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EQUIPAMIENTO AGRÍCOLA**

Ph. D. Thesis

**INNOVATIVE MINIMAL PROCESSING OF MINI BROCCOLI
FOR KEEPING QUALITY AND SAFETY AND ENHANCING
BIOACTIVE COMPOUNDS**

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La rama de conocimiento por la que esta Tesis ha sido desarrollada es Ingeniería y Arquitectura.

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*A mis padres,
por darme todo sin esperar nada a cambio,
por disfrutar y sufrir conmigo,
por enseñarme a vivir.*

“...Cuando creáis haber encontrado un hecho científico importante y os apremie el deseo de publicarlo, esperad unos días o unas semanas, o años; es preciso luchar, comprobar e incluso destruir los experimentos propios, es preciso agotar todas las hipótesis contrarias antes de proclamar el descubrimiento. Pero luego, al cabo de esfuerzos tan arduos, cuando la certeza llega, vuestra alegría será una de las más grandes que puede experimentar el alma humana...”.

Louis Pasteur (1822–1895)
Químico y biólogo francés.

*“La ciencia es respecto del alma
lo que es la luz respecto de los ojos,
y si las raíces son amargas,
los frutos son muy dulces”*

Aristóteles (384 AC–322 AC)
Filósofo griego.

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ABSTRACT

Broccoli has been described as the ‘super-vegetable’ in the media after a great number of epidemiological and laboratory studies on this *Brassica* specie. These studies have shown numerous health-promoting properties of broccoli such as chemopreventive, antioxidant, antitumor, antimutagenicity, anti-inflammatory, antimicrobial, antiviral and reduction of coronary heart disease risk. These properties are related to the high content of broccoli in phenolic compounds, glucosinolates (isothiocyanates –ITC–), carotenoids, vitamins, fatty acids, minerals, etc. For these reasons, the broccoli consumption has been promoted, unless the Spanish consumers are still reluctant to this vegetable due to its slightly bitter and astringent flavour and sulphur aromas (when cooked). In order to promote its consumption, the breeding companies have developed natural broccoli hybrids with other *Brassica* species with milder flavour than conventional varieties, such as the kailan or Chinese broccoli. This new kailan-hybrid broccoli is a vegetable with remarkable more pleasant flavour and aroma than the conventional broccoli varieties, and with a bioactive and nutritive profile similar to that of broccoli and kailan. Kailan-hybrid broccoli assembles the requirements for an excellent vegetable for the minimal processing or fresh-cut (FC) and fifth range product industries. However, the processing and storage of FC and fifth range commodities imply important changes on the physicochemical, microbial, sensory, bioactive and nutritional quality of them. Furthermore, the association of foodborne outbreaks with these products has considerably increased during the last years. In this way, and due to the undesirable by-products derived of the commonly used NaClO as sanitizer agent by the FC and fifth range industry, it is imperative to optimise the emerging NaClO-alternative sanitising treatments for the kailan-hybrid broccoli. Among them, the electrolysed water (EW), ultraviolet C light (UV-C) and the superatmospheric oxygen packaging (HO) have been studied. This Ph. D. Thesis covers many of the above mentioned research needs. In addition, the real health benefits of the consumption of this kailan-hybrid broccoli (raw or cooked) have been studied *in vivo*, obtaining a complete scientific-technologic knowledge of this particular vegetable from farm to human cells.

The research works have been performed within the Postharvest and Refrigeration Group and the Food Quality and Health Department of the Institute of Plant Biotechnology of the Universidad Politécnica de Cartagena (UPCT, Cartagena,

Spain). Some equipment of the Centro Tecnológico Nacional de la Conserva y la Alimentación (Molina de Segura, Murcia, Spain) and the Centro de Cualificación Turística (Murcia, Spain) have been also used in the experiments. The volunteer's recruitment and sampling for the *in vivo* studies were accomplished in the Nutrition Department of the Hospital General Universitario Reina Sofía (Murcia, Spain).

In the Chapter I, the physiological, physicochemical, microbial and sensory quality changes of the FC kailan–hybrid broccoli were compared with those of the conventional cv. Parthenon during 15 days at 2, 5 and 8 °C under air and modified atmosphere packaging (MAP). This cv. was selected due to be one of the most cultivated in Spain. As expected, the higher the temperature the higher the respiration rate and ethylene production, being higher for the kailan–hybrid broccoli than for the cv. Parthenon regardless of the temperature. MAP combined with low storage temperature resulted in a better microbial and sensory quality. Under MAP at 2 °C, decreases for mesophilic, psychophilic, enterobacteria and yeast and mould counts of 70, 88, 57 and 7 % for the cv. Parthenon and 43, 10, 57 and 10 % for the kailan–hybrid, respectively, were recorded at the end of storage compared to initial levels. After 15 days at 2 and 5 °C under MAP when compared to air–stored samples, undesirable sensory attributes changes, such as yellowness, off–flavour, and stem softening and bent, were retarded. Throughout conservation of air–stored samples at 8 °C, a reduction in total soluble solids content and an increase in yellowing were found. MAP storage at 2 °C (5–7 kPa O₂ + 14–15 kPa CO₂) or 5 °C (1.5–2.5 kPa O₂ + 15–16 kPa CO₂) provided for both broccoli cvs. an acceptable sensory quality and safety after 15 days.

Following the characterisation of the kailan–hybrid broccoli compared to cv. Parthenon reported in Chapter I, the Chapter II includes the effects of storage temperature and atmosphere composition on the nutritional quality and some bioactive compounds of both cvs. In this way, the Chapter II studies the nutritional quality and bioactive content changes of the FC kailan–hybrid broccoli compared with those of the cv. Parthenon throughout 15 days at 2, 5 and 8 °C under air and MAP. Florets showed higher dietary fibre content than stems. The total protein content of kailan–hybrid florets was 2.2–fold higher than that of cv. Parthenon. Higher amounts of S, Ca, Mg, Fe, Sr, Mn, Zn and Cu in the kailan–hybrid than those of cv. Parthenon were found. No data about the kailan–hybrid mineral content have been reported so far. Florets of broccoli cv. Parthenon registered higher initial total phenolic content than the kailan–hybrid,

followed by an increase throughout shelf-life favoured at 5 and 8 °C under MAP. MAP-stored samples at 8 °C showed higher individual phenolics content than MAP-stored samples at 2 °C. The initial total antioxidant capacity (TAC) of the kailan-hybrid florets was higher than that of the cv. Parthenon. As main conclusion, the kailan-hybrid florets generally showed healthier properties on the analysed bioactive and nutritive compounds compared to the conventional cv. Parthenon.

In Chapter III, the effects of several UV-C pretreatments (1.5, 4.5, 9 and 15 kJ m⁻²) on changes in physiological characteristics, sensory and microbial quality, and some bioactive compounds content over 19 days at 5 and 10 °C of FC kailan-hybrid broccoli were studied. Non-irradiated samples were used as controls. Low and moderate UV-C doses (1.5 and 4.5 kJ m⁻²) showed inhibitory effects on natural microflora growth. In relation to sensory quality, all treatments resulted in a shelf-life of 19 and 13 days at 5 and 10 °C, respectively, with the exception of 15 kJ m⁻² treated samples which resulted in a shorter shelf-life. These doses immediately induced an increase in total polyphenols content reaching 25 % more after 19 days at 5 °C compared to the initial value. All the hydroxycinnamoyl acid derivatives immediately increased after UV-C treatments, with values 4.8 and 4.5-fold higher for 4.5 and 9 kJ m⁻² treated samples, respectively, over the control. Changes in phenolic compounds were highly influenced by the storage temperature throughout shelf-life. The TAC generally followed the same pattern: the higher the UV-C doses, the higher the TAC values. Commonly, UV-C slightly reduced initial total chlorophyll content but delayed its degradation throughout shelf-life. It is concluded that a pretreatment of 4.5 kJ m⁻² is useful as a technique to improve epiphytic microbial quality and health-promoting compounds of FC kailan-hybrid broccoli.

In Chapter IV, the effects of neutral electrolysed water (NEW), UV-C and HO₂, in single or combined treatments, on the quality of FC kailan-hybrid broccoli up to 19 days at 5 °C were studied. As controls, washing with water and sanitation with NaClO were both used. Electrolyte leakage, sensory, microbial and nutritional quality, and bioactive content changes throughout shelf-life were studied. At day 15, the combined treatments achieved lower mesophilic and psychrophilic growth compared to the single ones. Single treatments induced higher ascorbate peroxidase (APX) activity reductions just after its application, while superoxide dismutase (SOD) activity showed the opposite behaviour. After 5 days at 5 °C, a great increase of APX and guaiacol

peroxidase (GPX) activity was found, achieving NEW+UV-C+HO and HO—including treatments the highest and the lowest APX activity increases, respectively. UV-C treatments induced the highest α -linolenic acid (ALA) decreases ranging around 35–38 % over control content on the processing day. Throughout shelf-life, NEW treatments greatly reduced ALA and stearic acid contents by 27–44 % and 31–61 %, respectively. During shelf-life, total phenolic content and TAC (1,415 mg chlorogenic acid equivalents–ChAE– kg^{-1}fw and 287 mg ascorbic acid equivalents antioxidant capacity–AAEAC– kg^{-1}fw , respectively) remained quite constant. In general, the studied treatments and their possible combinations seem to be promising techniques for keeping, or even enhancing, the quality of FC kailan–hybrid broccoli and, probably, other vegetables.

The aim of Chapter V was to study the microbial, physical, sensory and nutritional quality, and bioactive compounds content of boiled (vacuum and conventional), steamed, pressure-cooked, *sous vide*, microwaved (*sous vide* and conventional), deep-fried (vacuum and conventional) and grilled kailan–hybrid broccoli after cooking. *Sous vide* microwaving (*Sous vide*–MW) greatly decreased microbial counts, achieving very low psychrophilic and enterobacteria counts (1.1 and 0.2 log colony-forming unit –CFU– g^{-1} , respectively). Vacuum boiling and *sous vide* reduced the stem broccoli firmness by approximately 54–58 %, reaching a pleasant and moderate softening. *Sous vide*, grilling and steaming induced the lowest stem colour changes. Generally, all cooking treatments showed a good overall sensory quality. The total phenolic content (1,148 mg ChAE kg^{-1}fw) usually increased after cooking, with MW and grilled treatments registering the highest increases up to 2-fold. Commonly, the TAC (296.6 mg AAEAC kg^{-1}fw) increased after cooking by *sous vide*, MW and frying treatments showing the highest increments, by about 3.6-fold. Generally, the cooking process reduced the initial vitamin C content, with vacuum and conventional boiling showing the lowest and highest losses with 27 and 62 %, respectively, while vacuum deep frying preserved the initial value (1,737 mg kg^{-1}fw). As main conclusion, the studied grilling and vacuum-based cooking treatments resulted in better microbial quality, colour, stem firmness and sensory quality than the remaining ones. This maintained or even improved the TAC of the new kailan–hybrid broccoli studied.

In Chapter VI, the microbial, physical and sensory quality, and bioactive compounds changes of kailan–hybrid broccoli after industrial boiling, steaming, *sous*

vide, MW, *sous vide*–MW and grilling throughout 45 days at 4 °C were studied. Boiling, *sous vide*–MW and MW induced the highest total colour differences. Boiling and steaming produced the greatest stem softening. Based on the overall sensory quality, the commercial life was established in 45 days, except grilling (14 days) and *sous vide* (21 days). Apparently, cooking increased the total phenolic content up to 2.0 and 1.7–fold for grilling and MW, respectively, owing to a better extraction. *Sous vide*–MW, *sous vide* and MW induced the highest TAC increases around 5.4–4.7–fold, in contrast to the low enhancements of boiling and grilling (2.9–fold). The best chlorophylls retention was attained by boiling. The total carotenoids content was enhanced up to 1.5–2–fold. Conclusively, these treatments generally showed an excellent microbial reduction and health–promoting compounds content which, in some cases, was enhanced after 45 days.

The Chapter VII completes the Chapter VI in regard to the effects of those cooking treatments on other health–promoting compounds and nutrients of kailan–hybrid broccoli. In this way, the Chapter VII studies the glucosinolates, sulforaphane, vitamin C (sum of ascorbic and dehydroascorbic acids) and lutein contents after several industrial cooking methods on the kailan–hybrid broccoli and their changes during storage for 45 days at 4 °C. The glucosinolate profile firstly reported for kailan–hybrid revealed between 1.7 to 9.3–fold higher glucobrassicin content in their total edible fraction and florets (4.76 and 3.41 mg g⁻¹ dw, respectively) than that reported in florets from different conventional broccoli cvs. Boiling and *sous vide* induced the highest glucosinolate loss (80 %), while low pressure (LP) steaming, MW and *sous vide*–MW showed the lowest (40 %) loss. Glucoraphanin was the most thermo–stable. Throughout their commercial life, MW and grilled samples showed a decrease in total glucosinolates. Generally, myrosinase activity was completely inhibited after cooking with undetected sulforaphane contents. The initial total vitamin C dropped by up to 58 % after cooking and progressively decreased during storage, with *sous vide*–MW (92 %) and MW (21 %) showing the highest and lowest decrements, respectively. LP steaming and MW were the best industrial cooking methods for maintaining the glucosinolate and vitamin C contents, and enhancing up to 7.5–fold the initial lutein content. As far as we know, no studies on the effects of *sous vide*, *sous vide*–MW and grilling on glucosinolate, endogenous sulforaphane, vitamin C and lutein contents of broccoli have been reported, neither about their changes throughout the subsequent

commercial chilled storage. On the other hand, some works have studied the cooking effects on glucosinolate, but the information about the ITC levels (in particular sulforaphane, which is the biologically active form in humans) is very scarce.

The aim of Chapter VIII was to determine and compare the metabolic fate of ITC derived from glucosinolates after ingestion of 200-g raw or microwaved kailan-hybrid broccoli in 7 subjects in a cross-over design. The highest urinary ITC excretion rate of raw kailan-hybrid was found during the first 4 h after ingestion with 49.0 ± 7.0 %, over the total cumulative ITC excretion. From the other side, the highest urinary ITC excretion rate of the cooked samples was somehow delayed to the interval 4–10 h with 62.1 ± 4.8 %. The high ITC urinary excretion rates during the first 4 h are most likely due to the absorption in the small intestine, while the second peak (4–8 h) is presumably attributed to a ITC absorption in the colon. The excretion of the total amount of ITC ingested with raw kailan-hybrid (79.1 ± 18.8 %) was 2.5-fold higher than that from microwaved broccoli (32.0 ± 13.1 %). Our results show that the ITC absorption of the kailan-hybrid was 2-fold higher for raw and 3 to 9-fold higher for cooked samples than the values previously reported for conventional broccoli cvs. Consequently and as main conclusion, kailan-hybrid appears as a vegetable with great chemopreventive potential after ingestion than other *Brassica* species.

