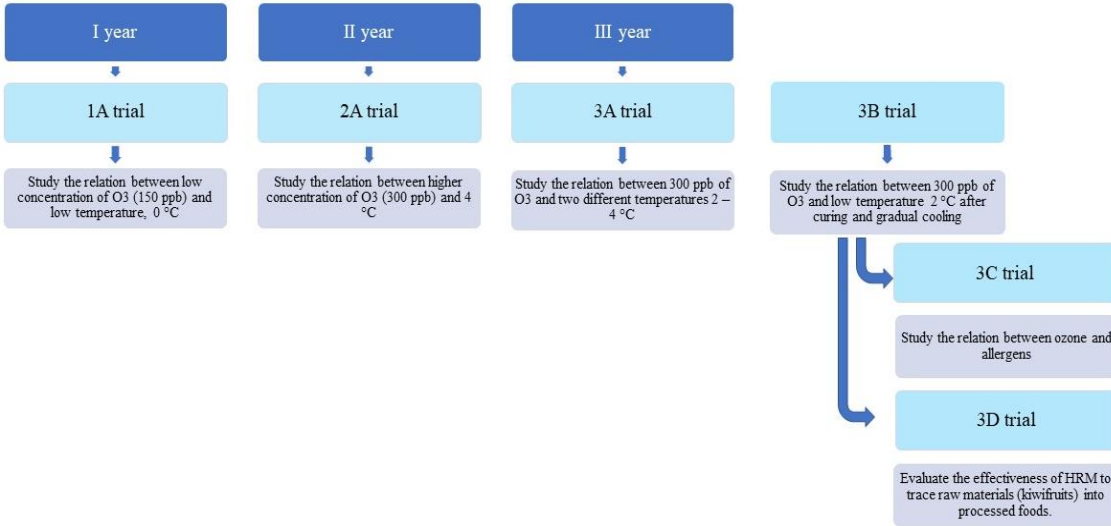


Table 2.1. Description of the trials conducted during the PhD years.

	1 YEAR	2 YEAR	3 YEAR			
	1A	2A	3A	3B	3C	3D
Studied cultivar	<i>A. chinensis</i> var. <i>deliciosa</i> and <i>chinensis</i>	<i>A. chinensis</i> var. <i>deliciosa</i> and <i>chinensis</i>	<i>A. chinensis</i> var. <i>chinensis</i>	<i>A. chinensis</i> var. <i>chinensis</i>	<i>A. chinensis</i> var. <i>chinensis</i>	<i>A. chinensis</i> var. <i>chinensis</i>
Aim of the trial	To study the relation between low dose of O ₃ and low temperature	To study the relation between the double dose of O ₃ and an increased temperature	To compare two low temperatures combined with O ₃ post-harvest treatment	To study the relation between low temperature and O ₃ after curing and gradual-cooling post-harvest treatments	To study the relation between O ₃ and allergens	To evaluate the effectiveness of HRM to trace raw materials (kiwifruit) into processed foods
Gaseous ozone concentration (ppb)	150 during the day 180 at night	300	300	300		
Flow	Continuous	Continuous	Continuous	Continuous		
Storage T (°C)	0 °C	4 °C	4 - 2 °C	2 °C		
Storage time (days)	42 days	60 days	60 days	60 days		
Additional post-harvest treatment				Curing (24h) Gradual cooling (7days)		
Shelf-life (days)	10 days at 18 °C	8 days at 18 °C				
Parameters analysed and analyses carried out	Epiphytes, F, SSC, TA, colour, bioactives	Epiphytes, F, SSC, TA, DM, WL, RR, colour, bioactives and antioxidants	Epiphytes, F, SSC, TA, colour, bioactives and antioxidant system	Bioactives and antioxidant system	PCR analysis	PCR and HMR analysis

2.2 MATERIAL AND METHODS

2.2.1 Fruit material

I and II Year

Actinidia chinensis var. *deliciosa* ‘Hayward’ fruit and *Actinidia chinensis* var. *chinensis* A137 x A134.41 (commonly known as Soreli) fruit from commercial orchards in Cisterna di Latina (LT), Italy, were used in this study. For each cultivar, fruit were harvested starting from the end of September for Soreli and from the mid of October for Hayward, corresponding to about 6.5 °Brix for Hayward and 8.0 °Brix for Soreli. Fruit (numbering 400) were selected for uniform size, absence of diseases, and lack of general defects, packed in single-layer trays and immediately transported to the postharvest laboratory of Dibaf of Università degli Studi della Tuscia, Viterbo, Italy.

III Year

3A, 3B and 3C Trial

Actinidia chinensis var. *chinensis* A137 x A134.41 (commonly known as Soreli) fruit from commercial orchards (AgricolliBio S.r.l company (41°30’33,84” N 13°4’33.17” E, 334 m above sea level) located in Cisterna di Latina, Lazio, Italy) were used in this study. Fruit were harvested starting at the end of September at ripening stage, corresponding to about 8.0 °Brix. Fruit were selected for uniform size, absence of disease and lack of general defects, packed in single layer trays and immediately transported to the Postharvest Laboratory, Dibaf, Università degli Studi della Tuscia, Viterbo, Italy. Fruit (numbering 400) were transported in a refrigerated van to the laboratory.

3D Trial

Actinidia chinensis var. *chinensis*, Soreli, were harvested at commercial maturity (8 °Brix and 12 N/mm) following the same procedures described above. Fruit were selected for uniform size, absence of disease and lack of general defects and processed and transformed in juice, biscuits and jam.

2.2.2 Storage conditions and applied treatments

I Year

Four homogeneous lots were got from the harvest. Two lots belonged to Hayward and others two to Soreli. Relative humidity of 90-95 % was maintained in all storage rooms. Three cold rooms, located at underground floor of Dibaf postharvest laboratory, side by side, were 2 m (l), 2.5 (w) and 2 m (h) of size. The first and second lots were monitored during the storage in presence of gaseous ozone in continuously at the concentration of 150 ppb during the day and 180 ppb during the night until the end of the storage (42 days). The ozone generator was C32-AG, provided by Industrie De Nora Spa, Milan, Italy. To compare the results provided by the treatment, a control was performed; so, the untreated samples were placed in normal atmosphere (second and four lots). The lots were placed in a room at 0 °C for 42 days. Sampling was done at 15 days' intervals as some previous researches have shown. After 42 days of storage conditions, fruit were placed in a shelf-life conditions: 18 °C for 10 days, sampling every 2 days to evaluate the behaviour of the kiwifruit during shelf-life.

For each sampling kiwifruit were subjected to the following analyses:

- 12 fruit destined to non-destructive-analysis: rheological analysis, firmness using Instron.
- 12 fruit destined to destructive analysis: qualitative: SSC, TA, firmness by manual penetrometer.
- 5 fruit destined to microbiological analysis at PhyDia laboratory belonged to UNITUS, Viterbo, Italy.
- 8 fruit were peeled, cut, frozen at -18 °C and used for the analysis of bioactive compounds.
- 3 biological replicates

II Year

Upon arrival, fruit were randomised, divided into two lots, and each kept in different conditions. Relative humidity of 90-95 % and 4 °C were maintained in all storage rooms. The cold rooms (no.3), located at underground floor of Dibaf postharvest laboratory, side by side, were 2 m, 2.5 and 2 m of size. The instrument was equipped with an oxygen concentrator (nominal production capacity of 32 g ozone h⁻¹) and the treatment applied was gaseous ozone in continuous flow at the concentration of 300 ppb during the night for 60 days for the whole duration of the trial. The ozone generator was C32-AG, provided by Industrie De Nora Spa,

Milan, Italy. To compare the results provided by the treatment, a control was performed so the untreated samples were placed in normal atmosphere.

Sampling was done at 15 days' intervals as some previous studies have shown. After 60 days of storage conditions at 4 °C, fruit were placed in a shelf-life conditions: 18 °C for 10 days, sampling every 2 days to evaluate the behaviour of the kiwifruit during shelf-life.

For each sampling kiwifruit were subjected to the following analyses:

- 12 fruit destined to non-destructive analysis: rheological analysis, firmness using Instron and respiration rate.
- 12 fruit destined to destructive analysis: qualitative: SSC, TA, colour, dry matter, weight loss and firmness by manual penetrometer.
- 5 fruit destined to microbiological analysis at PhyDia laboratory belonged to UNITUS, Viterbo, Italy.
- 8 fruit were peeled, cut, frozen at -18 °C and used for the analysis of bioactive compounds and antioxidant system.
- 3 biological replicates

III Year

3A Trial

Fruit were divided into four homogeneous lots (about 100 fruits each lot). The first and the second lots were treated by ozone gas applied for the whole duration of the trial at 300 ppb in a 9 m³ cold room using C32-AG ozone generator (provided by Industrie De Nora Spa, Milan, Italy) and maintained at 2 ± 0.05 and 4 ± 0.05 °C with ± 5 % RH for 60 days. The third and fourth lots were maintained at 2 ± 0.05 and 4 ± 0.05 °C with ± 5 % RH in normal atmosphere (control). All lots were stored for 60 days in the presence or absence of ozone in air. The cold rooms (no.3) located at underground floor of Dibaf postharvest laboratory, side by side, were 2 m, 2.5 and 2 m of size.

At harvest and every 15 days kiwifruit were subjected to the following analyses:

- 12 fruit destined to non-destructive-analysis, rheological analysis: firmness using Instron.
- 12 fruit destined to destructive analysis, qualitative: SSC, TA, firmness by manual penetrometer.

- 1381 - 5 fruit destined to microbiological analysis at PhyDia laboratory belonged to UNITUS,
1382 Viterbo, Italy.
- 1383 - 8 fruit were cut and frozen at -18 °C and used for the analysis of bioactive compounds
1384 and antioxidant system.
- 1385 - 3 biological replicates

1386
1387

1388 3B Trial

1389 Fruit were divided into two lots (150 fruits each lot). Kiwifruit underwent to curing, gradual
1390 cooling treatment and long-term storage. The post-harvest treatments have been realized for
1391 both lots.

1392

1393 Kiwifruit were stored for 24 hours at room temperature (curing) and subsequently they were
1394 gradually cooled (gradual cooling), reducing the temperature from 26 °C to 2 °C for 8 days
1395 (decreasing 3 °C from 26 °C to 23 °C, 20 °C, 17 °C, 14 °C, 11 °C, 8 °C, 5 °C, 2 °C after 1, 2,
1396 3, 4, 5, 6, 7, 8 days of storage, respectively).

1397

1398 After gradual cooling, the first lot was maintained at 2 ± 0.05 °C with 85 ± 5 % RH (control).
1399 The second lot was treated with ozone gas using the ozone generator C32-AG, Industrie De
1400 Nora Spa, Milan, Italy and maintained at 2 ± 0.05 °C with 85 ± 5 % RH. All kiwifruit lots
1401 were stored for 60 days. The cold rooms (no.3) located at underground floor of Dibaf
1402 postharvest laboratory, side by side, were 2 m, 2.5 and 2 m of size. Three biological replicates
1403 were realized per treatment and each one contained 10 fruits. All analyses were performed at
1404 harvest, after gradual cooling and every 15 days of cold storage.

1405 3C Trial

1406 Kiwifruit were keeping for 24 hours at room temperature, after they were gradually cooled,
1407 decreasing the temperature from 26 °C to 2 °C by 3 ± 0.05 °C at 1-day intervals for 8 days.
1408 Successfully, after gradual cooling fruit were randomly distributed into two samples: the first
1409 samples were maintained at 2 ± 0.05 °C with 85 ± 5 % RH (control), the second samples were
1410 treated with ozone gas at the concentration of 300 ppb using an ozone generator C32-AG,
1411 Industrie De Nora Spa, Milan, Italy and maintained at 2 ± 0.05 °C with 85 ± 5 % RH for 15
1412 days. The cold rooms (no.3) located at underground floor of Dibaf postharvest laboratory, side

by side, were 2 m, 2.5 and 2 m of size. The samples were powdered with liquid nitrogen and stored at -80 °C until RNA extraction.

3D Trial

Actinidia chinensis var. *chinensis*, Soreli, reached commercial maturity stage (above 15-16 °Brix), were transformed into juice, jam and biscuits after 60 days of cold storage at 2 °C under gaseous ozone and under normal atmosphere (control samples). The cold rooms (no.3) located at underground floor of Dibaf postharvest laboratory, side by side, were 2 m, 2.5 and 2 m of size. The processing of kiwifruit took place at at Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia agraria (CREA), Centro di ricerca per Olivicoltura, Frutticoltura e Agrumicoltura, Caserta, Italy.

Processing

A homogeneous pool of 20 Soreli kiwifruit was selected to the process of transformation on juice.

Kiwifruit were peeled, cut into slices and through the juice-extractor (Electric Juice Extractor, Mod. 7500 Angel Co.Ltd.) were transformed into juice, that was immediately frozen at -80°C (Figure 2.2).

Another homogenous pool of 30 fruits was selected and destined to the transformation into jam. Fruits were peeled, cut into slices, and boiled with sugar for above 2 hours at low flame. After it, the jam was stored at ambient temperature.

To get kiwifruit-contained biscuits, a homogeneous pool of 20 fruit was chosen and processed. Ripe fruit were peeled, cut into slices and mixed with egg white, sugars through kitchen robot. The mix was spread out into kitchen paper and put into the oven at 80 °C for 2 hours; after it, the biscuits were stored until the evaluation.



Figure 2.2. Extraction process of the Soreli's juice-by Electric Juice Extractor, Mod. 7500 Angel Co.Ltd.

2.2.3 Pomological, physico-chemical analyses

I. Total soluble solid content

The solid soluble content is expressed as SSC and the measure unity is °Brix, by measuring the refractive index of kiwifruit juice. It was determined squeezing 3 drops of juice on the lens of the digital refractometer (ATAGO, Palette PR-32, Japan). The calibration was made with deionised water and the lens was carefully rinsed between samples. Sugar is usually a major component of soluble solids in cell sap; it's much easier to measure soluble solid concentrations in extracted juice with a refractometer. These devices operate based on refraction of light in its passage through a thin sample of juice containing sugars, and variation in the density of juice according to its sugar content, respectively.

II. Titratable acidity

Titrateable acidity (TA) was determined as described by Garner et al. (1998). Each sample was titrated with an alkaline solution, 0.1 mol L⁻¹ NaOH, up to a pH end point of 8.2.

The results were expressed as milligrams of malic acid per 100 g FW. Malic acid is the predominant acid found in kiwifruit and its milliequivalent factor is 0.067. The total titratable acidity of the juice was calculated using the following formula:

$$\frac{\text{Volume NaOH (mL)} * 0.1M * 0.067}{\text{grams of sample}} * 100$$

Loss of acidity during maturation is often rapid. The sugar/acid or total soluble solids/acid ratio is often better related to palatability of the fruit than sugar or acid levels alone.

III. Colorimetric evaluation

Flesh colour was measured using a Minolta colorimeter (Minolta C2500; Konica Minolta, Ramsey, NY, USA) to determine chromaticity values L^* (lightness), a^* (green to red), and b^* (blue to yellow). The hue angle (HUE) was calculated from chromaticity values a^* and b^* as reported by McGuire (1992). Colour changes of kiwifruit samples during storage were evaluated through the colour difference ΔE and chroma in the colour space (L , a , b) (Figure 2.3).

The value of ΔE^* numerically expresses the difference between two colours as follows: imperceptible ($\Delta E^* < 1$), minimal ($1 \leq \Delta E^* < 2$), just perceptible ($2 \leq \Delta E^* < 3$), perceptible ($3 \leq \Delta E^* < 5$), strong difference ($5 \leq \Delta E^* < 12$) and different colour ($\Delta E^* \geq 12$).

$$\Delta E = \sqrt{(\Delta a^2) + (\Delta b^2) + (\Delta L^2)}$$

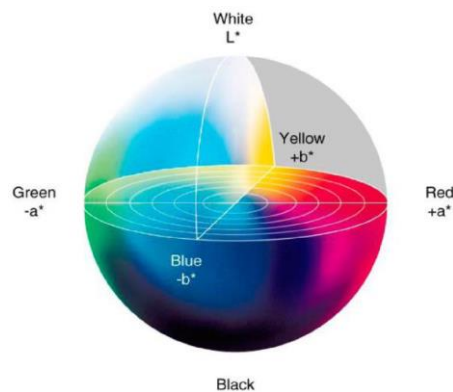


Figure 2.3. Color coordinates: L^* , a^* and b^* ; CIELab Method.

IV. Firmness

Instron Universal Testing Machine

Flesh firmness was evaluated non-destructively with an Instron Universal Testing Machine (mod. 4301; Instron Inc., Canton, MS, USA). It was assessed using a deformation test under a non-destructive force. Fruit (12) were placed width-wise over the plate of the machine and compressed with a flat compression anvil with a diameter of 55 mm at a bar speed of 25 mm min^{-1} and load deformation of 5 N. A preliminary test verified that 5 N did not induce tissue injury under the peel of the kiwifruit (Taglienti et al., 2009). The same 12 kiwifruit were used

at each sampling time, previously numbered to perform the analysis on the same fruits for the whole duration of the test. Fruit at 0 °C were rewarmed at ambient temperature before starting the Instron test, and afterwards restored in the cold rooms. Flesh hardness was expressed in newtons per millimetre, corresponding to flesh deformation at 5 N (pressure/deformation).

Fruit Pressure Tester

Flesh firmness was evaluated using table penetrometer (Fruit Pressure Tester 327, Effegi Gaifarine, Ravenna, Italy) and an Instron Universal Testing Machine (mod.4301 Instron Inc.Canton, MS, USA). It measures the pressure at which flesh yields to the penetration of a standard diameter plunger inserted to a standard depth. From a transversal section taken from a central part of the fruit, the peel was removed, and a pressure was applied up to complete pulp's break. The results were expressed as kg/cm².

V. Weight Loss

Weight Loss (%) was measured with an analytical balance using the follow formula:

$$WL (\%) = [(W_i - W_f) \div W_i] * 100$$

W_i= sample's weight at the harvest (day 0)

W_f = sample's weight after some days of sampling

VI. Respiration rate

It was measured by the portable analyser of gas O₂/CO₂ Helpy (Marvil Engineer SRL, Bolzano, Italy). It permits to measure the percentage of oxygen and carbonic anhydride produced. A glass jar (500 mL) was used, closing inside hermetically 4 fruit for 3 hours at ambient temperature (Figure 2.4).

$$RR = [(Vol_j - Ws_{grams}) \times \% CO_2] \div (Ws_{Kg} \times Th)$$

Vol j = volume jar empty

Ws gr = weight samples, grams

Ws kg = weight samples, kilograms

T h = closure time, hours



Fig.2.4. Display Helpy – Marvil engineering SRL.

VII. Dry matter

From each lot, some fruit were taken randomly, cut in a cube size and weighted and put into the stove for 24h; the day after, the fruits dried were re-weighted and applied the following formula:

$$DM (\%) = [(Wf - weight\ boat) \times Wj \times 100]$$

Dry matter content, which tends to increase as certain fruit mature, can be used for products in which there is a large increase in the amount of starch or sugar as the fruit mature (e.g. kiwifruit).

2.2.4 Analysis of bioactive compounds and antioxidant system: non-enzymatic and enzymatic

I. Ascorbic acid content

The AA content from kiwifruit was determined following the method of Malik and Singh (2005), with some modifications. Kiwifruit (2.5 g) was homogenized using 10 mL of 16 % (v/v) metaphosphoric acid solution containing 0.18 % (w/v) disodium ethylenediaminetetraacetic acid. The homogenate was centrifuged at 11 000×g for 10 min at 4 °C, filtered and collected. The assay mixture contained 200 µL supernatant (5:1, v/v) diluted Folin's reagent and 0.3 % (v/v) metaphosphoric acid in a final volume of 2 mL. The absorbance of the sample was recorded at 760 nm with a UV–visible spectrophotometer (Model V-630; Jasco, Japan). The AA concentration was calculated from a calibration curve, using AA as standard and expressed as milligrams of AA per 100 g FW. All analyses were performed in triplicate.

II. Pigment content

I year

All pigments (Chlorophyll a, Chlorophyll b and β - Carotene) in the sample were analyzed as described by Nagata & Yamashite (1992). Pigments were extracted with acetone-hexane (v:v 4:6). Then optical density of the supernatant was measured at 663 nm, 645 nm, 505 nm and 453 nm using a spectrophotometer. From these values, the content of chlorophyll a, b and β -carotene were estimated using the follow equations:

$$\text{Chlorophyll a (mg/100ml)} = 0.999A_{663} - 0.0989A_{645}$$

$$\text{Chlorophyll b (mg/100ml)} = - 0.328A_{663} + 1.77A_{645}$$

$$\beta\text{-carotene (mg/100ml)} = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453}$$

(A_{663} , A_{645} , A_{505} and A_{453} are absorbance at 663 nm, 645 nm, 505 nm, 453 nm, respectively).

II and III years

Total carotenoids (CAR) were extracted using 10 g of kiwifruit in methanol (1:10 w/v). The absorbance at 666, 653m and 470 nm was determined, and CAR was calculated using the Wellburn equation (Wellburn and Lichtenthaler, 1983). Results were expressed in micrograms of TC (Total Carotenoids) per 100 g FW.

