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EFFECT OF ABIOTIC STRESSES ON BIOACTIVE CONTENTS OF VEGETABLES AND HIGH-PRESSURE TECHNOLOGY IN RELATED FUNCTIONAL BEVERAGES

Advanced Techniques for Research and Development in Food and Agriculture (TAIDA)

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DT-16

CONFORMIDAD DE SOLICITUD DEAUTORIZACIÓN DE DEPÓSITO DE TESIS DOCTORAL POR LOS DIRECTORES DE LA TESIS

D. Francisco Artés Hernández, como Director y D. Ginés Benito Martínez Hernández, como Co-director de la Tesis doctoral EFFECT OF ABIOTIC STRESSES ON BIOACTIVE CONTENTS OF VEGETABLES AND HIGH-PRESSURE TECHNOLOGY IN RELATED FUNCTIONAL BEVERAGES.

INFORMA:

Que la referida Tesis Doctoral, ha sido realizada por Dª. Anna Carolina Formica de Oliveira, dentro del Programa de Doctorado en "Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario – TAIDA", dando nuestra conformidad para que sea presentada ante el Comité de Dirección de la Escuela Internacional de Doctorado para ser autorizado su depósito.

La rama de conocimiento en la que esta tesis ha sido desarrollada es:

- ☐ Ciencias
- ☐ Ciencias Sociales y Jurídicas
- ☒ **Ingeniería y Arquitectura**

En Cartagena, a 9 de enero de 2017

EL DIRECTOR DE LA TESIS — EL CO-DIRECTOR DE LA TESIS

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Fdo.: Francisco Artés Hernández — Fdo.: Ginés Benito Martínez Hernández

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CONFORMIDAD DE DEPÓSITO DE TESIS DOCTORAL POR LA COMISIÓN ACADÉMICA DEL PROGRAMA

Dª. Encarna Aguayo Giménez, Secretaria de la Comisión Académica del Programa "Técnicas Avanzadas en Investigación y Desarrollo Agrario y Alimentario – TAIDA"

INFORMA:

Que la Tesis Doctoral titulada, EFFECT OF ABIOTIC STRESSES ON BIOACTIVE CONTENTS OF VEGETABLES AND HIGH-PRESSURE TECHNOLOGY IN RELATED FUNCTIONAL BEVERAGES, ha sido realizada, dentro del mencionado Programa de Doctorado, por Anna Carolina Formica de Oliveira, bajo la dirección y supervisión de los Drs Francisco Artés Hernández y Ginés Benito Martínez Hernández.

En reunión de la Comisión Académica de fecha 10/01/2017, visto que en la misma se acreditan los indicios de calidad correspondientes y la autorización de los Directores de la misma, se acordó dar la conformidad, con la finalidad de que sea autorizado su depósito por el Comité de Dirección de la Escuela Internacional de Doctorado.

La Rama de conocimiento por la que esta tesis ha sido desarrollada es:

- ☐ Ciencias
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En Cartagena, a 11 de enero de 2017

LA SECRETARIA DE LA COMISIÓN ACADÉMICA

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COMITÉ DE DIRECCIÓN ESCUELA INTERNACIONAL DE DOCTORADO

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ABSTRACT

Phenolic compounds are phytochemicals with high health-promoting properties which are present in several fruit and vegetables such as carrots and broccoli. Carrot is a worldwide highly consumed vegetable although its phenolic content is relatively low regarding other horticultural products. Accordingly, the enhancement of phenolic levels in carrots, using i.e. abiotic stresses, will add value to this popular vegetable for the food and pharmaceutical industries. Chlorogenic acid represented 70 % of the sum of phenolic compounds in carrots. Total phenolic contents (TPC) of carrots during storage periods were highly correlated (R^2=0.82) to total antioxidant capacity (TAC) of these samples. It was found that shredding (wounding stress) and hyperoxia storage (80 kPa O_2) induced the highest phenylalanine ammonia-lyase (PAL), TPC and TAC enhancements in carrots during storage at 15 ºC for 72 h. Although pretreatment of shreds with an intermediate UV-C dose (9 kJ m^{-2}) reduced phenolic accumulation, 600 % increments were still registered in those samples stored under hyperoxia conditions for 72 h. These first findings of the PhD Thesis supply to the food and pharmaceutical industries useful and sustainable tools to obtain a great source of health-promoting compounds from this vegetable, and probable to others.

Subsequently, the effect of UV-B pretreatment (1.5 kJ m^{-2}) was studied on the PAL/TPC/TAC of shredded carrots and its combination with a low-intermediate (4 kJ m^{-2}) UV-C dose. UV-B treatment induced the highest PAL/TPC of 760/498 % after 72 h at 15 ºC, regarding their initial levels, while single and combined UV-C treatments induced a TPC accumulation of 440 %. Accordingly, the use of UV-C treatment, with high sanitizing interest for the fresh-cut industry, combined with UV-B radiation did not negatively affect the accumulation of bioactive compounds, achieving similar enhancements to untreated samples.

Broccoli cultivation leads to high volumes of plant by-products with high environmental impact which may be instead used as sources of health-promoting compounds for the food and pharmaceutical industries. Furthermore, such broccoli by-products may be revalorized through the increase of their health-promoting compounds with postharvest UV radiation as an abiotic stress. Accordingly, the effects of different postharvest UV-B radiation doses (5, 10 and 15 kJ m^{-2}), single or combined with UV-C treatment (9 kJ m^{-}

^{2}), were studied on the main bioactive compounds of Bimi® broccoli by-products (leaves and stalks) being compared to Bimi® edible florets. Leaves showed similar TPC and TAC, with 1716 and 552 mg kg^{-1} respectively, compared to florets. Furthermore, leaves showed 2.5/14.5 higher glucoraphanin/glucobrassicin contents than florets. UV postharvest treatments initially increased TPC and TAC levels of samples by 30-97 and 20-424 %, respectively. Particularly, UVB10+C treatment induced the highest TPC increase (110 %) in Bimi® leaves in the last 48 h of storage while UVB10 and UV10+C led to the highest TPC (709 and 680 mg kg^{-1}) of stalks at 48 h. Furthermore, UV10+C treatment increased glucobrassicin levels of leaves by 34 % while UVB15 and UVB15+C induced the highest glucoraphanin levels (131 and 117 mg kg^{-1}) in florets after 72 h. Accordingly, Bimi® leaves are hereby presented as a by-product that may be used as an excellent source of glucosinolates and phenolics, with high total antioxidant capacity, for the food and pharmaceutical industries. In addition, postharvest intermediate-high UV-B/C combined radiation treatments can highly revalorize such agricultural by-products and also add value to the edible fresh-cut Bimi® florets.

A functional smoothie containing the previously stressed (UV-C and/or high O_2) carrot shreds with high TPC/TAC contents was thermally treated (90 ºC for 30 s) and the beverage quality was studied during 14 days at 5 ºC being compared to CTRL samples. Heat-treated smoothies showed a good physicochemical and microbiological quality (< 6 log CFU g^{-1}) after 14 days at 5 ºC, although smoothies containing non-irradiated shreds registered lower psychrophilic and yeasts and moulds levels. Heat-treated smoothie containing non-irradiated O_2-shreds showed the highest TPC of 13.8 mg kg^{-1} after 14 days at 5 ºC. In this sense, a pre-enrichment incubation of carrot shreds under hyperoxia conditions allowed to obtain a functional smoothie with high phenolic levels and good microbiological and physicochemical quality up to 14 days at 5 ºC.

A non-thermal treatment, such as high pressure processing (HPP), of beverages may allow to obtain a product with better bioactive/nutritional and sensory quality, and extended shelf life regarding thermally treated samples. Accordingly, the physicochemical and microbial quality, and PAL/TPC of an orange smoothie (carrot and pumpkin) after different HPP treatments (CTRL, 300-600 MPa for 5 min at 23 ºC) and during storage for 7 days at 5ºC were studied. In general, quality parameters did not highly change after HPP treatments compared to CTRL samples on the processing day and after

7 days at 5 ºC. HPP reduced initial mesophilic counts (3.4 log CFU mL^{-1}) by 2.0-2.7 log units being these microbial counts well maintained after 7 days at 5 ºC contrary to CTRL samples. The physicochemical quality of the HPP-treated smoothies was highly maintained during storage. Generally, the 300 and 600 MPa HPP treatments induced the highest PAL activities after 7 days at 5 ºC. The initial TPC (712.1 mg ChAE kg^{-1}) was increased in the smoothies reaching the 300 and 400 MPa-treated samples the highest increments (1.6-fold) after storage.

The activity of some quality-degradative enzymes may be increased in determined beverages under low-moderate HPP treatments. For that reason, in the next study we tried to palliate such undesirable HPP effects by encapsulation with maltosyl-*β*-cyclodextrin (90 mM) using apple juice as beverage model due to the rapid enzymatic browning during processing of this fruit juice. Colour degradation of apple juice during 60 min at 22 ºC was well fitted to a fractional conversion model with root-mean-square error (RMSE) < 1.3. HPP treatments (300-600 MPa for 5 min at 22 ºC) did not affect the antibrowning effect of maltosyl-*β*-cyclodextrin. In that sense, maltosyl-*β*-cyclodextrin addition to apple juice prior to a low-moderate HPP treatment (300 MPa for 5 min at 22 ºC) highly controlled enzymatic browning.

A new food concept 'ready-to-blend' was firstly developed and studied in this PhD Thesis. Accordingly, the quality of a fresh-cut fruit/vegetables 'ready-to-blend' product was studied during storage at 5 ºC. The shelf life of the ready-to-blend product was highly extended to 9 days by a chitosan coating (10 g L^{-1}) being microbial levels and polyphenoloxidase activity well controlled. Added value of the product was enhanced by fortification with vitamin B12 (8.6 μg kg^{-1}) being of high interest for specific population sectors, such as vegetarians/vegans, elderly, etc., which have special needs for this vitamin. The prepared smoothies from the ready-to-blend portion showed a good quality during subsequent storage for 48 h at 5 ºC.

RESUMEN

Los compuestos fenólicos son fitoquímicos con propiedades beneficiosas para la salud, presentes en gran medida en las frutas y verduras, como la zanahoria o el brócoli. La zanahoria es una verdura ampliamente consumida en todo el mundo. Sin embargo, su contenido fenólico es relativamente bajo en comparación a otras verduras. Consecuentemente, el aumento de los niveles de compuestos fenólicos en zanahorias, usando estreses abióticos, por ejemplo, podría agregar un valor añadido a esta verdura para las industrias alimentaria y farmacéutica. El ácido clorogénico representó el 70 % de la suma de los compuestos fenólicos en las zanahorias estudiadas. Los compuestos fenólicos totales (TPC en inglés) estuvieron altamente correlacionados con la capacidad antioxidante total (TAC en inglés) con un R^2=0,82. Se comprobó que los estreses abióticos, como el cortado y la conservación en atmósfera de alto oxígeno (80 kPa O_2), indujeron un elevado aumento de la enzima fenilalanina amonio liasa (PAL en inglés), TPC y TAC en zanahorias durante una conservación a 15 ºC durante 72 h. Aunque el pretratamiento de la zanahoria rallada con una dosis intermedia (9 kJ m^{-2}) de radiación UV-C redujo la acumulación de compuestos fenólicos, se registró un incremento del 600 % en estas muestras almacenadas en alto oxígeno durante 72 h. Las primeras conclusiones de esta tesis doctoral, resultan útiles para las industrias farmacéutica y alimentaria, por obtener una gran fuente de compuestos bioactivos de esta verdura, y probablemente, aplicable en otros productos vegetales.

A continuación, se estudió el efecto de pretratamientos con radiación UV-B (1,5 kJ m^{-2}) sobre los contenidos de PAL/TPC/TAC en zanahoria rallada y su combinación con una dosis bajo-intermedia (4 kJ m^{-2}) de radiación UV-C. El pretratamiento con UV-B provocó el máximo aumento de PAL y TPC de 760 y 498 %, respectivamente, tras 72 h a 15 ºC, mientras que el tratamiento simple y combinado de UV-C indujo incrementos de un 440 %. Por consiguiente, el uso de UV-C, de alto interés como tratamiento sanitizante alternativo al cloro, combinado con la radiación UV-B no afectó negativamente la acumulación de compuestos bioactivos, consiguiendo aumentos similares a las muestras control (no tratadas).

El cultivo del brócoli conlleva la producción de altas cantidades de subproductos, con el consiguiente impacto medioambiental, los cuales podrían ser usados como fuentes de compuestos beneficiosos para la salud por las industrias de alimentación y farmacéuticas. Además, estos subproductos de brócoli podrían ser revalorizados mediante el incremento de sus compuestos beneficiosos para la salud mediante radiación UV como estrés abiótico. En esta línea, se estudiaron los efectos de diferentes tratamientos de radiación UV-B (5, 10 and 15 kJ m^{-2}), individual o combinados con una dosis de UV-C (9 kJ m^{-2}), sobre los principales compuestos bioactivos de los subproductos del brócoli Bimi® (hojas y tallos) siendo comparados con las partes comestibles de este vegetal, los floretes. Las hojas mostraron TPC y TAC similares a los de los floretes con niveles de 1716 y 552 mg kg^{-1}, respectivamente. Además, las hojas mostraron contenidos de glucorafanina/glucobrasicina 2,5/14,5 mayores que los floretes. Los tratamientos de UV incrementaron inicialmente los niveles de TPC y TAC de las muestras en un 30-97 y 20-424 %, respectivamente. Particularmente, el tratamiento UVB10+C indujo los mayores incrementos (110 %) de TPC en las hojas de Bimi® en las últimas 48 h de conservación mientras que UVB10 y UV10+C conllevaron a los niveles más altos de TPC (709 y 680 mg kg^{-1}) en los tallos a las 48 h. Además, el tratamiento UV10+C indujo los mayores incrementos de glucobrasicina del 34 % mientras que UVB15 y UVB15+C produjeron los contenidos más altos de glucorafanina (131 y 117 mg kg^{-1}) en los floretes después de 72 h. De esta forma, las hojas del brócoli Bimi® se muestran como un subproducto que puede ser utilizado como una fuente excelente de glucosinolatos y compuestos fenólicos (con alto poder antioxidante) para las industrias alimentarias y farmacéuticas. Además, el uso de tratamientos UV-B/C postcosecha combinados pueden revalorizar en gran medida estos subproductos agrícolas y también añadir valor a los floretes comestibles mínimamente procesados en fresco del brócoli Bimi®.

Se estudió la calidad de un batido (*smoothie*) funcional obtenido de zanahoria rallada, previamente estresada (UV-C y/o alto oxígeno), tratado térmicamente (90 ºC durante 30 s), durante 14 días a 5 ºC. El *smoothie* tratado térmicamente mostró una buena calidad fisicoquímica y microbiológica (< 6 log unidades formadoras de colonias (CFU en inglés) g^{-1}), tras los 14 días a 5 ºC. Sin embargo, en los *smoothies* que contenían zanahoria no irradiada se registraron recuentos más bajos de psicrófilos, mohos y levaduras. Los *smoothies* tratados térmicamente con zanahoria rallada no irradiada y almacenada en alto oxígeno mostraron los contenidos más altos de TPC con 13.8 mg de ácido clorogénico

kg^{-1} tras 14 días at 5 ºC. En ese aspecto, la incubación previa de la zanahoria rallada en condiciones de hiperoxia permitió obtener un *smoothie* funcional con alto contenido de compuestos fenólicos, así como una buena calidad microbiológica y fisicoquímica tras 14 días a 5 ºC.

La pasteurización fría de alimentos, como el procesado con alta presión (HPP en inglés), permite conseguir un producto con una mejor calidad nutricional, sin perder sus propiedades sensoriales, logrando también una vida útil más larga comparado con los tratamientos térmicos convencionales. De esta forma, se estudió la calidad fisicoquímica y microbiológica, y PAL/TPC de un *smoothie* naranja (con zanahoria y calabaza) tras diferentes tratamientos de HPP (control, 300-600 MPa durante 5 min a 23 ºC) y durante su conservación de 7 días a 5 ºC. En general, los parámetros de calidad no se vieron afectados por los tratamientos de HPP en comparación al control en el día de procesado ni tras 7 días a 5 ºC. HPP redujo los recuentos iniciales de mesófilos (3,4 log CFU mL^{-1}) a 2,0-2,7 unidades logarítmicas, manteniéndolas durante los 7 días a 5 ºC, a diferencia de las muestras control. Los parámetros de calidad fisicoquímica de las muestras tratadas con HPP se mantuvieron durante la conservación. En general, los tratamientos de 300 y 600 MPa provocaron una mayor actividad de PAL tras 7 días a 5 ºC. Los TPC de los *smoothies* tratados con 300 y 400 MPa mostraron los mayores incrementos (1,6 veces) tras la conservación.

La actividad de muchas enzimas alterantes de la calidad del producto puede aumentar debido a tratamientos de HPP de intensidad baja-intermedia en muchas bebidas. Por esta razón, en el siguiente experimento se contrarrestaron estos efectos debidos al HPP mediante la encapsulación con maltosil-*β*-ciclodextrina (90 mM) usando como modelo zumos de manzana debido a su rápido pardeamiento enzimático durante el procesado. La degradación del color del zumo de manzana durante 60 min a 22 ºC fue ajustada muy bien mediante un modelo de conversión fraccional con la raíz del error cuadrático medio (RMSE en inglés) inferior a 1,3. Los tratamientos de HPP (300-600 MPa durante 5 min a 22 ºC) no afectaron el efecto antipardeante de la maltosil-*β*-ciclodextrina. Por esta razón, la adición de maltosil-*β*-ciclodextrina en zumo de manzana, antes del tratamiento bajo-moderado de HPP (300 MPa durante 5 min a 22 ºC) puede controlar en gran medida el pardeamiento enzimático.

En esta Tesis doctoral también se desarrolló por primera vez el concepto "listo para licuar" (*ready-to-blend* en inglés). De esta forma, se estudió la evolución de los parámetros de calidad de una mezcla de ensalada de cuarta gama de frutas y verduras *ready-to-blend* durante su conservación a 5 ºC. La vida útil del producto se extendió hasta 9 días mediante una película comestible de quitosano (10 g L^{-1}) donde la actividad de la polifenoloxidasa y los niveles microbiológicos fueron muy bien controlados. Paralelamente se aumentó el valor añadido del producto mediante la fortificación con vitamina B12 (8.6 μg kg^{-1}). Esta vitamina es de gran interés para diversos sectores de la población, tales como vegetarianos/veganos, personas mayores etc., debido a las necesidades especiales de la misma. El *smoothie* preparado posteriormente de la mezcla *ready-to-blend* mostró en general una buena calidad durante su conservación a 5 ºC durante 48 h.

LIST OF ABBREVIATIONS

Δ*E*: total colour differences index

ºC: degree Celsius

µ: micro

3,5-CQA: 3,5-dicaffeoylquinic acid

3-CQA: chlorogenic acid (3-caffeoylquinic acid)

4,5-CQA: 4,5-dicaffeoylquinic acid

AA: ascorbic acid

ANOVA: analysis of variance

ATP: adenosine triphosphate

BI: browning index

BOPP: bi-oriented polypropylene

ChAE: chlorogenic acid equivalent

CDs: cyclodextrins

CFU: colony forming units

CIE Lab: Lab colour space

CTRL: control

cvs: cultivars

DNA: deoxyribonucleic acid

DPPH: 2,2-Diphenyl-1-picrylhydrazyl free radical

EU: European Union

FAO: Food and Agriculture Organization of the United Nations

FDA: Food and Drug Administration of the United States of America

fw: fresh weight

g: gram

GAPs: good agricultural practices

GC: gas chromatography

GMPs: good manufacturing practices

GPR: Grupo de Postrecolección y Refrigeración

h: hour

HACCP: hazard analysis critical control point

HPLC: high-performance (pressure) liquid chromatography

HPP: high hydrostatic pressures

HSP: heat shock protein

ITC: isothiocyanates

kJ: kilojoule

kg: kilogram

log: logarithm

m: meter

mg: milligram

min: minute

mL: milliliter

M: mol

MAP: modified atmosphere packing

MPa: megapascal

MPF: minimally processed foods

MS: mass spectrometry

NaOCl: sodium hypochlorite

Pa: pascal

PAL: phenylalanine ammonia-lyase

PE: polyethylene

PEF: pulse electric field

PEP: phosphoenolpyruvate

PG: polygalacturonase

PME: pectin methyl esterase

POD: peroxidase

PP: polypropylene

PPO: polyphenol oxidase

PS: polystyrene

RB: ready-to-blend

RD: Real Decreto

REL: relative electrolyte leakage

REPFED: refrigerated pasteurized foods of extended durability

RH: relative humidity

RMSE: root-mean-square error

RNA: ribonucleic acid

ROS: reactive oxygen species

RR: respiration rate

s: seconds

SD: standard deviation

SE: standard error

SOPs: standards operating procedures

SSC: soluble solids content

TA: titratable acidity

TAC: total antioxidant capacity

TPC: total phenolic content

TR: transmission rate

TTC: 2,3,5-triphenyltetrazolium chloride

UHPLC: ultra high-performance (pressure) liquid chromatography

UK: United Kingdom

UPCT: Universidad Politécnica de Cartagena

USA (US): United States of America

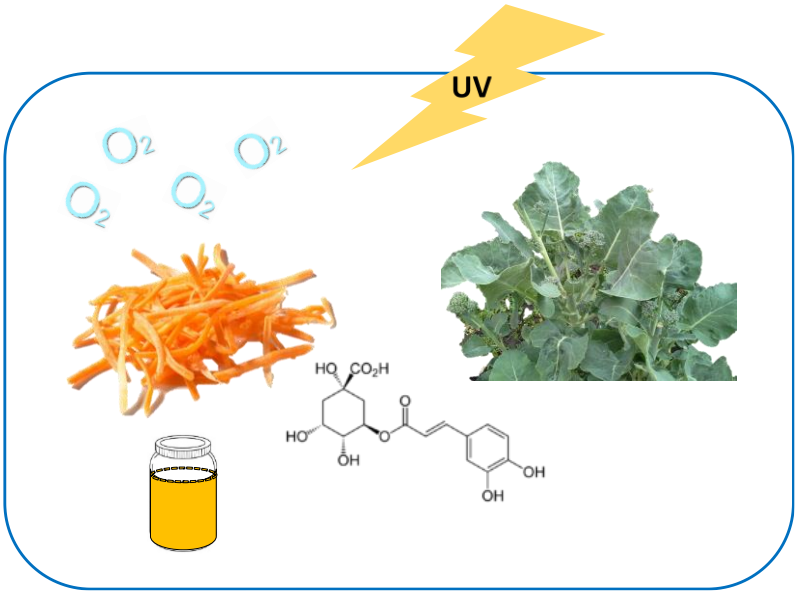
UV-B: ultraviolet B radiation

UV-C: ultraviolet C radiation

V: volts

WHO: World Health Organization

WI: whitening index



INTRODUCTION

1. BOTANICAL AND AGRONOMICAL CHARACTERISTICS OF CARROTS AND BIMI® BROCCOLI

1.1. Carrots

Carrots (*Daucus carota* L.) is a specie originary from Southeast Asian and Mediterranean countries. They have been cultivated and consumed from the ancient times by Greeks and Romans. The first cultivated carrots had purple colour being the actual orange carrots derived from plant breeding selections developed in 1700 in The Netherlands.

Carrot is a biannual plant. During the first year a rosette of few leaves and the root is formed. After a rest period, a short stem emerges in which the flowers are formed during the second growing station. The different carrot development stages can be observed in Figure 1.

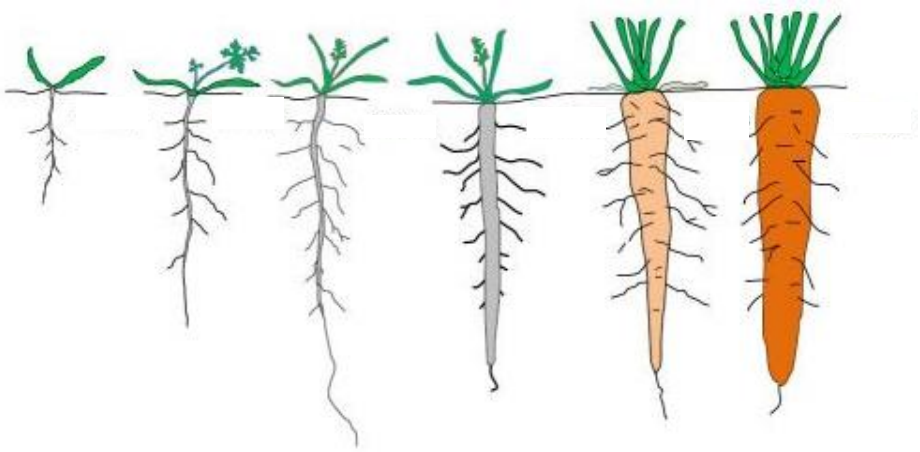


Figure 1. Development stages of carrots
(http://www.carrotmuseum.co.uk/cultivation.html)

Different zones can be observed in carrots when cut: an exterior zone, mainly formed by the phloem and another interior area constituted by the xylem, medulla and cambium (Figure 2). The most accepted carrots are those with a high proportion of phloem since the xylem is generally woody with low flavour. There are several visual and sensory properties of carrots which difference the diverse varieties of carrots for fresh and/or fresh-cut. In general, carrots must meet the following criteria:

- Firm (not flaccid or limp)
- Straight with a uniform taper from ‘shoulder' to ‘tip’
- Bright orange
- There should be little residual ‘hairiness’ from lateral roots
- No ‘green shoulders’ or ‘green core’ from exposure to sunlight during the growth phase
- Low bitterness from terpenoid compounds
- High moisture content and high reducing sugars are most desirable for fresh consumption

Quality defects include lack of firmness, non-uniform shape, roughness, poor colour, splitting or cracking, green core, sunburn, and poor quality of tops or trimming. Carrots can be harvested by three different ways: manual harvest (only in small fields), semi-mechanical harvest (using specified tools coupled to the tractor) and the mechanical harvest (the most used due to low personal needs which lead to lower harvesting costs).

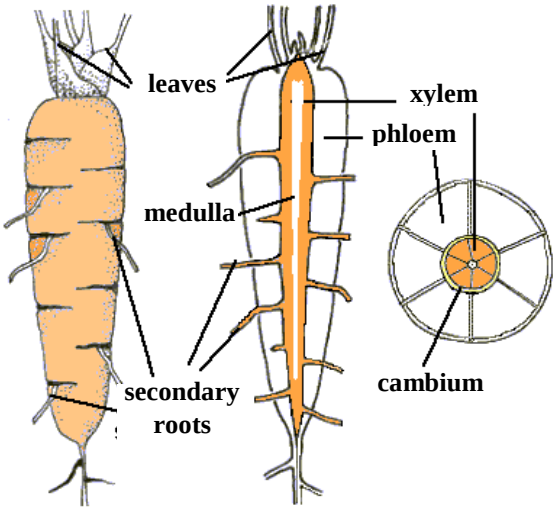


Figure 2. Parts of carrot root
(https://mibayra.wordpress.com/unidad-iv-organografia-de-la-raiz/)

There are several group of cultivars of carrots as it can be observed in Figure 3. Among them, Nantes is the most consumed carrot in Spain. The cultivated surface of carrots in Spain was 6.7 million of ha in 2015 with a production of 403.4 million tons (MAGRAMA, 2016a). The highest carrot producer is China with 17.3 million tons in 2014 (FAO, 2016).

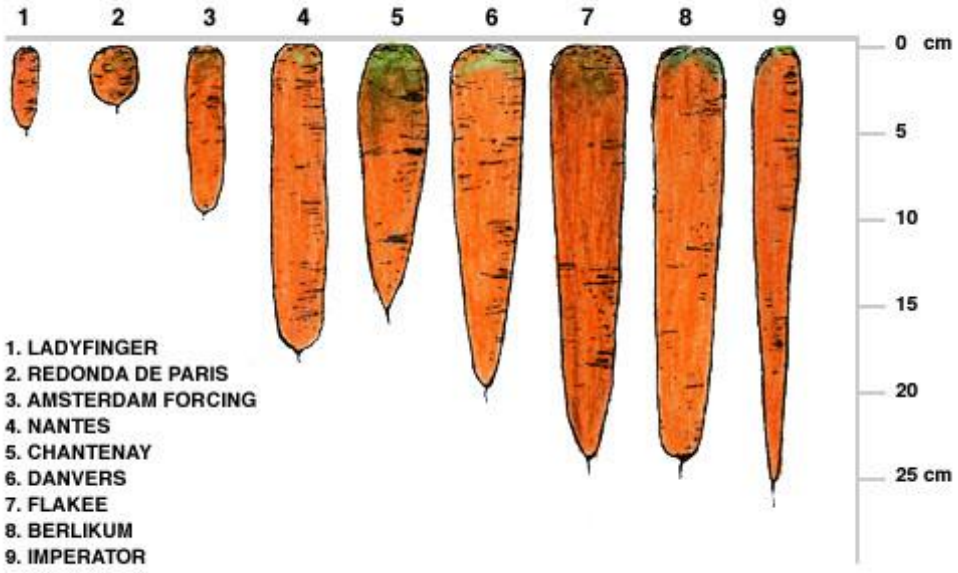


Figure 3. Groups of carrots cvs
(http://es.slideshare.net/reymundcosmocerno/cultivo-de-zanahoria).

1.2. Bimi® broccoli

Bimi® (Sakata Vegetables Europe) is a natural hybrid between conventional broccoli (*Brassica oleracea*, Italica group) and kailan (*B. oleracea*, Alboglabra group). This hybrid was firstly developed by the Sakata Seed Company of Yokohama (Japan). Other companies have developed different commercial kailan-hybrid broccoli varieties with registered trademarks: *Asparation* (Sakata Seed America), *Bellaverde* (Seminis Vegetable Seeds), *Broccolini* (Mann Packaging Company), *Tenderstem* (Marks and Spencer Plc.), etc. This vegetable was firstly commercialized by Sabon Incorporated, which made a commercial program to sell *Asparation* in México in 1994. Mann Packing Company introduced the new vegetable to the USA market in 1998. Lately, its cultivation has been extended to other countries such as the northern European countries, Brazil or Australia, among others.

This vegetable is characterized by a floret at the end of each stem (Figure 4). Similar to conventional broccoli, Bimi® broccoli has yellow flowers. The Bimi® broccoli has a flavour softer and more delicate than the conventional broccoli. Figure 5 shows the large differences in visual appearance between this new vegetable and broccoli.

Figure 4. Bimi® broccoli: A hybrid between conventional broccoli and kailan (http://www.bellaverde.co.uk/).

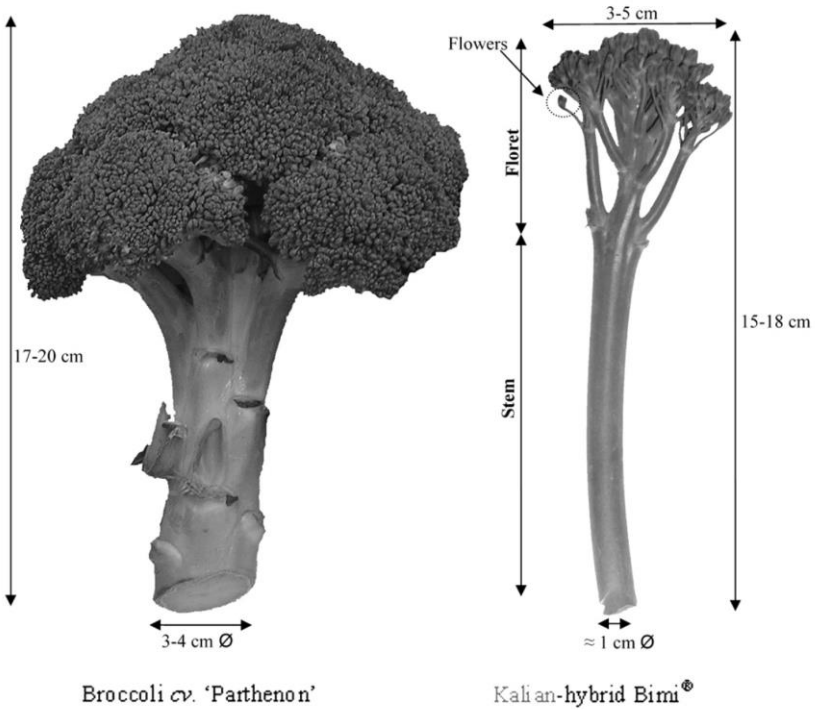


Figure 5. Conventional broccoli (cv. Parthenon) (left) and Bimi® broccoli (right) (Martínez-Hernández et al., 2013).

Bimi® broccoli, due to its delicate physical properties, is manually collected every day (in the first hours of the morning). In order to extend the postharvest shelf life of the Bimi® broccoli by minimizing the handling, its primary packaging is made on the fields. Due to the mild sensory characteristics of Bimi® broccoli, compared to the conventional

broccoli cv., it can be, apart from cooking, eaten either raw (i.e., in salads, *crudité*, etc.) or used as an excellent source of nutritional/bioactive compounds for smoothie preparation.

In Europe, the production of Bimi® broccoli is concentrated from October to June in warm areas of Spain, while in the summer months it is produced in northern locations as UK, The Netherlands, etc. However, the most important commercial production is located in Africa, where the production of this vegetable is assured all year around with intensive farming systems. Spain is among the main European producers of Bimi® broccoli with a cultivated surface (principally concentrated in the southeast area) of 150 ha (130 ha for exportation) for the campaign 2016/17 (data supplied by Sakata Seed Ibérica). Consumption of Bimi® broccoli has already begun in many European countries such as Belgium, UK, France, Germany, The Netherlands and the Scandinavian countries, but so far it does not practically present in the Spanish market.

2. NUTRITIONAL AND BIOACTIVE CONTENTS OF CARROTS AND BIMI® BROCCOLI

2.1. Relevant food and health terminology

Different terms are related to the health effects of phytochemicals on food products. Accordingly, they are defined:

- Phytochemicals: chemical compounds that naturally occur in plants.
- Nutrient or nutritional compound: are compounds that the organism needs to develop, in a normal form, the physiological and metabolic processes. They are divided in macronutrients (carbohydrates, fats, dietary fibre, proteins and water) and micronutrients (minerals and vitamins). Macronutrients provide the bulk energy that the organism's metabolic system needs to function while micronutrients provide the necessary cofactors for metabolism to be carried out. Micronutrients are used to build and repair tissues and to regulate body processes while macronutrients are converted to, and used for, energy (Regulation, 2006).

- Bioactive compounds: compounds which have the capability and the ability to interact with one or more component(s) of the living tissue by presenting a wide range of probable effects (Guaadaoui et al., 2014).
- Nutraceuticals: are diet supplements that deliver a concentrated form of a presumed bioactive compound from a food, presented in a non-food matrix (pills, extracts, tablets, etc.), and used with the purpose of enhancing health in dosages that exceed those that could be obtained from normal foods (Zeisel, 1999). There is not, as such, a regulatory framework for nutraceuticals in the EU Food Law contrary to USA where it is regulated by the FDA.
- Functional foods: the concept of functional food was developed in Japan in the mid-1980s. While a globally accepted definition has yet, functional foods can be defined as those foods that when consumed regularly exert a specific health-beneficial effect beyond their nutritional properties (i.e., a healthier status or a lower risk of disease) and this effect must be scientifically proven (Espin et al., 2007). A functional food can be a natural whole food, a food to which a component has been added, or a food from which a component has been removed by technological or biotechnological means. It can also be a food in which the nature of one or more components has been modified, or a food in which the bioavailability of one or more components has been modified, or any combination of these possibilities. A functional food may be targeted at the whole population or for particular groups, which may be defined, for example, by age or by genetic constitution (Regulation, 2006).
- Fortified foods: 'Fortification' or 'enrichment', is the 'addition of one or more essential nutrients to a food whether or not it is normally contained in it, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups' (Commission, 2015). For example, fortification of plant-derived products with vitamin B12 is a good alternative for vegetarians to uptake this essential vitamin without the need of dietary supplements or vitamin B12 pills. Accordingly, several studies, mainly from industrialized nations, have demonstrated the benefits of vitamin B12 supplementation in such susceptible population groups through fortified products (Allen et al., 2006; Molina et al., 2012).

2.2. Nutritional compounds of carrots and broccoli

Carrots and Bimi® broccoli are good sources of nutritional compounds as observed in Table 1. Both vegetables are excellent sources of dietary fibre and minerals while accounting low fat content. Particularly, Bimi® broccoli is higher than carrots in proteins, and vitamins C and B group; while carrots have higher levels of carotenoids (*β*-carotene 46 % of total carotenoids) and vitamin A.

Table 1. Main constituents of carrots and Bimi® broccoli (Martínez-Hernández, 2012; Miglio et al., 2008; Souci et al., 2000).

Constituents	Content (per 100 g^{-1} fresh weight)	
	Carrots	Bimi® broccoli
Water	88 g	90 g
Protein (*N×6.25*)	1.0 g	3.5 g
Fat	0.2 g	0.3 g
Carbohydrates	4.8 g	5.1 g
Total dietary fibre	3.6 g	3.2 g
Total carotenoids	14.1 mg	2.8 mg
β-carotene	6.5 mg	0.6 mg
Lutein	1.3 mg	1.5 mg
Vitamins		
Vitamin C	7.0 mg	174 mg
Vitamin A	1500 µg	177 µg
Vitamin B1	69 µg	99 µg
Vitamin B2	53 µg	178 µg
Folic acid (vit. B9)	26 µg	120 µg
Total minerals		
Major minerals (mg)		
P	35	112
Na	61	29
K	321	437
Ca	37	97
Mg	13	38
Cl	59	28
Trace minerals (µg)		
Fe	386	521
Mn	175	563
Zn	273	595
Al	169	98
Cu	50	70
Ni	5.7	18

2.3. Main bioactive compounds of carrots and broccoli

The dietary habits and disease risk relationship has been analysed in several epidemiological studies showing that plant-derived foods, fruit and vegetables in a great extent, have a direct impact on health (Slavin and Lloyd, 2012). Such health-promoting properties of fruit and vegetables have been mainly associated to the existence of bioactive compounds such as phenolic compounds, glucosinolates, carotenoids, chlorophylls, among others. Carrots and Bimi® broccoli are vegetables which may provide a large list of bioactive compounds, being phenolic compounds and glucosinolates of special interest due to their broad health-promoting properties.

2.3.1. Phenolic compounds: biosynthesis, classification and health-promoting properties

'Phenolic compounds' is a generic term that include a large number of compounds (more than 8,000) widely dispersed throughout the plant kingdom and characterized by having at least one aromatic ring with one or more hydroxyl groups attached (Figure 6). In nature, phenolics are usually found conjugated to sugars and organic acids (Cartea et al., 2011).

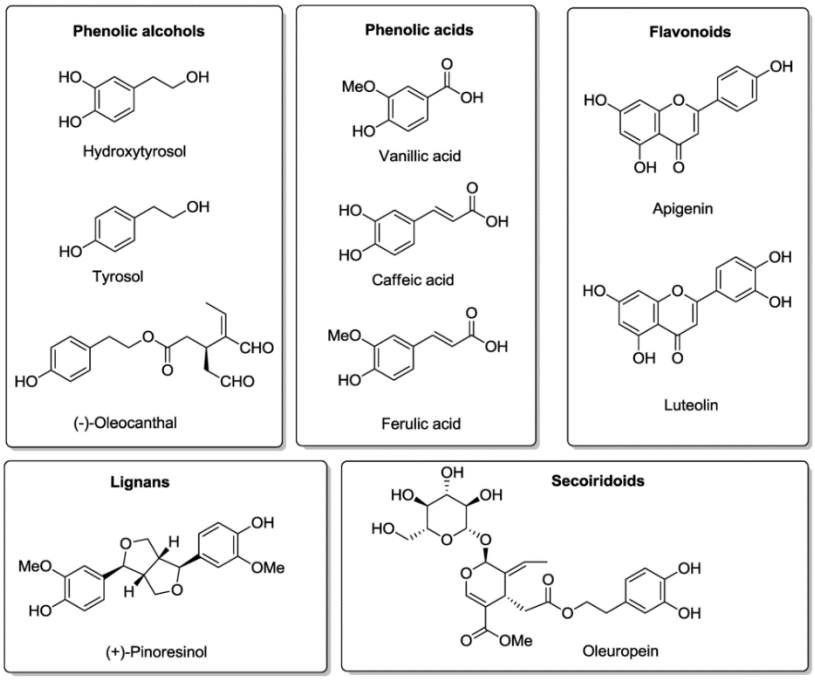


Figure 6. Phenolic compounds examples

The major biosynthetic routes to the various classes of phenolic compounds are summarized in Figure 7, which include the primary (shikimic acid) and secondary (phenylpropanoid) metabolic pathways. As it can be observed, the enzyme PAL plays a key role in the first stages of the pathway model. Particularly, PAL catalyses the non-oxidative deamination of L-phenylalanine resulting in trans-cinnamic acid and a free ammonium ion which is first step in the phenolic biosynthesis.

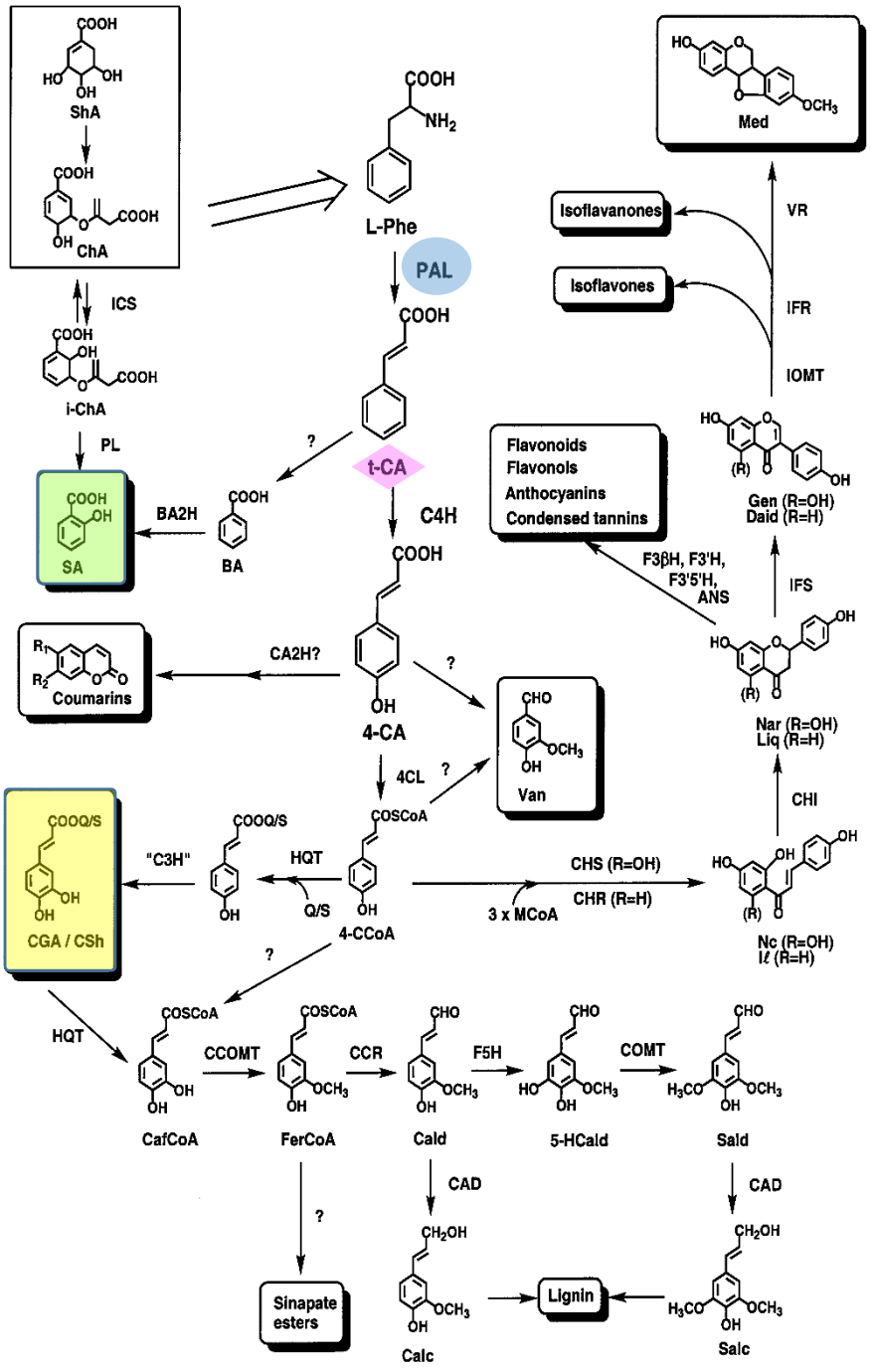


Figure 7. Biosynthetic pathways leading to phenolic compounds in plants (Dixon et al., 2002). The core reactions are shown in larger type. Abbreviations: BA, benzoic acid; BA2H, benzoic acid 2-hydroxylase;

t-CA, trans -cinnamic acid; 4-CA, 4-coumaric acid; CA2H, cinnamate 2-hydroxylase; Calc, coniferyl alcohol; Cald, coniferaldehyde; CafCoA, caffeoyl CoA; 4-CCoA, 4-coumaroyl CoA; **CGA, chlorogenic acid**; C3H, coumarate (coumaroyl quinate/shikimate) 3-hydroxylase; C4H, cinnamate 4-hydroxylase; ChA, chorismic acid; i-ChA, isochorismic acid; 4-CL, 4-coumarate:CoA ligase; CHR, chalcone reductase; CHS, chalcone synthase; COMT, caffeic acid O-methyltransferase; Csh, 4-coumaroyl shikimate; Daid, daidzein; FerA, ferulic acid; FerCoA, feruloyl CoA; Gen, genistein; 5-HCald, 5-hydroxyconiferaldehyde; HQT, hydroxycinnamoyl-oA:quinate hydroxycinnamoyl transferase; ICS, isochorismate synthase; IFR, isoflavone reductase; IFS, isoflavone synthase; Il, isoliquiritigenin; IOMT, isoflavone O-methyltransferase; Liq, liquiritigenin; MCoA, malonyl CoA; Med, medicarpin; Nar, naringenin; Nc, naringenin chalcone, **PAL, L-phenylalanine ammonia-lyase**; L-phe, L-phenylalanine; PL, pyruvate-lyase; SA, salicylic acid; Salc, sinapyl alcohol; Sald, sinapaldehyde; **ShA, shikimic acid**; Van, vanillin; VR, vestitone reductase.

Phenolic compounds can be classified based on the number and arrangement of their carbon atoms in flavonoids (flavonols, flavones, flavan-3-ols, anthocyanidins, flavanones, isoflavones and others) and non-flavonoids (phenolic acids, hydroxycinnamates, stilbenes and others) as it is described below (Cartea et al., 2011; Crozier et al., 2006) and shown in Figure 8.

- Phenolic acids have one carboxylic acid group and may be present in plants in free and bound forms. Phenolic acids are divided into two subgroups, the hydroxybenzoic and hydroxycinnamic acids and derivatives thereof. Hydroxybenzoic acids have in common the C6-C1 structure, and the hydroxycinnamic acids have aromatic compounds with a three-carbon side chain (C6-C3). The different phenolic acids differ in the number and position of the hydroxyl and methoxyl groups attached to the aromatic ring.
- Coumarins, or chromones, are derived from cinnamic acid by cyclization of the side chain of the o-coumaric acid. Commonly, they are present as glycosides.
- Flavonoids are the largest group of plant phenolic compounds. They have a structure of 15 carbon atoms (C6-C3-C6). The aromatic ring A is derived from the acetate/malonate pathway, and ring B is derived from phenylalanine through the shikimate pathway. Variations in substitution patterns to ring C (oxygenation, alkylation, glycosylation, acylation or sulfation) result in 13 flavonoid classes, being the most important flavonols, flavones, isoflavones, flavanones, flavanols (also called flavan-3-ols) and anthocyanidins or anthocyanins. The chalcones are intermediate in the biosynthesis of flavonoids.

- Stilbenes are a small group of phenolics characterized by a 1,2-diphenylethylene backbone. Most plant stilbenes are derivatives of the basic unit trans-resveratrol (3,5,4′-trihydroxy-trans-stilbene). In plants that naturally produce stilbenes, these metabolites are generally accumulated in both free and glycosylated forms.
- Lignans are formed of two phenylpropane units, which are commonly present in fruits, seeds, grains, trees and vegetables. Secoisolariciresinol and matairesinol were the first plant lignans identified, and later pinoresinol, lariciresinol and others.
- Tannins are phenolic compounds of molecular weight between 500 and 3000 D and may be subdivided into: hydrolysable, esters of gallic acid (gallo- and ellagitannins), condensed tannins (also known as proanthocyanidins), polymers of polyhydroxyflavan-3-ol monomers, and phlorotannins, found in brown seaweeds.

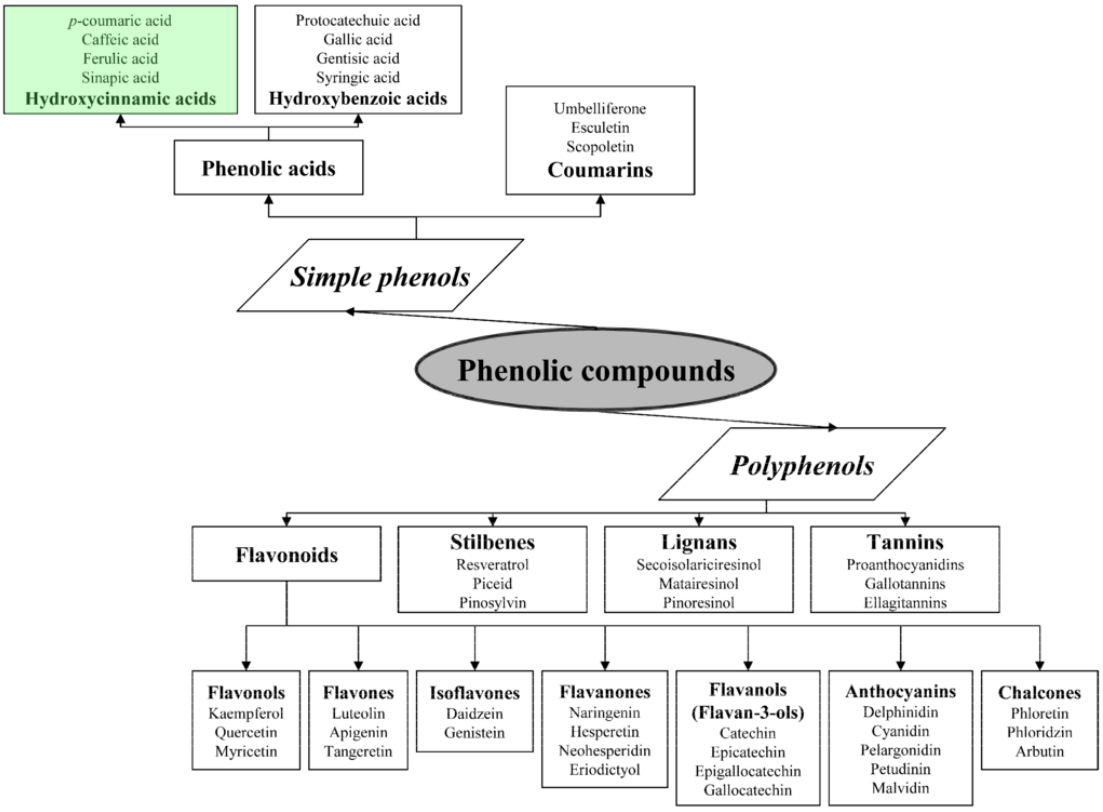


Figure 8. Classification of phenolic compounds (Soto et al., 2015)

The phenolic compounds contribute to flavour and colour of the plants. Nevertheless, the major of the intense research on them is focused on their many bioactive properties such as antimicrobial, antiviral, anti-inflammatory, antitumor, anticancer, antimutagenicity and reduction in coronary heart disease risk (Lule and Xia, 2005). Phenolic compounds

are strong antioxidants that potentiate the activity of antioxidant vitamins and enzymes serving as a defence system against oxidative stress, namely by the accumulation of reactive oxygen species (ROS). Phenolic compounds have what is considered the ideal chemical structure for free radical-scavenging activity since they gather the necessary properties to define an antioxidant, such as reactivity as a hydrogen/electron-donating agent and the transition metal-chelating potential (Tsao, 2010).

In carrots, the higher content of phenolic compounds is found in the peel followed by phloem and then xylem (Figure 9). The main phenolic compounds found in carrots are hydroxycinnamic acids and their derivatives, being chlorogenic acid (an ester of caffeic acid and quinic acid) the major compound as observed in Table 2 (Zhang and Hamauzu, 2004).

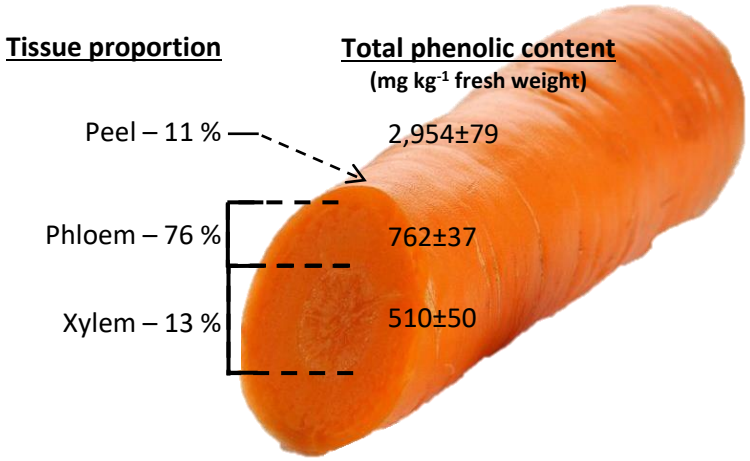


Figure 9. Tissue proportion and distribution of total phenolic content activity within the Nantes carrot tissue (elaborated from Alegria et al. (2016)).

Table 2. Main phenolic compounds of carrots (elaborated from Zhang and Hamauzu (2004))

Phenolic compound	**Phenolic content (mg kg^{-1} fresh weight)**		
	Peel	**Phloem**	**Xylem**
3'O-caffeoylquinic acid (chlorogenic acid)	150.4	18.3	1.9
Cis-5'caffeoylquinic acid	35.1	5.4	1.3
3'5'-dicaffeoylquinic acid	18.4	0.6	ND
4'p-coumaroylquinic acid	11.2	0.6	0.2
3'4'-dicaffeoylquinic acid	2.8	0.1	ND
Caffeic acid	1.1	0.7	0.1
3'O-caffeoylquinic acid	0.9	1.1	1.0

Bimi® broccoli has shown a total phenolic content (TPC) of 1148 mg kg^{-1} (Martínez-Hernández et al., 2013a). The higher phenolic contents in Bimi® broccoli is located in the floret with 3.1-fold higher content than the stem (Figure 10). The major phenolic compounds in broccoli are flavonoids (mainly flavonols but also anthocyanins) and the hydroxycinnamic acids (Table 3). TPC, and sinapic and caffeic acid derivates contents of 15.5-26.9, 4.2-7.9 and 0.4-3.2 mg g^{-1} dry weight, respectively, have been reported in 13 different broccoli cvs. (Redovniković et al., 2012). The phenolic compounds found in broccoli are summarized in Table 3. Furthermore, broccoli has been reported as one of the main dietary sources of lignans, comprising coumestans, the main group in this *Brassica* (de Kleijn et al., 2001).

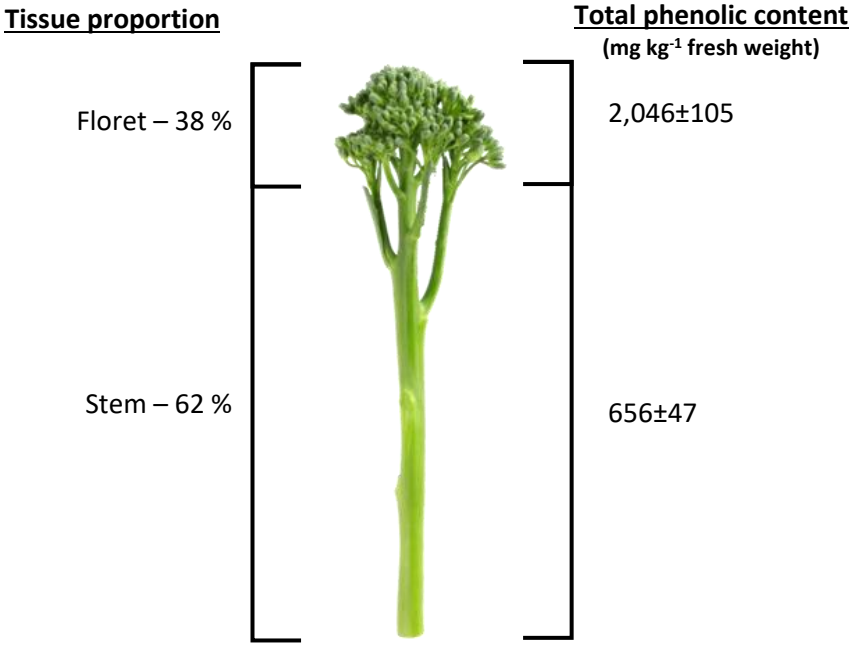


Figure 10. Tissue proportion and distribution of total phenolic content within Bimi® broccoli.

Table 3. Major phenolic compounds in broccoli (Vallejo et al., 2003).