LIST OF ABBREVIATIONS

®: registered trade mark	C₁: conductivity after 1 minute
μM: micromolar	C₁: conductivity after 60 minute
μmol: micromole	CA: California
λ_{max}: maximum wavelength	CAT: catalase
μg: microgram	cc: cubic centimeters
μm: micrometer	CFU: colony–forming unit
A: absorbance	ChAE = CAE: chlorogenic acid equivalent
AAEAC = AAE: ascorbic acid equivalent antioxidant capacity	Chl <i>a</i>: chlorophyll a
AC: after Christ	Chl <i>b</i>: chlrophyll b
ACMFS: UK Advisory Committee on the Microbiological Safety of food	cm: centimeter
AEW: acidic electrolised water	C_T: total conductivity
AFHORLA: Asociación Española de Frutas y Hortalizas Lavadas, Listas para su Empleo	cv.: cultivar
ALA: α–linolenic acid	CV: coefficient of variation
AM: <i>ante meridiem</i>	d: days
amu: atomic mass unit	DAD: diode array detector
AsA: ascorbate	DF: distrito federal
ANOVA: analysis of variance	DHA: dehydroascorbic acid
AOAC: Association of Official Analytical Chemists	DKG: diketogulonic acid
APX: ascorbate peroxidase	DNA: desoxyribonucleic acid
ASTM: American Society for Testing Materials	DPPH: 2,2–diphenyl–1–picrylhydrazyl
atm: atmosphere	dw: dry weight
AUC: area under the curve	EC: Enzyme Comission number
aw: water activity	EDTA: ethylenediaminetetraacetic acid
BMI: body mass index	EFSA: European Food Safety Authority
BOPP: bi–oriented polypropylene	EL: electrolyte leakage
C: Celsius	ESI–MS: electrospray ionization–mass spectrometry
	ESP: epithiospecifier protein
	EU: European Union
	eV: electron volt
	EW: electrolysed water
	FA: fatty acid

FAME: fatty acid methyl esters	kV: kilovolts
FC: fresh-cut	kW: kilowatts
FDA: Food and Drug Administration	L*: lightness
FL: Florida	L: liter
FRAP: ferric reducing antioxidant power	L-AA: L-Ascorbic acid
fw: fresh weight	LC: liquid chromatography
g: gram	log: logarithm
g: gravitational acceleration	LP: low pressure
GAP: good agricultural practices	LSD: least significant difference
GC: gas chromatography	LU: limit of usability
GMP: good manufacturing practices	m/z: mass-to-charge ratio
GPR: Grupo de Postrecolección y Refrigeración	m: meter
GPX: guaiacol peroxidase	M: molar
GR: guaiacol reductase	mA: milliamperes
GSH: glutathione	MAGRAMA: Ministerio de Agricultura, Alimentación y Medio Ambiente de España
GSSG: glutathione disulfide	MAP: modified atmosphere packaging
h: hour	mAU: milliabsorbance units
ha: hectare	MD: Maryland
HACCP: Hazard Analysis Critical Control Point	MDA: monodehydroascorbate
H°: Hue angle	mg: milligram
HO: superatmospheric oxygen packaging	MHz: megahertz
HP: high pressure	min: minute
HPLC: high-performance liquid chromatography	mL: milliliter
ISO: International Organization for Standardization	mm: millimeter
ITC: isothiocyanate	mM: millimolar
J: Joule	MO: Missouri
kg: kilogram	MP: minimally processed
kJ: kilojoules	MPa: megapascal
km: kilometres	MS: mass spectrometry
kPa: kilopascal	mV: millivolt
	MW: microwaving
	N: normal

List of abbreviations

NAD: dinucleotide phosphate	SOD: superoxide dismutase
NADH: monodehydroascorbate reductase	SOP: standard operation procedures
NADPH: nicotinamide adenine dinucleotide phosphate	SPE: solid phase extraction
NBT: nitro blue tetrazolium	spp.: species
ND: not detected	SSC: soluble solid content
NEW: neutral electrolysed water	SV: <i>sous vide</i>
nm: nanometer	SVAC: <i>Sous vide</i> Advisory Committee
nmol: nanomol	t: tons
NY: New York	TA: total acidity
ORP: oxide reduction potential	TAC: total antioxidant capacity
PA: palmitic acid	TAM: total aerobic mesophilic
PAD: photodiode array detector	TCD: thermal conductivity detector
PAL: phenylalanine ammonia lyase	TFA: trifluoroacetic acid
PCA: plate count agar	THM: tri-halo-methane
PET: polyethylene terephthalate	TP: total phenolics
pH: potential hydrogen	TTI: time-temperature integrators
PP: polypropylene	U: enzymatic unit
ppm: parts per million	UCDavis: University of California, Davis
PPO: polyphenol oxidase	UK: United Kingdom
PTFE: polytetrafluor ethylene	UPCT: Universidad Politécnica de Cartagena
RD: Real Decreto	USA: United States of America
REFPED: Refrigerated Pasteurized Foods of Extended Durability	USDA: United States Department of Agriculture
RH: relative humidity	UV: ultraviolet
ROS: reactive oxygen species	v/v: volume/volume
RR: respiration rate	V: volt
Rt: retention time	VA: Virginia
s: seconds	w/v: weight/volume
SA: stearic acid	W: watts
SAS: Statistical Analysis System	XRF: X-ray fluorescence
SD: standard deviation	
SE: standard error	
SEM: Scanning electron microscopy

INTRODUCTION

1. BROCCOLI, KAILAN AND KAILAN-HYBRID BROCCOLI BACKGROUND. AGRONOMICAL CHARACTERISTICS AND ECONOMIC ASPECTS

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1.1. Broccoli

Broccoli (*Brassica oleracea*, Italica group) is a vegetable of the *Brassicaceae* family. In this Family are also included other vegetables such as arugula, cauliflower, collards, bokchoy (Chinese cabbage), kailan (Chinese broccoli), kale, mustard greens, radish, turnips, watercress, rutabaga and Brussels sprouts. The word *broccoli* comes from the Italian plural of *broccolo*, diminutive of *brocco* (shoot), based on the Latin word *brocchus* (projecting). By 8,000 years ago, plants of the mustard family, to which broccoli phylogenetically belongs, were cultivated for food by the civilizations of Persia and China. The phylogenetic parent of broccoli was a wild cabbage taken to Persia after the Aryan invasion of Europe, from where they diffused it throughout the Mediterranean region. The first accurate knowledge about the genus *Brassica* begins with the Hellenic culture, 25 centuries ago. Lately, during the Roman Empire, this broccoli ancestor reached the Italian peninsula, where it was cultivated for human consumption from the first century AC. The present broccoli varieties have been developed from selections of desirable types which have mainly occurred in Italy within the past 2,000 years. During the past 300 years, due to the horticultural selection, some types of *Brassica* became highly improved, culminating in the cauliflowers and the heading broccoli. In the mid 20th century, the broccoli production spread from Italy to the rest of Europe and, lately, to the rest of the world (Buck, 1956).

Broccoli is a cool-season crop that grows preferably with extended cool weather in spring and fall (or during winter months in mild areas). The optimum daily temperatures for broccoli growth are ranged between 18 and 23 °C. The adult plant can reach 60–90 cm of height and 60 cm of diameter. The edible part of the plant corresponds to the inflorescence, which has a characteristic green–blue colour (Figure

1.1) and a special slight bitter taste. The broccoli head weight, for most commercial varieties, ranges between 550 g in cv. Bohr and 766 g in cv. Parthenon (Rogers, 2009).

Figure 1.1. Broccoli (cv. Parthenon).

Globally, the world produced 22,226,957 t of broccoli and cauliflower in 2009, with an increase of 5.3 % over the previous year. Between 2000 and 2009, worldwide production of broccoli and cauliflower grew by 33 %. China has been the top broccoli and cauliflower producer for many years, growing 9,428,012 t in 2009 (45 % more than in 2000). India harvested 7,315,832 t of these crops in 2009, 13 % more than in 2008. Other key producers are Italy, Spain, France and the USA with 515,942, 480,927, 448,000 and 338,744 t, respectively, of broccoli and cauliflower production in 2009. In Italy, Spain and the USA, the broccoli and cauliflower production fell in the course of this 10-year period, whereas the production in France increased (USDA, 2011). In Spain, the campaign 2011/2012 produced 358,300 t of broccoli with a cultivated surface of about 22,000 ha (MAGRAMA, 2012a).

The main broccoli production of Spain is located in the Región de Murcia, which registered the 46 % of the national production and a cultivated area of 10,618 ha in the 2010/11 campaign (MAGRAMA, 2012a). In terms of area and production, broccoli has gained importance in the Región de Murcia with respect to traditional crops, such as cotton, pepper (cv. Bola), cauliflower, etc. The latter production increase has been favoured by the easy culture conditions, short cycle and its good adaptation to all types of soil and water. The price has also influenced the augmented interest in this crop, since it has gradually increased every year. Due to the importance of this

vegetable in the Región de Murcia, some companies have been specialized in its production, shipping and processing (Martínez del Vas, 2002).

In spite of the high Spanish production, each Spanish consumer only purchased an average of 200 g year⁻¹ in 2011. Contrary to this low consumption, UK consumed 4 kg person⁻¹ year⁻¹ in the last campaign (data supplied by Sakata Seed Ibérica, S.L.U.). For this reason, associations like +Brócoli (<http://www.masbrocoli.com>) have been created in order to promote its consumption among the Spanish consumers. The breeding companies are also developing new varieties and hybrids with milder flavour than that of the traditional broccoli varieties.

1.2. Kailan

The origin of kailan (*B. oleracea*, Alboglabra group) is considered to be in Europe. However, it is most widely consumed in Asia. The Portuguese are said to have brought the vegetable to China during the early days of the Portuguese expansion and colonization. Historical evidences suggest that while the current broccoli form is most closely related to cabbages, such as the European Calabrese, kailan is most likely a direct offshoot of the Portuguese Tronchuda cabbage (*B. oleracea* cv. Costata). Some other names of kailan are Chinese broccoli, Chinese kale, jie-lan, gai-lan, leaf broccoli, etc. This vegetable is well adapted to cold temperatures and it grows well in a variety of soil and climate conditions. For these reasons, kailan is widely distributed in South China and other Southeast Asia areas, and relatively small quantities in Japan, Europe and America (Sun *et al.*, 2011).

Kailan is usually grown for its bolting stems (common edible part), which are pleasantly tender and crisp. This is a vegetable featuring thick, flat, glossy blue-green leaves with thick stems and a small number of tiny, almost vestigial flower heads similar to those of broccoli (Figure 1.2). The leaves, which may be smooth or wrinkled, are similar to those of broccoli.

Kailan grows best in well-drained and fertile soils with ample moisture. Most varieties grow to about 45 cm when mature. Its flavour is very similar to that of broccoli, but slightly more bitter than broccoli. For this reason, it has been naturally hybridized with broccoli to take advantage of this pleasant flavour. Although it is a perennial plant, it is commercially grown as an annual crop. It can spread up to 40 cm in

diameter and it can reach 45 cm in height. Kailan has some frost tolerance and can be grown all year around. However, it is crucial to select the right cv. for every season. Conducted trials have indicated that the late spring and summer are the best times to grow kailan. The growth of kailan is very slow from autumn onwards (Noichinda *et al.*, 2007).

Figure 1.2. Kailan.

1.3. Kailan–hybrid broccoli

Bimi[®] (Sakata Vegetables Europe) is a natural hybrid between broccoli and kailan. 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 1.3). Similarly to broccoli, kailan–hybrid broccoli has yellow flowers. The kailan–hybrid broccoli has a flavour softer and more delicate than the conventional broccoli. Figure 1.4 shows the large differences in visual appearance between this new vegetable and broccoli.

Figure 1.3. Bimi[®]: A kailan–hybrid broccoli (<http://www.bellaverde.co.uk/>).

Figure 1.4. Conventional broccoli (cv. Parthenon) (left) and kailan–hybrid broccoli (right) (Martínez–Hernández *et al.*, 2013).

Kailan–hybrid 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 kailan–hybrid broccoli by minimizing the handling, its primary packaging is made on the fields. Due to the mild sensory characteristics of kailan–

hybrid broccoli, compared to the conventional broccoli cv., it can be eaten either raw (i. e., in salads) or cooked. Kailan–hybrid broccoli can be cooked with mild cooking procedures due to its tenderness and small size (with better heat transmission during cooking). This is the great advantage that drastically reduces the inevitable nutritional losses during the cooking treatments, which are usually conducted for the consumption of the conventional broccoli cvs. due to their characteristic bitter and astringent flavour.

The production of this vegetable is concentrated from October to June in warm areas, while in the summer months it is produced in northern locations as UK, The Netherlands, etc. However, commercial production is mainly located in Africa, where production is assured all year around with intensive farming systems. Spain is among the main kailan–hybrid producers with a cultivated surface (principally concentrated in the south–east area) of 16 ha and a production of 65 t in the campaign 2010/11 (data supplied by Sakata Seed Ibérica). Consumption of kailan–hybrid 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. MAIN BIOACTIVE AND NUTRITIONAL COMPOUNDS OF BROCCOLI AND KAILAN

The relationship between dietary habits and disease risk has been analyzed in several epidemiological studies showing that plant–derived foods, fruit and vegetables in a great extent, have a direct impact on health (Tomás–Barberán and Gil, 2008). These health–promoting properties of fruit and vegetables have been mainly associated to the existence of bioactive compounds such as phenolic compounds, glucosinolates, carotenoids, chlorophylls, fatty acids and antioxidant enzymes, among others. Bioactive compounds are extra–nutritional constituents that typically occur in small quantities in foods (Kris–Etherton *et al.*, 2002). Nutritional compounds are those which the human 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). The main nutritional compounds of broccoli are described in Table 2.1 and in the following paragraphs. The information data concerning to the bioactive and nutritional contents of kailan–hybrid broccoli is very scarce and, from the scientific point of view, very poor. The bioactive

and nutritional compounds studied in the present Ph. D. Thesis are described in the following paragraphs.

Table 2.1. Main constituents of broccoli (Souci *et al.*, 2000).

Constituents	Content (per 100 g fw)
Water	89 g
Protein (N x 6.25)	3.5 g
Fat	0.2 g
Carbohydrates	2.7 g
Total dietary fibre	3.0 g
Total minerals	1.1 g
Vitamin C	100 mg
Vitamin B1	99 µg
Vitamin B2	178 µg
Vitamin B5	1.3 mg
Vitamin B6	280 µg
Vitamin E	621 µg
Vitamin K	154 µg
Vitamin PP	1.0 mg
Folic acid	114 µg

2.1. Nutritional compounds

2.1.1. Dietary fibre and proteins

The dietary fibre is mainly composed by macromolecules such as cellulose, hemicellulose, pectins, lignin, resistant starch and non-digestible oligosaccharides (Tarazona–Díaz, 2011). Broccoli is a rich source of dietary fibre with around 3.0 % fw (Souci *et al.*, 2000). However, the dietary fibre content can greatly vary among different broccoli cvs.

In animals, amino acids are obtained through the consumption of foods containing proteins. Generally, the protein content of fruit and vegetables is less than 1 % (fw). However, broccoli is an excellent protein source with quantities between 1 and 4 %, depending of the cv. (Zhuang *et al.*, 1997; Souci *et al.*, 2000).

2.1.2. Minerals

Minerals are micronutrients that, although they yield no energy, are necessary for the maintenance of certain essential physicochemical processes of the human

metabolism (Soetan *et al.*, 2010). Minerals may be broadly classified as macro (major) or micro (trace) elements. The macroelements (Na, K, Ca, Mg, Cl, P and S) are essential for human beings in amounts above 50 mg day⁻¹, while microelements (Fe, Zn, Cu, Mn, I, F, Se, Cr, Mo, Co and Ni) are essential in concentrations below 50 mg day⁻¹ (Moreno *et al.*, 2006).

Broccoli and kailan are good sources of all macro elements and some important microelements such as Fe, Zn, Mn and Cu (Rosa *et al.*, 2002) (Table 2.2). Although both vegetables have a similar mineral profile, kailan has approximately 7-fold higher Fe content than broccoli (Acikgoz, 2011; Hanson *et al.*, 2011). However, the mineral composition varies among cvs. and is also influenced by several preharvest factors like climate conditions, cultural practices, etc. (Rosa *et al.*, 2002).

Table 2.2. Mineral content ranges among different broccoli cultivars (Extracted from Rosa *et al.*, 2002; López–Berenguer *et al.*, 2007).