III. Total phenols and flavonoids content

Total phenolic and flavonoid extraction was carried out by homogenizing kiwifruit (2.5 g) in water–alcohol solution (methanol/water 80:20 v/v). The extract was centrifuged (14000×g for 5 min) and filtered through a Whatman No. 41 paper. The filtrate was combined and used for analysis of the total phenolics (POL) and total flavonoids (FLAV). The total POL and total FLAV contents were determined by the Folin–Ciocalteu (Singleton and Rossi, 1995) and aluminium chloride colorimetric method respectively. POL was expressed as milligrams of gallic acid equivalents (GAE) per 100 g FW, and FLAV as milligrams of catechin equivalent (CE) per 100 g FW.

IV. Total antioxidant activity (DPPH method)

Free-radical scavenging activity was carried out by homogenizing kiwifruit (5 g) in water–alcohol solution (methanol/water 80:20 v/v). The extract was centrifuged (10000×g for 10 min at 15 °C). The extract was used for the 1,1-diphenyl-2-picrylhydrazyl (DPPH) activity measured at 515 nm. The free-radical scavenging activity of kiwifruit extract was measured by DPPH according to the method of Pasquariello and coworkers (2015) with some modifications. The mixture assay consisted of kiwifruit extracts (20 µL) and 6.25×10^{-5} mol L⁻¹ solution of DPPH in methanol in a total volume of 1.5 mL. The percentage decrease in the DPPH concentration was calculated from the initial value after incubation for 15 min. A dose–response curve was generated using Trolox as a standard, and the antioxidant activity was expressed as micromoles of Trolox equivalent (TE) per gram FW.

V. Enzyme extraction and activity assays

Total soluble proteins were extracted by homogenizing 1.5 g of frozen fruit flesh powder in 3.5 mL of a buffer containing 0.1 M potassium phosphate (pH 7), 1 mM sodium-ethylenediaminetetraacetic acid (EDTA pH 7), polyethylene glycol (PEG) 6.25 mM, 5% (w/v) polyvinylpyrrolidone (PVPP), and 5 mM ascorbic acid (only for APX enzyme extraction). The homogenate was centrifuged at $14000 \times g$ for 20 min at 4 °C. The extract was used for dosing the total soluble proteins content by the Bradford assay, hydrogen peroxide content (H₂O₂), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and superoxide dismutase (SOD) activity.

VI. Superoxide dismutase activity

Superoxide dismutase (EC 1.15.1.1) activity was determined as described by Adiletta and coworkers (2019) with minor modifications. Reaction assay contained 50 mM potassium phosphate buffer pH 7.8, 0.1 mM sodium EDTA, 13 mM methionine, 75 µM nitro blue tetrazolium chloride (NBT), 0.5 µM riboflavin and 25 µL of crude enzyme extract. The reaction was started by adding riboflavin. After 10 min of incubation at room temperature under continuous light, absorbance value was measured at 560 nm. One SOD unit was defined as the amount of enzyme that inhibits the rate of NBT reduction by 50% below the assay conditions. SOD activity was expressed as U per g FW.

VII. Malondialdehyde content determination

Malondialdehyde (MDA) content was measured by the method of Pasquariello and coworkers (2015) with some modifications. Frozen fruit flesh powder (1 g) was re-suspended in

10 mL of 10% trichloroacetic acid (w/v), and 0.5% thiobarbituric acid dissolved in HCl 0.25 M. The solution was kept in a boiling water bath at 95 °C for 30 min and immediately cooled. The homogenate was centrifuged at 12000 x *g* for 10 min and the absorbance of supernatant was read at 450, 532 and 600 nm. The MDA content was expressed as nmol per g FW.

VIII. Ascorbate peroxidase activity

Ascorbate peroxidase (EC 1.11.1.11) activity was determined according to Petriccione and co-workers (2015) with some modifications. Assay mixture contained 100 mM potassium phosphate buffer (pH 7), 0.33 mM ascorbic acid, 0.35 mM H₂O₂, 0.66 mM sodium EDTA (pH 7) and 100 µL of crude enzyme extract. The oxidation of ascorbic acid was determined at 290 nm and APX activity was measured in µmol of ascorbate per g FW.

IX. Guaiacol peroxidase activity

Guaiacol peroxidase (EC 1.11.1.7) activity was determined according to Modesti and coworkers (2018) with slight modifications. The reaction assay contained 100 mM potassium phosphate buffer pH 7, 0.15 mM sodium-EDTA pH 7.0, 6.60 mM H₂O₂, 8 mM guaiacol and 100 µL of crude enzyme extract. Guaiacol peroxidase activity was detected spectrophotometrically at 470 nm, monitoring the tetraguaiacol formation. GPX activity was expressed in µmol per g FW.

X. Polyphenoloxidase activity

Polyphenoloxidase activity was determined with the method described by Adiletta and co-workers (2019b). Crude enzyme extract was realized homogenizing fruit flesh (1g) in sodium phosphate buffer (100 mM pH 6.4) containing 0.05 g of PVPP. PPO activity was measured in a buffered substrate (500 mM catechol in 100 mM sodium phosphate buffer pH 6.4) using crude enzyme extract (100 µL) and monitoring the increase in absorbance at 398 nm. PPO activity was expressed as µmol per g FW.

XI. Catalase activity

Catalase (EC 1.11.1.6) activity was assayed according to the method described by Adiletta and coworkers (2019) with minor modifications. Reaction assay contained 100 mM potassium phosphate buffer (pH 7), 20 mM H₂O₂ and 100 µL of crude enzyme extract. The reaction was induced by adding H₂O₂, was recorded a decrease in absorbance at 240 nm for 3 minutes. CAT activity was measured in µmol of H₂O₂ per g of fresh weight (FW).

XII. Lipoxygenase activity

Lipoxygenase activity was determined by the method described by Petriccione and coworkers (2018) with some modifications. Frozen fruit tissue powder (1 g) was homogenized in 3 mL of extraction buffer including 50 mM potassium phosphate buffer pH 7.8, 1 mM sodium-EDTA pH 7.2 % PVPP and centrifuged at 12500 x *g* for 10 min at 4 °C. Reaction assay contained sodium phosphate buffer 100 mM pH 6.5 mM linoleic acid sodium salt and crude enzyme extract (50 µL). LOX activity was measured spectrophotometrically by recording the increase in absorbance at 234 nm. LOX activity was expressed as nmol per g FW.

XIII. Hydrogen peroxide content determination

Hydrogen peroxide content was evaluated using the method described by Alexieva et al. (2001) Reaction mixture consisted of trichloroacetic acid 0,1%, 1M potassium iodide and 300 µL of crude enzyme extract. The H₂O₂ content was expressed as nmol per kg FW.

2.2.5 Microbiological analysis

Epiphytic analysis

To evaluate the presence of epiphytes, the analysis was performed in sterile conditions, homogenizing kiwifruit peel with 20 mL of sterile water in a sterile stomacher bag using a stomacher for 120 s. The total microbial growth (MG) count method was used to determine total aerobic bacteria, yeasts, and moulds. Serial dilutions of homogenate were realized, and the plates were incubated at 27 °C for 2 days.

The results are reported in the I trial as the number of colony forming units per surface (CFU/cm²); whereas, in the II and III trial as the number colony forming units per millilitre of cleaning water (CFU mL⁻¹).

Three technical replicates were realized for each biological replicate.

2.2.6 Molecular analysis

I. Total RNA extraction and cDNA synthesis

Total RNA was extracted from frozen powder of flesh kiwifruit using the RNeasy plant mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The concentration and integrity of RNA was measured by UV/visible spectroscopy and non-denaturing agarose gel, respectively. cDNA was synthesized from 4 µg of total RNA using oligo(dT)20 primer

and the iScript™ cDNA synthesis kit (BIORAD CA, USA) according to the manufacturer's protocol.

II. PCR primer design and amplification efficiency test

Allergen-related primers (Act C1, Act C2, Act C5 and Act C11) were designed using Primer expression software version 3. Reference genes, tubulin (TUB) and protein phosphatase 2A (PP2A), have been used in previous studies (Petriccione et al., 2015; Nardoza et al., 2013) (Table 3.10). The efficiency of each primer was established using a pool representative all of the cDNA samples, generated a five-point standard curve based on a ten-fold dilution series. The amplification of allergen-related primers was confirmed by a single band of appropriate size in a 2% agarose gel after electrophoresis (Fig. 3.29). The amplification efficiency (E) and correlation coefficient (R^2) of the primers were determined from the slope of the standard curve according to Radonic et al., 2004.

III. Real-time quantitative PCR analysis

Quantitative Real-time-PCR was carried out using a CFX Connect Real-time PCR detection system (Bio-Rad) to analyse the specific expression of allergens. Each sample was analysed in triplicate in 96-well plates using the SsoAdvanced™ SYBR® Green Supermix (Bio-Rad), with 100 ng of cDNA and 500 nM specific sense and anti-sense primers. The thermal cycling beginning with an initial step at 95 °C for 180 s, followed by 40 cycles of denaturation at 95 °C for 5 s and primer-dependent annealing at 59 °C for 30 s. Analysis of melt-curve was used to confirm the specificity of the amplification. The $2^{-\Delta\Delta CT}$ method was utilized to measure the relative fold changes in allergens expression and for normalizing through the reference genes.

IV. DNA extraction and quantification

DNA from all samples was extracted using Mericon Food kit (Quiagen, Hilden Germany) according to the manufacturer's instructions with minor modifications. Each sample (1g) was homogenized with 5 mL of food lysis buffer. An aliquot was incubated at 60 °C for 30 minutes, afterwards it was centrifuged at 13000 x *g* for 3 min. At the supernatant obtained was added at chloroform and centrifugate. After these steps, at the mixture obtained was added the binding buffer, subsequently it was transferred to the column supplied. Before using the elution buffer, various washes and centrifuges were performed to remove residues on the

membrane. The DNAs extract were quantified spectrophotometrically at 260 nm and they were stored at -20 °C.

V. Target gene selection and oligonucleotide primers

The DNA sequence corresponding to the gene encoding kiwellin and thaumatin like protein, allergens of kiwifruit. Specific primers Act02 (F:ACCGGCTGCATTTTTGATGG R:AAAGGCTTGGCATTGGAGGA) and Act05 (F:GTGGAGTGCCTTAGGGTTGG R:CATCACTTACGCCATGGACC) were designed starting from sequences reported in Genbank database submitted to a BLAST on 'Genome Kiwifruits Database' specific for *Actinidia chinensis* (Huang et al., 2013; <http://bioinfo.bti.cornell.edu/cgi-bin/kiwi/home.cgi>), which identifies regions of local similarity among homologous sequences from different species of *Actinidia spp.* and *A. chinensis* var. *chinensis*.

VI. Real-time PCR and HRM analysis

The amplifications by real-time PCR were carried out in 20 µl of total reaction volume containing 2 µl of DNA extract of kiwifruit pulp (*A. chinensis* var. *chinensis* and *A. chinensis* var. *deliciosa*), juice, jam and biscuits prepared with pulp of *A. chinensis* var. *chinensis*. The amplifications by real-time PCR were carried out at laboratory of Plant Physiology of CREA, using a CFX Connect Real-time PCR Detection System (Bio-Rad), in 20 µl of total reaction volume containing 2 µl of DNA extract of kiwifruit pulp (*A. chinensis* var. *chinensis* and *A. chinensis* var. *deliciosa*), juice, jam and biscuits prepared with pulp of *A. chinensis* var. *chinensis*, 10 µl of Precision Melt Supermix™ (Bio-Rad Laboratories, Hercules, CA, USA) and 200 nmol/ml of each primer. For HRM analysis, PCR products were denatured at 95 °C for 5 min and then annealed at 63 °C for 15 sec with a touchdown ranged from 57 °C to 72 °C for 40 cycles. These two steps were followed by melting curve ranging from 65 to 95 °C with temperature increments of 0.1 °C every 10 s. The fluorescence data were acquired by the end of each melting temperature.

2.3 STATISTICAL ANALYSIS

All results are expressed as the mean ± standard deviation. Statistical significance among untreated and treated kiwifruit are analysed by two-way analysis of variance (ANOVA) and Duncan's test at 5 % level is calculated to compare differences between means. Differences at $p < 0.05$ are considered significant and are indicated with different letters.

1720 Correlations among the evaluated parameter are analysed by Pearson's correlations ($p < 0.05$
1721 and < 0.01). A principal component analysis (PCA) is applied to describe the influences of
1722 ozone treatment on different antioxidant enzymes and to identify the components responsible
1723 to the main variations into dataset. All analyses are performed using the SPSS software
1724 package, version 24.0 (SPSS Inc., Chicago, IL, USA).

CHAPTER 3: RESULTS AND DISCUSSION

I YEAR

Kiwifruit were monitored during post-harvest cold storage in the absence (control) or presence of gaseous ozone at the concentration of 150 ppb during the day and 180 ppb at night of continuously at 0 °C on ‘Hayward’ and ‘Soreli’ kiwifruit over a period of 6 weeks; after that the postharvest treatment ended and kiwifruit were stored for 10 days of shelf-life at 18 °C. The untreated samples (control) were stored at the same storage conditions but in air.

During cold storage and shelf-life it is mainly effective in preserving color (ΔE color difference data), flesh firmness (FF) and total soluble solids concentration (SSC). They are important qualitative attributes indicating the eating quality and postharvest storability of kiwifruit. Whereas the microbiological data suggested that low concentration of ozone is not so effective in preventing microbiological decay. Moreover, no meaningful changes in bioactive compounds were observed between treated and untreated kiwifruit at the studied storage conditions.

The change in carbohydrate components, especially the shift from starch to various simple sugars, is one of the major phenomena observed during the ripening of kiwifruit. The change of soluble solids content (SSC) in Hayward during storage is observed in Figure 3.1. Control samples show a slower increase in soluble solids content than treated kiwifruit and the range of the SSC at the end of the test is around 13 °Brix. During the shelf life analysis, the SSC of the control increases up to 16 °Brix at 6 days of shelf life; conversely O₃ keeps the SSC content (13 °Brix) constantly. Thus, O₃ has positive effects on the maintenance of SSC during the shelf life (Figure 3.2). To confirm our data, it was observed in Hayward kiwifruit that both an ozone-enriched cold storage atmosphere (Minas et al., 2012) and shelf life at 20 °C (Minas et al., 2014), generally led to lower soluble solid contents compared with control fruit. The soluble solids content of Soreli shows an average of about 13.4 and 14.1 °Brix (Dicho et al., 2015). Data show an increasing trend of SSC (12 to 14 °Brix), without significant differences between the two treatments (Figure 3.3). During shelf-life the values shifted from about 14 to 16 °Brix without significant differences between the samples (Figure 3.4).

As confirmed by Minas and coworkers (2012), ozone doesn't affect the titratable acidity (TA), showing a similar trend between treated and untreated samples of Hayward (from 2.0 to 1.4 mg citric acid per 100 grams FW) and Soreli (from 1.7 to 1.3 mg citric acid per 100 grams FW).

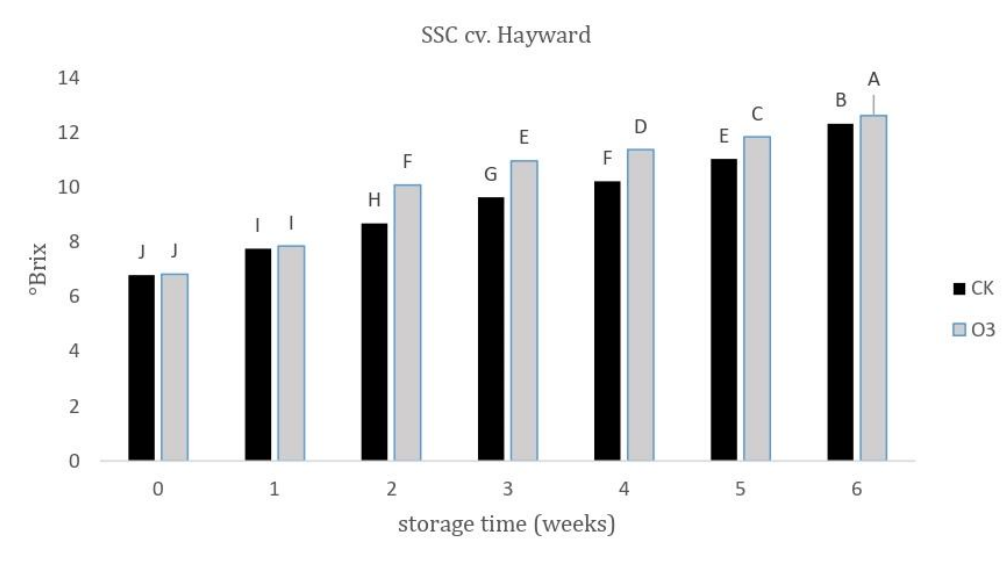


Figure 3.1. SSC of Hayward during cold storage. Means with the same letter are not significantly different ($P = 0.05$, Duncan test).

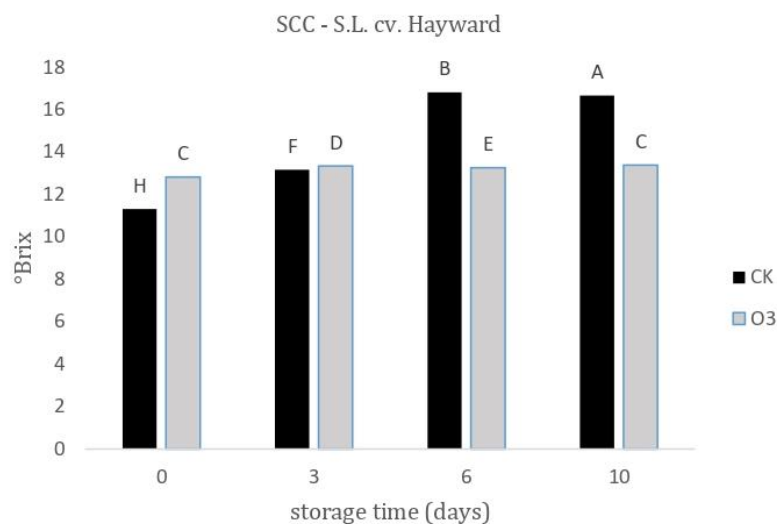


Figure 3.2. SSC of Hayward during shelf-life. Means with the same letter are not significantly different ($P = 0.05$, Duncan test).

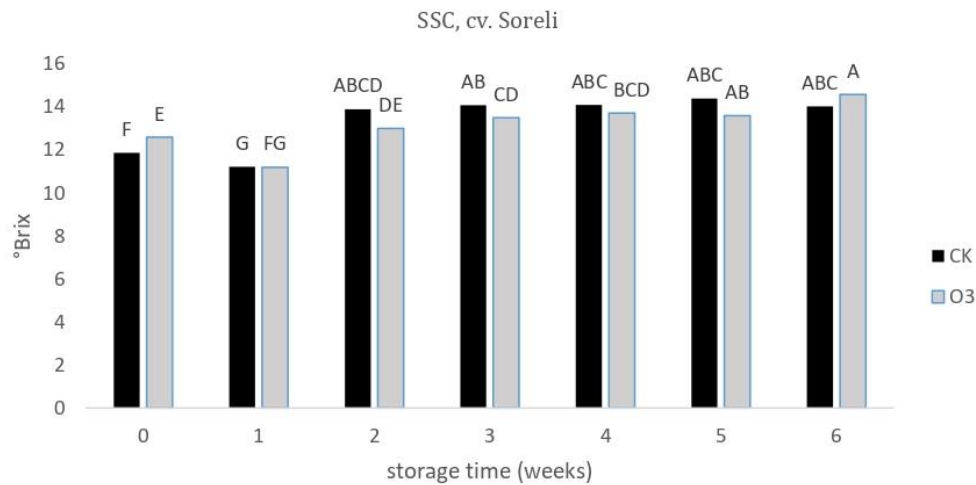


Figure 3.3. SSC of Soreli during cold storage. Means with the same letter are not significantly different ($P = 0.05$, Duncan test).

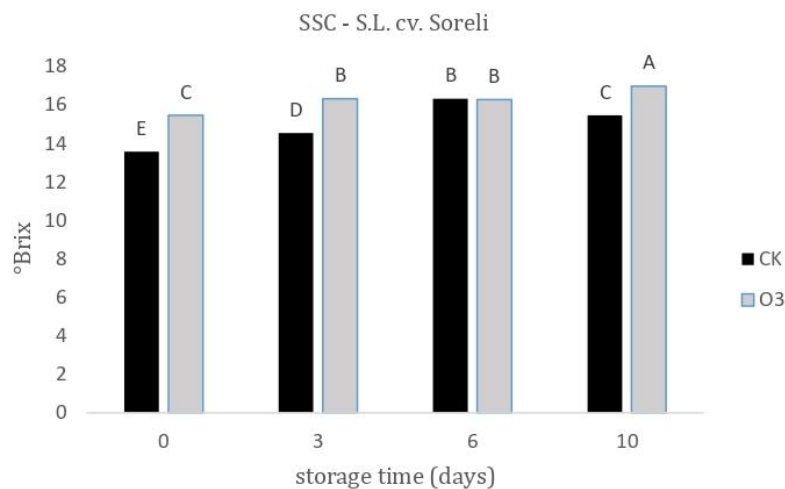


Figure 3.4. SSC of Soreli during shelf-life. Means with the same letter are not significantly different ($P = 0.05$, Duncan test).

The measurement of tissue firmness using Instron shows a similar trend between control and treated samples during storage at 0 °C of the yellow-flesh fruit (Figure 3.5).