Phenolic compound	Phenolic content (mg kg^{-1} fresh weight)
Caffeoylquinic derivatives	
Neochlorogenic acid	71.8
Chlorogenic acid	22.0
Sinapic acid derivatives	
1,2-Disinapoylgentiobiose	25.5
1-Sinapoyl-2-feruloylgentiobiose	32.7
1,2,2_-Trisinapoylgentiobiose	40.8
1,2_-Disinapoyl-2-feruloylgentiobiose	9.5
1-Sinapoyl-2,2_-diferuloylgentiobiose	2.4

1,2,2_-Trisinapoylgentiobiose	5.8
Feruloyl acid derivatives	
1,2-Diferuloylgentiobiose	51.1
Total flavonoids	659.3

2.3.2. Glucosinolates: biosynthesis, classification and health-promoting properties

The glucosinolates, previously known as mustard oils (from where they were discovered in the 17th century), are sulphur-containing compounds mainly found in the *Brassicaceae* family. Glucosinolates are derived from amino acids and can thus be divided into three groups according to their amino acid precursor: aromatic (derived from Leu, Ile, Val, and Met), aliphatic (Met), alkenyl (Met) and indoles glucosinolates (Trp). The glucosinolates biosynthesis in *Brassicas* proceeds through three independent stages: (a) chain elongation of selected precursor amino acids (only Met and Phe), (b) formation of the core glucosinolate structure, and (c) secondary modifications of the amino acid side chain (Figure 11). Together with side-chain elongation, secondary modifications are responsible for the >120 known glucosinolates structures (Fahey et al., 2001).

Myrosinase (thioglucoside glucohydrolase) is largely stored in separate cell compartments from the glucosinolates. When plant cells are damaged (i.e., during food preparation, mastication or injuries caused by predators, such as insects), glucosinolates come into contact with the myrosinase. Then, this enzyme catalyses the glucosinolates conversion to isothiocyanates (ITC) after several reactions described in Figure 12 (Cartea and Velasco, 2008). Contrary to previous studies, myrosinase-like activity in animal and human gut microflora has been recently reported to be inefficient and highly variable in humans (Bricker et al., 2014). Other products of the glucosinolate hydrolysis can be thiocyanates and nitriles, both without bioactive properties, depending on the pH or the presence of metal ions. Glucosinolates are water soluble, anionic, non-volatile and heat-stable compounds. It is believed that these molecules have no significant biological activity. Contrary to them, ITC are biologically active, typically lipophilic, highly reactive, volatile, malodorous and bitter (Fahey et al., 2001). A range of ITC, such as sulforaphane (derived from the glucosinolate glucoraphanin), have been considered strong chemopreventive compounds together with other antimicrobial properties and prevention of cardiovascular disease (reviewed by Traka and Mithen (2009)).

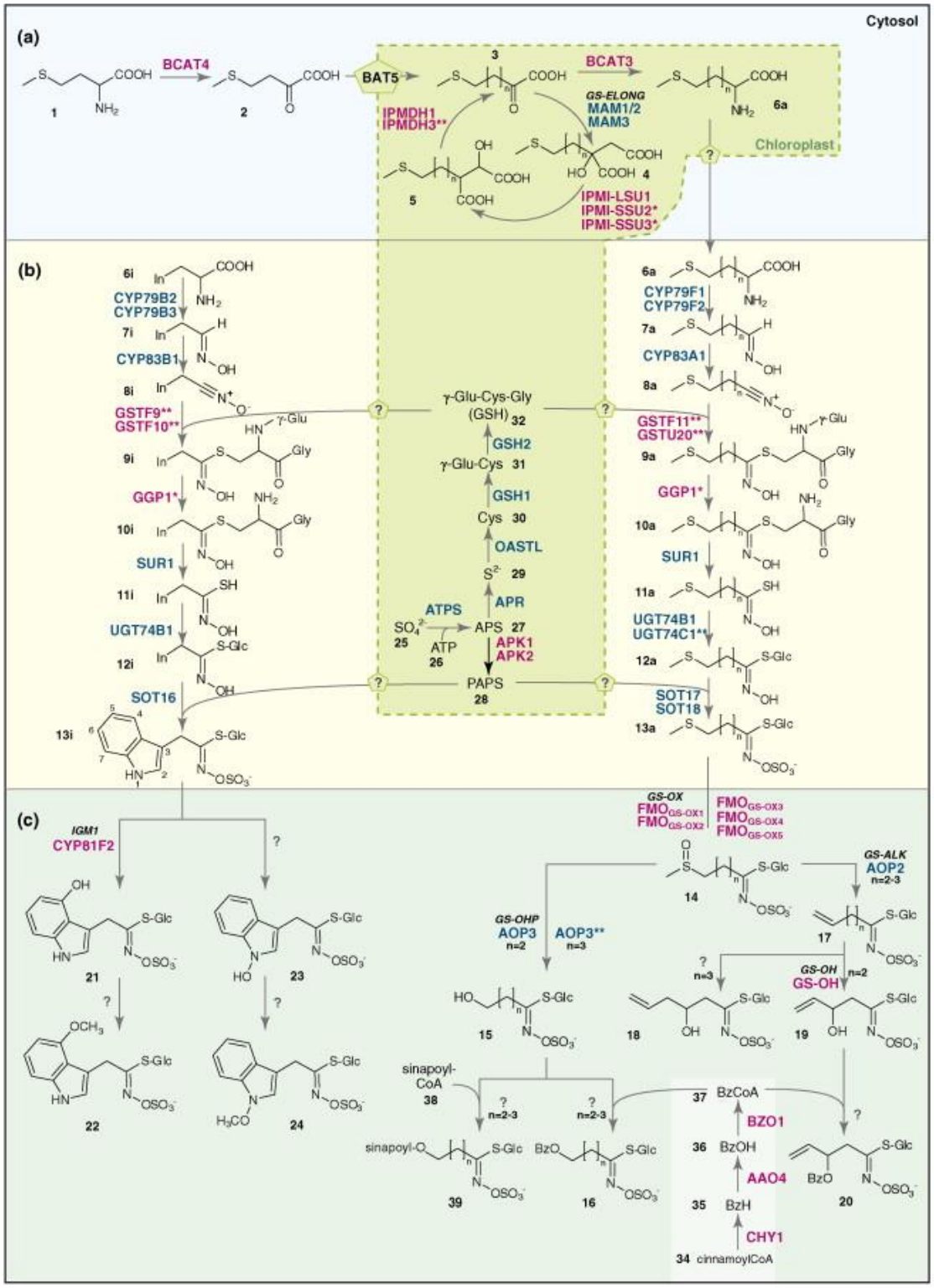


Figure 11. Aliphatic and indolic glucosinolate biosynthetic pathways in the *Brassica Arabidopsis thaliana* (Sonderby et al., 2010): (a) Chain elongation machinery; (b) Biosynthesis of core glucosinolate structure; (c) Secondary modifications. Abbreviations: APR, APS reductase; APS, adenosine-50-phosphosulfate; Bz, benzoyl; BzH, benzaldehyde; BzOH, benzoic acid; BzCoA, benzoyl-coenzyme A; In, 30-indolyl; OAS-TL, O-acetylserine(thiol)lyase; PAP, adenosine-30,50-bisphosphate; and PAPS, 30-phosphoadenosine-50-phosphosulfate.

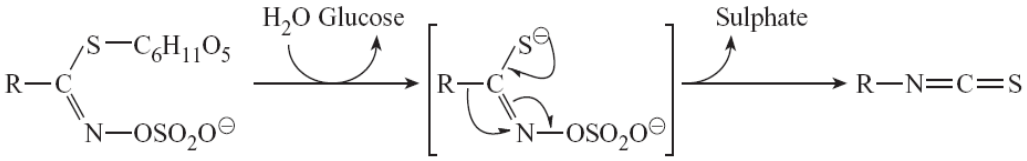


Figure 12. Enzymatic conversion of glucosinolates to ITC by plant myrosinase (Shikita et al., 1999).

Broccoli cvs. have high contents of glucosinolates (Kushad et al., 1999). The main glucosinolates found in Bimi® broccoli are glucoraphanin, glucobrassicin, gluconapin and proigoitrin as observed in Table 4 (Martínez-Hernández et al., 2013c).

Table 4. Glucosinolates distribution in parts of Bimi® broccoli (Martínez-Hernández et al., 2013c).

	Floret	Stem	Whole
Aliphatic			
Glucoraphanin (4-Methylsulphinylbutyl)	1.53	1.14	3.47
Glucobrassicanapin (4-Pentenyl)	0.71	0.24	1.45
Alkenyl			
Gluconapin (3-Butenyl)	0.02	0.02	0.05
Indolyl			
4-Hydroxyglucobrassicin (4-Hydroxy-3-indolylmethyl)	0.09	0.12	0.25
Glucobrassicin (3-Indolylmethyl)	3.41	0.46	4.76
4-Metoxyglucobrassicin (4-Methoxy-3-indolylmethyl)	1.40	0.52	1.74
Neoglucobrassicin (N-Methoxy-3-indolylmethyl)	0.26	0.20	0.60
Aromatic			
Gluconasturtiin (2-Phenylethyl)	0.23	0.13	0.41
TOTAL	7.66	2.82	12.73

2.3.3. Antioxidant compounds and their classification

ROS are chemically reactive molecules containing oxygen. ROS include oxygen ions (i.e., $^1O_2$), free-radicals (O_2^-, ·OH, NO·, etc.) and peroxides (H_2O, $ONOO^-$, etc.). ROS are highly reactive due to the presence of unpaired valence shell electrons. ROS form as a natural by-product of the normal metabolism of oxygen and have important roles in cell signalling and homeostasis. However, under exogenous (heat exposure, ultraviolet light, O_3, contaminants, additives, tobacco, drugs, etc.) or endogenous (monoelectronic O_2 reduction, auto-oxidation of carbon compounds, catalytic activation of several enzymes, etc.) stresses, ROS levels can increase dramatically. This may result in damage to cell structures. Cumulatively, this is known as oxidative stress. In this way, the antioxidants are compounds that at low concentrations, compared to the substrate, delay or prevent the

oxidation of that substrate during an oxidative stress. According to its nature, these compounds may be classified as enzymatic or non-enzymatic antioxidant compounds (Figures 13 and 14). The total antioxidant capacity (TAC) of a sample is determined by the synergistic interactions between different antioxidant compounds and for the specific reaction mechanism of each of them. The TAC can be influenced by physiological (i.e., ripening, senescence) and technological factors such as storage and processing conditions (Devasagayam et al., 2004).

Several epidemiological studies have shown that fruit and vegetables-rich diets reduce the incidence of cardiovascular and other chronic and degenerative diseases related to the oxidative damage (Balasundram et al., 2006; Dragsted, 2003). Thus, the protective effects of the fruit and vegetables consumption have been associated to the presence of antioxidant compounds, mainly polyphenols and vitamin C (Scalbert et al., 2005).

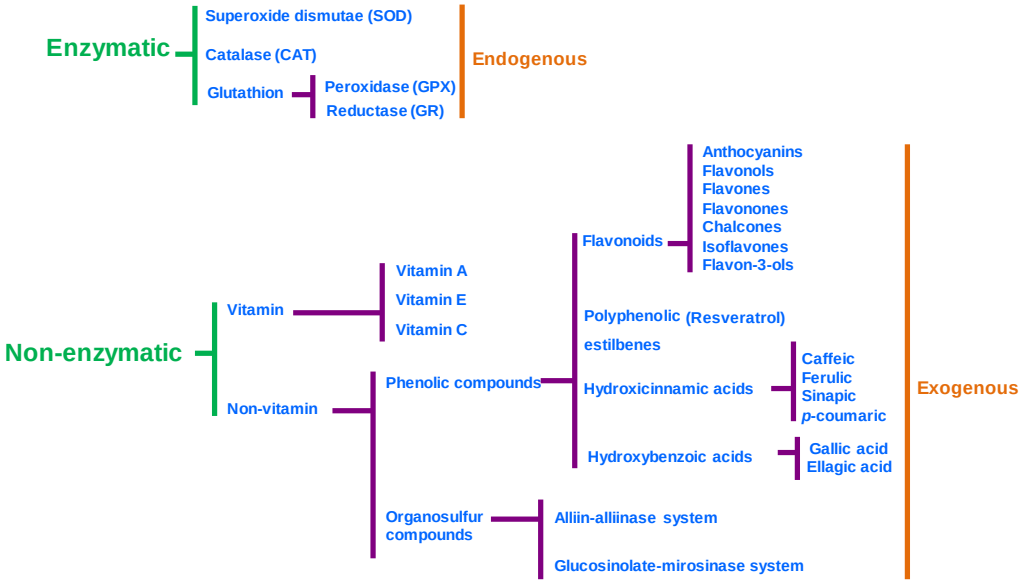


Figure 13. Antioxidant compounds classification (Artés-Hernández et al., 2009b).

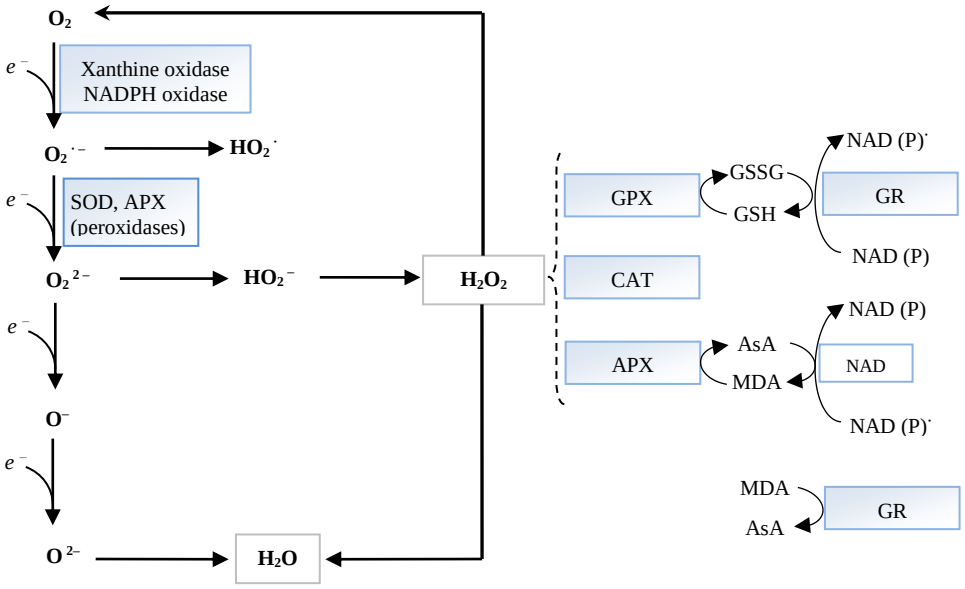


Figure 14. Antioxidant enzymes pathways (Gärtner and Wese, 1986). AsA: Ascorbate; APX: Ascorbate peroxidase (EC 1.11.1.11); CAT: Catalase (EC 1.11.1.6); GPX: Guaiacol peroxidase (EC 1.11.1.9); GR: Gluaiacol reductase (EC 1.6.4.2); GSH: Glutathione; GSSG: Glutathione disulfide; MDA: Monodehydroascorbate; NADH: Monodehydroascorbate reductase (EC 1.6.5.4); NAD: Nicotinamide adenine dinucleotide; NADPH: Nicotinamide adenine dinucleotide phosphate (EC 1.1.1.184); SOD: Superoxide dismutase (EC 1.15.1.1).

3. MINIMAL PROCESSING OF FRUIT AND VEGETABLES

3.1. Overview

The actual consumer profile is characterized with a high interest in a healthy diet but with little time to prepare the meals playing convenience a key factor in the consumer food choice. Furthermore, sensory aspect is also crucial, together with healthiness and convenience, in the final consumer decision. Traditionally, the use of intense thermal treatments (pasteurization, sterilization, etc.) to ensure the food safety has prevailed over the nutritional/bioactive and sensory properties of food. However, the interest of the food industry in mild thermal treatments, generally combined with low temperature storage, and non-thermal treatments (high pressure processing, irradiation, ultrasound, pulsed light and pulsed electric fields, etc.) is highly increasing with special focus in this research area. Furthermore, the consumer interest in food free from additives has led to an intense research on new preservatives of natural origin and specialized technology (essential oils, modified atmosphere packaging, nanotechnology, etc.). According to such scenario, new

food products, such as fresh-cut and fifth range products, are being developed with excellent nutritional/bioactive and sensory quality while meeting always the food safety aspects.

3.2. Fresh-cut products

The fresh-cut products are ready-to-eat products, elaborated free from additives by using light combined methods such as washing, cutting, sanitation, packaging (under modified atmosphere packaging, MAP) and chilling (Allende and Artés, 2005). These products usually do not need further processing prior to consumption. Depending of the country and preparation form, these products have received several denominations such as Fourth Range ('*4ème Game*') in the French-speaking countries, and minimally fresh processed, slightly processed, partially processed, ready-to-eat, ready-to-use or fresh-cut in the Anglo-Saxon countries. In Spain there is not a consolidated denomination, but 'minimally fresh processed' has been suggested (Artés, 2000).

New technologies and techniques are continuously being applied by the fresh-cut industry with the purpose of extending the shelf life of these products with the best sensory and nutritional/bioactive quality while meeting the regulations related to food safety. Thus, the high convenience of fresh-cut products together with their good sensory properties and high contents of health-promoting compounds present many advantages for consumers and food services (Artés, 2000; Wiley, 1994):

- Reduced preparation time (ready-to-eat)
- Similar characteristics to the original plant material (fresh-like)
- Provide a uniform and consistent high quality
- Easy supply of healthy products
- Reasonable price
- Easy to store with little storage space
- Low waste wage

3.3. Fifth range products

The application of heat treatments is one of the most ancient food preservation methods. The sterilization treatment has allowed the appearance of canned ready-to-eat meals from traditional recipes and with a long commercial life without refrigeration. Nevertheless, sterilization treatments are very aggressive with the nutritional and sensory properties of food, and especially with vegetables, leading to the potential rejection by the consumer. Consequently, the application of mild heat treatments with a subsequent refrigerated storage firstly defined the fifth range industry of vegetables.

Accordingly, the term of fifth range vegetable products was firstly attributed to vacuum-packaged boiled vegetables ready-to-heat and eat. Lately, when the offer of fifth range vegetable products increased they were defined as plant-based products which had been heat-treated to guarantee a conservation period of minimum 6 weeks (Tirilly and Bourgeois, 2002).

Nowadays, the fifth range products could be defined as those products which have received a mild thermal treatment after previous unit operations, in order to achieve the desirable organoleptic characteristics, which are kept in the refrigerator with/without adding preservative or dressings. In the Anglo-Saxon term the fifth range products are sometimes referred as 'Refrigerated pasteurized foods of extended durability' (REPFEDs) (Mossel and Struijk, 1991). The usual processing temperatures for such products is usually between 70 and 95 ºC. After heat treatment these food products are rapidly cooled ('blast chilling') and stored under refrigeration until consumption.

The fifth range vegetables can be described by the following characteristics:

- Heat-treated products, ready-to-eat and marketed under refrigeration.
- Preheated prior to consumption, usually in microwave or conventional oven.
- Usually packaged in plastic material.
- It is required to maintain the cold chain until consumption (processing, packaging, storage and distribution).
- The technology used to preserve consists of packaging, heat treatment and cooling.

3.4. Overview of fresh-cut and fifth range products market

The fresh-cut industry emerged in the early 1970s with the intention of suppling ready-to-eat salads to the fast food establishments. Lately, the fresh-cut market was expanded to other countries like Switzerland and Germany in the 1980s. In the middle of the 1980s, this market was extended to UK, France, The Netherlands and Italy. The introduction of the fresh-cut products appeared in Spain at the beginning of the 1990s (Artés and Artés–Hernández, 2003; Sánchez–Pineda, 2003).

The European fresh-cut industry has shown exponential growth since its appearance in the early 1980s. UK is the major fresh-cut produce consumer because the ready-to-eat product culture is deeply established in that country. In countries like Germany and Spain, in which fresh-cut fruit and vegetables market is still emerging, the market growth in the last years was higher than other countries in which this market is already established, for instance Italy and the Netherlands. In the 2015-2016 campaign the fresh-cut market in Spain increased in a 15 %. Concerning the features of the fresh-cut market, packaged salads appear to be the leader of fresh-cut products, in fact they hold about 50 % of total fresh-cut market volume. The other 50 % is shared by the fresh-cut fruit (10 %) and the other categories as ready-to-cook, crudités and other with 40 % (MAGRAMA, 2016b).

The market of fifth range products has highly increased in the recent years. Europe is one of the most active markets related to this sector, with a 50 % of worldwide market share, followed by USA with a 23 % (period January-June 2010). UK leads the top-five list of countries with the highest turnover of fifth range products. Although Spain is in the last position of the latter list, the consumption of these products in our country showed one of the highest growth rates. The delayed development of fifth range products in Spain, compared to France and UK, has been due to the sociodemographic differences with the latter countries. In this way, this food sector opens a market opportunity (Mintel, 2010).

In Spain, the consumption of the fifth range products increased in a 4.1 % from 2014 to 2015 %. The fifth range products consumption was 12.9 kg $year^{-1}person^{-1}$ in 2015, a 4.6 % higher than in 2014. The average spend was 53.1 € $year^{-1}$ $person^{-1}$ in 2015, a 4.2 % higher than in 2014 (MAGRAMA, 2016b). The new technologies have allowed to increase the offer of such products with better sensory and nutritional/bioactive quality,

which has also favoured the increase of their consumption. The fifth range products perfectly meet the following consumer expectations:

- High sensory and nutritional quality
- Adequate to their new habits
- Fresh and natural products
- Healthy properties
- Microbiologically safe

4. VEGETABLE-BASED SMOOTHIES: A CONVENIENT AND HEALTHY SOLUTION FOR THE ACTUAL CONSUMER

4.1. Definition of smoothie

A high consumption of fruit and vegetables has been related with a prevention of a wide array of diseases, such as degenerative disorders, cancer and cardiovascular among others (Slavin and Lloyd, 2012). Nevertheless, the present lifestyle makes difficult the preparation of these plant products. Thus, the consumption of fruit and vegetables should be promoted through the development of attractive ready-to-eat products that should be processed with minimal and non-aggressive treatments to preserve as much as possible the quality parameters of the raw materials (Artés et al., 2009). In this way, smoothies are an exceptional and convenient alternative to increase the daily consumption of fruit and vegetables (Rodríguez-Verástegui et al., 2015).

Smoothies are non-alcoholic beverages prepared from fresh or frozen fruit and/or vegetables, which are blended without filtering and usually mixed with crushed ice to be immediately consumed. Often, some smoothies may include other components like yogurt, milk, ice-cream, lemonade or tea. They have a milk shake-like consistency that is thicker than slush drinks (Castillejo et al., 2015).

4.2. Units operations during smoothie processing: carrots-based smoothies, a particular case

The unit operations during smoothie production depend of the fruit or vegetable used. Accordingly, different equipment is used for fruits like pear, watermelon, peach, pear, etc., or others like mandarins or oranges. However, the common unit operations for the production of fruit and vegetables smoothies are described in Figure 15.

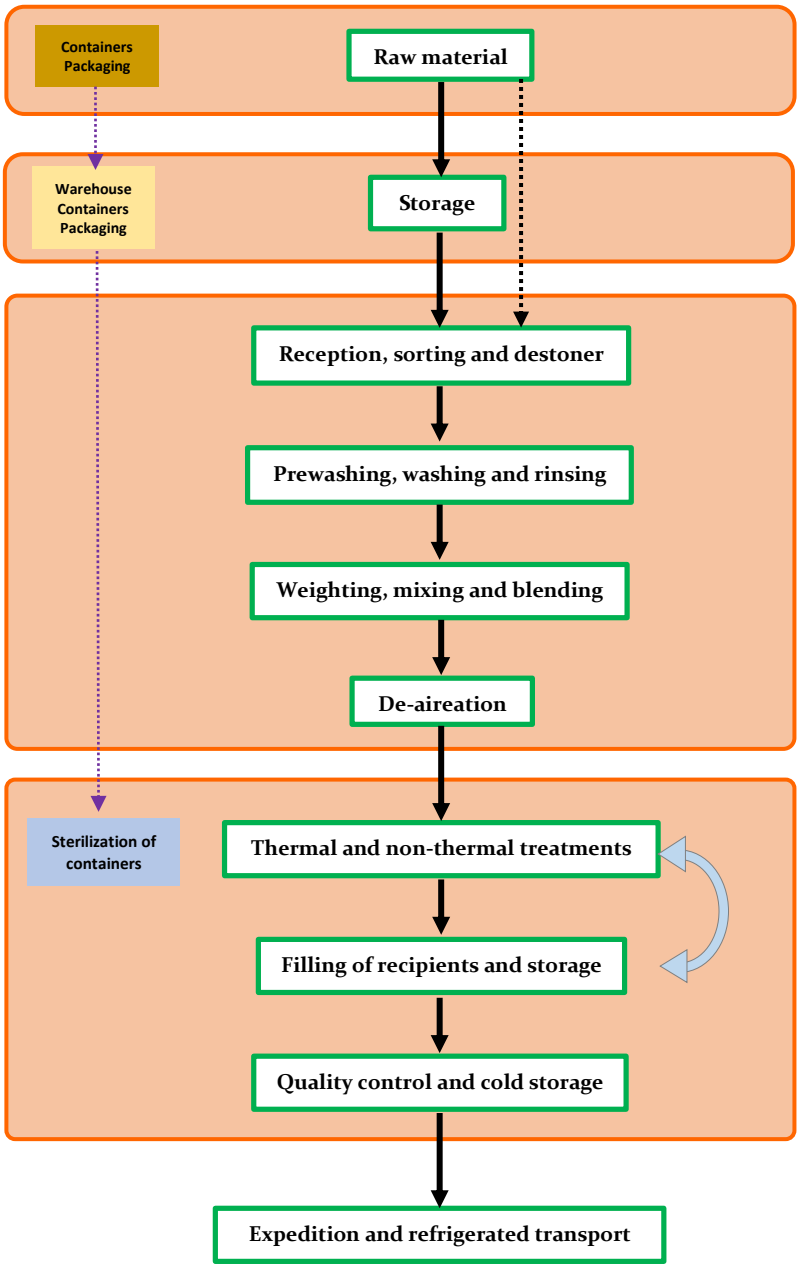


Figure 15. Common unit operations during smoothie processing

4.2.1. Raw material

The plant material must have a high quality at harvest which will contribute to obtain a smoothie with excellent quality. Generally, the quality of fruit and vegetables at harvest may be influenced by the following preharvest factors:

- Genetic factors: varieties, etc.
- Climate conditions: light, temperature, relative humidity (RH), pluviometry, etc.
- Soil conditions: soil type, pH, humidity, microbiota, mineral composition, etc.
- Agricultural practices: fertilization, pesticides, irrigation type, etc.

4.2.2. Reception, sorting and destoner

The first operation upon reception of the raw material at the processing plant is a quality inspection. If the plant material presents inadequate characteristics it must to be rejected to the producer. The weight of the classified raw material is necessary in order to control the processing steps, formulation of the product and quality control. In order to regularize the supply to the processing line, the raw material may be stored at 0-4 ºC and high RH (90-95 %).

Product sorting may be accomplished based on visual quality and uniformity of plant material. These parameters will facilitate all the subsequent processing steps, increasing the productivity and final quality of the smoothie. A de-stoner equipment is used to remove stones from the roots based on the centrifugal force that separate carrots to the peripheral area of the device while the stones remain in the centre.

4.2.3. Prewashing, washing and rinsing

The raw material is prewashed in the dirty area of the processing line with cold slightly-chlorinated water (5-10 ºC; 50 mg L^{-1} free chlorine). This step will eliminate the unwanted dirt, pesticide residues, plant debris, insects and foreign matter.

Washing of carrots can be done manually or with washer devices (rotary cylinders, bubbling washers or washers by aspersion). The washing and disinfection of carrots is

made with cold chlorinated water (1-2 ºC; 100-150 mg L^{-1} free chlorine), which is acidified (pH 6.5-7.5) with citric acid to augment the bacteriostatic effect of chlorine. The ideal contact time is around 2 min. However, NaClO may partially oxidize food constituents originating some carcinogenic compounds (Suslow, 1997). Accordingly, alternative sanitising treatments have been studied such as UV-C radiation, innocuous chemical substances (hydrogen peroxide, acetic acid, etc.) electrolyzed water, etc.

After washing, removal of residual leaves is conducted on conveyer belts. A preventive peeling of carrots may be conducted prior to blending.

4.2.4. Weighting, mixing and blending

The quantities of conditioned carrots and water, and other fruit and vegetables (depending of the smoothie composition), are measured and dumped into the blender machines. Blending intensity is regulated based on the desirable particle size which will depend of the fruit/vegetables used and consumer preferences.

4.2.5. De-aeration

Excessive air may be dissolved into the produced smoothie during blending. The oxygen from the air may lead to undesirable oxidation processes of the smoothie, vitamin C losses and enzymatic browning. Accordingly, de-aeration process will highly reduce the oxygen content from the smoothie. The de- aeration is conducted by passing the smoothie through a vacuum deposit leading to a mild boiling which eliminate the dissolved air. Bubbling nitrogen may be used also as a de- aeration system.

4.2.6. Thermal and non-thermal treatments

Beverages comprise the main direction of industrialization of fruit and vegetables. Such plant products must be properly treated and aseptically bottled since their composition represent a high risk of spoilage also compromising the food safety. Preservation treatments (thermal or non-thermal) may be conducted prior to filling or after filling. Filling of the product followed by treatment is preferred in order to avoid cross contamination during filling, which may be made under strict aseptic conditions.

The industry of fruit and vegetables beverages has been used intense thermal treatments (high temperatures and long treatment times) to ensure the inactivation of several enzymes and reduction of microbial loads in order to ensure the microbial quality and food safety of the product during a long shelf life even at room temperature.

However, the current industrial practices are turning into milder heat treatments (T^a < 90 ºC for a short time < 1 min) combined with a subsequent refrigerated storage. The higher cost of the refrigerated distribution and domestic conservation may be justified with the high sensory and nutritional/bioactive quality of the product. For example, a thermal treatment of 90 ºC for 45 s of a green vegetables smoothie ensured a shelf life of 45 days at 5 ºC being their nutritional/bioactive compounds highly preserved (Castillejo et al., 2016a). Furthermore, a 250-g portion of latter smoothie may highly cover the minimum daily recommended intakes of fibre, minerals and vitamin C (FAO/WHO, 2004) for different segments of the population with difficulties to consume fresh fruit and vegetables. On the other side, a treatment of 80 ºC for 3 min extended the shelf life of red vegetables smoothies from 20 to 40 days during storage at 5 ºC (Castillejo et al., 2015). Last treatment almost completely inactivated quality-degradative enzymes (polyphenol oxidase, PPO; peroxidase, POD; Pectinmethylesterase, PME; and pectingalacturonase, PG) being their activities under minimum levels during storage at 5 and 20 ºC (Rodríguez-Verástegui et al., 2015). Furthermore, last heat treatment enhanced the antioxidant compounds (up to 160 %) due to their higher extractability which a potential higher bioavailability (Bugianesi et al., 2004).

On the other side, non-thermal treatments of beverages have been developed in the last decades to obtain fruit and vegetables beverages with excellent microbial, sensory and nutritional/bioactive quality meeting always the legal regulations related to food safety. The most interesting non-thermal treatments used for fruit and vegetables smoothies are high pressures processing (HPP) and pulsed electric field processing (PEF).

HPP uses elevated pressures (100–1000 MPa), with or without the addition of heat. It is also known as high-hydrostatic-pressure processing since water is the most used pressure-transmitting fluid (Jung et al., 2011). It is claimed that this process is clean and energy-efficient compared with many conventional processes. HPP is used to achieve inactivation of microorganisms by damaging their membranes, induce some consumer-

desired food attributes (i.e. increased viscosity in some tomato products by inactivation of pectin-degradation enzymes), and/or avoid detrimental effects on other quality attributes (texture, flavour, and colour) specially appreciated by consumers (Martínez-Hernández et al., 2016b). HPP treatments may achieve high inactivation of pathogenic bacteria as recently reviewed (Rendueles et al., 2011). In Figure 16 can be observed the two possibilities of HPP: HPP+filling (A) and filling+HPP (B).

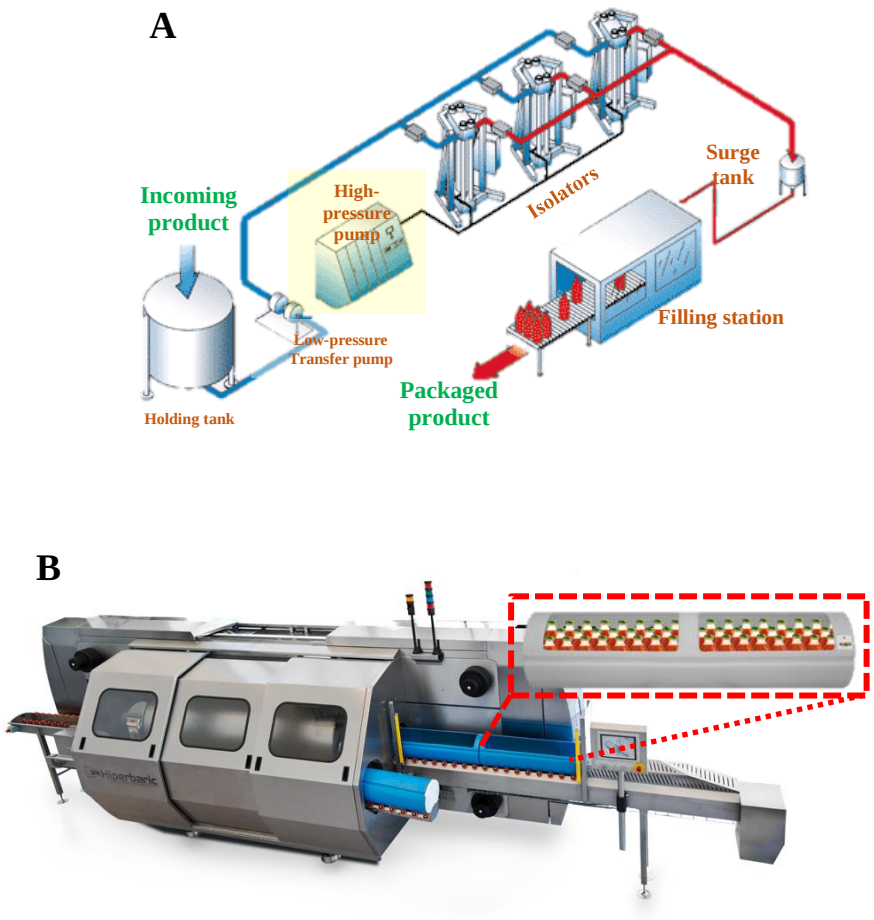


Figure 16. HPP of fruit and vegetables smoothies under two different processing alternatives: A) HPP+filling and B) filing+HPP.
(http://www.foodengineeringmag.com/articles/82608-fda-regs-spur-non-thermal-r
http://www.ipl-plastics.com/en/retail-packaging/products/file/sealpack-square/33)

Although there are still few HPP studies in smoothies, the reported data present this technology as an excellent alternative to conventional heat treatments. Accordingly, HPP treatments of fruit smoothies at 450-600 MPa at a moderate temperature (20 ºC) was proposed as good alternatives to conventional thermal processing (Keenan et al., 2010). In addition, the content of phenolic compounds, anthocyanins and vitamin C of a

strawberry purée were retained to a greater extent with HPP treatments of 400-600 MPa compared to conventional heat treatment (Keenan et al., 2012).

The PEF processing technology consists in the application of high-voltage pulses (20–80 kV cm^{-1}) for short periods of time (ms or μs) to a product placed in a treatment chamber confined between electrodes (Morales-de la Peña et al., 2011). Most studies using high-intensity PEF have been devoted to the design of pasteurization applications for liquid products to inactivate microorganisms and enzymes by maintaining the nutritional quality, antioxidant content, and freshness of liquid foods (Martínez-Hernández et al., 2016b). However, studies of PEF treatments in smoothies are very scarce. For example, the application of PEF (34 kV cm^{-1}, 60 μs) combined with a mild pre-heat treatment (55 ºC/60 s) extended the shelf life of a tropical fruit smoothie during storage at 4 ºC compared to a heat treatment at 72 ºC for 15 s (Walkling-Ribeiro et al., 2010). In addition, when this smoothie was inoculated with *Escherichia coli*, the combination of PEF (34 kV cm^{-1}, 60 μs) with the pre-heat treatment (55 ºC / 60 s) showed an additional effect reaching up to ≈7 CFU reductions of *E. coli*, an effect comparable to that of a treatment at 72 ºC for 15 s (Walkling-Ribeiro et al., 2008).

4.2.7. Filling of recipients and storage

The recipients are filled with the treated smoothie under aseptic conditions. The recipients are usually made of glass or plastic. Although transparent recipients are desired by consumers to visualize the smoothie colour, opaque recipients are preferred by producers in order to avoid oxidations by light.

After filling, the smoothies are cooled and stored. Storage and distribution of smoothies may be under refrigeration in the cases of mild heat treatments (in benefit of sensory and nutritional/bioactive quality) or room temperature when intense heat treatments are used.

4.2.8. Quality control and cold storage

Before the expedition of the product from the factory, it has to pass an effective quality control system to guarantee the safety, suitability and compliance of specifications. In

addition, it has to have a procedure of product recall when the specifications are not meet. The equipment has to be frequently revised, adjusted and calibrated.

4.3. History and overview of smoothies market

The Mediterranean, Eastern and Latin-American cultures have prepared beverages of fruit and vegetables from ancient times which were very similar to the current smoothies. The introduction of the fruit/vegetables shakes in the USA could be dated in the 1940-60s from Latin America. The reached popularity of fruit/vegetables shakes in the USA could be explained due to their good organoleptic properties as well as an excellent alternative to diet drinks. The elimination of milk from the fruit/vegetables shakes due to intolerances led to its substitution by ice, even improving the organoleptic properties with the ice-refreshing sense. The term 'smoothie' probably appeared in that time. Several companies emerged then in the USA which are currently sales leaders with smoothies franchises very popular and important providing to the consumers an infinite list of a variety of fruit/vegetables smoothies. The expansion of the healthy trend of smoothies to Europe arrived lately when several drink companies offered these innovative products like *gourmet* drinks, for a progressive inclusion in the even most conventional market.

The highest production of packaged smoothies sold under refrigeration is located in the northern countries of Europe. UK is the European leader with 84 million of litters per year (Figure 17), distantly followed by France, Germany and Poland with 8-15 million of litters per year in 2013 (AIJN, 2014b). The production of smoothies in those countries comprises between 9 and 16 % of total production of refrigerated beverages.

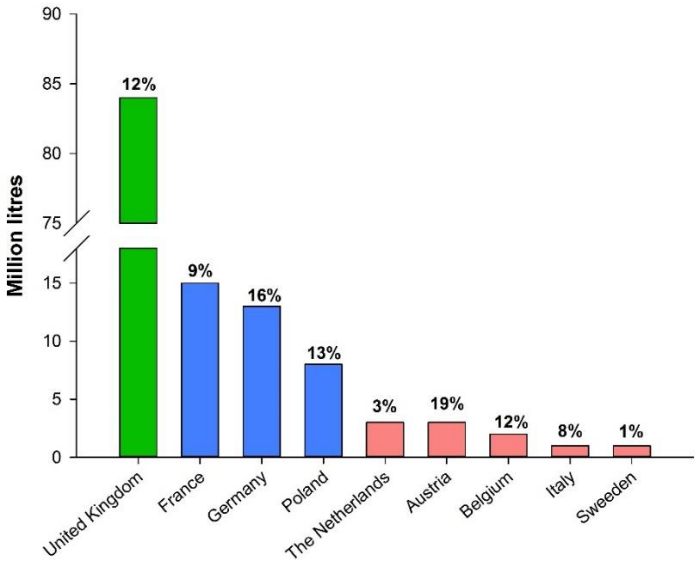


Figure 17. European production of smoothies in 2013. The values on each bar represent the percentage of smoothie production over the total production of refrigerated beverages (elaborated from AIJN (2014b)).

The Spanish production of smoothies is low and there is little information about it. A Spanish market study of 2012 revealed that smoothies represented a 9 % over a total of 240 selected (pasteurized or sterilized which does not include still water or carbonated drinks) commercialized beverages, being juices and nectars the major beverage group with a 77 % (Figure 18). However, the smoothies sales in the Spanish market has been highly incremented in the last years as it can be observed in the supermarkets, where the companies are highly introducing these products.

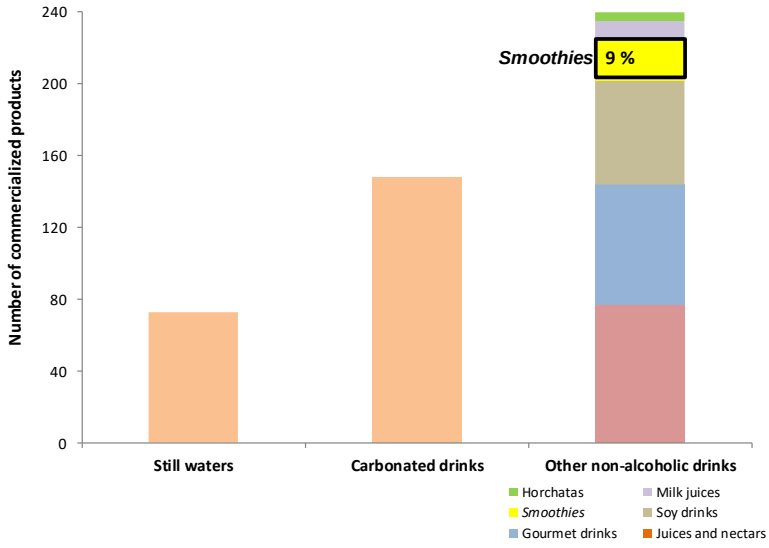


Figure 18. Market distribution over 240 selected (pasteurized or sterilized which does not include still water or carbonated drinks) commercialized beverages in Spain in 2012 (elaborated from Bardón-Iglesias et al. (2012)).

5. QUALITY DECAY OF PLANT PRODUCTS DURING PROCESSING AND STORAGE

The expected characteristics of plant-derived food products by consumers are freshness, optimum overall quality (general appearance, sensory quality, aroma and taste– and nutritional quality) and safety. However, during processing and retail period some physiological/biochemical, microbiological and nutritional/bioactive changes may occur, reducing the expected quality attributes of the product.

5.1. Physiological and biochemical changes

The fresh-cut processing steps may increase the metabolism of the plant material, reflected in higher respiration rates (RR) and C_2H_4 emission, which usually leads to a faster deterioration rate (Cantwell and Suslow, 2002a). Temperature is the most important factor that affects fresh-cut produce metabolism. When temperatures increase from 0 to 10 ºC, the respiration rate increases substantially (Watada et al., 1996). Produce types and cvs. differ in chilling sensitivity and, consequently, the optimum storage temperature will depend on the product itself and the exposure time. Fresh-cut vegetables are highly susceptible to weight loss because internal tissues are exposed if there is a lack of skin and cuticle. However, RH is generally very high within the package and dehydration is not a big problem. MAP can be beneficial for keeping RH and maintaining the product quality (Artés et al., 2009).

The action of the enzyme lipoxygenase, which catalyses peroxidation reactions, may result in the formation of aldehyde and ketone derivatives that are characteristic of many off–odours during the shelf–life of the fresh-cut product.

5.2. Nutritional and bioactive changes

The fresh-cut processing keys that highly influence the nutritional and bioactive contents are cutting, washing, dewatering, packaging, and processing and storage temperatures. The most important tool to extend the shelf–life and maintain the quality of the fresh-cut fruit and vegetables is the temperature management. Conditioning, washing and disinfection steps can favour the reduction of nutritional and bioactive contents by

lixiviation or elimination of some parts of the plant material. Glucosinolates levels do not necessarily decline rapidly after cutting and even induction can take place, but fresh-cut *Brassica* vegetables show optimal conditions for myrosinase activity with the conversion of glucosinolates to the biological active ITC.

Gas partial pressures during MAP or controlled atmospheres storage can highly influence the nutritional and bioactive profile of fresh-cut products. Conventional broccoli (cv. Marathon) stored during 7 days at 1 ºC in air–storage conditions (17 kPa O_2 + 2 kPa CO_2) showed a 71 % reduction of total glucosinolates (aliphatic and indoles). In the same conditions, significant flavonoids and hydroxycinnamic acid derivates decreases of 62 and 50–70 %, respectively, were found, contrary to the low stability of anthocyanins in fresh-cut vegetables (Vallejo et al., 2003). CO_2–enriched atmospheres with low O_2 content (0.5 kPa O_2 + 20 kPa CO_2) produced lower total glucosinolates increases (21 %) than air–stored samples (42 %) after 7 days at 10 ºC (Hansen et al., 1995).

5.3. Microbial quality and food safety

The application of sanitising treatments on fresh-cut products or thermal/non-thermal treatments on fruit and vegetables beverages is crucial to obtain a good microbial quality of products while meeting the food safety aspects. Furthermore, a high much more effort must be paid from a well–designed integrated production, handling, and processing to proper distribution chains, keeping appropriate chilling storage temperatures and optimal packaging conditions throughout the entire shelf life.

In addition, Good Agricultural Practices (GAPs) for suppliers of raw materials, Good Manufacturing Practices (GMPs), Standards Operating Procedures (SOPs) and an effective Hazard Analysis Critical Control Point (HACCP) program should be implemented and accomplished to minimize the risk of contamination by pathogens, assuring the safety of consumers. When the mentioned programs are not properly applied, outbreaks may occur with disastrous consequences. The microbiological risks of plant-derived products can be classified in two categories (Hurst, 2002):

- The contamination happened during cultivation or harvest of the plant material by indigenous pathogens.

- The microbiological risk is present during the fresh-cut processing, mainly in the cutting and washing steps, since the natural barriers of plant material (waxy outer skins) against microbiological invasion are damaged. Furthermore, cutting operation releases nutrients which can accelerate microbiological growth.

The previous Spanish regulation RD 3484/2000 (2000) which established the 'Regulation for the hygiene, elaboration, distribution and commercialization of ready-to-eat foods', which microbiological criteria was the spoilage and pathogenic microflora, was repealed by the Spanish Regulation RD 135/2010 (2011) in benefit of EU Regulation 1441/2007 (EC_1441/2007, 2007). The latter EU regulation establishes some pathogenic microorganisms as the unique microbiological criteria. Then, fresh-cut and fruit and vegetables beverages with mild heat treatments or non-thermal treatments are regulated by the EU 1441/2007 Regulation. Table 5 include the applicable microbial criteria of the latter Regulation for fresh-cut and fruit and vegetables beverages.

Table 5. Food safety criteria applied to the fresh-cut products food (Regulation EC 1441/2007, 2007).

Food category	Microorganism	Sampling plan [1]		Limits [2]		Stage where the criterion applies
		n	c	m	M	
Pre-cut fruit and vegetables (ready–to–eat).	*E. coli*	5	2	100 CFU g^{-1}	1,000 CFU g^{-1}	Manufacturing process.
Pre-cut fruit and vegetables (ready–to–eat).	*Salmonella*	5	0	Absence in 25 g		Products placed on the market during their shelf–life.
Ready–to–eat foods able to support the growth of *Listeria monocytogenes*, other than those intended for infants and for special medical purposes.	*L. monocytogenes*	5	0	100 CFU g^{-1} [3]		Products placed on the market during their shelf–life.
				Absence in 25 g [4]		Before the food has left the immediate control of the food business operator.

(1) n = number of units comprising the sample; c = number of sample units giving values between m and M // (2) For points 1.1–1.25 m = M. // (3) This criterion shall apply if the manufacturer is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit 100 CFU/g throughout the shelf–life. The operator may fix intermediate limits during the process that must be low enough to guarantee that the limit of 100 CFU/g is not exceeded at the end of shelf–life. // (4) This criterion shall apply to products before they have left the immediate control of the producing food business operator, when he is not able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 CFU/g throughout the shelf–life.

6. DETRIMENTAL EFFECTS OF HIGH PRESSURE PROCESSING ON APPLE BLEND COLOUR, A PARTICULAR CASE

Apple juice is the most worldwide consumed fruit juice, together with orange juice, due to its excellent sensory attributes and health-promoting related properties derived from the phytochemicals present in this fruit (AIJN, 2014a; Hyson, 2011; USDA, 2013). Food safety and the inactivation of oxidizing enzymes, have been conventionally controlled by heat treatments but in detriment of the sensory quality of the product. Accordingly, novel sustainable preservation technologies like HPP have emerged to maintain quality and bioactive/nutritional content of processed food guarantying the food safety (Artés et al., 2009). However, such mild-moderate HPP treatments at room temperature enhance the activity of quality degradative enzymes such as PPO, the main responsible of apple juice browning, as it is recently reviewed (Eisenmenger and Reyes-De-Corcuera, 2009).

Therefore, HPP treatment at 400 MPa for 5 min at 20 ºC induced a 65 % increase of the PPO activity in apple juice (Buckow et al., 2009). Reactivation of PPO and other enzymes has been also observed during shelf life of other HPP-treated products (Jacobo-Velazquez and Hernandez-Brenes, 2010). Furthermore, besides the evident sensory quality loss, application of high temperatures during HPP treatment has even shown an antagonistic effect between pressure and temperature on PPO inactivation in apple juice and other food products (Buckow et al., 2009; Ludikhuyze et al., 2002). Consequently, there is a need to find combined treatments of mild HPP treatment (low pressures/temperature/time), which are economically affordable by the food industry, and antibrowning agents.

Several antibrowning agents such as sulfiting agents, reducing agents (ascorbic acid and analogue substances, glutathione, *L*-cysteine), enzyme inhibitors (aromatic carboxylic acids, substituted resorcinols, anions, peptides), chelating agents (phosphates, EDTA, organic acids), acidulants (citric acid, phosphoric acid) and enzymes have been used to inhibit PPO activity in food products (compiled by Özoğlu and Bayındırlı (2002)). However, the actual consumer demands healthy food with natural ingredients free from additives. Cyclodextrins (CDs) are naturally occurring cyclic oligosaccharides from the bacterial digestion of starch, which have been studied as natural antibrowning agents in fruit and vegetable juices (Hicks et al., 1996; Iyengar and McEvily, 1992). They are

cylindrically shaped molecules with a cavity of hydrophilic outer surface, and hydrophobic internal surface which is able to form inclusion complexes with PPO substrates (Kalogeropoulos et al., 2010). Among CDs, maltosyl-β-CD (mβCD) has shown excellent antibrowning effect and solubility, while being safe even when administered parenterally contrary to parent β-CD (Del Valle, 2004; López-Nicolás et al., 2007a). To the best of our knowledge, there are no studies on application of CDs with HPP on fruit and/or vegetable beverages.

7. READY-TO-BLEND: A NEW FOOD CONCEPT BETWEEN FRESH-CUT AND SMOOTHIE PRODUCTS

The new product concept 'ready-to-blend' fruit and vegetables, firstly reported here, may combine the 'long' shelf life of a fresh-cut product with the convenient consumption of a beverage. A ready-to-blend product may be defined as a fresh-cut (which implies correspondent plant material preparation, sanitizing treatments and MAP combined with low storage temperature) fruit, vegetable, or a mix of them, which is packaged under specific proportions ready for domestic blending. Therefore, the microbial growth and enzymatic/non-enzymatic degradative reactions of fruit and vegetables blended beverages may be highly reduced during storage in ready-to-blend products. Furthermore, the needed processing treatments and/or food preservatives of blended beverages are avoided in ready-to-blend products since blending is made at home being the beverage immediately consumed. On the other side, fresh-cut fruit and vegetables have higher shelf life, compared to untreated related beverages, due to techniques such as MAP and edible coatings (i.e. with chitosan, among others) (Artés et al., 2009).

Chitosan, a deacetylated form of chitin, is a natural product with excellent antimicrobial properties and high potential to be used within the edible coatings of fresh-cut products to increase their shelf life (Lin and Chou, 2004). The most feasible hypothesis about the antimicrobial activity of chitosan is a change in cell permeability due to interactions between the polycationic chitosan and the electronegative charges on the cell surfaces. This interaction leads to the leakage of intracellular electrolytes and proteinaceous constituents leading to microbial cell death (Devlieghere et al., 2004).

8. METHODS TO INCREASE THE HEALTH-PROMOTING COMPOUNDS OF PLANT MATERIAL

Plant cells are able to respond to its own defence against imposed stresses which may be biotic or abiotic. This response is reached owed to the vegetable cell plasticity in respective metabolomics, proteomics and genomics which finally drives to the modulation of some defence metabolic pathways (Aghdam et al., 2013). The adaptive mechanisms of plants to respond to biotic and abiotic stresses are similar, viz. by activating a primary protection mechanism and by inducing the overexpression of specific proteins as a result of each stress (Timperio et al., 2008), as is observed in Figure 19.

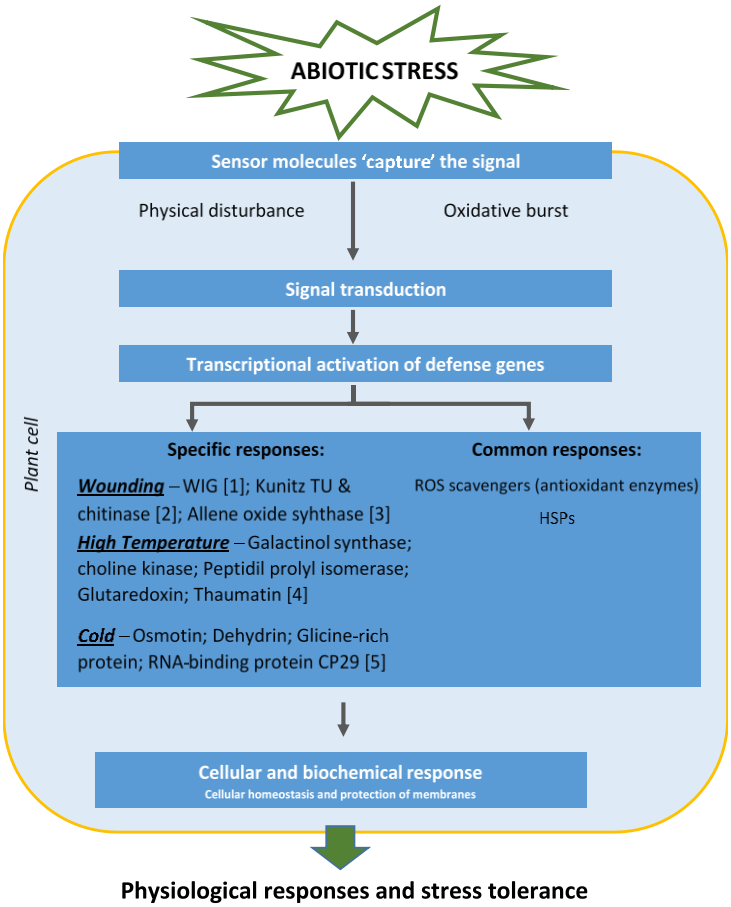


Figure 19. Specific and common stress induced responses in plants (Alegria, 2015).

Different protein families have been reported to be able to response to stresses by being again synthesized, stored or reduced, taking an important role in the antioxidant defence systems of plants as well. Abiotic stresses are able to induce protein dysfunctions by altering proteins structure, solubility or levels, where cell adaptation to conserve cellular homeostasis in stressful circumstances is indispensable to the own survival. To attain this

goal, one protein family involved in the stress response stands out, heat shock proteins (HSP) (Aghdam et al., 2013; Timperio et al., 2008).

The health-promoting compounds of several fruit and vegetables may be increased with biotic and abiotic stresses (Avena-Bustillos et al., 2012; Cisneros-Zevallos, 2003; Reyes et al., 2007). The use of such stresses have several advantages over genetic engineering being the latter method usually considered as potential biological hazards that generate an ecological imbalance (Colwell et al., 1985). Accordingly, the usage of stresses on plant products for the synthesis of secondary metabolites with health-promoting properties has a high interest (Jacobo-Velázquez and Cisneros-Zevallos, 2012). The use of several postharvest abiotic stresses (i.e. wounding, UV-light, hyperoxia, ethylene, methyl jasmonate, etc.) for the synthesis of phenolic compounds in fruits and vegetables has been approached in some studies. Carrots occupy the sixth place among the list of most consumed vegetables, although the TPC of this vegetable is almost the lowest one (Chun et al., 2005). Accordingly, new strategies such as stresses may be applied to increase the phenolic levels of this vegetable leading to higher ingestion of these antioxidant compounds from this popular and highly consumed root. Furthermore, the content of the anticancer glucosinolates compounds may be increase with the use of stresses as recently proposed (Villarreal-García et al., 2016).

8.1 Wounding

Lignin and suberin are synthesized and stored in wounded locations during wound healing to defend plants from water loss and pathogen attack (Dixon and Paiva, 1995). The great synthesis of hydroxycinnamic acids in wounded tissues has been observed in several plant products like carrots, lettuce and potato (Reyes et al., 2007). The phenolic synthesis can be linked with a greater rate of production than their polymerization since these phenolic compounds are the substrate used for the lignin and suberin production during wound healing (Figure 20).

The synthesis of soluble phenolics in stressed tissues has been proposed that is not restricted to the cell wall production but also it may function to protect cells under stress environments. The lignin and suberin biosynthesis starts with the production of hydroxycinnamic acids that are transformed to monolignols. These monolignols are then

transported to the cell wall where they are oxidized and polymerized. Several enzymes like laccases and peroxidases catalyse the dehydrogenative polymerization of monolignols. Hydrogen peroxide is used by peroxidases to oxidize monolignols while laccases use oxygen (Jacobo-Velázquez and Cisneros-Zevallos, 2012).