Mineral	Content
Na	2.0–3.2 ^a
K	23.9–33.8 ^a
Ca	2.1–6.8 ^a
Mg	1.3–2.0 ^a
Cl	4.1–54.0 ^a
P	4.9–9.6 ^a
S	8.9–18.7 ^a
Fe	79.35–96.1 ^b
Zn	35.2–52.8 ^b
Mn	30.1–32.4 ^b
Cu	4.0–13.0 ^b

^a mg g⁻¹ dry weight (dw)

^b µg g⁻¹ dw

2.1.3. Vitamin C

Among broccoli vitamins (C, B1, B2, B5, B6, E, K, PP, etc.), vitamin C is the most studied for its high levels in this vegetable. The majority of vertebrates are able to synthesize vitamin C (L-AA), except a few mammalian species including primates, humans and guinea pigs, being this deficiency localised to a lack of the terminal, flavo-enzyme, L-gulonolactone oxidase (Davey *et al.*, 2000). L-AA (the AA isomer that occurs in nature) is stable when dry, but solutions readily oxidise, especially in the

presence of trace amounts of Cu and alkali, may leading to the DHA formation. DHA is unstable itself and undergoes irreversible hydrolytic ring cleavage to 2,3-diketogulonic acid (2, 3-DKG) (without antiscorbutic activity) in aqueous solution. Factors such as concentration, temperature, light, pH, etc. influence the L-AA oxidation and DHA hydrolysis. The 2, 3-DKG and D-isoascorbic acid (AA stereoisomer) have little, if any, antiscorbutic activity (Davey *et al.*, 2000). Generally, three types of biological activity can be defined for L-AA: enzyme cofactor, radical scavenger and donor/acceptor in the electron transport either at the plasma membrane or the chloroplasts (Davey *et al.*, 2000). The L-AA dietary needs refer to a minimum intake of 60 mg day⁻¹, unless it was recommended to be increased to 75 mg day⁻¹ for women and 90 mg day⁻¹ for men (Padayatty and Levine, 2001).

Among vegetables, broccoli and kailan may be considered as excellent L-AA sources. Many broccoli cvs. have shown levels between 432 and 1,463 mg kg⁻¹ fw (Vallejo *et al.*, 2002a). Kailan also registered high L-AA content around 900 to 1,300 mg kg⁻¹ (Ismail and Fun, 2003; Hanson *et al.*, 2011).

2.1.4. Fatty acids

Fatty acids are carboxylic acids with a long aliphatic chain, which can be either saturated (no double bounds) or unsaturated (one or more double bounds). The ω -3 fatty acids are those which double bounds are located after the carbon 3 (starting from the -CH₃ extreme), while the ω -6 are those starting from carbon 6. Essential fatty acids are those which must be ingested by humans and other animals, due to the incapacity to synthesize them, in order to keep the body metabolism requirements. The ω -3 fatty acids can prevent cardiovascular inflammatory diseases and protect, or even enhance, the effect in medical treatments of diseases like Alzheimer, multiple sclerosis and cancer (Gogus and Smith, 2010). It has been reported the importance of a balanced intake of ω -6 and ω -3 fatty acids, with a ratio of 1-4:1, in order to achieve their health beneficial effects in the control of chronic diseases (Simopoulos, 1999).

The major fatty acids reported in broccoli are the two unsaturated fatty acids α -linolenic acid [(C18:3, $\Delta^{9,12,15}$) (53 % v/v) (ω -3)] and linoleic acid [(C18:2, $\Delta^{9,12}$) (18 % v/v) (ω -6)], and the saturated palmitic acid (C16:0, 16 % v/v). Broccoli also have small amounts of other unsaturated fatty acids (C16:1, C16:2, C16:3 and C18:1) and the

saturated stearic acid (C18:0) (Page *et al.*, 2001). The total fatty acids content in broccoli (cv. Marathon) was 34.2 $\mu\text{mol g}^{-1}$ fw, amounting α -linolenic and linoleic acids (the main polyunsaturated fatty acids of broccoli) approximately a 19 and 53 %, respectively, of the total fatty acids (Page *et al.*, 2001). Similarly, Zhuang *et al.* (1997) reported that the total polyunsaturated acids content of broccoli (cv. Iron Duke) represented a 68 % of the total fatty acids content.

2.2. Bioactive compounds

2.2.1. Phenolic compounds

Phenolics are characterized as having an aromatic ring bearing one or more hydroxyl groups, including their functional derivatives. In nature, phenolics are usually found conjugated to sugars and organic acids. Phenolic compounds can be divided in two groups: flavonoids and non-flavonoids. Among flavonoid phenolics (which share a basic structure consisting of two benzene rings linked through a heterocyclic pyrone C ring) are flavonols, flavanones, flavan-3-ols, anthocyanins and isoflavones (Ferrerres and Tomás-Barberán, 2012). In contrast, non-flavonoid phenolics include a more heterogeneous group of compounds including from the simplest of the class such as C₆-C₁ benzoic acids and C₆-C₃ hydroxycinnamates to more complex compounds such as C₆-C₂-C₆ stilbenes, C₆-C₃-C₃-C₆ lignans and hydrolysable tannins (gallotannins and ellagitannins) (Tomás-Barberán, 2003; Shahidi and Naczk, 2004). The phenolic compounds contribute to flavour and colour of the plants. Furthermore, these compounds have many bioactive properties such as antimicrobial, antiviral, anti-inflammatory, antitumor, anticancer, antimutagenicity, antioxidant potential and reduction in coronary heart disease risk (Lule and Xia, 2005).

The most widespread and diverse group of polyphenols in broccoli and kailan are the hydroxycinnamic acids, flavonols and anthocyanins (Table 2.3). Redovniković *et al.* (2012) studied the phenolic content of 13 different broccoli cvs., reporting data ranges for total phenolics, and sinapic and caffeic acid derivatives of 15.5–26.9, 4.2–7.9 and 0.4–3.2 mg g^{-1} dw, respectively. 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).

Table 2.3. Phenolic compounds present (marked as *x*) in broccoli and kailan [elaborated from data of Lin and Harnly (2009), and Cartea *et al.*, (2011)].

Phenolic compounds	Broccoli	Kailan
<i>Hydroxycinnamic acids</i>		
3- <i>p</i> -coumaroyl quinic acid	<i>x</i>	<i>x</i>
5- <i>p</i> -coumaroyl quinic acid		<i>x</i>
5-feruloyl quinic acid		<i>x</i>
Caffeic acid		<i>x</i>
3-caffeoyl quinic acid (chlorogenic acid)	<i>x</i>	<i>x</i>
4-caffeoyl quinic acid		<i>x</i>
5-caffeoyl quinic acid (neochlorogenic acid)	<i>x</i>	<i>x</i>
Sinapic acid		<i>x</i>
1,2-disinapoylgentiobiose	<i>x</i>	<i>x</i>
1,2-diferuloylgentiobiose	<i>x</i>	<i>x</i>
1-sinapoyl-2-feruloylgentiobiose	<i>x</i>	<i>x</i>
1-sinapoyl-2,2'-diferuloylgentiobiose	<i>x</i>	<i>x</i>
1-disinapoyl-2-feruloylgentiobiose		<i>x</i>
1,2,2'-trisinapoylgentiobiose	<i>x</i>	<i>x</i>
1,2'-disinapoyl-2-feruloylgentiobiose	<i>x</i>	<i>x</i>
<i>Isorhamnetin (I) derivatives</i>		
I-O-3-sophorotrioside-7-glucoside	<i>x</i>	
I-O-3-sophorotrioside-7-sophoroside	<i>x</i>	
I-O-3,7-di-O-glucoside	<i>x</i>	
I-O-diglucoside		<i>x</i>
I-O-glucoside		<i>x</i>
<i>Quercetin (Q) derivatives</i>		
Q-3-O-sophorotrioside-7-O-sophoroside	<i>x</i>	
Q-3-O-sophorotrioside-7-O-glucoside	<i>x</i>	
Q-3-O-sophoroside-7-O-glucoside	<i>x</i>	
Q-3,7-di-O-glucoside	<i>x</i>	<i>x</i>
Q-3-O-sophoroside	<i>x</i>	
Q-3-O-glucoside	<i>x</i>	
Q-3-O-glucoside-7-O-sophoroside	<i>x</i>	
Q-3-O-diglucoside		<i>x</i>
Q-3-O-(caffeoyl)-sophorotrioside-7-O-glucoside	<i>x</i>	
Q-3-O-(caffeoyl)-diglucoside-7-O-glucoside		<i>x</i>
Q-3-O-(sinapoyl)-sophorotrioside-7-O-glucoside	<i>x</i>	
Q-3-O-(sinapoyl)-sinapoyldiglucoside		<i>x</i>
Q-3-O-(feruloyl)-sophorotrioside-7-O-glucoside	<i>x</i>	
Q-3-O-(<i>p</i> -coumaroyl)-sophorotrioside-7-O-glucoside	<i>x</i>	
Q-3-O-(feruloyl)-diglucoside-7-O-glucoside		<i>x</i>
Q-3-O-(caffeoyl)-sophoroside-7-O-glucoside	<i>x</i>	
Q-3-O-(<i>p</i> -coumaroyl)-sophoroside-7-O-glucoside	<i>x</i>	
Q-3-O-(feruloyl)-sophoroside	<i>x</i>	

<i>Kaempferol (K) derivatives</i>		
K-3-O-tetraglucoside-7-O-sophoroside		
K-3-O-(hydroxyferuloyl)-diglucoside-7-O-glucoside		x
K-3-O-(caffeoyl)-diglucoside-7-O-glucoside		x
K-3-O-sinapoyltriglucoside-7-O-diglucoside		x
K-3-O-sinapoyldiglucoside-7-O-glucoside		x
K-3-O-sophorotrioside-7-O-sophoroside	x	
K-3-O-sophorotrioside-7-O-glucoside	x	
K-3-O-sophoroside-7-O-diglucoside	x	
K-3-O-sophoroside-7-O-glucoside	x	
K-3-O-sophoroside-7-O-sophoroside	x	
K-3,7-di-O-glucoside	x	
K-3-O-sophoroside	x	
K-3-O-triglucoside-7-O-diglucoside		x
K-3-O-glucoside	x	
K-3-O-glucoside-7-O-sophoroside	x	
K-3-O-(caffeoyl)-sophorotrioside-7-O-sophoroside	x	
K-3-O-(methoxycaffeoyl)-sophorotrioside-7-O-sophoroside	x	
K-O-(sinapoyl)-sophorotrioside-7-O-sophoroside	x	
K-O-(feruloyl)-sophorotrioside-7-O-sophoroside	x	
K-3-O-(sinapoylhydroxyferuloyl)-triglucoside-7-O-diglucoside		x
K-3-O-(feruloylhydroxyferuloyl)-triglucoside-7-O-diglucoside		x
K-3-O-(<i>p</i> -coumaroyl)-diglucoside-7-O-glucoside		x
K-3-O-(feruloyl)-triglucoside-7-O-glucoside		x
K-3-O-(diferuloyl)-triglucoside-7-O-diglucoside		x
K-3-O-(feruloyl)-diglucoside-7-O-glucoside		x
K-3-O-(<i>p</i> -coumaroyl)-sophorotrioside-7-O-sophoroside	x	
K-3-O-(<i>p</i> -coumaroyl)-glucoside-7-O-sophoroside		x
K-3-O-(caffeoyl)-sophorotrioside-7-O-glucoside	x	
K-3-O-(methoxycaffeoyl)-sophorotrioside-7-O-glucoside	x	
K-O-(sinapoyl)-sophorotrioside-7-O-glucoside	x	
K-O-(feruloyl)-sophorotrioside-7-O-glucoside	x	
K-3-O-(caffeoyl)sophoroside-7-O-glucoside	x	
K-3-O-(methoxycaffeoyl)-sophoroside	x	
K-3-O-(sinapoyl)-diglucoside		x
K-3-O-(disinapoyl)-triglucoside-7-O-diglucoside		x
K-3-O-(feruloyl)-diglucoside		x

2.2.2. Glucosinolates and isothiocyanates

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. Four types of glucosinolates can be found in *Brassicas*: aromatic

(derived from phenylalanine), aliphatic, alkenyl (the last two derived from methionine) and indoles glucosinolates (derived from tryptophan) (Wallsgrove and Bennett, 1995). Myrosinase (thioglucoside glucohydrolase; EC 3.2.3.1) 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 catalyzes the glucosinolates conversion to ITC after several reactions (Figure 2.1). 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 (Fahey *et al.*, 1999). Glucosinolates are water soluble, anionic, non-volatile and heat-stable compounds. It is believed that these molecules have no significant biological activity (Fahey *et al.*, 1997). Contrary to them, ITC are biologically active, typically lipophilic, highly reactive, volatile, malodorous and bitter (Fahey *et al.*, 1999).

Figure 2.1. Enzymatic conversion of glucosinolates to ITC by plant myrosinase (Shikita *et al.*, 1999).

Glucosinolates profiles may vary among *Brassica* species and, in turn, the glucosinolate content differs among different cvs. Glucosinolate content is highly influenced by genetics, but its levels can also be significantly altered by the environment that the crop was grown under (Jeffery *et al.*, 2003). The main glucosinolates found in broccoli and kailan are glucoraphanin, glucobrassicin, gluconapin and progoitrin (Table 2.4).

The most bioactive ITC found in broccoli are sulforaphane (derived from glucoraphanin), allyl isothiocyanate (derived from sinigrin) and indole-3-carbinol (derived from glucobrassicin) (Jones *et al.*, 2006). ITC have antibacterial and antifungal activities in plants, and provide important protection from insect and herbivore attack (Rosa *et al.*, 1997). A range of ITC, such as sulforaphane, inhibits Phase I enzymes, responsible for the activation of carcinogens, and induces Phase II detoxification enzyme systems, thereby increasing the cancer defence mechanisms of the body, as it

has been previously reported *in vitro* (Zhang *et al.*, 1992; Talalay *et al.*, 1995; Munday and Munday, 2004). ITC have also been implicated in the inhibition of cancer cell proliferation and induction of apoptosis (Musk *et al.*, 1995; Huang *et al.*, 1998; Smith *et al.*, 1998), as well as inhibition of *Helicobacter pylori*, the bacteria responsible for stomach ulcers (Fahey *et al.*, 2002). Furthermore, sulforaphane derived from broccoli sprouts has been linked to prevention of cardiovascular disease in an animal model study using rats (Wu *et al.*, 2004).

Table 2.4. Main glucosinolates of broccoli (cvs. Emperor, Shogun, Marathon and Viola) and kailan (Schonhof *et al.*, 2004).

Trivial name	Systematic name	Class	Content in mg 100 g ⁻¹ fw (% relative abundance)	
			Broccoli	Kailan
Glucoraphanin	4-Methylsulphinylbutyl	Aliphatic	6.6–33.9 (27.6–52.3)	39.7 (26.5)
Glucobrassicin	3-Indolylmethyl	Indolyl	6.9–12.1 (11.9–42.6)	5.7 (3.4)
Gluconapin	3-Butenyl	Alkenyl	1.0 (1.6)	76 (57.7)
Progoitrin	(2R) 2-Hydroxy-3-butenyl	Alkenyl	0.6–10.3 (3.7–15.8)	19.1 (3.7)
4-Methoxyglucobrassicin	4-Methoxy-3-indolylmethyl	Indolyl	0.4–1.3 (1.4–3.9)	0.6 (0.4)
4-Hydroxyglucobrassicin	4-Hydroxy-3-indolylmethyl	Indolyl	0.06–0.36 (0.4–1.0)	1.5 (0.7)
Neoglucobrassicin	N-Methoxy-3-indolylmethyl	Indolyl	0.9–6.3 (3.1–9.1)	2.1 (1.4)
Sinigrin	2-Propenyl	Alkenyl	0.9 (1.5)	0.9 (0.9)
Glucoiberin	3-Methylsulphinylpropyl	Aliphatic	0.3–3.8 (1.6–14.8)	1.5 (1.7)

2.2.3. Chlorophylls

Chlorophylls are natural pigments found in the chloroplasts of plants, algae and some cyanobacteria. Several factors such as cultivar, preharvest conditions, postharvest handling and processing may vary the chlorophyll content of vegetables. In this way, there is a wide range of the chlorophyll content previously reported in broccoli with 260 to 970 mg kg⁻¹ fw, reporting chlorophyll *a* around 3-fold higher levels than chlorophyll *b* (Funamoto *et al.*, 2002; Lemoine *et al.*, 2008). Contrary, kailan reported 4.6-fold higher chlorophyll *b* content than *a* over a total chlorophyll content of approximately 400 mg kg⁻¹ fw (Noichinda *et al.*, 2007).