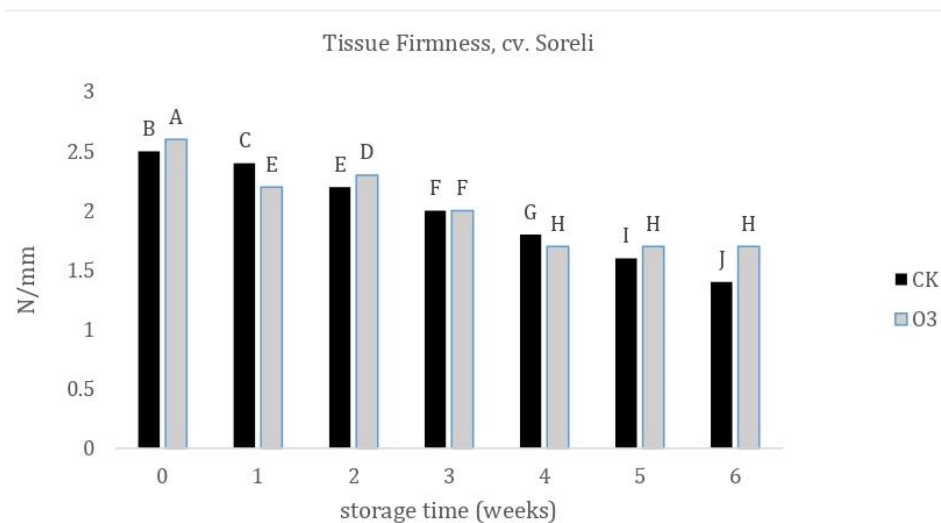
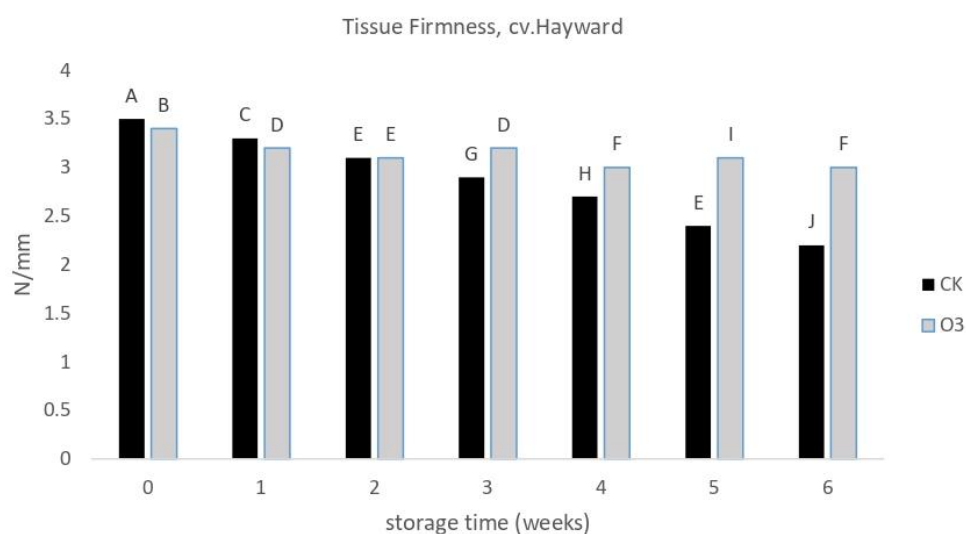


Figure 3.5. Firmness (measured by Instron instrument) of Soreli during cold storage. Means with the same letter are not significantly different ($P = 0.05$, Duncan test).

Studying the storage at 0 °C and during shelf-life of the green-flesh-fruit and the shelf-life only of the Soreli fruit, the values show that O₃ is effective in maintaining the texture compared to untreated kiwifruit (Figures 3.6; 3.7; 3.8). Likewise, Minas and coworkers (2014) observed that ozone treatment (0.3 uL L⁻¹ for 2 and 4 months of cold storage followed by 10 days of shelf life at 20 °C) is efficient in promoting higher firmness retention and, in addition, it blocks ethylene production, delays ripening, and stimulates antioxidant and antiradical activities of kiwifruit.

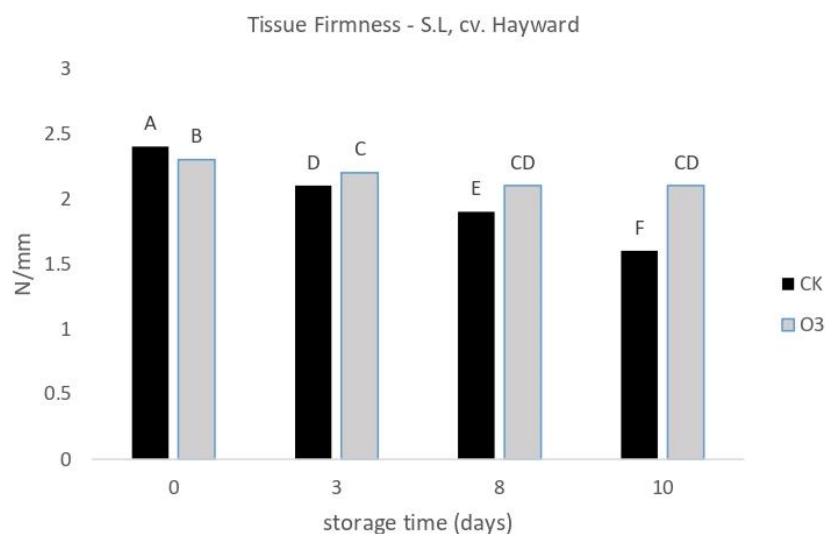
Chroma and Hue data of green- and yellow-flesh kiwifruit do not show significant differences during cold storage and throughout shelf-life (data not shown). The CIELab colour differences (ΔE^*) allow precise measurement and better understanding of the colour development of kiwifruit that occurred during storage. For the Soreli and, to a limited extent, for Hayward at 0 °C and during shelf-life, the ozone sample shows better positive effects on the maintenance of the colour during the storage time compared to untreated samples (Figures 3.9; 3.10; 3.11; 3.12).



1792

1793 Figure 3.6. Firmness (measured by Instron instrument) of Hayward during cold storage.

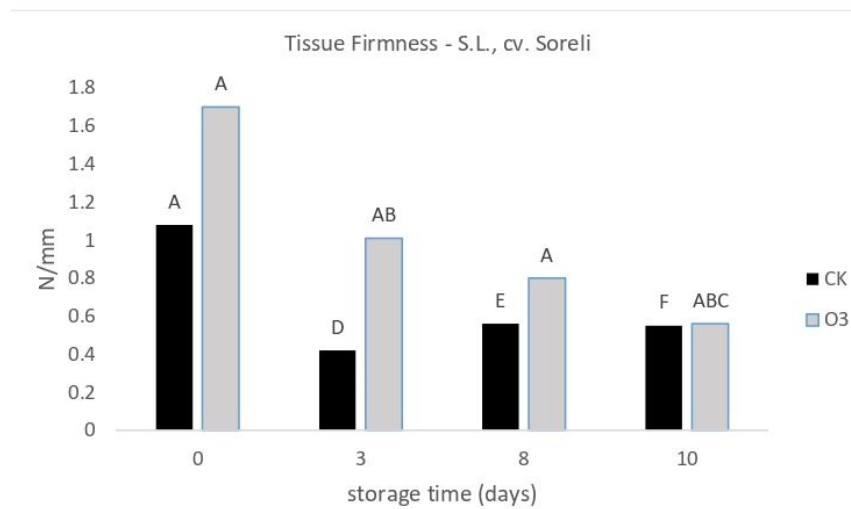
1794 Means with the same letter are not significantly different ($P = 0.05$, Duncan test).



1795

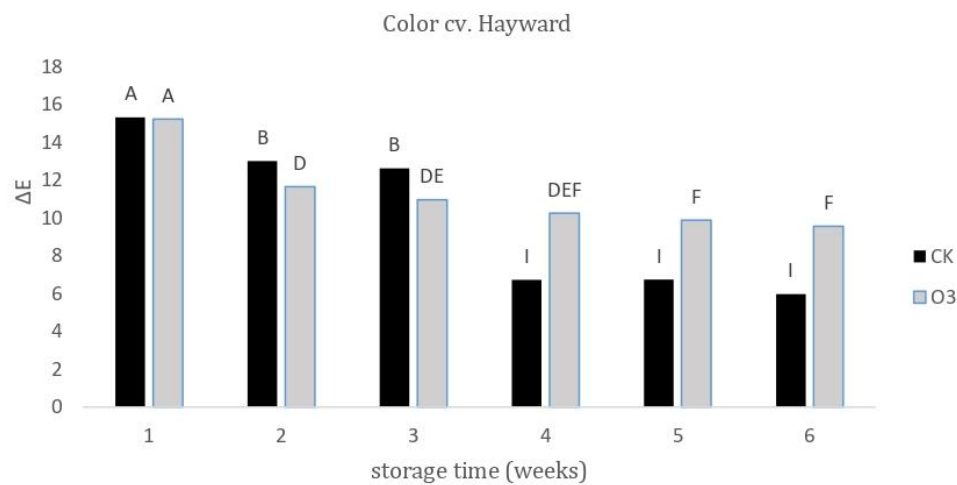
1796 Figure 3.7. Firmness (measured by Instron instrument) of Hayward during shelf-life. Means

1797 with the same letter are not significantly different ($P = 0.05$, Duncan test).



1798

1799 Figure 3.8. Firmness (measured by Instron instrument) of Soreli during shelf-life. Means
 1800 with the same letter are not significantly different ($P = 0.05$, Duncan test).



1801

1802 Figure 3.9. The CIELab color difference (ΔE) trend of Hayward during cold storage. Means
 1803 with the same letter are not significantly different ($P = 0.05$, Duncan test).

1804

1805

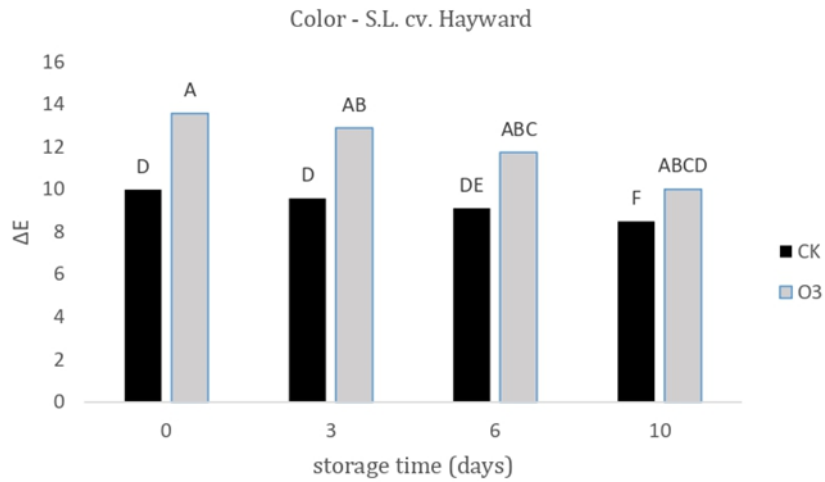


Figure 3.10. The CIELab color difference (ΔE) trend of Hayward during shelf-life. Means with the same letter are not significantly different ($P = 0.05$, Duncan test).

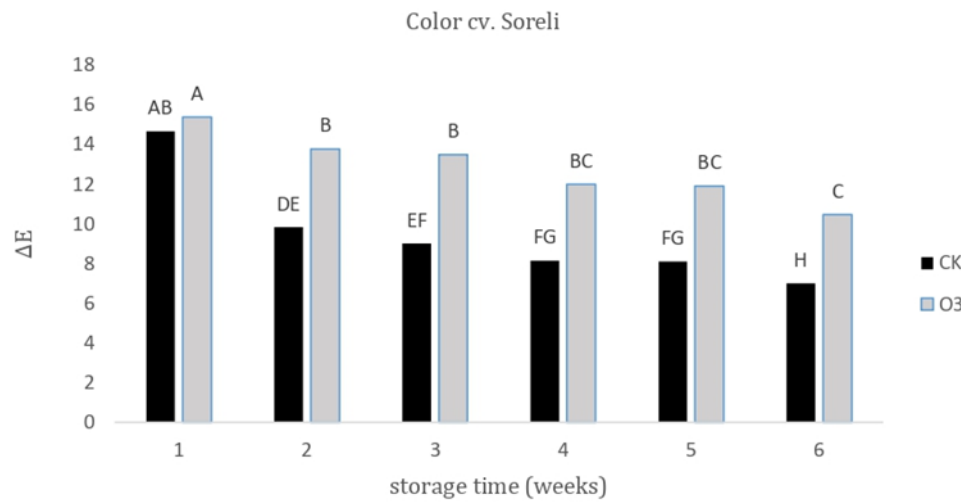


Figure 3.11. The CIELab color difference (ΔE) trend of cv. Soreli during cold storage. Means with the same letter are not significantly different ($P = 0.05$, Duncan test).

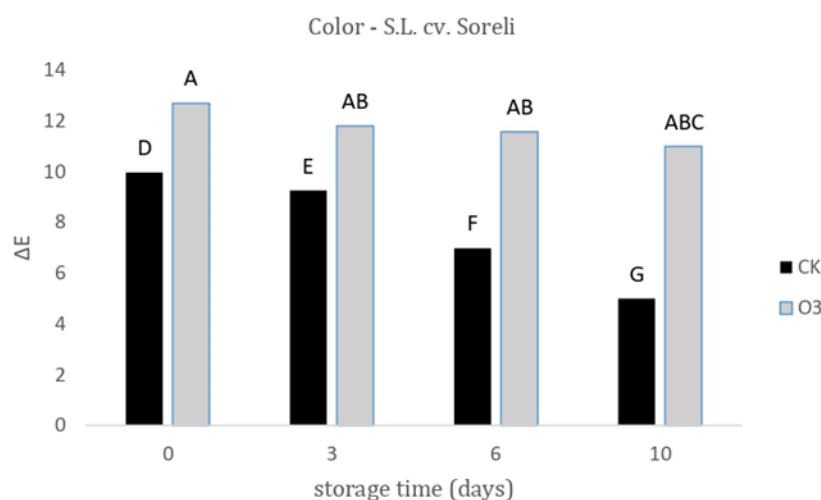


Figure 3.12. The CIELab color difference (ΔE) trend of cv. Soreli during shelf-life. Means with the same letter are not significantly different ($P = 0.05$, Duncan test).

Data evidence that ozone treatment is not effective in controlling total bacterial count, in Hayward and Soreli kiwifruit (Table 3.1). Discrepancies on the positive or negative sanitizing effects of ozone in various commodities can be found in literature (Pérez et al.,1999).

Table 3.1. Total microbial count (CFU/cm²) of Hayward and Soreli during cold storage and shelf-life.

Theses/ Storage (days)	Total microbial count			
	0 d	10 d	0 d S.L.	10 d S.L.
CK “Hayward”	6.82E+04	4.56E+04	3.39E+04	4.24E+04
O ₃ “Hayward”	2.13E+04	1.43E+04	3.39E+04	7.34E+04
CK “Soreli”	7.04E+03	7.80E+03	2.70E+04	4.47E+03
O ₃ “Soreli”	8.38E+03	1.60E+04	2.70E+04	6.19E+03

Analysing the content of bioactive compounds such as chlorophyll a, b, β -carotene and vitamin C, the data evidence that ozone does not negatively influence the content of bioactive compounds in green and yellow-flesh fruit; indeed the table 3.2 does not show statistical differences between treated and untreated samples.

Table 3.2. Comparison of bioactive content of the four thesis of Hayward and Soreli kiwifruit at the beginning and at the end of storage at 0 °C and in shelf-life conditions (18 °C for 10 days); it is expressed by mg/100g FW.

Theses/Bioactives	Chlorophyll A		Chlorophyll B		B-carotene		Ascorbic acid	
COLD STORAGE	mg/100g FW		mg/100g FW		mg/100g FW		mg/100g FW	
<u>Storage (days)</u>	0 d	42 d	0 d	4d	0 d	42 d	0 d	42 d
CK Hayward	0.09	0.13	0.16	0.19	0.04	0.01	70.3	43.2
O3 Hayward	0.12	0.12	0.16	0.19	0.04	0.0015	68.3	45.7
CK Soreli	0.05	0.04	0.14	0.09	0.04	0.009	35.8	19.9
O3 Soreli	0.05	0.04	0.14	0.10	0.04	0.03	35.0	20.5
SHELF-LIFE								
<u>Storage (days)</u>	0 d	10 d	0 d	10 d	0 d	10 d	0 d	10 d
CK Hayward	0.15	0.13	0.17	0.17	0.011	0.037	42.6	31.9
O3 Hayward	0.15	0.09	0.17	0.11	0.0015	0.009	43.9	32.9
CK Soreli	0.055	0.057	0.15	0.09	0.042	0.017	19.3	10.5
O3 Soreli	0.055	0.023	0.15	0.28	0.0015	0.16	19.2	10.22

Regarding the main bioactive compounds of kiwifruit, ascorbic acid is an important nutritional component. Ascorbic acid content was reported to be in the range 30-100 mg ascorbic acid per 100 g FW (Matsumoto et al., 1983). It is dependent on the season, growing conditions and the degree of ripening when the kiwifruit were harvested.

Our data showed that these values in yellow-flesh kiwifruit at the beginning of the cold storage were lower than those in green fruits (35.4 versus 69 mg ascorbic acid per 100 grams FW). For the two cv, data of storage time at 0 °C and during shelf-life, indicate that the content of vitamin C is slightly higher in ozone-treated samples than in the untreated. Due to the high oxidative capacity of ozone and its ability to generate toxic molecular species, it acts as a potent phytotoxic agent that elicits plant defense reactions. In this sense, ozone and ozone-derived oxyradicals may be scavenged by low molecular weight antioxidants of the plant cell such as ascorbic acid or polyamines (Pérez et al.,1999). The changes in ascorbic acid content in ozone-treated fruit could be the result of an antioxidative system that promotes the biosynthesis of vitamin C from carbohydrate reserves of the fruit (Pérez et al.,1999).

The evidence from the first trial suggest that ozone, as post-harvest treatment, is a suitable choice for the preservation of ‘Hayward’ and ‘Soreli’ kiwifruit. Ozone is not so effective in preventing microbiological growth.

In Hayward kiwifruit, O₃ has positive effect on the maintenance of SSC during shelf-life for

10 days at 18 °C. In Soreli an increase of SSC is highlighted without significant differences. Ozone treatment maintains higher tissue firmness in Hayward fruit and it does not influence negatively in Soreli. Ozone is effective in preserving colour (ΔE^*) during cold storage of green and gold kiwifruit. No significant changes in titratable acidity and bioactive compounds in ozonated samples were observed.

The data got from the current work were published: Goffi V., Modesti M., Forniti R. and Botondi R. (2018). Quality of green (*Actinidia chinensis* var. *deliciosa* 'Hayward') and yellow (*A. chinensis* var. *chinensis* 'Soreli') kiwifruit during cold storage at 0 °C in normal atmosphere and with gaseous ozone. Acta Hortic. 1218, 473-480. <https://doi.org/10.17660/ActaHortic.2018.1218.65>

II YEAR

During the second year a continuous gaseous ozone flow (nominal production capacity of instrument: 32 g ozone h⁻¹) at the concentration of 300 ppb was applied during storage at 4 °C on 'Hayward' and 'Soreli' kiwifruit over a period of 60 days, followed of 8 days of shelf-life at 18 °C. The untreated samples were stored at the same storage conditions but in air.

The findings from the trial put on evidence a well-known phenomenon: ozone is a strong oxidizing agent, so, it induces oxidative stress. Some physiological responses have been observed: synthesis of secondary metabolites (Figure 3.13).

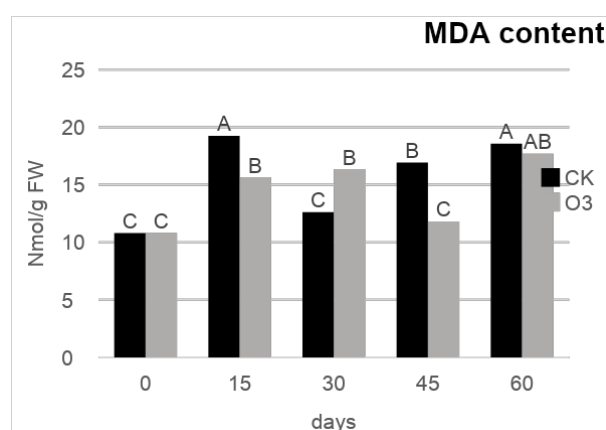


Figure 3.13. Malondialdehyde content (MDA) of Soreli untreated (CK) and treated (O₃) kiwifruit during cold storage (60 days). Means with the same letter are not significantly different (P = 0.05, Duncan test).

A-SORELI

The radical scavenging activity (DPPH) of treated Soreli does not drop during the storage, differently from control samples (Figure 3.14.).