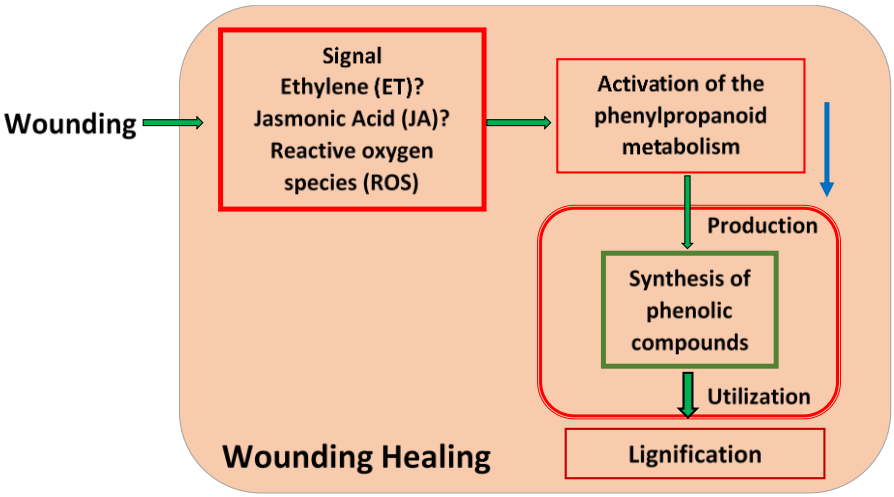


Figure 20. Synthesis of phenolic compounds during the wound healing process (Jacobo-Velázquez and Cisneros-Zevallos, 2012).

The wound-induced biosynthesis of hydroxycinnamic acids, suberin and lignin in plants is linked with the activation of metabolic processes related on the supplementation of carbons skeletons to the phenylpropanoid metabolism. Carbohydrates (starch and sucrose) are the substrates required for the phenolic biosynthesis. The synthesis of phenolics compounds, like respiration, initiates in the cytosol of plant cells where sucrose is cleaved to induce the production of fructose 6-P and glucose 6-P. A portion of the hexose-P pool is transported to the plastid and transformed to erythrose 4-P by the oxidative pentose-phosphate pathway. The other portion of the hexose-P pool is transformed to glycerone-P by glycolysis in the cytosol. Glycerone-P is transported to the plastid and transformed to phosphoenolpyruvate (PEP). PEP and erythrose 4-P are the substrates for the shikimate pathway which take places in the plastid. The shikimate pathway induces the chorismate synthesis, which is used by the chorismate mutase to produce prephanate. Then, prephanate is transformed to arogenate, which is successively converted to L-phenylalanine. PAL uses L-phenylalanine to start the phenolic biosynthesis as part of the phenylpropanoid metabolism that takes place in the endoplasmic reticulum of plant cells (Jacobo-Velázquez and Cisneros-Zevallos, 2012).

8.2. UV radiation

UV radiation (10-400 nm) may be considered as an abiotic stress since it is able to induce synthesis and/or accumulation of phytochemicals in the plant, which leads to enhanced antioxidant activity. UV radiation might act on the pathways involved in the biosynthesis of the three principal groups of secondary metabolites: phenolics, terpenes and nitrogen-containing compounds (Cisneros-Zevallos, 2003). Low UV doses may cause repairable DNA damage which would activate repair mechanisms for radiation-induced DNA damage. Accordingly, UV radiation may stimulate vital processes inside the cells and create a positive change in the homeostasis of plant tissues (Luckey, 1980). The metabolic responses of plant cells are dependent on the applied dose, irradiation time, the sensitivity of the antioxidant/phytochemicals to irradiation, and the raw material used (Alothman et al., 2009).

UV-B radiation (280-315 nm) has been reported to induce significant enhancements of bioactive compounds such as phenolics (such as hydroxycinnamic acids), and respective antioxidant capacity, and glucosinolates in carrots and broccoli, respectively (Avena-Bustillos et al., 2012; Ruhland et al., 2005). The signal transduction pathways by which UV-B controls the gene expression are poorly known. Nevertheless, the available studies have shown a pivotal role for reactive oxygen species as key second messengers acting up-stream of a number of pathways involving the plant hormones salicylic acid, jasmonic acid and ethylene (Mackerness, 2000). The application of UV-B treatments to grapes has led to an increase of resveratrol levels through the activation of PAL (Cantos et al., 2000). Furthermore, UV-B treatments (1.3 to 12 kJ m^{-2}) have been able to induce phenolic accumulation (mainly 5-caffeoylquinic acid) in carrots (Avena-Bustillos et al., 2012).

UV-C (100-280 nm) radiation has been proposed as an alternative sanitizing treatment to NaOCl in fresh-cut products (Artés et al., 2009). The germicidal effect of UV-C doses on fresh-cut products (0.5-20 kJ m^{-2}) is explained by the UV-C-induced formation of pyrimidine dimers in the microbial genetic material that alter the DNA helix and block microbial cell replication (Nakajima et al., 2004). However, plant cells have likewise presented metabolic responses to some UV-C doses through the synthesis of bioactive compounds. Accordingly, low UV-C doses (0.5 kJ m^{-2}) have induced an increase in PAL activity, while high doses (2.5 kJ m^{-2}) lowered such enzyme activity (Nigro et al., 2000).

Some cell cultures synthetize UV-protective flavonoids and phytoalexins when are irradiated with UV-C (Mercier et al., 1994). Low UV-C radiation induce a number of changes in plant products including the production of antifungal compounds and ripening delay (El Ghaouth et al., 2003). A fungal resistance appeared after UV-C radiation apparently related with PAL activity in the peel of fruit and vegetables exposed to UV-C (Chalutz et al., 1992; Shama and Alderson, 2005). Moreover, UV-C has prompted the synthesis of phytoalexins, being these compounds elicitors of other protection mechanisms leading to modifications in the cell wall, increasing the biosynthesis and activity of several enzymes and by enhancing the antioxidant capacity.

Conclusively, UV radiation is a technology with high interest to increase the health-promoting compounds while it does not leave any residue, does not have legal restrictions, is easy to use and lethal to most types of microorganisms (Bintsis et al., 2000). However, combination UV-B and UV-C treatments for different hurdle technologies has not been studied yet. Accordingly, the combination of UV-B and UV-C is an excellent challenge of achieve excellent germicidal effects (UV-C) and enhancement of health-promoting compounds of fresh-cut products.

8.3. Hyperoxia atmospheres

Hyperoxia atmosphere packaging (over 60 kPa) of fresh-cut products has been described as an effective technique to inhibit enzymatic browning, prevent anaerobic fermentation and moisture and odour losses and reduce aerobic and anaerobic microbial growth (Artés et al., 2009). A number of factors may explain the toxicity of hyperbaric O_2, like the adverse effects on the redox potential of the system, the oxidation of enzymes having sulfhydryl groups or disulfide bridges, and the accumulation of injurious ROS (Kader and Ben-Yehoshua, 2000).

Hyperoxia atmospheres have been considered as an abiotic stress inducing accumulation of phenolic compounds in carrots (Jacobo-Velázquez et al., 2011). Nevertheless, such effect was not detected on the phenolic levels of Bimi® broccoli (Martínez-Hernández et al., 2013d). The enhancement of those antioxidant compounds under hyperoxia conditions may be explained by an increment of ROS (due to hyperoxia levels) by both incrementing the respiration rate and partially inhibiting the activation of the antioxidant

enzymes ascorbate peroxidase and catalase (Figure 21). These increased ROS levels in the tissue treated with hyperoxia induced a higher activation of PAL and thus higher accumulation of phenolic compounds.

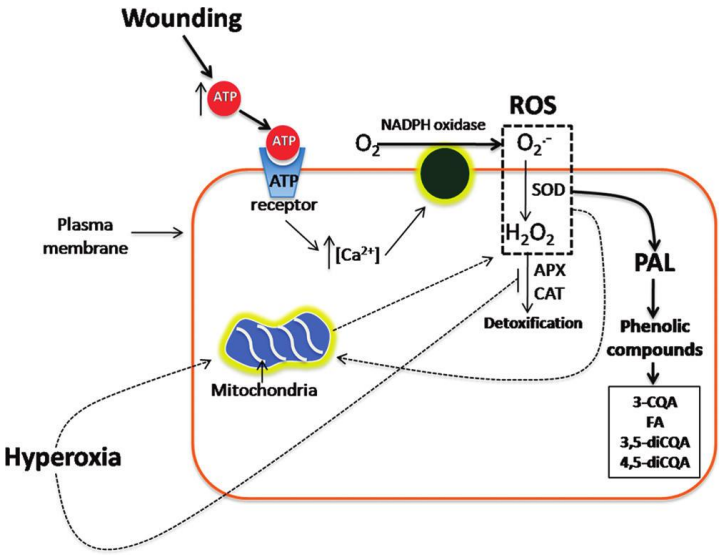


Figure 21. Hypothetical model explaining the role of ROS on the stress-induced production of phenolic compounds in carrots (Jacobo-Velázquez et al., 2011).

9. BY-PRODUCTS REVALORIZATION: BROCCOLI, AS A PARTICULAR CASE.

9.1. Overview of world food wastes and revalorization of crops by-products

FAO has recently published that about 1.3 billion tons of food is worldwide wasted or lost per year. Food losses during preharvest varies from 10-20 % Figure 22. Particularly, losses in agricultural production of developing countries dominate over total food losses throughout the food supply chains.

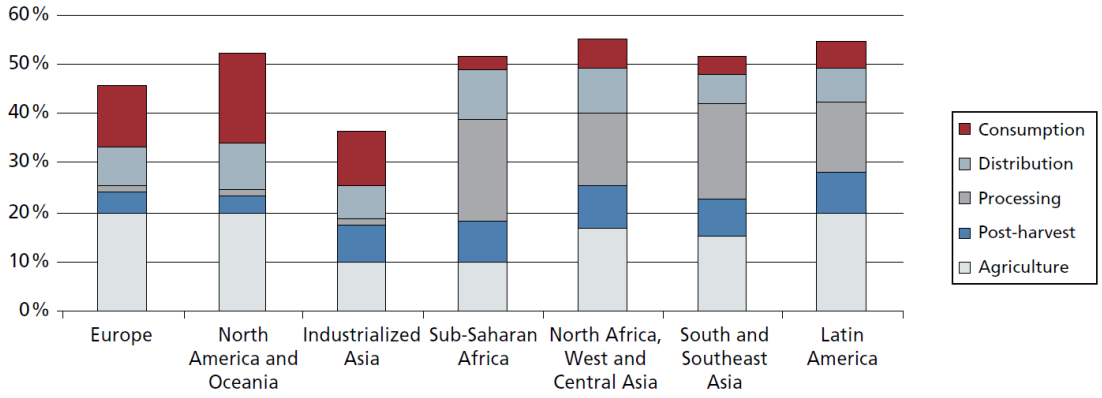


Figure 22. Part of the initial production lost or wasted at different stages of the food supply chains for fruit and vegetables in different regions (FAO, 2015).

The world population is expected to reach 8.5 billion by 2030 and 9.7 billion in 2050 (FAO, 2015). It means that food supplies would need to increase by 60 % either with any of the following options:

- Increasing production
- Improving distribution
- Reduction of losses

Focusing on the last option, revalorization of plant by-products wasted during preharvest stage appear as an interesting challenge. Development of by-products supports the low carbon economy using renewable resources, offering environmental and economic benefits and improve efficiency in food industry. Furthermore, the use of by-products increases the added value from a given unit of agricultural raw material, stimulating economic growth. Crop by-products could be used in animal feed, production of essential oils, extraction of bioactive compounds for the pharmaceutical and cosmetic industries, and innovative ingredients with high contents of bioactive contents for functional foods.

9.2. By-products production during Bimi® broccoli culture

The marketable part from Bimi® broccoli plant is the floret (inflorescence) being stalks and leaves discarded (Figure 23). The stalks and leaves of Bimi® broccoli represent 80 % (in dry weight) of total above-ground plant biomass. This constitutes a great amount of waste, with a negative effect on the agricultural environment. Moreover, the sometimes

abnormally high temperatures in the winter and spring seasons of warmer cultivation areas may induce premature flowering, resulting in the total loss of the marketable yield (florets), and converting all the biomass into an unprofitable by-product. The increase in broccoli cultivation in the last few years has made it difficult to find uses for the total amount of by-products generated (≈0.13 × 106 tons in 2008 in the Murcia Region) (Dominguez-Perles et al., 2010; Vargas-García et al., 2014).

Figure 23. Details of Bimi® broccoli at harvest (Courtesy of Sakata Seed Ibérica).

The traditional management of such residues implies their incorporation into the field without any previous treatment. Another usual practice is the composting of such residues to obtain manure, or its application for cattle feeding. The silage residues of broccoli present high contents of protein (34.7 %) and fibre (neutral and acid detergent fibre levels of 18.8 and 12.9 %, respectively) with a high digestibility by cattle.

Currently, the use of broccoli by-products is restricted to flour and fibre (Campas-Baypoli et al., 2009) and glucosinolate standard extraction or characterization (Campas-Baypoli et al., 2010; West et al., 2004). The potential use of broccoli by-products as a source of bioactive compounds is getting the attention of the scientific community (Dominguez-Perles et al., 2010; Dominguez-Perles et al., 2011; Mahro and Timm, 2007). Furthermore, the previously commented high percentage of broccoli by-products points this vegetable as an economically interesting source of bioactive compounds from their by-products.

9.3. Use of abiotic stresses to increase health-promoting compounds of broccoli: UV-C and UV-B

Broccoli stalks and leaves have important contents of glucosinolates and phenolic compounds with high myrosinase activities (Dominguez-Perles et al., 2010; Dosz and Jeffery, 2013). Furthermore, the application of postharvest abiotic stresses may even enhance those levels of bioactive compounds. However, latter research area in broccoli by-products is still unexplored. Application of abiotic stresses during preharvest stage of broccoli plant for revalorization of by-products is also very scarce. Interestingly, preharvest saline stress (80 mM NaCl) applied in broccoli plants led to increased glucosinolates and phenolic contents in leaves and stalks of some broccoli cvs. (Dominguez-Perles et al., 2010).

Application of postharvest abiotic stresses (wounding, exogenous ethylene and methyl jasmonate) to increase glucosinolate contents of marketable broccoli florets has been recently reported (Villarreal-García et al., 2016). Contrary, latter authors reported that such stresses impeded phenolic compounds enhancement in broccoli florets. Accordingly, research on application of abiotic stresses to broccoli by-products is of high interest being proposed UV radiation (B and C) as an excellent stress-inducing technology due to the absence of residues, no legal restrictions, easy to use, and low and cheap maintenance.

OBJECTIVES

OBJECTIVES

The general objective of this PhD Thesis was to develop functional carrot-based smoothies with high bioactive compounds content and long shelf life by using novel non-thermal pasteurization to retain overall food quality, and with revalorization of by-products of agricultural crops to reduce waste.

This is due to the fact that there is an increasingly growing market for nutraceuticals and functional foods, and enhancing the health benefit properties of fruit and vegetables will add value and create new opportunities for growers and processors by reaching health-oriented markets. To achieve this goal, there is a need to provide innovative techniques or combination or selected techniques to assure food safety and quality.

For this reason, post-harvest abiotic stresses, single and combined, are a novel way to enhance bioactive compounds in fruit and vegetables, and consequently, improving human well-being.

Such general objective was achieved by the following specific objectives:

- Enhancement of bioactive compounds of carrots and Bimi® using different abiotic stresses (mainly phenolics and other antioxidant compounds).
- Selection and optimization of the best abiotic stress combination/s to obtain maximum enhancements of bioactive properties of the vegetables, as well as its by-products.
- Optimization of the smoothie formula from stressed vegetables according to sensory tests.
- Study and optimization of high hydrostatic pressure as non-thermal treatment to extend the shelf life of functional smoothies, at refrigerated and room temperatures, with minimal degradation of nutritional and bioactive compounds, meeting always the applicable food safety regulations.
- Develop a novel concept of fresh-cut ready-to-blend horticultural product and monitoring its shelf life quality and food safety changes.

- Study and optimization of high hydrostatic pressure and maltosyl-β-cyclodextrin as a combination technology to control browning in apple juices as a beverage model.
- Use of antimicrobial food coatings to control microbial growth and increase the nutritional/bioactive properties.

CHAPTER I

UV-C and hyperoxia abiotic stresses to improve healthiness of carrots. Study of combined effects

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I.1. INTRODUCTION

The actual interest of better eating habits might be explained by the increasing of life expectancy or the high costs of health care, and need to satisfy population groups with special needs such as elderly and children (Siró et al., 2008). High intakes of fruit and vegetables have been proven to prevent a grand array of diseases such as degenerative disorders, cancer, cardiovascular among others (Slavin and Lloyd, 2012). Nowadays, foods are not only intended to feed, but also to prevent chronic and nutritional-related diseases as well as to improve overall human well-being, mainly linked to the crescent consumer's knowledge on functional foods. Enhancement of the health-promoting properties of fruit and vegetables will add value and create new opportunities, even with recent economical drawbacks. Therefore, there is a need to provide technologies to handle fresh products with enhanced health-promoting properties (Jongen, 2002).

Carrot is a popular vegetable among broad strata of the population. The popularity of this vegetable is mainly due to its sensory characteristics and nutritional compounds. Furthermore, carrots do not contribute with high calories intake, however they play a significant source of nutrients, such as carotenoids, vitamins (A, E) and antioxidants on human diet (Sharma et al., 2011). Phenolic compounds are great antioxidants related to several health-promoting properties such as anti-inflammatory, antitumoral, as well as preventing neurodegenerative and chronic disorders. Moreover, those compounds contribute to sensory features to food products. Nowadays, health recommendations rely on a diet rich in multiple antioxidant compounds than one used based on a single antioxidant (Shahidi and Ambigaipalan, 2015). Plant products such as carrots have been proposed as biofactories of phenolic compounds through different mechanisms induced by abiotic stresses (Cisneros-Zevallos, 2003). Concisely, PAL is the key enzyme of primary (shikimate) and secondary (phenylpropanoid) pathways and is, therefore, involved in the biosynthesis of polyphenolic compounds (Dixon and Paiva, 1995). It is well reported that this enzyme is induced by an array of biotic and abiotic stress-induced mechanisms, such as wounding, radiation exposure, hyperoxia storage, water stress, chilling injury, low minerals, hormones and pathogen attack, among others (Alegria et al., 2012; Avena-Bustillos et al., 2012; Becerra-Moreno et al., 2012; Jacobo-Velázquez et al., 2011). Consequently, such postharvest abiotic stresses enhance the levels of phenolic compounds like caffeoylquinic (CQA) acid, ferulic acid and their derivates as a

defence mechanism of the plant (Jacobo-Velázquez et al., 2011). Previous studies have shown that single application of wounding, low UV-C doses and hyperoxia storage enhanced phenolic content on carrots and other plant products (Artés-Hernández et al., 2009; Cisneros-Zevallos, 2003; Martínez-Hernández et al., 2013d; Martínez-Hernández et al., 2011; Sánchez-Rangel et al., 2013). Nonetheless, to the best of our knowledge, the combined effect of wounding, moderate UV-C radiation and hyperoxia atmospheres on the phenolic compounds levels and related TAC has not been studied yet. Accordingly, this work studied the singular and combined effects of UV-C pretreatment and hyperoxia storage on PAL activity, phenolic compounds and related TAC during storage of whole and shredded carrots at 15 ºC.

I.2. MATERIALS AND METHODS

I.2.1. Plant material preparation

Fresh carrots (cvs. group Nantes, cv. Soprano) were bought in a local market (Cartagena, Spain) on April 6th. According to producer specifications, carrots were harvested on the first week of April in Villena area (northwest area of Alicante region, Spain) without any postharvest treatment, but washing, previous expedition to the market. Carrots were transported to the Pilot Plant of the Technical University of Cartagena where they were stored in a cold room at 5 ºC until the next day when the experiment was conducted. Plant material was carefully inspected, selecting those with similar visual appearance and size (14-15 cm long and 2-3 cm diameter). Then, carrots (unpeeled) were sanitized in a cold room (8 ºC) with chlorine (100 mg L^{-1} NaClO; 5 ºC; pH 6.5±0.1) for 2 min, rinsed with tap water at 5 ºC for 1 min and drained in a perforated basket for 1 min. A ratio of 300 g plant material: 5 L chlorine was used. Carrots were wounded to shreds (2 mm × 3 mm × 40-60 mm) with a food processor (FreshExpress+, Moulinex, Lyon, France). Unwounded carrots were used as control (hereinafter 'whole'). Approximately 9 kg of shreds and 9 kg of whole carrots were prepared for the experiment. Immediately after wounding all samples were submitted to wounding, UV-C radiation and hyperoxia storage treatments as described below.

I.2.2. Abiotic stress treatments

I.2.2.1. UV-C pretreatment

The UV-C treatment chamber consisted of a reflective stainless steel chamber with two banks (one bank suspended horizontally over the radiation vessel and the other placed below it) being fitted to each bank 15 unfiltered germicidal emitting lamps (>80 % emitted spectrum at λ=254.7 nm; TUV 36W/G36 T8, Philips, Eindhoven, The Netherlands) which has been previously described (Artés-Hernández et al., 2009a). Whole or shredded carrots were placed between the two lines of UV-C lamps at 17.5 cm above and below over a 35 mm thick bi-oriented polypropylene (PP) film mounted on a polystyrene (PS) net (130 × 68 cm) that minimized blockage of the UV-C radiation. The applied UV-C intensity of 67.6 W m^{-2} was calculated as the mean of 18 UV-C readings on each side of the net using a VLX 254 radiometer at λ=254 nm (Vilber Lourmat, Marne la Vallee, France). Thus, both sides received the same UV-C intensity. The UV-C light intensity was kept constant and the applied dose was varied by altering the exposure time at the fixed distance. A UV-C radiation treatment of 9 kJ m^{-2} (exposure time of 139 s) was applied. Non-irradiated samples were used as 'control'.

I.2.2.2. Hyperoxia storage

Samples to be stored under hyperoxia conditions were placed in plastic containers (30 cm diameter, 60 cm height) connected to a humidified air-flow-through system of either air or a gas mixture containing 80 kPa O_2 (balanced with N_2). The gas entering the containers was previously passed through a water trap giving a humidity close to saturation in order to greatly minimize water losses (Jacobo-Velázquez et al., 2011; McLaughlin and O'Beirne, 1999) and avoid additional phenolic biosynthesis owed to water stress (Becerra-Moreno et al., 2015). In order to ensure a good air flow through carrot shreds, these samples were distributed in opened plastic petri dishes (8.5 cm diameter, 1 cm height). The CO_2 partial pressures were kept < 0.15 kPa to avoid any physiological effect exerted by CO_2 such as anaerobic metabolism (Surjadinata and Cisneros-Zevallos, 2003). Gas treatments were applied at 15 ºC for up to 72 h in darkness. Sampling was conducted every 12 h with 3 replicates per treatment. Every replicate per treatment and sampling time consisted of approximately 100 g of carrots (in the case of whole carrots each 100

g-replicate was composed from three different carrot units). Samples were stored in reclosable PP bags zipper-locking at -80 ºC until further analysis.

I.2.3. Analyses

I.2.3.1. UV-C transmittance through carrot tissue

Carrot sections (1.5 cm × 1.5 cm) with different thickness (0.10 to 3.00 mm) from internal and external tissue were prepared with a scalpel. Thickness of carrot sections was measured with a digital calliper (500-302 Series, Mitutoyo, Aurora IL, USA). Subsequently, carrot sections were carefully attached over the radiometer, placed on the net of the UV-C treatment chamber, and UV-C intensity was measured with (I) and without (I_0) the carrot section. For every thickness, five identical (±0.02 mm) sections were prepared representing five replicates. UV-C transmittance (T) for every carrot thickness was calculated using equation (I.1).

$$T(\%) = \left(\frac{I}{I_0}\right) \times 100 \quad \text{(I.1)}$$

I.2.3.2. Relative electrolyte leakage

Relative electrolyte leakage (REL) was measured according to the method described by Martínez-Hernández et al. (2016a) but with modifications. A 10-g carrot shreds portion was placed in a glass bottle (100 mL capacity) and 70 mL of 0.2 M mannitol (Sigma Aldrich, Steinheim, Germany) were added. For whole samples, a carrot was placed in a glass bottle (1 L capacity) and 800 mL of 0.2 M mannitol were added. The electrical conductivity of the bathing mannitol solution was measured with an electrical conductivity meter (GLP32, Crison, Alella, Spain) after 60 min (C_0) of incubation with orbital shaking (Stuart SSL1, Osa, UK) at a speed of 60 cycles min^{-1}. Then, the samples were heated at 121 ºC for 20 min in an autoclave and the conductivity (C) of the bathing mannitol solution was measured after cooling at room temperature. The REL was calculated using equation (I.2). Three replicates per treatment were analysed.

$$REL\ (\%) = \left(\frac{C_0}{C}\right) \times 100 \quad \text{(I.2)}$$

I.2.3.3. Colour

Colour was determined using a colorimeter (Minolta CR-300 Series, Japan) calibrated with a white reference plate (light source C), 2º observer and 8-mm viewing aperture. Measurements were recorded using the standard tristimulus parameters (*L**, *a**, *b**) of the *CIE Lab* system on three equidistant points of each replicate. Three colour readings were taken on three parts of the same sample and all three measurements were automatically averaged by the device and recorded.

Whitening and browning and are the main colour degradation processes occurred in wound (fresh-cut) carrots. Accordingly, whitening index (*WI*) and browning index (*BI*) were calculated from *CIE Lab* parameters according to equations (I.3) and (I.4) as previously described (Castillejo et al., 2015; Martínez-Hernández et al., 2016a; Palou et al., 1999).

$$WI\ (\%) = 100 - \sqrt{\left[(100 - L^*)^2 + a^{*\,2} + b^{*2}\right]} \tag{I.3}$$

$$BI = \frac{100 \times \left[\left[\frac{[a^{*2} + (1.75 \times L^*)]}{[(5.645 \times L^*) + a^{*2} - (3.012 \times b^*)]}\right] - 0.31\right]}{0.172} \tag{I.4}$$

Complimentary, total colour differences (Δ*E*) is a colorimetric parameter extensively used to characterize the variation of colours during processing and storage of food products (Martínez-Hernández et al., 2013a). Δ*E* was calculated according to equation (I.5).

$$\Delta E = \sqrt{(L^* - L^*{}_0)^2 + (a^* - a^*{}_0)^2 + (b^* - b^*{}_0)^2} \tag{I.5}$$

I.2.3.4. Phenylalanine ammonia-lyase

Phenylalanine ammonia-lyase (PAL) activity was analysed according to Ke and Saltveit (1986) with modifications. Concisely, 2 g carrot tissue samples were mixed with polyvinylpolypyrrolidone (Sigma, St Louis, MO, USA) (0.2 g) and homogenized (Ultra Turrax® model 18T, IKA-Werke GmbH & Co. KG, Germany) in cold 50 mM borate

buffer (pH 8.5) containing 400 µL L^{-1} *β*-mercaptoethanol (Sigma, St Louis, MO, USA). Homogenates were filtered through four layers of cheesecloth and then centrifuged at 10,000 × g for 20 min at 4 ºC. Supernatants were used as enzyme extract. Two sets of UV-Star 96-well plates (Greiner Bio-One, Frickenhausen, Germany) containing 69 µL of PAL extract plus 200 µL ultrapure water were prepared for every sample and pre-incubated at 40 ºC for 5 min. Afterwards, 30 µL of either water (blank) or 100 mM L-phenylalanine substrate solution (freshly prepared before assay) were added to each of the well for every sample set. The absorbances of sample sets were measured at 290 nm, using a Multiscan plate reader (Tecan Infininte M200, Männedorf, Switzerland), at time 0 and after 1 h of incubation at 40 ºC. The PAL activity was calculated as µmol of *t*-cinnamic acid synthesized kg^{-1} fresh weight (fw) h^{-1} using a *t*-cinnamic acid (Sigma, St Louis, MO, USA) standard curve (0-6.75 mM). Each of the three replicates was analysed in duplicate.

I.2.3.5. Phenolic compounds

Extraction to determine phenolic compounds and TAC extract was conducted by homogenization (Ultra Turrax®) of 2 g of sample in 8 mL methanol (Sigma, St Louis, MO, USA) for 20 s under ice-water bath. Subsequently, extracts were centrifuged at 13,500 × g for 20 min at 4 ºC and supernatants were collected and analysed. Extracts for individual phenolic compounds were further filtered through a 0.22 µm polyethersulphone filter and stored at −80 ºC in amber vials until Ultra High-Performance liquid chromatography (UHPLC) analysis.

TPC was analysed by Folin–Ciocalteu reagent method as previously described (Martínez-Hernández et al., 2011). Briefly, a 19 µL aliquot of TPC extract was placed on a PS flat bottom 96-well plate (Greiner Bio-One, Frickenhausen, Germany) and 29 µL of Folin–Ciocalteu reagent 1 N (Sigma, St Louis, MO, USA) were added. Samples were incubated for 3 min in darkness at room temperature. After incubation, 192 µL of a solution containing Na_2CO_3 (4 g L^{-1}) and NaOH (20 g L^{-1}) were added and the reaction was carried out for 1 h at room temperature in darkness, measuring the absorbance at 750 nm using the Multiscan plate reader. TPC was expressed as chlorogenic acid equivalents (ChAE) in mg kg^{-1} fw. Each of the three replicates was analysed in triplicate.

Analyses of individual phenolic compounds were conducted as previously described (Alegria, 2015) with some modifications. Briefly, samples of 20 µL were analysed using an UHPLC instrument (Shimadzu, Kyoto, Japan) equipped with a DGU-20A degasser, LC-30AD quaternary pump, SIL-30AC autosampler, CTO-10AS column heater and SPDM-20A photodiode array detector. The UHPLC system was controlled by the software LabSolutions (Shimadzu, v. 5.42 SP5). Chromatographic analyses were carried out onto a Kinetex C18 column (100 mm × 4.6 mm, 2.6 µm particle size; Phenomenex, Macclesfield, UK) with a KrudKatcher Ultra HPLC guard column (Phenomenex, Macclesfield, UK). The column temperature was maintained at 25 ºC. The mobile phase was acidified water (A; formic acid to final pH 2.3) and acidified methanol (B; formic acid to final pH 2.3). The flow rate was 1.5 mL min^{-1}. Gradient program used was 0/88, 1.2/88, 2.4/85, 8.3/70, 9.4/50, 11.8/50, 20.8/55, 22.0/60 (min/% phase A). Then, column equilibration was conducted at 0 % A for 2.2 min. Chromatograms were recorded at 320 nm. Phenolic acids were quantified as standards of chlorogenic acid (3-CQA), ferulic acid (Sigma, St Louis, MO, USA), isochlorogenic acid A (3,5-CQA) and C (4,5-CQA) (ChromaDex, Irvine, CA, USA). The calibration curves were made with at least six data points. The results were expressed as mg kg^{-1} fw. Each of the three replicates was analysed in duplicate.

I.2.3.6. Total antioxidant capacity

The extracts were analysed for TAC according to Brand-Williams et al. (1995) with slight modifications (Martínez-Hernández et al., 2013d). Briefly, a solution of 0.7 mM 2,2-diphenyl-1-picrylhydrazil (DPPH) (Sigma, St Louis, MO, USA) in methanol was prepared 2 h before the assay and adjusted to 1.1 (nm) immediately before use. A 21 µL aliquot of the previously described extract was placed on a PS flat-bottom 96-well plate and 194 µL of DPPH was added. The reaction was carried out for 30 min at room temperature in darkness and the absorbance at 515 nm was measured using the Multiscan plate reader. Results were expressed as mg Trolox (Sigma, St Louis, MO, USA) equivalent kg^{-1} fw. Each of the three replicates was analysed in triplicate.

I.2.3.7. Statistical Analyses

A complete randomized design in triplicate, with two-way ANOVA (treatment × storage), by Post Hoc Tuckey HSD tests (p=0.05), were used with SPSS software (v. 21, IBM, USA). Possible synergistic effects of the stresses combinations were studied with Limpel's formula (equation I.6) according to (Richer, 1987), where the effectiveness of a combination of treatments exceeds the prediction of the effectiveness of their additive action.

$$E_e = X + Y - \left(\frac{XY}{100}\right) \quad \text{(I.6)}$$

I.3. RESULTS

I.3.1. UV-C transmittance through carrot tissue

UV-C radiation showed a low transmittance through carrot tissue. Accordingly, carrot sections prepared with the lowest thickness (0.1 mm) showed a transmittance < 20 %. Carrot external tissue showed a low UV-C transmittance compared to internal tissue (Figure I.1). Accordingly, external tissue sections of 0.1 mm thickness showed a transmittance of 1.25 % while the same thickness for internal tissue showed a transmittance of 18.7 %.

I.3.2. Relative electrolyte leakage

Initial REL of whole and shredded carrots was 0.9±0.2 and 36.9±0.9 %, respectively, without significant ($p < 0.05$) differences among irradiated and non-irradiated samples on processing day (Figure I.2). No significant ($p < 0.05$) REL differences among whole carrots were found at 72 h (data not shown). Attending to shredded samples, REL values of air-stored ones showed 13-19 lower units compared to hyperoxia conditions. Among air-stored shreds, irradiated ones showed the lowest REL with 14.9±3.5 %. Non-irradiated shreds stored under air conditions registered intermediate REL of 32.8±1.6 % at 72 h.

I.3.3. Colour

Whole carrots did not show significant ($p < 0.05$) colour changes throughout storage (data not shown). Attending to shredded samples, the applied UV-C dose only induced mild colour changes of ΔE=6.2 with a slight browning of ΔBI=80.4 on processing day (data not shown). Initial WI of 22.3 was not significantly ($p < 0.05$) changed after 72 h with final ΔWI ranging from 2-10 (Figure I.3). Irradiated shreds showed higher ΔE (23-25) and browning (ΔBI=80-101) after 72 h compared to control carrots regardless of gas treatments (Figure I.3).

I.3.4. Phenylalanine ammonia-lyase activity

Carrots showed an initial PAL activity of 39.5±9.6 µmol cinnamic acid formed kg^{-1} h^{-1} fw without significant ($p < 0.05$) differences among treatments (Figure I.4). PAL activity of hyperoxia-stored shreds early increased after 12 h registering an activity 71 % higher compared to their respective initial levels. However, the remaining shredded samples did not achieve significant changes of PAL activity after 12 h. In general, PAL activity of shreds registered a continuous increase throughout storage reaching maximum levels at 60 h. Accordingly, air-stored shreds registered PAL activities 820 % higher after 60 h while hyperoxia-stored shreds showed 1050 % enhanced PAL activities after 60 h regarding their initial levels.

UV-C pretreatment resulted in 4 and 3-fold reduced enhancements of PAL activities after 60 h under air and hyperoxia conditions, respectively, compared to non-irradiated carrots. PAL activity of non-irradiated shreds was approximately 30 % reduced from 60 to 72 h regardless of the storage atmosphere. PAL activities of UV-C-pretreated shreds followed a continuous increment throughout storage, although in a lower rate compared to non-irradiated shreds. In the same way, UV-C-pretreated shreds stored under air conditions registered a 94 % increment of PAL activity from 60 to 72 h, while the PAL activity of the same samples remained unchanged under hyperoxia conditions during that period.

I.3.5. Phenolic compounds

The initial TPC of whole carrots was 166.4±11.7 mg ChAE kg fw^{-1} (Figure I.5). The major individual phenolic compounds identified were 3-CQA, 3,5-CQA, 4,5-CQA and ferulic acid (Table I.1). These phenolic compounds accounted 67.5, 15.5, 14.8 and 2.2 % of the sum of individual phenolics, respectively. The phenolic contents of carrots were unchanged ($p < 0.05$) immediately after wounding and UV-C radiation on processing day, although these levels increased throughout storage of stressed samples.

Regarding whole carrots, a UV-C pretreatment induced maximum TPC enhancements of 220 and 315 % after 36 and 48 h in air and hyperoxia storage conditions, respectively, and then followed by a general decrease. This behaviour was also observed in non-irradiated shreds, which registered ≈ 400-410 % higher TPC contents after 48 h compared to initial levels regardless of the atmosphere conditions. Whole irradiated samples showed 120 and 340 % higher TPC contents after 72 h under air and hyperoxia storage conditions, respectively, compared to their respective initial levels. TPC of non-irradiated whole carrots remained unchanged ($p < 0.05$) throughout storage. Correspondingly, the highest 3-CQA enhancements were registered by irradiated whole samples with 585 (air) and 636 % (hyperoxia) after 72 h.

Wounding of non-irradiated carrots enhanced TPC by ≈1,490 % after 72 h under air conditions. Hyperoxia benefit on TPC was only significantly observed after 60 h reaching non-irradiated samples the maximum TPC enhancements, ≈2,000 %, after 72 h. Similarly, non-irradiated shreds stored under hyperoxia conditions registered approximately 5-fold higher 3,5-CQA enhancements after 72 h compared to air-stored non-irradiated shreds. Furthermore, the rest of phenolic compounds of non-irradiated shreds registered 2-3-fold higher accumulation under hyperoxia conditions compared to air conditions after 72 h. The combination of wounding and hyperoxia stresses showed a synergistic effect after 72 h since the observed TPC enhancement (2,000 %) was higher than that calculated according to Limpel's formula (1,620 %).

The UV-C pretreatment, in shreds, induced lower TPC accumulation throughout storage, contrary to non-irradiated shreds. Accordingly, UV-C-pretreated shreds registered 1.5 and 3.2-fold lower TPC enhancements after 72 h under air and hyperoxia conditions,

respectively, compared to non-irradiated shreds. Similarly, 3,5-CQA/4,5-CQA registered 2 and 6-fold lower accumulation in irradiated shreds under air and hyperoxia conditions, respectively, compared to non-irradiated shreds. However, irradiated shreds under hyperoxia conditions showed 1.4-fold higher 3-CQA content compared to non-irradiated samples stored under air conditions for 72 h. Meanwhile, no great differences between air and hyperoxia conditions were observed in the UV-C-induced lower levels of the rest of individual phenolic compounds.

I.3.6. Total antioxidant capacity

The initial TAC of whole carrots was 135.7±45.4 mg Trolox kg^{-1} fw (Table I.2). Wounding of carrots increased initial TAC by ≈610 % just after processing on day 0. Similarly, UV-C treatment of whole and shredded samples induced 269 and 16 % higher TAC on processing day. The combination of wounding and UV-C did not show a synergistic effect since the observed TAC enhancement of carrots treated with both stresses combined (720 %), compared to untreated whole ones, was lower than TAC enhancement calculated according to Limpel's formula (870 %) being then considered as an additive effect.

TAC of all carrots showed a constant increase throughout storage, registering maximum increases in the last 24 h of storage. Whole samples registered maximum TAC increases of 7.0 and 2.4-2.8-fold regarding their respective initial levels in non-irradiated air-stored and hyperoxia and/or UV-C stressed ones, respectively. However, final TAC of shreds was higher than that of whole shreds. Accordingly, shreds stored under air and hyperoxia registered TAC of 2,050 and 2,668 mg Trolox kg^{-1} fw at 72 h, respectively. However, UV-C pretreatment of shreds induced 30 and 40 % lower TAC levels at 72 h under air and hyperoxia conditions, respectively, compared to the respective non-irradiated samples at 72 h. TAC changes throughout storage were highly correlated (R^2= 0.70) to TPC.

I.4. DISCUSSION

Carrot has been widely used as a model system to understand the effect of different postharvest abiotic stresses on the phenylpropanoid metabolism due to the great enhancement of phenolic compounds observed, with high antioxidant capacity, compared to other vegetables (Cisneros-Zevallos, 2003). UV-C is a sanitizing method used in fresh-cut products as a sustainable alternative to conventional NaOCl (Martínez-Hernández et al., 2015b). Food safety legislation of fresh-cut products is regulated for *E. coli*, *Salmonella* spp. and *Listeria monocytogenes* (EC_1441/2007, 2007). Inactivation kinetics of these three pathogens by UV-C has been recently modelled in fresh-cut products (Martínez-Hernández et al., 2015a). Therefore, intermediate doses of 9-10 kJ m^{-2} are needed to ensure the legislated food safety criteria of a fresh-cut product (Martínez-Hernández et al., 2011; Martínez-Hernández et al., 2015a). In that sense, a UV-C dose of 9 kJ m^{-2} was selected in this experiment achieving a sanitizing effect of approximately 1.5 log units for mesophiles and yeasts and moulds (data not shown). The effect of this moderate UV-C dose, single or combined with other abiotic stresses like hyperoxia storage and wounding, on the phenolic content and related antioxidant capacity and PAL activity are firstly reported in this study, to the best of our knowledge.

The initial TPC of non-wounded carrot (166.4 mg ChAE kg fw^{-1}) was similar to previous data being hydroxycinnamic acids and their derivatives the major phenolic compounds found (Alegria et al., 2012). According to phenolic profile, chlorogenic acid was the major compound found accounting approximately 70 % of the sum of individual phenolic compounds. As expected, application of the studied abiotic stresses did not immediately affect PAL activity of samples on processing day, and consequently the phenolic contents. However, TAC was apparently increased immediately after UV-C radiation and, in a greater extend, after wounding (showing the combination of both stresses an additive effect according to Limpel's formula). TAC was highly correlated (R^2=0.70) to TPC throughout storage of samples as previously found (Cisneros-Zevallos, 2003). Accordingly, the observed higher TAC immediately after wounding and UV-C pretreatment may be an experimental artifact resulted from higher extraction of other antioxidant compounds of carrots such as carotenoids due to increased cell wall depolymerization (Alegria et al., 2012; Bhat et al., 2007).

Wounding and hyperoxia storage of carrots at 15 ºC induced phenolic compounds enhancements which were well correlated to TAC and explained by the observed changes of PAL activity. The phenolic compounds accumulation after wounding and hyperoxia stresses has been related to PAL activation being proposed ATP and reactive oxygen species as signalling molecules (Jacobo-Velázquez et al., 2011). Furthermore, phenolic compounds in wounded plants are produced in part as a mechanism to support the biosynthesis of lignin (Becerra-Moreno et al., 2015). Accordingly, a REL decrease was observed in air-stored shreds after 72 h possibly due to such lignification process being such lignification probably inhibited in hyperoxia-stored samples. This study shows a detailed register of phenolic contents and related antioxidant capacity in stressed carrots in 12-h intervals. Accordingly, phenolic accumulation in shredded carrots during storage at 15 ºC could be divided into three different phases: 1st phase, < 24 h: unchanged phenolic compounds levels with minimum PAL activity; 2nd phase, 24-48 h: moderate phenolic increments (≈600-700 mg ChAE kg^{-1} accumulated in 24 h) concurring with the greatest increase of PAL activity; 3nd phase, 48-72 h: high phenolic increments (≈1,600-2,700 mg ChAE kg^{-1} accumulated in 24 h) while a moderate increment of PAL activity was registered. The observed lower increase of PAL activity from 48 to 60 h and subsequent intense decrease in non-irradiated shreds may be a result of a feedback modulation or due to the diversion of the synthetic capacity of the cell to the production of other proteins (Alegria, 2015; Boerjan et al., 2003; Saltveit, 2000). Maximum phenolic content of non-irradiated shreds at 72 h may be the delayed consequence of maximum PAL activity which is the first key enzyme in the phenylpropanoid pathway. The combination of wounding and hyperoxia stresses showed a synergistic effect with phenolic accumulation of 2,000 % after 72 h previously described in carrot shreds (Jacobo-Velázquez et al., 2011).

Combination of moderate UV-C dose and subsequent hyperoxia storage reduced TPC increments throughout storage compared to non-irradiated samples. However, the content of chlorogenic acid in irradiated samples under hyperoxia was slightly higher (1.4-fold) compared to non-irradiated samples after 72 h under high hyperoxia. Similarly, UV-C-irradiated carrot bagasse showed higher 3-CQA content compared to non-irradiated bagasse after 48 h at 25 ºC being not correlated to TPC data which were statistically similar between non-irradiated and irradiated samples (Sánchez-Rangel et al., 2013). Low UV-C doses (≤2.5 kJ m^{-2}) applied as single treatment in carrot shreds induced phenolic

accumulations of approximately 20-35 % after 5-8 days at 5 ºC (Alegria et al., 2012). On the other side, UV-C radiation treatments (4.5-6 kJ m^{-2}) and hyperoxia storage (90-100 kPa O_2 balanced with nitrogen) of fresh-cut broccoli Bimi® and tatsoi baby leaves did not induce significant TPC and TAC which were even reduced during storage at 5 ºC up to 19 days (Martínez-Hernández et al., 2013d; Tomás-Callejas et al., 2012). Latter finding may be explain by the high ascorbic acid content of broccoli Bimi® and tatsoi (Samuolienė et al., 2012) contrary to carrot, showing vegetables with low ascorbic acid content higher phenolic accumulation after abiotic stresses (Reyes et al., 2007). A possible explanation for the hereby found lower phenolic accumulation in irradiated samples with moderate UV-C dose, compared to non-irradiated samples, may be a partial PAL denaturation by such UV-C dose delaying the stress-enhanced activity of this enzyme. A subsequent PAL reactivation may occur as observed in air-stored irradiated shreds from 60 to 72 h which agrees with enhanced phenolic levels of these samples in that period. However, this great PAL reactivation from 60 to 72 h was not observed in irradiated shreds stored under hyperoxia conditions. That absence or delayed PAL reactivation beyond 72 h may be a consequence of oxidative detrimental effects of hyperoxia storage on this enzyme. In contrast to shredded samples, whole UV-C-pretreated carrots experimented a mild phenolic accumulation peak early during storage, contrary to unchanged phenolic contents of non-irradiated samples. Carrot peel has a very high UV-C protective effect since peel with 0.1 mm-thickness only allowed penetration of 1.25 % of total UV-C applied. However, a layer of internal tissue with 0.1 mm-thickness allowed UV-C penetration of 18.7 %. Accordingly, carrot peel in whole samples reduced hypothetical damage to PAL but somehow allowed transmission of stress-induced signal with consequent observed phenolic accumulation. The hereby found phenolic peak of irradiated whole samples was observed later in hyperoxia-stored samples compared to air conditions due to previously supposed partial damage of PAL after UV-C radiation.

Colour is the main sensory parameter decisive on the visual consumer election of fresh-cut carrots on retail surfaces. Whitening and browning and are the main colour degradation processes occurred in wound (fresh-cut) carrots. Carrot browning was early related to enzymatic oxidation of polyphenolic compounds (Chubey and Nylund, 1969) being its occurrence in UV-C-treated products owed to the increased peroxidase (POD) activity (Tomás-Barberán and Espín, 2001). On the other side, whitening mechanism was

deeply studied in carrots being attributed in a first reversible physical stage to dehydration and lately to an irreversible physiological response involving activation of phenolic metabolism and production of lignin (Cisneros-Zevallos et al., 1995). The hereby registered WI ranges were not visually detected as previously reported by a sensory panel test (Alegria et al., 2012). The low whitening changes (ΔWI) after 72 h registered in carrots were greatly reduced by the supply of humidified gas to the containers containing carrot samples during storage avoiding samples dehydration. The slightly higher colour changes due to browning in irradiated samples after 72 h of storage may be owed to the pre-activated POD during UV-C pretreatment (Tomás-Barberán and Espín, 2001). Orange colour of carrots is owed to its high carotenoid content representing β-carotene approximately 80 % of the total content of these natural pigments (Rodriguez-Concepcion and Stange, 2013). Total carotenoids contents have been reported to be increased by 2-3-fold after wounding, heat shock or UV-C radiation in shredded carrots (Alegria et al., 2012). However, last authors did not report noticeable visual colour differences in samples during storage comparing to freshly-cut carrots. Conclusively, no colour changes were detected in whole carrots while mild colour changes were registered in shredded samples after 72 h which would not lead to an organoleptic rejection of these stressed samples.

Microbiological analyses of stressed carrots after 72 h revealed total mesophilic and yeasts and moulds loads of whole and shredded samples lower than 6.0 and 6.5 log CFU g^{-1}, respectively, without significant differences among treatments (data not shown). Accordingly, these microbiological levels were below the threshold limit (7 log CFU g^{-1}) to define fresh-cut products shelf life (Gilbert et al., 2000).

Chlorogenic acid, the main phenolic compound in carrots, is an ester of caffeic acid with quinic acid with great antioxidant capacity compared to other phenolic compounds (Castelluccio et al., 1995). To prevent or slow the oxidative damage in humans induced by free radicals sufficient amounts of phenols as antioxidants need to be consumed with foods. Carrots occupy the sixth place among the list of most consumed vegetables in the American diet, although the TPC of this vegetable is almost the lowest one (Chun et al., 2005). Accordingly, proposed postharvest abiotic stresses can highly increase the phenolic levels of carrots leading to greater ingestion of these antioxidant compounds from this popular and highly consumed vegetable.

I.5. CONCLUSIONS

Wounding and moderate UV-C pretreatment of carrots greatly increased the activity of the PAL, the key enzyme in the biosynthesis pathway of phenolic compounds, with subsequent increments of 1,000-1,500 % of total phenolic content after 72 h at 15 ºC. A hyperoxia storage even augmented those total phenolic increments up to 2,000 %, being also partially benefited by a mild water stress, although the pretreatment with UV-C reduced PAL activity favoured by a higher electrolyte leakage. Accordingly, this study provides a detailed photograph (12 h intervals) of phenolic accumulation after synergistic effects of those postharvest abiotic stresses. The application of such stresses may be used as a postharvest tool to greatly increase the health-promoting properties of carrots meeting food safety aspects related to the moderate UV-C dose used.

Table I.1. Individual phenolic compounds of carrots treated with different postharvest abiotic stresses (wounding, UV-C and hyperoxia storage) during storage up to 72 h at 15 ºC (n=3±SD). Different capital letter denotes significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letter denotes significant differences ($p < 0.05$) among different sampling times for the same treatment.

Storage (h)	0	12	24	36	48	60	72
3-CQA (mg kg^{-1} fw)							
Whole							
AIR	36.6±13.3 Bc	65.6±15.0 Abc	74.6±0.7 Cb	60.4±6.8 Fbc	59.7±2.4 Fbc	48.1±8.7 Fbc	150.7±49.8 Da
AIR-UVc	48.2±2.3 Ad	42.7±3.5 Bd	115.1±9.1 Bd	250.0±33.4 Bd	149.8±5.2 Ca	196.4±54.6 BCb	330.3±10.5 BCc
HO*	36.6±13.3 Be	36.2±1.5 Be	85.2±19.3 Ccd	364.9±25.1 Aa	79.7±5.0 Ed	98.1±16.1 EFc	124.6±0.5 Db
HO-UVc	48.2±2.3 Ae	80.3±19.7 Ad	158.4±27.8 Ac	161.8±6.9 Cc	304.3±1.6 Ab	319.3±14.0 Ab	354.9±13.8 Ba
Shredded							
AIR	27.5±0.3 Cd	34.9±4.0 Bd	69.5±9.5 CDc	71.5±6.1 Fc	160.4±11.8 Ca	133.6±19.6 DEb	146.6±6.6 Dab
AIR-UVc	43.5±2.2 ABd	38.3±0.5 Bd	51.8±2.0 DEd	58.7±8.2 Fd	200.4±19.5 Ba	164.7±7.1 CDb	117.1±29.4 Dc
HO	27.5±0.3 Cf	42.9±8.6 Bef	69.2±1.0 CDe	137.7±1.1 Dd	190.4±4.4 Bc	249.5±61.3 Bb	299.3±7.3 Ca
HO-UVc	43.5±2.2 ABd	46.0±1.8 Bd	42.9±0.9 Ed	115.6±16.0 Ec	125.8±18.6 Dc	197.0±14.0 BCb	421.2±12.2 Aa
Ferulic acid (mg kg^{-1} fw)							
Whole							
AIR	1.2±0.9 ABb	1.5±0.9 Ab	1.6±0.2 DEb	0.2±0.2 Cb	2.1±0.7 Cb	2.2±0.1 Eb	12.6±3.0 Aa
AIR-UVc	2.2±0.4 Ac	1.3±0.6 BCc	3.9±0.5 Bb	7.2±3.0 Aa	3.9±0.6 Bb	5.1±0.6 Bb	7.8±0.4 BCa
HO	1.2±0.9 ABb	1.3±0.0 BCb	0.0±0.0 Db	2.2±1.6 Bb	4.4±1.1 Ba	0.0±0.3 Ca	3.8±0.0 CDa
HO-UVc	2.2±0.4 Ad	2.3±0.0 Ad	7.6±0.9 Ab	2.3±0.2 Bd	8.6±0.9 Aa	5.1±0.4 Bc	8.7±0.9 ABa
Shredded							
AIR	0.4±0.0 Bd	0.3±0.0 Ed	0.9±0.6 DEcd	1.1±0.2 BCc	2.9±0.7 BCb	3.1±0.8 Db	4.4±0.5 BCDa
AIR-UVc	1.0±0.2 Bd	0.7±0.1 DEd	0.7±0.5 Ed	1.2±0.0 BCcd	3.6±1.1 BCa	2.9±0.8 DEab	2.1±0.2 Dbc
HO	0.4±0.0 Be	0.7±0.5 DEe	2.9±0.4 Cc	1.6±0.2 BCd	2.2±0.7 Ccd	6.2±0.5 Ab	9.0±0.0 ABa
HO-UVc	1.0±0.2 Bd	1.0±0.1 CDd	1.0±0.2 DEcd	2.1±0.3 Bbcd	2.2±1.7 Cbc	3.1±0.3 Db	6.8±0.2 BCa

3,5-CQA (mg kg^{-1} fw)							
Whole							
AIR	8.4±0.0 Be	8.7±0.3 Acd	9.0±0.2 BCb	9.2±0.3 BCa	8.7±0.1 Acd	8.5±0.1 Cde	8.8±0.1 Dbc
AIR-UVc	8.7±0.0 Bc	8.6±0.1 ABc	8.7±0.2 BCbc	15.1±0.7 BCa	8.6±0.0 Ac	8.7±0.0 Cc	9.4±0.8 CDb
HO	8.4±0.0 Bc	8.7±0.0 ABbc	8.7±0.1 Cbc	11.4±1.2 Ca	9.9±2.0 Ab	8.5±0.1 Cbc	8.5±0.0 Dbc
HO-UVc	8.7±0.0 Bbc	8.7±0.1 Abc	9.5±0.5 Aab	10.3±1.0 Aa	9.3±0.1 Ab	8.3±0.8 Cc	8.1±0.6 Dc
Shredded							
AIR	9.0±0.3 Abc	8.6±0.0 ABc	8.7±0.0 BCc	8.7±0.1 BCc	9.1±0.5 Abc	9.5±0.1 Bb	10.3±0.4 Ca
AIR-UVc	8.6±0.2 Ba	8.6±0.0 Ba	8.7±0.1 Ca	8.6±0.2 Ca	9.4±0.3 Aa	9.6±0.5 Ba	9.4±1.8 CDa
HO	9.1±0.3 Acd	8.6±0.0 ABd	8.9±0.1 BCcd	8.9±0.1 BCcd	9.4±0.4 Ac	10.6±0.6 Ab	16.4±1.2 Aa
HO-UVc	8.6±0.2 Bd	8.7±0.0 ABd	9.0±0.0 Bd	10.2±0.8 Bb	9.1±0.4 Acd	9.7±0.1 Bbc	11.9±0.6 Ba
4,5-CQA (mg kg^{-1} fw)							
Whole							
AIR	8.0±0.3 Ab	4.9±0.7 Bc	3.7±0.2 CDd	2.7±0.1 Cc	4.9±0.0 Dc	4.0±0.2 EFd	18.0±0.1 Aa
AIR-UVc	8.7±0.1 Bc	4.0±0.6 Bd	4.7±1.0 Bd	15.5±0.6 Aa	6.0±1.0 Cc	6.6±1.4 Bc	7.7±1.3 Fb
HO	8.0±0.3 Ac	4.9±0.9 Bd	4.3±0.1 BCd	8.6±1.6 Ba	6.8±0.0 Cbc	7.1±0.1 Abc	7.5±0.1 Db
HO-UVc	8.7±0.1 Bcd	8.0±3.5 Abc	9.0±0.4 Ab	3.0±0.1 Ee	16.7±1.2 Aa	4.4±0.3 Ede	5.9±0.1 Ecd
Shredded							
AIR	3.4±0.2 Cb	3.3±0.1 Bb	3.6±0.2 DEb	3.2±0.1 DEb	4.9±0.9 DEa	3.8±0.0 Fb	5.5±0.5 EFa
AIR-UVc	3.8±0.4 Cc	3.5±0.1 Bc	3.1±0.1 Ec	3.1±0.1 Ec	8.0±0.5 Ba	5.4±0.2 Cb	4.9±1.0 CDb
HO	3.4±0.2 Ccd	3.7±0.5 Bcd	3.3±0.1 DEd	3.9±0.2 CDc	4.7±0.2 DEb	4.9±0.3 Db	9.2±0.7 Ba
HO-UVc	3.8±0.4 Ccd	3.4±0.1 Bd	3.6±0.1 DEcd	3.4±0.3 DEd	4.0±0.5 Ec	4.8±0.3 Db	8.4±0.1 BCa

*HO: hyperoxia storage; 3-CQA: 3-caffeoylquinic acid; 3,5-CQA: 3,5-dicaffeoylquinic acid; 4,5-CQA: 4,5-dicaffeoylquinic acid

Table I.2. Total antioxidant capacity of carrots treated with different postharvest abiotic stresses (wounding, UV-C and hyperoxia storage) during storage up to 72 h at 15 ºC (n=3±SD). Different capital letter denotes significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letter denotes significant differences ($p < 0.05$) among different sampling times for the same treatment.