Chlorophylls have been associated with many health benefits such as anti-inflammatory, antioxidant, anticancer properties and kidney stones prevention (Tawashi *et al.*, 1982; Dashwood *et al.*, 1998; Egner *et al.*, 2003; Lanfer-Marquez *et al.*, 2005).

2.2.4. Carotenoids

Carotenoids are tetraterpenes derived from a symmetrical C₄₀ skeleton. Carotenoids are natural pigments that can be classified into two great groups: carotenes, which are strictly hydrocarbons, and xanthophylls, derived from the former that contains oxygenated functions. Structurally, the carotenoids may be acyclic (i. e., lycopene) or contain a ring of five or six carbons at one or both ends of the molecule (i. e., β -carotene). Carotenoids play a very important role as protectors of the chlorophylls and the photosynthetic apparatus in general by blocking reactive forms of triplet chlorophylls (³Chl) and singlet oxygen (¹O₂) formed during the capture of light energy. They also take an active part in the plant's photoprotective and antioxidant action (Artés *et al.*, 2002). Xanthophylls contain oxygen in their structure while carotenes are purely hydrocarbons, which have not oxygen. The xanthophyll group includes, among others, lutein, zeaxanthin, neoxanthin, violaxanthin, and α - and β -cryptoxanthin. The α -, β - and γ -carotenes are the main carotene types, which are very important as precursors of the vitamin A (Maiani *et al.*, 2009). Only β -carotene, β -cryptoxanthin, α -carotene, lycopene, lutein and zeaxanthin represent more than 95 % of total blood carotenoids in humans. Lutein, zeaxanthin and other xanthophylls are believed to function as protective antioxidants in the macular region of the human retina. The latter carotenoids have also shown protection against cataract formation, coronary heart diseases and stroke (Snodderly, 1995; Ribaya-Mercado and Blumberg, 2004; Chrong *et al.*, 2007). β -carotene has shown other health benefits, may be related to their antioxidative potential such as enhancement of the immune system function (Bendich, 1989), protection from sunburn (Mathews-Roth, 1990) and inhibition of the development of certain types of cancers (Nishino, 1998).

The main carotenoids found in broccoli are lutein and β -carotene with ranges of 707–3,300 and 291–1,750 $\mu\text{g } 100 \text{ g}^{-1} \text{ fw}$, respectively (Leth *et al.*, 2000; Murkovic *et al.*, 2000; Larsen and Christensen, 2005). Lutein is also the major carotenoid found in kailan ranging the lutein/zeaxanthin content between 2,800 and 3,220 $\mu\text{g } 100 \text{ g}^{-1} \text{ fw}$. However, previous studies on kailan have shown high quantities of violanxathin,

neoxanthine and β -carotene with ranges of 1,240–2,140, 900–1,230 and 610–1,360 μg 100 g^{-1} fw, respectively (Hanson *et al.*, 2011).

2.2.5. Antioxidant compounds and their classification

The reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. The ROS include oxygen ions (i. e., $^1\text{O}_2$), free-radicals (O_2^- , $\cdot\text{OH}$, $\text{NO}\cdot$, etc.) and peroxides (H_2O_2 , 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 (Devasagayam *et al.*, 2004). According to its nature, these compounds may be classified as enzymatic or non-enzymatic antioxidant compounds (Figures 2.2 and 2.3). The 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 (Tarazona-Díaz, 2011).

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 (Dragsted, 2003; Balasundram *et al.*, 2006). 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 2.2. Antioxidant compounds classification.

Figure 2.3. 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 BROCCOLI

3.1. Introduction

The actual consumer, with scarce time to prepare a suitable meal (which usually does not meet the nutritional dietary requirements), looks for convenient fresh foods with no additives, high nutritional value and antioxidant properties to be consumed both at home and food services (Artés, 2000; Artés *et al.*, 2009).

In order to supply these consumer requirements, the minimally processed (MP) or FC vegetables have appeared in the last years. The FC vegetables are ready-to-eat products, elaborated free from additives by using light combined methods such as washing, cutting, sanitation, packaging (under MAP) and chilling. These products usually do not need further processing prior to consumption (Artés, 2000; Artés and Allende, 2005).

The FC vegetable industry is continuously applying new technologies in order to extend the shelf-life of these products keeping the best sensory, microbial and nutritional quality. In this way, the high suitability and nutritional value of the FC vegetables present many advantages for consumers and food services (Wiley, 1994; Artés, 2000, 2004; Beuchat, 2002; Bruhn, 2002):

- Reduced preparation time (ready-to-eat).
- Similar characteristics to the original vegetable (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.

3.2. Current status of the minimally processed products industry

In order to supply salads to the fast food establishments, the FC industry emerged at the beginning of the 70s in the USA. In the 80s, this market was introduced in Switzerland and Germany. In the middle of this decade, it extended to UK, France,

The Netherlands and Italy. The FC products appeared in Spain at the beginning of the 90s (Artés and Artés–Hernández, 2003; Sánchez–Pineda, 2003).

UK is the major FC produce consumer because the ready–to–eat product culture is deeply established in that country. France, which has occupied for many years the second place in the FC produce rank, has recently been overcome by Italy. In 2008, the Spanish FC vegetable industry registered a total production of 61,593 t (79 % for distribution and 21 % for hotels and restaurants) with a turnover of approximately 200 millions €. The data from 2008 implies an increase over the previous year of the 3.2 %. In 2008, 7.7 million Spanish households purchased FC produce once a year, at least, reaching an average purchase frequency around 2.8 kg year⁻¹ (AFHORLA, 2012).

The number of products that are being incorporated into the FC vegetable market is increasing while this sector progressively sits on the market.

3.3. General processing and unit operations

The minimal processing of kailan–hybrid broccoli comprises several unit operations. The Figure 3.2 shows the general unit operations and the maximum recommended temperatures for each processing step in the production line of FC kailan–hybrid broccoli.

3.3.1. Raw material

The physiological behaviour and the suitability for the FC vegetable processing that ensure a good postharvest quality and shelf–life may be influenced by the following preharvest factors (Yildiz, 1994):

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

Figure 3.2. Flow chart of the FC kailan–hybrid broccoli processing (elaborated from Artés–Hernández, 2012).

Kailan–hybrid broccoli is hand harvested due to the sensitivity to damages of this crop and mechanization difficulties. The following manual harvesting forms are commonly used (Figure 3.3):

- Whole head (A): all stems are used with good uniformity.
- Advanced stems from the main head (B): only the most advanced stems are cut and the rest are harvested in the following harvest time.
- Regrowths (C).

Figure 3.3. Harvesting forms of kailan–hybrid broccoli (Courtesy of Sakata Seed Ibérica S.L.U.).

Vegetables like broccoli are inflorescences harvested in early maturation stages. This implies a great content of plant storage products and high metabolic activity. In this way, broccoli has one of the highest respiration rates (RR) among fruit and vegetables ($> 60 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ at 5°C). In general, the shelf–life of FC vegetables is

inversely proportional to its RR. Thus, in order to minimize its RR, the following handling practices during kailan–hybrid broccoli harvesting should be conducted:

- Harvesting in the first hours of the morning when temperatures are low.
- Avoid sun exposure of the product.
- In order to keep the product humidity, the use of wet fibre beds in the harvest boxes is adequate.
- Time between harvest and cooling may be less than one hour.

The optimum maturity indices of kailan–hybrid broccoli at harvest, which will ensure a good quality of the FC produce, are:

- No floret yellowness.
- Stem form: first category (without nodes) and second category (four nodes maximum).
- Diameter: 8–15 mm.
- Long: 120–220 mm.

Figure 3.4. Details of kailan–hybrid broccoli at harvesting (Courtesy of Sakata Seed Ibérica S.L.U.).

The maturity indices for conventional broccoli are head diameter (different for every variety) and compactness. Furthermore, all florets should be closed.

3.3.2. Transportation and reception

The transportation time from the farm to the processing plant may be less than one hour. If this transportation time is surpassed is strongly recommended to precool the product with crushed-ice or use refrigerated transport (5–10 °C).

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.

3.3.3. Precooling and storage

The objective of the precooling is to quickly remove the heat that the plant material brings from the field after harvesting. The best precooling method for conventional and kailan–hybrid broccoli is the ice–crushed beds on the box of the product (Figure 3.5). This step is very important to extend the shelf–life of these high–metabolic–rate vegetables. Therefore, the stress generated during the minimal processing operations will be retarded, maintaining the produce quality. Precooling horticultural produce has many advantages like (Artés, 1987):

- Reducing weight loss and wilting.
- Lowering microbial growth.
- Lowering RR and decay.
- Lowering ethylene emission and its sensitivity.

Figure 3.5. Kailan–hybrid broccoli ice–crushed precooled.

In order to regularize the supply to the processing line, the cooled raw material is stored at 0–4 °C and high relative humidity (90–95 % RH) for a few hours or one day (Artés and Artés–Hernández, 2003).

3.3.4. Sorting, classification and conditioning

Product sorting may be accomplished based on visual quality and uniformity of florets and stems. These parameters will facilitate all the subsequent processing steps, increasing the productivity and final quality of the FC product. The recommended sorting parameters are (Figure 3.6):

- No stem damages.
- No yellowing buds.
- No more than 4 nodes in the stem.
- Maximum diameter of the top and side shoots of 15 and 12 mm, respectively.
- Minimum stem length of 15–22 cm.
- No pathological or physiological disorders.
- No insects.

Kailan–hybrid broccoli conditioning implies the elimination of the remaining leaves from the stems. The long stems will be adapted, by manual cutting (with sharp knives), to the length range of 15–20 cm. Generally, manual cutting is considered as the method that provides the maximum quality and efficiency (Somsen *et al.*, 2004). The cutting equipment should be cleaned, disinfected and sharpened at regular intervals every working day. Standards Operating Procedures (SOPs) for cleaning and maintenance of the cutting equipment must be established by the industry. Cutting of kailan–hybrid broccoli stems implies an increase of the plant material RR, ethylene emission and tissue injuries. Consequently, this step also increases the rate of tissue senescence and reduces the product resistance to microbial spoilage (Artés *et al.*, 2009).

In order to minimize these undesirable effects of cutting, a low processing temperature (maximum 8 °C) may be kept in this area (Artés–Hernández, 2012). The conditioned product will be conducted directly from the dirty to the clean area for further processing.

Figure 3.6. Kailan–hybrid broccoli quality specifications (Courtesy of Sakata Seed Ibérica S.L.U.).

3.3.5. Prewashing

The raw material is prewashed in the dirty area of the processing line with cold slightly-chlorinated water (5–10 °C; 50 mg L⁻¹ free Cl). This step will eliminate the unwanted dirt, pesticide residues, plant debris, insects and foreign matter. Besides, the prewashing will retard the enzymatic discoloration reactions and the natural microflora will be reduced (Artés, 2000; Artés and Artés-Hernández, 2003; Allende and Artés, 2005).

3.3.6. Washing, disinfection and rinsing

The superficial microbial load of the plant material will be effectively reduced by the washing and disinfection steps (Suslow, 1997; Artés *et al.*, 2009). Fresh produce used as raw materials may be a vehicle for transmitting infectious diseases (Leistner and Gould, 2002; Harris *et al.*, 2003; Artés and Allende, 2005). Chlorine (in various forms) has been widely used as a disinfectant in the FC industry. NaClO is the most commonly used disinfectant in the FC industry due to its strong oxidizing properties, antimicrobial activity and low cost. In this way, the washing and disinfection of kailan-hybrid broccoli are made with cold chlorinated water (1–2 °C; 100–150 mg L⁻¹ free Cl), 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.

A rinsing step of the product with cold tap water (1–2 °C) will ensure free Cl levels lower than 5 ppm. A range of washing and rinsing water volumes of 6–12 L per kg of product are commonly used (Artés and Artés-Hernández, 2003). The washing and rinsing efficacy can be improved by the generation of turbulences by pressure-air injection in the water baths, although pressure showers, drive chains or rotating drums can be alternatively used (Artés-Hernández and Artés, 2005).

However, NaClO may partially oxidize food constituents originating unhealthy by-products such as chloroform (CHCl₃), haloacetic acids or other trihalomethanes, that have known or suspected carcinogenic or mutagenic potential effect with proved toxicity to liver and kidney (Nieuwenhuijsen *et al.*, 2000; Hrudey, 2009; Ölmez and Kretzschmar, 2009). At the same time, at pH over 7.5 NaClO reacts with organic N-based materials to produce chloramines (Suslow, 1997). These negative aspects related with chlorine have induced some European countries (Germany, The Netherlands,

Denmark, Switzerland and Belgium) to forbid the use of NaClO for disinfection of FC produces (Artés *et al.*, 2011). In addition, it has been demonstrated that effectiveness of NaClO is limited to some products (Beuchat and Brackett, 1990). In fact, it was not very effective for inhibiting *L. monocytogenes* growth in shredded lettuce or Chinese cabbage (Ahvenainen, 1996). Actually, chlorine compounds are helpful in reducing the aerobic microbial counts in many leafy vegetables, but not necessarily in root vegetables.

Consequently, the food industry is now looking for alternatives to chlorine which may assure the safety of the FC products and maintain the quality and shelf-life, while also reducing the rate of water consumption during processing. Thus, sustainable sanitising alternatives to NaClO have been proposed (Artés *et al.*, 2009). Among these techniques are UV-C radiation, EW and HO, which have been studied in this Ph. D. Thesis.

3.3.6.1. Electrolysed water

EW is generated by electrolysis of a dilute salt solution (NaCl, usually about 0.1 %) of pure water in an electrolysis chamber where anode and cathode electrodes are separated by a membrane (Figure 3.7). On the anode side, acidic EW (AEW) is generated and has strong bactericidal effect on most known pathogenic bacteria, due to its low pH, high oxidation-reduction potential (ORP) (about 1100 mV) and the presence of HOCl. The cathode area produces NEW (Ongeng *et al.*, 2006; Artés *et al.*, 2011). HOCl is present in EW at pH 6.8 and it is generated by electrolysis of the NaCl solution, since the HCl formed at the anode site neutralizes the NaOH at the cathode site (Artés *et al.*, 2011).

The acidic type was the first EW form developed and accepted by the food industry in Japan as disinfectant for food processing equipment (Izumi, 1999). Due to its neutral pH, NEW could be less aggressive to the corrosion of processing equipment compared to AEW. EW has a strong bactericidal effect against pathogens and spoilage microorganisms as it has been reported in many FC produce (reviewed by Artés *et al.*, 2011). These good microbial reductions were attained with reduced or no impact on the physical and sensory parameters of the FC product. The bactericidal effect has been attributed to its high ORP (Izumi, 1999; Koseki and Itoh, 2001; Bari *et al.*, 2003).

A theory for the inactivation mechanism of bacteria by EW proposed that ORP could firstly affect and damage the redox state of glutathione disulfide–glutathione couple, and then penetrate the outer and inner membranes of *E. coli* O157:H7, resulting the necrosis (Liao *et al.*, 2007). Recently, Hao *et al.* (2012) stated that, considering the lower chlorine concentration of EW compared with traditional chlorine disinfectants, the existing form of chlorine compounds rather than the hydroxyl radicals played important role in the disinfection efficacy of EW.

Figure 3.7. Diagram of the electrolysed water production
(www.pic2fly.com/Electrolyzed+Water.html).

Attending to nutritional quality, a better polyphenol and chlorophyll retention with an increment of the CAT (EC 1.11.1.6) activity was observed in AEW–treated (40 ppm free chlorine, pH 3.0) Mizuna baby leaves than a conventional 100 ppm NaClO treatment throughout 11 days at 5 °C (Tomás–Callejas *et al.*, 2011).