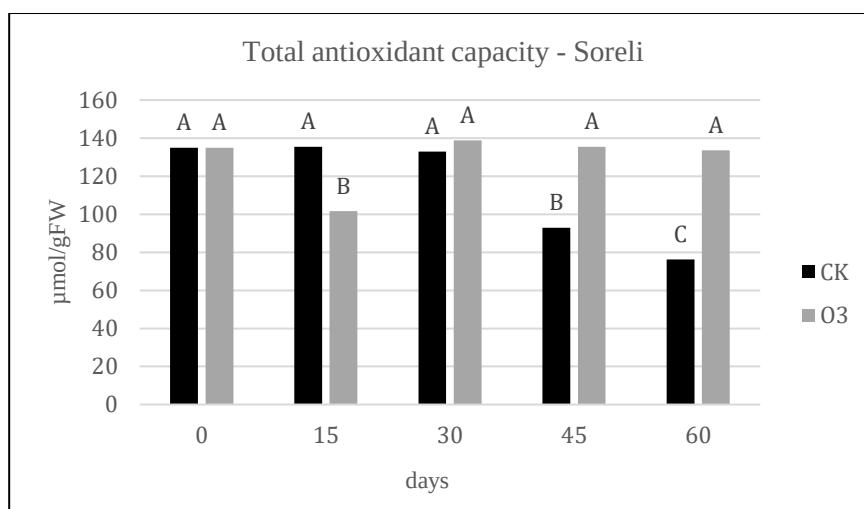


Figure 3.14. Radical scavenging activity (DPPH) of Soreli untreated (CK) and treated (O₃) kiwifruit during cold storage (60 days). Means with the same letter are not significantly different (P = 0.05, Duncan test).

Firmness values drop significantly after 30 days for both untreated and treated samples (Figure 3.15). Whereas the color in ozone treated samples dropped compared to control after 30 days of storage (Figure 3.16).

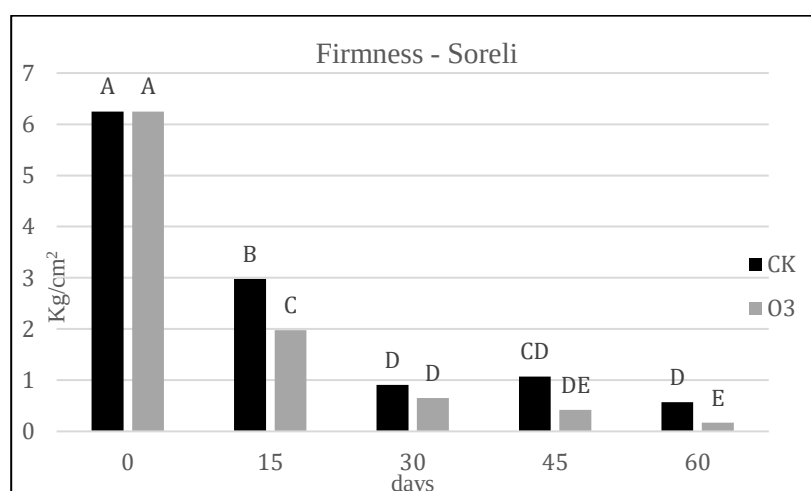


Figure 3.15. Firmness (measured by penetrometer) of Soreli untreated (CK) and treated (O₃) kiwifruit during cold storage (60 days). Means with the same letter are not significantly different (P = 0.05, Duncan test).

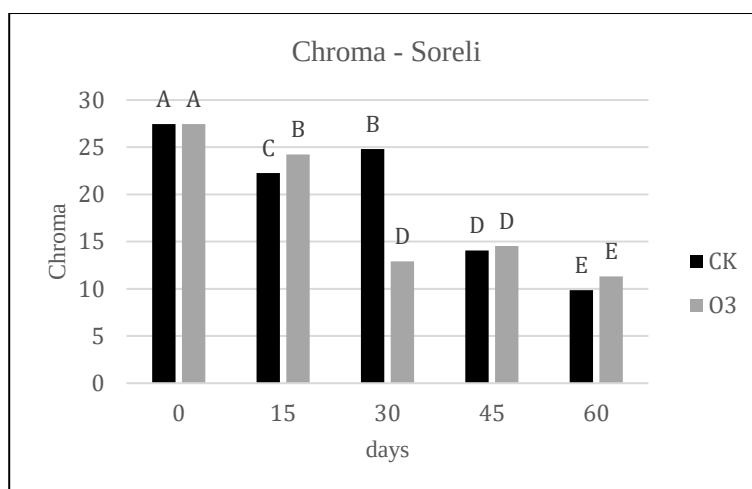


Figure 3.16. Chroma of Soreli untreated (CK) and treated (O₃) kiwifruit during cold storage (60 days). Means with the same letter are not significantly different (P = 0.05, Duncan test).

Two-dimensional principal component analysis plot of qualitative and nutraceutical traits in Soreli kiwifruit (Figure 3.17) explains 42.9% of data variability. The first principal component (PC1) explains storage trends. At 1 month of storage (T2, 30 days) physiological change occurs into untreated samples compared to treated ones during cold storage. The second principal component (PC2) underlines the differences between treated and untreated samples during shelf-life. Treated kiwifruit show different trend compared to the control (positively correlated to PC2). Flesh firmness (FF), lightness (L*), colour difference (DE), total microbial count (MG) and carotenoids (CAR) are positively correlated to PC1 and negatively to PC2; whereas acidity (TA), chlorophylls A and B (Chl a; Chl b) and ascorbic acid (AA) are positively correlated to PC2.

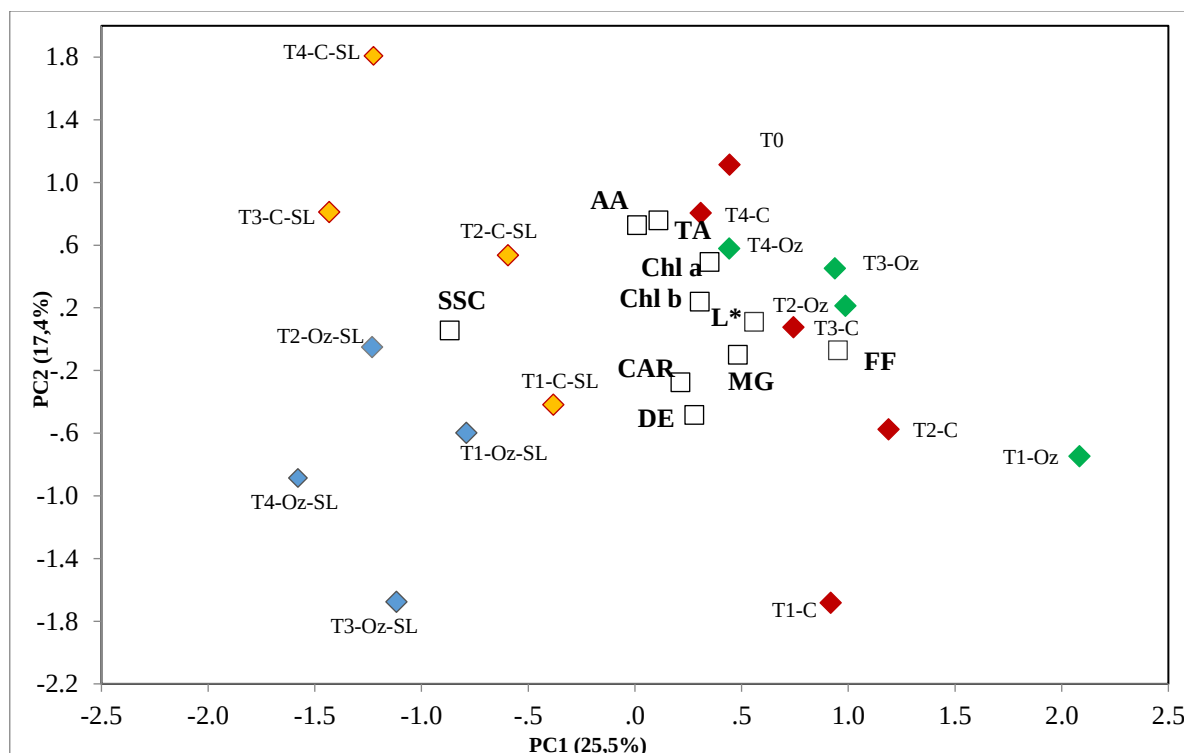


Figure 3.17. Two dimensional principal component analysis plot of qualitative and nutraceutical traits in 'Soreli' kiwifruit, at harvest (T0), after 15 days (control fruit C-T1, ozone-treated fruit OZ-T1), after 30 days (control fruit C-T2, ozone-treated fruit OZ-T2), after 45 days (control fruit C-T3, ozone-treated fruit OZ-T3) and after 60 days (control fruit C-T4, ozone-treated fruit OZ-T4) of storage at 4 °C; and during shelf-life conditions, after 2 days (control fruit T1-C-SL and ozone-treated fruit T1-Oz-SL), after 4 days (control fruit T2-C-SL and ozone-treated fruit T2-Oz-SL), after 6 days (control fruit T3-C-SL and ozone-treated fruit T3-Oz-SL) and after 8 days (control fruit T4-C-SL and ozone-treated fruit T4-Oz-SL).

CAR: total carotenoids; AA: ascorbic acid; TA: titratable acidity; Chl a: chlorophyll a; Chl b: chlorophyll b; MG: microbial growth; TA: titratable acidity, L*: lightness, FF: flesh firmness, ΔE : difference color. The different colour of the symbols represents the different theses: red is the control (untreated) stored at 4 °C, green represents the treated samples stored at 4 °C, blue-light symbolizes the treated samples stored at shelf-life conditions and yellow represents the control stored at shelf-life conditions.

B- HAYWARD

Ozone negatively affects Hayward kiwifruit during storage. MDA content is higher in treated samples than untreated already after 15 days of storage (Figure 3.18). The Radical scavenging activity (Figure 3.19) is not significantly different along the storage time and between

treatments. The firmness drops during cold storage without showing significant differences between untreated and treated kiwifruit (Figure 3.20); while the colour is more affected by ozone treatment during cold storage (Figure 3.21).

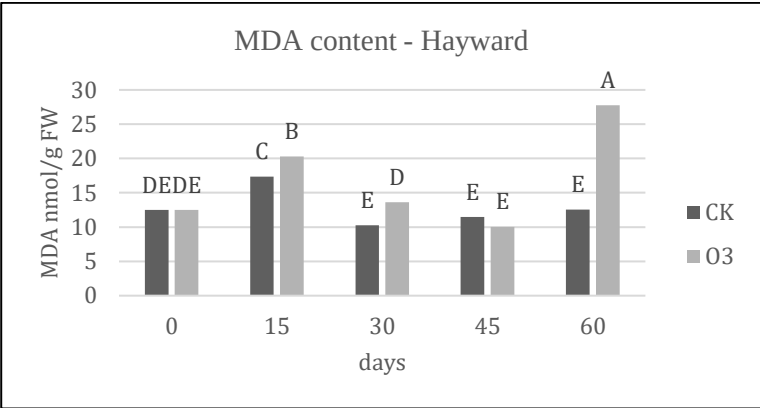


Figure 3.18. Malondialdehyde content (MDA) of Hayward untreated (CK) and treated (O₃) kiwifruit during cold storage (60 days). Means with the same letter are not significantly different (P = 0.05, Duncan test).

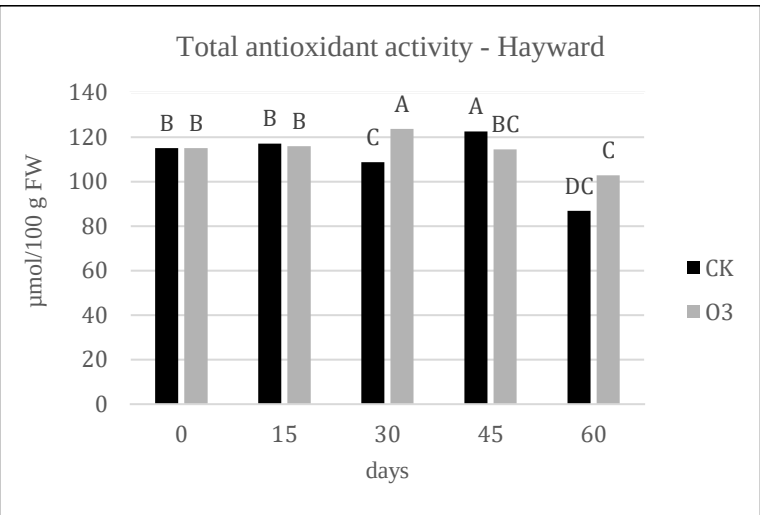


Figure 3.19. Radical scavenging activity (DPPH) of Hayward untreated (CK) and treated (O₃) kiwifruit during cold storage (60 days). Means with the same letter are not significantly different (P = 0.05, Duncan test).

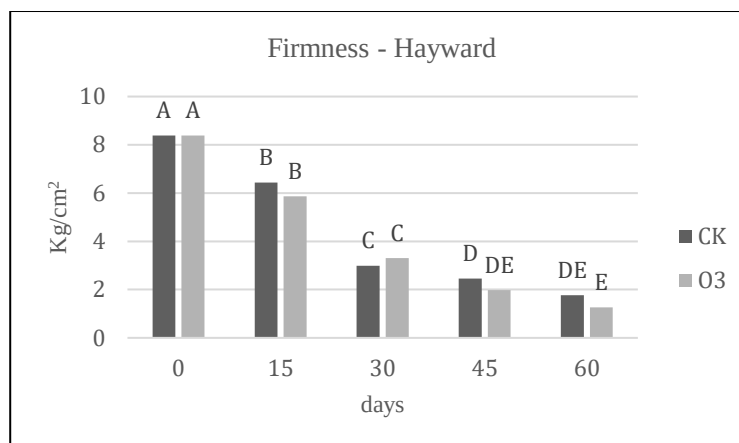


Figure 3.20. Firmness (measured by penetrometer) of Hayward untreated (CK) and treated (O₃) kiwifruit during cold storage (60 days). Means with the same letter are not significantly different (P = 0.05, Duncan test).

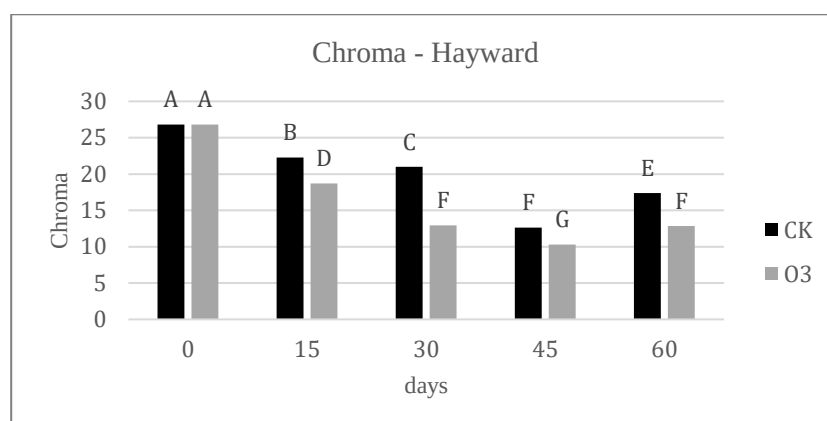


Figure 3.21. Chroma of Hayward untreated (CK) and treated (O₃) kiwifruit during cold storage (60 days). Means with the same letter are not significantly different (P = 0.05, Duncan test).

Two-dimensional principal component analysis plot of qualitative and nutraceutical traits in Hayward kiwifruit (Figure 3.22) explains 43.6 % of the total variability of data. PCA scores show that samples are subjected to physical-chemical changes during cold storage. Firmness (FF), lightness (L*), carotenoids (CAR), chlorophylls B (Chl b) are highly positively correlated with the first principal component (PC1), whereas SSC, colour difference (DE), ascorbic acid (AA), chlorophylls A (Chl a) are negatively correlated with PC1. The second axis is driven only by ascorbic acid and total microbial count (MG). Along the second principal component (PC2) shelf-life is well spatially separated from cold storage, but more similar between the treated and untreated samples during shelf-life. Regarding to cold storage, lightness, firmness and carotenoids show a change after 30 days (T2) for untreated and treated samples.

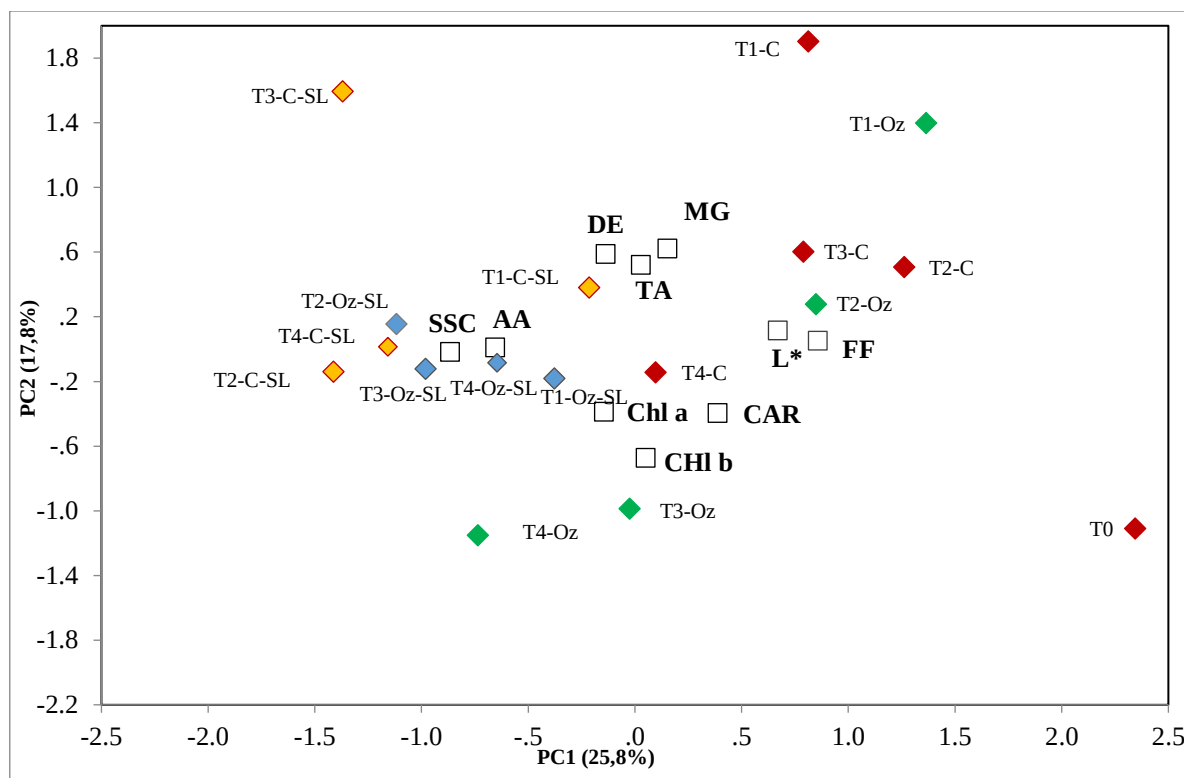


Figure 3.22. Two dimensional principal component analysis plot of qualitative and nutraceutical traits in Hayward kiwifruit, at harvest (T0), after 15 days (control fruit C-T1, ozone-treated fruits OZ-T1), after 30 days (control fruit C-T2, ozone-treated fruits OZ-T2), after 45 days (control fruit C-T3, ozone-treated fruits OZ-T3) and after 60 days (control fruit C-T4, ozone-treated fruits OZ-T4) of storage at 4 °C; and during shelf-life conditions, after 2 days (control fruits T1-C-SL and ozone-treated fruits T1-Oz-SL), after 4 days (control fruits T2-C-SL and ozone-treated fruits T2-Oz-SL), after 6 days (control fruits T3-C-SL and ozone-treated fruits T3-Oz-SL) and after 8 days (control fruits T4-C-SL and ozone-treated fruits T4-Oz-SL).

CAR: total carotenoids; AA: ascorbic acid; TA: titratable acidity; Chl a: chlorophyll a; Chl b: chlorophyll b; MG: microbial growth; TA: titratable acidity, L*: lightness, FF: flesh firmness, ΔE : difference color. The different colour of the symbols represents the different theses: red is the control (untreated) stored at 4 °C, green represents the treated samples stored at 4 °C, blue-light symbolizes the treated samples stored at shelf-life conditions and yellow represents the control stored at shelf-life conditions.

In conclusion, as ozone can accelerate or inhibit the senescence and elicit the antioxidant activity, it is relevant, into commercial handling of kiwifruit, a more precise parameters management and a better understanding of cultivar-specific responses of fruit to specific

conditions, in order to be better tailored to match the fruit's capacity to respond to, or withstand, specific temperatures and ozone concentration.

III YEAR

3A trial

The aim of this work was to describe the yellow-flesh kiwifruit 'Soreli' response to storage temperatures (2 °C and 4 °C) and gaseous ozone postharvest treatment in order to find the most suitable and specific management of cold postharvest treatment to prolong the quality of this cultivar.

Getting into the details the effect of the interaction of the temperatures and gaseous ozone postharvest on physico-chemical and microbiological parameters of Soreli showed that the physico-chemical parameters are important predictive indicators that reflect the kiwifruit quality during storage. In this study, the analyzed physico-chemical parameters were affected by the temperature, by the ozone treatment and by their interactions in some case. The firmness decreased during the storage time (days) with no significant differences ($p < 0.05$) among ozone-treated kiwifruit samples compared to control ones for the two tested temperatures of storage (Tables 3.3 and 3.4). The flesh softening is associated with fruit ripening, fruit injury and is mainly caused by pectin degradation (Meena et al., 2018). Firmness is a criterion for establishing the end of the storage at the optimum eating window. Some reports suggested that the firmness values should be between $0.5-1.5 \text{ kg} \times (0.5 \text{ cm}^2)^{-1}$ for good eating quality (Barboni et al., 2010).