Storage (h)	0	12	24	36	48	60	72
Whole							
AIR	135.7±45.4 Dd	234.5±35.2 Dcd	351.8±74.1 Ebcd	481.6±24.5 Dabc	619.3±59.1 Cab	368.8±44.1 Gbcd	740.7±92.6 Ea
AIR-UVc	500.0±44.0 Cc	475.6±41.6 Cc	470.6±42.1 DEc	314.2±31.5 DEcd	241.3±42.1 Dd	844.4±39.8 Eb	1,511.9±43.1 CDa
HO*	135.7±45.4 Dc	365.2±32.6 Dbc	476.1±54.0 DEab	349.5±43.1 Ebc	197.9±39.6 Dbc	619.5±76.8 Fa	732.6±70.4 Ea
HO-UVc	500.0±44.0 Cc	515.6±49.5 Cc	548.7±88.2 CDEc	378.2±21.0 DEd	169.7±29.7 Dd	1,071.7±55.4 Db	1,432.8±33.9 Da
Shredded							
AIR	959.6±103.1 Bb	847.2±54.5 Bc	742.1±47.2 CDd	841.3±45.6 Cc	983.3±32.4 Bb	1,490.4±42.8 Ca	1,580.2±41.4 CDa
AIR-UVc	1112.7±152.3 Ab	1,004.2±87.6 Ab	1,046.6±52.9 ABb	1,198.5±87.2 Ab	1229.0±26.4 Ab	1,878.7±24.7 Ba	2,049.6±115.8 Ba
HO	959.6±103.1 Bd	898.2±87.5 Bd	816.3±49.6 BCd	1,042.2±92.1 BCcd	1158.4±43.1 Ac	2,063.2±64.4 Ab	2,668.1±128.1 Aa
HO-UVc	1112.7±152.3	1,085.3±95.6 Abc	1,232.1±34.0 Ab	1,106.2±36.8 ABbc	967.3±32.2 Bc	1,336.8±42.7 Cb	1,863.2±55.3 BCa

*HO: hyperoxia storage

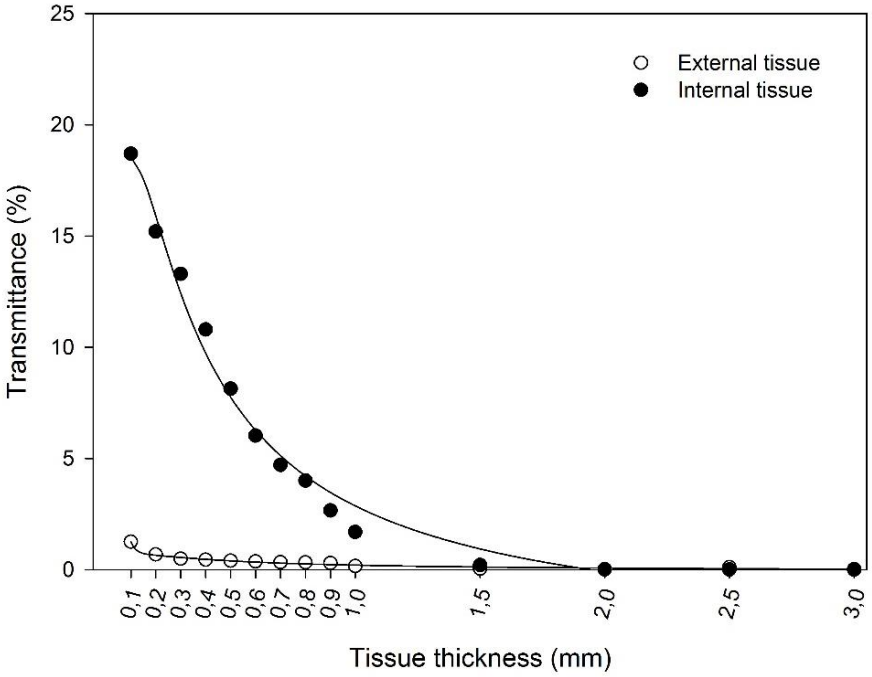


Figure I.1. UV-C transmittance of internal and external carrot tissue sections with different thickness. Symbols represent experimental data and lines represent fitted data with polynomial inverse third order (R^2> 0.98).

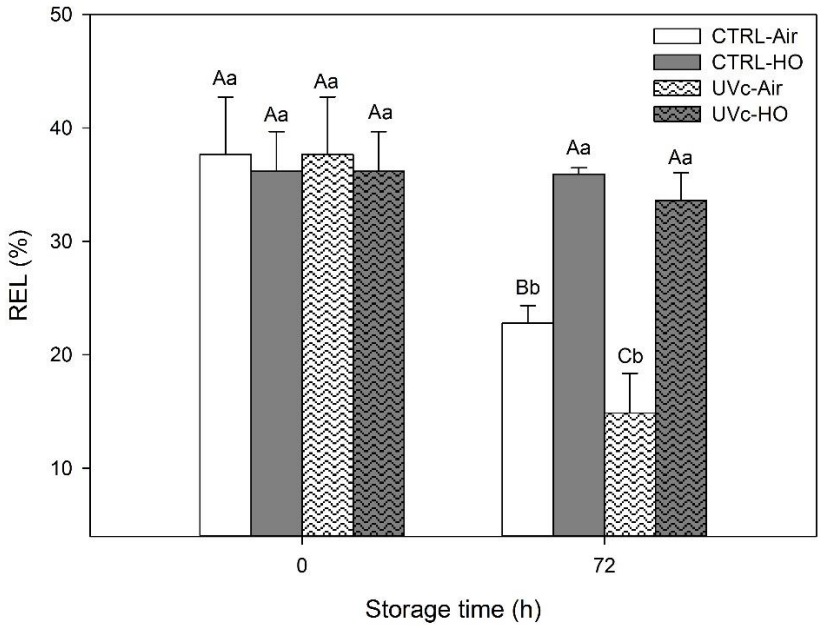


Figure I.2. Relative electrolyte leakage (REL) of shredded carrots treated with different postharvest abiotic stresses (UV-C and hyperoxia storage) at time 0 and after 72 h of storage at 15 ºC (n=3±SD). Different capital letter denotes significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment.

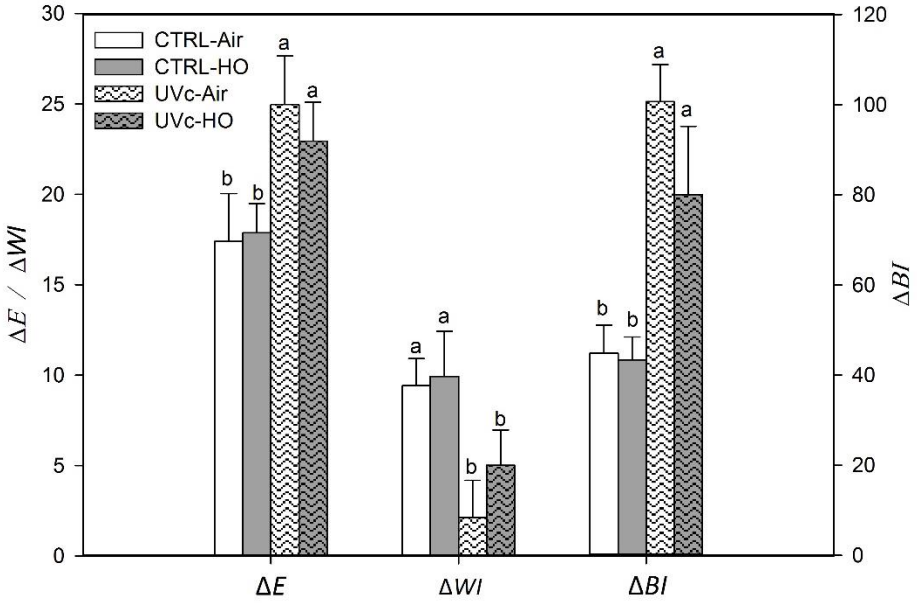


Figure I.3. Total colour (Δ*E*), whitening (Δ*WI*) and browning differences (Δ*BI*) of shredded carrots treated with different postharvest abiotic stresses (UV-C and hyperoxia storage) after 72 h of storage at 15 ºC (n=3±SD). Different letters denote significant differences ($p < 0.05$) among different treatments.

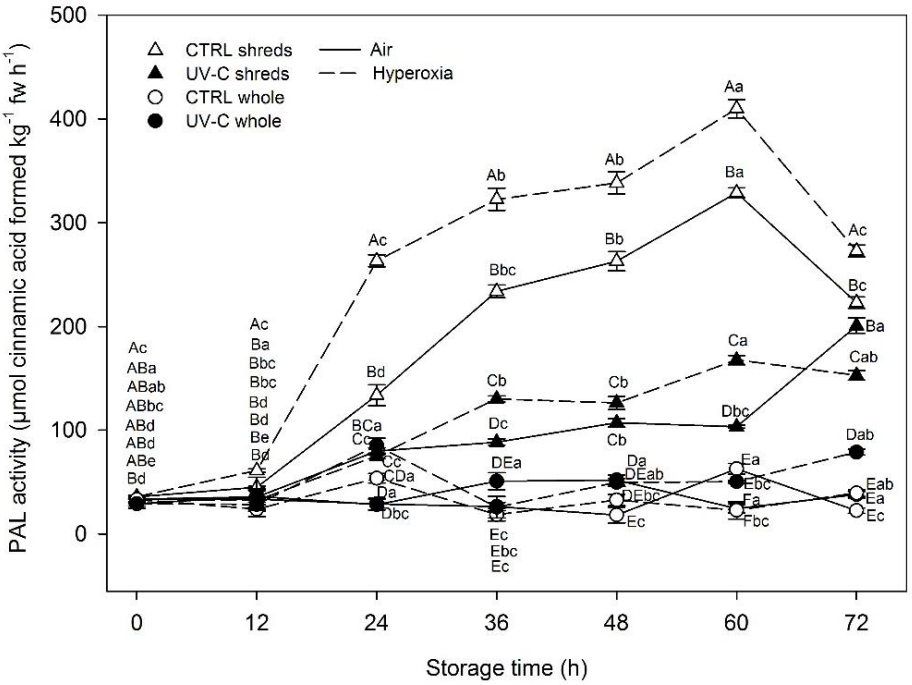


Figure I.4. Phenylalanine ammonia-lyase (PAL) activity of carrots treated with different postharvest abiotic stresses (wounding, UV-C and hyperoxia storage) during storage up to 72 h at 15 ºC (n=3±SD). Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment.

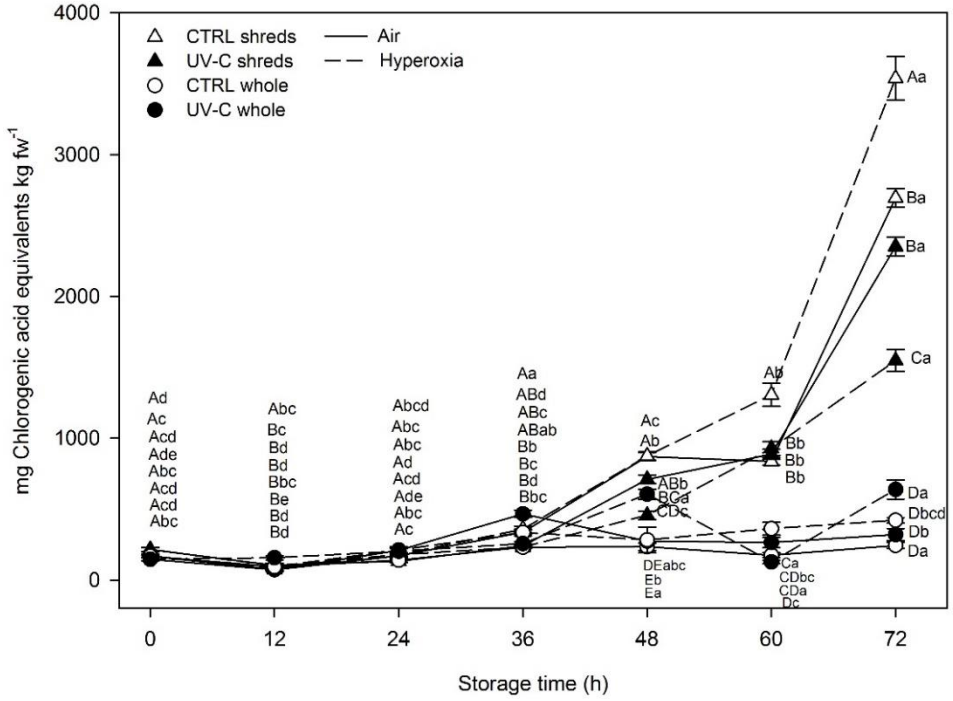
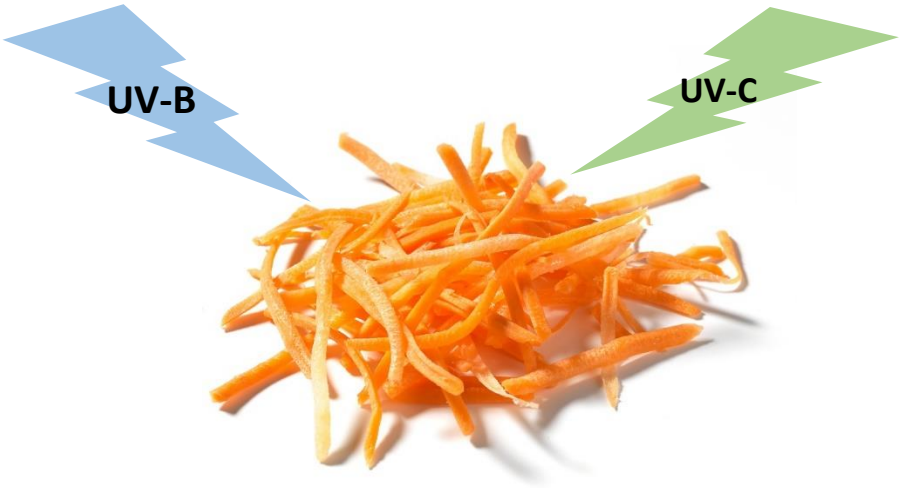


Figure I.5. Total phenolic content of carrots treated with different postharvest abiotic stresses (wounding, UV-C and hyperoxia storage) during storage up to 72 h at 15 ºC (n=3±SD). Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment.



CHAPTER II

Effects of UV-B and UV-C combination on phenolic compounds biosynthesis in fresh-cut carrots

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II.1. INTRODUCTION

Nowadays, food is not only intended to feed, but also to prevent chronic and nutritional-related diseases as well as to improve overall human well-being, mainly linked to the crescent consumer's knowledge on functional foods. The high contents of phytochemicals from fruit and vegetables have been proven to prevent a grand array of diseases such as degenerative disorders, cancer, cardiovascular among others related to the consumption of these plant products (Slavin and Lloyd, 2012). Enhancement of the health-promoting properties of fruit and vegetables will add value and create new opportunities, even with recent economical drawbacks. Therefore, there is a need to provide technologies to handle fresh products with enhanced health-promoting properties (Jongen, 2002).

Carrot is a popular vegetable among broad strata of the population. The popularity of this vegetable is mainly due to its sensory characteristics and nutritional compounds. Furthermore, carrots do not contribute with high calories intake, however they play a significant source of nutrients, such as carotenoids, vitamins (A, E) and antioxidants on human diet (Sharma et al., 2011). Phenolic compounds are great antioxidants related to several health-promoting properties such as anti-inflammatory, antitumoral, as well as preventing neurodegenerative and chronic disorders. Moreover, those compounds contribute to sensory features of food. Currently, health recommendations rely on a diet rich in multiple antioxidant compounds than one used based on a single antioxidant (Shahidi and Ambigaipalan, 2015). Plant products have been proposed as biofactories of phenolic compounds through different mechanisms induced by abiotic stresses. Particularly, carrot has been widely used as a model system to understand the effect of different postharvest abiotic stresses on the phenylpropanoid metabolism due to the observed high enhancement of phenolic compounds, with high antioxidant capacity, compared to other vegetables (Cisneros-Zevallos, 2003). Concisely, PAL is the key enzyme of primary (shikimate) and secondary (phenylpropanoid) pathways and is, therefore, involved in the biosynthesis of polyphenolic compounds (Dixon and Paiva, 1995). It is well reported that this enzyme is induced by an array of biotic and abiotic stress-induced mechanisms, such as wounding, radiation exposure, hyperoxia storage, water stress, chilling injury, low minerals, hormones and pathogen attack, among others (Alegria et al., 2016; Avena-Bustillos et al., 2012; Becerra-Moreno et al., 2012; Jacobo-

Velázquez et al., 2011). Consequently, such postharvest abiotic stresses enhance the levels of phenolic compounds like caffeoylquinic acid, ferulic acid and their derivates as a defence mechanism of the plant (Jacobo-Velázquez et al., 2011).

Application of UV-B radiation (280–320 nm) has been proposed as a friendly and cheap non-molecular tool to enhance the phenolic compounds in carrots and other horticultural crops during postharvest life (Castagna et al., 2014; Du et al., 2012; Scattino et al., 2014). On the other side, the high germicidal properties of UV-C radiation (100-280 nm) have justified its use as a sustainable alternative to chlorine washing treatment in some fresh-cut products while also being able to stress plant tissues in certain conditions (Artés et al., 2009). Then, the application of UV-B radiation just after wounding could highly enhance phenolic accumulation, while its combination with UV-C may reduce the initial microbial load of fresh-cut products extending their shelf life. Nonetheless, to the best of our knowledge, such combined UV treatment has not already been studied in fresh-cut products. Accordingly, this work studied the single and combined effect of UV-B and UV-C pre-packaging treatments on PAL activity, phenolic compounds and related TAC of shredded fresh-cut carrots during storage at 15 ºC.

II.2. MATERIALS AND METHODS

II.2.1. Plant material preparation

Fresh carrots (cvs. group Nantes, cv. Soprano) were bought in a local market (Cartagena, Spain) on the third week of April 2016. According to the producer specifications, carrots were harvested on the first week of April in Villena area (northwest area of Alicante region, Spain) without any postharvest treatment, but washing, previous expedition to the market. Carrots were transported to the Pilot Plant of the Universidad Politécnica de Cartagena where they were stored in a cold room at 5 ºC until the next day when the experiment was conducted. Plant material was carefully inspected, selecting those with similar visual appearance and size (14-15 cm long and 2-3 cm diameter). Then, carrots (unpeeled) were sanitized in a cold room (8 ºC) with chlorine (150 mg L^{-1} NaClO; 5 ºC; pH 6.5±0.1) for 2 min, rinsed with tap water at 5 ºC for 1 min and drained in a perforated basket for 1 min. A ratio of 300 g plant material: 5 L chlorine was used. Carrots were wounded to shreds (2 mm × 3 mm × 40-60 mm) with a food processor (FreshExpress+,

Moulinex, Lyon, France). Approximately 9 kg of carrot shreds were prepared for the experiment. All samples were submitted to radiation treatments immediately after wounding.

II.2.2. Radiation treatments and incubation conditions

The radiation chamber consisted of a reflective stainless steel chamber with two lamp banks (one bank suspended horizontally over the radiation vessel and the other one placed below it) being fitted to each bank 6 UV-B and 7 UV-C (alternatively positioned) unfiltered emitting lamps (TL 40W/01 RS and TUV 36W/G36 T8, respectively; Philips, Eindhoven, The Netherlands). UV-B and UV-C radiations were separately applied controlled by two general keys that switched all UV-C or UV-B at the same time. The radiation chamber also had a ventilator continuously switched on during treatments to renovate the air from inside of the chamber with the cold air from the cold room (8 ºC). Shredded carrots were placed between the two lines of lamps at 17.5 cm above and below over a bi-oriented PP film (thickness: 35 mm) mounted on a PS net (130 × 68 cm) that minimized blockage of the radiation. The applied UV-B and UV-C intensities of 9.27 and 25.21 W m^{-2}, respectively, were calculated as the mean of 18 readings on each side of the net using LP 471 UVB (Delta OHM, Italy) and VLX 254 radiometers, respectively (Vilber Lourmat, Marne la Vallee, France). Thus, both sides received the same radiation intensities. The equipment is based on that previously described for UV-C illumination (Artés-Hernandez et al., 2009). The light intensities were kept constant and the applied doses were varied by altering the exposure time at the fixed distance. Applied treatments were:

- CTRL: No radiation treatment used as control.
- UV-B: 1.5 kJ m^{-2} UV-B (162 s). The UV-B dose was selected based on our previous experiments and the data reported by Avena-Bustillos et al. (2012) in order to obtain maximum phenolic accumulation in carrots while minimizing heating and evaporation processes during treatment, which may affect the quality of the product.
- UV-C: 4.0 kJ m^{-2} UV-C (159 s). The UV-C dose was selected based on our previous studies in order to achieve a proper microbial reduction while keeping

quality and safety of the product (Formica-Oliveira et al., 2016a; Martínez-Hernández et al., 2015a).

- UV-B+UV-C: 1.5 kJ m^{-2} UV-C followed by 4.0 kJ m^{-2} UV-B.
- UV-C+UV-B: 4.0 kJ m^{-2} UV-B followed by 1.5 kJ m^{-2} UV-C.

Approximately 150 g of treated sample was placed in a rectangular PP basket (170 mm × 120 mm × 60 mm) and covered with a plastic polyethylene (PE) bag to reduce water loss. Three baskets (replicates) per treatment and sampling time were prepared. Samples were stored at 15 ºC (90–95 % RH) up to 72 h (sampling times: 0, 24, 48 and 72 h). Samples were stored at –80 ºC until further analyses of PAL activity, phenolic compounds and TAC were conducted.

II.2.3. Phenylalanine ammonia-lyase

PAL activity was analysed according to Ke and Saltveit (1986) but with modifications (Formica-Oliveira et al., 2016a). Concisely, 2 g of carrot tissue was mixed with 0.2 g of polyvinylpolypyrrolidone (Sigma, St Louis, MO, USA) and homogenized (Ultra Turrax®) in cold 50 mM borate buffer (pH 8.5) containing 400 μL L^{-1} of *β*-mercaptoethanol (Sigma, St Louis, MO, USA). Homogenates were filtered through four layers of cheesecloth and then centrifuged at 10,000 × g for 20 min at 4 ºC. Supernatants were used as enzyme extracts. Two sets of UV-Star flat-bottom 96-well plates containing 69 μL of PAL extract plus 200 μL ultrapure water were prepared for every sample and pre-incubated at 40 ºC for 5 min. Afterwards, 30 μL of either water (blank) or 100 mM L-phenylalanine substrate solution (freshly prepared before assay) was added to each of the wells for every sample set. The absorbances of the sample sets were measured at 290 nm using the Multiscan plate reader at time 0 and after 1 h of incubation at 40 ºC. The PAL activity was calculated as μmol kg^{-1} fw h^{-1} of *t*-cinnamic acid synthesized. Each of the three replicates was analysed in triplicate.

II.2.4. Phenolic compounds

Extraction to determine phenolic compounds and TAC was conducted by homogenization (Ultra Turrax®) of 2 g of sample in 8 mL of methanol (Sigma, St Louis, MO, USA) for 20 s in an ice-water bath. Subsequently, extracts were centrifuged at 13,500 × g for 20

min at 4 ºC and supernatants were collected and analysed. Extracts for individual phenolic compounds were further filtered through a 0.22 µm polyethersulphone filter and stored in amber vials at −80 ºC until UHPLC analysis was conducted.

TPC was analysed by the Folin–Ciocalteu reagent method (Singleton and Rossi, 1965) but with modifications (Martínez-Hernández et al., 2011). Briefly, 19 µL of TPC extract was placed on a PS flat-bottom 96-well plate and 29 µL of 1 N Folin–Ciocalteu reagent was added. Samples were incubated for 3 min in darkness at room temperature. Then, 192 µL of a solution containing Na_2CO_3 (4 g L^{-1}) and NaOH (20 g L^{-1}) was added and the reaction was carried out for 1 h at room temperature in darkness. The absorbance was measured at 750 nm after incubation using the Multiscan plate reader. TPC was expressed as chlorogenic acid equivalents in mg kg^{-1} fw. Each of the three replicates was analysed in triplicate.

Analysis of individual phenolic compounds was conducted as previously described (Formica-Oliveira et al., 2016a). Briefly, samples of 20 µL were analysed using an UHPLC instrument (Shimadzu, Kyoto, Japan) equipped with a DGU-20A degasser, LC-30AD quaternary pump, SIL-30AC autosampler, CTO-10AS column heater and SPDM-20A photodiode array detector. The UHPLC system was controlled by the software LabSolutions (Shimadzu, v. 5.42 SP5). Chromatographic analyses were carried out onto a Kinetex C18 column (100 mm × 4.6 mm, 2.6 µm particle size; Phenomenex, Macclesfield, UK) with a KrudKatcher Ultra HPLC guard column (Phenomenex, Macclesfield, UK). The column temperature was maintained at 25 ºC. The mobile phase was acidified water (A; formic acid to final pH 2.3) and acidified methanol (B; formic acid to final pH 2.3). The flow rate was 1.5 mL min^{-1}. Gradient program used was 0/88, 1.2/88, 2.4/85, 8.3/70, 9.4/50, 11.8/50, 20.8/55, 22.0/60 (min/% phase A). Then, column equilibration was conducted at 0 % A for 2.2 min. Chromatograms were recorded at 320 nm. Phenolic acids were quantified with standards of chlorogenic acid, ferulic acid (Sigma, St Louis, MO, USA), 3,5-CQ) and 4,5-CQA (ChromaDex, Irvine, CA, USA). The results were expressed as mg kg^{-1} fw. Each of the three replicates was analysed in duplicate.

II.2.5. Total antioxidant capacity

The extracts were analysed for TAC based on Brand-Williams et al. (1995) but with modifications (Martínez-Hernández et al., 2013d). Briefly, a solution of 0.7 mM DPPH (Sigma, St Louis, MO, USA) in methanol was prepared 2 h before the assay and adjusted to 1.1±0.02 nm immediately before use. A 21 µL aliquot of the previously described extract was placed on a PS flat-bottom 96-well plate and 194 µL of DPPH was added. The reaction was carried out for 30 min at room temperature in darkness and the absorbance was measured at 515 nm using the Multiscan plate reader. Results were expressed as mg Trolox kg^{-1} fw. Each of the three replicates was analysed in triplicate.

II.2.6. Statistical Analyses

A complete randomized design in triplicate with two-way ANOVA (treatment × storage) by Post Hoc Tuckey HSD tests (p=0.05) was conducted using the SPSS software (v. 21, IBM, USA). Possible synergistic effects of the combined treatments were discarded according to Limpel's formula (Equation II.1) (Richer, 1987), wherever the effectiveness of a combination of treatments exceeds the prediction of the effectiveness of their additive action.

$$E_e = X + Y - \left(\frac{XY}{100}\right) \quad \text{(II.1)}$$

II.3. RESULTS AND DISCUSSION

II.3.1. Phenylalanine ammonia-lyase activity

Carrots showed an initial PAL activity of 19.7±4.9 µmol kg^{-1} h^{-1} (Figure II.1). Similar PAL activity has been previously reported for the same carrot cultivar (Formica-Oliveira et al., 2016a). PAL activity did not significantly ($p < 0.05$) change after radiation treatments on processing day.

In general, PAL activity of shredded carrots increased throughout storage. Latter finding may be explained since PAL is induced by an array of biotic and abiotic stress-induced mechanisms such as the applied wounding (Cisneros-Zevallos, 2003). PAL activity of

CTRL and UV-B samples early increased (214 and 352 %, respectively) after 24 h reaching the highest PAL increments (1,013 and 804 %, respectively) among the rest of treatments at 48 h. PAL activity of CTRL and UV-B samples decreased after such high enhancements with levels of 155-160 μmol kg^{-1} h^{-1} at 72 h without significant ($p < 0.05$) differences among them. A similar increment (750 %) of PAL activity after 72 h at 15 ºC has been reported in shredded carrots irradiated with a UV-B dose of 1.3 kJ m^{-2} (Du et al., 2012). Such data is in accord to the detailed photograph (12 h intervals) of PAL activity and phenolic accumulation in stressed (wounding and UV-C) carrots recently reported (Formica-Oliveira et al., 2016a). Latter photograph showed that PAL activity and phenolic accumulation in stressed carrots could be divided into three different phases during storage at 15 ºC: 1st phase, < 24 h: early PAL activity increments; 2nd phase, 24-48 h: moderate phenolic increments concurring with the highest increase of PAL activity; 3nd phase, 48-72 h: high phenolic increments while a moderate increment of PAL activity is registered.

Treatments including UV-C clearly reduced the PAL activity. However, the behaviour of PAL activity of UV-C samples was similar to UV-B with the maximum increase (267 %) at 48 h followed by a decrease to initial levels after 72 h. The lower increase of PAL activity observed in UV-C samples compared to UV-B may be a result of a feedback modulation or due to the diversion of the synthetic capacity of the cell to the production of other proteins not observed with the UV-B radiation (Alegria, 2015; Boerjan et al., 2003; Saltveit, 2000). Enzymes have long been known to be inactivated on exposure to UV radiation (Pattison et al., 2012). Accordingly, another possible explanation may be a partial PAL denaturation by UV-C (a UV radiation with higher photon energy than UV-B) delaying the stress-enhanced activity of this enzyme. The inhibiting effect of UV-C on PAL activity of shredded carrots has been also recently observed (Formica-Oliveira et al., 2016a).

Combined treatments showed PAL activity increments of 115-144 % after 72 h. However, such increases were lower than those observed in CTRL and UV-B-treated samples due to UV-C radiation. On the other side, the application order for the combined treatments did not affect the PAL activity of samples since no significant ($p < 0.05$) differences between UV-B+UV-C and UV-C+UV-B were found throughout all storage period.

II.3.5. Phenolic compounds

Initial TPC of CTRL carrots was 207.4±43.0 mg kg^{-1} (Table II.1). The major individual phenolic compounds identified were 3-CQA, 3,5-CQA, 4,5-CQA and ferulic acid (Table II.2). These phenolic compounds accounted 69.6, 11.0, 9.8 and 9.5 % of the sum of individual phenolic compounds, respectively. Similar initial TPC of carrot has been previously reported being hydroxycinnamic acids and their derivatives the major phenolic compounds found (Alegria et al., 2016; Formica-Oliveira et al., 2016a; Jacobo-Velázquez et al., 2011). The 3-CQA content of samples was proportionally much lower than TPC, although 3-CQA was the main phenolic compound. Similar observation could be also deduced from similar studies with shredded carrots (Alegria et al., 2016; Formica-Oliveira et al., 2016a; Heredia and Cisneros-Zevallos, 2009; Jacobo-Velázquez et al., 2011). Latter finding can be explained since other antioxidant compounds of carrots may react with the Folin-Ciocalteu reagent overestimating the TPC. As expected, radiation treatments did not immediately change ($p < 0.05$) the phenolic compounds levels as similarly observed for PAL activity.

Phenolic levels of all samples progressively increased throughout storage. Such increase of these phytochemicals is a response to the applied postharvest abiotic stresses like wounding and radiation (Avena-Bustillos et al., 2012; Cisneros-Zevallos, 2003; Formica-Oliveira et al., 2016a). This phenolic biosynthesis has been reported to be a consequence of PAL activation after such abiotic stresses, as previously discussed, being proposed ATP and reactive oxygen species as signalling molecules (Jacobo-Velázquez et al., 2011). UV-B showed the highest TPC increases regarding its initial content with 90, 215 and 498 % after 24, 48 and 72 h at 15 ºC, respectively, which is 20 % higher than the TPC enhancement reached by CTRL samples (Table II.1). The maximum TPC observed at 72 h may be the delayed consequence of maximum PAL activity observed at 48 h as previously reported (Formica-Oliveira et al., 2016a). In general, different responses to low or high doses of UV-B have been observed in plants either by stimulating protection mechanisms or by activating repair mechanisms (Frohnmeyer and Staiger, 2003). Biosynthesis of UV absorbing compounds is the most common protective mechanism against potentially damaging radiation (Hahlbrock and Scheel, 1989). These secondary metabolites accumulate in the vacuoles of epidermal cells in response to UV-B irradiation

and attenuate the penetration of the UV-B into deeper cell layers (Avena-Bustillos et al., 2012).

The highest PAL activity of CTRL samples was not correlated to TPC of these samples, which was similar to the UV-C-including treatments. Latter fact could be explained by a higher content of other antioxidant compounds in CTRL samples which could react within the TPC method. Similarly, contrary to the observed high reduction of PAL activity after single UV-C treatment and UV combinations, TPC accumulations in these samples were only slightly reduced (4-12 % after 72 h) regarding the single UV-B treatment with similar values to CTRL samples.

The treatments including UV-C radiation (single or combined) showed similar TPC enhancements to CTRL samples throughout storage. This finding is in accordance to the observed PAL inhibition in treatments including UV-C radiation as previously discussed. Likewise, no TPC enhancements were observed in samples treated by a low UV-C dose (0.8 kJ m^{-2}) contrary to non-irradiated samples after 10 days at 5 ºC (Alegria et al., 2012). Furthermore, shredded carrots treated at higher UV-C dose (9 kJ m^{-2}) showed lower TPC accumulation compared to non-irradiated samples after 72 h at 15 ºC (Formica-Oliveira et al., 2016a). Accordingly, as UV-C radiation dose increases the TPC accumulation (determined by Folin-Ciocalteu method) seems to be reduced. On the other side, single UV-C treatment showed 50-170 % higher 3-CQA than CTRL samples after 48-72 h (Table II.2). Latter finding was not observed from TPC data probably owed to the previously commented interference of other antioxidant compounds with the TPC method. However, the enhanced 3-CQA contents observed in the samples treated by single treatments were not observed in the combined treatments probably to the longer times of these treatments. In general, the contribution of 3,5-CQA, 4,5-CQA and ferulic acid to TPC was minimum with no significant ($p < 0.05$) changes throughout storage of all samples.

Chlorogenic acid, the main phenolic compound in carrots, is an ester of caffeic acid with quinic acid with high antioxidant capacity regarding other phenolic compounds (Castelluccio et al., 1995). Carrots occupy the sixth place among the list of most consumed vegetables in the American diet, although the TPC of this vegetable is almost the lowest one (Chun et al., 2005). Hence, the enhancement of those antioxidant

compounds during storage could be favoured by UV-B treatment as hereby observed and according to previous data (Avena-Bustillos et al., 2012; Du et al., 2012). UV-C radiation is mainly used in fresh-cut products due to the high germicidal properties of this UV radiation being considered as a sustainable alternative to conventional chlorine washings (Martínez-Hernández et al., 2015b). Accordingly, moderate UV-C doses initially reduced by approximately 1.5 log units mesophiles and yeasts and moulds loads in shredded carrots being such microbial loads below the threshold limit (7 log units), which defines the shelf life of fresh-cut products, after 72 h at 15 ºC (Formica-Oliveira et al., 2016a). In this sense, the combination of UV-B with UV-C may reduce microbial loads of shredded fresh-cut carrots while still allowing high phenolic compounds accumulation (approximately 440 % after 72 h regarding its initial values) similarly to CTRL samples.

II.3.6. Total antioxidant capacity

The initial TAC of CTRL carrots was 121.1±79.8 mg kg^{-1} (Table II.1). Radiation treatments did not immediately change ($p < 0.05$) TAC except UV-C+UV-B treatment which showed 3-fold higher TAC than CTRL samples. Latter data may be an experimental artifact resulted from higher extraction of other antioxidant compounds of carrots due to increased cell wall depolymerization.

TAC of all samples increased throughout storage similar to TPC data. Carrots have a high antioxidant capacity mainly due to their content of phenolic compounds. In this sense, TAC was highly correlated to TPC with R^2 of 0.82 as previously found (Cisneros-Zevallos, 2003). UV-B samples early showed the highest TAC increment with levels 47 % higher than CTRL samples at 24 h. In the same line, UV-B samples showed the highest TAC levels with 3,705 mg kg^{-1} at 72 h compared to the other radiation treatments. The rest of treatments showed final TAC levels of 2,537-2,890 mg kg^{-1} at 72 h without significant ($p < 0.05$) differences among them.

Sufficient antioxidants compounds need to be consumed with foods to prevent or slow the oxidative damage in humans induced by free radicals. UV-B treatment is hereby shown as an excellent sustainable and cheap treatment to be applied by the food industry to even enhance the accumulation of phenolic compounds, and consequently the antioxidant capacity, in wounded carrots. The combination with UV-C is recommended

as a sustainable sanitizing treatment alternative to conventional chlorine washings. Furthermore, the accumulation of such antioxidant compounds still occurred in the treatments including UV-C radiation although in a lower degree compared to single UV-B. No significant TAC differences were found relating to the application order of radiation treatments. However, a combined UV-C+UV-B treatment can be recommended to quickly reduce initial microbial loads by UV-C illumination of samples just after wounding.

II.4. CONCLUSIONS

The total phenolic compounds accumulation in shredded fresh-cut carrots after 72 h at 15 ºC could be increased up to 30 % with a UV-B pre-packaging treatment of 1.5 kJ m^{-2}. Particularly, UV-B-treated samples achieved an accumulation of phenolic compounds of approximately 500 % after 72 h at 15 ºC while non-irradiated samples showed a 380 % accumulation regarding their respective initial levels. UV-C has been proposed as a sanitizing treatment in fresh-cut products alternative to conventional NaOCl. According to the hereby reported data, the use of the sanitizing UV-C treatment in combination with a UV-B treatment did not highly affect the phenolic accumulation, still allowing an accumulation of 440 % after 72 h. Such combined UV-C+UV-B pre-treatment seems to be a good tool for the food industry to diversify its product offer for the actual consumer, which is increasingly interested in high antioxidant content products while meeting the food safety issues

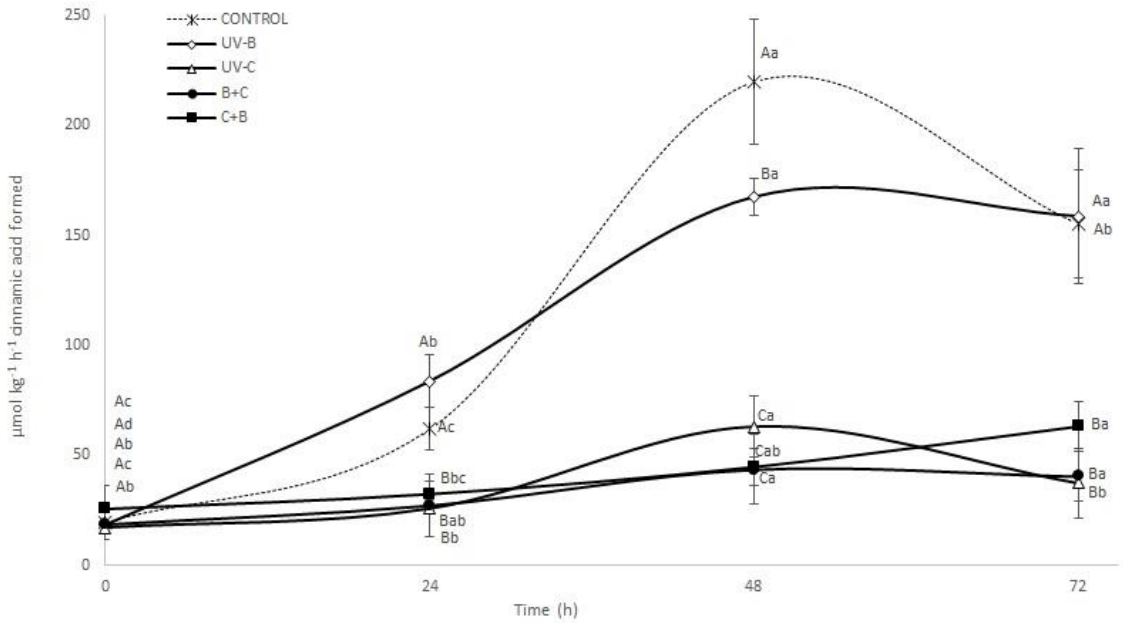


Figure II.1. Phenylalanine ammonia-lyase activity of shredded fresh-cut carrot treated with UV-C and UV-B, and their combinations, during storage up to 72 h at 15 ºC (n=3±SD). Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment.

Table II.1. Total phenolic content and total antioxidant capacity of shredded fresh-cut carrot treated with UV-C and UV-B, and their combinations, during storage up to 72 h at 15 ºC (n=3±SD). Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment.

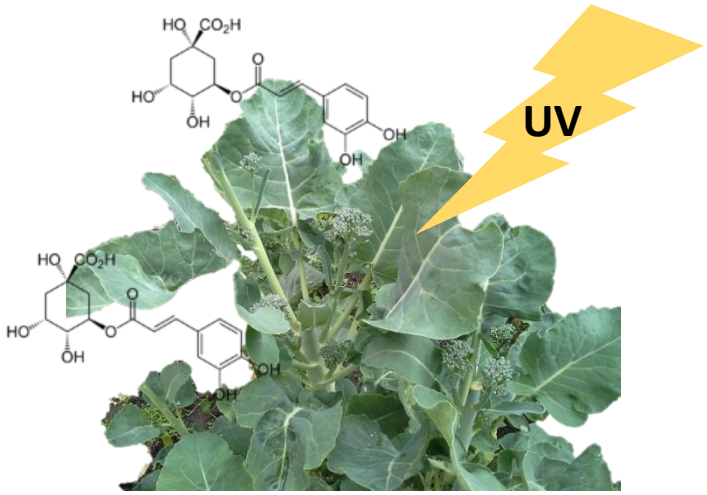
Storage (h)	0	24	48	72
Total phenolic content*				
CTRL	207.4±43.0 Ac	314.0±37.6 Ac	458.5±10.4 Bb	1,001.2±116.3 Ba
UV-B	205.3±49.5 Ad	389.2±92.4 Ac	647.8±76.2 Ab	1,228.1±71.1 Aa
UV-C	187.8±8.9 Ac	327.1±11.2 Ab	411.3±65.8 Bb	1,007.5±95.7 Ba
UV-B+UV-C	173.1±21.8 Ad	314.3±30.1 Ac	472.2±71.5 Bb	975.3±62.6 Ba
UV-C+UV-B	175.0±39.1 Ac	331.2±27.5 Ac	469.2±5.4 Bb	1,008.3±153.2 Ba
Total antioxidant capacity**				
CTRL	121.1±79.8 Bd	1,263.1±262.3 Bc	1,758.6±326.9 Cb	3,680.1±17.7 Aa
UV-B	245.0±109.9 ABc	1,862.6±145.6 Ab	2,438.2±144.9 Ab	3,704.8±10.6 Aa
UV-C	191.9±78.5 ABd	1,487.1±165.6 ABbc	2,233.4±120.1 ABa	2,889.6±281.1 Ba
UV-B+UV-C	213.5±76.3 ABd	1,169.4±42.8 Bc	2,445.8±38.4 Ab	2,820.6±332.2 Ba
UV-C+UV-B	363.2±131.4 Ad	1,323.3±297.9 ABc	2,090.8±117.1 Bb	2,537.1±219.4 Ba

*mg kg^{-1} chlorogenic acid equivalents; ** mg kg^{-1} Trolox equivalents

Table II.2. Individual phenolic compounds of shredded fresh-cut carrot treated with UV-C and UV-B, and their combinations, during storage up to 72 h at 15 ºC (n=3±SD). Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment.

Storage (h)	0	24	48	72
3-CQA (mg kg^{-1})				
CTRL	56.7±19.4 Ab	107.6±12.8 Ba	92.5±17.7 Ba	41.0±9.9 Db
UV-B	56.0±4.6 Ab	152.1±14.2 Aa	175.4±26.7 Aa	146.3±12.4 Aa
UV-C	58.5±10.1 Ac	110.4±11.2 Bb	139.6±14.4 Aa	110.7±15.0 Bb
UV-B+UV-C	57.0±13.5 Aa	95.1±36.3 Ba	84.0±28.3 Ba	96.9±14.3 BCa
UV-C+UV-B	43.4±1.5 Ac	101.5±16.9 Ba	42.6±7.9 Cc	76.6±1.9 Cb
Ferulic acid (mg kg^{-1})				
CTRL	7.8±1.8 Aa	10.7±0.6 Aa	9.6±4.4 Aa	7.7±0.9 Aa
UV-B	5.1±0.4 Ba	8.5±1.0 Ba	8.3±6.6 ABa	6.1±1.6 Aa
UV-C	7.6±0.2 Aa	6.6±0.6 Cab	7.0±4.3 ABab	3.2±0.9 Bb
UV-B+UV-C	4.6±1.1 Ba	4.5±1.5 Da	4.3±0.4 ABab	2.3±0.6 Bb
UV-C+UV-B	2.7±0.3 Cb	4.9±0.1 Da	1.7±0.5 Bc	2.6±0.7 Bb
3,5-CQA (mg kg^{-1})				
CTRL	9.0±0.3 Aa	9.2±0.2 Aa	9.0±0.1 ABa	9.0±0.1 Aa
UV-B	9.0±0.1 Aa	9.4±0.2 Aa	9.3±0.4 Aa	9.3±0.7 Aa
UV-C	9.0±0.1 Aa	9.1±0.3 Aa	9.1±0.3 ABa	9.3±0.7 Aa
UV-B+UV-C	9.0±0.3 Aa	9.4±0.5 Aa	9.2±0.3 Aa	9.3±0.5 Aa
UV-C+UV-B	8.7±0.3 Aa	9.2±0.1 Aa	8.7±0.2 Ba	9.7±1.0 Aa
4,5-CQA (mg kg^{-1})				
CTRL	8.0±1.6 Aa	8.0±0.9 Aa	7.8±1.6 Aa	6.8±1.2 Aa
UV-B	7.6±0.1 ABa	6.7±0.8 Aa	7.9±1.4 Aa	6.8±1.9 Aa
UV-C	7.3±0.3 ABa	8.1±0.5 Aa	8.3±1.7 Aa	7.6±2.1 Aa
UV-B+UV-C	8.6±1.5 Aa	8.9±1.4 Aa	8.1±1.0 Aa	6.4±2.0 Aa
UV-C+UV-B	5.8±0.2 Bb	7.7±0.3 Aab	6.2±0.5 Aab	8.0±2.1 Aa

3-CQA: 3-caffeoylquinic acid; 3,5-CQA: 3,5-dicaffeoylquinic acid; 4,5-CQA: 4,5-dicaffeoylquinic acid



CHAPTER III

Postharvest UV-radiation treatments to revalorize broccoli by-products and edible parts

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III.1. INTRODUCTION

Bimi® broccoli is a new natural hybrid between Chinese broccoli, also called kailan or gailan, and conventional broccoli. Bimi® broccoli is characterized by a small floret with a long (15-18 cm) slender stem which has a mild sweeter taste compared to conventional broccoli varieties, being completely edible (raw or cooked) (Martínez-Hernández et al., 2013b). Bimi® broccoli is rich in phenolic compounds, glucosinolates, vitamin C and other antioxidant compounds (Martínez-Hernández et al., 2013a; Martínez-Hernández et al., 2013c). Glucoraphanin and glucobrassicin are the main glucosinolates present in Bimi® broccoli (Martínez-Hernández et al., 2013c) being their cognate isothiocyanates, sulforaphane and indol-3-carbinol, extensively studied for their potent induction of mammalian detoxication (phase 2) enzyme activity and anti-cancer agent (Traka and Mithen, 2008).

FAO has recently published that about 1.3 billion tons of food is worldwide wasted or lost per year being 10-20 % attributed to preharvest losses (FAO, 2015). Use of plant by-products supports the low carbon economy using renewable resources, offering environmental and economic benefits and improve efficiency in food industry. The non-edible parts of Bimi® plant (stalks and leaves) may have high contents of bioactive compounds like similarly found in conventional broccoli varieties (Aguiló-Aguayo et al., 2014; Dosz et al., 2014). Accordingly, the use of Bimi® plant by-products wasted during preharvest stage appears as an interesting source of health-promoting compounds for the food and pharmaceutical industries. The leaves and stalks of Bimi® broccoli represent 75.5 % (in dry weight basis) of total above-ground plant biomass. This constitutes a high amount of waste, with a negative effect on the agricultural environment, in the Región de Murcia (Southeast of Spain) that is the main European Bimi® broccoli producer with 150 ha cultivated in the last campaign (data supplied by Sakata Seeds Ibérica). Moreover, the sometimes abnormally high temperatures in the winter and spring seasons of warmer production areas may induce early flowering, resulting in the total loss of the saleable yield (florets), and converting all the biomass into an unprofitable by-product.

Plant products have been proposed as biofactories of bioactive compounds through different induced abiotic stresses-mechanisms such as UV radiation, wounding, etc. (Cisneros-Zevallos, 2003; Formica-Oliveira et al., 2017). Application of UV radiation (B

and C) has been proposed as a friendly and cheap non-molecular tool to enhance the phenolic compounds of horticultural crops during postharvest life (Castagna et al., 2014; Du et al., 2012; Formica-Oliveira et al., 2017; Scattino et al., 2014). Furthermore, UV-C radiation has a high germicidal effect which may reduce microbial growth during storage of UV-treated samples. In that way, the use of UV+B and UV-C radiation treatments may be used to revalorize broccoli plant by-products.

To our knowledge there have been no prior studies regarding the use of single and combined UV-B and UV-C postharvest treatments on the phenolic compounds and glucosinolates of broccoli by-products and edible parts. Accordingly, this work studied the singular and combined effects of UV-B and UV-C pre-treatments on TPC, and related TAC, and main glucosinolates (glucoraphanin and glucobrassicin) of Bimi® broccoli leaves, stalks and florets during storage at 15 ºC for up to 72 h.

III.2. MATERIALS AND METHODS

III.2.1. Plant material

Bimi® broccoli plants were grown under open air cultivation in fields located in the Region of Murcia, in the Southeast Mediterranean Spanish area, in the spring growing cycle (planting in March) and were harvested randomly at the end of May (average temperature intervals of 4–27 ºC). Plant material was grown according to integrated pest management cultural practices. Bimi® plants were hand-harvested at commercial ripening stage of Bimi® florets (head diameters of 3–5 cm and stem lengths of 15–18). Immediately after harvesting, Bimi® plants were pre-cooled with crushed ice and transported by car about 80 km to the Pilot Plant of our Research Group in the Universidad Politécnica de Cartagena, where it was stored at 4 ºC and 90–95 % RH until next day.

III.2.2. Sample preparation

Preparation of plant material was conducted in a disinfected cold room at 8 ºC. Leaves were removed from the plant main stalk using a sharp knife. Bimi® florets were cut in about 15-cm-long spears. The obtained three Bimi® broccoli parts (florets, stalks and leaves) were then washed with chlorinated water (150 mg L^{-1} free chlorine; pH 6.5; 5 ºC)

for 2 min and rinsed with tap water at 5 ºC for 1 min. Once drained, plant material was carefully dried with towel paper and disposed in plastic trays until UV treatments were applied.

III.2.3. Radiation treatments and storage conditions

The radiation chamber consisted of a reflective stainless steel chamber with two lamp banks (one bank suspended horizontally over the radiation vessel and the other one placed below it) being fitted to each bank 6 UV-B and 7 UV-C (alternatively positioned) unfiltered emitting lamps (TL 40W/01 RS and TUV 36W/G36 T8, respectively; Philips, Eindhoven, The Netherlands). UV-B and UV-C radiations were separately applied controlled by two general keys that switched all UV-C or UV-B at the same time. The radiation chamber also had a ventilator continuously switched on during treatments to renovate the air from inside of the chamber with the cold air from the cold room (8 ºC). Plant material was placed between the two lines of lamps at 17.5 cm above and below over a bi-oriented PP film (thickness: 35 μm) mounted on a PS net (130 × 68 cm) that minimized blockage of the radiation. The applied UV-B and UV-C intensities of 9.27 and 25.21 W m^{-2}, respectively, were calculated as the mean of 18 readings on each side of the net using LP 471 UVB (Delta OHM, Italy) and VLX 254 (Vilber Lourmat, Marne la Vallee, France) radiometers, respectively. Thus, both sides received the same radiation intensities. The radiation chamber is based on that previously described (Artés-Hernandez et al., 2009). The light intensities were kept constant and the applied doses were varied by altering the exposure time at the fixed distance. Applied treatments were:

- CTRL: No radiation treatment used as control.
- Single UV-B treatments: 5 (540 s), 10 (1,080 s) and 15 kJ m^{-2} UV-B (1,619 s). Such UV-B doses were selected based on previous experiments and according to Avena-Bustillos et al. (2012) in order to obtain maximum phenolic accumulation while minimizing heating and evaporation processes during UV-B treatment.
- Combined UV-B+UV-C treatments: the previous UV-B doses were applied followed by a UV-C dose of 9 kJ m^{-2} (357 s). Such UV-C was selected based on Martínez-Hernández et al. (2011) and previous tests in order to obtain maximum bioactive increases.

The treated plant material was then placed in rectangular plastic trays and covered with a black PE bag to reduce water loss. Samples were stored at 15 ºC (90–95 % RH) up to 72 h (sampling times: 0, 24, 48 and 72 h). Three replicates per treatment and sampling time were prepared. Samples were frozen in liquid nitrogen at every sampling time and stored at –80 ºC until further TPC, TAC and glucosinolates analyses were conducted. Frozen samples were ground to fine powder prior to analyses using liquid N_2 with a mincer (IKA, A 11 basic, Berlin, Germany) at 12,700 × g for 10 s.

III.2.4. Total phenolic content and total antioxidant capacity

Extraction to determine TPC and TAC was conducted by homogenization (Ultra Turrax®) of 0.5 (floret), 0.25 (leaves) or 1 g (stalk) ground frozen sample in 3 mL of methanol for 20 s in an ice-water bath. Subsequently, extracts were centrifuged at 13,500 × g for 20 min at 4 ºC and supernatants were collected and analysed. TPC was analysed by the Folin–Ciocalteu reagent method (Singleton and Rossi, 1965) but with modifications (Martínez-Hernández et al., 2011). Briefly, 19 µL of TPC extract was placed on a PS flat-bottom 96-well plate and 29 µL of 1 N Folin–Ciocalteu reagent (Sigma, St Louis, MO, USA) was added. Samples were incubated for 3 min in darkness at room temperature. Then, 192 µL of a solution containing Na_2CO_3 (4 g L^{-1}) and NaOH (20 g L^{-1}) was added and the reaction was carried out for 1 h at room temperature in darkness. The absorbance was measured at 750 nm after incubation using the Multiscan plate reader. TPC was expressed as chlorogenic acid equivalents in mg kg^{-1} fw. Each of the three replicates was analysed in triplicate.

TAC extracts were analysed based on Brand-Williams et al. (1995) but with modifications (Martínez-Hernández et al., 2013d). Briefly, a methanolic solution of 0.7 mM DPPH (Sigma, St Louis, MO, USA) was prepared 2 h before the assay and adjusted to 1.1±0.02 nm immediately before use. A TAC extract aliquot of 35 µL was placed on a PS flat-bottom 96-well plate and 180 µL of adjusted DPPH solution was added. The reaction was carried out for 30 min at room temperature in darkness and the absorbance was measured at 515 nm using the Multiscan plate reader. Results were expressed as mg Trolox kg^{-1} fw. Each of the three replicates was analysed in triplicate.

III.2.5. Glucoraphanin and glucobrassicin contents

A frozen ground sample of 2 g was mixed with 5 mL of hot (70 ºC) 70 % methanol and extraction was conducted for 15 min in an agitated water bath at 70 ºC to inactivate myrosinase enzyme. Then, the extracts were centrifuged (3,000 × g, 5 min, 4 ºC), and the supernatants were collected and used as glucosinolates extracts after filtration (0.20 μm syringe polytetrafluoroethylene filters). Analysis and identification of glucoraphanin and glucobrassicin were conducted according to Francisco et al. (2009). An UHPLC instrument (Shimadzu, Kyoto, Japan) equipped with a DGU-20A degasser, LC-30AD quaternary pump, SIL-30AC autosampler, CTO-10AS column heater and SPDM-20A photodiode array detector was used. Chromatographic analyses were carried with a C18 column (Gemini NX 250mm × 4.6 mm, 5 μm; Phenomenex, Torrance CA, USA). The mobile phase was a mixture of (A) trifluoroacetic acid (TFA) 0.1% and (B) acetonitrile/TFA (99.9/0.1). The flow rate was 1 mL min^{-1} in a linear gradient starting with 0 % B at 0–5 min, reaching 17 % B at 15–17 min, 25 % B at 22 min, 35 % B at 30 min, 50 % B at 35 min, 100 % B at 50 min and 0 % B at 55–65 min. Readings were recorded at 229 nm. Glucoraphanin and glucobrassicin were identified on the basis of retention time and UV spectra as compared with authentic standards (PhytoLab GmbH & Co. KG, Vestenbergsgreuth, Germany). The results were expressed as mg kg^{-1} fw. Each of the three replicates was analysed in duplicate.

III.2.6. Statistical analyses

A complete randomized design in triplicate with two-way ANOVA (treatment × storage) was conducted. Statistical significance was assessed at the level p=0.05, and Tukey's multiple range test was used to separate means with SPSS software (v. 21, IBM, USA).

III.3. RESULTS AND DISCUSSION

III.3.1. Phenolic compounds

Bimi® florets, leaves and stalks showed initial TPC of 1,328, 1,716 and 360 mg kg^{-1}, respectively (Table III.1). As observed, Bimi® leaves is a by-product that can be considered as an excellent source of phenolics with 1.3-fold higher TPC than florets. Furthermore, leaves showed 5-fold higher TPC than stalks. In general, TPC of samples

increased after UV treatments on processing day probably owed to a better extraction of phenolic compounds after plant cells disruption due to UV radiation as previously reported (Escalona et al., 2010; Martínez-Hernández et al., 2011). Therefore, 27 and 50 % higher TPC levels were initially observed in florets treated with the most intense radiation doses of UVB15 and UVB15+C, respectively. The TPC increments of UV-treated leaves and stalks ranged among 31-97 and 30-75 %, respectively. Contrary to florets, no clear trend of the dose intensity/combination on the TPC enhancements was observed in UV-treated leaves and stalks. Latter finding may be explained since Bimi® florets are the youngest parts of the plant, being their plant cells more sensible to UV incidence, leading to the observed dose-dependent higher phenolics extractability compared to leaves and stalks.