Safety is the main advantage of EW. In contrast with the NaClO problems (i.e., skin and membrane irritation and toxicity) NEW and AEW are not corrosive to skin, mucous membranes, or organic material. In addition, when EW comes in contact with organic matter, or is diluted by tap water or reverse osmosis water, it becomes ordinary water again, being more eco–friendly than NaClO (Tomás–Callejas *et al.*, 2011).

Consequently, EW has many potential uses for the food industry being advantageous because it involves on–site production of the disinfectant, which means

there are no chemicals to store or handling costs for dealing with them. However, issues such as gas emission, strong acidity of the AEW, metal corrosion, free chlorine content, short shelf-life (about 2 weeks) and by-products formation need to be supported for further research (Artés *et al.*, 2011).

3.3.6.2. UV-C radiation

The use of non-ionizing, germicidal and artificial UV at a wavelength of 190–280 nm (UV-C) could be effective for surface decontamination of FC products (Allende and Artés, 2003a). The UV-C induces the formation of pyrimidine dimers which alter the DNA helix and block microbial cell replication (Kuo *et al.*, 1997; Lucht *et al.*, 1998). Consequently, cells that are unable to repair radiation-damaged DNA die and sub-lethally injured cells are often subject to mutations (Lado and Yousef, 2002). It was established that bacterial spores and stationary phase cells are more resistant to UV-C than vegetative and exponential phase cells (Warriner *et al.*, 2009). Strains sensitivity to UV-C changes depends on the microorganism (Gardner and Shama, 2000). Related to temperature, UV-C seems to be effective in a range from 5 to 37 °C.

UV-C light has been evaluated in many FC produce as an alternative for surface disinfection of natural microflora as well as the main foodborne pathogens (Allende and Artés, 2003a; Artés *et al.*, 2011). However, the structure and surface of the vegetable have an influence on the incident irradiation (Bintsis *et al.*, 2000; Gardner and Shama, 2000; Lado and Yousef, 2002).

An interesting benefit of UV-C application is that it stimulates plant defence mechanisms increasing resistance to pathogens. But UV-C may modify cell permeability on vegetables increasing electrolytes, amino acids and carbohydrates leakage, which consequently may stimulate bacterial growth (Nigro *et al.*, 1998). For this reason, it is very important to find a safe dose which greatly weakens microbial development without damaging the product.

The UV-C radiation has been reported to affect many physiological parameters such as the RR and C₂H₄ production (Artés *et al.*, 2011). Attending to sensory quality, yellowing of FC broccoli heads was retard after UV-C light (Civello *et al.*, 2006), although surface browning and softening in other UV-C-treated FC products have been reported (Allende *et al.*, 2006). Robles *et al.* (2007) indicated that UV-C-treated

tomatoes showed retarded ripening and kept better firmness and sensory attributes than those air-stored. This process could be related to an increased enzymatic activity caused by membrane disruption with the consequent loss of compartmentalization (Gómez *et al.*, 2010).

Abiotic stresses, like UV light, may enhance the bioactive content of fresh fruit and vegetables (Cisneros-Zevallos, 2003). The effects of UV radiation processing on several bioactive compounds (anthocyanins, total phenolics, lycopene, ascorbic acid, chlorophylls, antioxidants enzymes, etc.) of plant produce have been reviewed (Allothman *et al.*, 2009).

The UV-C radiation offers some advantages to the food industry since it has no residues, no legal restrictions, lethal to most types of microorganisms, is easy to use and does not require extensive safety equipment to be implemented (Bintsis *et al.*, 2000; Allende and Artés, 2003a; Yaun *et al.*, 2004).

The best application of the UV-C technique to the FC industry is the UV-C tunnels, where the product would circulate on a conveyor belt. There are other discontinuous systems (UV-C drums, UV-C hoods, etc.) used at pilot plant scale or for the small production industries. The Figure 3.8 shows a kailan-hybrid broccoli sanitation in a UV-C hood.

Figure 3.8. Kailan-hybrid broccoli sanitation with UV-C light at a pilot plant scale (discontinuous system).

3.3.6.3. Superatmospheric oxygen

The combined effect of high O₂ concentrations (over 60 kPa –HO–) and CO₂ around 10 to 20 kPa may provide adequate suppression of microbial growth increasing produce shelf-life (Artés *et al.*, 2011). The HO could be effective for inhibiting enzymatic browning, preventing anaerobic fermentation, reducing moisture and odour losses, and decreasing aerobic and anaerobic microbial growth (Day, 2001).

A number of factors may explain the toxicity of hyperbaric O₂, like the adverse effects on the ORP of the system, the oxidation of enzymes having sulfhydryl groups or disulfide bridges, and the accumulation of injurious ROS (Kader and Ben-Yehoshua, 2000). CO₂ causes a decrease in the intra and extracellular pH and interferes with the cellular metabolism with a stronger inhibitory effect at low temperature due to enhanced CO₂ solubility (Dixon and Kell, 1989).

Some studies have been carried out for analysing the effect of high O₂ and CO₂ levels on the quality and safety of FC plant commodities (reviewed by Artés *et al.*, 2011). Related to microbial growth, it seems to be that exposure to HO did not strongly inhibit it, while high CO₂ reduced the growth to some extent in most cases. Contrary to HO, the antimicrobial activity of CO₂ at high concentrations is already well known.

Attending to the physiological parameters, Kader and Ben-Yehoshua (2000) have found some contradictory results since HO may stimulate, have no effect or reduce RR and C₂H₄ emission. These effects depend of factors such as the commodity, ripening stage, storage time and temperature, and O₂, CO₂ and C₂H₄ concentrations in the atmosphere. The addition of CO₂ is unnecessary when HO are injected in barrier film packages, because the respiratory activity of produce generates antimicrobial CO₂ levels reaching 20 kPa.

Superatmospheric O₂ has been considered as an abiotic stress (Jacobo-Velázquez *et al.*, 2011). Different HO effects on the TAC (increment, decrease or invariable), and total phenolic and chlorophyll contents of many FC produce have been reported (Zheng *et al.*, 2007; Jacobo-Velázquez *et al.*, 2011; Tomás-Callejas *et al.*, 2012). In this way, the different effects of HO on the nutritional quality of the FC produce may vary depending on the commodity, O₂ partial pressure, storage time and temperature (Zheng *et al.*, 2007). From *in vitro* studies of polyphenol oxidase (PPO)

(the main enzyme responsible of browning in fresh produce) kinetics, it seems to be that the substrate concentration, as well as the O₂ level, had a clear inhibitory effect on the reaction rate. Moreover, the inhibitory effect of O₂ was more evident at low final product concentration (Gómez *et al.*, 2006).

Regarding sensory quality, acceptable scores were found in FC treated with HO atmospheres, retarding surface browning, flesh softening, and off-flavour, as reviewed by Artés *et al.* (2011) in many FC produce. In practice, the use of HO in MAP for keeping the FC produce quality is still emergent and needs to be supported by more research.

The application of combined sanitising treatments, according to the hurdle technology (Leistner and Gould, 2002), could have a synergistic effect leading to a better microbial reduction. Combination of MAP and UV-C had a positive effect by reducing psychrotrophic bacteria, coliform, and yeast growth in FC lettuce without adversely affects sensory quality (Allende and Artés, 2003b). A triple combination of MAP, UV-C and HO showed lower mesophilic counts in Tatsoi baby leaves than individual treatments after 11 days at 5 °C (Tomás-Callejas *et al.*, 2012).

3.3.7. Dewatering

The water from the vegetable surface may be carefully eliminated after the washing and rinsing steps to reduce the moisture content of the product and remove cell leakage, which can promote microbial growth and enzymatic activity (Simons and Sanguansri, 1997; Soliva-Fortuny and Martín-Belloso, 2003). Some dewatering systems recommended for FC kailan-hybrid are centrifugal spin driers, vibrating racks, hydro-sieves and forced-air spinless drying tunnels. Cold air injection over a perforated conveyor belt is also a good alternative, although its efficacy is low for high productions (Artés and Artés-Hernández, 2003).

3.3.8. Weight and packaging

The correct quantity of product may be weighed and disposed in bags or trays in the packaging step. The most extended packaging method for preparing FC vegetables is MAP (Figure 3.9). This is a technique that reduces the O₂ and elevates the CO₂ concentrations within the packages, compared to air, prolonging the shelf-life of the FC

product. These gas concentrations are commonly attained by using a permeability–selective plastic film. This film must be selected according to its gas permeability to the gases of physiological interest (O_2 , CO_2 , C_2H_4 , and H_2O vapour) (Artés, 1993; Artés *et al.*, 1998). The optimal gas balance inside the package is maintained by the diffusion of these gases throughout the film, which are generated during the respiration of the commodity under a concentration gradient (Artés, 1993; Allende *et al.*, 2000; Kader, 2002; Artés *et al.*, 2006).

The passive MAP is achieved if an appropriate atmosphere can passively evolve within a hermetically sealed packaged through consumption of O_2 and production of CO_2 by respiration. The gas permeability of the selected film must allow O_2 to enter the package at a rate offset by the consumption of O_2 by the commodity. Similarly, CO_2 must be vented from the package to offset the production of CO_2 by the commodity (Artés, 1993; Tomás–Callejas, 2011). Ideal gas concentrations for storage of broccoli under controlled atmosphere are 1–2 % O_2 with 5–10 % CO_2 at a temperature range of 0–5 °C (Cantwell, 2002). Although such low O_2 levels extend product shelf–life under controlled conditions, temperature fluctuations during commercial handling make this risky since broccoli can easily produce offensive sulphur–containing volatiles. In this way, most MAP of broccoli is designed to maintain O_2 at 3–10 % and CO_2 at approximately 7–10 % to avoid the mentioned off–odour volatiles development (Cantwell and Suslow, 1997). These O_2 concentrations within the package ensure that the point of fermentation will not be reached, which would induce anaerobic respiration with the potentially tissue injuries (Watada *et al.*, 1996). The main benefits of MAP are (Artés, 2000):

- Reduction of RR.
- Reduction of heat emission from respiration.
- Lowering ethylene activity and subsequent senescence.
- Lowering sugar, vitamin and organic acid losses.
- Total or partial limitation of physiological changes, such as chilling injuries, scalding, browning, etc.
- Lowering microbial growth.

Alternatively, active MAP can be accomplished by creating a slight partial vacuum and replacing the main proportion of the package atmosphere with the above–

recommended gas mixture. This mixture can be further adjusted through the use of absorbing substances in the package to scavenge O₂, CO₂, or C₂H₄. Although active MAP implies some additional costs, its main advantage is that it ensures the rapid establishment of the desired atmosphere with a longer shelf-life of the FC product (Artés *et al.*, 2006).

Figure 3.9. Detail of kailan–hybrid broccoli under MAP.

Finally, a proper labelling of the packages has to be done. These labels have to include the processing date, expiration date, net weight, producer details and the lot code, which will ensure a good traceability of the final product.

3.3.9. Quality control and cold storage

Before the expedition of the product from the FC 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 met. The equipment has to be frequently revised, adjusted and calibrated.

The bags or trays of the FC product will be disposed in boxes which will be briefly stored at 0–1 °C until the refrigerated distribution.

3.3.10. Cold transportation and distribution

The recommendable temperature range throughout distribution chain is 0 to 5 °C. However, it is practically impossible to guarantee that this range will be always maintained during transit, distribution and retail display. Temperature is the most important factor that influences the shelf-life of FC products. For retail sale, the FC products must be placed in special display cabinets at 0–5 °C (although 0–1 °C is preferable) in the food supply chains. However, it has already been demonstrated that FC products are often subjected to temperature abuse of about 12 °C in the display cabinets of supermarkets. The inadequate temperature management during distribution and marketing, coupled with excessive temperature fluctuations during storage, can result in changes of the gas partial pressures within the MA packages. These MA alterations induce a consequent RR increment, heat production and water condensation within the package. In this way, the latter effects will reduce the shelf-life of the FC product with high microbial spoilage risk. Sometimes, time-temperature integrators are used on packages to prevent abusive temperatures during transport and distribution. However, financial and environmental costs have limited the implantation of this technique (Artés and Artés-Hernández, 2003).

3.4. Overall quality and safety of FC vegetables

The expected characteristics of FC products by consumers are freshness, optimum overall quality (general appearance, sensory quality –texture/firmness, aroma and taste– and nutritional quality) and safety (Artés and Artés-Hernández, 2003). However, during FC processing and retail period some physiological, physical and nutritional changes may occur, reducing the expected quality attributes of the FC products. Furthermore, some pathological disorders can appear in the FC products, which can highly limit the shelf-life and safety of the FC product.

3.4.1. Physiological, physical and pathological disorders

The FC processing increases the metabolism of the plant material, reflected in higher RR and C₂H₄ emission, which usually leads to a faster deterioration rate (Cantwell and Suslow, 2002). Temperature is the most important factor that affects FC produce metabolism. When temperatures increase from 0 to 10 °C, the RR 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. FC 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, 2000). 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 FC product.

These changes in the metabolism of FC kailan-hybrid broccoli may be reflected in the following physiological disorders:

- Floret yellowing: the florets are the most perishable part of the broccoli head. Floret yellowing may be due to overmaturity at harvest, high storage temperatures and/or ethylene exposure. Any development of yellow colours ends commercial marketability.
- Stem bent: this disorder is very characteristic from kailan-hybrid broccoli. This stem bent is thought to be produced by an enzymatic degradation of the cell structure. Both physiological disorders of kailan-hybrid broccoli can be observed in Figure 3.10.

Figure 3.10. Stem bent and floret yellowing of kailan-hybrid broccoli.

Physical disorders like rough handling at harvest, which can damage the florets and increase decay, should be also minimised.

The main pathological disorders that can appear during the postharvest life of FC kailan–hybrid broccoli are:

- Bacterial decay: various soft–rot causing organisms (*Erwinia carotovora* – Figure 3.11–, *Pseudomonas*) may affect kailan–hybrid broccoli shelf–life. Decay due to these organisms is usually associated with physical injuries.

Figure 3.11. *Erwinia carotovora* in broccoli
(http://gardener.wikia.com/wiki/Bacterial_soft_rot).

- Fungal pathogens: although not as common as bacterial rots, grey mould rot (*Botrytis cinerea*) (Figure 3.12) and black mould (*Alternaria* spp.) can attack broccoli heads; this may occur under rainy and cool growing conditions.

Figure 3.12. Grey mould rot (*Botrytis cinerea*) in broccoli.

3.4.2. Nutritional and bioactive compounds changes

The FC processing keys that highly influence the nutritional and bioactive contents are cutting, washing, dewatering, packaging, and processing and storage temperatures (Francis *et al.*, 2012). The most important tool to extend the shelf-life and maintain the quality of the FC fruit and vegetables is the temperature management. Storage at 2 °C for 3 weeks of six broccoli cvs. achieved high L-AA retentions ranging between 56 and 98 % (Albrecht *et al.*, 1990). Broccoli florets (cv. Marathon) stored simulating a commercial transport and distribution (7 days at 1 °C + 3 days at 15 °C) reported losses of 71–80 % for total glucosinolates, 62–59 % for total flavonoids, 51–44 % for sinapic acid derivatives and 73–74 % for caffeoyl–quinic acid derivatives at the end of the storage period (Vallejo *et al.*, 2002b).

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. According to this, cutting produced an L-AA reduction around 36 % during the FC processing of conventional broccoli (Martínez *et al.*, 2004). Glucosinolates levels do not necessarily decline rapidly after cutting and even induction can take place, but FC *Brassica* vegetables show optimal conditions for myrosinase activity with the conversion of glucosinolates to the biological active ITC. An UV-C treatment (8 kJ m⁻²) retarded yellowing and chlorophyll degradation in FC broccoli (cv. Cicco) florets stored for 21 days at 4 °C. Furthermore, this sanitising method produced higher phenolics, L-AA and soluble sugars contents, as well as higher TAC than controls, although no differences in the content of soluble proteins were found (Lemoine *et al.*, 2007).