SSC, an indicator of fruit ripening and intrinsic quality, increased during the days of storage due to the conversion from starch to soluble sugars (Atkinson et al., 2011). The ozone induced the ripening process, and, it was evident at the end of the storage with higher SSC values of 15 ± 0.64 and 13.82 ± 0.45 for ozone-treated fruits at 4 °C and 2 °C, respectively (Tables 3.3 and 3.4). The SSC at 4 °C at T3 (45 d) and T4 (60 d) showed higher values compared to 2 °C (Table 3.4). Two-way ANOVA revealed that there were significant effects of postharvest treatment and storage time for SSC as shown in the p-value ($p < 0.001$) (Table 3.5). Barboni and coworkers (2010) exhibited that the ozone treatment had a stronger influence on reducing sugar than cold storage in 'Hayward'.

Table 3.3. Physico-chemical parameters (firmness (N mm⁻¹), SSC (° Brix) and TA: titratable acidity (mg citric acid g⁻¹ fresh weight) and total microbial growth (MG-CFU/mL) in control (CTRL) and ozone-treated fruits (Ozone) of ‘Soreli’, at harvest (T0), after 15 days (T1), after 30 days (T2), after 45 days (T3) and after 60 days (T4) of cold storage at 2 °C. Three technical replicates were realized for each biological replicate. Means followed by same letters do not significantly differ in CTRL and Ozone (P=0.05, Duncan test) for the same parameters at the same storage time. Means within each row with the same lowercase letter are not significantly different (P > 0.05) from each other for the specific treatment.

Parameters	Treatments	T0	T1	T2	T3	T4
Firmness	CTRL	12.71 ± 2.00 (d)	7.19 ± 2.19 (c)	4.63 ± 1.68 (b)	2.70 ± 0.69 (a)	2.60 ± 0.43 (a)
	Ozone	12.71 ± 2.00 (d)	6.77 ± 1.89 (c)	3.19 ± 0.78 (a)	2.77 ± 0.73 (a)	2.12 ± 0.46 (a)
SSC	CTRL	8.74 ± 0.41 (a)	10.82 ± 0.55 (b)	14.25 ± 0.52 (g)	12.82 ± 0.57 (cd)	13.64 ± 0.42 (ef)
	Ozone	8.74 ± 0.41 (a)	12.63 ± 0.56 (c)	13.53 ± 0.4 (ef)	13.22 ± 0.48 (de)	13.82 ± 0.45 (fg)
TA	CTRL	1.35 ± 0.06 (cd)	1.36 ± 0.11 (d)	1.2 ± 0.04 (ab)	1.21 ± 0.16 (abc)	1.14 ± 0.005 (a)
	Ozone	1.35 ± 0.06 (cd)	1.20 ± 0.08 (ab)	1.33 ± 0.06 (bcd)	1.33 ± 0.05 (bcd)	1.28 ± 0.05 (abcd)
MG	CTRL	7320 ± 71.55 (h)	6720 ± 40.0 (g)	4453.3 ± 23.1 (e)	5733.33 ± 305.5(f)	5666.7 ± 152.7 (f)
	Ozone	7320 ± 71.55 (h)	1306 ± 54.0 (b)	1040 ± 40.0 (a)	2946.6 ± 151.4(d)	2683.3 ± 160.7 (c)

Table 3.4. Physico-chemical parameters (firmness (N mm⁻¹), SSC (°Brix) and TA: titratable acidity (mg citric acid g⁻¹ fresh weight) and total microbial growth (MG-CFU mL⁻¹) in control (CTRL) and ozone-treated fruits (Ozone) of Soreli, at harvest (T0), after 15 days (T1), 30 days (T2), 45 days (T3) and 60 days (T4) of cold storage at 4 °C. Three technical replicates were realized for each biological replicate. Means followed by same letter do not significantly differ (P=.05, Duncan test) for the same parameters at the same storage time. Means within each row with the same lowercase letters are not significantly different (P > 0.05) from each other for the specific treatment.

Parameters	Treatments	T0	T1	T2	T3	T4
Firmness	CTRL	12.71 ± 2.00 (c)	7.1 ± 1.11 (b)	3.84 ± 0.15 (a)	3.15 ± 0.29 (a)	2.67 ± 0.29 (a)
	Ozone	12.71 ± 2.00 (c)	6.38 ± 1.19 (b)	3.5 ± 0.27 (a)	3.10 ± 0.19 (a)	2.96 ± 0.28 (a)
SSC	CTRL	8.73 ± 0.41 (a)	12.66 ± 0.73 (b)	14.3 ± 0.63 (d)	13.81 ± 0.58 (cd)	13.5 ± 1.06 (c)
	Ozone	8.73 ± 0.41 (a)	12.83 ± 0.76 (b)	13.74 ± 0.68 (cd)	14.4 ± 0.58 (d)	15 ± 0.64 (e)
TA	CTRL	1.35 ± 0.065 (a)	1.30 ± 0.062 (a)	1.26 ± 0.009 (a)	1.27 ± 0.10 (a)	1.27 ± 0.009 (a)
	Ozone	1.35 ± 0.065 (a)	1.28 ± 0.047 (a)	1.30 ± 0.066 (a)	1.29 ± 0.09 (a)	1.32 ± 0.031 (a)
MG	CTRL	7320 ± 71.55 (g)	6983.3 ± 125.83(f)	4533.3 ± 251.6 (d)	5983.3 ± 161 (e)	5766.7 ± 152.7 (e)
	Ozone	7320 ± 71.55 (g)	1983.3 ± 160.7 (b)	1700 ± 200.0 (a)	1970 ± 125.3 (b)	2433.3 ± 76.3 (c)

2054

Table 3.5. The p-values obtained in the two-way ANOVA for each postharvest treatment (T-control and ozone) during cold storage (D-days) and their interaction (PT x D) in 'Soreli' at 2 °C (T2) and 4 °C (T4). (MG: total microbial growth; POL: polyphenol content; FLAV: flavonoid content; AA: acid ascorbic content; CAR: carotenoid content and DPPH: radical scavenging activity).

Parameters	T2			T4		
	P-value			P-value		
	Postharvest treatment	Days	PT×G	Postharvest treatment	Days	PT×G
Firmness	0.095	<0.001	0.402	0.034	<0.001	0.788
SSC	0.001	<0.001	<0.001	0.003	<0.001	0.001
TA	0.151	0.095	0.024	0.446	0.284	0.867
L*	0.673	<0.001	0.412	<0.001	<0.001	<0.001
ΔE	0.92	<0.001	0.106	<0.001	<0.001	<0.001
Chroma	0.013	<0.001	0.012	<0.001	<0.001	<0.001
Hue Angle	0.864	<0.001	0.998	0.519	<0.001	0.227
MG	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
AA	0.411	<0.001	0.037	0.957	<0.001	0.49
CAR	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
POL	0.661	<0.001	0.734	0.003	<0.001	0.105
FLAV	0.001	<0.001	<0.001	0.016	<0.001	0.055
DPPH	<0.001	<0.001	<0.001	0.177	<0.001	0.134

2060

2061 Titratable acidity (TA), the content of organic acids in fruits, did not exhibit significant
2062 changes both during cold storage (D) and between the treatments (T) at 2 °C and 4 °C. So TA,
2063 the index that influences the flavor of fruit (Ainalidou et al., 2015), in average range of 1.40-
2064 1.10 mg citric acid g⁻¹ FW, was not affected by the storage conditions; Barboni and coworkers
2065 (2010) found no significant effects induced by storage method.

2066
2067 Several studies demonstrated that suitable refrigeration temperatures combined to ozone-
2068 postharvest treatment maintained the qualitative features of blueberries, peaches, table grapes
2069 and citrus fruits during storage (Palou et al., 2001; Palou et al., 2002; Concha-Meye et al.,
2070 2015). In addition, our results are not in agreement with the study of Minas and coworkers
2071 (Minas et al., 2012), which proposed an increase in the ethylene synthesis and a decrease in
2072 ripening process due to ozone postharvest treatment in green kiwifruit by proteomic approach.

2073
2074 To measure microbiological quality index, the microbial growth (MG) was observed. The
2075 tables 3.3 and 3.4 suggested that MG exhibited significant differences for post-harvest
2076 treatment (PT), and time of storage (D) and their interaction PT x D ($p < 0.001$). After the
2077 harvest time (T₀), the MG delayed under gaseous ozone storage compared to control fruit.
2078 The lowest storage temperature (2 °C) was more effective to preserve the microbial decay
2079 compared to 4 °C.

2080 Ozone is applied as postharvest treatment to control the pathogens growth on fruits and
2081 vegetables for its antimicrobial properties (Tzortzakis et al., 2017). Gaseous ozone exposure
2082 (0.3 µL L⁻¹) delayed the development of stem-end rot disease, caused by *Botrytis cinerea*, on
2083 ‘Hayward’ kiwifruit during cold storage (0 °C, RH 95 %) for 4 months (Minas et al., 2010).

2084
2085 The study of the effect of the interaction of the temperatures and gaseous ozone treatment on
2086 qualitative parameters of ‘Soreli’ emerged the following considerations: kiwifruit is well
2087 known as a rich basket of bioactive compounds with health benefits (Richardson et al., 2018).
2088 Yellow-flesh kiwifruit such as ‘Huayou’, ‘Jintao’ and ‘Zesy002’ had a high AA content
2089 (159.08, 148.82 and 161.3 mg 100g⁻¹ FW respectively) compared with the globally cultivated
2090 green-flesh ‘Hayward’ (85 mg 100g⁻¹ FW) (Ma et al., 2017). In our study, the AA content
2091 decreased during the storage with no significant differences between the postharvest
2092 treatments in both temperatures (2 °C and 4 °C), differently of the storage time (Tables 3.6
2093 and 3.7).

Table 3.6. Qualitative parameters (ascorbic acid (AA- mg 100 g⁻¹ fresh weight), carotenoids content (CAR-µg 100 g⁻¹ fresh weight), polyphenol content (POL-mg GAE 100 g⁻¹ fresh weight); flavonoid content (FLAV - mg CE 100 g⁻¹ fresh weight) and total antioxidant activity (as expressed by DPPH in µg TE 100 g⁻¹ fresh weight)) in control (CTRL) and ozone-treated fruit (Ozone) of ‘Soreli’, at harvest (T0), after 15 days (T1), 30 days (T2), 45 days (T3) and 60 days (T4) of cold storage at 2 °C. Three technical replicates were realized for each biological replicate. Means with the same letter are not significantly different (P = 0.05, Duncan test) for the same parameters at the same storage time. Means within each row with the same lowercase letters are not significantly different (P > 0.05) from each other for the specific treatment.

Parameters	Treatments	T ₀	T ₁	T ₂	T ₃	T ₄
AA	CTRL	146.685 ± 16.65 (b)	132.80 ± 4.54 (b)	113.47 ± 1.61 (a)	107.67 ± 5.61 (a)	103.36 ± 2.96 (a)
	Ozone	146.685 ± 16.65 (b)	108.34 ± 0.6 (a)	112.74 ± 3.22 (a)	109.85 ± 0.70 (a)	112.81 ± 3.57 (a)
CAR	CTRL	95.67 ± 0.11 (e)	69.0 ± 3.2 (b)	77.1 ± 1.9 (c)	63.4 ± 4.7 (a)	69.7 ± 0.3 (b)
	Ozone	95.67 ± 0.11 (e)	83.7 ± 0.53 (d)	74.96 ± 2.61 (c)	95.21 ± 0.8 (e)	61.3 ± 0.43 (a)
POL	CTRL	140.9 ± 15.6 (b)	77.9 ± 6.02 (a)	76.4 ± 5.03 (a)	83.47 ± 1.8 (a)	80.41 ± 0.73 (a)
	Ozone	140.9 ± 15.6 (b)	74.22 ± 0.77(a)	80.23 ± 1.94 (a)	81.7 ± 0.31 (a)	88.71 ± 0.97 (a)
FLAV	CTRL	4.02 ± 0.03 (e)	3.28 ± 0.3 (ab)	4.26 ± 0.06 (f)	3.12 ± 0.04 (a)	3.44 ± 0.12 (bcd)
	Ozone	4.02 ± 0.03 (e)	3.66 ± 0.14 (d)	3.56 ± 0.14 (cd)	4.55 ± 0.17 (g)	3.35 ± 0.17 (bc)
DPPH	CTRL	6.55 ± 0.08 (c)	6.55 ± 0.001 (c)	6.03 ± 0.05 (a)	6.23 ± 0.004 (b)	6.73 ± 0.26 (d)
	Ozone	6.55 ± 0.08 (c)	6.05 ± 0.07 (a)	6.55 ± 0.05 (c)	7.07 ± 0.05 (e)	7.03 ± 0.01 (e)

Table 3.7. Qualitative parameters (ascorbic acid (AA. Mg 100 g⁻¹ fresh weight), carotenoids content (CAR-µg 100 g⁻¹ fresh weight), polyphenol content (POL-mg GAE 100 g⁻¹ fresh weight); flavonoid content (FLAV - mg CE 100 g⁻¹ fresh weight) and total antioxidant activity (as expressed by DPPH in µg TE 100 g⁻¹ fresh weight)) in control (CTRL) and ozone-treated fruits (Ozone) of ‘Soreli’, at harvest (T0), after 15 days (T1), 30 days (T2), 45 days (T3) and 60 days (T4) of cold storage at 4 °C. Three technical replicates were realized for each biological replicate. Means with the same letter are not significantly different (P = 0.05, Duncan test) for the same parameters at the same storage time. Means within each row with the same lowercase letters are not significantly different (P > 0.05) from each other for the specific treatment.

Parameters	Treatments	T0	T1	T2	T3	T4
AA	CTRL	146.68 ± 16.65 (c)	138.61 ± 9.8 (bc)	50.73 ± 0.10 (a)	59.12 ± 7.08 (a)	57.47 ± 1.1 (a)
	Ozone	146.68 ± 16.65 (c)	122.71 ± 26.02 (b)	62.43 ± 6.56 (a)	59.93 ± 9.74 (a)	59.58 ± 3.38 (a)
CAR	CTRL	95.67 ± 0.11 (c)	70.32 ± 0.24 (b)	175.89 ± 0.26 (h)	137.83 ± 0.28 (f)	95.00 ± 0.62 (c)
	Ozone	95.67 ± 0.11 (c)	104.04 ± 0.82 (d)	66.42 ± 1.20 (a)	139.05 ± 0.45 (g)	112.70 ± 0.48 (e)
POL	CTRL	140.88 ± 15.6 (d)	132.06 ± 2.76 (cd)	117.45 ± 7.9 (abc)	105.81 ± 10.35 (ab)	122.30 ± 10.63 (bcd)
	Ozone	140.88 ± 15.6 (d)	95.87 ± 8.93 (a)	109.57 ± 18.46 (ab)	99.23 ± 9.92 (a)	100.15 ± 3.98 (a)
FLAV	CTRL	4.02 ± 0.027 (e)	2.01 ± 0.014 (d)	1.96 ± 0.029 (d)	1.75 ± 0.029 (bc)	1.53 ± 0.14 (a)
	Ozone	4.02 ± 0.027 (e)	1.85 ± 0.029 (cd)	1.73 ± 0.14 (bc)	1.58 ± 0.17 (ab)	1.62 ± 0.11 (ab)
DPPH	CTRL	6.55 ± 0.08 (c)	4.51 ± 0.007 (ab)	4.43 ± 0.017 (ab)	4.31 ± 0.17 (a)	4.35 ± 0.35 (a)
	Ozone	6.55 ± 0.08 (c)	4.35 ± 0.0072 (a)	4.62 ± 0.007 (b)	4.51 ± 0.01 (ab)	4.45 ± 0.86 (ab)

The AA concentration, that did not change significantly, was confirmed also by Tavarini and coworkers (2008). Ascorbic acid is an instable chemical compound that can be degraded during processing and storage. When fruit become overripe, vitamin C content declines, concurrently with the degradation of fruit tissue (Wilhelmina K, 2005). Indeed, it is an indicator of the suitability of the treatment. Tabakoglu and coworkers (2018) observed the decrease of the AA content of black mulberry fruit during the cold storage at 2 °C. Minas and coworkers (2012) indicated higher levels of AA in ozone treated samples compared with untreated kiwifruit after 1 day of shelf life, suggesting the ozone stimulation on the antioxidant synthesis; and so, confirming the ineffectiveness of ozone on preserving AA content during cold storage. Furthermore, a decrease in the levels of ascorbic acid and in the activities of antioxidant enzymes in strawberry fruit kept in cold storage with ozone gas (Zhang et al., 2011). Exposure to ozone for long time induced a reduction in ascorbic acid

content probably due to the radical scavenging mechanism against free radicals of auto-decomposed ozone and activation of ascorbate oxidase (Kumar M. and Sabikhi L., 2019). The decrease in ascorbic acid content, during cold storage, could be also due to the onset of senescence processes associated to a loss of cellular integrity and compartmentalization as demonstrated by Spinardi and Mignani (2018).

Carotenoid content exhibited not a linear trend; it decreased during the storage time at 2 °C, whereas at 4 °C it increased and returned to an initial amount after 60 d (T4) (Tables 3.6 and 3.7). The not linear behaviour of the carotenoid content in ‘Soreli’ fruit was explained by the stimulation of the defense mechanisms in the response to ozone treatment. Exposure of ozone-enriched atmosphere (0.3 L L⁻¹, 0 °C, RH 95 %) of green kiwifruit reduced the carotenoid content by 9.6 % after 2 hours and increased it by 5.7 % and 8.7 % after 72 and 144 h hours, respectively (Minas et al., 2010).

Kiwifruit are rich in polyphenols. Ma et al. (2017) found that the polyphenol content in green and yellow-flesh kiwifruit ranged from 58.45 to 152 mg GAE 100g⁻¹ FW. In this study, the polyphenol content of kiwifruit ranged from 70 to 140 mg GAE 100g⁻¹ FW. A decrease during cold storage at 2 °C and 4 °C was observed (Tables 3.6 and 3.7). At 2 °C, there was a significant decrease in polyphenol content after 15 days of cold storage in untreated and treated kiwifruit, with no significant changes among them. From 30 to 60 days of cold storage, the polyphenol content remained constant. The POL content was lower in ozone-treated samples than the untreated samples during the storage at 4 °C at T1 (15d) and T4 (60d); with a lower decreasing trend at 4 °C compared to 2 °C. The decreasing trend observed may be due to degradation of the polyphenolic compounds, following a change in the pattern of polyphenoloxidase activity that may contribute to the oxidation of phenolic substrates to quinones (Xia et al., 2016).

The total flavonoids (FLAV) exhibited different trends at 2 °C and 4 °C. At 2 °C TF decreased during the days of storage both in the untreated and ozone-treated samples. At 4 °C the content maintained constant after 15d when stored with gaseous ozone treatment, whereas, after 30 d (T2), it showed significant changes in the untreated fruit. No significative differences were detected between the treatments (Tables 3.6 and 3.7). The content of flavonoids detected in fruits of the ‘Soreli’ ranged from 1.53 to 4.26 mg CE 100g⁻¹ FW. Ma and coworkers (2017) analyzed 16 phenolic compounds in different cultivars with yellow and green flesh kiwifruit,

observing that the amount of (+/-)-catechin varied from 0.2 to 9 mg catechin 100 g⁻¹ FW. The yellow flesh fruit ‘Jintao’ contained 4-5 mg catechin 100 g⁻¹ FW, similarly to the TF value of ‘Soreli’ detected in the current study.

Two-way ANOVA showed the impact of the ozone treatment and the storage temperatures and their interaction on the antioxidant activity of ‘Soreli’ during cold storage (Table 3.5). The DPPH assay pointed out that fruits stored at 2 °C in an ozone-enriched atmosphere for 60 d exhibited a higher antioxidant activity than untreated. Whereas at 4 °C there were no significant differences for T-CTRL and T-ozone and for its interaction with the D-days; but it was possible to get some differences for the storage time. The trend of the DPPH assay indicated that the gaseous treatment could stimulate the antioxidant capacity during the cold storage at 2 °C. These results are validated also from Minas and coworkers (Minas et al., 2012) that, detecting the soluble antioxidants in organic solvents, found a higher antioxidant activity than CTRL in an ozone-enriched cold atmosphere (0.3 µL L⁻¹) for 1-5 months and then exposed at 20 °C for 6 days. Some studies suggested that generally the cold storage negatively influenced the total antioxidant capacity of kiwifruit (Tavarini et al., 2008). Shivashankara and coworkers (Shivashankara et al., 2004) linked the increase of antioxidant capacity during low-temperatures storage with the contribution of total phenolics in which fruits polyphenols are greater than ascorbic acid content. Kiwifruit is well known as excellent source of ascorbic acid (Tavarini et al., 2008).

Regarding the effect of the interaction of the temperatures and gaseous ozone treatment on the color of ‘Soreli’, it is necessary consider that the ripening of the yellow fleshed kiwifruit as ‘Soreli’ is characterized by de-greening. It is correlated to Hue angle (HUE) and Chroma (CH).