TPC of CTRL leaves, stalks and florets increased by 30, 44 and 15 %, respectively, after 24 h. Similar phenolic increments have been reported in broccoli stored for 24 h at 20 ºC (Starzyńska et al., 2003). The observed phenolic enhancements in broccoli plant material may be owed to a transient increment of the PAL activity, the key enzyme in the biosynthesis of phenolic compounds, as previously reported (Baclayon et al., 2007). However, the latter transient TPC enhancement in leaves during the first 24 h was delayed as the UVB dose increased showing unchanged TPC levels for UVB15-treated samples after 24 h. Such initial downregulating effect of UVB on initial TPC enhancement was even enhanced when combined with UV-C. Accordingly, TPC reductions of 20-35 % were observed in leaves treated with UVB+C treatments after 24 h comparing to their respective initial levels. However, a general TPC increment for all samples was observed from 24 to 72 h. CTRL and UVB5/10 showed the lowest TPC increments of 30-55 % in leaves from 24 to 72 h. On the other side, the highest UVB dose (15 kJ m^{-2}) and all the UVB+C combinations induced TPC enhancements in leaves of 80-110 % from 24 to 72 h, comparing to their respective initial levels, registering UVB10+C the highest phenolic increment. Attending to stalks, no high TPC changes were generally observed during incubation period. However, UVB10 and UVB10+C induced the highest TPC increments in stalk samples at 48 h with 709 and 680 mg kg^{-1}. Nevertheless, the high TPC levels of stalks at 48 h were reduced at 72 h. UV radiation has been reported to have low penetration (< 1mm) in plant cells (Formica-Oliveira et al., 2016a). Accordingly, the observed low TPC in stalks may be owed to the small surface:volume ratio of stalks (3-4 cm diameter) compared to leaves. Bimi® florets showed a similar behaviour comparing

to leaves and stalks through storage period. Thus, all UVB+C treatments induced an initial TPC decrease in florets followed by an increment with maximum levels at 72 h. Nevertheless, the TPC increments of florets was 2-5-fold lower than those observed in leaves showing UVB10+C the highest TPC increment of 55 % from 24 to 72 h.

In conclusion, Bimi® leaves can be used as an excellent source of phenolics for the food and pharmaceutical industries with levels 1.3-fold higher than florets. Furthermore, the use of postharvest UV treatments may revalorize this Bimi® by-product with TPC increments up to 110 % with a combined UV-B (10 kJ m^{-2}) and UV-C (9 kJ m^{-2}) treatment during storage at 15 ºC. The latter combined UV dose may be also used to increase the TPC of fresh-cut Bimi® florets supplying to the consumer a product with higher health-promoting properties. Furthermore, postharvest UVB10 and UVB10+C treatments are recommended to revalorize Bimi® stalks due to the TPC increments observed after 48 h at 15 ºC.

III.3.2. Total antioxidant capacity

Bimi® florets, leaves and stalks showed initial TAC of 577, 552 and 77 mg kg^{-1}, respectively (Table III.2). As observed, and contrary to phenolic data, leaves showed similar TAC to florets. Phenolic compounds are known to have strong antioxidant capacity being the main contributors to TAC of several vegetables (Cisneros-Zevallos, 2003; Reyes et al., 2007). Furthermore, Bimi® broccoli has high vitamin C content (Martínez-Hernández et al., 2013a). Therefore, the similar TAC levels between Bimi® leaves and florets may be due to the vitamin C contribution to TAC. UV treatments generally increased TAC of samples possibly owed to a higher extraction of antioxidant compounds as discussed for TPC. Bimi® broccoli florets and leaves showed TAC increments of 20-120 % after UV treatments. However, stalks showed TPC increases of 170-420 % after UV treatments likely due to a higher extraction of some particular antioxidant compounds.

TAC behaviour of samples during storage was similar to TPC. Accordingly, TAC of all UV-treated leaves showed a decrease from 0 to 24 h followed by an increment of 100-130 % at 72 h. UV-treated leaves, stalks and florets showed 1-1.2-fold higher TAC than CTRL samples after 72 h of storage without high differences between radiation

treatments. The biosynthesis of UV-absorbing compounds is the most common protective mechanism against potentially damaging radiation. Accordingly, these secondary metabolites, which may be antioxidant compounds like phenolics, accumulate in the vacuoles of epidermal cells in response to UV radiation treatments (Hahlbrock and Scheel, 1989). As previously discussed, Bimi® leaves can be considered as an excellent by-product according to initial TAC levels: 7.2-fold higher than stalks and similar to florets. Furthermore, this Bimi® by-product may be revalorized with the use of postharvest UV treatments.

III.3.3. Glucoraphanin and glucobrassicin contents

Bimi® florets, leaves and stalks showed initial glucoraphanin/glucobrassicin contents of 35.7/18.1, 30.0/1.0 and 88.1/263.0 mg kg^{-1}, respectively (Figures III.1-2, Table III.3). As observed, Bimi® leaves showed 2.5/14.5-fold higher glucoraphanin/glucobrassicin levels than florets. Such high glucosinolates levels in Bimi® leaves may lead to a high enzymatic (myrosinase) conversion to their respective bioactive products, isothiocyanates (sulforaphane/indole-3-carbinol, respectively), as previously reported (Bertelli et al., 1998) together with the higher myrosinase activity reported in broccoli leaves compared to florets or stalks (Dosz et al., 2014). Lower glucoraphanin/glucobrassicin levels have been reported in stalks of conventional broccoli compared to florets (Aguiló-Aguayo et al., 2014).

Glucoraphanin levels of Bimi® leaves and stalks were initially increased by 14-25 % after UVB5, UVB10 and UVB15+C treatments while the rest of UV treatments induced lower or none glucoraphanin enhancements. Glucobrassicin showed higher enhancements than glucoraphanin after UV treatments ranging from 20 to 85 %, inducing UVB10 the highest glucobrassicin increments in florets and stalks of 71 and 85 %, respectively. Furthermore, the UVB10+C combination was the only treatment able to increase (34 %) the glucobrassicin content of Bimi® leaves. The observed glucoraphanin/glucobrassicin initial increments after UV treatments may be explained by a higher compound extractability owed to radiation-disruption of plant cells as previously discussed for TPC and TAC.

Glucoraphanin and glucobrassicin contents of CTRL florets increased during storage showing 121 and 534 % higher values, respectively, after 72 h at 15 ºC (Figures III.1-2). Glucosinolates increments in broccoli and other brassicas during storage at similar temperatures have been also reported associated with an enhanced synthesis or release of bound glucosinolates during storage (Hansen et al., 1995). However, glucosinolates levels have been reported to start to decrease when product senescence processes initiate explained by the membrane damage and cell rupture, conditions favourable for hydrolytic breakdown of glucosinolates by myrosinase-catalysed hydrolysis or autolysis (Chong and Bérard, 1983; Hansen et al., 1995; Sørensen, 1990). The observed higher glucobrassicin increment during storage compared to glucoraphanin of CTRL florets is in accord to previous data of the brassica *Arabidopsis thaliana* where genes related to indole (i.e., glucobrassicin) glucosinolates biosynthesis are more predisposed to be induced in wound-stressed tissues than those of aliphatic (i.e., glucoraphanin) glucosinolates (Mikkelsen et al., 2003). Glucoraphanin and glucobrassicin levels of CTRL Bimi® leaves and stalks also increased after 72 h although in a lower degree (18-98 % increments) compared to florets probably owed to higher metabolism rates in the latter younger plant organs compared to leaves and stalks as previously discussed. All UV treatments induced higher glucoraphanin/glucobrassicin contents after storage compared to non-irradiated samples in such Bimi® young organs inducing UVB15/UVB15+C and UVB10/UVB10+C the highest glucoraphanin and glucobrassicin levels, respectively, after 72 h (Figures III.1-2). However, UVB5 induced the highest glucoraphanin/glucobrassicin increments of 584/1,334 % in Bimi® florets after 72 h comparing to their respective initial levels. Accordingly, the low UVB dose of 5 kJ m^{-2}, and its combination with UV-C, induced higher glucosinolates enhancements in florets during storage although higher glucosinolates levels were observed for the intermediate-high doses (10-15 kJ m^{-2}) owed to its higher respective glucosinolates initial contents. Similarly, low UV-B doses induced higher glucoraphanin and glucobrassicin enhancements in broccoli sprouts compared to higher UV-B doses (Mewis et al., 2012). The glucosinolates biosynthesis pathway has been well studied in the brassica *A. thaliana* with their associated genes although little is known about glucosinolates-specific genes in broccoli (Mewis et al., 2012; Sonderby et al., 2010). It has been hypothesized that UV-B radiation might slow down the glucosinolate degradation rate in broccoli sprouts being inversely correlated with the UV-B dose (Mewis et al., 2012). Accordingly, the expression of some *A. thaliana*-homologous genes involved in the glucosinolate biosynthesis were up-regulated by UV-

B in broccoli sprouts (Mewis et al., 2012). MYB28 is the dominant transcription factor which regulates the activity of the enzyme ATP sulfurylase 1 involved in the aliphatic glucosinolates biosynthesis pathway of *A. thaliana* (Sønderby et al., 2010). Accordingly, homologous-MYB28 relative expression and glucoraphanin content were higher in Bimi® broccoli treated with moderate UV-C radiation compared to a higher dose (unpublished data).

Lower glucoraphanin/glucobrassicin changes were observed during storage of Bimi® by-products compared to florets were UV-mediated up-regulation seemed to be higher in such young plant organs. However, UVB-15 treatment induced glucobrassicin enhancements in Bimi® leaves and stalks of 135 and 83 %, respectively, after 72 h. Accordingly, UVB-15 treatment may be used to enhance the glucobrassicin levels of leaves and stalks to revalorize these Bimi® by-products while such UVB-15 and its combination with UV-C may also lead the highest glucosinolates levels of fresh-cut Bimi® broccoli florets.

III.4. CONCLUSIONS

Bimi® leaves are hereby presented as a potential source of bioactive compounds such as phenolic compounds and glucosinolates showing levels up to 15-fold higher than edible florets. Furthermore, postharvest UV-B and UV-C radiation treatments are proposed in this study like an innovative and eco-friendly tool to revalorize Bimi® leaves and stalks, and increase healthiness of edible parts, through the enhancement of their bioactive compounds. Particularly, intermediate UV-B/C (10/9 kJ m^{-2}) treatments induced the highest phenolics enhancements and glucobrassicin contents of Bimi® leaves after storage. Furthermore, high UV-B (15 kJ m^{-2}) treatment, single or combined, increased the healthiness of Bimi® edible florets with the highest glucoraphanin levels after storage.

Table III.1. Total phenolic content (mg kg^{-1}) of Bimi® broccoli by-products (leaves and stalks) and florets treated with UV-B treatments, and combination with UV-C, during incubation up to 72 h at 15 ºC (n=3±SD). Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment.

	Incubation time (h) at 15 ºC			
	0	**24**	**48**	**72**
Leaves				
CTRL	1,716±232 Ec	2,230±171 Bb	3,292±299 Aa	3,453±128 Ca
UV-B5	2,624±271 BCc	2,896±76 Ac	3,285±205 Ab	3,664±192 BCa
UV-B10	3,372±78 Ab	2,660±235 Ac	3,364±120 Ab	3,895±122 ABa
UV-B15	2,248±126 Dc	2,246±266 Bc	3,192±153 Ab	3,954±273 ABa
UV-B5+C	2,563±200 CDb	2,063±224 Bc	2,688±75 Bb	3,749±179 ABCa
UV-B10+C	2,918±164 BCb	1,895±215 Bc	2,825±247 Bb	3972±15 Aa
UV-B15+C	2,956±277 Bb	2,021±224 Bc	2,534±212 Bb	3546±192 Ca
Stalks				
CTRL	360±17 Db	518±49 BCa	537±58 CDa	538±45 ABa
UV-B5	468±44 Cb	493±46 BCb	598±42 Ca	461±31 CDb
UV-B10	490±8 Cbc	542±3 Bb	709±33 Aa	488±45 BCDc
UV-B15	605±69 ABa	563±19 Ba	553±31 CDa	576±34 Aa
UV-B5+C	628±29 Aa	493±17 BCb	607±38 BCa	475±25 CDb
UV-B10+C	557±43 Bb	667±58 Aa	680±72 ABa	439±8 Dc
UV-B15+C	564±4 ABa	463±53 Cb	477±8 Db	508±38 BCab
Florets				
CTRL	1,328±15 Cc	1,528±57 Bb	1,830±134 ABa	1,744±116 Ba
UV-B5	1,006±47 Dc	1,418±66 BCb	1,932±168 Aa	2,018±193 ABa
UV-B10	1,400±48 Cb	1,541±161 Bb	1,968±114 Aa	2,025±178 Aa
UV-B15	1,686±45 Bb	1,960±189 Aa	1,594±92 BCb	2,082±131 Aa
UV-B5+C	1,589±127 Bb	1,612±80 Bb	1,574±185 Cb	2,020±129 Aa
UV-B10+C	1,402±71 Cb	1,237±64 Cb	1,646±48 BCa	1,923±198 ABa
UV-B15+C	1,994±52 Aa	1,581±141 Bb	1,534±172 Cb	1,835±130 ABa

Table III.2. Total antioxidant capacity (mg kg^{-1}) of Bimi® broccoli by-products (leaves and stalks) and florets treated with UV-B treatments, and combination with UV-C, during incubation up to 72 h at 15 ºC (n=3±SD). Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling time and plant part. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment and plant part.

	Incubation time (h) at 15 ºC			
	0	**24**	**48**	**72**
Leaves				
CTRL	552±57 Cc	656±55 BCc	1,221±50 ABa	1,031±145 Cb
UV-B5	889±76 Bb	980±55 Ab	1,291±30 Aa	1,233±122 Ba
UV-B10	1,196±117 Ab	718±70 Bc	1,267±84 Ab	1,429±50 ABa
UV-B15	669±34 Cc	662±66 BCc	1,188±95 ABCb	1,461±113 Aa
UV-B5+C	841±98 Bc	646±51 BCd	1,042±41 CDb	1,477±143 Aa
UV-B10+C	864±40 Bc	604±55 Cd	1,103±116 BCDb	1,242±53 Ba
UV-B15+C	820±38 Bbc	632±87 BCc	968±131 Db	1,350±129 ABa
Stalks				
CTRL	77±5 Ec	169±31 Cc	483±31 Cb	1,182±94 Aa
UV-B5	231±18 CDc	216±32 Bc	622±83 Bb	1,114±17 ABa
UV-B10	256±10 Cc	208±25 BCc	943±31 Ab	1,151±106 Aa
UV-B15	203±10 Dc	116±18 Dd	522±24 Cb	1,001±65 BCa
UV-B5+C	401±14 Ac	234±23 Bd	644±61 Bb	825±70 Da
UV-B10+C	360±37 Bc	318±25 Ac	525±44 Cb	1,170±76 Aa
UV-B15+C	347±35 Bc	220±28 Bd	513±45 Cb	925±52 CDa
Florets				
CTRL	577±58 DEb	683±75 DEb	1,103±108 ABa	1,021±76 Da
UV-B5	369±89 Fb	826±124 BCDb	1,128±97 Aa	1,232±125 Ca
UV-B10	684±16 CDc	737±115 CDEc	1,061±102 ABCb	1,421±37 ABCa
UV-B15	773±43 BCc	987±50 Ab	790±99 Dc	1,467±107 Aa
UV-B5+C	523±88 Ec	867±16 ABCb	939±103 BCDb	1,453±158 ABa
UV-B10+C	802±81 Bc	632±62 Ec	1,018±122 ABCb	1,249±150 BCa
UV-B15+C	1,206±52 Aa	889±77 ABb	905±85 CDb	1,337±124 ABCa

Table III.3. Glucoraphanin and glucobrassicin contents (mg kg^{-1}) of Bimi® broccoli by-products (leaves and stalks) treated with UV-B treatments, and combination with UV-C, during storage up to 72 h at 15 ºC (n=3±SD). Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling time and plant part. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment and plant part.

	Glucoraphanin		**Glucobrassicin**	
	0 h	**72 h**	**0 h**	**72 h**
Leaves				
CTRL	88.1±3.7 Bb	149.3±17.5 ABa	263.0±114.0 Ba	201.5±73.9 Ca
UV-B5	117.0±8.7 Aa	90.7±23.9 Ba	256.5±17.8 Bb	410.0±52.4 Aa
UV-B10	110.2±10.1 Aa	80.8±34.1 Bb	396.1±36.1 Aa	376.5±100.8 ABa
UV-B15	104.0±4.4 Aa	133.8±13.7 ABb	131.8±3.7 Cb	309.7±72.2 Ba
UV-B5+C	95.1±10.6 ABa	102.4±34.2 ABCa	119.4±6.8 Ca	170.9±134.5 Ca
UV-B10+C	103.4±8.5 Aa	110.5±17.7 ABa	269.2±20.3 Bb	369.5±44.5 Ba
UV-B15+C	113.3±1.6 Aba	98.4±12.7 Ba	248.6±2.1 Ba	106.3±41.4 Cb
Stalks				
CTRL	30.0±3.7 Ba	35.5±6.9 Ba	1.0±0.2 Ca	2.0±1.1 Ba
UV-B5	35.0±4.0 BDa	1.8±0.4 Db	6.4±0.4 Aa	3.5±0.8 Ab
UV-B10	35.9±3.0 Ba	37.3±12.5 BCa	6.8±0.7 Aa	3.4±0.3 Ab
UV-B15	30.0±3.2 Ba	30.7±0.3 Ca	2.4±0.2 Bb	4.4±1.1 Aa
UV-B5+C	29.7±2.9 Ba	31.9±0.7 Ba	1.2±0.1 Ca	0.6±0.1 Ca
UV-B10+C	50.7±3.5 Aa	42.5±7.3 ABa	1.4±0.2 Ca	0.8±0.1 Ca
UV-B15+C	29.4±4.4 Bb	52.7±9.9 Aa	2.2±0.2 Ba	1.2±0.6 BCb

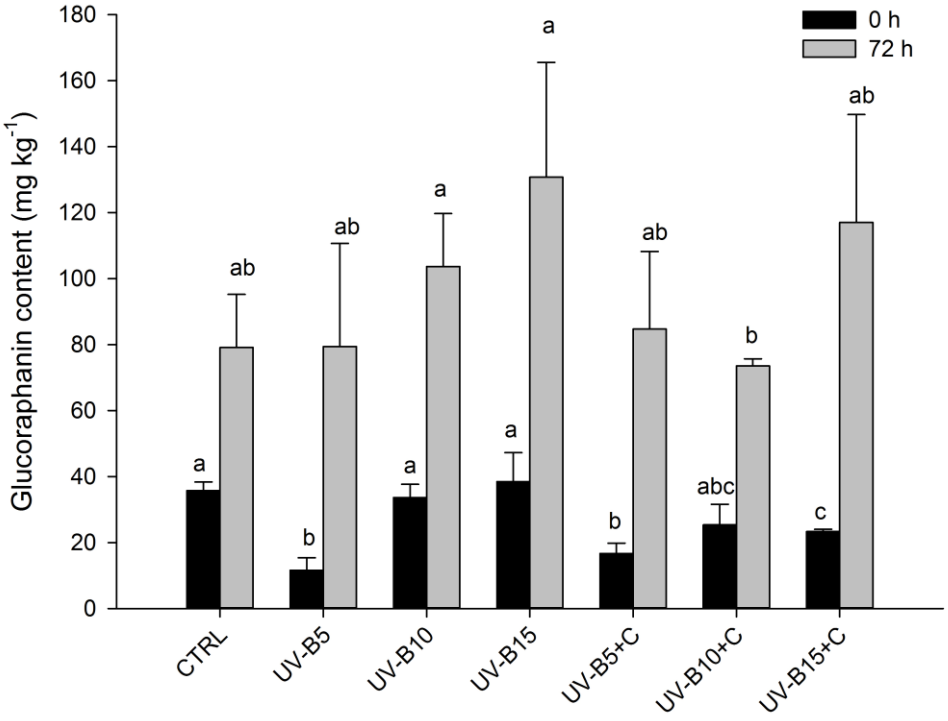


Figure III.1. Glucoraphanin contents of Bimi® broccoli florets treated with UV-B treatments, and combination with UV-C, during storage up to 72 h at 15 ºC (n=3±SD). Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment.

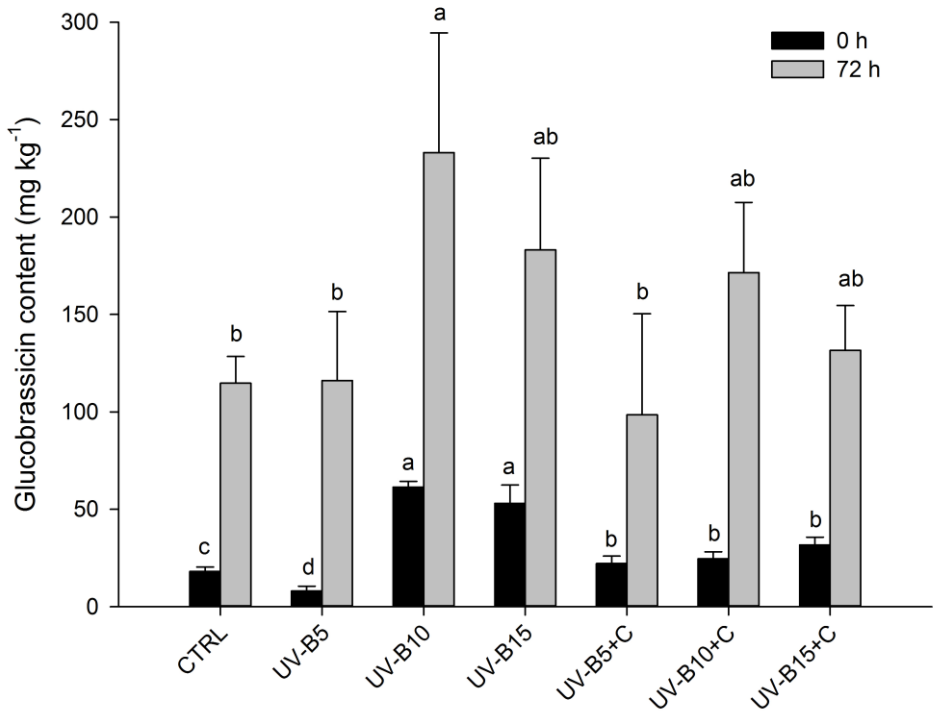
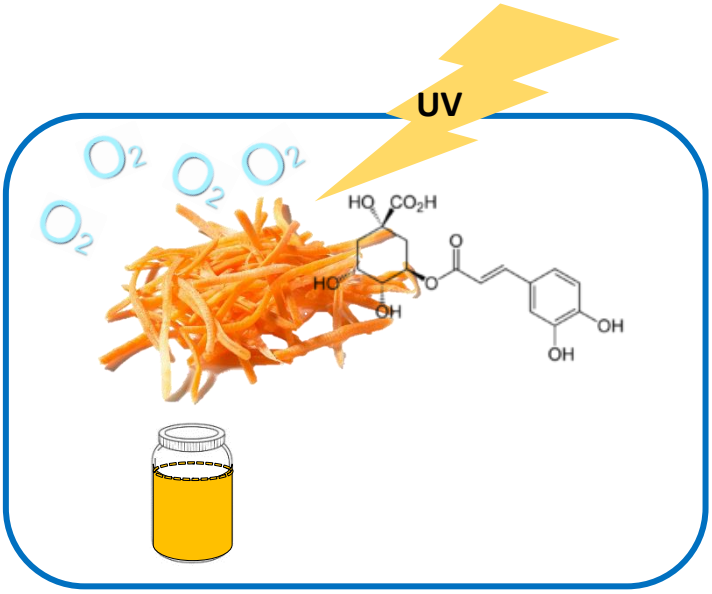


Figure III.2. Glucobrassicin contents of Bimi® broccoli florets treated with UV-B treatments, and combination with UV-C, during storage up to 72 h at 15 ºC (n=3±SD). Different capital letters denote significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letters denote significant differences ($p < 0.05$) among different sampling times for the same treatment.



CHAPTER IV

A functional smoothie from carrots with induced enhanced phenolic content

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IV.1. INTRODUCTION

The crescent consumer's knowledge on functional foods has led to an increasing interest in foods not only intended to feed, but also to prevent chronic and nutritional-related diseases as well as to improve overall human well-being. High intake of fruit and vegetables has been proved to prevent a grand array of diseases, such as degenerative disorders, cancer and cardiovascular among others (Slavin and Lloyd, 2012). However, the current lifestyle turns difficult the preparation of these plant products. Thus, their consumption should be promoted through the development of attractive ready-to-eat products that should be processed with minimal and non-aggressive treatments to preserve as much as possible the quality parameters of the raw materials (Artés et al., 2009). Accordingly, smoothies represent an excellent and convenient alternative to promote the daily consumption of fruits and vegetables (Rodríguez-Verástegui et al., 2015). Smoothies are non-alcoholic beverages prepared from fresh or frozen fruit and/or vegetables, which are blended without filtering and usually mixed with crushed ice to be immediately consumed. Often, some smoothies may include other components like yogurt, milk, ice-cream, lemonade or tea. They have a milk shake-like consistency that is thicker than slush drinks (Castillejo et al., 2015). Fruits and vegetables are rich in phenolic compounds among other bioactive compounds. Phenolic compounds are excellent antioxidants related to several health-promoting properties such as anti-inflammatory, antitumoral, as well as preventing neurodegenerative and chronic disorders (El Gharras, 2009). PAL is the key enzyme of primary (shikimate) and secondary (phenylpropanoid) pathways and is, therefore, involved in the biosynthesis of polyphenolic compounds (Dixon and Paiva, 1995). It is well reported that PAL activity may be enhanced by an array of biotic and abiotic stress-induced mechanisms, such as wounding, radiation exposure, hyperoxia storage, water stress, ultrasounds, chilling injury, low minerals, hormones and pathogen attack, among others (Cisneros-Zevallos, 2003; Cuéllar-Villarreal et al., 2016). Previous studies have shown that wounding, low UV-C doses and hyperoxia storage, singly, enhance phenolic content on carrots and other foodstuff (Alegria et al., 2012; Avena-Bustillos et al., 2012; Becerra-Moreno et al., 2012; Jacobo-Velázquez et al., 2011). Carrots occupy the sixth place among the list of most consumed vegetables in the American diet, although its TPC is almost the lowest among them (Chun et al., 2005). Accordingly, the health benefits derived from carrots could be increased by enhancing their phenolics levels during a controlled pre-enrichment incubation by using

postharvest abiotic stresses. Furthermore, synergistic effects on phenolics increments after combined application of different stresses may occur. Nevertheless, the effects on phenolic/antioxidant levels after combined application of wounding, intermediate UV-C dose and hyperoxia atmosphere on carrots has not been studied yet. Therefore, a functional phenolic-enriched carrot smoothie may be developed previously applying abiotic stresses on carrot material, singled or combined, during a pre-enrichment incubation prior to smoothie preparation. Moreover, a mild heat treatment of the smoothie may guarantee the food safety criteria and physicochemical quality of this functional carrot smoothie during refrigerated storage.

Accordingly, the aim of this study was to optimize a pre-enrichment treatment of carrots to increase the phenolic/antioxidant levels in order to obtain a functional carrot smoothie with enhanced phenolic/antioxidants contents. Furthermore, the effects of a mild heat treatment and subsequent refrigerated storage on the enriched phenolic/antioxidant levels of the functional carrot smoothie were also studied.

IV.2. MATERIALS AND METHODS

IV.2.1. Plant material

Fresh carrots (cvs. group Nantes, cv. Soprano) were purchased at a local market in Cartagena (Southeast of Spain) in April 2015. Carrots were carefully inspected, selecting only those with similar size (14-15 cm long and 2-3 cm diameter) and visual appearance free from defects (blemishes, wounds, etc.). Subsequently, carrots were sanitized in a cold room (8 ºC) with chlorine (100 mg L^{-1} NaClO; 5 ºC; pH 6.5±0.1) for 2 min, rinsed with tap water at 5 ºC for 1 min and drained in a perforated basket for 1 min. A ratio of 300 g plant material: 5 L chlorinated water was used. Carrots were wounded to shreds (2mm × 3mm × 40-60 mm) with a food processor (FreshExpress+, Moulinex, Lyon, France). Pre-enrichment treatments were conducted immediately after wounding.

IV.2.2. Pre-treatments and incubation of plant material

The UV-C treatment chamber used was previously detailed (Artés-Hernández et al. (2009a). Carrot shreds were placed between the two lines of UV-C lamps at 17.5 cm

above and below over a 35 mm thick bi-oriented PP film mounted on PS net (130 × 68 cm) that minimized blockage of the UV-C radiation. The applied UV-C intensity of 67.6 W m^{-2} was calculated as the mean of 18 UV-C readings on each side of the net using a VLX 254 radiometer (Vilber Lourmat, Marne la Vallee, France). Thus, both sides received the same UV-C intensity. The UV-C light intensity was kept constant and the applied dose was adjusted by the exposure time at the fixed distance. A UV-C radiation treatment of 4 kJ UV-C m^{-2} (exposure time of 139 s) was applied. Non-irradiated samples were used as control (hereinafter 'CTRL').

Samples to be stored under hyperoxia conditions (hereinafter 'HO') were placed in plastic containers (30 cm diameter, 60 cm height) and connected to an air-flow-through system supplied with humidified flows of either air or a gas mixture containing 80 % O_2 (balanced with N_2). In order to ensure a good air flow through carrot shreds, these samples were distributed in opened plastic petri dishes (8.5 cm diameter, 1 cm height). CO_2 partial pressures were kept < 0.15 kPa to avoid any physiological effect exerted by CO_2 such as anaerobic metabolism. Samples stored under air conditions were used as control (hereinafter 'Air'). Gas treatments were applied at 15 ºC for 72 h in darkness. Pre-enrichment incubation of carrots, as well as smoothie preparation and subsequent storage, is summarized in Figure IV.1.

IV.2.3. Carrot smoothie preparation, heat treatment and storage conditions

Carrot smoothie was prepared in a food processor (Robot Cook®, Robot Coupe, Vincennes Cedex, France) using sterilized water in a relation of 1:1 (carrot weight: water volume). Heat treatment of carrot smoothie was applied using a Mastia thermoresistometer as previously described (Conesa et al. (2009). Immediately after smoothie blending, the sterilized vessel of the thermoresistometer was filled with 400 mL of the smoothie. The thermoresistometer was programmed to increase the initial smoothie temperature (8±2 ºC) with a heating rate of 30 ºC/min to 90 ºC, then maintained for 30 s and cooled down to a final temperature of 40 ºC (cooling rate of 30 ºC/min). After heat treatment, the smoothie temperature was cooled down to 5 ºC submerging the vessel in an ice-water bath while continuously agitation was programmed in the thermoresistometer. Subsequently, 15-mL aliquots of heat-treated samples were taken in aseptic conditions in sterile Falcon tubes through the thermoresistometer sampling port.

Samples were stored in darkness at 5 ºC. Non heat-treated carrot smoothie was used as control. Visual appearance, flavour, texture, off-colours, off-odours, lumpiness, turbidity, precipitation/phase separation and overall quality of heat-treated smoothie conducted by an informal sensory panel test of 8 persons were reported to be over the limit of acceptability up to 14 days at 5 ºC. Sampling was conducted on processing day (0) and after 7 and 14 days at 5 ºC. Five replicates per treatment and sampling day were prepared.

IV.2.4. Analyses

IV.2.4.1. Physiochemical analyses

The pH, titratable acidity (TA), total soluble solids content (SSC) and colour (*CIE Lab*) of samples were determined as previously described (Castillejo et al. (2015). TA and SSC were expressed as g citric acid 100 mL^{-1} and ºBrix, respectively. ΔE and ΔBI were calculated from *CIE Lab* data according to equations previously described (Palou et al., 1999).

IV.2.4.2. Microbial analysis

Mesophilic, psychrophilic and yeast and moulds (Y+M) growth was determined using standard enumeration methods as previously described (Castillejo et al. (2015). All microbial counts were reported as log colony forming units per gram of product (log CFU g^{-1}). The detection limits were 1 log CFU mL^{-1} for mesophilic/psychrophilic and 2 log CFU mL^{-1} for Y+M. Each of the five replicates was analysed in duplicate. The presence of *Salmonella* spp., *L. monocytogenes* and generic *E. coli* was monitored throughout storage of smoothies according to the European legislation (EC_1441/2007, 2007).

IV.2.4.3. Phenylalanine ammonia-lyase

PAL activity was analysed as previously described (Ke and Saltveit, 1986) with modifications (Jacobo-Velázquez et al., 2011). Concisely, 2 g of sample was mixed with polyvinylpolypyrrolidone (0.2 g) and homogenized (Ultra Turrax®) in cold 50 mM borate buffer (pH 8.5) containing 400 μL L^{-1} β-mercaptoethanol. Homogenates were filtered through four layers of cheesecloth and then centrifuged at 10,000 × g for 20 min at 4 ºC.

Supernatants were used as enzyme extract. Two sets of UV-Star 96-well plates containing 69 µL of PAL extract plus 200 µL ultrapure water were prepared for every sample and pre-incubated at 40 ºC for 5 min. Afterwards, 30 µL of either water (blank) or 100 mM L-phenylalanine substrate solution (freshly prepared before assay) were added to each of the well for every sample set. The absorbances of sample sets were measured at 290 nm using the Multiscan plate reader at time 0 and after 1 h of incubation at 40 ºC. The PAL activity was expressed as µmol of *t*-cinnamic acid synthesized kg^{-1} fw h^{-1} using a *t*-cinnamic acid standard curve (0-6.75 mM). Each of the three replicates was analysed in duplicate.

IV.2.4.4. Phenolic compounds

Extraction to determine phenolic compounds and TAC extract was conducted by homogenization (Ultra Turrax®) of 2 g of sample in 8 mL methanol for 20 s under ice-water bath. Subsequently, extracts were centrifuged at 13,500 × g for 20 min at 4 ºC and supernatants were collected and analysed. Extracts for individual phenolic compounds were further filtered through a 0.22 µm polyethersulphone filter and stored at −80 ºC in amber vials until UPLC analysis.

The TPC was analysed by Folin–Ciocalteu reagent method as previously described (Martínez-Hernández et al., 2011). Briefly, a 19 µL aliquot of TPC extract was placed on a PS flat bottom 96-well plate and 29 µL of Folin–Ciocalteu reagent 2 N (Sigma, St Louis, MO, USA) were added. Samples were incubated for 3 min in darkness at room temperature. After incubation, 192 µL of a solution containing Na_2CO_3 (4 g L^{-1}) and NaOH (20 g L^{-1}) were added and the reaction was carried out for 1 h at room temperature in darkness. Subsequently, absorbance was read at 750 nm using the same Multiscan plate reader as described before. TPC was expressed as chlorogenic acid (Sigma, St Louis, MO, USA) equivalents (ChAE) in mg kg^{-1} fw. Each of the three replicates was analysed in duplicate.

Analyses of individual phenolic compounds were conducted as previously described (Alegria, 2015) with some modifications. Briefly, samples of 20 µL were analysed using an UHPLC instrument (Shimadzu, Kyoto, Japan) equipped with a DGU-20A degasser,

LC-30AD quaternary pump, SIL-30AC autosampler, CTO-10AS column heater and SPDM-20A photodiode array detector. The UHPLC system was controlled by the software LabSolutions (Shimadzu, v. 5.42 SP5). Chromatographic analyses were carried out onto a Kinetex C18 column (100 mm × 4.6 mm, 2.6 µm particle size; Phenomenex, Macclesfield, UK) with a KrudKatcher Ultra HPLC guard column (Phenomenex, Macclesfield, UK). The column temperature was maintained at 25 ºC. The mobile phase was acidified water (A; formic acid to final pH 2.3) and acidified methanol (B; formic acid to final pH 2.3). The flow rate was 1.5 mL min^{-1}. Gradient program used was 0/88, 1.2/88, 2.4/85, 8.3/70, 9.4/50, 11.8/50, 20.8/55, 22.0/60 (min/% phase A). Then, column equilibration was conducted at 0 % A for 2.2 min. Chromatograms were recorded at 320 nm. Phenolic acids were quantified as standards of chlorogenic acid, ferulic acid (Sigma, St Louis, MO, USA), 3,5-CQA and 4,5-CQA (ChromaDex, Irvine, CA, USA). The calibration curves were made with at least six data points. The results were expressed as mg kg^{-1} fw. Each of the three replicates was analysed in duplicate.

IV.2.4.5. Total antioxidant capacity

The extracts were analysed for TAC as previously described (Brand-Williams et al., (1995) with modifications (Martínez-Hernández et al., 2013d). Briefly, a solution of 0.7 mM DPPH in methanol was prepared 2 h before the assay and adjusted to 1.10±0.02 nm immediately before use. A 21 µL aliquot of the above described extract was placed on a PS flat-bottom 96-well plate and 194 µL of DPPH was added. The reaction was carried out for 30 min at room temperature in darkness and the absorbance at 515 nm was measured using the Multiscan plate reader. Results were expressed as mg Trolox equivalent antioxidant capacity kg^{-1} fw. Each of the three replicates was analysed in duplicate.

IV.2.5. Statistical Analyses

A complete randomized design in triplicate with two-way ANOVA (abiotic stress treatment × storage) was conducted. Statistical significance was assessed at the level p=0.05, and Tukey's multiple range test was used to separate means with SPSS software (v. 21, IBM, USA).

IV.3. RESULTS

IV.3.1. Physicochemical quality

Carrot shreds showed an initial pH and TA of 6.32-6.37 and 0.32-0.48 % (Table IV.1), respectively, similar to previous data (Pushkala et al., 2012). The pH of the shreds significantly ($p < 0.05$) decreased and TA increased ($p < 0.05$) during pre-enrichment incubation as a combined effect of microbial growth and phenolic acids enrichment as shown later. Accordingly, pH/TA of non-irradiated and irradiated shreds significantly ($p < 0.05$) decreased to 3.20-4.05/4.75-5.22 and 4.87-5.02/3.42-4.02, respectively, after pre-enrichment incubation. The higher acidification observed in non-irradiated samples may be explained by the higher phenolic acids content of these samples as it will be discussed later. No clear influence of the atmosphere storage conditions on pH and TA of carrot shreds after pre-enrichment incubation was observed. Similarly, no significant ($p < 0.05$) differences on pH and TA values were observed among air or hyperoxia-stored (80 % O_2) blueberry fruit during storage up to 35 days at 4 ºC (Zheng et al., 2003). Carrot smoothies from CTRL-Air, CTRL-HO, UV-C-Air and UV-C-HO carrot shreds showed initial pH/TA values of 3.35/2.05, 4.13/1.07, 5.03/0.68 and 5.15/1.00, respectively. Heat treatment did not change ($p < 0.05$) initial pH and TA of carrot smoothies. Quality of carrot beverages is difficult to maintain during storage due to its low acidity. The pH of carrot beverages is usually acidified with citric acid, or other acidulants, to approximately 3.8 as a general commercial practice by the food industries in order to reduce microbial growth and degradative enzymatic and non-enzymatic reactions during storage (Quitão-Teixeira et al., 2009; Talcott and Howard, 1999). Alternatively, acidification of carrot juice through fermentation has been proposed as a preservation method combined with pasteurization (Tamminen et al., 2013). Accordingly, the spontaneous fermentation occurred during pre-enrichment incubation of carrot shreds allowed to naturally reduce the pH extending the shelf life with a desirable mild acidic taste. In general, heat-treated carrot smoothies did not show high pH/TA changes throughout storage at 5 ºC with final pH/TA values of 3.8-4.5/0.87-1.14. Similarly, acidified blanched carrot juice showed more stable pH and TA values than non-acidified juices up to 21 days of storage at 4 ºC (Yu and Rupasinghe, 2012).

Carrot shreds showed initial SSC of 7.85-7.92 (Table IV.1) similar to previous data (Martínez-Hernández et al., 2016a). Carrot shreds stored under hyperoxia conditions presented significantly higher ($p < 0.05$) SSC compared to air-stored samples after pre-enrichment incubation. Similarly, SSC of blueberry fruit increased during hyperoxia (80 % O_2) storage at 5 ºC (Zheng et al., 2003). The latter behaviour may be explained by the reduced microbial growth under hyperoxia conditions, as shown later, and consequently lower microbial sugars consumption. SSC of carrot smoothies was not highly changed after heat treatment. In general, SSC of non-heat-treated carrot smoothies decreased through storage as consequence of microbial growth. Contrary, SSC of heat-treated smoothies generally did not register high changes due to the lower microbial loads. Unchanged SSC in pasteurized carrot juice after 15 days at 5 ºC was also reported (Kaur and Sharma (2013).

Colour is an important parameter for conformity determination of carrot beverages quality. ΔE is a colorimetric parameter extensively used to characterize the variation of colours during processing and storage of food products. *BI* represents the purity of brown colour and is reported as an important parameter in processes where enzymatic or non-enzymatic browning take place (Palou et al., 1999). For that reason, ΔE and *BI* have been satisfactorily used to assess colour quality of carrot beverages after processing treatments and subsequent storage (Kaur and Sharma, 2013). UV-C pre-treatment of carrot shreds induced initial mild browning (ΔE=5.6, ΔBI= 89.4; data not shown). Browning observed in some fruits and vegetables after UV-C radiation has been attributed to the increased POD activity (Tomás-Barberán and Espín, 2001). However, such browning of carrots shreds after the low UV-C dose used was not visually observed. Irradiated shreds showed higher BI after pre-enrichment incubation which may be owed to the pre-activated POD during UV-C pre-treatment (Table IV.1). Furthermore, pre-enrichment incubation under hyperoxia conditions induced slightly higher ΔE and ΔBI compared to carrot shreds incubated under air conditions. β-carotene, the main pigment responsible of the bright orange colour of carrots, is very susceptible to isomerization and oxidation (Knockaert et al., 2012). Furthermore, POD activity may increase under hyperoxia storage as previously reported (Yang et al., 2009). Accordingly, the observed higher colour degradation under hyperoxia compared to air conditions may be explained by a β-carotene degradation and incremented POD activity. Heat treatment of carrot smoothies induced low colour changes ($\Delta E < 25$, $\Delta BI < 96$) which correspond to undetected visual colour changes by a

trained panel test (Kaur and Sharma, 2013). Accordingly, β-carotene degradation in carrot puree and juice was very low, or even enhanced, due to higher extractability after such heat treatment as previously modelled (Lemmens et al., 2010; Marx et al., 2003; Quitão-Teixeira et al., 2009). Colour changes of heat-treated carrot smoothies during storage were lower compared to untreated smoothies which may be owed to heat inactivation of colour-degradative enzymes and reduced β-carotene degradation under such low storage temperature. Accordingly, only 5.5 % residual POD activity was reported in carrot juice after a similar heat treatment and it was even reduced to 2 % after 14 days at 4 ºC (Quitão-Teixeira et al., 2009). Attending to pre-enrichment treatments, all smoothies from irradiated carrots shreds showed slightly higher colour changes after 14 days of storage at 5 ºC. Nevertheless, all heat-treated smoothies from stressed carrot shreds (CTRL-HO, UV-C-Air and UV-C-HO) presented a good physicochemical quality after 14 days of storage at 5 ºC.

IV.3.2. Microbiological quality

Carrot shreds showed initial mesophilic, psychrophilic and Y+M loads of 5.4, 5.1 and 4.8 log CFU g^{-1}, respectively (Table IV.2). UV-C pre-treatment significantly ($p < 0.05$) reduced initial microbial loads of carrots shreds by 1.1-1.3 log units. Similar microbial reductions have been reported in Bimi® broccoli after a UV-C dose of 4.5 kJ m^{-2} (Martínez-Hernández et al., 2011). UV-C is a non-ionizing radiation able to alter microbial DNA through dimer formation (Bintsis et al., 2000). If the damage goes unrepaired, the accumulation of DNA photoproducts can be lethal to cells through the blockage of DNA replication and RNA transcription, which ultimately result in reproductive cell death. However, it has been also reported that UV-C may lead to the conversion of bacteria in the viable but non-cultivable state as a strategy of protection against the germicide effect of UV-C (to economize on energy, induction of repair mechanisms, inhibit the generation of mutant bacteria, etc.) (Ben Said et al., 2010). Pre-enrichment incubation of shreds under air conditions led to mesophilic/psychrophilic and Y+M growth of 3.2/2.8 and 4.7 log units, respectively, after 72 h. However, pre-enrichment incubation of non-irradiated shreds under HO conditions highly limited mesophilic/psychrophilic and Y+M growth to only 1.1/1.0 and 2.7 log units, respectively, after 72 h. Likewise, total viable counts were better controlled under hyperoxia (90 %) compared to air storage (Amanatidou et al., 2000). The observed microbicidal effects

during hyperoxia storage may be explained by several factors such as the unfavourable effects on the oxidation–reduction potential of the system, the oxidation of enzymes having sulfhydryl groups or disulfide bridges, and the accumulation of injurious reactive O_2 species (Kader and Ben-Yehoshua, 2000). The sanitizing effects of UV-C radiation and hyperoxia storage in other fresh-cut fruit and vegetables have been previously reviewed (Artés et al., 2009). UV-C irradiated shreds showed significantly ($p < 0.05$) higher microbial growth compared to non-irradiated samples during pre-enrichment incubation. Latter detrimental effect of UV-C pre-treatment during storage, contrary to benefit from initial sanitation, may be explained by the plant cell disruption caused by UV-C radiation leading to leakage of electrolytes (Martínez-Hernández et al., 2013d) such as sugars which favours microbial growth.

In general, heat treatment reduced initial microbial loads of carrot smoothies (7-8 log units) below detection limits. Accordingly, the applied heat treatment was enough to achieve pasteurization levels. Microbial loads of untreated smoothies were over 10 log CFU mL^{-1} after 7 days at 5 ºC (data not shown). Mesophilic counts of heat-treated carrot smoothies increased during storage registering final loads of 3.5-4.1 log CFU mL^{-1}, without significant ($p < 0.05$) differences among pre-treatments, after 14 days at 5 ºC. Smoothies from UV-C-HO and UV-C-Air pre-treated shreds showed the highest psychrophilic growths with 4.7 and 3.5 log units increments, respectively, after 14 days at 5 ºC. Meanwhile, smoothies from CTRL-HO and CTRL-Air shreds registered psychrophiles increments of 2.9 and 1.4 log units, respectively, after 14 days at 5 ºC. As observed, psychrophilic growth in pasteurized smoothies was higher as the stress level from pre-enrichment incubation augmented following this order: HO>UV-C>UV-C+HO. Latter behaviour may be explained since as the stress level increased surviving microorganisms after heat treatment acquired higher adaptation to grow under unfavourable conditions such as low temperature storage. Similarly, heat-treated smoothies from UV-C-HO and UV-C-Air shreds significantly ($p < 0.05$) registered 0.8 and 1.3 log CFU mL^{-1} increments, respectively, while the other two pre-treatments did not register significant ($p < 0.05$) changes after 14 days at 5 ºC. *Salmonella* spp., *L. monocytogenes* and generic *E. coli* were monitored throughout storage of smoothies with absence meeting the European legislation limits (EC, 2007). Phenolic acids are known to exhibit antimicrobial activity against a variety of microorganisms (Wen et al., 2003). In the same line, carrot juice have shown high antilisterial properties (Beuchat and Brackett,

1990). Application of combined preservative factors (called hurdles) is used by food industries according to the hurdle technology to achieve effective preservation of foods (Leistner, 2000). Consequently, the good microbiological quality (microbial loads < 6 log units) of all heat-treated carrot smoothies after 14 days of storage may be owed to the combination of achieved acidic pH, enhanced antimicrobial compounds (phenolic acids) and low storage temperature.

IV.3.3. Phenylalanine ammonia-lyase activity

PAL is the key enzyme between primary (shikimate pathway) and secondary (phenylpropanoid) metabolism pathways involved in the biosynthesis of polyphenolic compounds (Dixon and Paiva, 1995). Carrot shreds showed initial PAL activity of 12.5-16.2 µmol cinnamic acid formed kg^{-1} h^{-1} fw (Table IV.3) similar to previous data (Martínez-Hernández et al., 2016a). UV-C pre-treatment did not induce significant ($p < 0.05$) changes in the PAL activity of carrot shreds at day 0. PAL activity of shredded carrots highly increased ($p < 0.05$) after pre-enrichment period. CTRL-HO carrot shreds showed the highest increments with a PAL activity of 224.9 µmol cinnamic acid formed kg^{-1} h^{-1} fw after 72 h of pre-enrichment period. The rest of pre-treatments showed PAL activities ranging from 86.2 to 102.7 after pre-enrichment period without significant ($p < 0.05$) differences among them. PAL activation after wounding and hyperoxia storage has been reported as an abiotic stress response being proposed ATP and reactive oxygen species as signalling molecules (Jacobo-Velázquez et al., 2011). Fresh carrot smoothie from CTRL-HO shreds showed an initial PAL activity of 112.43 µmol cinnamic acid formed kg^{-1} h^{-1} fw while the activity of this enzyme ranged from 40.6 to 52.5 µmol cinnamic acid formed kg^{-1} h^{-1} fw or the rest of smoothies. Pasteurization of carrot smoothie highly reduced PAL activity by 81-95 % without significant differences ($p < 0.05$) among pre-treatments. Likewise, heat treatment (70 ºC for 3 min) of vegetables red smoothies (pH 4.4) led to significant ($p < 0.05$) reductions of PAL activities of 65-70 % (Rodríguez-Verástegui et al., 2015). In general, PAL activity of smoothies decreased throughout storage registering final activities of 22.4/11.7 µmol cinnamic acid formed kg^{-1} h^{-1} fw for smoothies from CTRL-HO shreds while the rest of samples ranged among 1.0-5.7 µmol cinnamic acid formed kg^{-1} h^{-1} fw. PAL activation due to wounding stress occurred during smoothie preparation may be highly reduced at low storage temperatures. Accordingly, no PAL activation was observed in the carrot smoothies in this storage

period of 14 days at 5 ºC. Similarly, PAL activity of red vegetables smoothies highly incremented ($p < 0.05$) after 20 days at 5 ºC (Rodríguez-Verástegui et al., 2015). Thus, the decrease of PAL activity observed in the carrot smoothies throughout storage may be owed to the low storage temperature and acidic pH conditions as previously reported in PAL preparations (Rees and Jones, 1996).

IV.3.4. Phenolic compounds

Carrot shreds reported initial TPC of 187.3 mg CHA kg^{-1} fw (Table IV.3). Similar TPC have been reported for the same carrot cultivar (Alegria et al., 2010). The major individual phenolic compounds identified were 3-CQA, 3,5-CQA, 4,5-CQA and ferulic acid (Table 4). The phenolic contents of carrots were significantly unchanged ($p < 0.05$) immediately after UV-C pre-treatment. The TPC of carrot shreds increased ($p < 0.05$) by approximately 2,060, 1,510, 1,170 and 760 % in CTRL-HO, CTR-Air, UV-C-Air and UV-C-HO samples, respectively, after 72 h of pre-enrichment incubation. Postharvest abiotic stresses such as wounding, UV-C radiation and hyperoxia storage have been reported to highly increment the contents of phenolic compounds in carrots during subsequent storage (Alegria et al., 2012; Martínez-Hernández et al., 2011). This phenolic biosynthesis has been reported to be a consequence of PAL activation after these abiotic stresses being proposed ATP and reactive oxygen species as signalling molecules (Jacobo-Velázquez et al., 2011). UV-C-HO showed the lowest phenolic accumulation ($p < 0.05$) during pre-enrichment incubation among the rest of treatments probably owed to a partial PAL denaturation by such UV-C treatment delaying the stress-enhanced activity of this enzyme. The pre-enrichment incubation of carrot shreds allowed to obtain carrot smoothies with TPC of 710.4-1925.7 mg CHA kg^{-1} fw, representing 3-CQA the 87.3 % of the sum of phenolic compounds. 3-CQA, an ester of caffeic acid with quinic acid with high antioxidant capacity compared to other phenolic compounds, has been reported as the main phenolic compound in carrots (Castelluccio et al., 1995). The identified minor phenolic compounds 3,5-CQA, 4,5-CQA and ferulic acid accounted 7.8, 2.4 and 2.5 % of the sum of phenolic compounds, respectively. Heat treatment of carrot smoothies did not induce significant ($p < 0.05$) changes of TPC or individual phenolic compounds. Consistently, no TPC changes were reported between untreated and heat-treated carrot purees and juices (Patras et al., 2009a; Quitão-Teixeira et al., 2009).

The TPC of untreated smoothies registered a mild TPC increment of 10-25 % at day 7 showing the smoothie from CTRL-HO shreds the highest increment. This TPC increment at day 7 is in accordance to the ferulic acid and 3,5-CQA increments (Table IV.4) and to the higher PAL activity observed of these samples regarding the rest of smoothies (Table IV.3). However, heat-treated smoothies did not show the same behaviour at day 7. Similar phenolic increments have been reported in red vegetables smoothies during low temperature storage (Rodríguez-Verástegui et al., 2015). Interestingly, high TPC increments of approximately 610-850 % were registered in heat-treated smoothies at day 14 comparing to their respective initial levels. Heat-treated smoothies from non-irradiated air/HO shreds showed the highest ($p < 0.05$) TPC at day 14 with approximately 10,960/13,824 mg CHA kg^{-1} fw. This high TPC enhancement of non-irradiated samples were due to 3-CQA and ferulic acids enhancements. The higher phenolic biosynthesis observed in smoothies from air-incubated carrot shreds is in accordance to the still higher PAL activities of these smoothies at day 14. However, PAL activity of those samples at day 14 may not explain such high increments of phenolic compounds observed in heat-treated smoothies. Accordingly, this enhanced biosynthesis of phenolic compounds in heat-treated smoothies at day 14 may be owed to other enzymes different to PAL involved in the phenylpropanoid pathway. Heat treatment (100 ºC for 45 s) of carrots has been reported to induce TPC enhancements during subsequent storage of carrot shreds at 5 ºC comparing to untreated samples (Alegria et al., 2012). Accordingly, the heat treatment applied to the carrot smoothies could trigger signals related to other enzymes different from PAL involved in the phenylpropanoid pathway although the activation of these enzymes could be retarded until day 14 due to the low storage temperature.

IV.3.5. Total antioxidant capacity

The initial TAC of carrot shreds was 1,102.3±97.3 µmol Trolox kg^{-1} fw (Table IV.3). TAC increased during pre-enrichment incubation being highly correlated (R^2=0.90) to TPC. Among pre-enrichment treatments, non-irradiated carrot shreds stored under hyperoxia conditions registered the highest TAC enhancements as observed for TPC. Similar high TAC-TPC correlations have been previously reported after wounding and hyperoxia storage of carrots (Jacobo-Velázquez et al., 2011). The specific antioxidant capacity (ratio of total antioxidant capacity over total soluble phenolics) has been proposed as a useful index to provide information of the effectiveness of phenolic

compounds to neutralize free radicals (Cisneros-Zevallos, 2003; Heredia and Cisneros-Zevallos, 2009). A higher specific antioxidant capacity means phenolic compounds have a higher capacity to stabilize free radicals (Reyes et al., 2007). Irradiated samples showed significantly ($p < 0.05$) higher specific antioxidant capacities compared to non-irradiated samples reporting UV-C-HO shreds the highest value with 1010.7 µmol Trolox kg^{-1} fw. Similar specific antioxidant activity was reported by induced carrot phenolics after postharvest abiotic stresses (Cisneros-Zevallos, 2003).

Smoothies from carrots without UV-C pre-treatment showed the highest ($p < 0.05$) TAC levels with 1,462.2 (hyperoxia) and 1,343.8 (air) µmol Trolox kg^{-1} fw. On the other side, smoothies from irradiated carrots showed lower TAC levels with 829.1 (hyperoxia) and 1,056.9 (air) µmol Trolox kg^{-1} fw. Similar to TPC, heat treatment of carrot smoothies did not induce significant ($p < 0.05$) changes of TAC. TAC of smoothies increased by 64-227 % after 14 days of storage at 5 ºC. The highest TAC increments after 14 days in un-heated smoothies was observed in those samples from non-irradiated carrots. However, the highest TAC increments in heat-treated smoothies were registered by UV-C pretreated samples. Latter behaviour may be explained since UV-C pre-treatment was able to compensate subsequent reduction of activities of enzymes involved in the phenylpropanoid pathway after heat treatment.

IV.4. CONCLUSIONS

Carrot is a vegetable highly consumed which low phenolic levels could be naturally increased leading to a phenolic/antioxidant enriched plant material to produce a functional carrot smoothie. The phenolic levels of shredded carrots used for the smoothie preparation were highly enhanced after pre-enrichment incubation for 72 h at 15 ºC up to 2,060 % in those non-irradiated shreds stored under hyperoxia conditions. The total antioxidant capacity was highly correlated to total phenolic content. The high temperature-short time heat treatment reduced microbial loads below the detection limits with low growth during subsequent refrigerated storage. The physicochemical quality was good for all smoothies at the end of storage. UV-C pretreatment of carrot shreds resulted in carrot smoothies at the end of storage with higher psychrophilic and yeasts and moulds loads and lower phenolic levels. Accordingly, pre-enrichment incubation of carrot shreds under hyperoxia conditions allowed to obtain a functional smoothie with

high phenolic levels and good microbiological and physicochemical quality up 14 days at 5 ºC.