Gas partial pressures during MAP or controlled atmospheres storage can greatly influence the nutritional and bioactive profile of FC products. Conventional broccoli (cv. Marathon) stored during 7 days at 1 °C in air-storage conditions (17 % O₂ + 2 % CO₂) showed a 71 % reduction of total glucosinolates (aliphatic and indoles). In the same conditions, significant flavonoids and hydroxycinnamic acid derivatives decreases of 62 and 50–70 %, respectively, were found, contrary to the low stability of anthocyanins in FC vegetables (Vallejo *et al.*, 2003). The low O₂ levels attained during MAP modify the cell metabolism and favour the L-AA oxidation and/or inhibit the DHA reduction to L-AA. It has been observed a change in the AA–DHA equilibrium

favouring the AA reduction and the DHA increase, with a better total vitamin C (L-AA + DHA) retention in MAP than air-stored samples (Barth *et al.*, 1993). Greater RH inside the packages possibly served to better preserve vitamin C content in packaged broccoli spears (Lee and Kader, 2000). Controlled atmospheres did not alter the lutein and β -carotene contents in green beans (Cano *et al.*, 1998). CO₂-enriched atmospheres with low O₂ content (0.5 % O₂ + 20 % CO₂) produced lower total glucosinolates increases (21 %) than air-stored samples (42 %) after 7 days at 10 °C (Hansen *et al.*, 1995).

3.4.3. Safety aspects of FC vegetables

The application of a sanitising treatment does not mean a sanitation program. It implies much more effort from a well-designed integrated production, handling, and processing to proper distribution chains, keeping appropriate chilling storage temperatures and optimal MAP conditions throughout the entire commercial life. In addition, Good Agricultural Practices (GAPs) for suppliers of raw materials, Good Manufacturing Practices (GMPs), 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 (Artés, 2004; Tomás-Callejas, 2011). Furthermore, AFHORLA issued the 'Guía de buenas prácticas de producción de IV Gama' in 2006 for the Spanish FC industry. Although the use of these guides is not mandatory, its adoption by the industry is strongly recommended in order to minimize microbial food safety hazards and ensure food safety to consumers. When the mentioned programs are not properly applied, outbreaks may occur with disastrous consequences (Table 3.1). According to this, these outbreaks associated with FC products have pointed microbiological safety as the major issue of concern in the FC industry.

The microbiological risks which may occur in the FC products can be classified in two categories (Hurst, 2002):

- The contamination of the plant material happens during cultivation or harvest by indigenous pathogens.
- The microbiological risk is present during the FC 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.

Spain has adopted the EU legislation (Regulation EC 1441/2007) with the RD 135/2010 (2010), derogating the previous RD 3484/2000 (2001), on microbiological criteria for food safety, which is mandatory and has a direct application to the FC industry (Table 3.2).

Table 3.1. Outbreaks linked to fresh and FC produce from 2005 to 2011 (Olaimat and Holley, 2012).

Location	Year	Pathogen	Produce	Cases (deaths)
Canada	2005	<i>Salmonella</i>	Mung bean	592
USA	2005	<i>Salmonella</i>	Tomatoes	459
USA	2006	<i>E. coli</i> O157:H7	Spinach	199 (3)
Australia	2006	<i>Salmonella</i>	Alfalfa sprouts	125
USA, Canada	2006	<i>Salmonella</i>	Fruit salad	41
USA	2006	<i>Salmonella</i>	Tomatoes	183
USA	2006	<i>E. coli</i> O157:H7	Lettuce	81
Australia	2006	<i>Salmonella</i>	Cantaloupe	115
USA	2006	<i>E. coli</i> O157:H7	Spinach	22
Europe	2007	<i>Salmonella</i>	Baby spinach	354
North America, Europe	2007	<i>Salmonella</i>	Basil	51
Australia, Europe	2007	<i>Shigella sonnei</i>	Baby carrots	230
Europe	2007	<i>Salmonella</i>	Alfalfa sprouts	45
USA, Canada	2008	<i>Salmonella</i>	Peppers	1442 (2)
USA, Canada	2008	<i>E. coli</i> O157:H7	Lettuce	134
UK	2008	<i>Salmonella</i>	Basil	32
USA	2008	<i>Salmonella</i>	Cantaloupe	51
USA, Canada	2008	<i>Salmonella</i>	Peanut butter	714 (9)
USA	2009	<i>Salmonella</i>	Alfalfa sprouts	235
USA	2010	<i>E. coli</i> O145	Lettuce	26
USA	2010	<i>Salmonella</i>	Alfalfa sprouts	44
USA	2010	<i>L. monocytogenes</i>	FC celery	10 (5)
USA	2011	<i>Salmonella</i>	Alfalfa and	140
USA	2011	<i>Salmonella</i>	Cantaloupe	20
USA	2011	<i>Salmonella</i>	Papaya	106
Europe	2011	<i>E. coli</i> O104:H4	Vegetable	3911 (47)
USA	2011	<i>L. monocytogenes</i>	Cantaloupe	146 (31)
USA	2011	<i>E. coli</i> O157:H7	Strawberries	15 (1)
USA	2011	<i>E. coli</i> O157:H7	Lettuce	60

Table 3.2. Food safety criteria applied to the FC products food (Regulation EC 1441/2007, 2007).

Food category	Microorganism	Sampling plan ¹ n	c	Limits ²		Stage where the criterion applies
				m	M	
Precut fruit and vegetables (ready-to-eat).	<i>E. coli</i>	5	2	100 CFU g ⁻¹	1,000 CFU g ⁻¹	Manufacturing process.
Precut fruit and vegetables (ready-to-eat).	<i>Salmonella</i>	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 <i>L. monocytogenes</i> , other than those intended for infants and for special medical purposes.	<i>L. monocytogenes</i>	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.

4. FIFTH RANGE VEGETABLES: COOKING METHODS

4.1. Introduction

In the last few years, the ready-to-eat vegetable industry has developed new products in order to diversify the production and augment the offer for a consumer who is constantly demanding new healthy and natural products with high quality but low preparation time. The fifth range industry has found in the cooked vegetables sector a good market opportunity. Some of this demand comes also from the catering services and fast food chains, which require larger volumes of these products.

The application of heat treatments is the most widespread food preservation technique. The sterilization treatment has allowed the appearance of canned ready-to-eat meals from traditional recipes and with a long commercial life without refrigeration. However, this treatment is very aggressive with the nutritional and sensory properties of food, and especially with vegetables, causing rejection of consumers. In order to solve these technological problems, mild cooking techniques with a subsequent refrigerated storage have appeared to configure the fifth range industry of vegetables.

The technology used to preserve the fifth range vegetables includes packaging, heat treatment and cooling and can be described by the following characteristics:

- Heat-treated products, ready-to-eat and marketed under refrigeration.
- Heated prior to consumption, usually in microwave or conventional oven.
- Usually packaged in plastic materials.
- Keeping the cold chain until consumption (processing, packaging, storage and distribution).

The name of ‘fifth range vegetables’ has been attributed primarily to vacuum-packaged boiled vegetables ready-to-heat and eat accordingly to the evolution of preservative food techniques. Lately, the fifth range vegetables have been described as plant-based products which have been heat-treated to guarantee a conservation period of minimum 6 weeks (Tirilly and Bourgeois, 2002). In the Anglo-Saxon term, the fifth range products are sometimes referred as ‘Refrigerated Pasteurized Foods of Extended Durability’ (REPFEDs) (Mossel and Struijk, 1991). These products are characterized by a processing at 65–95 °C. The higher temperature range (> 70 °C) is usually applied to

vegetables. After heat treatment, food is rapidly cooled and stored under refrigeration until consumption.

4.2. Current status of the fifth range products industry

The fifth range products market has considerably grown in the recent years. Europe is largely the most active market in this sector, with a 50 % of worldwide market share, followed by USA with a 23 % (period January–June 2010) (Mintel, 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 (Mintel, 2002). In this way, this food sector opens a market opportunity.

In Spain, the consumption of the fifth range products has increased in a 65 % since 2001, reaching a consumption of 11.9 kg year⁻¹ person⁻¹ in 2011 (MAGRAMA, 2012b). Among the common cooking styles used by the Spanish consumers in 2011, the fifth range products represent a 10.0 %, achieving the fifth range products with less than 20 min of preparation the 77.8 % of the latter food sector. Furthermore, among the fifth range products consumed in Spain, the vegetable–based ones represented the 15.3 % of the total volume in 2011 (MAGRAMA, 2012c). The evolution of the consumption of these products over the period 2001–2011 is shown in Figure 4.1. This consumption increment has been allowed since this kind of food perfectly meets the following consumer expectations:

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

The new technological advances have permitted to obtain new products with better nutritional and sensory quality, which has also favoured the increase of their

consumption. From the fifth range vegetables, the sautéed (stir-frying) dishes represent a 30.1 % over the total turnover (Sainz *et al.*, 2008).

Figure 4.1. Evolution of consumption *per capita* of the fifth range products between 2001 and 2011 (MAGRAMA, 2011).

4.3. Conventional and innovative cooking methods

The actual increasing demand of low and easy-preparation foods has attached a considerably long expiration date, usually of several weeks, expected by the consumers. Along the recent and emerging trajectory of the fifth range vegetable industry, conventional cooking techniques, such as boiling, steaming, deep-frying and grilling, have been applied. Together with the latter cooking methods, new techniques like vacuum cooking (*cook vide*), *sous vide* ('under vacuum' from French) and MW are being adapted to the fifth range vegetable industry in order to:

- Diversify the product offer.
- Minimise the nutritional losses during thermal treatments.
- Maximize the sensory properties of the product.
- Reduce the production costs (versatile and effective equipment, less energetic cost, etc).

These new techniques allow cooking at lower temperature and reduced time in order to obtain products with the aforementioned characteristics.

4.4. General processing operations prior to cooking

The current manufacturing technology of fifth range products is based on the ‘cook and chill’ term. Furthermore, when this kind of food is destined to catering services, it is also necessary to implement effective packaging techniques which guarantee its safety and long commercial distribution. The ‘cook and chill’ is a system where food is prepared and subjected to a sufficient heat treatment, packaging (prior or after cooking), fast cooling (blast chilling) and chilling storage until consumption. The rapid decrease in temperature inhibits the growth of spoilage and pathogenic microorganisms in their vegetative and spores forms (Díaz–Molins, 2009). The steps of the ‘cook and chill’ technology are summarized in the Figure 4.2.

Figure 4.2. Flow chart of the ‘cook and chill’ technology in the fifth range industry.

★ Critical factors affecting the commercial life of the fifth range products.

4.4.1. Raw material reception and storage

The first step once the raw material arrives to the factory is a quality control which ensures that this material accomplishes the quality attributes necessary for the subsequent fifth range processing. If the required quality standards are met by the material, it will go for further processing or will be stored (0–4 °C; 90–95 % RH), depending of the factory supply requirements.

4.4.2. Selection, classification and conditioning

The selection, classification and conditioning for the fifth range processing of kailan–hybrid broccoli are similar to those previously described for the FC processing.

4.4.3. Cooking

4.4.3.1. Boiling

Conventionally, the most used cooking method for vegetables (although it has application for all kind of food) has been boiling. The advantages and disadvantages of boiling are detailed in Table 4.1.

Table 4.1. Advantages and disadvantages of boiling.

Advantages	Disadvantages
<ul style="list-style-type: none">• Safe and simple method.• Appropriate for large–scale cookery.• Boiling makes digestible older and tougher meat and poultry.• Nutritious, well flavoured stock is produced.• Maximum colour is retained when cooking green vegetables.	<ul style="list-style-type: none">• Loss of nutritional value of the product (if the water is discarded), such as hydrosoluble vitamins.• Some boiled foods can look unattractive.• Can be a slow method of cooking.

In the fifth range industry, boiling treatment can be performed in big pots (discontinuous system) or in conveyor belts (carrying the product) submerged in boiling water (continuous system).

4.4.3.2. Steaming

Steaming technique cooks food by moist heat (steam) under varying degrees of pressure. Depending of the degree of pressure used, there are two methods of steaming: atmospheric or low pressure (LP) and high pressure (HP ≈ 0.1 MPa) steaming. Steaming has its origin in China back about 3,000 years with rudimentary stoneware steamers. This is a cooking method which can be employed for almost all kind of food. In Western cooking, steaming is most often used to cook vegetables (it is rarely used to cook meats), while this cooking method is commonly used for seafood and meat dishes in the Oriental cuisine.

Steaming is widely considered as a healthy cooking technique, especially for vegetables, which allows obtaining a high nutritive, light and easy-to-digest product. Furthermore, steaming is an excellent technique to retain the colour and flavour of vegetables.

In the fifth range industry, the continuous LP steaming method consists of special tunnels where the product, which circulates over a conveyor belt, is cooked by the vapour injected by an external generator. The discontinuous steaming (LP and HP) method is made in industrial steamers similar to an autoclave (Figure 4.3). Usually, the discontinuous equipment has a pressure valve which allows steaming the product at LP (opened) or HP (closed).

Figure 4.3. Discontinuous steamer (pilot plant scale).

4.4.3.3. Deep-frying

The deep-frying is an ancient and popular cooking method originated in the Mediterranean area due to the olive oil influence (Varela, 1988). The frying temperatures range between 130 and 190 °C, although the most common is the range 170–190 °C (Bouchon, 2006). The frying equipment used in the industries consists of a long recipient with oil (at the desired frying temperature) where the product is transported with a conveyor belt during the frying process (continuous system).

4.4.3.4. Grilling

Grilling is a cooking method which involves dry heat applied to the surface of the food product, commonly from above or below. Heat transfer to the food can be made via thermal radiation or by direct conduction, depending if the product is or not in contact, respectively, with the grilling source. Direct heat grilling can expose food to temperatures over 260 °C . Grilled products acquire distinctive roast colour marks (Figure 4.4) and aroma from the Maillard reactions, which take place at temperatures over 155 °C (Schröder, 2003).

Figure 4.4. Grilling of kailan-hybrid broccoli.

Grilling is often presented as a healthy alternative to cooking with oil (deep-frying), although the fat and juices lost by grilling may contribute to a dry product.

However, grilling (together with frying) can lead to the formation of the carcinogenic heterocyclic amines, benzopyrenes and polycyclic aromatic hydrocarbons (Sugimura *et al.*, 2004). Nevertheless, it has been found that meat marinating (with red wine or beer) may reduce the formation of these compounds (Melo *et al.*, 2008).

Industrial grills (continuous or discontinuous) are coupled with a sear marker device (Figure 4.5) in order to obtain the characteristic grilling marks on the final product.

Figure 4.5. A) SearMaker device; B) Detail of the marking device of the SearMaker of Omar Associates (http://www.omarassociates.net/grill_markers.html).

4.4.3.5. Vacuum cooking (*'cook vide'*): frying and boiling

Vacuum cooking, or *cook vide*, is defined as the cooking process that is carried out under pressures well below atmospheric level. Owing to the low temperature and oxygen content during the process, the vacuum cooking presents some advantages for the fifth range product, including better preservation of natural colour, flavours and nutritional value (Andrés-Bello *et al.*, 2009). The vacuum frying is used for the value-added products, such as fruit and vegetables (i. e., potato, banana and mango chips). Kailan-hybrid broccoli is an excellent vegetable suitable for the vacuum cooking.

Over the last decade, chefs have introduced these innovative cooking techniques into their kitchens creating the binomial 'scientific-kitchen'. In this way, they have developed equipment like the Gastrovac[®] (ICC Company), which allows frying or boiling under controlled vacuum-pressure conditions (Figure 4.6).

Figure 4.6. Vacuum boiling of kailan–hybrid broccoli in the Gastrovac[®].