The figures 3.23 showed that during the storage at 2 °C and 4 °C there was a decrease of the lightness (L*), (Fig. 3.23 A), of the saturation of the color (CH), (Fig. 3.23 C) and of the hue angle (HUE), (Fig. 3.23 D); whereas the color difference (ΔE) increased (Figure 3.23 B). The lightness of the flesh color of ‘Soreli’ decreased more at 4 °C than at 2 °C. Two-way ANOVA showed that the postharvest treatment and its interaction at 2 °C did not show significant differences, reported in the table 3.5. Higher temperature (4 °C) exhibited greater influence on the parameter L* during the storage time and on ozone-treated samples, showing higher reduction of L* values; the same behaviour is observed for the saturation of the color (CH).

After 15 days (T1) the ΔE increased very markedly in all samples regardless of the treatments, especially in samples stored at 4 °C, causing the higher loss in the typical color of yellow flesh kiwifruit (Fig. 3.3.1). These results are inconsistent with what observed by Goffi and coworkers (2018) in ‘Soreli’ fruit treated with gaseous ozone (150 ppb during the day and 180 ppb during the night) and stored at 0 °C for 42 days, in which the ozone treated samples showed better positive effects on the maintenance of the color compared to untreated samples. The color’s trend highlighted in our trial suggested that the relation between 4 °C and the highest concentration of gaseous ozone generated stress to the kiwifruit, triggering metabolic activity as enzymatic browning responsible to color changes in flesh fruit.

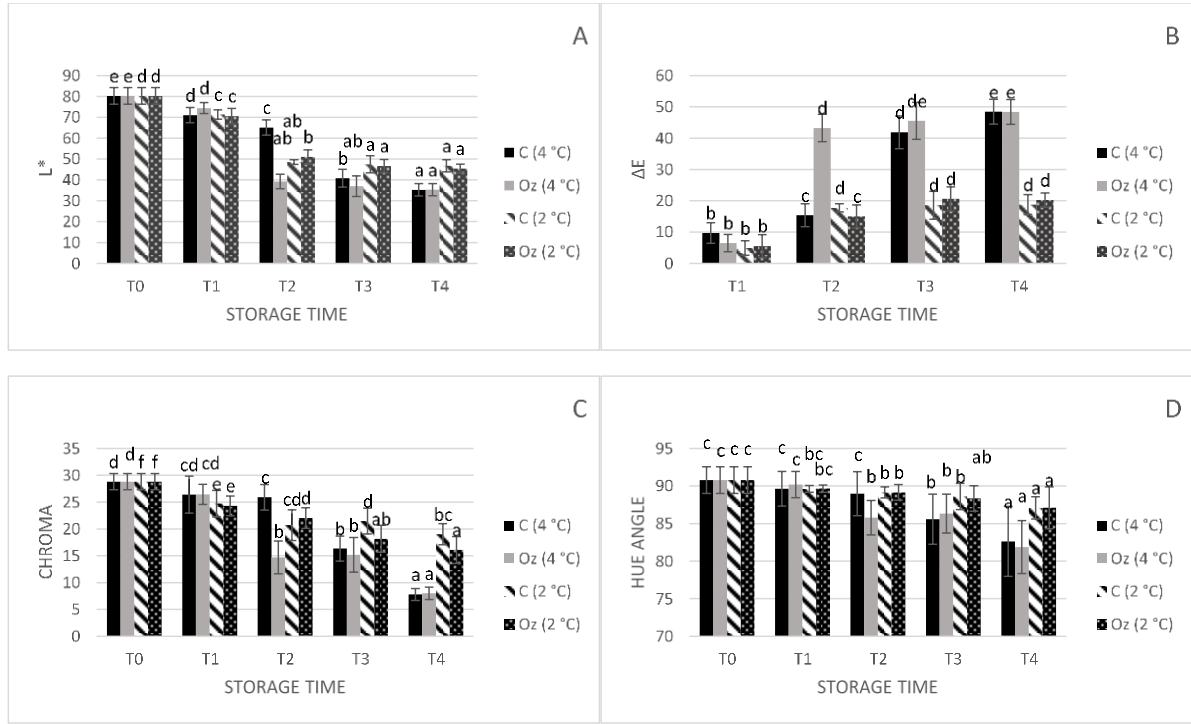


Fig.3.23. Color parameters, L*: Lightness (A), ΔE : Color difference (B); Chroma (C) and Hue angle (D), in ‘Soreli’ at harvest (T0) after 15 days (T1), 30 days (T2), 45 days (T3) and 60 days (T4) of storage at the two different temperatures (2 °C and 4 °C). Histograms represent the mean $\pm$ SD. Three technical replicates were realized for each biological replicate (Duncan test $P \leq 0.05$). Means with the same letter are not significantly different ($P = 0.05$, Duncan test) in CTRL and Ozone ($P=0.05$, Duncan test) for the same parameters at the same storage time. Means within each row followed by the same lowercase letter are not significantly different ($P > 0.05$) from each other for the specific treatment.

Two-dimensional principal component analysis plot of qualitative and nutraceutical traits in Soreli kiwifruit explains the 38.37 % for the first principal component (PC1) and 25.85 % for the second principal component (PC2) of the variance of all analyzed traits.

Colour parameters such as lightness (L; $r^2 = 0.921$), hue angle (HUE; $r^2 = 0.573$) and chroma (CH; $r^2 = 0.839$), firmness ($r^2 = 0.858$), microbial growth (MG; $r^2 = 0.489$) and ascorbic acid content (AA; $r^2 = 0.700$) were positively correlated to PC1; whereas SSC ($r^2 = -0.777$) and ΔE ($r^2 = -0.831$) were negatively correlated to PC1. Flavonoid content (FLAV, $r^2 = 0.830$) and DPPH ($r^2 = 0.878$) showed positive correlation to PC2, while, TA ($r^2 = -0.263$), carotenoids (CAR, $r^2 = -0.671$) and polyphenols (POL, $r^2 = -0.778$) were negatively correlated to PC2.

The behaviour of ‘Soreli’ during the cold storage was shown distinctly (Fig.3.24). After the harvest time there was a shift of the samples stored at 2 °C and 4 °C along PC1, but without underling significant differences between the treated and untreated samples. The storage at 2 °C was located at the upper axis, whereas the storage at 4 °C at the lower axis (Fig.3.24).

The 2D-plot suggested that the temperature management still played an important role in the storage compared to the gaseous ozone treatment. The fruit stored at 2 °C exhibited a slow rate of deterioration (e.g. of the ΔE) in comparison with 4 °C. The antioxidant compounds content varied differently during two months’ storage at the two temperatures; indeed, kiwifruit stored at 4 °C showed a high carotenoids and polyphenols content, while ascorbic acid and flavonoids contents were higher at 2 °C.

The differences between the treated and the control samples were not significant compared to the storage time (days). Kiwifruit stored at 2 °C preserved qualitative changes and delayed the oxidative stress compared to the storage at 4 °C. PCA is an useful technique to monitor the postharvest behavior of the kiwifruit during cold storage. PCA is a widely multivariate analysis used in several studies to evaluate the effectiveness of postharvest treatments on several fruits (Mgaya-Kilima et al., 2014; Petriccione et al., 2015; Modesti et al., 2018).

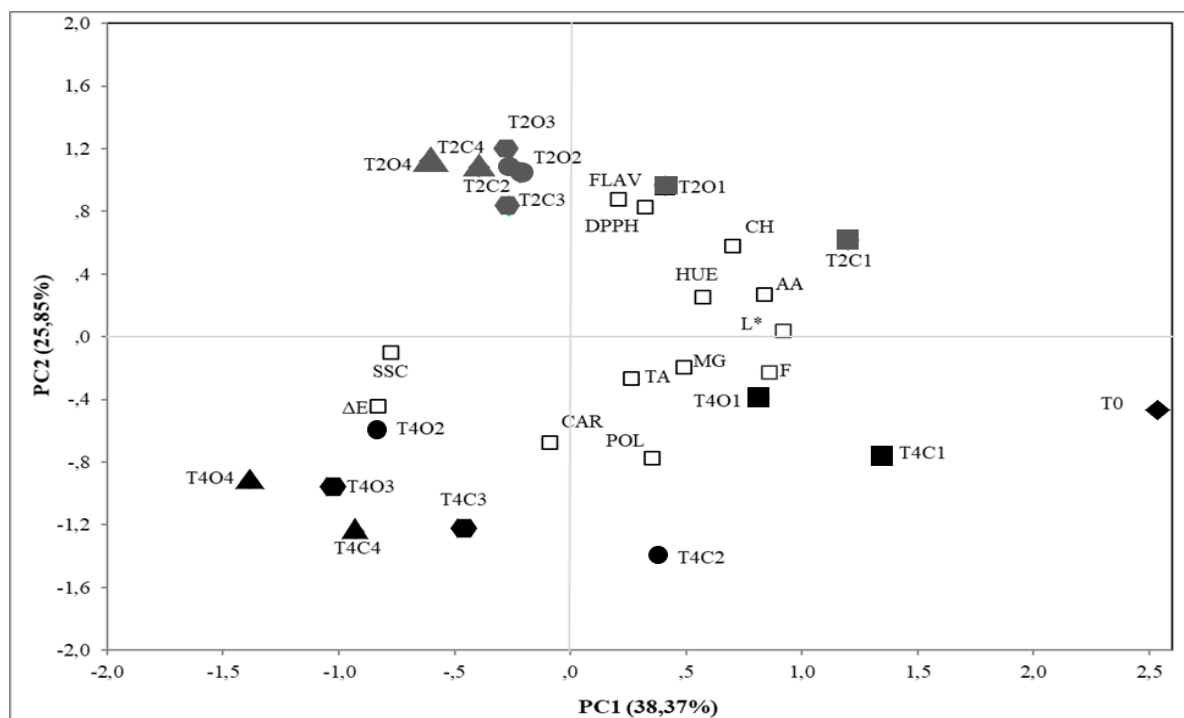


Fig. 3.24. Two-dimensional principal component analysis plot of qualitative and nutraceutical traits in Soreli kiwifruit, at harvest (T0), after 15 days (C1 control fruit, O1 ozone-treated fruit), 30 days (C2 control fruit, O2 ozone-treated fruit), 45 days (C3 control fruit, O3 ozone-treated fruit) and 60 days (C4 control fruit, O4 ozone-treated fruit) of storage at two different temperatures T2 (2 °C) and T4 (4 °C).

(F: firmness; SSC: soluble solid content; TA: total titratable acidity; L: lightness; HUE: hue angle; CH: Chroma; ΔE: color difference; MG: total microbial growth; POL: polyphenol content; FLAV: flavonoid content; AA: acid ascorbic content; CAR: carotenoid content; DPPH: radical scavenging activity). The symbols represent the different storage time (♦ is T0, ■ is C1 and O1, ● is C2 and O2, ● is C3 and O3 and ▲ is C4 and O4); the different colours of the symbols indicate the two different temperatures: black represents T4 and grey T2.

In the current study it was found that ozone treatment induced the ripening process; this effect was evident at the end of the storage. Soluble solids content (SSC) for ozone-treated fruit at 2 °C and 4 °C were higher. Storage temperature and gaseous ozone treatment influenced in a different manner the bioactive compounds, such as polyphenols, flavonoids, ascorbic acid and carotenoids. Additionally, under gaseous ozone storage microbial growth was delayed, improving the microbial quality index when fruit were stored at the lowest storage temperature (2 °C). Nevertheless, the factor that affected mainly the physico-chemical and bioactive compounds was the storage temperature than the post-harvest treatment, as shown by the principal component analysis.

Soreli showed different physico-chemical and nutraceutical behaviors correlated to storage temperatures and ozone postharvest treatments. Ozone treatment induced the ripening process and a decrease in bioactive compounds content during the first period of cold storage (15 days) at the two tested temperatures. Furthermore, ozone treatment controlled the microbial growth at the two tested storage temperatures. The low temperature, 4 °C, is not suitable for preserving kiwifruit quality during storage; compared to the low temperature close to 0 °C (2 °C) in ‘Soreli’ because 4 °C was less effective at preserving microbial decay and involved more bioactive compounds, such as carotenoids, in the defense against oxidative stress. The gaseous ozone treatment at 300 ppb in continuous flow combined to 2 °C can be used as an effective postharvest treatment of ‘Soreli’. It delayed the microbial decay, affected the reduction of sugars (SSC) and it controlled better the natural decay of bioactive compounds.

The data obtained from the current work were published: Goffi, V., Zampella, L., Forniti, R., Petriccione M. and Botondi R. (2019). Effects of ozone postharvest treatment on physicochemical and qualitative traits of *Actinidia chinensis* ‘Soreli’ during cold storage. Journal of Science and Food Agriculture, 99, 5654-5661. doi:10.1002/jsfa.9823.

3B TRIAL

The aim of this second activity (3B) was to investigate the influence of ozone, after gradual cooling treatment, on antioxidant defence system in *Actinidia chinensis* var. *chinensis*, ‘Soreli’.

The trial was conducted with a treatment of continuous gaseous ozone at the concentration of 300 ppb during storage at 2 °C over a period of 60 days on ‘Soreli’, after “curing” (gradual cooling): 10 days during which 24h fruit are stocked at ambient temperature, and after it, for 7 days the temperature is gradually reduced up to 2 °C.

In the current study it was found the effect of ozone postharvest treatment on non-enzymatic and enzymatic antioxidant system. Ozone is a strong and natural oxidant agent, that has many potential applications in food industry. Indeed, ozone-based treatments have been tested during storage and processing to extend the shelf-life and delay the microbial growth of bacteria and other microorganisms in several fruits (Zhang et al., 2005; Karaca et al., 2007; Horvitz et al., 2012 and Toti et al., 2018).

Bioactive compounds content decreased during cold storage and no significant difference has been registered ($p < 0.05$) between ozone-treated and untreated samples (Table 3.8).

Table 3.8. Antioxidant non-enzymatic content, ascorbic acid (AA-mg/100 gFW), polyphenol content (POL-mg GAE/100 gFW); flavonoid content (FLAV-mg CE/100 gFW) in ozone-treated (Ozone) and untreated (CTRL) kiwifruit cv Soreli at harvest (T0-H), after gradual cooling (T0-GC) and after 15 d (T1), 30 d (T2), 45 d (T3) and 60 d (T4) of cold storage at 2 °C.

Means with the same capital letters in a column (among treatments) and lowercase letters on the lines (during storage time) do not differ significantly by the Tukey test ($p < 0.05$).

Several studies have been reported that ascorbic acid is an instable compound that can be degraded during kiwifruit storage due to ripening and senescence processes caused by the loss

Traits	Treatments	T0-H	T0-GC	T1	T2	T3	T4
AA	CTRL	159,27 ± 0,93 (hA)	140,74 ± 0,84 (gA)	132,51 ± 0,51 (fB)	114,33 ± 0,40 (eA)	109,37 ± 9,23 (cA)	102,82 ± 0,13 (aA)
	Ozone	159,27 ± 0,93 (hA)	140,74 ± 0,84 (gA)	106,98 ± 0,6 (bA)	112,30 ± 0,13 (dA)	110,43 ± 0,21 (cA)	114,73 ± 0,11 (eB)
POL	CTRL	133,92 ± 8,73 (dA)	101,35 ± 1,91 (cA)	80,63 ± 0,58 (abB)	74,91 ± 0,31 (aA)	81,93 ± 0,24 (abB)	79,39 ± 0,34 (abA)
	Ozone	133,92 ± 8,73 (dA)	101,35 ± 1,91 (cA)	75,97 ± 0,73 (aA)	80,76 ± 0,27 (abB)	80,34 ± 0,24 (abA)	85,68 ± 0,30 (bB)
FLAV	CTRL	5,38 ± 0,23 (eA)	4,13 ± 0,17 (cdA)	3,58 ± 0,10 (aA)	4,33 ± 0,11 (dB)	3,98 ± 0,02 (bcA)	4,11 ± 0,01 (cdB)
	Ozone	5,38 ± 0,23 (eA)	4,13 ± 0,17 (cdA)	3,83 ± 0,03 (abB)	3,62 ± 0,04 (aA)	4,26 ± 0,08 (cdB)	3,75 ± 0,04 (abA)

of cell integrity (Miller et al., 2013). This AA decrease could be due to scavenge of the free radicals formed during the decomposition of ozone or to activation of ascorbate oxidase involved in the degradation of AA to dehydroascorbic acid (Lee et al., 2000).

The polyphenols content, that include several compounds such as phenolic acids, flavonoids, stilbenes and lignans, is influenced by ozonation such as demonstrated by different studies (Miller et al., 2013). In our previous study has been observed no significant effect of ozone treatment in ‘Soreli’ on polyphenol content during cold storage (Goffi et al., 2019). The degradation of polyphenols during ozonation can be due to several chemical reactions. Those reactions may be direct reactions of ozone with polyphenols due to hydroxyl radicals produced through ozone decomposition catalyzed mainly by the hydroxide ion (OH) or mainly catalyzed by polyphenol oxidase (Miller et al., 2013).

Postharvest oxidative stress is involved in potential storability and susceptibility to development of physiological disorders during storage of several fruits. SOD is the first enzyme involved in antioxidant defense during oxidative stress, that catalyzes the dismutation of radical superoxide ($O_2^{\cdot-}$) into either hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). SOD activity increased significantly during gradual cooling ($p<0.05$) and in the first 15 days of cold storage, followed by a decline until the end of storage (Fig. 3.25A). Ozone-treated fruits showed significant higher values compared to untreated samples (Fig. 3.25A).

CAT is involved in the detoxification of H_2O_2 , produced by SOD activity (Pasquariello et al., 2015). CAT activity increased, after gradual cooling compared to harvest, followed by a decrease during cold storage with significant higher values in ozone-treated samples (Fig.3.25B). Another scavenger of ROS is APX, an important key enzyme of ascorbic acid metabolism. Gradual cooling and cold storage induced a decrease in APX activity in untreated and treated samples (Fig. 3.25C) that could be due to reduction of ascorbic acid content during storage (Table 3.8). The H_2O_2 content exhibited a constant trend during storage time. Ozone-treated samples showed a significant lower H_2O_2 content compared to untreated samples ($p<0.05$) (Fig.3.25D). The increase in SOD and CAT activities could enhance both the ability of kiwifruit to convert $O_2^{\cdot-}$ into H_2O_2 , and detoxification of H_2O_2 in ozone-treated fruit. CAT and APX, are two key enzymes, involved in H_2O_2 detoxification with different kinetic features. CAT shows a high turnover rate and low affinity for substrate while APX is activated at high H_2O_2 dose (Adiletta et al., 2019). CAT acts during gradual cooling with high activity due to cold stress condition and both enzymes maintain their activity during cold storage to reduce H_2O_2 content. These results have been confirmed by Modesti and coworkers (2018) in ozone treated wine grape.

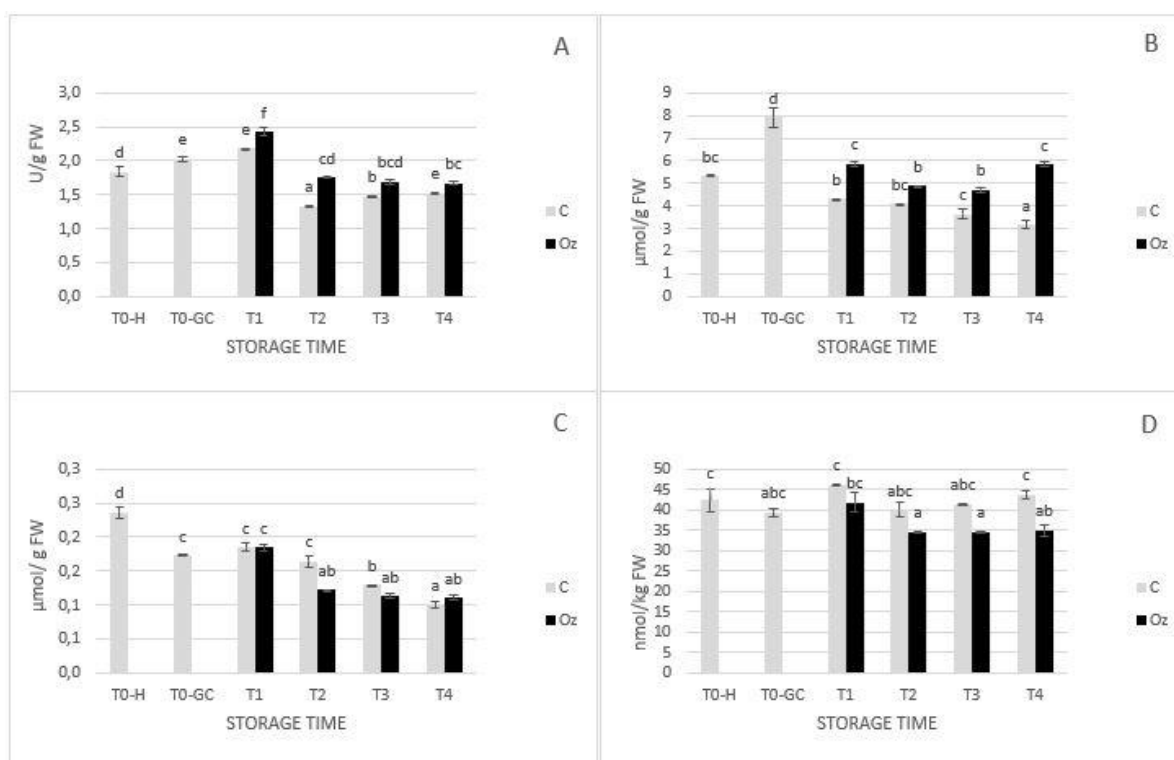


Figure 3.25. Superoxide dismutase (A), catalase (B), ascorbate peroxidase (C) activities and H₂O₂ content (D) in untreated (C) and ozone-treated (Oz) kiwifruit at harvest (T0-H), after gradual cooling (T0-GC) and at 15 days (T1), 30 days (T2), 45 days (T3) and 60 days (T4) of cold storage at 2 °C.