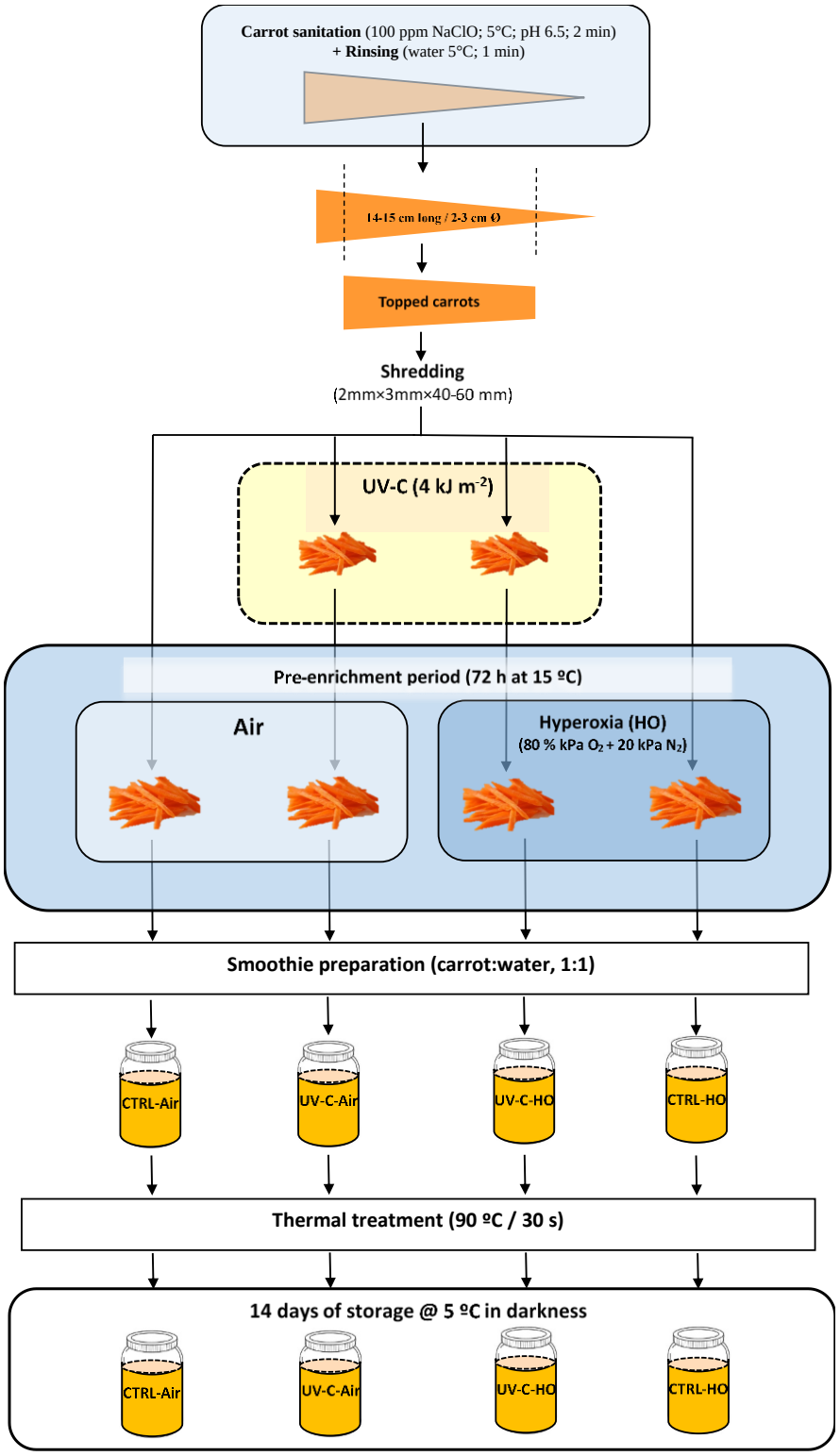

Figure IV.1. Flow diagram of pre-enrichment incubation of carrots, smoothie preparation and storage conditions.

Table IV.1. pH, titratable acidity (TA; %), soluble solids content (SSC; º Brix), total colour differences (ΔE) and browning index differences (ΔBI) changes of carrot shreds after different treatments (hyperoxia, UV-C radiation and controls) during pre-enrichment incubation (72 h at 15 ºC) and subsequent storage at 5 ºC of prepared non-treated or heat-treated (90 ºC for 30 s) smoothie (n=5±SD). Different capital letter denotes significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letter denotes significant differences ($p < 0.05$) among different sampling times for the same treatment.

	Carrot shreds incubation		**Non-treated smoothie**			**Heat-treated smoothie**		
	0 h	**72 h**	**0 d**	**7 d**	**14 d**	**0 d**	**7 d**	**14 d**
pH								
CTRL-Air	6.32±0.31 Aa	3.20±0.28 Cb	3.35±0.15 Ca	3.47±0.26 Ba	3.52±0.28 Ba	3.40±0.32 Ca	3.51±0.21 Ca	3.51±0.22 Ca
CTRL-HO	6.32±0.31 Aa	4.05±0.33 Bb	4.13±0.24 Ba	4.08±0.11 Aa	4.22±0.34 Aa	4.11±0.27 Ba	4.17±0.18 Ba	4.19±0.26 ABa
UV-C-Air	6.37±0.42 Aa	4.87±0.52 Ab	5.03±0.21 Aa	4.20±0.31 Ab	4.10±0.19 Ab	4.85±0.37 Aa	4.84±0.34 Aa	4.53±0.37 Aa
UV-C-HO	6.37±0.42 Aa	5.02±0.34 Ab	5.15±0.36 Aa	4.02±0.48 Ab	3.93±0.21 Ab	5.05±0.51 Aa	4.21±0.16 Bb	3.84±0.24 Bb
TA								
CTRL-Air	0.32±0.02 Ab	5.22±0.41 Aa	2.05±0.08 Ac	4.48±0.38 Aa	3.63±0.26 Bb	1.86±0.26 Ab	1.11±0.15 Ac	2.83±0.16 Aa
CTRL-HO	0.32±0.02 Ab	4.75±0.22 Aa	1.07±0.06 Bc	3.12±0.34 Ba	2.44±0.15 Cb	1.00±0.06 Bb	1.15±0.02 Aa	0.91±0.08 Bb
UV-C-Air	0.48±0.04 Ab	3.42±0.29 Ca	0.68±0.04 Cb	3.32±0.39 Ba	3.56±0.29 Ba	0.72±0.07 Ca	0.76±0.03 Ba	0.87±0.04 Ba
UV-C-HO	0.48±0.04 Ab	4.02±0.34 Ba	1.01±0.11 Bc	4.52±0.32 Ab	5.04±0.27 Aa	0.84±0.04 Cb	1.08±0.16 Aa	1.14±0.08 Ba
SSC								
CTRL-Air	7.92±0.52 Aa	5.65±0.31 Db	2.70±0.23 Dc	3.10±0.08 Bb	3.50±0.15 Aa	2.80±0.38 Bb	3.20±0.09 Ca	3.21±0.23 Ca
CTRL-HO	7.92±0.52 Aa	9.50±0.29 Ab	4.90±0.34 Aa	3.30±0.31 Bb	2.10±0.26 Cc	3.70±0.18 Ab	3.60±0.21 Bb	4.00±0.14 Aa
UV-C-Air	7.85±0.24 Aa	6.42±0.34 Cb	3.20±0.12 Ca	2.50±0.16 Cb	2.90±0.31 Bc	2.70±0.34 Bc	4.00±0.11 Aa	3.60±0.16 BCb
UV-C-HO	7.85±0.24 Aa	8.42±0.27 Bb	4.30±0.28 Bb	5.20±0.14 Aa	3.60±0.22 Ac	3.80±0.26 Aa	3.81±0.27 ABa	3.92±0.24 ABa
Δ*E*								
CTRL-Air	-	9.48±0.67 C	-	17.89±0.74 Cb	48.16±4.52 Aa	-	2.00±0.31 Cb	6.17±0.41 Ca
CTRL-HO	-	11.89±1.75 B	-	20.49±1.12 Bb	30.09±3.62 Ba	-	3.48±0.26 Bb	9.08±0.26 Ba
UV-C-Air	5.88±0.42 A	7.65±0.42 D	-	12.16±0.62 Db	22.40±1.88 Ca	-	2.96±0.31 Cb	12.85±0.94 Aa
UV-C-HO	5.88±0.42 A	15.53±1.02 A	-	32.21±1.84 Aa	32.16±3.26 Ba	-	6.96±0.42 Ab	10.05±1.21 Ba

ΔBI								
CTRL-Air	-	71.97±8.11 B	-	60.60±7.12 Cb	64.49±4.32 Ca	-	12.65±1.81 Bb	51.62±4.08 Ba
CTRL-HO	-	100.21±12.68 A	-	47.03±5.62 Db	78.44±6.01 Ba	-	20.79±1.36 Ab	61.65±5.13 Aa
UV-C-Air	89.39±11.12 A	74.84±5.11 B	-	79.67±9.52 Bb	98.18±7.60 Aa	-	8.55±0.96 Cb	29.73±1.62 Ca
UV-C-HO	89.39±11.12 A	151.11±12.65 A	-	108.03±9.11 Ab	116.80±10.34 Aa	-	14.30±1.12 Bb	66.53±4.23 Aa

Table IV.2. Mesophilic, psychrophilic and yeasts and moulds counts (log CFU g^{-1}) of carrot shreds after different treatments (hyperoxia, UV-C radiation and controls) during pre-enrichment incubation (72 h at 15 ºC) and subsequent storage at 5 ºC of prepared non-treated or heat-treated (90 ºC for 30 s) smoothie (n=5±SD). Different capital letter denotes significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letter denotes significant differences ($p < 0.05$) among different sampling times for the same treatment.

	Carrot shreds incubation		**Non-treated smoothie**	**Heat-treated smoothie**		
	0 h	**72 h**		**0 d**	**7 d**	**14 d**
Mesophiles						
CTRL-Air	5.3±0.4 Ab	8.6±0.6 Aa	8.1±1.0 A	< DT*	3.4±0.1 Aa	3.5±0.2 Aa
CTRL-HO	5.3±0.4 Ab	6.5±0.4 Ba	6.3±0.4 B	1.5±0.7 Bb	3.4±0.4 Aa	3.8±0.2 Aa
UV-C-Air	4.1±0.5 Bb	8.5±0.9 Aa	8.4±0.7 A	2.4±0.2 Ab	3.5±0.4 Aa	3.9±0.1 Aa
UV-C-HO	4.1±0.5 Bb	8.3±0.7 Aa	8.2±0.7 A	< DT	3.3±0.5 Aa	4.1±0.5 Aa
Psychrophiles						
CTRL-Air	5.1±0.4 Ab	7.9±0.9 Ba	8.0±0.7	2.8±0.3 Ab	3.1±0.2 Aa	4.2±0.6 Ba
CTRL-HO	5.1±0.4 Ab	6.6±0.7 Ca	6.4±1.0	< DT	2.8±0.1 Ab	3.9±0.4 Ba
UV-C-Air	4.0±0.3 Bb	8.6±0.2 Aa	7.6±0.4	2.5±0.4 Ab	3.1±0.4 Ab	6.0±0.8 Aa
UV-C-HO	4.0±0.3 Bb	7.8±0.8 Ba	7.2±0.3	< DT	3.0±0.2 Ab	5.7±0.2 Aa
Y+M**						
CTRL-Air	4.8±0.6 Ab	9.5±1.1 Aa	8.5±0.6	2.3±0.4	2.0±0.0 Aa	2.0±0.0 Ba
CTRL-HO	4.8±0.6 Ab	7.5±0.9 Ba	7.3±0.4	< DT	2.0±0.0 Aa	2.0±0.0 Ba
UV-C-Air	3.5±0.2 Bb	7.6±0.4 Ba	7.2±0.8	< DT	2.0±0.0 Aa	2.8±1.2 ABa
UV-C-HO	3.5±0.2 Bb	7.1±0.5 Ba	6.4±0.4	< DT	2.8±0.7 Aa	3.3±0.4 Aa

*DT: Detection limit (1 log CFU mL^{-1} for mesophilic/psychrophilic and 2 log CFU mL^{-1} for Y+M); /**

Table IV.3. Phenylalanine ammonia-lyase activity (PAL; μmol *t*-cinnamic acid synthesized kg^{-1} fw h^{-1}), total phenolic content (TPC; Chlorogenic acid equivalent kg^{-1} fw) and total antioxidant capacity (TAC; mg Trolox equivalents kg^{-1} fw) of carrot shreds after different treatments (hyperoxia, UV-C radiation and controls) during pre-enrichment incubation (72 h at 15 ºC) and subsequent storage at 5 ºC of prepared non-treated or heat-treated (90 ºC for 30 s) smoothie (n=5±SD). Different capital letter denotes significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letter denotes significant differences ($p < 0.05$) among different sampling times for the same treatment.

	Carrot shreds incubation		**Non-treated smoothie**			**Heat-treated smoothie**		
	0 h	**72 h**	**0 d**	**7 d**	**14 d**	**0 d**	**7 d**	**14 d**
PAL								
CTRL-Air	16.2±1.1 Ab	102.7±8.6 Ba	46.3±13.4 Ba	3.1±0.4 Bb	1.8±0.6 Bb	2.4±0.7 B	5.5±1.3 Ca	5.7±0.3 Ba
CTRL-HO	16.2±1.1 Ab	224.9±11.1 Aa	112.4±3.1 Aa	32.2±9.8 Ab	22.4±1.5 Ab	4.2±3.2 ABb	10.6±1.3 Aa	11.7±3.5 Aa
UV-C-Air	12.5±0.9 Ab	86.2±7.1 Ba	40.6±6.0 Ba	3.9±0.6 Bb	1.6±0.4 Bb	7.8±0.6 Aa	6.2±2.5 BCa	1.6±0.4 Cb
UV-C-HO	12.5±0.9 Ab	97.6±6.3 Ba	52.5±3.1 Ba	3.2±1.3 Bb	1.0±0.2 Bb	5.6±2.4 ABa	9.0±1.3 ABa	2.2±0.6 Cb
TPC								
CTRL-Air	187.3±12.5 Ab	3012.3±411.2 Ba	1486.7±67.8 Ba	1636.2±48.7 Ba	1667.4±159.1 Aa	1396.4±260.3 Bb	1332.9±53.1 Bb	10958.1±749.6 Ba
CTRL-HO	187.3±12.5 Ab	4050.8±362.2 Aa	1925.7±93.5 Ab	2410.6±299.0 Aa	1727.4±22.9 Ab	1955.3±222.2 Ab	1889.5±54.2 Ab	13823.5±285.7 Aa
UV-C-Air	177.5±15.1 Ab	2253.2±120.1 Ca	989.3±64.7 Cb	1117.3±51.4 Ca	1001.3±80.0 Bab	981.8±69.9 Cc	1150.4±34.2 Cb	8633.9±123.3 Ca
UV-C-HO	177.5±15.1 Ab	1562.2±132.0 Da	710.4±43.9 Db	832.9±5.1 Ca	733.9±7.7 Cb	744.6±107.5 Cb	749.2±25.2 Db	7102.3±509.1 Da
TAC								
CTRL-Air	1102.3±97.3 Ab	2701.3±185.2 Aa	1343.8±48.3 Bc	1812.2±20.5 Cb	2562.4±145.7 Ba	1462.5±116.9 Ab	2359.5±98.7 Ba	2472.3±88.4 Ba
CTRL-HO	1102.3±97.3 Ab	3012.0±181.2 Aa	1462.2±103.2 Ac	4640.1±140.1 Aa	3651.2±45.6 Ab	1238.6±140.7 Bc	2287.3±23.0 Cb	2522.2±58.0 Ba
UV-C-Air	952.2±42.6 Ab	2095.2±106.2 Ba	1056.9±28.9 Cc	1851.9±30.8 Cb	2252.8±73.4 Ca	1115.5±67.8 Bc	2561.7±47.6 Aa	2480.7±57.9 Bb
UV-C-HO	952.2±42.6 Ab	1542.6±97.5 Ca	829.1±22.6 Dc	2701.1±17.8 Ba	2106.7±66.0 Cb	830.0±60.0 Cc	1976.8±35.3 Db	2710.7±51.2 Aa

Table IV.4. Individual phenolic contents (mg kg^{-1} fw) of carrot shreds after different treatments (hyperoxia, UV-C radiation and controls) during pre-enrichment incubation (72 h at 15 ºC) and subsequent storage at 5 ºC of prepared non-treated or heat-treated (90 ºC for 30 s) smoothie (n=5±SD). Different capital letter denotes significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letter denotes significant differences ($p < 0.05$) among different sampling times for the same treatment.

	Carrot shreds incubation		Non-treated smoothie			Heat-treated smoothie		
	0 h	72 h	0 d	7 d	14 d	0 d	7 d	14 d
3-CQA								
CTRL-Air	45.32±4.26 Bb	302.10±21.02 Ba	146.50±6.03 Ba	131.58±1.30 Ab	116.16±0.84 Ac	155.86±12.89 ABb	177.32±8.08 Ba	186.57±3.78 Ca
CTRL-HO	45.32±4.26 Bb	385.25±11.26 Aa	199.11±13.00 Aa	115.59±5.70 Bb	46.04±2.81 Cc	186.78±30.28 Bb	228.57±23.79 Aab	247.85±9.80 Aa
UV-C-Air	60.26±9.42 Ab	375.29±40.89 Aa	180.18±12.91 Aa	112.55±10.52 BCb	67.59±13.51 Bc	209.60±7.96 Aa	178.48±8.33 Bb	162.21±3.96 Dc
UV-C-HO	60.26±9.42 Ab	350.68±20.25 ABa	158.28±7.95 Ba	103.62±1.75 Cb	52.05±2.37 Cc	156.90±3.22 Bb	133.73±9.16 Cc	225.33±8.90 Ba
3,5-CQA								
CTRL-Air	29.16±1.12 Aa	35.16±4.11 Aa	16.06±0.64 Ba	10.39±0.18 Ba	14.53±0.28 Ba	15.36±0.44 Ba	15.63±0.25 Ba	15.51±0.28 Ca
CTRL-HO	29.16±1.12 Ab	40.26±1.82 Aa	18.73±0.30 Ab	21.17±1.38 Aa	14.18±1.63 Bc	17.66±1.69 Ab	18.44±0.43 Aab	20.20±0.13 Aa
UV-C-Air	20.09±3.65 Bb	36.26±2.05 Aa	15.25±0.38 Bb	21.33±1.08 Aa	17.42±0.37 Ab	16.82±0.65 ABb	18.96±0.92 Aa	17.19±1.18 Bab
UV-C-HO	20.09±3.65 Ba	28.15±2.72 Ba	12.28±0.45 Cc	15.73±0.40 ABa	13.36±0.14 Bb	12.70±0.07 Cb	11.34±0.42 Cc	14.21±0.10 Da
4,5-CQA								
CTRL-Air	6.04±0.86 Ab	9.85±0.45 ABa	5.11±0.32 Ba	3.32±0.88 Aa	4.73±0.16 Aa	3.17±0.12 Ca	4.75±0.11 Ba	4.65±0.15 Ba
CTRL-HO	6.04±0.86 Ab	12.36±1.16 Aa	5.55±0.25 Aa	5.20±0.23 Aa	3.80±0.04 Bb	5.25±0.55 Aa	5.23±0.08 Aa	5.73±0.03 Aa
UV-C-Air	5.57±0.98 Ab	8.98±0.71 Ba	4.13±0.05 Cb	4.68±0.28 Aa	4.02±0.33 Bb	4.53±0.14 ABa	4.70±0.22 Ba	4.43±0.27 Ba
UV-C-HO	5.57±0.98 Ab	8.27±0.63 Ba	3.99±0.09 Cb	4.37±0.09 Aa	3.76±0.08 Bc	4.15±0.09 BCb	3.73±0.18 Cc	4.58±0.05 Ba
Ferulic acid								
CTRL-Air	0.88±0.11 Bb	9.45±0.38 Ba	4.65±0.24 ABa	3.72±3.22 Ba	6.06±0.26 Aa	7.70±1.18Ab	9.93±0.29 Ba	10.25±0.26 Ba
CTRL-HO	0.88±0.11 Bb	12.06±1.02 Aa	5.92±1.32 Aa	7.34±0.82 Aa	3.33±0.16 Bb	8.50±1.94 Ab	10.74±0.43 Aab	12.27±0.50 Aa
UV-C-Air	1.85±0.21 Ab	6.82±0.42 Ca	2.91±0.97 Ca	4.35±0.47 ABa	2.71±1.03 Ba	4.67±0.41 Bb	5.43±0.44 Ca	4.87±0.14 Cab
UV-C-HO	1.85±0.21 Ab	7.06±0.81 Ca	3.05±0.58 BCb	4.75±0.01 ABa	2.54±0.39 Bb	2.99±1.17 Bb	2.92±0.34 Db	4.70±0.08 Ca

3-CQA: 3-caffeoylquinic acid (Chlorogenic acid); 3,5-CQA: 3,5-dicaffeoylquinic acid (Isochlorogenic acid A); 4,5-CQA: 4,5-dicaffeoylquinic acid (Isochlorogenic acid C).

CHAPTER V

High hydrostatic pressure treatments for keeping quality of orange vegetables smoothies

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V.1. INTRODUCTION

The current worldwide drive for a healthier lifestyle has led to a rising demand for convenient fresh foods, free from additives, with high nutritional value, including antioxidant and free-radical scavenging properties, to be consumed both at home and in food services (Artés et al., 2009). Accordingly, the fresh-cut produce market has undergone a rapid growth within the food industry, caused by trends in life style, diet, importance for health, and healthy alternative in restaurants. Fresh-cut produce are more perishable than intact vegetables because they have been subjected to several physical stress, such as cutting, slicing, peeling, shredding, trimming, and coring (Iqbal et al., 2008). In the past years, consumption of fruit and vegetable juices, and more recently smoothies, are increasing due to its practicability being a potential alternative to whole fruit and vegetable intake. Depending on the type of beverage, a range of phytochemicals, such as phenolic compounds, may also be present with the related health-promoting properties (Di Cagno et al., 2011; Ruxton, 2008). Phenolic compounds, and specifically, hydroxycinnamic acids, are well known for its high antioxidant capacity being an important tool to prevent degenerative diseases. PAL catalyses the first step on phenylpropanoid pathway, regulating the propagation of secondary metabolites, being consequently involved in the biosynthesis of phenolic compounds.

Novel preservation technologies have emerged to maintain quality and bioactive/nutritional content of processed food guarantying always the food safety (Artés et al., 2009). HPP is a novel non-thermal technology which uses water as a medium to transmit pressures usually up to 800 MPa. One of the main advantages of HPP is the almost instantaneous isostatic pressure transmission to the product, independent of size, shape and food composition yielding highly homogeneous products. Food treated by HPP has been shown to keep almost its original bioactive/nutritional compounds, freshness, flavour, taste and colour changes are minimal (Patras et al., 2009b; Pereira and Vicente, 2010).

To the best of our knowledge, research on the effects of HPP on phenolic content and PAL activity of carrot smoothies is very scare. The main objective of the present study was evaluating physicochemical and microbial quality changes, as well as PAL

activity and TPC, of a carrot smoothie after different HPP treatments and 7 days of cold storage.

V.2. MATERIAL AND METHODS

V.2.1. Plant material and smoothie preparation

Carrots, butternut squash and oranges were bought in a local supermarket in Cartagena (Spain). Plant material was carefully inspected, selecting those with similar visual appearance and size. Minimally processing was performed in a disinfected cold room at 8 ºC at the Pilot Plant of the Universidad Politécnica de Cartagena. Carrots were topped and butternut squash was peeled. Subsequently, plant material was sanitized with cold chlorinated water (100 mg L^{-1} NaOCl; 5 ºC; pH 6.5±0.1) for 2 min, rinsed with tap water at 5 ºC for 1 min and drained in a perforated basket for 1 min. A ratio of 300 g plant material: 5 L chlorine was used.

Smoothie preparation was conducted with a food processor (Robot Cook®, Robot Coupe, Vincennes Cedex, France) in proportions of 350 g carrots, 200 g butternut squash and 500 mL orange juice (fresh squeezed). Approximately 80 g of smoothie were filled under aseptic conditions into a sterile squeeze PVC pouch (9 cm × 13 cm; 118 mL; Infantino, San Diego, USA) immediately after smoothie preparation. Subsequently, pouches filled with the smoothie were immediately treated in a HPP equipment (HP Pilot Food Processor, Stansted Fluid Power Ltd., Essex, U.K.) at 300, 400, 500 or 600 MPa (hereinafter T1, T2, T3 and T4, respectively) for 5 min at 23 ºC. Samples without processing were used as control (hereinafter CTRL). Sampling was conducted on processing day (0) and after 7 days at 5 ºC. Three replicates per treatment and sampling day were prepared.

V.2.2. Physicochemical analyses

The pH, TA, SSC and colour of smoothies were determined according to Castillejo et al. (2015). Briefly, a pH-meter (Basic20, Crison, Alella, Spain) was used to determine the pH. The SSC of the juice was determined by a digital hand-held refractometer (Atago N1, Tokyo, Japan) at 20 ºC and expressed as ºBrix. TA was determined by titration (0.1 M

NaOH to final pH 8.1) of 7 mL of smoothie plus 33 mL of distilled water and expressed as % (g malic acid 100 mL^{-1}). Colour of smoothies was determined using a colorimeter (Chroma Meter CR-300, Minolta, Japan). Colour measurements were recorded using the standard tristimulus parameters (*L**, *a**, *b**) of the *CIE Lab* system and Hue angle parameter was calculated. Each of the three replicates was analysed in duplicate.

V.2.3. Microbiological analyses

Total mesophilic load was evaluated using standard enumeration methods according to previously described method (Castillejo et al., 2015). All microbial counts were reported as log colony forming units per gram of product (log CFU g^{-1}). Each of the three replicates was analysed in duplicate.

V.2.4. Total phenolic content

TPC extraction was conducted by homogenization (Ultra Turrax®) of 2 g of sample in 8 mL methanol for 20 s under ice-water bath. Subsequently, extracts were centrifuged at 13,500 × g for 20 min at 4 ºC and supernatants were collected and used as TPC extract. TPC was analysed by Folin–Ciocalteu reagent method previously described with some modifications (Martínez-Hernández et al., 2013d; Swain and Hillis, 1959). Briefly, a 19 µL extract was placed on a PS flat bottom 96-well plate (Greiner Bio-One, Frickenhausen, Germany) and 29 µL of Folin–Ciocalteu reagent 1 N was added. Samples were incubated for 3 min in darkness at room temperature. After incubation, 192 µL of a of a buffer solution (37.7 mM Na_2CO_3; 0.5 M NaOH) was added and the reaction was carried out for 1 h at room temperature in darkness, measuring the absorbance at 750 nm using the Multiscan plate reader. TPC was expressed as mg chlorogenic acid equivalents (ChAE) kg^{-1} fw. Each of the three replicates was analysed in duplicate.

V.2.5. Phenylalanine ammonia-lyase activity

PAL activity was analysed according to previously described method with slight modifications (Jacobo-Velázquez et al., 2011). Concisely, smoothie samples (2 g) were mixed with polyvinylpolypyrrolidone (100 g L^{-1}) and homogenized in cold 50 mM borate

buffer (pH 8.5; 5 ºC) containing 400 µL L^{-1} β-mercaptoethanol. Homogenates were filtered through four-layers cheesecloth and then centrifuged at 10,000 × g for 20 min at 4 ºC. Supernatants were used as PAL extract. Two sets of UV Star 96-well plates containing 69 µL of PAL extract plus 200 µL ultrapure water were prepared for every sample and pre-incubated at 40 ºC for 5 min. Afterwards, 30 µL of either water (blank) or 100 mM L-phenylalanine substrate solution (freshly prepared before assay) were added to each of the well for every sample set. The absorbances of the sample sets were measured at 290 nm at time 0 and after 1 h of incubation at 40 ºC in the Multiscan plate reader. The PAL activity was calculated as µmol of t-cinnamic acid synthesized kg^{-1} fw h^{-1}.

V.2.6. Statistical analyses

A complete randomized design in triplicate, with two-way ANOVA (treatment × storage) was conducted. Statistical significance was assessed at the level p=0.05, and Tukey's multiple range test was used to separate means with SPSS software (v. 21, IBM, USA).

V.3. RESULTS AND DISCUSSION

Smoothie showed initial pH, TA and SSC of 4.48, 3.46 % and 11.1 ºBrix, respectively, which were decreased by 0.10-0.25 units without significant ($p < 0.05$) differences among treatments (Table V.1). Latter quality parameters of smoothies did not greatly change after 7 days of storage at 5 ºC.

Colour is one of the main characteristics that is strongly associated with the concept of quality (Wibowo et al., 2015). In general, HPP treatments did not induce great colour changes ($\Delta E < 10$) except T4 treatment which registered ΔE=42 mainly due to reduction of smoothie luminosity and redness (Table V.1). This might indicate an enhancement on colour compounds to lighter tones (López Camelo and Gómez, 2004) and consequently losses on red colour. In accordance to previous research (Melendez-Martinez et al., 2003), our smoothie belonged to ranged standards regarding L^* parameter for orange juices (58-60) after 7 days of storage. Although no significant ($p < 0.05$) colour changes were observed after 7 days of storage, T4 samples showed the highest ΔE of 48, comparing to

its initial level, while $\Delta E < 20$ were registered for the rest of treatments. Those values are in accordance to previous studies on orange juices (Meléndez-Martínez et al., 2011).

The initial mesophilic load of CTRL samples of 3 log CFU g^{-1} was greatly reduced (> 2 log units) after HPP treatments, without significant ($p < 0.05$) differences among them, with values close or below the detection limit (1 log CFU g^{-1}) (Table V.2). Total mesophilic loads of HPP-treated samples remained unchanged ($p < 0.05$) after 7 days at 5 ºC while it was increased by 4.1 log units in CTRL samples. Similar results have been observed in previous studies with different food matrix (Bull et al., 2004; Considine et al., 2008), indicating an enzymatic denaturation and disruption of microbial cell membranes (Yordanov and Angelova, 2010).

PAL regulates phenolic compounds as a response to metabolic pathways (Cisneros-Zevallos, 2003). CTRL samples initiated with 206.4 µmol cinnamic acid formed kg^{-1} h^{-1} which was reduced by 56-83 % after HPP treatments, except for T3 samples that showed 20 % increased PAL activity (Table V.2). PAL activity was also reduced after thermal treatment on red smoothies being explained by the low pH of the smoothie affecting to the signal for triggering this enzyme (Rodríguez-Verástegui et al., 2015). Contrary to initial PAL changes after HPP treatments, T1 smoothie registered a PAL activity increase of 4.5-fold after storage while T3 samples showed the highest reduction (71 %). PAL activity of CTRL samples was reduced by 88 % after storage similar to minimally processed carrots (Jacobo-Velázquez et al., 2011). T4 smoothies presented a steady behaviour with 99.2 µmol cinnamic acid formed kg^{-1} h^{-1} after 7 days of storage at 5 ºC. Similar PAL behaviour during storage was observed in fresh-cut carrot slices after microwave treatments (Martínez-Hernández et al., 2016a).

Phenolic compounds are one of major antioxidant constituents on orange vegetables (Kjeldsen et al., 2003). CTRL samples showed initial TPC of 712.1 mg ChAE kg fw^{-1} being reduced by 31, 14 and 7 % after T1, T2 and T3/T4 treatments, respectively (Table V.2). However, longer (15 min) HPP treatments (500 MPa and 600MPa) induced TPC increases in blackberry puree probably owed to a better extractability of phenolic acids (Patras et al., 2009a). TPC of CTRL/T1/T2 and T3/T4 samples increased by 1.5-1.6 and 1.1-1.3-fold, respectively. T2 samples registered the highest TPC after storage. The observed TPC enhancement after storage may be explained by a combined effect of: 1)

progressive higher phenolic extractability after storage due to further plant cells disruption during storage and 2) phenolic biosynthesis during storage.

V.4. CONCLUSIONS

In general, studied HPP treatments did not change the initial physiochemical quality which was well maintained after 7 days of storage at 5 ºC. HPP treatments greatly reduced initial microbial loads being kept during storage. HPP did not induce significant reductions on PAL activity and TPC content comparing to control smoothies. Smoothies treated with 400 MPa achieved the highest TPC after storage.

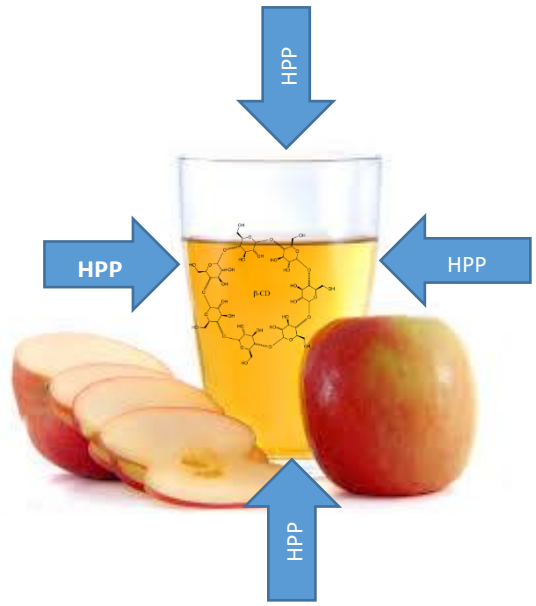
Table V.1. Soluble solids content (SSC), pH, titratable acidity (TA) and colour changes of orange smoothie after different high hydrostatic processing treatments and after 7 days of storage at 5 ºC.

Treatment	Processing day						
	SSC (ºBrix)	**pH**	**TA (%)**	***L****	***a****	***b****	**Δ*E***
CTRL	11.1	4.48	3.46	61.68	7.26	62.28	-
T1	11.3	4.33	3.20	62.73	7.62	63.09	0.9
T2	11.0	4.28	3.28	62.45	7.83	63.84	1.7
T3	11.0	4.28	3.29	62.63	8.63	66.32	9.6
T4	11.0	4.25	2.75	57.80	4.05	54.64	41.9
	7 days at 5 ºC						
	SSC (ºBrix)	**pH**	**TA (%)**	***L****	***a****	***b****	**Δ*E***
CTRL	12.1	4.33	3.14	64.53	7.61	61.56	4.4
T1	12.2	4.38	3.36	65.32	7.82	58.51	13.9
T2	13.0	4.38	3.72	63.64	6.34	58.57	15.7
T3	12.0	4.31	3.22	64.88	7.76	60.38	20.1
T4	12.4	4.41	3.05	64.79	7.32	60.70	48.1

Table V.2. Total mesophilic count (TMC), phenylalanine ammonia-lyase (PAL) and total phenolic content (TPC) of orange smoothie after different high hydrostatic processing treatments and after 7 days of storage at 5 ºC.

Treatment	Processing day		
	TMC*	**PAL*****	**TPC****
CTRL	3.4	206.4	712.1
T1	< 1	34.7	491.1
T2	1.3	91.2	609.0
T3	< 1	248.5	662.6
T4	1.2	71.4	662.1
	7 days at 5 ºC		
	TMC*	**PAL*****	**TPC****
CTRL	7.4	25.2	1032.6
T1	< 1	156.4	787.5
T2	1.4	1.0	914.6
T3	< 1	70.9	748.8
T4	1.4	99.2	851.6

*TMC expressed as log CFU g^{-1}; ** TPC expressed as mg chlorogenic acid equivalent kg^{-1} fw; ***PAL expressed as μmol of t-cinnamic acid synthesized kg^{-1} fw h^{-1}



CHAPTER VI

Browning control in high pressure-treated apple juice by maltosyl-*β*-cyclodextrin

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VI.1. INTRODUCTION

Apple juice is the most worldwide consumed fruit juice, together with orange juice, due to its excellent sensory attributes and health-promoting related properties derived from the phytochemicals present in this fruit (AIJN, 2014a; Hyson, 2011; USDA, 2013). Food safety and the inactivation of oxidizing enzymes, have been conventionally controlled by heat treatments but in detriment of the sensory quality of the product. Accordingly, novel sustainable preservation technologies have emerged to maintain quality and bioactive/nutritional content of processed food guarantying the food safety (Artés et al., 2009). HPP is a non-thermal technology which uses water as a medium to transmit pressures usually up to 800 MPa. One of the main advantages of HPP is the almost instantaneous isostatic pressure transmission to the product, independently of size, shape and food composition yielding highly homogeneous products. HPP treatments may achieve high inactivations of pathogenic bacteria as recently reviewed (Rendueles et al., 2011). Excellent microbial reductions have been achieved by HPP treatments in fruit and vegetables juices, with just mild treatments like 300 MPa for 5 min at 22 ºC (Formica-Oliveira et al., 2016b), while preserving, in a great content, its original bioactive/nutritional compounds, freshness, flavour and taste (Baron et al., 2006; Patras et al., 2009a). However, such mild-moderate HPP treatments at room temperature enhance the activity of quality degradative enzymes such as polyphenoloxidase (PPO), the main responsible of apple juice browning, as it is recently reviewed (Eisenmenger and Reyes-De-Corcuera, 2009). Accordingly, HPP treatment at 400 MPa for 5 min at 20 ºC induced a 65 % increase of the PPO activity in apple juice (Buckow et al., 2009). Reactivation of PPO and other enzymes has been also observed during shelf life of other HPP-treated products (Jacobo-Velazquez and Hernandez-Brenes, 2010). Furthermore, besides the evident sensory quality loss, application of high temperatures during HPP treatment has even shown an antagonistic effect between pressure and temperature on PPO inactivation in apple juice and other food products (Buckow et al., 2009; Ludikhuyze et al., 2002). Accordingly, there is a need to find combined treatments of mild HPP treatment (low pressures/temperature/time), which are economically affordable by the food industry, and antibrowning agents.

Several antibrowning agents such as sulfiting agents, reducing agents (ascorbic acid and analogues, glutathione, l-cysteine), enzyme inhibitors (aromatic carboxylic acids,

substituted resorcinols, anions, peptides), chelating agents (phosphates, EDTA, organic acids), acidulants (citric acid, phosphoric acid) and enzymes have been used to inhibit PPO activity in food products (compiled by Özoğlu and Bayındırlı (2002)). However, the actual consumer demands healthy food with natural ingredients free from additives. Cyclodextrins (CDs) are naturally occurring cyclic oligosaccharides from the bacterial digestion of starch, which have been studied as natural antibrowning agents in fruit and vegetable juices (Hicks et al., 1996; Iyengar and McEvily, 1992). They are cylindrically shaped molecules with a cavity of hydrophilic outer surface, and hydrophobic internal surface which is able to form inclusion complexes with PPO substrates (Kalogeropoulos et al., 2010). Among CDs, maltosyl-*β*-CD (m*β*CD) has shown excellent antibrowning effect and solubility, while being safe even when administered parenterally contrary to parent *β*-CD (Del Valle, 2004; López-Nicolás et al., 2007a). Enzymatic browning in apple juice has been satisfactorily observed and characterized by colorimetric methods (López-Nicolás et al., 2007a; López-Nicolás et al., 2007b). To the best of our knowledge, there are no studies on application of CDs with HPP on fruit and/or vegetable juices.

The aim of this study was the study of enzymatic browning in apple juice treated with m*β*CD after HPP treatments.

VI.2. MATERIAL AND METHODS

VI.2.1. Plant material and juice preparation

Apples (*Malus domestica* cv. Royal Gala) were purchased from a local supermarket in February and stored at 5 ºC until the next day when they were processed. Apple juicing was conducted with a juicer (MP75, Braun, Germany). Immediately after juicing, 25 mL of the apple juice were introduced in a 150 mL plastic squeeze pouch (Fill *n* Squeeze, Lucro Liquido Ltd, Huddersfield, UK) and m*β*CD and HPP treatments were immediately applied within the first minute after juicing. Accordingly, minute 1 was considered as time 0.

VI.2.2. Maltosyl-*β*-cyclodextrin and HPP treatments

Maltosyl-*β*-CD (Alfa Chemistry, Stony Brook NY, USA) was pre-dissolved in 5 mL of nanopure water and added to the 25 mL of pre-packaged apple juice, giving a final concentration of 90 mM (according to López-Nicolás et al. (2007a)), capped and homogenized by hand for 5 s. As control (CTRL), 5 mL of nanopure water was used.

HPP treatments were conducted immediately after processing with a HPP device (HP Pilot Food Processor, Stansted Fluid Power Ltd., Essex, UK) at 0, 300 and 600 MPa for 5 min at 22 ºC. HPP treatments were applied to CTRL (CTRL-HPP0, CTRL-HPP300 and CTRL-HPP600) and m*β*CD samples (m*β*CD-HPP0, m*β*CD-HPP300 and m*β*CD-HPP600). Colour changes of samples were measured for 60 min at 22 ºC after preparation.

VI.2.3. Colour changes

Colour changes were determined using a colorimeter (Chroma Meter CR-300, Minolta, Japan) calibrated with a white reference plate (light source C), 2º observer and 8-mm viewing aperture. Samples were introduced in a special glass tube mounted on a device connected to the colorimeter. Three colour readings were taken turning the tube every caption and all three measurements were automatically averaged by the device and recorded. Measurements were recorded using the standard tristimulus parameters (L^*, a^*, b^*) of the *CIE Lab* system.

Browning is the main colour degradation process occurred in apple juice. Accordingly, *BI* was calculated from *CIE Lab* parameters according to equation (VI.1) as previously described (Palou et al., 1999).

$$BI = \frac{100 \times \left[\left[\frac{[a^{*2}+(1.75 \times L^*)]}{[(5.645 \times L^*)+a^{*2}-(3.012 \times b^*)]}\right]-0.31\right]}{0.172} \quad \text{(VI.1)}$$

Complimentary, ΔE is a colorimetric parameter extensively used to characterize the variation of colours during processing and storage of food products (Lante and Zocca, 2010; Martínez-Hernández et al., 2013a). ΔE was calculated according to equation (VI.2) as previously described (Walkling-Ribeiro et al., 2010).

$$\Delta E = \sqrt{(L^* - L^*{}_0)^2 + (a^* - a^*{}_0)^2 + (b^* - b^*{}_0)^2} \quad \text{(VI.2)}$$

VI.2.4. Mathematical modelling of colour changes

Experimental data were fitted to a fractional conversion model according to equation (VI.3) as previously proposed by Soliva-Fortuny et al. (2001) and López-Nicolás et al. (2007a) by nonlinear regression procedures of the Statgraphics Plus (version 5.1) software.

$$e^{-k\,t} = \frac{(\Delta E_f - \Delta E)}{(\Delta E_f)} \qquad \text{(VI.3)}$$

where ΔE is current value of total colour difference, ΔE_f is the non-zero value of the parameter upon prolonged time, t is the time after treatments and k is the first-order kinetic constant.

VI.3. RESULTS AND DISCUSSION

VI.3.1. Colour changes after HPP treatments

Colorimetric studies are considered as a satisfactory method for observing and characterizing enzymatic browning in apple juice (López-Nicolás et al., 2007a; López-Nicolás et al., 2007b). ΔE is a colorimetric parameter extensively used to characterize the variation of colours during processing and storage of food products. BI represents the purity of brown colour and is reported as an important parameter in processes where enzymatic or non-enzymatic browning take place (Palou et al., 1999). Colour data were registered in this experiment for the first hour after juicing since browning mainly occurs in this initial period of time (López-Nicolás et al., 2007a). Furthermore, 83 % of browning have been registered within the first hour in some other foods after long incubation periods (Cheng and Crisosto, 1995). Data modelling provides important kinetic parameters to clarify and predict quality changes in these kind of products, which is still one of the most challenging goals for food processors. Colour changes of apple juice were well modelled with the previous model proposed by Soliva-Fortuny et al. (2001) to study the influence of enzymatic browning on the colour parameters of fresh-cut apples in several conditions. Colour data were well fitted with this model with root-mean-square error (RMSE) of 0.15-1.28 (Table VI.1). Among HPP treatments, no relevant RMSE differences were observed among treatments. In general, CTRL-HPP0 and CTRL-HPP600 showed similar colour kinetic behaviour with an initial exponential high colour

change followed by a lag phase (Figures VI.1a and VI.2a). However, CTRL-HPP300 showed a lower exponential colour change but it was constant in the period that data were registered being not clearly observed a lag phase as for CTRL-HPP0 and CTRL-HPP600 samples (Figures VI.1a and VI.2a). Exponential colour changes were faster in CTRL samples showing after 14 min similar lag phase as that reached by CTRL-HPP600 only after 24 min. Accordingly, CTRL-HPP300 showed the lowest *k* values followed by CTRL-HPP600 and CTRL-HPP0 (Table VI.1). The kinetic colour change behaviour observed in these samples could be explained by high PPO and POD activities in the exponential phase which were highly reduced in the lag phase when free substrates contents were used reaching minimum levels. Such initial colour degradation, retarded in HPP samples, was owed to HPP enzymatic inactivation. Accordingly, PPO inactivation after several HPP treatments (0.1-700 MPa; 20-60 ºC; up to 30 min) has been previously modelled in apple juice (Buckow et al., 2009). The faster colour degradation observed in CTRL-HPP600 compared to CTRL-HPP300 samples could be owed to a higher accessibility of phenolic compounds for PPO and POD enzymes due to plant cell disruption achieved by such high pressure. However, CTRL-HPP300 treatment induced lower phenolic extractability while applied pressure was enough to reduce PPO and/or POD activities. Nevertheless, the low colour degradation in CTRL-HPP300 was constant for the registered time leading to higher colour changes after long time periods. Furthermore, a higher PPO and/or POD activation could occur under such low pressure. Accordingly, CTRL-HPP300 juice showed darker colour after 90 min regarding CTRL-HPP600 and CTRL-HPP0 (Figure VI.3). HPP treatment is a non-thermal technology, which highly control microbial growth in several food products although there is much evidence about their limitations for fruit and vegetables juices susceptible to browning, being apple juice the main challenging goal for food processors. Accordingly, enzymatic activation in fruit and vegetables juices after HPP treatments has been recently reviewed (Eisenmenger and Reyes-De-Corcuera, 2009). Latter authors stated three possible hypotheses for this fact: (1) reversibility in enzyme conformation or rearrangement of the active sites facilitating the enzymatic reaction; (2) changes in substrate or media properties; or (3) favouring the equilibrium toward enzyme release against inhibition from their conjugated complex.

Colour degradation of apple juice is mainly due to browning (Nicolas et al., 1994; Pathare et al., 2013). Attending to colour parameters studied, all of them could be considered as

good browning indicators in apple juice since a similar kinetic behaviour was observed for all treatments with such colour parameters. The used model was better adjusted for ΔE with RMSE values 0.10-0.40 units lower compared to BI (Table VI.1). However, modelled *BI* data still reached low values which ensured a good data fit to this model.

VI.3.2. Colour changes after HPP treatments using maltosyl-β-cyclodextrin

Colour changes of apple juice treated with m*β*CD are presented in Figures VI.1b and VI.2b. Data of m*β*CD-treated samples were also well fitted in the applied model with RMSE of 0,15-0,54 (Table VI.1). Colour changes were highly reduced by m*β*CD treatment as observed in Figure VI.4. m*β*CD-HPP300 showed the lowest colour changes while m*β*CD-HPP600 registered the highest colour differences among m*β*CD-treated samples (Figure VI.1b, VI.2b, VI.4). Contrary to CTRL-HPP300, colour was very well preserved in m*β*CD-HPP300 samples registering the lowest browning. As previously commented, HPP600 treatment could lead to higher extractability of phenolic compounds being more available for PPO and/or POD enzymes unless previous CD treatment of those samples highly formed inclusion complexes with such free phenolic compounds leading to the observed low colour changes. The excellent visual quality of m*β*CD-HPP300 could be owed to a low phenolic extraction and consequently better efficiency in the formation of inclusion complexes with the available free phenolics compounds. Furthermore, the formation of inclusion complexes of m*β*CD with PPO and/or POD in apple juice has been previously modelled according to colour data (López-Nicolás et al., 2007a). *β*-CD has been the most commercially successful of the CD family, while *α*- and *γ*-CD have considerably smaller markets, although its use in the food industry is limited due to its low solubility in aqueous medium (Martina and Cravotto, 2015). The substitution of the hydroxyl groups of CD causes dramatic improvements in aqueous solubility and guest specificity, particularly in the case of *β*-CD providing the maltosyl substitution group a more intense bonding with better stabilization of the inclusion (Acarturk et al., 1993; López-Nicolás et al., 1995; Veiga et al., 2002). Accordingly, (López-Nicolás et al. (2007a)) reported lower colour changes with m*β*CD compared to the unmodified *β*-CD showing 90 mM excellent control of colour changes as it was corroborated in our experiments.

As hereby observed, HPP treatments may not modify the inclusion complexes formed leading to the observed low colour changes in the apple juice. Further research must be conducted in order to study the shelf life of the juice which may be much longer under low temperature storage.

VI.4. CONCLUSIONS

HPP is a non-thermal technology able to reduce microbial loads under moderate pressures although enzymatic browning may be considerably enhanced in apple juice. Colour degradation of apple juice treated at low-moderate and high pressure was well modelled with a fractional conversion model with RMSE < 1.3. The addition of maltosyl-*β*-cyclodextrin (90 mM) highly controlled enzymatic browning in HPP treated (0, 300 and 600 MPa for 5 min at 22 ºC) apple juice during 1 h at room temperature. Accordingly, the addition of maltosyl-*β*-cyclodextrin to apple juice, prior to a HPP treatment at 300 MPa for 5 min at 22 ºC, is presented as an excellent combined treatment for apple juice to control enzymatic browning.

Table VI.1. Parameter values of the fractional conversion model used to describe the enzymatic browning in apple juice after maltosyl-*β*-cyclodextrin (m*β*CD) and high pressure processing treatments (0, HPP0; 300, HPP300; and 600 MPa, HPP600 for 5 min at 22 ºC) during 60 min at 22 ºC.

		ΔE		ΔBI	
		k	RMSE	*k*	RMSE
CTRL	HPP0	0.1361	0.86	0.1125	1.28
	HPP300	0.0294	1.22	0.0343	1.15
	HPP600	0.0698	0.75	0.0555	1.00
mβ-CD	HPP0	0.0865	0.29	0.0292	0.44
	HPP300	0.2093	0.15	0.0973	0.38
	HPP600	0.0587	0.22	0.0480	0.54

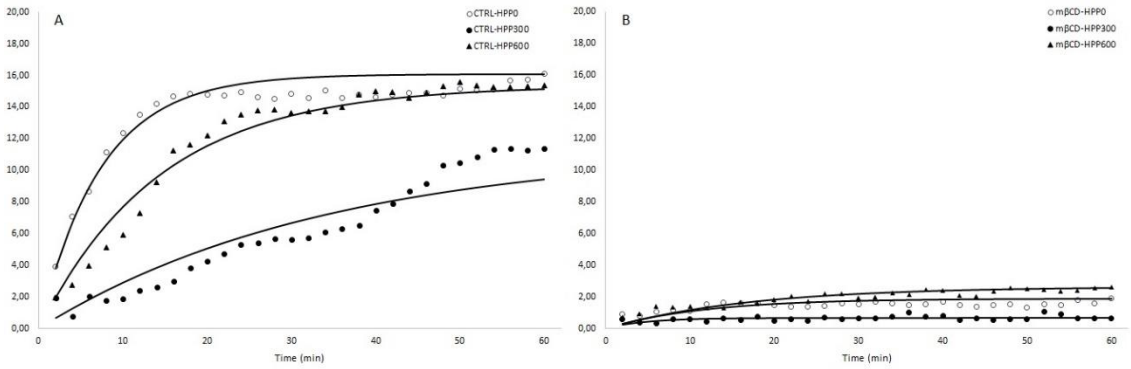


Figure VI.1. Evolution of total colour differences (Δ*E*) in control (CTRL; A) and maltosyl-*β*-cyclodextrin (m*β*CD; B) apple juice after high pressure processing treatments (0, HPP0; 300, HPP300; and 600 MPa, HPP600 for 5 min at 22 ºC) during 60 min at 22 ºC. Points represent experimental data (n=3). Lines represent fitted data.

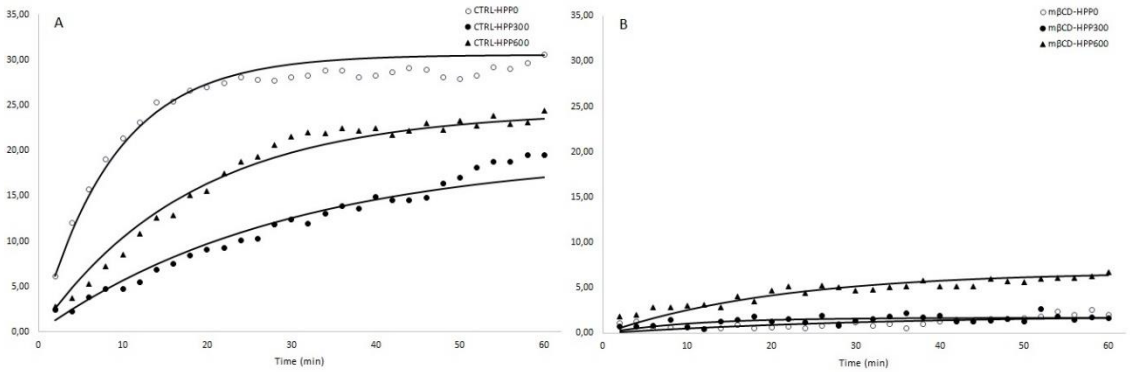


Figure VI.2. Evolution of browning index differences (Δ*BI*) in control (CTRL; A) and maltosyl-*β*-cyclodextrin (m*β*CD; B) apple juice after high pressure processing treatments (0, HPP0; 300, HPP300; and 600 MPa, HPP600 for 5 min at 22 ºC) during 60 min at 22 ºC. Points represent experimental data (n=3). Lines represent fitted data.

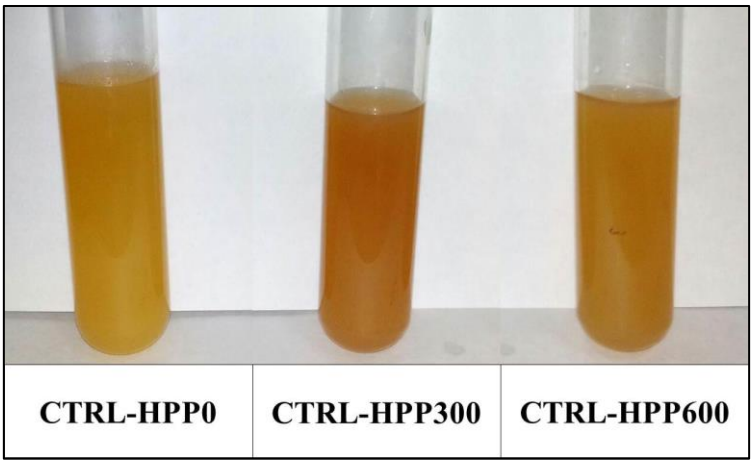


Figure VI.3. Apple juice treated with different high pressure processing treatments (0, HPP0; 300, HPP300; and 600 MPa, HPP600 for 5 min at 22 ºC) after 90 min at 22 ºC.

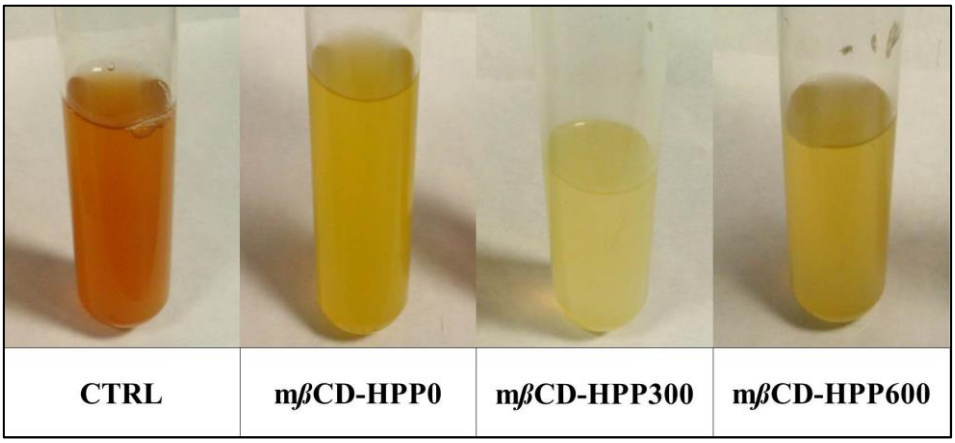
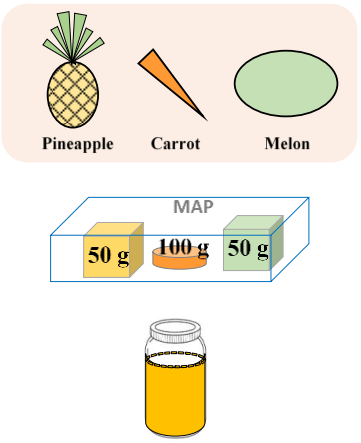


Figure VI.4. Apple juice pre-treated with maltosyl-*β*-cyclodextrin (m*β*CD) and different high pressure processing treatments (0, HPP0; 300, HPP300; and 600 MPa, HPP600 for 5 min at 22 ºC) after 90 min at 22 ºC.



CHAPTER VII

Improved quality of a vitamin B12-fortified 'ready-to-blend' fresh-cut mix salad with chitosan

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VII.1. INTRODUCTION

Fruit and vegetables have a high content of phytochemicals like polyphenols, vitamins, carotenoids, etc. (Sánchez-Moreno, 2002) which have been proved to prevent a grand array of diseases, such as degenerative disorders, cancer and cardiovascular diseases among others (Slavin and Lloyd, 2012). Nevertheless, fruit and vegetables worldwide consumption is below the recommended daily intake (WHO/FAO, 2003). Accordingly, fruit and vegetables intake may be enhanced through the development of more attractive and ready/easy-to-eat products such as blended beverages. Nevertheless, microbial growth and enzymatic/non-enzymatic degradative reactions of fruit and vegetables blended beverages are highly enhanced during storage leading to limited shelf life (Cheftel, 1995). Blended fruit and vegetables beverage may still have a high plant cell viability with consequent physiological behaviour. Furthermore, several processing treatments and/or food preservatives of blended beverages are avoided in order to preserve their sensory quality. On the other side, fresh-cut fruit and vegetables have higher shelf life, compared to untreated related beverages, due to techniques such as MAP and edible coatings, among others (Artés et al., 2009). In this sense, the new product concept 'ready-to-blend' (RB) fruit and vegetables, firstly reported here, may combine the 'long' shelf life of a fresh-cut product with the convenient consumption of a beverage. A RB product may be defined as a fresh-cut (which implies correspondent plant material preparation, sanitizing treatments and MAP combined with low storage temperature) fruit, vegetable, or a mix of them, which is packaged under specific proportions ready for domestic blending.