4.4.3.6. *Sous vide and its variant sous vide–microwaving*

The *sous vide* is a technique developed by the French cooker George Pralus in 1967. This cooking technique is derived from the traditional cooking method known as *papillote*. The *sous vide* technology is widely used in France (present in around 10–15 % of French kitchens) and Belgium. In Spain, the *sous vide* application to the fifth range industry has not been consolidated probably due to two reasons: primarily, to poor acceptance by the general consumer associated to a higher price of these products, information lacking for the consumer and opposition to new products; and secondly, to the necessary investments in investigation and research required by this system, compared to traditional systems used in the food industry. However, this technique is being used by world known Spanish chefs (Ferran Adrià and Joan Roca I Fontané), who recently developed *sous vide* equipment like Roner[®] (Villarino–Rodríguez, 2009).

The most complete definition for *sous vide* was the proposed by the *Sous vide* Advisory Committee (SVAC) as follows: *Catering discontinuous system in which a food, raw or precooked, is vacuum packed in bags or containers of laminate plastic, subjected to a heat treatment under controlled conditions, cooled rapidly, chilled stored and reheated prior to consumption* (SVAC, 1991). The *sous vide* technique is usually applied to vegetables since they do not require a pre-cooking step, such as meat or fish.

The increasing interest of the fifth range industries in the *sous vide* technique has led to a wide commercial offer of *sous vide* equipment. Among these devices are those based on: air–steam combination, heating/cooling with water (Thermix and Afrem[®] systems from the company Armor Inox), streaming water (Steriflow[®] from the company Barriquand steriflow) and microwave heating (*sous vide*–MW).

The *sous vide*–MW uses as heating source the microwave technology. This technique habitually uses the Darfresh[®] and Cryovac[®] (Figure 4.7) packaging systems (Cryovac Inc. company).

Figure 4.7. A) Kailan–hybrid broccoli packaged prior *sous vide* processing. B) Fresh asparagus packaged under Cryovac[®] system (<http://southernspecialties.com/southern-selects/>).

The election of the temperature/time binomial is very important during the cooking procedures to achieve a good sensory quality and safety. Depending of the product, the shelf–life of the *sous vide* products may vary from 6 to 45 days. In particular, this binomial acquires a crucial connotation in the *sous vide* method since the relatively low cooking temperatures used may jeopardize the product safety. In comparison with other products, such as meat and fish, vegetables need higher processing temperatures (90–100 °C, depending of the vegetable) for acceptable texture (Ghazala, 1998). After previous studies in our laboratory, the optimum temperature/time for kailan–hybrid broccoli was 90 °C/15 min, which accomplishes the minimum heat treatment requirement for pasteurization (Sheard and Rodger, 1995).

4.4.3.7. Microwaving

Microwaves have a determined frequency of 2,450 MHz, sometimes, in Europe 896 and in the USA 915 MHz (Fellows, 1994). The Figure 4.8 shows a diagram of a microwave system with the four common parts (1, Magnetron; 2, Waveguide; 3, Applicator; 4, Strips).

Figure 4.8. Schematic diagrams of microwave systems.

The advantages and disadvantages of microwave cooking are shown in Table 4.2.

Table 4.2. Advantages and disadvantages of microwaving.

Advantages	Disadvantages
<ul style="list-style-type: none"> • Allow in-pack or out-pack cooking. • Minimal damage to the structure and nutritional value of the product. • Quick heat penetration and maximum heating rate. • Low energy consumption. • Allow continuous processing. 	<ul style="list-style-type: none"> • Integration in the processing lines. • Limited heating penetration in some products. • Uniformity of heating: need of computerized design methods for controlling the heating on edges and corners. • Validation and process control: variability of heating times and final temperature controls.

The most used in-pack microwave cooking systems by the fifth range industries are MicVac[®] (Figure 4.9) and MES[®] technologies (MicVac Company).

Figure 4.9. MicVac[®] system (MicVac Company).

4.4.4. Packaging

The packaging step is crucial for the fifth range products due to its importance for inhibiting the chemical and microbiological deterioration during the processing and storage (Rodgers, 2007). The container may prevent the exit of water and volatile compounds (own aromas and flavours from food). Apart from these characteristics, the container may satisfy some technical, commercial and legal properties.

The following main types of packaging technologies are used in the fifth range industry (Rodgers, 2007):

1. Vacuum: the air from the container is completely evacuated.
2. Controlled atmosphere: a single gas or mixture of gases is injected in the container after the removal of the air. It is subjected to a constant control during the storage period. The most used gases are CO₂ and N₂.
3. Modified atmosphere: similar to the latter but there is no control during the storage period.

The vacuum packaging has an inhibitory effect on the aerobic microorganism (spoilage and pathogens) growth and fat oxidation. The low O₂ concentrations achieved within the container of the fifth range products inhibit the growth of mesophilic aerobic microorganisms. The applied partial vacuum (total vacuum is avoided due to the collapse risk of the tray) is usually compensated with N₂ and CO₂ residual concentrations with the purpose of preventing oxidation and microbiological alterations

(Díaz–Molins, 2009). This packaging method allows obtaining products with a long commercial life compared to other conventional cook–chill methods (Church and Parsons, 2000).

Figure 4.10. Vacuum chamber during kailan–hybrid broccoli packaging.

The heat–sealed bags and plastic trays are the preferred presentations for the fifth range products. Vacuum chambers (for bags, Figure 4.10) and tray sealers (Figure 4.11) are the most used packaging equipment for these products. These devices allow packaging under vacuum or injecting the desired gas.

Figure 4.11. Tray sealer during kailan–hybrid broccoli packaging.

4.4.5. Blast chilling

The temperature and RH conditions reached after the cooking step might favour a rapid microbial growth in the fifth range products. The 12–50 °C range presents the highest growth risk of the spore-forming bacteria resistant to the thermal treatment (Gaze *et al.*, 1998). With the aim of avoiding the undesired microbial growth, the maximum time to start the blast chilling must be inferior to 30 min. Furthermore, an internal product temperature of 0 to 3 °C must be reached in a maximum time of 90 min (Anonymous, 1989). Air, water and plates blast chillers, and cold rooms are the most used devices to decrease the temperature of the cooked product (Zhang and Sun, 2006). However, new technologies for blast chilling like vacuum cooling have shown excellent results in cooked broccoli and carrots (Sun and Wang, 2001).

4.4.6. Cold storage, transportation and distribution

The refrigeration is crucial for the conservation of the fifth range products. The main hazard associated with the chilled storage is the possible germination and growth of spores that have survived to the thermal treatment. The storage temperatures of these products may be in the range 1–4 °C, being very important an accuracy of the cold room of ± 0.5 °C (Díaz-Mollins, 2009). The cold room may be close to the blast chiller to avoid excessive temperature fluctuations. In this way, the refrigerated transport and distribution will retard the microbial growth and a long shelf-life of 6–42 days can be achieved, depending of the commodity and cooking method used.

4.4.7. Regeneration

The regeneration is the warming-up of the fifth range product prior to consumption. The purpose of this process is to reach a temperature over 65 °C. The maximum time for consumption after regeneration is about 1 h (at room temperature) or 2 h (at refrigerated storage). After consumption, the remaining product may be discarded. The most used regeneration systems are MW, convection and infrared ovens, water baths (bain-Marie) (for soups and sauces) and regeneration cars.

4.5. Nutritional and sensory quality changes

The cooking procedures may produce the highest nutritional quality losses of the products of the food industry. In this way, the characteristics of the commodity may

point the most recommendable cooking method to obtain a fifth range product which meets sensory and nutritional expectations of the consumer.

The first work referring the effect of cooking methods in the nutritional quality of vegetable-based products refers to broccoli (Buckley, 1987). This study showed vitamin C retentions of 86, 9.4 and 7.3 % for *sous vide*, boiled or HP steamed broccoli, respectively, after 5 days of chilled storage. Table 4.3 also shows the nutritional components changes of some vegetables after *sous vide* cooking. As observed in Table 4.3, vitamin C is among the vitamins that attain the highest losses during cooking owed to its high thermolabile and hydrosoluble nature.

Table 4.3. Vitamin retention in *sous vide* processed vegetables after 21 days of chilled storage and subsequent regeneration (Watier and Belliot, 1991).

Compound	Retention (%)			
	Carrots	Green beans	Cauliflower	Potatoes
β -carotene	–	89	100	–
Vitamin E	–	100	100	–
Vitamin C	63	86	40	69
Vitamin B1	86	67	86	56
Vitamin B2	100	100	71	86
Vitamin B5	84	63	90	37
Vitamin B3	48	62	65	61
Vitamin B6	80	100	79	63
Vitamin B9	100	100	85	100

Petersen (1993) studied the effect of *sous vide*, steaming and boiling on the L-AA, vitamin B6 and folacin retention of broccoli (cv. Shogun). Generally, L-AA, carotenoids and phenolics in broccoli all decreased significantly with time during boiling or MW cooking (Zhang and Hamauzu, 2004). Among the different cooking methods studied to the present, boiling has been reported as the method which produces the highest nutritive and bioactive compounds losses (phenolics, glucosinolates, carotenoids, vitamins, minerals, etc.) due to lixiviation processes. However, steaming has been widely reported to achieve good retention of those compounds owed to the short cooking time and limited cooking liquids used (Petersen, 1993; Zhang and Hamauzu, 2004; Galgano *et al.*, 2007; López-Berenguer *et al.*, 2007).

The aliphatic glucosinolates are generally more stable than indole glucosinolates during postharvest and cooking treatments of *Brassica* vegetables (Cieslik *et al.*, 2007; Rungapamestry *et al.*, 2007). Vacuum pressure of 97 % is recommended for *sous vide* processing of broccoli since the L-AA retention (100 °C for 40 min) was significantly enhanced compared to a low vacuum level (92 %), meanwhile vitamin B6 was unaffected (Petersen, 1993).

Some mild cooking treatments achieve better bioactive content retention than aggressive cooking methods (Matusheski *et al.*, 2001). This study showed that heating broccoli at 60 °C for 5 min, or more, inactivated the epithiospecifier protein (which favours nitrile production over ITC in broccoli under certain conditions), resulting in a higher sulforaphane conversion from glucosinolates. This fact occurs while myrosinase is not inactivated, which happens after a thermal treatment of 100 °C for 5–15 min. Other studies reported apparent increased TAC, total phenolic and lutein contents in a range of 1.2–1.3-fold after boiling, steaming and MW of broccoli (Yamaguchi *et al.* 2001; Zhang and Hamazu, 2004; Turkmen *et al.*, 2005). However, the latter increases were attributed to a cellular tissue disruption, which would release the bioactive compounds from the insoluble portion of the plant tissue.

The good nutritional quality attained by mild treatments may be greatly reduced if the subsequent steps such as blast chilling, refrigerated storage and regeneration are not properly conducted (Ghazala, 1998).

4.6. Overall quality of fifth range broccoli

Cooking is a food culinary procedure which may attain good sensory characteristics of the product due to some physical–chemical processes (matrix softening, pH changes, undesirable compounds elimination, etc.), obtaining a product with milder flavour. However, it is difficult to draw general conclusions regarding the sensory quality of cooked products for the following reasons (Creed, 1995):

1. The experiments are designed to assess changes in specific sensory attributes (caused by different storage temperatures and different cooking times) or to make comparisons between the innovative and traditional cooking for the same food or other processing systems.

2. Some studies applied different treatment conditions for the same cooking method, which cannot allow a proper comparison with other studies.
3. Scales, attributes, procedures and statistical tests used in the studies are not the same, which makes sometimes quite difficult to compare the results.
4. Some experiments have reproducibility problems when making comparisons. For example, when a freshly prepared sample with many ingredients is repeated to compare it with another sample stored.

Sous vide treatment emerged as a great cooking method able to keep in a better way the sensory properties of the food products after cooking. For this reason, many of the studies, with scientific relevance, referring to the effect of cooking on the sensory properties of food products compared the *sous vide* treatment with other traditional methods. Attending to this culinary technique, the following general conclusions may be stated:

- In terms of texture, aroma, flavour and appearance, significant differences between chilled *sous vide* and frozen *sous vide* products are found.
- When comparing traditional cooking methods with *sous vide*, flavour is further enhanced by *sous vide* treatment, although this sensory parameter achieves undesirable scores during storage.
- High processing temperatures are needed for vegetables to ensure an acceptable texture for consumers.
- The shelf-life of *sous vide* products is very long compared to other more aggressive methods, which provokes excessive matrix disruption of the product with more nutrients available for the spoilage microorganisms.

Petersen (1993) studied the effects of boiling, LP steaming and *sous vide* on the sensory quality of broccoli (cv. Shogun), reporting that the LP steamed and *sous vide* samples showed better acceptability than the boiled samples. Furthermore, the *sous vide* treatment had a better effect on the flavour. However, the *sous vide* treatment enhanced the bitter taste, which was not the case for the boiled samples. Varoquaux *et al.* (1995) established that a *sous vide* treatment not exceeding 90 °C for 2 h was sufficient to obtain the best sensory quality for lentils. Thus, any heat treatment above 90 °C, or processing time exceeding 2 min, caused excessive lentils softening. Goto *et al.* (1995) compared the *sous vide* method with different traditional Japanese dishes like

takikomigohan (steamed rice with other ingredients), *saba-nituke* (boiled mackerel with soy sauce) and cabbage rolls. The appearance, colour, taste, smell, smoothness and overall preference of the latter dishes did not show significant differences compared to the *sous vide* samples. When comparing *sous vide* with other dishes, such as *nikujaga* (cooked meat and potatoes with soy sauce) and boiled chicken with fig cream, the *sous vide* treatment was preferred (Goto *et al.*, 1995). The latter work also studied the effect of the cooking methods on some fruit-based products such as the kiwi sauce, reporting the *sous vide* samples better colour than the traditional methods. In the same study field, Goto *et al.* (1995) found that the aroma and flavour of the *sous vide*-processed (100 °C for 1.5 min) mitsuba (*Cryptotaenia japonica*), an aromatic plant Japanese used in the preparation of soups, were better than the boiled samples (1 min). Werlein (1998) reported that *sous vide* carrots (98 °C for 20 min) were also preferred, even at the end of storage (21 days at 2 °C), than boiled samples (12 min).

4.7. Safety aspects of fifth range vegetables

The fifth range products are considered as potentially hazardous since such foods can support the growth of pathogenic and spoilage bacteria. The temperature, gas composition within the package, product characteristics (mainly pH and water activity – *aw*–) and storage period may select the growth of different bacterial groups, their activity and growth rate in this kind of fifth range products. The cooking processing may eliminate most of the vegetative cells but a possible recontamination of the product after the thermal treatment may enhance the microbiological deterioration and, therefore, its commercial life. Furthermore, the mild cooking treatments can be sufficient to eliminate almost all the vegetative bacterial cells but not all the spore forms or thermophilic microbial cells. The Table 4.4 shows the thermal resistance of the main bacterial spores and bacteria. A rapid blast chilling is necessary in order to reduce the temperatures after cooking which may favour a rapid microbial growth.

The vacuum packaging and the protective-MAP cannot ensure O₂ levels below 1 to 5 % within the package. Firstly, these low-O₂ atmospheres can allow the facultative anaerobic bacteria growth (i. e., *Bacillus cereus*) and, lately, the remaining O₂ content gradually decrease until null levels which allocate the strict anaerobic bacteria growth (i. e., *Clostridium botulinum*, *C. perfringens*, etc). This mechanism explains why the strict anaerobic microorganisms, such as the *Clostridium* group, only can be isolated

after a long storage period, while the microaerophilic microorganisms, such as *Lactobacillus* spp., grow in the first storage stages of these products (Martens, 1995). The MAP with 20 % CO₂ + 80 % N₂ avoids the spoilage caused by aerobic microorganisms, such as *Pseudomonas* spp. (Gill and Molin, 1991). In this way, the film selection is a crucial point which conditions the commercial life of these products, since the O₂ permeation through the package can produce the aerobic microflora growth.