Error bars indicate standard deviations and values with different letters are significantly different (p < 0.05; Tukey test).

GPX, utilizing hydrogen peroxide as electron acceptor, catalyses the oxidation of several antioxidant compounds (Xia et al., 2016). Gradual cooling treatment induced a significant increase in GPX activity, whereas a lower GPX activity in untreated kiwifruit and the end of storage have been registered (Fig. 3.26A).

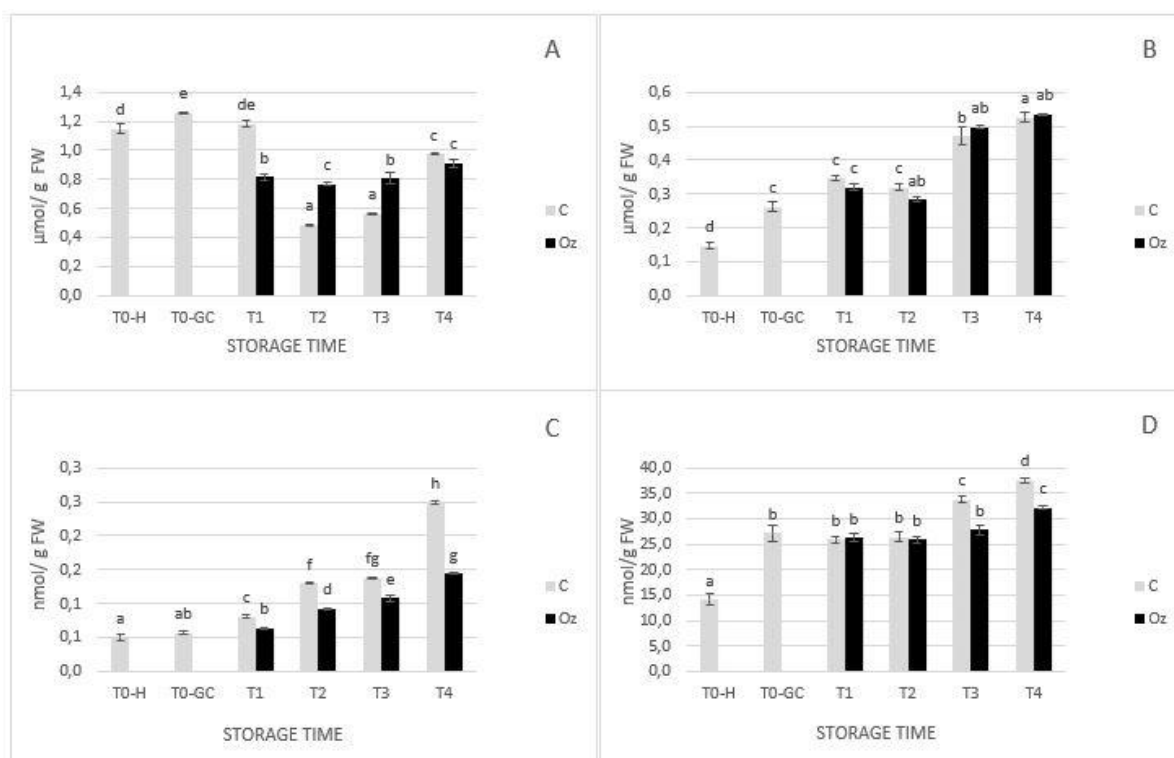


Figure 3.26. Guaiacol peroxidase (A), polyphenol oxidase (B), lipoxigenase activities (C) and malondialdehyde content (D) in untreated (C) and ozone-treated (Oz) kiwifruit at harvest (To-H), after gradual cooling (T0-GC) and at 15 days (T1), 30 days (T2), 45 days (T3) and 60 days (T4) of cold storage at 2 °C. Error bars indicate standard deviations and values with different letters are significantly different ($p < 0.05$; Tukey test).

Two-way ANOVA showed the effects of ozone treatment and the storage time and their interaction on the antioxidant system of ‘Soreli’ during cold storage. CAT and SOD were affected ($p < 0.001$) by all the considered factors (PT, D, and $PT \times D$) (Table 3.9).

Table 3.9. The p-values obtained in the two-way ANOVA for each postharvest treatment (PT) during cold storage at 2 °C (D-days) and their interaction (PT x D) in kiwifruit cv Soreli (superoxide dismutase activities (SOD), catalase (CAT), ascorbate peroxidase (APX) and H₂O₂ content (H₂O₂), guaiacol peroxidase (GPX), polyphenoloxidase (PPO), lipoxygenase activities (LOX), malondialdehyde content (MDA), ascorbic acid (AA), polyphenol content (POL) and flavonoids content (FLA).

Parameters	P-value		
	Postharvest treatment	Days	PT×D
CAT	<0.001	<0.001	<0.001
APX	0.002	<0.001	0.003
SOD	<0.001	<0.001	<0.001
H ₂ O ₂	<0.001	0.002	0.062
GPX	0.232	<0.001	<0.001
PPO	0.532	<0.001	0.183
LOX	<0.001	<0.001	<0.001
MDA	0.003	<0.001	0.005
POL	0.658	<0.001	0.728
FLA	0.001	<0.001	<0.001
AA	0.409	<0.001	0.040

Gradual cooling is an adaptation process to cold storage conditions used to maintain fruit quality and to alleviate chilling injury in kiwifruit industry and to delay *Botrytis cinerea* growth (Bautista-Baños et al., 1997). In Soreli kiwifruit, gradual cooling induced an increase in antioxidant enzymes activities, our results are in agree with previous studies that assessed as SOD, CAT, APX and POD activities in ‘Hayward’ kiwifruit were induced by low-temperature conditioning (Yang et al., 2012).

Several studies have demonstrated that postharvest treatments in kiwifruit can improve the antioxidant enzymes system (Yang et al., 2013; Meena et al., 2018). Xu and co-workers (2019) demonstrated that lysozyme and 1-methylcyclopropene (1-MCP) treatments prolonged the shelf-life of ‘Hayward’ kiwifruit improved APX, SOD and CAT activities with higher values in treated kiwifruit than untreated samples. Gradual postharvest cooling, from 15 °C to 0 °C, in ‘Hongyang’ kiwifruit exhibited higher SOD, CAT, APX, and GPX activities than those treated by direct cooling during storage (Yang et al., 2012). Furthermore, nitric oxide postharvest treatment reduced POD activity but increased the CAT and SOD activities in

‘Xuxiang’ kiwifruit during storage at 4 °C for 80 days (Xu et al., 2019). Although many studies have been conducted on ozone postharvest treatments in different fruit species, no of these reported the effects on antioxidant enzymes in kiwifruit (Pérez et al., 1999; Zhu et al., 2008). Ozone treatment induced an increase in SOD, CAT, and APX activities in papaya and pear fruits (Pasquariello et al., 2013; Zhao et al., 2013; Ong et al., 2014). Modesti and coworkers (2018) highlighted that the postharvest treatments by methyl jasmonates and ozone in wine grape induced an initial increase of SOD, APX, CAT, GPX. Ozone at low concentration can active the antioxidant system reduces intracellular ROS content and protecting the membrane lipid peroxidation (Modesti et al., 2018; Vettraino et al., 2019). Ozone treatment (4 ppm) inhibited the decrease of POD and CAT activities, and reduced membrane oxidative damage in strawberry during 20 days of cold storage (Zhang et al., 2011).

It was studied also the effect of ozone postharvest treatment on enzymatic browning and membrane damage. Browning in flesh kiwifruit is due to enzymatic oxidation. PPO catalyses the oxidation of phenols to quinones that are responsible of undesirable brown pigments (Petriccione et al., 2015). The PPO activity showed a significant increase after gradual cooling ($p<0.05$) with no significant differences between ozone-treated and untreated kiwifruit during cold storage (Fig. 3.26B). PPO use polyphenols as substrates in the browning reactions and this cause a decrease in polyphenol contents during storage (Table 3.8).

MDA content and LOX activity were analysed to evaluate the oxidative membrane damages after gradual cooling and during cold storage (Xu et al., 2019). MDA content showed a significant increase after gradual cooling, moreover, untreated kiwifruit exhibited higher value after 45 and 60 days of cold storage ($p<0.05$) (Fig. 3.26D). LOX activity showed an increase during cold storage with significantly higher values ($p<0.05$) in untreated samples. No significant differences were highlighted during gradual cooling in kiwifruit. An inhibition in LOX activity was confirmed by low MDA content in ozone treated samples as demonstrated in other studies (Modesti et al., 2018). In order, to evaluate the effect of storage time and ozone treatment on enzymatic browning and membrane damage, a two-way ANOVA analyses was performed (Table 3.9).

Several studies have demonstrated that postharvest treatments such as methyl jasmonates and ozone induced a significant decrease of PPO and LOX activities in wine grape (Modesti et al., 2018) while nitric oxide reduced MDA and ROS content, LOX activity in kiwifruit (Zhu et al., 2008).

Concerning PCA and Pearson correlation after ozone treatment, PCA has been used to determine the principal components (PCs) that explained the overall variance in all analyzed traits (47.1% and 19.0% for PC1 and PC2, respectively) (Fig. 3.27). CAT ($r^2=0,731$), GPX ($r^2=0,579$), SOD ($r^2=0,715$) and APX ($r^2=0,782$) activity were positively correlated to PC1; whereas MDA ($r^2= - 0,829$), LOX ($r^2= - 0,912$) and PPO ($r^2= - 0,840$) were negatively with the first principal component. H₂O₂ content ($r^2=0,916$) showed positive correlation to PC2. The multivariate space of the first two PCs showed that after gradual cooling the average PC scores in kiwifruit shifted from higher values to lower values along positive PC1. After 15 days of cold storage, ozone-treated and untreated fruit showed a shift from positive values to negative ones along PC1 with higher scores along PC2. As cold storage progressed all samples displayed a shift from positive values to negative ones along PC2. After 60 days of cold storage only ozone-treated kiwifruit showed a shift along negative values of PC1 and positive ones of PC2. The non-enzymatic system (POL, FLAV and AA) was positively correlated to the samples at harvest (T0-H) and after gradual cooling (T0-GC), while the antioxidant enzymes (APX, SOD, GPX and CAT) were positively correlated to the samples at harvest (T0-H) and after gradual cooling (T0-GC), to the untreated and ozone-treated samples after fifteen days of storage (T1-C, T1-OZ), and to ozone-treated sample after thirty days of storage (T2-OZ). Membrane oxidative damage indicators (MDA and LOX) and the main enzyme involving in fruit flesh browning (PPO) were correlated to the sample at the end of storage and significantly to untreated samples after thirty (T2-C), forty-five (T3-C) and sixth (T4-C) days of storage, respectively. The 2-D plot showed that the gaseous ozone treatment delayed the oxidative stress compared to untreated samples. Moreover, cold storage determined the decrease in bioactive compounds content while the ozone induced an increase of the antioxidant enzymes activities, that underwent to senescence process during long-term storage. Ozone postharvest treatment was effective to preserve the kiwifruit quality during cold storage (Villa-Rodriguez et al., 2015). PCA can be a valid tool to evaluate the effect of postharvest treatment on ‘Soreli’ kiwifruit evaluating antioxidant system as demonstrated by other studies (Petriccione et al., 2015a, b; Goffi et al., 2018).

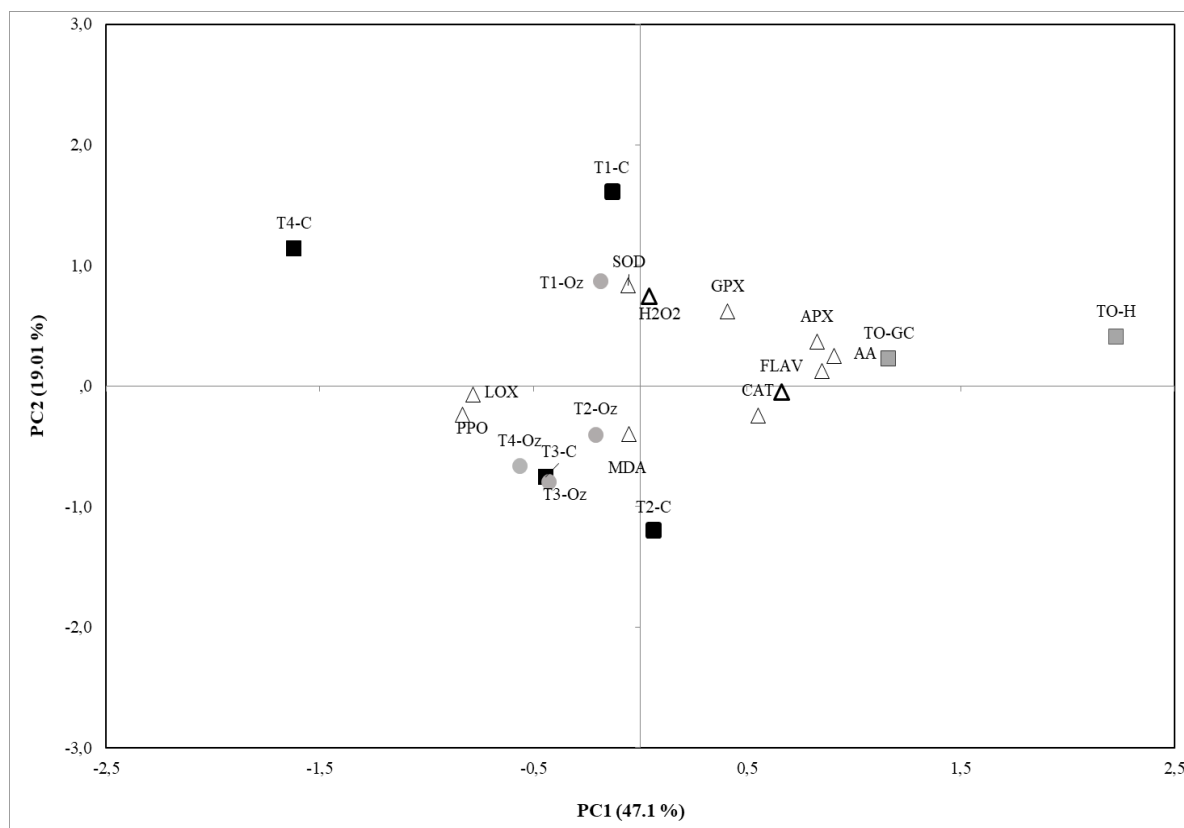


Figure 3.27. Two dimensional principal component analysis plot of antioxidant system in untreated (C) and ozone-treated (Oz) kiwifruit ‘Soreli’ at harvest (T0-H) and after gradual cooling (T0-GC) and at 15 days (T1), 30 days (T2), 45 days (T3) and 60 days (T4) of cold storage at 2 °C (Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and H₂O₂ content (H₂O₂), guaiacol peroxidase (GPX), polyphenoloxidase (PPO), lipoxigenase activities (LOX), malondialdehyde content (MDA), ascorbic acid (AA), polyphenol content (POL) and flavonoids content (FLA).

Pearson matrix underlines the inter-correlations among the antioxidant enzymes. MDA is positively correlated to LOX ($r = 0.784$; $p \leq 0.01$) and PPO ($r = 0.748$; $p \leq 0.01$) (Figure 3.28). This suggest that during kiwifruit storage the lipid peroxidation due to LOX activity leads to the oxidative membrane damages with an increase in MDA content. Furthermore, the loss of cellular compartmentalization induced an increase in PPO activity and a decrease in polyphenols content with a negative correlation among them ($r = -0.604$; $p \leq 0.01$). Other studies have demonstrated that lipid peroxidation leads to membrane deterioration and is highly correlated with LOX activity during fruit storage (Zheng et al., 2008).

SOD activity showed a significant positive correlation with APX ($r = 0.458$; $p \leq 0.05$) and CAT activity ($r = -0.545$ $p \leq 0.01$). In kiwifruit the combined action, of H₂O₂-generating SOD and the H₂O₂-metabolizing APX or CAT, is responsible to the oxidant resistance during

storage. Furthermore, SOD, APX and CAT are negatively correlated with MDA content and LOX activity, suggesting that oxidative stress is partially responsible for membrane damage during kiwifruit storage. APX was positively correlated to ascorbic acid content ($r = 0.783$; $p \leq 0.01$) being its reducing substrate of reaction.

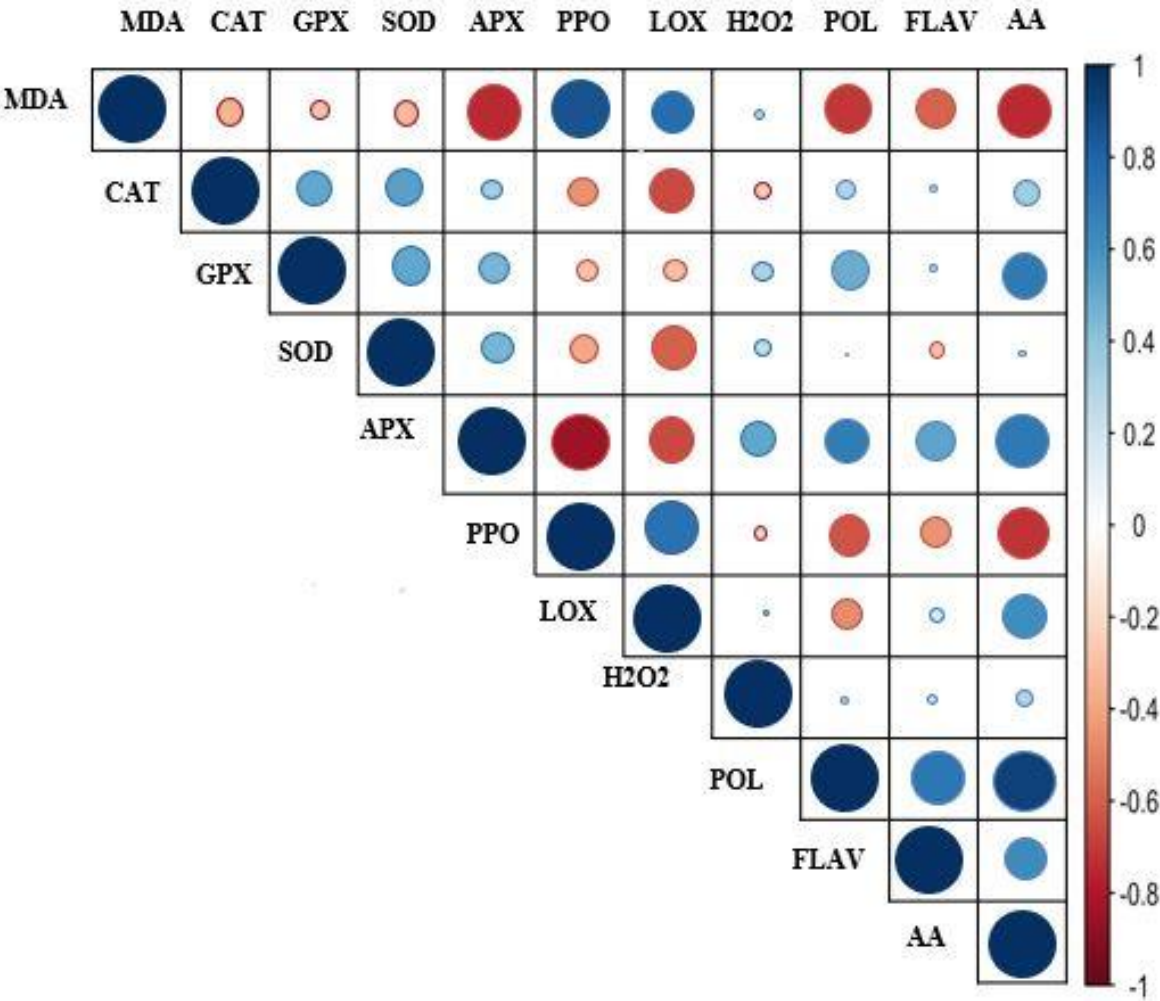


Figure 3.28. Pearson correlation matrix between superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and H₂O₂ content (H₂O₂), guaiacol peroxidase (GPX), polyphenoloxidase (PPO), lipoxygenase activities (LOX), malondialdehyde content (MDA), ascorbic acid (AA), polyphenol content (POL) and flavonoids content (FLA). The correlation coefficients are proportional to the circular size and colour intensity. Positive correlation is displayed in blue and negative in red.

Eco-friendly promising strategies, that allow to improve the antioxidant system to maintain the cellular compartmentalization and preservation of nutritional components and antioxidants, are valid tools in postharvest to improve the storability of fruits (Cao et al., 2009; Carletti et al., 2013; Sachadyn-król et al., 2016).

In conclusion ozone postharvest treatments have great commercial interest due to its non-residual property and flexibility of application methods. Our results suggest that gradual cooling and ozone treatment combined by cold storage enhanced the levels of antioxidant enzymes, such as APX, SOD and CAT, extending the postharvest life of ‘Soreli’ kiwifruit. Indeed, untreated fruit shown higher levels in MDA content and LOX activity than ozone-treated kiwifruit. Ozone treatment induced a rapid response of detoxification system by reducing oxidative stress.