Chitosan, a deacetylated form of chitin, is a natural product with excellent antimicrobial properties and high potential to be used within the edible coatings of fresh-cut products to increase their shelf life (Wang et al., 2007). The most feasible hypothesis about the antimicrobial activity of chitosan is a change in cell permeability due to interactions between the polycationic chitosan and the electronegative charges on the cell surfaces. This interaction leads to the leakage of intracellular electrolytes and proteinaceous constituents leading to microbial cell death (Devlieghere et al., 2004). Vitamin B12 deficiency may lead to serious health consequences such as impair the utilization of folate, neurological deterioration, megaloblastic anaemia, elevated plasma homocysteine and possibly, impaired immune function. It is mainly found in animal products so there

is a need to be supplemented in specific populations groups such as vegetarians/vegans, elderly, etc. Accordingly, several studies, mainly from industrialized nations, have demonstrated the benefits of vitamin B12 supplementation in such susceptible population groups through fortified products (Allen et al., 2006; Molina et al., 2012). 'Fortification' or 'enrichment', is the 'addition of one or more essential nutrients to a food whether or not it is normally contained in it, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups' (Commission, 2015). Hence, a RB product fortified with vitamin B12 may be an excellent solution with good microbial and nutritional/bioactive quality which ensures the daily vitamin B12 recommended intakes.

The objective of this work was to investigate the effect of a chitosan coating fortified with B12 on a RB fruit/vegetable mix stored at 5 ºC on the physicochemical, microbial and bioactive quality of beverages prepared on different blending days (BD: 0, 4, 7 and 9 d). Moreover, quality changes of such beverages were assayed during a subsequent storage at 5 ºC for up to 48 h (beverage sampling times: 0 h, 24 h and 48 h) simulating a domestic storage of an eventual beverage leftover from a blending day. Quality parameters of the RB fresh-cut fruit/vegetables mix were studied on the prepared beverage in order to provide data of the product really ingested by the consumer.

VII.2. MATERIAL AND METHODS

VII.2.1. Plant material, preparation, treatments and storage conditions

Pineapple (*Ananas comosus* cv. Smooth cayenne), melon (*Cucumis melo* cv. Piel de sapo) and carrots (cvs. group Nantes, cv. Soprano) were obtained from a local supermarket (Cartagena, Spain) and stored at 5 ºC and 90–95 % RH until the next day, when they were processed. Minimal processing was accomplished in a disinfected cold room at 10 ºC. Plant material was previously pre-washed with cold tap water (1 min; 5 ºC). Subsequently, pineapples and melons were peeled and the core was removed. Carrots were topped. Then, plant material was washed in chlorinated cold water (100 mg L^{-1}; 5 ºC; pH 6.5; 2 min; 300 g plant material:5 L chlorinated water), rinsed with tap water (1 min; 5 ºC) and drained in a perforated basket. Sanitized melons and pineapples were cut into 2 × 2 × 2 cm cubes while carrots were cut into slices of 8 mm thickness using a

manual slicer. The cutting material was previously sharpened and disinfected with 700 g L^{-1} ethanol. Subsequently, plant material was submerged in the following coating treatments for 1 min:

- CTRL: acidified water with acetic acid to pH=5.1. Such pH was selected in order avoid interferences due to acidic conditions of latter treatments.
- Chitosan (CH): The CH coating solution contained 10 g L^{-1} of chitosan (75–85 % deacetylated; medium molecular weight), 10 mL L^{-1} of potato starch, 10 mL L^{-1} of glycerol and 5 mL L^{-1} of acetic acid (Durango et al., 2006). The final pH of CH coating treatment was 5.1.
- Chitosan + vitamin B12 (CH+B12): CH coating solution was enriched with 25 mg L^{-1} vitamin B12 supplement powder (cyanocobalamin 1 %; Bulk suppkements.com, Henderson, USA). The final pH of CH+B12 coating treatment was 5.1.

Coated plant material was subsequently dried under forced air into a cleanroom (5 ºC, 90 % RH) for 2 h. Then, approximately 50 g of coated pineapple cubes, 100 g of coated carrot slices and 50 g of coated melon cubes were weighed into a rectangular basket (170 mm × 120 mm × 60 mm). Such fresh-cut fruit/vegetable proportions per each RB basket were determined based on preliminary informal sensory tests in order to obtain an orange fresh blended beverage using 200 mL of water. RB baskets were then thermally sealed on the top with a bi-oriented two-layer PP: PE film of 25+25 µm thickness (Plásticos del Segura, Murcia, Spain) in order to generate a MAP. O_2 transmission rate (TR) and CO_2 TR at 23 ºC and 0 % RH was similar with 500 $cm^3\ m^{-2}\ d^{-1}\ atm^{-1}$ and water vapour TR at 23 ºC and 85 % RH was 0.5 g $m^{-2}\ d^{-1}\ atm^{-1}$ (data provided by the supplier). Baskets were stored at 5 ºC (90-95 % RH) in darkness after 0 (BD0), 4 (BD4), 7 (BD7) and 9 d (BD9). Five replicates per treatment and BD were prepared.

Beverages were prepared using all the product contained in a RB basket, with addition of 200 mL of still mineral water, with a food processor (3.7 L Robot Cook®, Robot Coupe, Montceau-en-Bourgogne, France) for 1.5 min at 1,450 × g followed by 0.5 min at 3,260 × g. Every RB fruit/vegetables basket resulted in three beverage portions of approximately 140 mL each. Domestic storage of beverages, prepared at different BD, was simulated by introducing every beverage portion (140 mL) in a 150-mL transparent glass bottle and stored up to 48 h at 5 ºC in darkness using each of the three prepared

beverages for a different beverage sampling time: 0 h, 24 h and 48 h. The experiment layout is described in Figure VII.1 for better clarification.

VII.2.2. Respiration rate and gas analysis within modified atmosphere packages

The respiration rate (RR) of each fresh-cut RB fruit/vegetables portion was determined using a closed system as previously described (Martínez-Hernández et al., 2011). Three replicates per treatment were placed within 750 mL glass jars at 5 ºC up to 9 days. The increases in CO_2 were monitored after closing the jars for 2 h. Headspace gas samples (1 mL) were withdrawn from the jars with a gas-tight syringe and analysed in a gas chromatograph (GC; PerkinElmer Precisely Clarus 500, Massachusetts, USA). Three replicates were made from each jar every evaluation day.

The gas composition (O_2 and CO_2) within RB packages was also monitored throughout storage at 5 ºC. Headspace gas samples (1 mL) were withdrawn and analysed in the GC described above from three replicates for each treatment and evaluation period. Three replicates per basket were analysed every sampling day prior to beverage preparation.

VII.2.3. Cell viability of prepared beverages

Cell viability in the fresh blended beverage was determined according to the stain 2,3,5-triphenyltetrazolium chloride (TTC) as previously described (Silva et al., 2012). Briefly, 600 mg of the beverage was mixed with 18 mL of TTC [prepared at 6 g L^{-1} in 50 mmol L^{-1} phosphate buffer (pH 7.4)] and allowed to incubate for 24 h at 28 ºC in darkness. Subsequently, 42 mL of ethanol 95 % (v/v) were added. Formed formazan was extracted by incubating samples at 100ºC for 4 min followed by centrifugation at 6,000 × g for 20 min at 20 ºC. Absorbance of samples was measured at 490 nm using an UV-visible spectrophotometer (Hewlett Packard, model 8453, Columbia, USA).

VII.2.4. Physiochemical quality

Physicochemical quality was determined based on pH, TA, SSC and *CIE Lab* colour parameters determined with the same equipment and methodology described by Castillejo

et al. (2016a). Total colour differences (ΔE) throughout storage, compared to their respective initial values, were calculated as previously described (Tontul et al., 2016).

VII.2.5. Microbial quality

Epiphytic microflora (mesophilic, psychrophilic, *Enterobacteriaceae*, lactobacilli (LAB) and yeast and mould (Y+M)) growth was determined using standard enumeration methods according to Martínez-Hernández et al. (2013a). All microbial counts were reported as log colony forming units per gram of product (log CFU g^{-1}). Each of the five replicates was analysed in duplicate. The presence of *Salmonella* spp., *L. monocytogenes* and generic *E. coli* was monitored according to the European legislation (Regulation EC 1441/2007, 2007).

VII.2.6. Enzymatic analyses

VII.2.6.1. Polyphenoloxidase and peroxidase

PPO and POD extraction and analysis were conducted according to Rodríguez-Verástegui et al. (2015). PPO and POD activities ($\Delta A min^{-1}$) were estimated by the initial velocity method from the linear portion of the curves. One enzyme unit of activity (U) refers to the increase in absorbance of 1 min^{-1}. PPO and POD activities were expressed as U g^{-1} fw. Each of the five replicates was analysed by duplicate.

VII.2.6.2. Pectin methylesterase

PME extraction and analysis was conducted according to Cameron et al. (1992) with modifications. Briefly, a beverage sample of 2 g was homogenized (UltraTurrax®) with 3.5 mL of cold NaCl buffer (88 g L^{-1}) containing 31.4 g L^{-1} polyvinylpolypyrrolidone. pH of homogenate was adjusted to 7.5 with NaOH. Subsequently, the homogenate was filtered (four-layer cheesecloth) and centrifuged at 15,000 × g for 20 min at 4 ºC. The supernatant was collected and used as PME extract. PME was analysed by mixing 40 µL of enzyme extract with 150 µL of pectin (5 g L^{-1}; pH 7.5) and 15 µL bromothymol blue [250 mg L^{-1} prepared in phosphate buffer 3 mmol L^{-1} (pH 7,5)] in a PS flat-bottom 96-well plate. The increase in absorbance at 620 nm at 25 ºC for 20 min with the Multiscan

plate reader. PME activity was estimated and expressed as explained for PPO and POD. Each of the five replicates was analysed by duplicate.

VII.2.7. Vitamin B12

The vitamin B12 content of beverages was determined with a commercial kit (RIDASCREEN®FAST Vitamin B_{12}, R-Biopharm, Darmstadt, Germany). Vitamin B12 extraction was conducted by homogenization (Ultra Turrax®) for 5 s of 1 mL of beverage and 4 mL of phosphate buffered saline buffer (supplied by the kit). Then, samples were heated at 100 ºC in a water bath for 3 min and immediately cooled to room temperature with an ice-water bath. Subsequently, samples were centrifuged (4,000 × g for 10 min at 15 ºC). Ascorbic acid was removed from the supernatant using an ascorbate oxidase spatula (Roche Diagnostics GmbH, Mannheim, Germany) in order to avoid interferences with the kit. Finally, latter supernatant was used as vitamin B12 extract and analysis was conducted according to the commercial kit protocol. The results were expressed as µg kg^{-1} fw. Each of the five replicates was analysed by duplicate.

VII.2.8. Total phenolic content and antioxidant capacity

Extraction to determine TPC and TAC was conducted according to Rodríguez-Verástegui et al. (2015) but with slight modifications. Briefly, a beverage samples of 2 g was homogenized (UltraTurrax®) in 8 mL methanol. Subsequently, supernatant from centrifuged extract (13,500 × g, 20 min, 4 ºC) was collected and used as TPC and TAC extract. TPC and TAC were determined as previously described (Martínez-Hernández et al., 2013a). TPC was expressed as chlorogenic acid equivalents (ChAE) in mg kg^{-1} fw. TAC was expressed as mg Trolox kg^{-1} fw. Each of the five replicates was analysed in duplicate.

VII.2.9. Statistical Analysis

The experiment was a two-factor (treatment × storage time) design subjected to analysis of variance (ANOVA) using Statgraphics Plus software (vs. 5.1, Statpoint Technologies Inc., Warrenton, USA). Statistical significance was assessed at the level $p = 0.05$, and Tukey's multiple range test was used to separate means.

VII.3. Results and discussion

VII.3.1. Respiration rate

The RB fruit/vegetable mix unit showed an initial RR of 43.9-61.5 nmol CO_2 kg^{-1} s^{-1} (Figure VII.2). There were no significant ($p < 0.05$) RR differences among treatments throughout cold storage. RR of samples increased through storage registering a first great RR increment on day 3 although the maximum RR values were observed on day 6 with levels of 389.4-465.3 nmol CO_2 kg^{-1} s^{-1}. After latter respiration maximum, RR decreased to similar levels registered on days 1-2. Increased respiration after some storage period in non-climacteric tissues may be caused by the onset of decay by microorganisms. Furthermore, wounding during minimal processing of fresh-cut products induces elevated C_2H_4 production rates that may stimulate respiration and consequently accelerate deterioration and senescence in vegetative tissues (Fonseca et al., 2002). The observed RR behaviour is in accordance to microbial growth monitored after 4-7 days of storage, as shown later, and the delayed, due to the low storage temperature, wounding-induced abiotic response of the plant tissue. Such maximum RR is also in accordance to the reduction trend of SSC and TA, directly related to carbohydrates and organic acids consumption respectively, during aerobic respiration as observed in samples on day 7 (see microbial data). This RR behaviour has been reported in other non-climateric fresh-cut fruit and vegetables as widely reported and reviewed (Fonseca et al., 2002).

Although no significant ($p < 0.05$) RR differences were found between coated and uncoated samples throughout storage, a lower RR trend was observed in uncoated samples compared to coated ones. Edible coatings are used as a protective barrier to reduce RR which is attributed to a modification of the internal gas atmosphere of the fruit and vegetables surface. In this sense, chitosan coating is likely to modify the internal gas atmosphere of the plant product without observed anaerobic respiration since chitosan coatings are more selectively permeable to O_2 than to CO_2 (Bai et al., 1988). Accordingly, RR reductions have been observed in fresh-cut products coated with chitosan (Hernández-Muñoz et al., 2008; Kittur et al., 2001; Valenzuela et al., 2015).

VII.3.2. Modified atmosphere

The gas composition within the RB fruit/vegetable mix portion during the passive MAP generation is shown in Figure VII.3. According to RR data, no significant ($p < 0.05$) gas partial pressure differences among coated or uncoated samples were observed throughout storage. As expected, CO_2 partial pressures increased and O_2 decreased throughout passive MAP generation starting to reach the equilibrium state from days 4-5 until the end of storage with O_2/CO_2 ranges of 3-5/13-17 kPa. Latter gas equilibrium is in accordance to the recommended gas partial pressures in MAP of fresh-cut carrot, pineapple and melon, combined with low storage temperature and high RH, to extend their shelf lives (Cantwell and Suslow, 2002b).

VII.3.3. Cell viability of fresh blended beverages prepared from fresh-cut samples

Tetrazolium salts such as TTC are well-known compounds with high redox potentials which can be inserted into the mitochondrial respiratory chain and are often reduced to deeply coloured formazans. In this sense, they have been frequently used to determine cell viability with the advantage over other methods that the amount of the end product (formazan) can be determined spectrophotometrically. Accordingly, TTC method is a more objective method when compared to counting individual cells, which are frequently difficult to distinguish by microscopic observation, after vital staining (i.e., fluorescein 3,6-diacetate; FDA). Fresh prepared beverage showed an absorbance of 0.25 at 490 nm. According to Silva et al. (2012), TTC absorbance value of 0.25 approximately corresponds to 40 % of viable cells. The high viable cell percentage includes such beverages in the postharvest physiology area contrary to other fruit/vegetables beverages which are supplemented with ingredients/processing coadjutants and are frequently treated (thermal or non-thermal treatments) leading to high, or total, reduction of the viability of their plant cells. However, further investigations need to be conducted to deeply study the changes in the viable cell percentages of the beverages depending of the blending mode/device, time and speed, being correlated to other studied physical (particle size, viscosity, etc.) and physiological properties (RR, etc.).

VII.3.4. Physicochemical quality

The physicochemical quality (SSC, pH and TA) of beverages is presented in Figure VII.4. Accordingly, samples had initial SSC, pH and TA of 4.9 %, 4.8 and 0.182 %, respectively, on BD0-0h (blending day 0-beverage storage 0 h). Attending to treatments, beverages from coated samples showed SSC and TA values of 0.2-0.3 and 0.043-0.067 units lower, respectively, and related 0.2 pH units increment compared to beverages from uncoated samples on BD0-0h. Chitosan with a partial positive charge has been shown to possess acid-binding properties and to be effective in aiding the separation of colloidal and dispersed particles from food processing wastes (No et al., 2007). Latter chitosan properties may explain the reduced TA and SSC values according to similar observations reported in chitosan-treated carrot and apple juice (Imeri and Knorr, 1988). In general, no significant ($p < 0.05$) differences on *CIE Lab* colour parameters were observed between beverages from uncoated or coated RB samples on BD0-0h with initial values of luminosity (L^*)=41.6-42.3, redness (a^*)=14.1-14.9 and yellowness (b^*)=31.3-33.7. The vitamin B12 fortification of CH+B12 treatment did not significantly ($p < 0.05$) affect the physicochemical parameters of DB0-0h samples regarding CH coating. Similarly, chitosan (89.9 % deacylation) coatings enriched with higher quantities of vitamin E (2 g L^{-1}) did not induce initial pH and TA changes on strawberries (Han et al., 2004).

Attending to physicochemical differences between blending days, no high SSC and pH variations were observed after 9 d of storage (Figure VII.4). Colour differences of samples increased according to storage time (Table VII.1). Hence, BD4-0h, BD7-0h and BD9-0h samples showed ΔE mean values of 1.3, 6.3 and 11.0, respectively, compared to their respective values on BD0-0h. Latter colour differences throughout storage were mainly due to b^* and L^* increases ranging from 4 to 9 ΔE increment units after 9 days. Similar changes have been previously reported in fresh-cut pineapples, carrots and melons (Martínez-Hernández et al., 2016a; Montero-Calderón et al., 2008; Saftner et al., 2003). No significant ($p < 0.05$) colour differences between treatments were observed on BD4-0h, BD4-0h and BD7-0h samples. However, CH beverages showed slightly higher L^* and b^* values after 48 h compared to CTRL and CH+B12 samples which were similar ($p < 0.05$) among them. Similar colour protecting effects, related to lower water losses with chitosan coatings, have been observed in several fruits as previously reviewed (No et al., 2007). However, such colour protecting effect was not observed in CH+B12 samples

probably since negatively charged vitamins added into the chitosan coating solutions might absorb, to some extent, chitosan molecules (Koide, 1998), thus reducing the colour stabilization ability of this coating (Han et al., 2004). Attending to TA, the reduction of 0.087 units observed in CTRL samples after 9 days was approximately 2-fold reduced in coated samples with no influence of vitamin B12 fortification. Similarly, Hernández-Muñoz et al. (2008) reported lower TA reductions in strawberries coated with chitosan (high molecular weight) compared to uncoated samples owed to a greater water loss by uncoated samples since TA is given as a percentage of citric acid per sample wet weight.

The physicochemical quality of beverages was not highly changed during subsequent storage up to 48 h at 5 ºC. Accordingly, SSC was not significantly ($p < 0.05$) changed after 48 h of storage for any of the beverages prepared from the RB samples stored up to 9 days. Similarly, pH changes lower than 0.25 units were observed for all beverages after 48 h. In general, TA was only slightly (< 0.012 units) changed after 48 h in all beverages prepared from 9 d-RB samples. TA of beverages prepared from 9 d-samples remained unchanged after 24 h, although it increased by 0.058 units after 48 h. The TA increase of latter samples is in accord to microbial growth observed in those samples after 9 days (as shown later). Colour of beverages prepared from 0-7 d RB samples showed low colour changes after 48 h with $\Delta E < 6.0$. However, and comparing to last samples, colour changes after 48 h in beverages prepared from 9 d-RB samples were doubled (ΔE = 12.0-16.3) without high differences among treatments. Latter colour changes were mainly due to L^* and b^* reductions (5-10 and 8-10 lower units) after 48 h, respectively, in accordance to high PPO and POD activities observed in those samples (as shown later).

Conclusively, coating treatments did not highly affect the physicochemical quality of prepared beverages. Furthermore, low physicochemical differences between 0 h-samples were observed as well as during subsequent 48 h storage.

VII.3.5. Microbial quality

Microbial quality (psychrophiles, mesophiles, *Enterobacteriaceae*, LAB, Y+M) of samples is shown in Figure VII.5 and Supplementary material VII.1. Psychrophilic (Figure VII.5A) and mesophilic (Supplementary material VII.1) initial loads of BD0-0h samples were below the detection limit (1 log CFU g^{-1}). It could be explained by the high

NaOCl effectiveness to reduce the initial loads of these microbial groups. LAB (Figure VII.5B), *Enterobacteriaceae* (Figure VII.5C) and Y+M (Figure VII.5D) counts of BD0-0h samples were 2.0, 2.6 and 2.9 log CFU g^{-1}, respectively. CH treatment greatly reduced *Enterobacteriaceae* loads of B0-0h CTRL samples by > 1.6 log units (counts below the detection limit). Nevertheless, LAB counts were only decreased by 0.3 log units while Y+M loads remained unchanged ($p < 0.05$) after CH treatment. The higher antimicrobial activity of chitosan against *Enterobacteriaceae* may be found in the gram-negative nature of this microbial group compared to LAB, gram-negative bacteria. Accordingly, the leakage of intracellular microbial material after chitosan treatment in gram-negative was found superior to that reported in gram-positive bacteria (Chung and Chen, 2008; Helander et al., 2001). Similarly, LAB were less susceptible to chitosan while gram-negative bacteria, including *Enterobacteriaceae*, were more susceptible to this natural antimicrobial compound (Devlieghere et al., 2004).

Attending to microbial counts of samples at different blending days, a similar behaviour was generally observed for all microbial groups. Hence, microbial loads of 0 h-samples were not greatly changed for the first 7 days storage with < 0.7 log units increments. Furthermore, psychrophilic and mesophilic loads on days 4 and 7 were similar to the rest of microbial groups apart from their initial low levels. A general high microbial growth of 2.2-3.6, 1.5-2.3 and 0.3-1.2 log units for mesophilic/psychrophilic/LAB, *Enterobacteriaceae* and Y+M, respectively, was registered from day 7 to day 9. Higher microbial loads were registered in CTRL compared to CH and CH+B12 samples at day 9. Accordingly, CH and CH+B12 samples showed 0.5-1.2 log units lower microbial increments from day 7 to day 9 compared to uncoated samples.

As expected, microbial loads of prepared beverages at different blending days increased during subsequent storage up to 48 h. However, microbial levels of samples were below 6 log CFU g^{-1} after 48 h, even in beverages prepared from 9 d-RB samples, except for mesophilic, psychrophilic and LAB counts of CTRL samples. In general, CH and CH+B12 beverages showed lower microbial counts compared to CTRL samples after 48 h.

Conclusively, CH coating of RB fruit/vegetable mix led to lower microbial growth of prepared beverages during their subsequent storage up to 48 h. Accordingly, beverages

prepared from chitosan-coated samples showed psychrophilic/LAB and *Enterobacteriaceae*/Y+M loads below 6 and 5 log CFU g^{-1} after 48 h.

VII.3.6. Enzymatic activity

Initial PPO and POD activities of BD0-0h CTRL samples were 0.15 and 3.10 U g^{-1}, respectively (Figures VII.6A and VII.6B), showing CH/CH+B12 beverages approximately 2-fold higher ($p < 0.05$) PPO/POD activities. The higher PPO/POD activity in chitosan-containing samples could be probably owed to a better enzymatic extraction in these samples due to the positive charge of chitosan. However, PPO and POD activities of chitosan-containing samples on BD0, BD4 and BD7 were markedly reduced while enzymatic activities from CTRL samples were increased. The control of enzymatic browning in beverages by chitosan has been also previously described in filtered apple and pear juices. Latter effect could be probably attributed to the ability of the positively charged polymer to coagulate suspended solids to which PPO and POD are bound (Sapers, 1992). PPO/POD activities of all samples were increased from BD7 to BD9 without significant ($p < 0.05$) differences among treatments. A similar PPO activity behaviour was observed between all treatments during storage up to 48 h. POD activity of CTRL and CH beverages remained unchanged ($p < 0.05$) during 48 h of storage. However, POD activity of CH+B12 samples was highly reduced (30-40 %) after 24 h remaining stable for the 24-48 h period. Accordingly, the lower POD activity in CH+B12 beverages throughout storage could be owed to a hypothetical inhibiting effect of vitamin B12 on the POD activity. Latter hypotheses is also in accord to the observed lower colour differences in CH+B12 beverages prepared from 9 d-stored RB samples regarding CH treatment (Table VII.1).

Initial PME activity of BD0-0h samples was 0.35 U g^{-1} without significant ($p < 0.05$) differences among treatments (Figure VII.6C). PME activity increased from BD0-0h to BD4-0h by 60-80 % followed by a decrease, registering on BD7-0h and BD9-0h PME activity values 50-80 % lower compared to BD0-0h. No significant ($p < 0.05$) differences among treatments were found on latter samples. PME activity throughout storage of beverages generally increased registering higher increments as the storage time of the RB samples did, regardless of the treatment.

PPO/POD and PME enzymes are the main quality degrading enzymes affecting to colour and firmness, respectively, of plant products limiting their shelf lives. The activities of latter enzymes are even enhanced when cell disruption occurs, for example after blending, when enzymes come in contact with their respective subtracts. Then, undesirable browning products and degradation of firmness-related molecules occurs. The hereby applied chitosan coating led to lower activities of those browning-related enzymes during storage of the RB fruit/vegetable mix. Furthermore, vitamin B12 fortification enhanced latter effect particularly for POD while did not negatively affect to PME or PPO activities.

VII.3.7. Vitamin B12

The vitamin B12 content of CH+B12 samples on BD0 was 8.6 μg kg^{-1} (data not shown). The vitamin B12 increased throughout storage of the CH+B12 RB portions registering significant ($p < 0.05$) increments of 40 and 50 % on BD7-0h and BD9-0h, respectively, compared to BD0-0h CH+B12 samples. Latter vitamin B12 increment could be owed to a better extraction favoured by the product storage. There were no significant ($p < 0.05$) vitamin B12 changes during the 48 h-storage of beverages for any of the blending times. Accordingly, a 200 mL dose of the vitamin B12-beverage may ensure the daily vitamin B12 intake of 2.0 μg day^{-1} recommended by the FAO/WHO (2004). In this sense, this particular beverage may be considered a fortified food, which may supplement/cover vitamin B12 deficiencies in selected populations groups such as vegetarians/vegans, elderly, individuals with disorders of malnutrition, etc.

VII.3.8. Total phenolic content and antioxidant capacity

TPC of BD0-0h samples was 246.9 mg ChAE kg^{-1} without significant ($p < 0.05$) differences among treatments (Figure VII.7A). In general, no high TPC changes (< 15 %) were observed between different blending days. However, BD0-0h CH+B12 samples showed an 80 % TPC increment after 9 days regarding their respective TPC levels on BD0-0h. A latter TPC increment of 100-110 % was lately observed in BD9-24h beverages, while no significant ($p < 0.05$) TPC changes were registered for the remaining samples at 48 h. The phenolic compounds accumulation after wounding stress has been related to PAL activation being proposed ATP and reactive oxygen species as signalling molecules (Reyes et al., 2007). Accordingly, TPC increments in red vegetables smoothies

after 10 days at 5 ºC were well correlated to increments of PAL activity owed to the wounding stress occurred during smoothie preparation (Rodríguez-Verástegui et al., 2015).

TAC of BD0-0h beverages was 180.2 mg ChAE kg^{-1} without significant ($p < 0.05$) differences among samples (Figure VII.7B). TAC only decreased by approximately 20 % after 7 days although a high TAC descend of approximately 90 % was reached on BD9-0h samples, without significant ($p < 0.05$) differences among treatments. Latter TAC decrease may be correlated to the vitamin C degradation of samples since high TAC-vitamin C correlations of 75 and 34 % have been reported in melon and pineapple, respectively (Guo et al., 2003). TAC of BD0 and BD4 beverages decreased by 10-23 % after 48 h. Correspondingly, vitamin C of red vegetable smoothies was decreased by 40 % after 4 days at 5 ºC (Castillejo et al., 2016b). Such TAC losses after 48 h were even aggravated in BD7 samples with a TAC reduction of 40-50%. However, TAC behaviour in stored beverages from BD9 RB samples showed a different behaviour. Interestingly, TAC of CTRL samples remained unchanged ($p < 0.05$) after 48 h while TAC levels of CH and CH+B12 samples highly increased by approximately 100-140 %, without significant ($p < 0.05$) differences among both coating treatments. Latter TAC increment of CH and CH+B12 samples is correlated to TPC enhancements observed in those samples. However, unchanged TAC of CTRL samples may be owed to a high vitamin C degradation which was somehow reduced with the chitosan coating as previously reported (Xiao et al., 2010).

VII.4. CONCLUSIONS

The MAP of the ready-to-blend fruit/vegetable mix, jointly with a chitosan coating, greatly maintained the quality of the product up to 9 days at 5 ºC. The beverage prepared from the ready-to-blend fruit/vegetable mix showed ≈ 40 % viable cells. The activities of the main quality-degradative enzymes in the beverages from the ready-to-blend samples were reduced while epiphytic microflora loads were kept below 6 log units. Chitosan coating and vitamin B12 fortification did not negatively affect the total phenolic content neither the total antioxidant capacity of beverages which were even enhanced after 24 h in those beverages prepared from ready-to-blend samples stored for 9 days at 5 ºC. The vitamin B12 fortification of the ready-to-blend fruit/vegetable mix ensured the

recommended daily vitamin B12 intake in a 200 mL beverage dose, avoiding pills and other vitamin B12 supplements in selected populations groups such as vegans/vegetarians, elderly, individuals with disorders of malnutrition, etc.

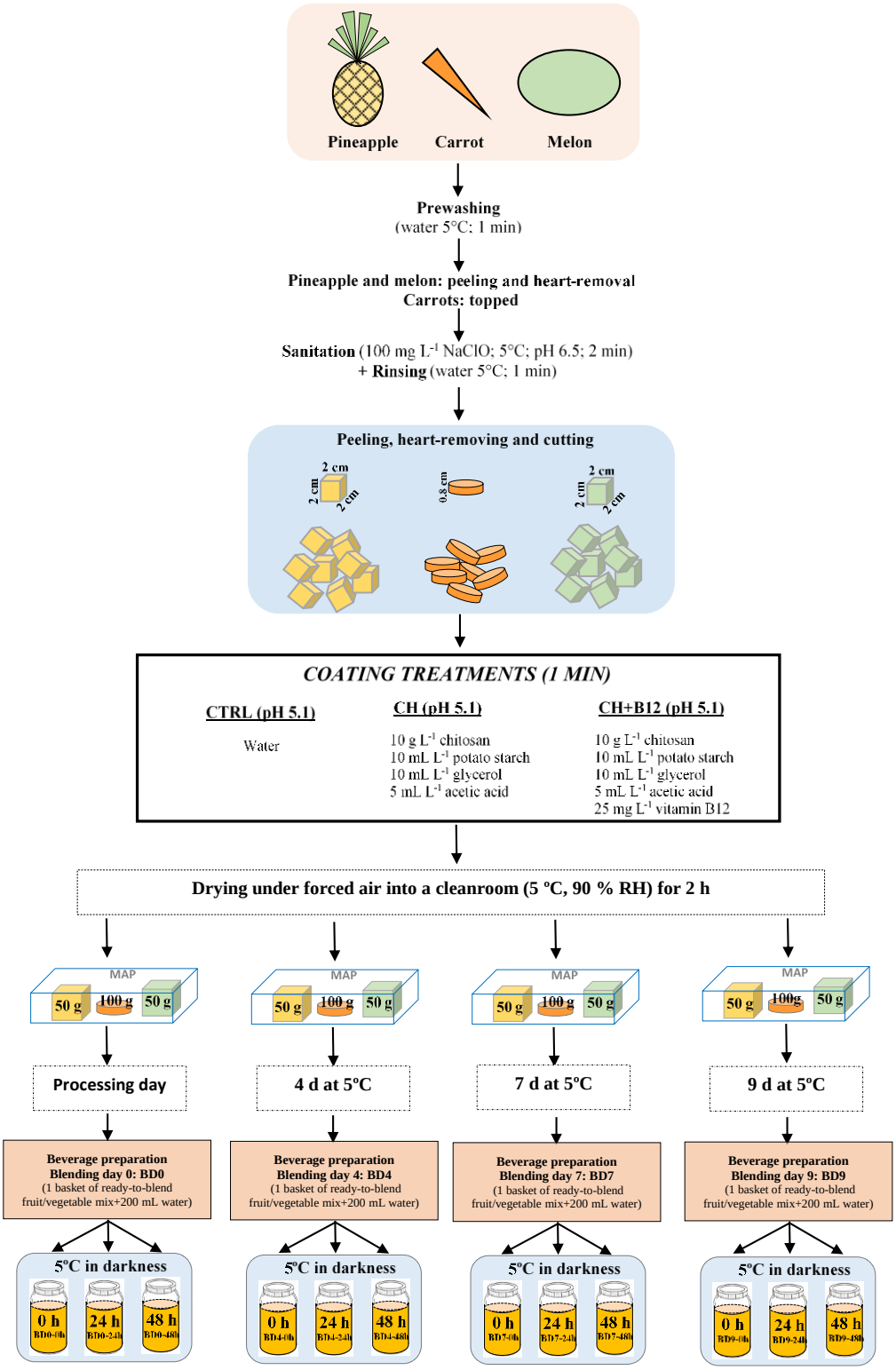


Figure VII.1. Flow diagram of the experiment including preparation of the ready-to-blend fresh-cut portion, beverage preparation and storage conditions.

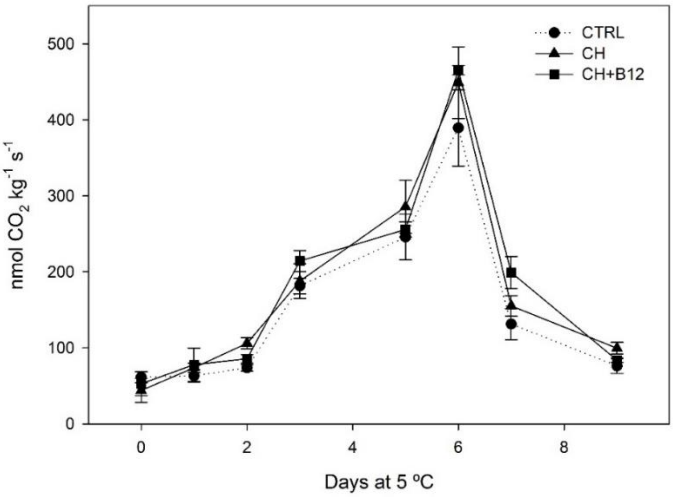


Figure VII.2. Respiration rate of ready-to-blend fresh-cut fruit/vegetable mix stored up to 9 days at 5 ºC (n=5±SD). Different capital letter denotes significant differences (p < 0.05) among different treatments for the same sampling time. Different lowercase letter denotes significant differences (p < 0.05) among different sampling times for the same treatment.

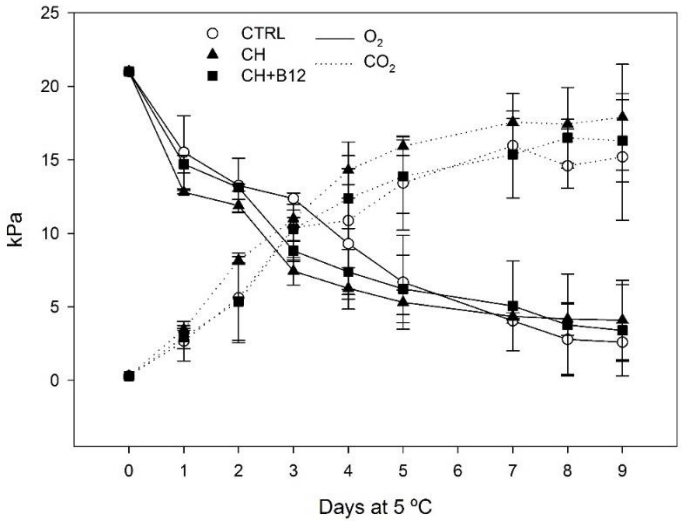


Figure VII.3. Gas partial pressures of a fresh-cut fruit/vegetable mix stored up to 9 days at 5 ºC (n=5±SD). Different capital letter denotes significant differences (p < 0.05) among different treatments for the same sampling time. Different lowercase letter denotes significant differences (p < 0.05) among different sampling times for the same treatment.

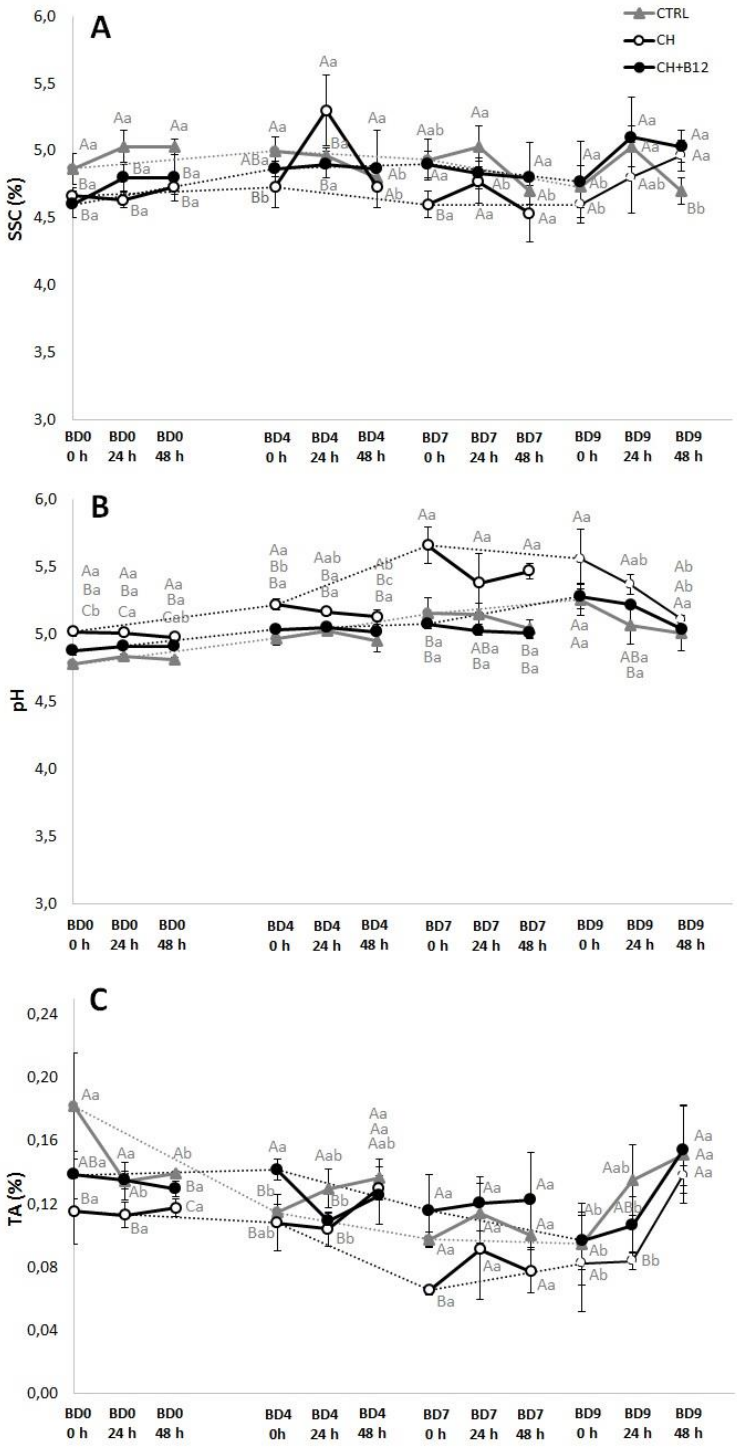


Figure VII.4. Soluble solids content (SSC; %), pH and titratable acidity (TA; %) of fresh blended beverages, stored up to 48 h at 5 ºC, prepared on different blending days (BD) from a fresh-cut fruit/vegetable mix stored up to 9 days at 5 ºC (n=5±SD). Different capital letter denotes significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letter denotes significant differences ($p < 0.05$) among different sampling times for the same treatment.

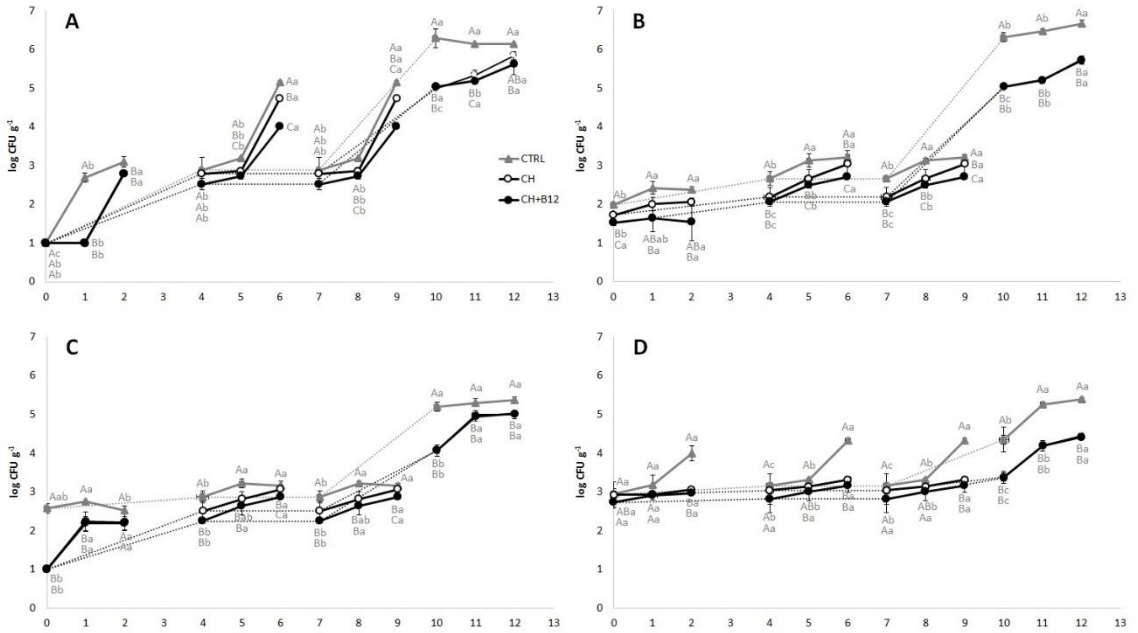


Figure VII.5. Psychrophilic (A), lactobacilli (B), *Enterobacteriaceae* (C) and yeasts and moulds (D) counts of fresh blended beverages, stored up to 48 h at 5 ºC, prepared on different blending days (BD) from a fresh-cut fruit/vegetable mix stored up to 9 days at 5 ºC (n=5±SD). Different capital letter denotes significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letter denotes significant differences ($p < 0.05$) among different sampling times for the same treatment.

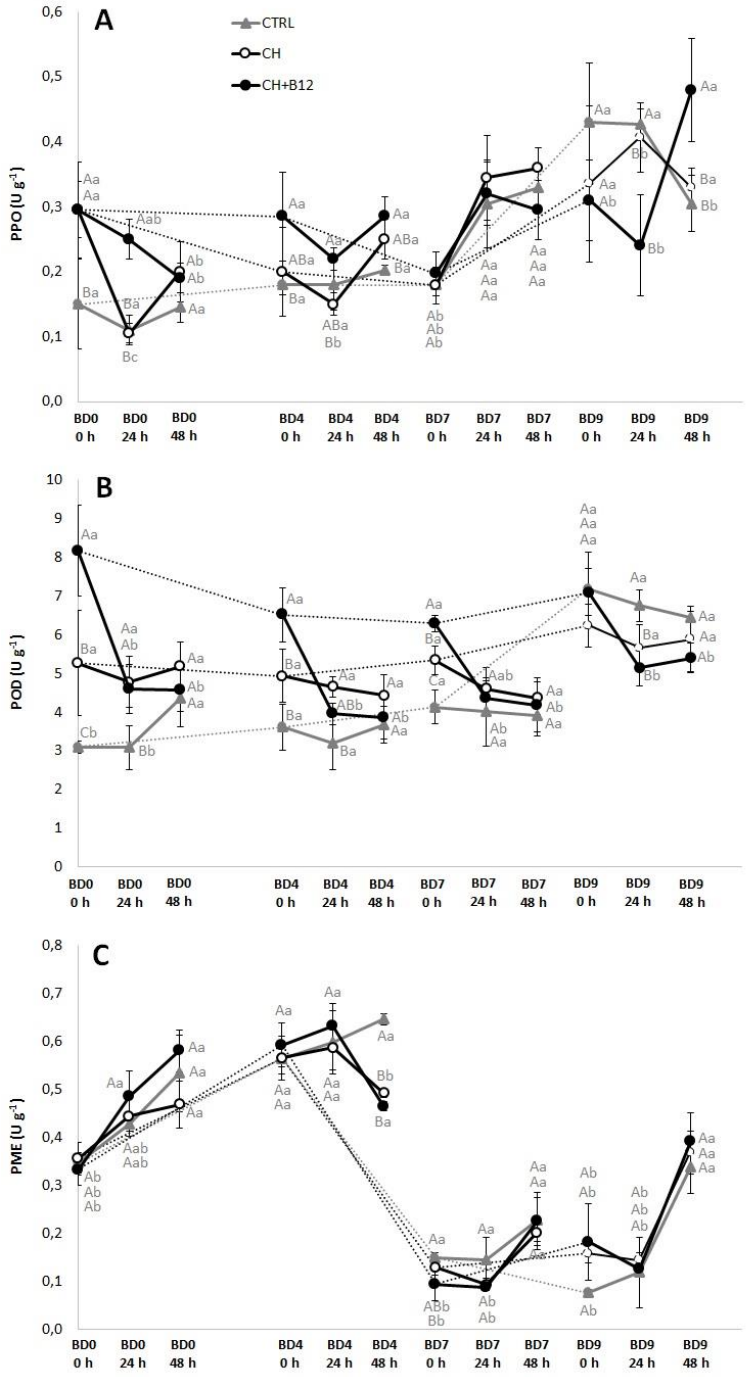


Figure VII.6. Polyphenoloxidase (PPO; A), peroxidase (POD; B) and pectin methylesterase (PME; C) activities of fresh blended beverages, stored up to 48 h at 5 ºC, prepared on different blending days (BD) from a fresh-cut fruit/vegetable mix stored up to 9 days at 5 ºC (n=5±SD). Different capital letter denotes significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letter denotes significant differences ($p < 0.05$) among different sampling times for the same treatment.

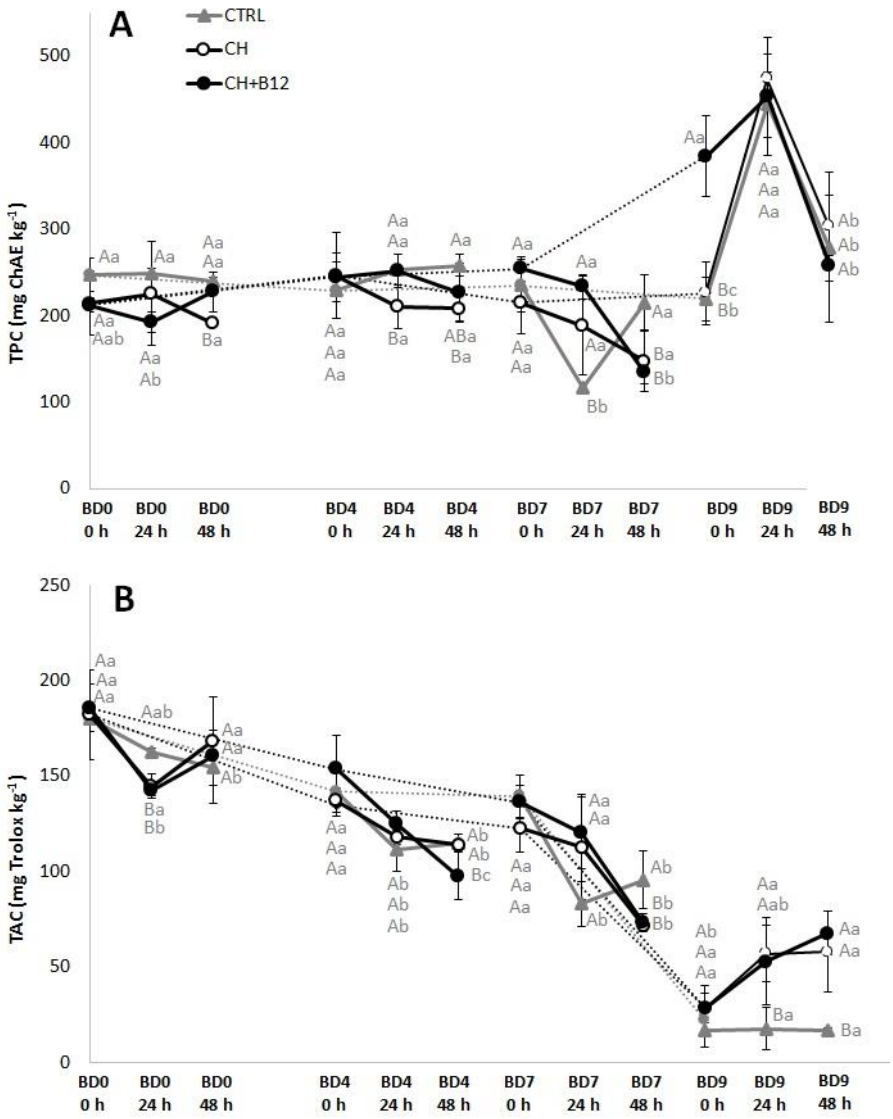
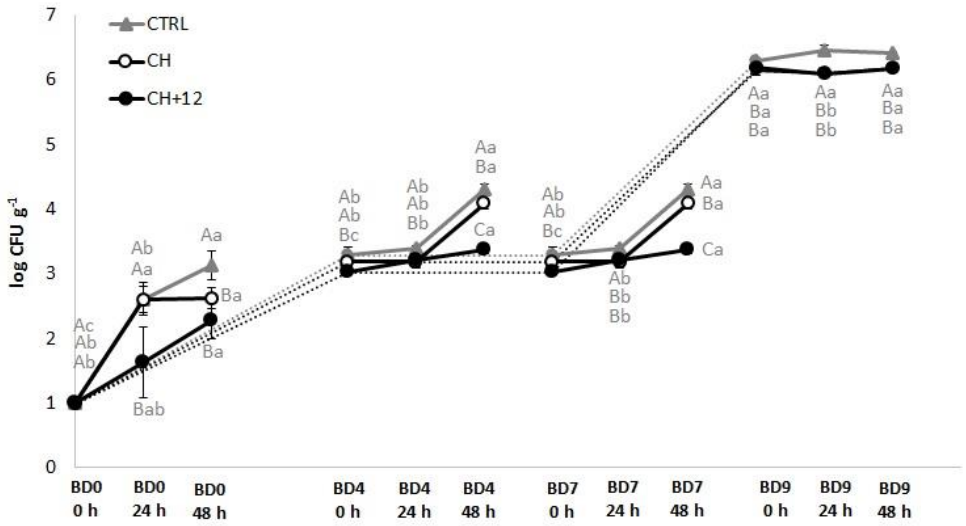


Figure VII.7. Total phenolic content (TPC; A) and total antioxidant capacity (TAC; B) of fresh blended beverages, stored up to 48 h at 5 ºC, prepared on different blending days (BD) from a fresh-cut fruit/vegetable mix stored up to 9 days at 5 ºC (n=5±SD). Different capital letter denotes significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letter denotes significant differences ($p < 0.05$) among different sampling times for the same treatment.



Supplementary material VII.1. Mesophilic counts of fresh blended beverages, stored up to 48 h at 5 ºC, prepared on different blending days (BD) from a fresh-cut fruit/vegetable mix stored up to 9 days at 5 ºC (n=5±SD). Different capital letter denotes significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letter denotes significant differences ($p < 0.05$) among different sampling times for the same treatment.

Table VII.1. Total colour differences of fresh blended beverages, stored up to 48 h at 5 ºC, prepared on different blending days (BD) from a fresh-cut fruit/vegetable mix stored up to 9 days at 5 ºC (n=5±SD). Different capital letter denotes significant differences ($p < 0.05$) among different treatments for the same sampling time. Different lowercase letter denotes significant differences ($p < 0.05$) among different sampling times for the same treatment.

	CTRL	**CH**	**CH+B12**
BD0			
0 h	-	1.7±0.9 Aa	2.9±0.9 Aa
24 h	1.0±0.7 Aa	1.3±0.5 Aa	0.9±0.2 Ab
48 h	1.2±0.7 Aa	1.8±0.7 Aa	1.7±0.1 Ab
BD4			
0 h	-	1.8±0.5 Ab	2.5±0.1 Ab
24 h	1.4±0.6 Ab	1.6±0.4 Ab	2.1±1.5 Ab
48 h	4.1±1.0 Aa	4.3±0.9 Aa	5.3±1.9 Aa
BD7			
0 h	-	2.2±0.8 Ab	1.7±0.8 Ab
24 h	3.7±1.1 ABa	2.8±1.5 Bb	5.8±1.9 Aa
48 h	6.0±2.7 Aa	5.8±1.9 Aa	6.0±1.5 Aa
BD9			
0 h	-	7.7±1.5 Ab	4.2±1.2 Bc
24 h	12.0±1.8 Ba	16.3±1.1 Aa	13.4±1.1 Ba
48 h	9.3±1.1 Ca	14.7±0.4 Aa	10.8±0.6 Bb

CONCLUSIONS

CONCLUSIONS

Different postharvest abiotic stresses, such as wounding, hyperoxia storage and UV radiation were studied in this PhD Thesis to induce the activation of PAL enzyme and related antioxidant phenolic contents in carrots. We found increases up to 2,000 % after 72 h at 15 ºC, being this finding of special interest for the food and pharmaceutical industries. Phenolic accumulations up to 500 % were also reached by UV-B irradiated samples after 72 h at 15 ºC. Revalorization of Bimi® broccoli by-products (leaves and stalks which represent the 75 % of the plant) was also possible by UV irradiation. Innovative carrot-based smoothies were developed from such phenolic-enhanced carrots being their microbial and physicochemical quality well preserved by mild thermal treatment during 14 days at 5 ºC. A non-thermal treatment (HPP) was also studied on orange-coloured smoothies leading to an excellent shelf life of such beverages. HPP treatments may contrary induce enzymatic browning in determined beverages. Accordingly, apple juice was used as a beverage model being the enzymatic browning highly controlled by maltosyl-*β*-cyclodextrin, which was well modelled with a fractional conversion model, being not affected such encapsulation by the HPP treatment. A new 'ready-to-blend' concept was firstly developed in this PhD Thesis by studying the quality changes of a fresh-cut ready-to-blend fruit/vegetable mix during storage and the related prepared smoothies at different blending times. The shelf life of such ready-to-blend product was extended by a chitosan coating and added value was increased to the product by fortification with vitamin B12, being of high interest for determined populations groups (vegetarians/vegans, elderly, etc.) with special needs of this vitamin.

SCIENTIFIC PUBLICATIONS DERIVED FROM THE PRESENT PHD THESIS

Original papers published in peer–reviewed journals of the Institute for Scientific Information (ISI) of the Journal Citation Reports (JCR).

Formica-Oliveira, A. C., Martínez-Hernández, G. B., Aguayo, E., Gómez, P. A., Artés, F. and Artés-Hernández, F. 2016. UV-C and hyperoxia abiotic stresses to improve healthiness of carrots. Study of combined effects. Journal of Food Science and Technology, 53(9), 3465-3476.

Formica-Oliveira, A. C., Martínez-Hernández, G. B., Díaz-López, V., Artés, F. and Artés-Hernández, F. 2017. Effects of UV-B and UV-C combination on phenolic compounds biosynthesis in fresh-cut carrots. Postharvest Biology and Technology, 127, 99-104.

Formica-Oliveira, A. C., Martínez-Hernández, G. B., Aguayo, E., Gómez, P. A., Artés, F. and Artés-Hernández, F. 2016. A functional smoothie from carrots with induced enhanced phenolic content. Food and Bioprocess Biotechnology. 10, 491-502.

Artés-Hernández, F., Formica-Oliveira, A. C., Artés, F. and Martínez-Hernández, G. B. Improved quality of a vitamin B12-fortified 'ready-to-blend' fresh-cut mix salad with chitosan. Food Science and Technology International. *Accepted.*

Original papers submitted to peer–reviewed journals if the ISI-JCR

Martínez-Hernández, G. B., Formica-Oliveira, A. C., Artés, F. and Artés-Hernández, F. Browning control in high pressure-treated apple juice by maltosyl-*β*-cyclodextrin. *Submitted, September 2016.*

Formica-Oliveira, A. C., Martínez-Hernández, G. B., Artés, F. and Artés-Hernández, F. Postharvest UV-radiation treatments to revalorize broccoli by-products and edible parts. *Submitted, January 2017.*

Original papers published in Proceedings of relevant Symposiums with DOI.

Formica-Oliveira, A. C., Martínez-Hernández, G. B., Díaz-López, V., Otón, M., Artés, F. and Artés-Hernández, F. 2017. High hydrostatic pressure treatments for keeping quality of orange vegetables smoothies. Proceedings of the VIII International Postharvest Symposium: Enhancing Supply Chain and Consumer Benefits - Ethical and Technological Issues. Acta Horticulturae. Edit: ISHS. Accepted.