The data collected from the current work were published: Goffi V., Magri A., Botondi R., Petriccione M. (2020). Response of antioxidant system to ozone postharvest treatment in ‘Soreli’ kiwifruit. *Journal of Science and Food Agriculture*. [https://doi: 10.1002/jsfa.10055](https://doi.org/10.1002/jsfa.10055).

3C TRIAL

The third activity aimed to investigate the influence of cold storage (2 °C) and ozone postharvest treatment (300 ppb) during the storage of ‘Soreli’ kiwifruit on a representation of allergens with important consequences at food consumption level. It was quantified the relative level of four major allergens in ‘Soreli’ (Table 3.10). The study evaluates the relationship between kiwifruit allergens and fruit behaviour at harvest, after gradual cooling and after postharvest treatment with gaseous ozone and at 15 days of cold storage (2 °C) to consider the different expression patterns in ozone-treated and untreated kiwifruit.

Allergens have an important role as defence-associated proteins in response to biotic and abiotic stresses (Petriccione et al., 2017) and these stress conditions induce the synthesis of allergens (Popovic et al., 2013).

In the preliminary analysis, to determine the specificity of the primer pairs used in this study, melting curve analysis and agarose gel electrophoresis were performed following the RT-qPCR experiment. The standard curve method using a pool of all the cDNA samples was performed to calculate the PCR efficiency (E) and the correlation coefficient (R^2) of each primer pair. Average E values ranged from 96.2 to 104.2%, with R^2 varying from 0.997 to 0.999 (Table 3.10). The results showed that all the primer pairs were suitable for RT-qPCR analysis. In addition, a single band with the expected size was detected in a single PCR product (Fig. 3.29). RT-qPCR was used to quantify the mRNA levels of four target genes encoding allergens (Act C1, Act C2, Act 5 and Act C11).

Ozone postharvest treatment could act on proteins synthesis, delaying their content as suggested by Minas and coworkers (2018). After gradual cooling, Act C1, Act C2 and Act C11 were overexpressed 1.35-fold, 1.18-fold, and 2.16-fold compared to kiwifruit at harvest, respectively, while Act C5 are slightly down-regulated (0.92-fold) (Figure 3.30). Furthermore, several studies demonstrated that ozone regulates the expression of ethylene pathways and consequently the production of some allergens (Minas et al., 2012). In fact, Minas and coworkers reported a down-regulation of proteins such as kiwellin and Bectv1 after ozone treatment (Minas et al., 2018). Few studies reported the relationship between allergens expression and postharvest techniques (Petriccione et al., 2017). Kiewning and coworkers (2013) have evaluated the influence of long-term storage, modified atmosphere storage condition and 1-MCP treatment on the Mal d1 production in different cultivars of apples. They have demonstrated that the utilizing of 1-MCP combined of a long-term storage as treatment

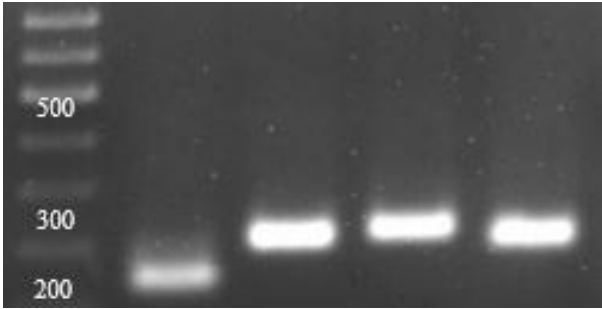
2576 postharvest reduces the production of Mal d1 in every cultivar considered except for
 2577 ‘Boskoop’ (Kiewning et al., 2013). Another study reported the effects of ethylene and 1-MCP
 2578 on apple fruit allergens. In particular, Mal d1.01, Mal d1b, Mal d1d and Mal d1e turn out to
 2579 be up-regulated by ethylene treatment and down-regulated by 1-MCP, while these treatments
 2580 have no effect on the synthesis of Mal d1.03 and Mal d1.04b (Yang et al., 2013).

2581

2582 Table 3.10. Descriptions of allergen-related genes and reference genes in *Actinidia chinensis*
 2583 var *chinensis*, ‘Soreli’.

Gene name (symbols) and Accession number	Primer sequence (5'-3')	Amplicon length (bp)	PCR efficiency (%)	Regression coefficient (R2)	References
Gene reference					
Tubulin (TUB) FG411346	F: CCGTTCATCTTGGTACTGC R: GGGAGAAGGAATGGACGAGA	92	104.2	0.997	Petriccione et al., 2015
Protein phosphatase 2A (PP2A) FG522516	F: GCAGCACATAATTCCACAGG R: TTTCTGAGCCCATAACAGGAG	110	102.3	0.999	Nardozza et al., 2013
Allergen-related gene					
Actinidin (Act C1) M21335.1	F: ATGCCATCTTACCCGTCAA R: TCCTAAGCGCTGTACCTCTG	136	101.6	0.997	This study
Thaumatococin-like protein (Act C2) JX905282.1	F: AAACGAGCAGTGTCCGAATG R: CCCCAGGGCATGTAAATGTG	197	96.2	0.999	This study
Kiwelin (Act C5) JX905292.1	F: CCTCCATCTCCTCCTGCAAT R: CGAGCAATCGTAGGTAGGGT	206	100.1	0.999	This study
Betv1-like (Kirola) (Act C11) FG456801.1	F: ACCGTGGCTCTGTCATCTT R: ACCGAGTTGTGTTCCCTTT	191	99.9	0.998	This study

2584



2585

2586 Figure 3.29. Agarose gel electrophoresis of fragments obtained from RT-qPCR for each
 2587 allergen-related gene using cDNA as a template. (Lane 1: Molecular size marker 100bp (DNA
 2588 ladders, Microtech, Milano, Italy); lane 2: Act C1; lane 3: Act C2; lane 4: Act C5 and lane 5:
 2589 Act C11).

2590

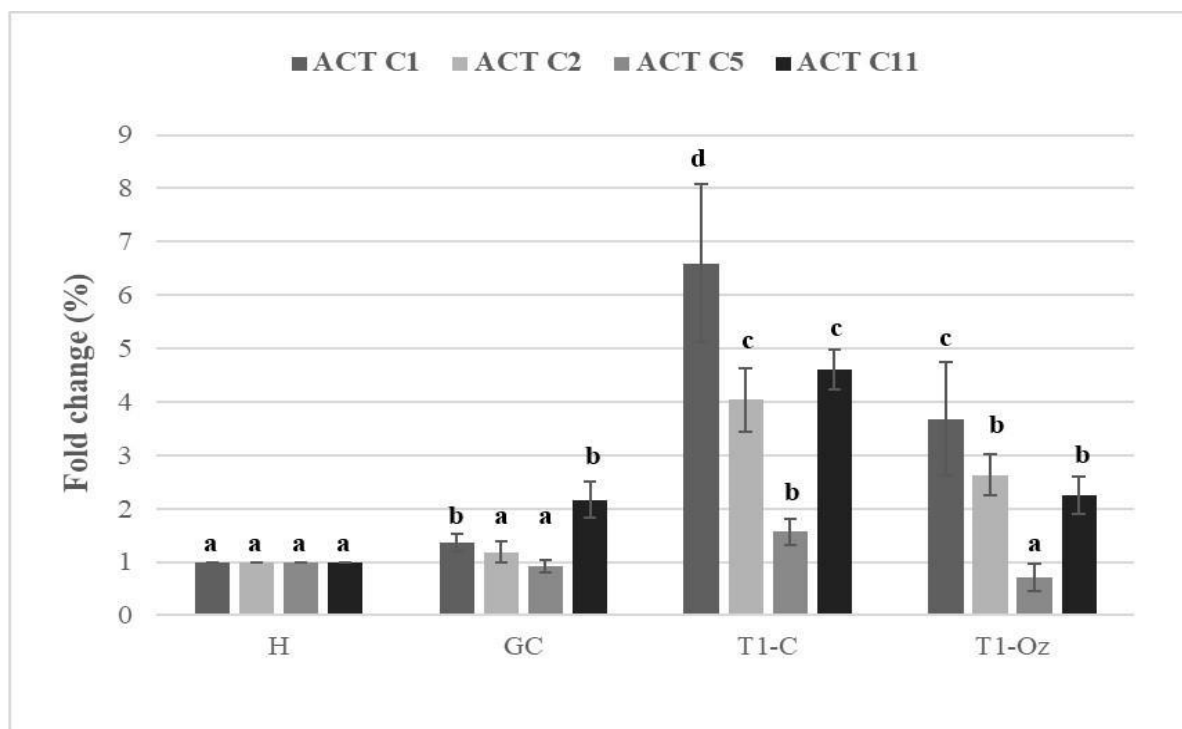


Figure 3.30. Fold changes of *A. chinensis* allergen gene expression normalized with the most stable reference genes (PP2A and TUB) using the comparative $2^{-\Delta\Delta C_t}$ method in kiwifruit. (Act C1: actinidin; Act C2: thaumatin-like protein; Act C5: kiwellin; Act C11: betv1-like protein; H: harvest; GC: after gradual cooling; T1-C: after 15 d of cold storage at 2 °C; T1-Oz: after 15 d of cold storage at 2 °C and treated with ozone gaseous). Mean with the same letters are not statistically different ($P = 0.05$, Duncan test).

This study demonstrates that all tested allergen genes are mostly expressed, with high transcript levels, in untreated kiwifruit stored for 15 days at 2 °C while the transcript levels decrease following by ozone treatment. Further studies should provide the quantification of allergens in kiwifruit by proteomic approaches responsible to oral allergy syndrome.

3D TRIAL

This preliminary study was carried out to evaluate the effectiveness of high-resolution DNA melting for the traceability of a raw material, as ‘Soreli’ kiwifruit, in a food chain. Soreli kiwifruit were processed into juice, jam and biscuits.

High-Resolution DNA melting technology has been successfully applied in various food sector, for olive oil and wine authentication (Pereira et al., 2017), for identification, adulteration and quantification of major Greek and Mediterranean bean species (Madesis et al., 2012). The current work shows the formation of four different clusters. DNA, derived from Hayward pulp, was considered as a standard of distinction with Soreli pulp and processed products.

In the figure 3.31 and 3.32 it is possible to visualize the clustering deriving from DNA amplification with the Act02 and Act05 primer, respectively. There is a red cluster for Soreli pulp and juice, a green one for Hayward pulp, a blue one for Soreli biscuits and a pink one for Soreli jam.

The different detected clusters even in products prepared with Soreli pulp may be due to a non-purification of DNA. Being processed foods with other raw materials it is possible to find, with the same sequence, different HRM curves, as reported by Słomka and coworkers (2017). This preliminary study allowed us to understand that DNA, from processed food, needs to be purified, in order to be able to distinguish the different kiwifruit cultivars used in the food industry for the preparation of processed products.

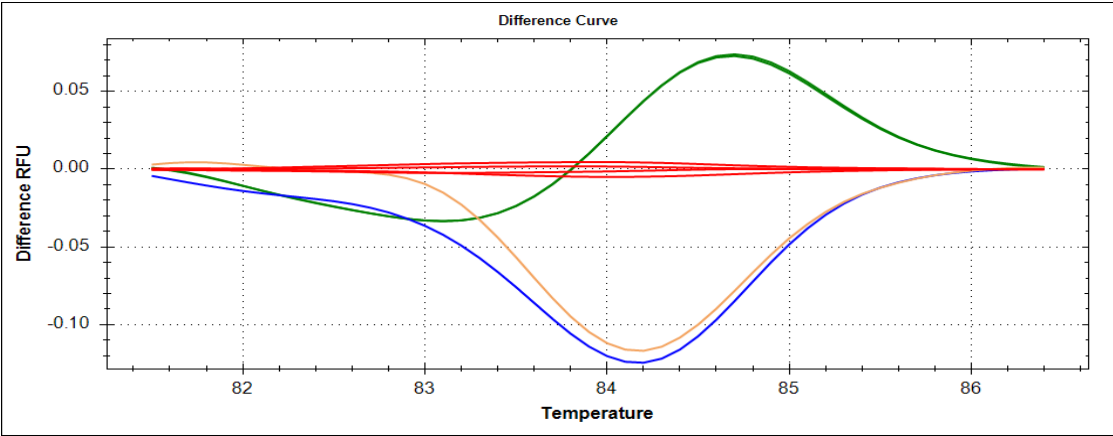


Figure 3.31. Barcoding of kiwifruit pulp and food process using HRM analysis with Act02 molecular marker.

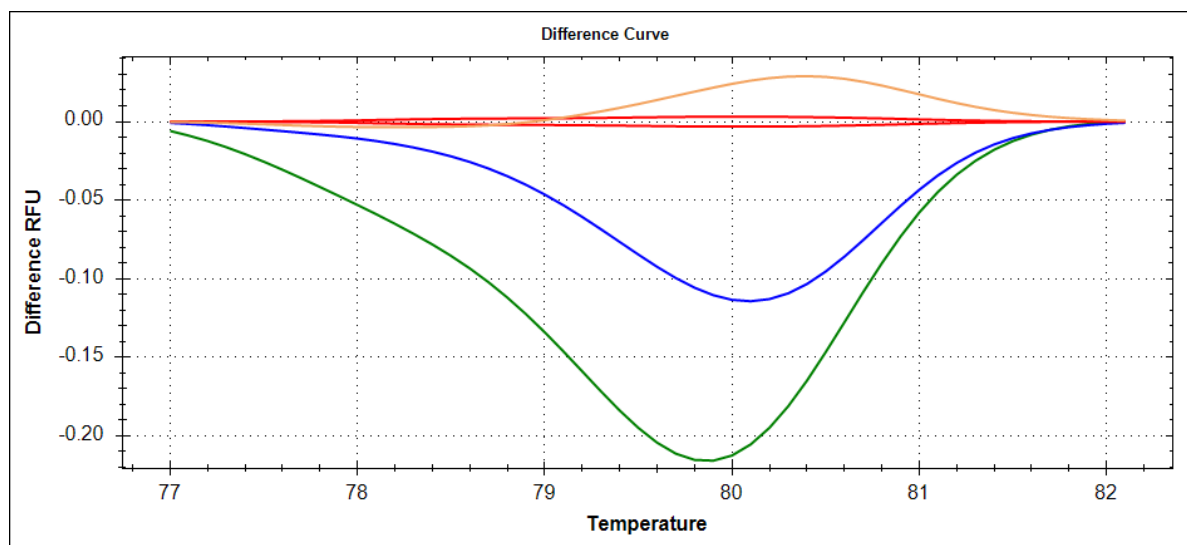


Figure 3.32. Barcoding of kiwifruit pulp and food process using HRM analysis with Act05 molecular marker.

CHAPTER 4: FINAL CONSIDERATIONS AND FUTURE PERSPECTIVE

Kiwifruit are considered a functional fruit with a high content of non-enzymatic antioxidants (ascorbic acid, polyphenol, flavonoids) that are able to counteract the high concentration of the ROS alleviating the biochemical imbalances (Richardson et al., 2018). Nowadays, many natural compounds such as chitosan, jasmonates, some volatiles compounds like ozone, nitric oxide and ethylene have been tested as postharvest treatments and some of them can cause alteration of the antioxidant system status (Gonzalez-Aguilar et al., 2010; Gao et al., 2013). The oxidative damage to the membranes, caused by the failure of the antioxidant system, is considered an early signal of fruit senescence (Mittler et al., 2002; Zheng et al., 2008).

Ozone efficacy can be influenced by factors affecting the solubility, stability and reactivity of ozone. The simultaneous contribution of solubility/stability and reactivity to efficacy of ozone can change with the environmental conditions. In addition, the nature and composition of the food and the type and load of microbial contamination influence greatly the effectiveness of ozone.

The applied gaseous concentration of 150/180 ppb in continuous flow during 60 days of 0 °C storage is not effective in preventing microbial growth both in ‘Hayward’ nor in ‘Soreli’, because the amount of ozone is less to exert an oxidation. Indeed, the bioactive compounds did not show any change during the storage between ozone treated samples and untreated ones. The color of the kiwifruit seem not to be affected by the ozone treatment, too.

Increasing the temperature storage conditions to 4 °C and the concentration of gaseous ozone to 300 ppb in continuous flow, Hayward and Soreli kiwifruit are strongly affected by oxidizing action of ozone. Softening and oxidative stress are detected since at the first stages of the storage; it stands out the synergic effect of the temperature with ozone. Ozone can cause oxidation of feruloylated cross-linkages or phenolic cross-linkages among cell wall pectin, structural proteins or other polymers and thereby changes the firmness of the kiwifruit, as well as others fresh commodities. High temperature increases the decomposition rate of ozone, and the decomposed ozone releases free radicals that may exert higher oxidation, interacting with the organic matrix of kiwifruit. The mechanism of ozone consists in the entrance of ozone through lenticels to intercellular spaces and be converted to some radical oxygen species

(ROS), superoxide anion O_2^- , hydroxyl radicals $HO^\cdot$ and H_2O_2 . Unless efficiently metabolized, ROS alters metabolism (Pell and Dann, 1991). The metabolism of ROS occurs by invoking the endogenous antioxidant defence system, which consists of low molecular weight antioxidants, as well as several antioxidant enzymes. Thus, ROS can react with membrane lipids, generating lipid peroxides, initiating series of damaging reactions, accelerating senescence.

Hayward kiwifruit shows less sensitive behaviour to different storage conditions probably due to its greater adaptability of cold temperatures compared to yellow flesh-kiwifruit, that is characterized by susceptibility to low temperatures.

Therefore, the findings induced the investigation to focus just on Soreli kiwifruit the study of a comparison between the temperatures of 2°C and 4°C, in order to have a validation or not of previously obtained data. Ozone treatment at 300 ppb controlled microbial growth in Soreli fruit when stored at 2 °C and 4 °C for 60 days, underlying that the used amount is effective to exert oxidizing action against the glycoproteins and glycolipids of the cell membranes of microorganisms, compared to 150/180 ppb, but the temperature of 2 °C is more effective to delay microbial decay compared to 4 °C. In this regard, as reported in literature, food components interfere with the bactericidal properties of ozone. Organic substances, surrounding the cells, constituting the food matrix, may compete with microorganism for ozone, reducing ozone efficiency.

The temperature of 2 °C controlled the natural decay of bioactive compounds, and the conversion of sugars. The soluble solids content (SSC) is an indicator of fruit ripening and increases during the storage due to the conversion of starch into soluble sugars. Kiwifruit stored at 4 °C showed higher values at the end of the storage compared to fruits stored at 2 °C, underlying an effect of modulation of the catabolic reactions by the synergic action between gaseous ozone and 2 °C.

The following step was to consider only 2 °C as temperature, being suitable to preserve the quality of ‘Soreli’ and being able to induce the synthesis of bioactive compounds; therefore, the aim of a subsequent study was to investigate the response of antioxidant system to postharvest ozone treatment (300 ppb) in ‘Soreli’ fruit, after gradual cooling for 810 days.

Precooling is a useful process to reduce the rate of biochemical and microbiological changes because the heat of the field is removed from fruits after harvest; the heat of field results in

high respiration rate and rapid growth and reproduction of microorganisms on the fruit surface (Han et al., 2017).

Gradual cooling and ozone treatment combined by cold storage enhanced the levels of antioxidant enzymes, such as ascorbate peroxidase (APX), superoxide dismutase (SOD) and catalase (CAT), extending the postharvest life of ‘Soreli’ kiwifruit. Indeed, untreated fruit showed higher levels in MDA content and lipoxygenase activity (LOX) than ozone-treated kiwifruit, underlying the ability of the chosen postharvest treatment to preserve membrane integrity by inhibiting lipoxygenase activity and malondialdehyde accumulation. Ozone treatment induced a rapid response of detoxification system by reducing oxidative stress. Thus, kiwifruit, living biological system, respond to storage-induced oxidative stress by activating a number of antioxidative stress-related defense mechanism.

Thereby, ozone can be considered as a postharvest elicitor if applied at the right concentration and time, increasing the enzymatic and non-enzymatic antioxidant system in fruits; that means that besides, living biological systems respond to biotic and abiotic stresses through chemical defence, synthesizing specific metabolites, some of them recognized as healthy compounds.

Ozone showed a potential inhibitory effect on the synthesis of allergens during cold storage at 2 °C: the transcript level decreased after continuous flow of gaseous ozone at the rate of 300 ppb for the entire storage period.

The High-Resolution DNA Melting Technology (HRM) could be a potential tool to evaluate the effectiveness of high-resolution DNA melting for the traceability of raw food matrix such as ‘Soreli’ in the food chain. According to the data it is necessary purifying the DNA from processed foods in order to distinguish the different kiwifruit cultivars used in food industry for the preparation of processed foods.

Overall, it can be concluded that gaseous ozonation can be a potential useful postharvest treatment for storage fresh commodities, but it is important to choose the right ozone concentration and storage conditions, thus, modelling according to the specific cultivar and to its commercial purpose.

Future perspective may look to the study and application of different integrated approaches to increase knowledge on physiological development, active metabolic pathways and molecular processes associated with postharvest treatment of yellow-flesh kiwifruit. In addition, it is

recommended in-depth analyses of the relationship between the concentrations of kiwifruit allergens, ripening stages during postharvest storage and postharvest treatment such as gaseous ozone, in order to understand the variability of the expression of kiwifruit allergens. Novel processing technologies also should be developed and/or in-depth studied to help in the reduction of kiwifruit allergens concentration.

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