REFERENCES

Acarturk, F., Imai, T., Saito, H., Ishikawa, M., & Otagiri, M. (1993). Comparative study on inclusion complexation of maltosyl-beta-cyclodextrin, heptakis(2,6-di-O-methyl)-beta-cyclodextrin and beta-cyclodextrin with fucosterol in aqueous and solid state. *Journal of Pharmacy and Pharmacology, 45*(12), 1028-1032.

Aghdam, M. S., Sevillano, L., Flores, F. B., & Bodbodak, S. (2013). Heat shock proteins as biochemical markers for postharvest chilling stress in fruits and vegetables. *Scientia Horticulturae, 160*, 54-64.

Aguiló-Aguayo, I., Hossain, M. B., Brunton, N., Lyng, J., Valverde, J., & Rai, D. K. (2014). Pulsed electric fields pre-treatment of carrot purees to enhance their polyacetylene and sugar contents. *Innovative Food Science and Emerging Technologies, 23*, 79-86.

AIJN (2014a). Liquid Fruit 2014. *Market Report of the European Fruit Juice Association, 2014*.

AIJN (2014b). Liquid fruit market report. Brussels, Belgium: European Juice Fruit Association (AIJN).

Alegria, C. (2015). Heat shock and uv-c abiotic stress treatments as alternative tools to promote fresh-cut carrot quality and shelf-life. *PhD Thesis. University of Lisbon*.

Alegria, C., Gonçalves, E. M., Moldão-Martins, M., Cisneros-Zevallos, L., & Abreu, M. (2016). Peel removal improves quality without antioxidant loss, through wound-induced phenolic biosynthesis in shredded carrot. *Postharvest Biology and Technology, 120*, 232-239.

Alegria, C., Pinheiro, J., Duthoit, M., Gonçalves, E. M., Moldão-Martins, M., & Abreu, M. (2012). Fresh-cut carrot (cv. Nantes) quality as affected by abiotic stress (heat shock and UV-C irradiation) pre-treatments. *LWT - Food Science and Technology, 48*(2), 197-203.

Alegria, C., Pinheiro, J., Gonçalves, E. M., Fernandes, I., Moldão, M., & Abreu, M. (2010). Evaluation of a pre-cut heat treatment as an alternative to chlorine in minimally processed shredded carrot. *Innovative Food Science and Emerging Technologies, 11*(1), 155-161.

Alothman, M., Bhat, R., & Karim, A. A. (2009). Effects of radiation processing on phytochemicals and antioxidants in plant produce. *Trends in Food Science & Technology, 20*(5), 201-212.

Allen, L., Benoist, B., Dary, O., & Hurrell, R. (2006). Guidelines on food fortification with micronutrients. WHO/FAO.

Allende, A., & Artés, F. (2005). Minimal fresh processing of vegetables, fruits and juices. In D. W. Sun (Ed.), *Emerging Technologies for Food Processing* (pp. 675–715). Oxford, UK: Elsevier–Academic Press.

Amanatidou, A., Slump, R. A., Gorris, L. G. M., & Smid, E. J. (2000). High Oxygen and High Carbon Dioxide Modified Atmospheres for Shelf-life Extension of Minimally Processed Carrots. *Journal of Food Science, 65*(1), 61-66.

Artés-Hernández, F., Escalona, V. H., Robles, P. A., Martínez-Hernández, G. B., & Artés, F. (2009a). Effect of UV-C radiation on quality of minimally processed spinach leaves. *Journal of the Science of Food and Agriculture, 89*(3), 414-421.

Artés-Hernández, F., Tomás-Callejas, A., Garre, J., & Artés, F. (2009b). Fortalecimiento de la capacidad antioxidante de las frutas y hortalizas durante su conservación refrigerada. *CTC. Revista de agroalimentación e industrias afines, 41*, 26-34.

Artés-Hernández, F., Escalona, V. H., Robles, P. A., Martínez-Hernández, G. B., & Artés, F. (2009). Effect of UV-C radiation on quality of minimally processed spinach leaves. *Journal of the Science of Food and Agriculture, 89*(3), 414-421.

Artés, F. (2000). Productos vegetales procesados en frescos. In M. Lamúa (Ed.), *Aplicación del frío a los alimentos* (Vol. 127–141). Madrid, Spain: Mundi Prensa.

Artés, F., & Artés–Hernández, F. (2003). Principios y diseño de industrias de procesado en fresco de hortalizas. In *Envasado y comercialización de frutas y hortalizas mínimamente procesadas* (pp. 67–92). La Rioja, Spain: Gobierno de La Rioja.

Artés, F., Gómez, P., Aguayo, E., Escalona, V., & Artés-Hernández, F. (2009). Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. *Postharvest Biology and Technology, 51*(3), 287-296.

Avena-Bustillos, R. J., Du, W. X., Woods, R., Olson, D., Breksa, A. P., 3rd, & McHugh, T. H. (2012). Ultraviolet-B light treatment increases antioxidant capacity of carrot products. *Journal of the Science of Food and Agriculture, 92*(11), 2341-2348.

Baclayon, D. P., Matsui, T., Suzuki, H., & Kosugi, Y. (2007). Changes in activity and gene expression of phenylalanine ammonia-lyase in broccoli branchlets during early postharvest senescence. *Journal of Biological Sciences, 7*(4), 614-619.

Bai, R.-K., Huang, M.-Y., & Jiang, Y.-Y. (1988). Selective permeabilities of chitosan-acetic acid complex membrane and chitosan-polymer complex membranes for oxygen and carbon dioxide. *Polymer Bulletin, 20*(1), 83-88.

Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry, 99*(1), 191-203.

Bardón-Iglesias, R., Cruz-Pérez, M., Fúster-Lorán, F., Gómez-Mateo, J. V., Marino- Hernando, E., & Ribes-Ripoll, M. A. (2012). Estudio del sector de las bebidas analcohólicas comercializadas en la comunidad de Madrid. Madrid, Spain: Dirección General de Ordenación e Inspección, Consejería de Sanidad de la Comunidad de Madrid.

Baron, A., Dénes, J.-M., & Durier, C. (2006). High-pressure treatment of cloudy apple juice. *LWT - Food Science and Technology, 39*(9), 1005-1013.

Becerra-Moreno, A., Benavides, J., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2012). Plants as Biofactories: Glyphosate-Induced Production of Shikimic Acid and Phenolic Antioxidants in Wounded Carrot Tissue. *Journal of Agricultural and Food Chemistry, 60*(45), 11378-11386.

Becerra-Moreno, A., Redondo-Gil, M., Benavides, J., Nair, V., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2015). Combined effect of water loss and wounding stress on gene activation of metabolic pathways associated with phenolic biosynthesis in carrot. *Frontiers in Plant Science, 6*, 837.

Ben Said, M., Masahiro, O., & Hassen, A. (2010). Detection of viable but non cultivable Escherichia coli after UV irradiation using a lytic Qβ phage. *Annals of Microbiology, 60*(1), 121-127.

Bertelli, D., Plessi, M., Braghiroli, D., & Monzani, A. (1998). Separation by solid phase extraction and quantification by reverse phase HPLC of sulforaphane in broccoli. *Food Chemistry, 63*(3), 417-421.

Beuchat, L. R., & Brackett, R. E. (1990). Inhibitory effects of raw carrots on Listeria monocytogenes. *Applied and Environmental Microbiology, 56*(6), 1734-1742.

Bhat, R., Sridhar, K. R., & Tomita-Yokotani, K. (2007). Effect of ionizing radiation on antinutritional features of velvet bean seeds (Mucuna pruriens). *Food Chemistry, 103*(3), 860-866.

Bintsis, T., Litopoulou-Tzanetaki, E., & Robinson, R. K. (2000). Existing and potential applications of ultraviolet light in the food industry – a critical review. *Journal of the Science of Food and Agriculture, 80*(6), 637-645.

Boerjan, W., Ralph, J., & Baucher, M. (2003). LIGNIN BIOSYNTHESIS. *Annual Review of Plant Biology, 54*(1), 519-546.

Brand-Williams, W., Cuvelier, M., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology, 28*(1), 25-30.

Bricker, G. V., Riedl, K. M., Ralston, R. A., Tober, K. L., Oberyszyn, T. M., & Schwartz, S. J. (2014). Isothiocyanate metabolism, distribution, and interconversion in mice following consumption of thermally processed broccoli sprouts or purified sulforaphane. *Molecular Nutrition & Food Research, 58*(10), 1991-2000.

Buckow, R., Weiss, U., & Knorr, D. (2009). Inactivation kinetics of apple polyphenol oxidase in different pressure–temperature domains. *Innovative Food Science and Emerging Technologies, 10*(4), 441-448.

Bugianesi, R., Salucci, M., Leonardi, C., Ferracane, R., Catasta, G., Azzini, E., et al. (2004). Effect of domestic cooking on human bioavailability of naringenin, chlorogenic acid, lycopene and beta-carotene in cherry tomatoes. *European Journal of Nutrition, 43*(6), 360-366.

Bull, M. K., Zerdin, K., Howe, E., Goicoechea, D., Paramanandhan, P., Stockman, R., et al. (2004). The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice. *Innovative Food Science and Emerging Technologies, 5*(2), 135-149.

Cameron, R. G., Buslig, B. S., & Shaw, P. E. (1992). Adaptation of a Spectrophotometric Assay for Pectinmethylesterase to a Kinetic Microplate Reader. *Journal of Food Science, 57*(4), 1006-1008.

Campas-Baypoli, O. N., Sanchez-Machado, D. I., Bueno-Solano, C., Nunez-Gastelum, J. A., Reyes-Moreno, C., & Lopez-Cervantes, J. (2009). Biochemical composition and physicochemical properties of broccoli flours. *International Journal of Food Sciences and Nutrition, 60 Suppl 4*, 163-173.

Campas-Baypoli, O. N., Sanchez-Machado, D. I., Bueno-Solano, C., Ramirez-Wong, B., & Lopez-Cervantes, J. (2010). HPLC method validation for measurement of sulforaphane level in broccoli by-products. *Biomedical Chromatography, 24*(4), 387-392.

Cantos, E., Garcia-Viguera, C., de Pascual-Teresa, S., & Tomas-Barberan, F. A. (2000). Effect of postharvest ultraviolet irradiation on resveratrol and other phenolics of cv. Napoleon table grapes. *Journal of Agricultural and Food Chemistry, 48*(10), 4606-4612.

Cantwell, M., & Suslow, T. V. (2002a). Postharvest handling systems: fresh–cut fruits and vegetables. In K. A.A. (Ed.), *Postharvest technology of horticultural crops* (3 ed., Vol. 3311, pp. 445–464). Oakland, USA: University of California, Agriculture and Natural Resources.

Cantwell, M. I., & Suslow, T. V. (2002b). Postharvest handling systems: Fresh-cut fruits and vegetables. In A. A. Kader (Ed.), *Postharvest Technology of Horticultural Crops* (3rd ed.). Oakland (CA, USA): University of California, Agriculture and Natural Resources.

Cartea, M. E., Francisco, M., Soengas, P., & Velasco, P. (2011). Phenolic Compounds in Brassica Vegetables. *Molecules, 16*(1), 251.

Cartea, M. E., & Velasco, P. (2008). Glucosinolates in Brassica foods: bioavailability in food and significance for human health. [journal article]. *Phytochemistry Reviews, 7*(2), 213-229.

Castagna, A., Dall'Asta, C., Chiavaro, E., Galaverna, G., & Ranieri, A. (2014). Effect of Post-harvest UV-B Irradiation on Polyphenol Profile and Antioxidant Activity in Flesh and Peel of Tomato Fruits. [journal article]. *Food and Bioprocess Technology, 7*(8), 2241-2250.

Castelluccio, C., Paganga, G., Melikian, N., Bolwell, G. P., Pridham, J., Sampson, J., et al. (1995). Antioxidant potential of intermediates in phenylpropanoid metabolism in higher plants. *FEBS Letters, 368*(1), 188-192.

Castillejo, N., Martínez-Hernández, G. B., Gómez, P. A., Artés, F., & Artés-Hernández, F. (2015). Red fresh vegetables smoothies with extended shelf life as an innovative source of health-promoting compounds. [journal article]. *Journal of Food Science and Technology*, 1-12.

Castillejo, N., Martinez-Hernandez, G. B., Monaco, K., Gomez, P. A., Aguayo, E., Artes, F., et al. (2016a). Preservation of bioactive compounds of a green vegetable smoothie using short time-high temperature mild thermal treatment. *Food Science and Technology International*.

Castillejo, N., Martínez-Hernández, G. B., Monaco, K., Gómez, P. A., Aguayo, E., Artés, F., et al. (2016b). Preservation of bioactive compounds of a green vegetable smoothie using short time–high temperature mild thermal treatment. *Food Science and Technology International*.

Cisneros-Zevallos, L. (2003). The Use of Controlled Postharvest Abiotic Stresses as a Tool for Enhancing the Nutraceutical Content and Adding-Value of Fresh Fruits and Vegetables. *Journal of Food Science, 68*(5), 1560-1565.

Cisneros-Zevallos, L., Saltveit, M. E., & Krochta, J. M. (1995). Mechanism of Surface White Discoloration of Peeled (Minimally Processed) Carrots During Storage. *Journal of Food Science, 60*(2), 320-323.

Colwell, R. K., Norse, E. A., Pimentel, D., Sharples, F. E., & Simberloff, D. (1985). Genetic engineering in agriculture. *Science, 229*(4709), 111-112.

Commission, C. A. (2015). General principles for the addition of essential nutrients to foods CAC/GL 09-1987 (amended 1989, 1991). Rome, Italy.

Conesa, R., Andreu, S., Fernandez, P. S., Esnoz, A., & Palop, A. (2009). Nonisothermal heat resistance determinations with the thermoresistometer Mastia. *Journal of Applied Microbiology, 107*(2), 506-513.

Considine, K. M., Kelly, A. L., Fitzgerald, G. F., Hill, C., & Sleator, R. D. (2008). High-pressure processing--effects on microbial food safety and food quality. *FEMS Microbiology Letters, 281*(1), 1-9.

Crozier, A., Jaganath, I. B., & Clifford, M. N. (2006). Phenols, polyphenols and tannins: An overview. In A. Crozier, M. Clifford, & H. Ashihara (Eds.), *Plant secondary metabolites: occurrence, structure and role in the human diet* (pp. 1-24). Oxford, UK: Blackwell.

Cuéllar-Villarreal, M. d. R., Ortega-Hernández, E., Becerra-Moreno, A., Welti-Chanes, J., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2016). Effects of ultrasound treatment and storage time on the extractability and biosynthesis of nutraceuticals in carrot (Daucus carota). *Postharvest Biology and Technology, 119*, 18-26.

Chalutz, E., Droby, S., Wilson, C. L., & Wisniewski, M. E. (1992). UV-induced resistance to postharvest diseases of citrus fruit. *Journal of Photochemistry and Photobiology B: Biology, 15*(4), 367-371.

Cheftel, J. C. (1995). Review : High-pressure, microbial inactivation and food preservation / Revisión: Alta-presión, inactivación microbiológica y conservación de alimentos. *Food Science and Technology International, 1*(2-3), 75-90.

Cheng, G. W., & Crisosto, C. H. (1995). Browning Potential, Phenolic Composition, and Polyphenoloxidase Activity of Buffer Extracts of Peach and Nectarine Skin Tissue. *Journal of the American Society for Horticultural Science, 120*(5), 835-838.

Chong, C., & Bérard, L. S. (1983). Changes in glucosinolates during refrigerated storage of cabbage. *Journal of the American Society for Horticultural Science, 108*, 688-691.

Chubey, B. B., & Nylund, R. E. (1969). SURFACE BROWNING OF CARROTS. *Canadian Journal of Plant Science, 49*(4), 421-426.

Chun, O. K., Kim, D.-O., Smith, N., Schroeder, D., Han, J. T., & Lee, C. Y. (2005). Daily consumption of phenolics and total antioxidant capacity from fruit and vegetables in the American diet. *Journal of the Science of Food and Agriculture, 85*(10), 1715-1724.

Chung, Y.-C., & Chen, C.-Y. (2008). Antibacterial characteristics and activity of acid-soluble chitosan. *Bioresource Technology, 99*(8), 2806-2814.

de Kleijn, M. J., van der Schouw, Y. T., Wilson, P. W., Adlercreutz, H., Mazur, W., Grobbee, D. E., et al. (2001). Intake of dietary phytoestrogens is low in postmenopausal women in the United States: the Framingham study(1-4). *Journal of Nutrition, 131*(6), 1826-1832.

Del Valle, E. M. M. (2004). Cyclodextrins and their uses: a review. *Process Biochemistry, 39*(9), 1033-1046.

Devasagayam, T. P., Tilak, J. C., Boloor, K. K., Sane, K. S., Ghaskadbi, S. S., & Lele, R. D. (2004). Free radicals and antioxidants in human health: current status and future prospects. *J Assoc Physicians India, 52*, 794-804.

Devlieghere, F., Vermeulen, A., & Debevere, J. (2004). Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. *Food Microbiology, 21*(6), 703-714.

Di Cagno, R., Minervini, G., Rizzello, C. G., De Angelis, M., & Gobbetti, M. (2011). Effect of lactic acid fermentation on antioxidant, texture, color and sensory properties of red and green smoothies. *Food Microbiology, 28*(5), 1062-1071.

Dixon, R. A., Achnine, L., Kota, P., Liu, C.-J., Reddy, M. S. S., & Wang, L. (2002). The phenylpropanoid pathway and plant defence—a genomics perspective. *Molecular Plant Pathology, 3*(5), 371-390.

Dixon, R. A., & Paiva, N. L. (1995). Stress-Induced Phenylpropanoid Metabolism. *The Plant Cell, 7*(7), 1085-1097.

Dominguez-Perles, R., Martinez-Ballesta, M. C., Carvajal, M., Garcia-Viguera, C., & Moreno, D. A. (2010). Broccoli-derived by-products--a promising source of bioactive ingredients. *Journal of Food Science, 75*(4), C383-392.

Dominguez-Perles, R., Moreno, D. A., Carvajal, M., & Garcia-Viguera, C. (2011). Composition and antioxidant capacity of a novel beverage produced with green tea and minimally-processed byproducts of broccoli. *Innovative Food Science and Emerging Technologies, 12*(3), 361-368.

Dosz, E. B., & Jeffery, E. H. (2013). Commercially produced frozen broccoli lacks the ability to form sulforaphane. *Journal of Functional Foods, 5*(2), 987-990.

Dosz, E. B., Ku, K. M., Juvik, J. A., & Jeffery, E. H. (2014). Total myrosinase activity estimates in brassica vegetable produce. *Journal of Agricultural and Food Chemistry, 62*(32), 8094-8100.

Dragsted, L. O. (2003). Antioxidant actions of polyphenols in humans. *Int J Vitam Nutr Res, 73*(2), 112-119.

Du, W. X., Avena-Bustillos, R. J., Breksa, A. P., 3rd, & McHugh, T. H. (2012). Effect of UV-B light and different cutting styles on antioxidant enhancement of commercial fresh-cut carrot products. *Food Chemistry, 134*(4), 1862-1869.

Durango, A. M., Soares, N. F. F., & Andrade, N. J. (2006). Microbiological evaluation of an edible antimicrobial coating on minimally processed carrots. *Food Control, 17*(5), 336-341.

EC (2007). Comission Regulation (EC) No 1441/2007. *Official Journal of the European Union, 322*, 12-29.

EC_1441/2007 (2007). Commission regulation on microbiological criteria for foodstuffs. *Official Journal of the European Union* (Vol. 32).

Eisenmenger, M. J., & Reyes-De-Corcuera, J. I. (2009). High pressure enhancement of enzymes: A review. *Enzyme and Microbial Technology, 45*(5), 331-347.

El Ghaouth, A., Wilson, C. L., & Callahan, A. M. (2003). Induction of Chitinase, beta-1,3-Glucanase, and Phenylalanine Ammonia Lyase in Peach Fruit by UV-C Treatment. *Phytopathology, 93*(3), 349-355.

El Gharras, H. (2009). Polyphenols: food sources, properties and applications – a review. *International Journal of Food Science & Technology, 44*(12), 2512-2518.

Escalona, V. H., Aguayo, E., Martínez-Hernández, G. B., & Artés, F. (2010). UV-C doses to reduce pathogen and spoilage bacterial growth in vitro and in baby spinach. *Postharvest Biology and Technology, 56*(3), 223-231.

Espin, J. C., Garcia-Conesa, M. T., & Tomas-Barberan, F. A. (2007). Nutraceuticals: facts and fiction. *Phytochemistry, 68*(22-24), 2986-3008.

Fahey, J. W., Zalcmann, A. T., & Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry, 56*(1), 5-51.

FAO (2015). World population Prospects: The 2015 revision, methodology of the United Nations population estimates and projections. In P. d. Department of economic and social affairs (Ed.), (Vol. ESA/P/WP.242). New York, USA: United Nations.

FAO (2016). FAOSTAT Statistic data. http://www.fao.org/faostat/. Accessed December 2016 2016.

FAO/WHO (2004). *Vitamin and mineral requirements in human nutrition* (2ed., Code of Federal Regulations). Bangkok, Thailand: World Health Organization and Food and Agriculture Organization of the United Nations.

Fonseca, S. C., Oliveira, F. A. R., & Brecht, J. K. (2002). Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. *Journal of Food Engineering, 52*(2), 99-119.

Formica-Oliveira, A. C., Martínez-Hernández, G. B., Aguayo, E., Gómez, P. A., Artés, F., & Artés-Hernández, F. (2016a). UV-C and hyperoxia abiotic stresses to improve healthiness of carrots: study of combined effects. [journal article]. *Journal of Food Science and Technology, 53*(9), 1-12.

Formica-Oliveira, A. C., Martínez-Hernández, g. B., Díaz-López, V., Artés, F., & Artés-Hernández, F. (2017). Effects of UV-B and UV-C combination on phenolic compounds biosynthesis in fresh-cut carrots. *Postharvest Biology and Technology, 127*, 99-104.

Formica-Oliveira, A. C., Martínez-Hernández, G. B., Díaz-López, V., Otón, M., Artés, F., & Artés-Hernández, F. (2016b). *High hydrostatic pressure treatments for keeping quality of orange coloured vegetables smoothies.* Paper presented at the VIII International Postharvest Symposium, Cartagena, 21-24 June 2016

Francisco, M., Moreno, D. A., Cartea, M. E., Ferreres, F., Garcia-Viguera, C., & Velasco, P. (2009). Simultaneous identification of glucosinolates and phenolic compounds in a representative collection of vegetable Brassica rapa. *Journal of Chromatography A, 1216*(38), 6611-6619.

Frohnmeyer, H., & Staiger, D. (2003). Ultraviolet-B Radiation-Mediated Responses in Plants. Balancing Damage and Protection. *Plant Physiology, 133*(4), 1420-1428.

Gilbert, R. J., de Louvois, J., Donovan, T., Little, C., Nye, K., Ribeiro, C. D., et al. (2000). Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. PHLS Advisory Committee for Food and Dairy Products. *Communicable disease and public health, 3*(3), 163-167.

Guaadaoui, A., Benaicha, S., Elmajdoub, N., Bellaoui, M., & Hamal, A. (2014). What is a bioactive compound? A combined definition for a preliminary consensus. *International Journal of Nutrition and Food Sciences, 3*(3), 174-179.

Guo, C., Yang, J., Wei, J., Li, Y., Xu, J., & Jiang, Y. (2003). Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. *Nutrition Research, 23*(12), 1719-1726.

Hahlbrock, K., & Scheel, D. (1989). Physiology and Molecular Biology of Phenylpropanoid Metabolism. *Annual Review of Plant Physiology and Plant Molecular Biology, 40*(1), 347-369.

Han, C., Zhao, Y., Leonard, S. W., & Traber, M. G. (2004). Edible coatings to improve storability and enhance nutritional value of fresh and frozen strawberries (Fragaria × ananassa) and raspberries (Rubus ideaus). *Postharvest Biology and Technology, 33*(1), 67-78.

Hansen, M., Moller, P., Sorensen, H., & De Trejo, M. C. (1995). Glucosinolates in broccoli stored under controlled atmosphere. *Journal of the American Society for Horticultural Science, 120*, 1069-1074.

Helander, I. M., Nurmiaho-Lassila, E. L., Ahvenainen, R., Rhoades, J., & Roller, S. (2001). Chitosan disrupts the barrier properties of the outer membrane of Gram-negative bacteria. *International Journal of Food Microbiology, 71*(2–3), 235-244.

Heredia, J. B., & Cisneros-Zevallos, L. (2009). The effect of exogenous ethylene and methyl jasmonate on pal activity, phenolic profiles and antioxidant capacity of carrots (Daucus carota) under different wounding intensities. *Postharvest Biology and Technology, 51*(2), 242-249.

Hernández-Muñoz, P., Almenar, E., Valle, V. D., Velez, D., & Gavara, R. (2008). Effect of chitosan coating combined with postharvest calcium treatment on strawberry (Fragaria × ananassa) quality during refrigerated storage. *Food Chemistry, 110*(2), 428-435.

Hicks, K. B., Haines, R. M., Tong, C. B. S., Sapers, G. M., El-Atawy, Y., Irwin, P. L., et al. (1996). Inhibition of Enzymatic Browning in Fresh Fruit and Vegetable Juices by Soluble and Insoluble Forms of β-Cyclodextrin Alone or in Combination with Phosphates. *Journal of Agricultural and Food Chemistry, 44*(9), 2591-2594.

Hurst, W. C. (2002). Safety aspects of fresh–cut fruits and vegetables. In O. Lamikanra (Ed.), *Fresh–cut fruits and vegetables: science, technology and market* (pp. 46–85). Boca Raton, USA: CRC Press.

Hyson, D. A. (2011). A Comprehensive Review of Apples and Apple Components and Their Relationship to Human Health. *Advances in Nutrition: An International Review Journal, 2*(5), 408-420.

Imeri, A. G., & Knorr, D. (1988). Effects of Chitosan on Yield and Compositional Data of Carrot and Apple Juice. *Journal of Food Science, 53*(6), 1707-1709.

Iqbal, T., Rodrigues, F. A., Mahajan, P. V., Kerry, J. P., Gil, L., Manso, M. C., et al. (2008). Effect of minimal processing conditions on respiration rate of carrots. *Journal of Food Science, 73*(8), E396-402.

Iyengar, R., & McEvily, A. J. (1992). Anti-browning agents: alternatives to the use of sulfites in foods. *Trends in Food Science & Technology, 3*, 60-64.

Jacobo-Velázquez, D. A., & Cisneros-Zevallos, L. (2012). An Alternative Use of Horticultural Crops: Stressed Plants as Biofactories of Bioactive Phenolic Compounds. *Agriculture, 2*(3), 259.

Jacobo-Velazquez, D. A., & Hernandez-Brenes, C. (2010). Biochemical changes during the storage of high hydrostatic pressure processed avocado paste. *Journal of Food Science, 75*(6), S264-270.

Jacobo-Velázquez, D. A., Martínez-Hernández, G. B., del C. Rodríguez, S., Cao, C.-M., & Cisneros-Zevallos, L. (2011). Plants as Biofactories: Physiological Role of Reactive Oxygen Species on the Accumulation of Phenolic Antioxidants in Carrot Tissue under Wounding and Hyperoxia Stress. *Journal of Agricultural and Food Chemistry, 59*(12), 6583-6593.

Jongen, W. (2002). *Fruit and Vegetable Processing: Improving Quality*. Cambridge (England): Woodhead PublishingLimited

Jung, S., Samson, C. T., & Lamballerie, M. (2011). High hydrostatic pressure food processing. In A. Proctor (Ed.), *Alternatives to conventional food processing* (pp. 254–306). London, UK: Royal Society of Chemistry Publishing.

Kader, A. A., & Ben-Yehoshua, S. (2000). Effects of superatmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. *Postharvest Biology and Technology, 20*(1), 1-13.

Kalogeropoulos, N., Yannakopoulou, K., Gioxari, A., Chiou, A., & Makris, D. P. (2010). Polyphenol characterization and encapsulation in β-cyclodextrin of a flavonoid-rich Hypericum perforatum (St John's wort) extract. *LWT - Food Science and Technology, 43*(6), 882-889.

Kaur, M., & Sharma, H. K. (2013). Effect of enzymatic treatment on carrot cell wall for increased juice yield and effect on physicochemical parameters. *African Journal of Plant Science, 7*(6), 234-243.

Ke, D., & Saltveit, M. E. (1986). Effects of calcium and auxin on russet spotting and phenylalanine ammonia-lyase activity in iceberg lettuce. *HortScience, 21*(5), 1169-1171.

Keenan, D. F., Brunton, N. P., Gormley, T. R., Butler, F., Tiwari, B. K., & Patras, A. (2010). Effect of thermal and high hydrostatic pressure processing on antioxidant activity and colour of fruit smoothies. *Innovative Food Science and Emerging Technologies, 11*(4), 551-556.

Keenan, D. F., Rößle, C., Gormley, R., Butler, F., & Brunton, N. P. (2012). Effect of high hydrostatic pressure and thermal processing on the nutritional quality and enzyme activity of fruit smoothies. *LWT - Food Science and Technology, 45*(1), 50-57.

Kittur, F., Saroja, N., Habibunnisa, & Tharanathan, R. (2001). Polysaccharide-based composite coating formulations for shelf-life extension of fresh banana and mango. [journal article]. *European Food Research and Technology, 213*(4), 306-311.

Kjeldsen, F., Christensen, L. P., & Edelenbos, M. (2003). Changes in Volatile Compounds of Carrots (Daucus carota L.) During Refrigerated and Frozen Storage. *Journal of Agricultural and Food Chemistry, 51*(18), 5400-5407.

Knockaert, G., Lemmens, L., Van Buggenhout, S., Hendrickx, M., & Van Loey, A. (2012). Changes in β-carotene bioaccessibility and concentration during processing of carrot puree. *Food Chemistry, 133*(1), 60-67.

Koide, S. S. (1998). Chitin-chitosan: Properties, benefits and risks. *Nutrition Research, 18*(6), 1091-1101.

Kushad, M. M., Brown, A. F., Kurilich, A. C., Juvik, J. A., Klein, B. P., Wallig, M. A., et al. (1999). Variation of Glucosinolates in Vegetable Crops of Brassica oleracea. *Journal of Agricultural and Food Chemistry, 47*(4), 1541-1548.

Lante, A., & Zocca, F. (2010). Effect of β-cyclodextrin addition on quality of precooked vacuum packed potatoes. *LWT - Food Science and Technology, 43*(3), 409-414.

Leistner, L. (2000). Basic aspects of food preservation by hurdle technology. *International Journal of Food Microbiology, 55*(1–3), 181-186.

Lemmens, L., De Vleeschouwer, K., Moelants, K. R. N., Colle, I. J. P., Van Loey, A. M., & Hendrickx, M. E. (2010). β-Carotene Isomerization Kinetics during Thermal Treatments of Carrot Puree. *Journal of Agricultural and Food Chemistry, 58*(11), 6816-6824.

Lin, H.-Y., & Chou, C.-C. (2004). Antioxidative activities of water-soluble disaccharide chitosan derivatives. *Food Research International, 37*(9), 883-889.

López-Nicolás, J. M., Bru, R., Sánchez-Ferrer, A., & García-Carmona, F. (1995). Use of 'soluble lipids' for biochemical processes: linoleic acid-cyclodextrin inclusion complexes in aqueous solutions. *Biochemical Journal, 308*(1), 151-154.

López-Nicolás, J. M., Núñez-Delicado, E., Sánchez-Ferrer, A., & García-Carmona, F. (2007a). Kinetic model of apple juice enzymatic browning in the presence of cyclodextrins: The use of maltosyl-β-cyclodextrin as secondary antioxidant. *Food Chemistry, 101*(3), 1164-1171.

López-Nicolás, J. M., Pérez-López, A. J., Carbonell-Barrachina, A., & García-Carmona, F. (2007b). Kinetic study of the activation of banana juice enzymatic browning by the addition of maltosyl-beta-cyclodextrin. *Journal of Agricultural and Food Chemistry, 55*(23), 9655-9662.

López Camelo, A. F., & Gómez, P. A. (2004). Comparison of color indexes for tomato ripening. *Horticultura Brasileira, 22*, 534-537.

Luckey, T. (1980). *Hormesis with ionizing radiation.* Boca Raton, USA: CRC Press.

Ludikhuyze, L., Van Loey, A., Indrawati, Denys, S., & Hendrickx, M. (2002). Effects of high pressure on enzymes related to food quality. In M. Hendrickx, & D. Knorr (Eds.), *Ultra high pressure treatments of foods* (pp. 115–166). New York: Kluwer Academic Plenum Publisher.

Lule, S. U., & Xia, W. (2005). Food Phenolics, Pros and Cons: A Review. *Food Reviews International, 21*(4), 367-388.

Mackerness, S. A.-H.-. (2000). Plant responses to ultraviolet-B (UV-B: 280–320 nm) stress: What are the key regulators? [journal article]. *Plant Growth Regulation, 32*(1), 27-39.

MAGRAMA (2016a). Avances de superficies y producciones de cultivos. Madrid, Spain: MAGRAMA.

MAGRAMA (2016b). Informe del consumo de alimentación en España. Madrid, Spain: MAGRAMA.

Mahro, B., & Timm, M. (2007). Potential of Biowaste from the Food Industry as a Biomass Resource. *Engineering in Life Sciences, 7*(5), 457-468.

Martina, K., & Cravotto, G. (2015). Cyclodextrin as a Food Additive in Food Processing. In *Functional Polymers in Food Science* (pp. 267-288): John Wiley & Sons, Inc.

Martínez-Hernández, G. B. (2012). *Innovative minimal processing of mini broccoli for keeping quality and safety and enhancing bioactive compounds*. Universidad Politécnica de Cartagena, Cartagena, Spain.

Martínez-Hernández, G. B., Amodio, M. L., & Colelli, G. (2016a). Potential use of microwave treatment on fresh-cut carrots: physical, chemical and microbiological aspects. *Journal of the Science of Food and Agriculture, 96*(6), 2063-2072.

Martínez-Hernández, G. B., Artés-Hernández, F., Colares-Souza, F., Gómez, P. A., García-Gómez, P., & Artés, F. (2013a). Innovative cooking techniques for improving the overall quality of a kailan-hybrid broccoli. [journal article]. *Food & Bioprocess Technology, 6*(8), 2135-2149.

Martínez-Hernández, G. B., Artés-Hernández, F., Gómez, P. A., & Artés, F. (2013b). Comparative behaviour between kailan-hybrid and conventional fresh-cut broccoli throughout shelf-life. *LWT - Food Science and Technology, 50*(1), 298-305.

Martínez-Hernández, G. B., Artés-Hernández, F., Gómez, P. A., & Artés, F. (2013c). Induced changes in bioactive compounds of kailan-hybrid broccoli after innovative processing and storage. *Journal of Functional Foods, 5*(1), 133-143.

Martínez-Hernández, G. B., Artés-Hernández, F., Gómez, P. A., Formica, A. C., & Artés, F. (2013d). Combination of electrolysed water, UV-C and superatmospheric O2 packaging for improving fresh-cut broccoli quality. *Postharvest Biology and Technology, 76*, 125-134.

Martínez-Hernández, G. B., Boluda-Aguilar, M., Taboada-Rodríguez, A., Soto-Jover, S., Marín-Iniesta, F., & López-Gómez, A. (2016b). Processing, Packaging, and Storage of Tomato Products: Influence on the Lycopene Content. [journal article]. *Food Engineering Reviews, 8*(1), 52-75.

Martínez-Hernández, G. B., Gómez, P. A., Pradas, I., Artés, F., & Artés-Hernández, F. (2011). Moderate UV-C pretreatment as a quality enhancement tool in fresh-cut Bimi® broccoli. *Postharvest Biology and Technology, 62*(3), 327-337.

Martínez-Hernández, G. B., Huertas, J.-P., Navarro-Rico, J., Gómez, P. A., Artés, F., Palop, A., et al. (2015a). Inactivation kinetics of foodborne pathogens by UV-C radiation and its subsequent growth in fresh-cut kailan-hybrid broccoli. *Food Microbiology, 46*, 263-271.

Martínez-Hernández, G. B., Navarro-Rico, J., Gómez, P. A., Otón, M., Artés, F., & Artés-Hernández, F. (2015b). Combined sustainable sanitising treatments to reduce Escherichia coli and *Salmonella Enteritidis* growth on fresh-cut kailan-hybrid broccoli. *Food Control, 47*, 312-317.

Marx, M., Stuparic, M., Schieber, A., & Carle, R. (2003). Effects of thermal processing on trans–cis-isomerization of β-carotene in carrot juices and carotene-containing preparations. *Food Chemistry, 83*(4), 609-617.

McLaughlin, C. P., & O'Beirne, D. (1999). Respiration Rate of a Dry Coleslaw Mix Affected by Storage Temperature and Respiratory Gas Concentrations. *Journal of Food Science, 64*(1), 116-119.

Meléndez-Martínez, A. J., Gómez-Robledo, L., Melgosa, M., Vicario, I. M., & Heredia, F. J. (2011). Color of orange juices in relation to their carotenoid contents as assessed from different spectroscopic data. *Journal of Food Composition and Analysis, 24*.

Melendez-Martinez, A. J., Vicario, I. M., & Heredia, F. J. (2003). Application of tristimulus colorimetry to estimate the carotenoids content in ultrafrozen orange juices. *Journal of Agricultural and Food Chemistry, 51*(25), 7266-7270.

Mercier, J., Arul, J., & Julien, C. (1994). Effect of food preparation on the isocoumarin, 6-methoxymellein, content of UV-treated carrots. *Food Research International, 27*(4), 401-404.

Mewis, I., Schreiner, M., Nguyen, C. N., Krumbein, A., Ulrichs, C., Lohse, M., et al. (2012). UV-B Irradiation Changes Specifically the Secondary Metabolite Profile in Broccoli Sprouts: Induced Signaling Overlaps with Defense Response to Biotic Stressors. *Plant and Cell Physiology, 53*(9), 1546-1560.

Miglio, C., Chiavaro, E., Visconti, A., Fogliano, V., & Pellegrini, N. (2008). Effects of Different Cooking Methods on Nutritional and Physicochemical Characteristics of Selected Vegetables. *Journal of Agricultural and Food Chemistry, 56*(1), 139-147.

Mikkelsen, M. D., Petersen, B. L., Glawischnig, E., Jensen, A. B., Andreasson, E., & Halkier, B. A. (2003). Modulation of CYP79 genes and glucosinolate profiles in Arabidopsis by defense signaling pathways. *Plant Physiology, 131*(1), 298-308.

Mintel (2010). Prepared meals. London, UK: Mintel Reports.

Molina, V., Médici, M., Font de Valdez, G., & Taranto, M. P. (2012). Soybean-based functional food with vitamin B12-producing lactic acid bacteria. *Journal of Functional Foods, 4*(4), 831-836.

Montero-Calderón, M., Rojas-Graü, M. A., & Martín-Belloso, O. (2008). Effect of packaging conditions on quality and shelf-life of fresh-cut pineapple (Ananas comosus). *Postharvest Biology and Technology, 50*(2–3), 182-189.

Morales-de la Peña, M., Elez-Martínez, P., & Martín-Belloso, O. (2011). Food Preservation by Pulsed Electric Fields: An Engineering Perspective. [journal article]. *Food Engineering Reviews, 3*(2), 94-107.

Mossel, D. A., & Struijk, C. B. (1991). Public health implication of refrigerated pasteurized ('sous-vide') foods. *International Journal of Food Microbiology, 13*(3), 187-206.

Nakajima, S., Lan, L., Kanno, S., Takao, M., Yamamoto, K., Eker, A. P., et al. (2004). UV light-induced DNA damage and tolerance for the survival of nucleotide excision repair-deficient human cells. *Journal of Biological Chemistry, 279*(45), 46674-46677.

Nicolas, J. J., Richard-Forget, F. C., Goupy, P. M., Amiot, M. J., & Aubert, S. Y. (1994). Enzymatic browning reactions in apple and apple products. *Critical Reviews in Food Science and Nutrition, 34*(2), 109-157.

Nigro, F., Ippolito, A., Lattanzio, V., Di Venere, D., & Salerno, M. (2000). Effect of ultraviolet-C light on postharvest decay of strawberry. *Journal of Plant Pathology, 82*(1), 29-37.

No, H. K., Meyers, S. P., Prinyawiwatkul, W., & Xu, Z. (2007). Applications of Chitosan for Improvement of Quality and Shelf Life of Foods: A Review. *Journal of Food Science, 72*(5), R87-R100.

Özoğlu, H., & Bayındırlı, A. (2002). Inhibition of enzymic browning in cloudy apple juice with selected antibrowning agents. *Food Control, 13*(4–5), 213-221.

Palou, E., López-Malo, A., Barbosa-Cánovas, G. V., Welti-Chanes, J., & Swanson, B. G. (1999). Polyphenoloxidase Activity and Color of Blanched and High Hydrostatic Pressure Treated Banana Puree. *Journal of Food Science, 64*(1), 42-45.

Pathare, P. B., Opara, U. L., & Al-Said, F. A.-J. (2013). Colour Measurement and Analysis in Fresh and Processed Foods: A Review. [journal article]. *Food and Bioprocess Technology, 6*(1), 36-60.

Patras, A., Brunton, N., Da Pieve, S., Butler, F., & Downey, G. (2009a). Effect of thermal and high pressure processing on antioxidant activity and instrumental colour of tomato and carrot purées. *Innovative Food Science and Emerging Technologies, 10*(1), 16-22.

Patras, A., Brunton, N. P., Da Pieve, S., & Butler, F. (2009b). Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innovative Food Science & Emerging Technologies, 10*(3), 308-313.

Pattison, D. I., Rahmanto, A. S., & Davies, M. J. (2012). Photo-oxidation of proteins. [10.1039/C1PP05164D]. *Photochemical & Photobiological Sciences, 11*(1), 38-53.

Pereira, R. N., & Vicente, A. A. (2010). Environmental impact of novel thermal and non-thermal technologies in food processing. *Food Research International, 43*(7), 1936-1943.

Pushkala, R., Parvathy, K. R., & Srividya, N. (2012). Chitosan powder coating, a novel simple technique for enhancement of shelf life quality of carrot shreds stored in macro perforated LDPE packs. *Innovative Food Science and Emerging Technologies, 16*, 11-20.

Quitão-Teixeira, L. J., Odriozola-Serrano, I., Soliva-Fortuny, R., Mota-Ramos, A., & Martín-Belloso, O. (2009). Comparative study on antioxidant properties of carrot juice stabilised by high-intensity pulsed electric fields or heat treatments. *Journal of the Science of Food and Agriculture, 89*(15), 2636-2642.

Redovniković, I. R., Repajić, M., Fabek, S., Delonga, K., Toth, N., & Furač, J. V. (2012). Comparison of selected bioactive compounds and antioxidative capacity in different broccoli cultivars. *Acta Alimentaria, 41*(2), 221-232.

Rees, D. G., & Jones, D. H. (1996). Stability of l-phenylalanine ammonia-lyase in aqueous solution and as the solid state in air and organic solvents. *Enzyme and Microbial Technology, 19*(4), 282-288.

Regulation, E. (2006). Nutrition and health claims made on foods In E. p. a. o. t. council (Ed.), *L 404/9* (Vol. 1924/2006).

Rendueles, E., Omer, M. K., Alvseike, O., Alonso-Calleja, C., Capita, R., & Prieto, M. (2011). Microbiological food safety assessment of high hydrostatic pressure processing: A review. *LWT - Food Science and Technology, 44*(5), 1251-1260.

Reyes, L. F., Villarreal, J. E., & Cisneros-Zevallos, L. (2007). The increase in antioxidant capacity after wounding depends on the type of fruit or vegetable tissue. *Food Chemistry, 101*(3), 1254-1262.

Richer, D. L. (1987). Synergism—a patent view. *Pesticide Science, 19*(4), 309-315.

Rodriguez-Concepcion, M., & Stange, C. (2013). Biosynthesis of carotenoids in carrot: An underground story comes to light. *Archives of Biochemistry and Biophysics, 539*(2), 110-116.

Rodríguez-Verástegui, L. L., Martínez-Hernández, G. B., Castillejo, N., Gómez, P. A., Artés, F., & Artés-Hernández, F. (2015). Bioactive Compounds and Enzymatic Activity of Red Vegetable Smoothies During Storage. [journal article]. *Food and Bioprocess Technology, 9*(1), 137-146.

Ruhland, C. T., Xiong, F. S., Clark, W. D., & Day, T. A. (2005). The influence of ultraviolet-B radiation on growth, hydroxycinnamic acids and flavonoids of Deschampsia antarctica during Springtime ozone depletion in Antarctica. *Photochemistry and Photobiology, 81*(5), 1086-1093.

Ruxton, C. H. S. (2008). Smoothies: one portion or two? *Nutrition Bulletin, 33*(2), 129-132.

Saftner, R. A., Bai, J., Abbott, J. A., & Lee, Y. S. (2003). Sanitary dips with calcium propionate, calcium chloride, or a calcium amino acid chelate maintain quality and shelf stability of fresh-cut honeydew chunks. *Postharvest Biology and Technology, 29*(3), 257-269.

Saltveit, M. E. (2000). Wound induced changes in phenolic metabolism and tissue browning are altered by heat shock. *Postharvest Biology and Technology, 21*(1), 61-69.

Samuolienė, G., Brazaitytė, A., Sirtautas, R., Sakalauskienė, S., Jankauskienė, J., Duchovskis, P., et al. The impact of supplementary short-term red led lighting on the antioxidant properties of microgreens. In*, 2012* (956 ed., pp. 649-656): International Society for Horticultural Science (ISHS), Leuven, Belgium.

Sánchez-Moreno, C. (2002). Review: Methods Used to Evaluate the Free Radical Scavenging Activity in Foods and Biological Systems. *Food Science and Technology International, 8*(3), 121-137.

Sánchez-Rangel, J. C., Benavides, J., & Jacobo-Velázquez, D. A. PRODUCTION OF NUTRACEUTICALS IN CARROT BAGASSE USING ABIOTIC STRESSES. In*, 2013* (1012 ed., pp. 1475-1479): International Society for Horticultural Science (ISHS), Leuven, Belgium.

Sánchez–Pineda, M. T. (2003). *Procesos de elaboración de alimentos y bebidas*. Madrid, Spain: AMV–Ediciones Mundi Prensa.

Sapers, G. M. (1992). Chitosan Enhances Control of Enzymatic Browning in Apple and Pear Juice by Filtration. *Journal of Food Science, 57*(5), 1192-1193.

Scalbert, A., Manach, C., Morand, C., Remesy, C., & Jimenez, L. (2005). Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition, 45*(4), 287-306.

Scattino, C., Castagna, A., Neugart, S., Chan, H. M., Schreiner, M., Crisosto, C. H., et al. (2014). Post-harvest UV-B irradiation induces changes of phenol contents and corresponding biosynthetic gene expression in peaches and nectarines. *Food Chemistry, 163*, 51-60.

Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review. *Journal of Functional Foods, 18, Part B*, 820-897.

Shama, G., & Alderson, P. (2005). UV hormesis in fruits: a concept ripe for commercialisation. *Trends in Food Science & Technology, 16*(4), 128-136.

Sharma, K. D., Karki, S., Thakur, N. S., & Attri, S. (2011). Chemical composition, functional properties and processing of carrot—a review. [journal article]. *Journal of Food Science and Technology, 49*(1), 22-32.

Shikita, M., Fahey, J. W., Golden, T. R., Holtzclaw, W. D., & Talalay, P. (1999). An unusual case of 'uncompetitive activation' by ascorbic acid: purification and kinetic properties of a myrosinase from Raphanus sativus seedlings. *Biochemical Journal, 341*(Pt 3), 725-732.

Silva, L. C., Paiva, R., Vargas, D. P., da Silva, D. P. C., Herrera, R. C., Barbosa, S., et al. (2012). Cell viability of *Byrsonima intermedia* A Juss Calli. *Journal of Agricultural Science and Technology B, 2*, 713-720.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture, 16*(3), 144-158.

Siró, I., Kápolna, E., Kápolna, B., & Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance—A review. *Appetite, 51*(3), 456-467.

Slavin, J. L., & Lloyd, B. (2012). Health Benefits of Fruits and Vegetables. *Advances in Nutrition: An International Review Journal, 3*(4), 506-516.

Soliva-Fortuny, R. C., Grigelmo-Miguel, N., Odriozola-Serrano, I., Gorinstein, S., & Martín-Belloso, O. (2001). Browning Evaluation of Ready-to-Eat Apples as Affected by Modified Atmosphere Packaging. *Journal of Agricultural and Food Chemistry, 49*(8), 3685-3690.

Sønderby, I. E., Burow, M., Rowe, H. C., Kliebenstein, D. J., & Halkier, B. A. (2010). A Complex Interplay of Three R2R3 MYB Transcription Factors Determines the Profile of Aliphatic Glucosinolates in Arabidopsis. *Plant Physiology, 153*(1), 348-363.

Sonderby, I. E., Geu-Flores, F., & Halkier, B. A. (2010). Biosynthesis of glucosinolates--gene discovery and beyond. *Trends in Plant Science, 15*(5), 283-290.

Sørensen, H. (1990). Glucosinolates: Structure-Properties-Function. In F. Shahidi (Ed.), *Canola and Rapeseed: Production, Chemistry, Nutrition and Processing Technology* (pp. 149-172). Boston, MA: Springer US.

Soto, M., Falqué, E., & Domínguez, H. (2015). Relevance of Natural Phenolics from Grape and Derivative Products in the Formulation of Cosmetics. *Cosmetics, 2*(3), 259.

Souci, S. W., Fachmann, W., & Kraut, H. (2000). *Food Composition and nutrition tables*. Stuttgart, Germany: Medpharm Scientific Publishers Stuttgart.

Starzyńska, A., Leja, M., & Mareczek, A. (2003). Physiological changes in the antioxidant system of broccoli flower buds senescing during short-term storage, related to temperature and packaging. *Plant Science, 165*(6), 1387-1395.

Surjadinata, B., & Cisneros-Zevallos, L. (2003). Modeling wound-induced respiration of fresh-cut carrots (Daucus carota L.). *Journal of food science, 68*(9), 2735-2740.

Suslow, T. V. (1997). Postharvest chlorination- Basic properties and key points for effective disinfection. Davis (CA, USA): University of california, Division of Agriculture and Natural Resources.

Swain, T., & Hillis, W. E. (1959). The phenolic constituents of Prunus domestica. I.—The quantitative analysis of phenolic constituents. *Journal of the Science of Food and Agriculture, 10*(1), 63-68.

Talcott, S. T., & Howard, L. R. (1999). Phenolic Autoxidation Is Responsible for Color Degradation in Processed Carrot Puree. *Journal of Agricultural and Food Chemistry, 47*(5), 2109-2115.

Tamminen, M., Salminen, S., & Ouwehand, A. C. (2013). Fermentation of carrot juice by probiotics: Viability and preservation of adhesion. *International Journal of Biotechnology for Wellness Industries, 2*, 10-15.

Timperio, A. M., Egidi, M. G., & Zolla, L. (2008). Proteomics applied on plant abiotic stresses: role of heat shock proteins (HSP). *J Proteomics, 71*(4), 391-411.

Tirilly, Y., & Bourgeois, C. M. (2002). *Tecnología de las hortalizas*. Zaragoza, Spain.: Acribia.

Tomás-Barberán, F. A., & Espín, J. C. (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *Journal of the Science of Food and Agriculture, 81*(9), 853-876.

Tomás-Callejas, A., Otón, M., Artés, F., & Artés-Hernández, F. (2012). Combined effect of UV-C pretreatment and high oxygen packaging for keeping the quality of fresh-cut Tatsoi baby leaves. *Innovative Food Science and Emerging Technologies, 14*, 115-121.

Tontul, I., Topuz, A., Ozkan, C., & Karacan, M. (2016). Effect of vegetable proteins on physical characteristics of spray-dried tomato powders. *Food Science and Technology International*.

Traka, M., & Mithen, R. (2008). Glucosinolates, isothiocyanates and human health. [journal article]. *Phytochemistry Reviews, 8*(1), 269-282.

Traka, M., & Mithen, R. (2009). Glucosinolates, isothiocyanates and human health. [journal article]. *Phytochemistry Reviews, 8*(1), 269-282.

Tsao, R. (2010). Chemistry and Biochemistry of Dietary Polyphenols. *Nutrients, 2*(12), 1231-1246.

USDA (2013). Most commonly consumed fruits among U.S. consumers. In E. R. Service (Ed.).

Valenzuela, C., Tapia, C., López, L., Bunger, A., Escalona, V., & Abugoch, L. (2015). Effect of edible quinoa protein-chitosan based films on refrigerated strawberry (Fragaria × ananassa) quality. *Electronic Journal of Biotechnology, 18*(6), 406-411.

Vallejo, F., Tomas-Barberan, F., & Garcia-Viguera, C. (2003). Health-promoting compounds in broccoli as influenced by refrigerated transport and retail sale period. *Journal of Agricultural and Food Chemistry, 51*(10), 3029-3034.

Vargas-García, M. C., Andreu-Rodríguez, F. J., Fernández-Güelfo, L. A., Fernández-Morales, F. J., & García-Morales, J. L. (2014). Producción de residuos agrícolas. In *De residuo a recurso. El camino hacia la sostenibilidad*. Madrid, Spain: Grupo Mundi-Prensa.

Veiga, M. D., Merino, M., Fernández, D., & Lozano, R. (2002). Characterization of Some Cyclodextrin Derivatives by Thermal Analysis. [journal article]. *Journal of Thermal Analysis and Calorimetry, 68*(2), 511-516.

Villarreal-García, D., Nair, V., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2016). Plants as Biofactories: Postharvest Stress-Induced Accumulation of Phenolic Compounds and Glucosinolates in Broccoli Subjected to Wounding Stress and Exogenous Phytohormones. *Frontiers in Plant Science, 7*, 45.

Walkling-Ribeiro, M., Noci, F., Cronin, D. A., Lyng, J. G., & Morgan, D. J. (2008). Inactivation of Escherichia coli in a tropical fruit smoothie by a combination of heat and pulsed electric fields. *Journal of Food Science, 73*(8), M395-399.

Walkling-Ribeiro, M., Noci, F., Cronin, D. A., Lyng, J. G., & Morgan, D. J. (2010). Shelf life and sensory attributes of a fruit smoothie-type beverage processed with moderate heat and pulsed electric fields. *LWT - Food Science and Technology, 43*(7), 1067-1073.

Wang, J., Wang, B., Jiang, W., & Zhao, Y. (2007). Quality and Shelf Life of Mango (Mangifera Indica L. cv. `Tainong') Coated by Using Chitosan and Polyphenols. *Food Science and Technology International, 13*(4), 317-322.

Watada, A. E., Ko, N. P., & Minott, D. A. (1996). Factors affecting quality of fresh-cut horticultural products. *Postharvest Biology and Technology, 9*(2), 115-125.

Wen, A., Delaquis, P., Stanich, K., & Toivonen, P. (2003). Antilisterial activity of selected phenolic acids. *Food Microbiology, 20*(3), 305-311.

West, L. G., Meyer, K. A., Balch, B. A., Rossi, F. J., Schultz, M. R., & Haas, G. W. (2004). Glucoraphanin and 4-hydroxyglucobrassicin contents in seeds of 59 cultivars of broccoli, raab, kohlrabi, radish, cauliflower, brussels sprouts, kale, and cabbage. *Journal of Agricultural and Food Chemistry, 52*(4), 916-926.

WHO/FAO (2003). Population nutrient intake goals for preventing diet-related chronic diseases. In *Diet, nutrition and the prevention of chronic diseases* (Vol. 916, WHO Technical Report Series).

Wibowo, S., Vervoort, L., Tomic, J., Santiago, J. S., Lemmens, L., Panozzo, A., et al. (2015). Colour and carotenoid changes of pasteurised orange juice during storage. *Food Chemistry, 171*, 330-340.

Wiley, R. C. (1994). Preservation methods for minimally processed refrigerated fruits and vegetables. In R. C. Wiley (Ed.), *Minimally processed refrigerated fruits and vegetables* (pp. 66–134). New York, USA: Chapman & Hall.

Xiao, C., Zhu, L., Luo, W., Song, X., & Deng, Y. (2010). Combined action of pure oxygen pretreatment and chitosan coating incorporated with rosemary extracts on the quality of fresh-cut pears. *Food Chemistry, 121*(4), 1003-1009.

Yang, Z., Zheng, Y., & Cao, S. (2009). Effect of High Oxygen Atmosphere Storage on Quality, Antioxidant Enzymes, and DPPH-Radical Scavenging Activity of Chinese Bayberry Fruit. *Journal of Agricultural and Food Chemistry, 57*(1), 176-181.

Yordanov, D. G., & Angelova, G. V. (2010). High Pressure Processing for Foods Preserving. *Biotechnology & Biotechnological Equipment, 24*(3), 1940-1945.

Yu, L. J., & Rupasinghe, H. P. V. (2012). Effect of acidification on quality and shelf-life of carrot juice. *Canadian Journal of Plant Science, 92*(6), 1113-1120.

Zeisel, S. H. (1999). Regulation of "nutraceuticals". *Science, 285*(5435), 1853-1855.

Zhang, D., & Hamauzu, Y. (2004). Phenolic compounds and their antioxidant properties in different tissues of carrots (*Daucus carota* L.). *Food, Agriculture & Environment, 2*(1), 95-100.

Zheng, Y., Wang, C. Y., Wang, S. Y., & Zheng, W. (2003). Effect of High-Oxygen Atmospheres on Blueberry Phenolics, Anthocyanins, and Antioxidant Capacity. *Journal of Agricultural and Food Chemistry, 51*(24), 7162-7169.