Table 4.4. Thermal resistance of the main bacterial spores and bacteria (Martens, 1995).

Microorganism	Temperature (°C)	D value* (min)
Yeast and moulds	70	3
<i>Campylobacter jejuni</i>	55	0.74–1.00
<i>Brucella</i> spp.	65.5	0.1–0.2
<i>Salmonella senftenberg</i> 775W	65.5	0.8–1.0
<i>Salmonella</i> spp.	65.5	0.02–0.25
<i>Staphylococcus aureus</i>	65.5	0.2–2.0
<i>Listeria monocytogenes</i>	66.1	16.7–16.9
<i>Enterococcus faecalis</i>	70	3
Spores from aerobic mesophilic bacteria		
<i>Bacillus cereus</i>	100	5.0
<i>Bacillus subtilis</i>	100	11.0
<i>Bacillus polymyxa</i>	100	0.1–0.5
Spores from anaerobic mesophilic bacteria		
<i>Clostridium butyricum</i>	100	0.1–0.5
<i>Clostridium perfringens</i>	100	0.3–20.0
<i>Clostridium botulinum</i>		
Type A and Type B proteolytic	100	50.0
Type E and Types B and F no proteolytic	80	1
Spores from aerobic thermophilic bacteria		
<i>Bacillus coagulans</i>	120	0.1
<i>Bacillus stearothermophilus</i>	120	4.0–5.0
Spores from anaerobic thermophilic bacteria		
<i>Clostridium thermosaccharolyticum</i>	120	3–4
<i>Clostridium nigrificans</i>	120	2–3

*D value is the time required to reduce the number of survivor bacteria to the 10% of the initial value.

The refrigeration during distribution and storage are critical for these fifth range products. Only a 10 % of the bacterial population present prior to the thermal treatment is able to grow at chilled temperatures, and a low fraction of them are spoilage microorganisms.

Generally, the fifth range products have high aw and pH values, and are formulated with little or no additives. Thus, the low O₂ availability, high pH and aw and absence of competing microorganisms can allow the development of pathogenic microorganisms to dangerous levels, without necessarily any alteration sign on the product.

In conclusion, the crucial points during the processing of the fifth range products (always processed with GMP and under a HACCP plan) are a sufficiently intense heat treatment and a chilled storage (< 3.3 °C). These practices, combined with the use of additional barriers to the microbial growth (pH and/or aw reduction, addition of organic salts, etc.) would effectively prevent the microbial growth and pathogenicity in the fifth range products.

Among all outbreaks of strong evidence caused by cooked food in Europe in 2010, *Salmonella* spp. were the main causative effect (43.3 %), being reported 17 cases (from the 97 total cases) in Spain. In 2005, a *Salmonella* spp. outbreak in fifth range eat roast chicken (company SADA) originated 302 hospitalizations and one dead. According to these evidences, some agencies responsible for the food regulation, such as the ACMFS and the US–FDA, established good manufacturing practices that help to ensure the safety of this kind of products (EFSA, 2012).

The Spanish Legislation RD 135/2010 of 12 February 2010 derogated the previous RD 3484/2000 of 29 December 2000, which established the hygienic indications for manufacturing, distribution and commerce of the ready-to-eat meals. Consequently, the legislation for the fifth range products in Spain is also regulated by the above cited European Legislation N° 1441/2007 (2007).

5. BIOAVAILABILITY

The bioactive and nutritional compounds from food may induce potential beneficial effects on health. However, these compounds not always reach human cells at

the specified optimum concentrations, since a proportion of them could be directly excreted (via renal) or transformed in other non-health-promoting compounds. For this reason, in order to determine the complete biological activity of these compounds when they are ingested, their bioavailability and metabolic fate might also be studied (Kroon *et al.*, 2004). Thus, it is not only important to know the bioactive content of the food, but that the absorbed quantity too.

Bioavailability for food compounds can be defined as the proportion of the administered substance capable of being absorbed and available for use or storage in target tissues. The concept of nutrient bioavailability encompasses the processes of ingestion, absorption, distribution, utilisation and loss (Davey *et al.*, 2000). The bioavailability of a substance can be determined by both *in vivo* (the compound is administered to a living organism: human or animal) or *in vitro* methods (simulations of the digestive tract by isolated gut cells or gut segments) (Tarazona-Díaz, 2011). There are several physiological factors that are very difficult to reproduce in a laboratory by *in vitro* methods. For this reason, the *in vivo* method is the most convenient way to determine the bioavailability of an ingested compound (Van Campen and Glahn, 1999).

There are two types of bioavailability: absolute or relative bioavailability. The absolute bioavailability (estimated as AUC) compares the bioavailability following extravascular administration of the compound (oral) with bioavailability following intravenous administration in a dose-normalized way. On the other side, the relative bioavailability can compare the bioavailability of different food samples (Davey *et al.*, 2000). It would be very difficult to determine, in an accurate form, with a simple blood test the total quantity of the compound in blood after the food ingestion. According to this, and strictly speaking, the bioavailability of a food compound is determined by the absolute bioavailability. As an alternative to the complicated determination of the absolute bioavailability, the study of the human metabolic fate of some substances can be made indirectly by biomarkers, if they have been previously elucidated and validated.

Numerous epidemiological studies indicate that consumption of large quantities of fruits and vegetables, particularly cruciferous vegetables (i. e., broccoli, cabbage, kale and Brussels sprouts), is associated with a reduced incidence of cancer (Traka and

Mithen, 2009). The observation that dietary glucosinolates are not detected in urine suggests they are not absorbed (Shapiro *et al.*, 1998). There is a controversy if some enteric microflora strains have myrosinase activity (Fahey *et al.*, 2001). This fact could lead to a complete glucosinolate–isothiocyanate conversion when plant myrosinase is thermally degraded by cooking treatments. ITC are metabolized by sequential enzymatic reactions of the mercapturic acid pathway to be finally excreted in urine as mercapturic acids (Figure 5.1).

ITC and their metabolites through the mercapturic acid pathway (known as dithiocarbamates) react quantitatively with 1,2–benzenedithiol in the cyclocondensation assay giving as product 1,3–benzo–dithiole–2–thione. This method has been validated as a sensitive urinary biomarker for the uptake of dietary ITC in humans (Chung *et al.*, 1998).

Figure 5.1. Metabolic fate of glucosinolates in the human organism.

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OBJECTIVES

OBJECTIVES

The research works conducted in this Ph. D. Thesis have been focused on keeping, or even enhancing, the bioactive compounds content and nutritional value of a new kailan–hybrid broccoli as a FC and fifth range product. Furthermore, the quality and food safety during the processing of these kinds of products have been studied. This vegetable is a new natural hybrid between broccoli and kailan. It has been developed by the breeding companies in order to reach a better acceptance by consumers for its sensory characteristics in attempt to increase the current low consumption of conventional broccoli cvs. in several countries like Spain. In fact, kailan–hybrid broccoli has a more pleasant flavour and aroma than the conventional broccoli cvs. likened to an asparagus. In addition, kailan–hybrid broccoli has great morphological differences compared to conventional broccoli cvs. Among those, the most relevant are a long slender stem and reduced florets (compared to conventional cvs.) which makes it ideal for minimal processing purposes.

The optimization of the FC and fifth range processing for this vegetable has been studied. In this way, new sanitizing alternatives to disinfection with NaClO (commonly used in the FC industry and forbidden in some European countries, which may lead to new regulatory restrictions in the near future) such as EW, UV–C radiation and HO packaging have been tackled. Furthermore, in order to obtain a fifth range product with high health–promoting properties, long shelf–life and always safe for the consumers new cooking techniques have been studied. Finally, with the aim of having a whole knowledge of these health–promoting properties of the kailan–hybrid broccoli (raw or cooked) *in vivo* studies about their bioavailability have been also conducted.

This Ph. D. Thesis supplies useful scientific and technological data for the FC and fifth range industries to introduce a new product with excellent health–promoting compounds, which real human cell fate has been also hereby tested.

The specific objectives of this Ph. D. Thesis are:

- To know the nutritional, bioactive compounds content, physico–chemical, microbiological and sensory characterization of FC kailan–hybrid broccoli compared to that of the conventional cv. Parthenon, one of the most cultivated in Spain.

- To assess the effects of several UV–C radiation doses in health–promoting compounds as well as in the main quality parameters changes throughout shelf–life of FC kailan–hybrid broccoli.
- To evaluate and optimize alternative sanitizers (EW, UV–C radiation and HO packaging) to NaClO as single or combined treatments. The effects of them over the health–promoting compounds and quality of FC kailan–hybrid broccoli during shelf–life have been also studied.
- To apply, develop and optimize several conventional and innovative cooking methods on the kailan–hybrid broccoli. The effects of these treatments and subsequent chilling storage on health–promoting properties, overall quality and safety of the cooked products were also studied.
- To study the *in vivo* metabolic fate of glucosinolates of the kailan–hybrid broccoli once it is ingested. The comparison between raw and cooked product has also been accomplished.

CHAPTER I

Comparative behaviour between kailan-hybrid and conventional fresh-cut broccoli throughout shelf-life

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ABSTRACT

The physiological, physicochemical, microbial and sensory quality changes of fresh-cut kailan-hybrid were compared with those of the conventional 'Parthenon' cv. during 15 days at 2, 5 and 8 °C under air and modified atmosphere packaging (MAP). As expected, the higher the temperature the higher the respiration rate and ethylene production, being higher for the kailan-hybrid than for the 'Parthenon' cv. regardless of the temperature. MAP, combined with low storage temperature, resulted in a better microbial and sensory quality. Under MAP at 2 °C, decreases of 70, 88, 57 and 7 % for the 'Parthenon' cv. and 43, 10, 57 and 10% for the kailan-hybrid were recorded for mesophilic, psychrophilic, enterobacteria and yeasts and moulds counts, respectively. Undesirable sensory attributes changes, such as yellowness, off-flavour, and stem softening and bent, were retarded under MAP after 15 days at 2 and 5 °C. In air-stored samples at 8 °C a reduction in total soluble solids content and an increase in yellowing were found. MAP storage at 2 °C (5–7 kPa O₂ + 14–15 kPa CO₂) or 5 °C (1.5–2.5 kPa O₂ + 15–16 kPa CO₂) provided for both broccoli cultivars an acceptable sensory quality and safety after 15 days of storage.

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1. Introduction

The current worldwide lifestyle desire for healthier, safer, and more convenient fresh food diets correlates these targets by the consumer's desire for high overall quality products. Fresh-cut (FC) fruit and vegetables meet these consumers' needs and, consequently, its market has enlarged greatly all around the world. The consumer's increasing interest in broccoli is mainly due to its considerable relevance as a health-promoting fresh produce (Jeffery et al., 2003; Lemoine, Chaves, & Martínez, 2010; Podsędek, 2007). Additionally, broccoli is a convenient vegetable with specific sensorial characteristics which commonly requires minimal or no cooking preparation prior to consumption.

New broccoli cultivars with milder flavour than the conventional ones have recently appeared in the market, in attempt to increase its consumption. A good example is the hybrid between kailan (*Brassica oleracea*, Alboglabra group), also called kai-lan, gai-

lan or kailan, and conventional broccoli (*B. oleracea*, Italica group), commercially called Bimi® or Tenderstem®. This morphologically distinct kailan-hybrid looks like a broccoli with a long slender stem and has a mild sweeter taste, being completely edible (both as whole raw and after soft cooking methods) and quite well adapted for FC purposes (Martínez-Hernández, Gómez, Pradas, Artés, & Artés-Hernández, 2011; Martínez-Hernández et al., 2012).

The major postharvest preservation techniques applied to prevent or delay undesirable quality changes of FC commodities are chilling storage and MAP, combined with sanitising treatments (Artés, Gómez, Aguayo, Escalona, & Artés-Hernández, 2009; Lemoine et al., 2010). Its use to delay the postharvest deterioration (yellowness, off-flavours, off-odours, softening, etc.) and to keep the visual quality of some FC conventional broccoli cultivars has been reported (Serrano, Martínez-Romero, Guillén, Castillo, & Valero, 2006; Soliva-Fortuny & Martín-Belloso, 2003). However, no scientific information on the kailan-hybrid broccoli behaviour under these techniques has been published so far.

The aim of this work was to characterize the behaviour of the new FC kailan-hybrid broccoli throughout its shelf-life compared to that of the conventional 'Parthenon' cv. In this way, the effect of three storage temperatures under MAP and air on the main physiological, physicochemical, microbial and sensory quality attributes throughout shelf-life was studied.

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2. Materials and methods

2.1. Plant material

Kailan-hybrid (*B. oleracea* Italica Group × *Alboglabra* Group) of about 15–18 cm length (Fig. 1) and 'Parthenon' cv. (*B. oleracea* Italica Group) at commercial maturity stage (about 350–400 g/head) were hand-harvested from open air cultivated parcels located in Murcia Region (Campo de Lorca, Juan Marín S.L.), in the Southeast Mediterranean Spanish area. Broccoli was immediately crushed, ice pre-cooled, and transported 80 km to the Pilot Plant at the Universidad Politécnica de Cartagena, where it was stored at 1 °C and 90–95% RH until next day.

2.2. Sample preparation, minimal processing and storage conditions

Minimal processing was accomplished in a disinfected cold room at 8 °C. Plant material was carefully inspected, selecting those free from defects and with similar visual appearance. Small leaves of kailan-hybrid broccoli stems (when they were present) were eliminated with a sharp knife. The kailan-hybrid broccoli was cut in about 15 cm lengths and the 'Parthenon' heads were cut into florets.

Cut pieces were then washed for 2 min with chlorinated water (5 °C, 100 ppm NaClO at pH 6.5) and rinsed for 1 min with cold (5 °C) tap water. Once drained, 120 g broccoli were disposed in 1.5 L (kailan-hybrid) and 2 L ('Parthenon') rigid polypropylene (PP) trays (12 × 17 cm) which were thermally sealed on the top with an antimist 30 µm thick bioriented PP film (Plásticos del Segura S.L., Murcia, Spain). The film permeability was 900 cm³ O₂ m⁻² day⁻¹ atm⁻¹ and 1100 cm³ CO₂ m⁻² day⁻¹ atm⁻¹ at 23 °C and 0% RH (data from the supplier). As air control, a 40 µm thick macro-perforated PP film was used. Three replicates of one tray per

treatment and sampling time were prepared, and trays were kept at 2, 5 and 8 °C (90–95% RH). Quality analyses were conducted on the processing day and after 6, 9, 12 and 15 days of shelf-life. Such temperatures simulate ideal, maximum recommended, and mis-used, respectively, throughout shipping, distribution and retail sale of broccoli in Europe (Artés, Vallejo, & Martínez, 2001).

2.3. Respiration rate, ethylene emission and gas analysis within packages

The RR and C₂H₄ emission were determined using a closed system (Escalona, Aguayo, Gómez, & Artés, 2004) with three replicates. C₂H₄, O₂ and CO₂ partial pressures within packages were analysed according to Tomás-Callejas, Boluda, Robles, Artés, and Artés-Hernández (2011). For each treatment and evaluation period, three replicates were analysed.

2.4. Microbial analysis

To determine mesophilic, psychrophilic, enterobacteria and yeasts and moulds counts, standard enumeration methods were used (Tomás-Callejas et al., 2011). All microbial counts were reported as log₁₀ colony forming units per gram (log CFU g⁻¹). *Salmonella* spp., *Listeria monocytogenes* and generic *Escherichia coli* were monitored according to the European legislation for FC vegetables (Regulation EC 1441/2007, 2007). All analyses were made by triplicate.

2.5. Sensory evaluation

Sensory quality was evaluated on the processing day and after 6, 9, 12 and 15 days of shelf-life by seven trained panelists aged 24–63 years. A five-point scale of damage incidence and severity was

Fig. 1. Visual morphological differences and different parts of broccoli 'Parthenon' and kailan-hybrid Bimi®.

scored for off-odours, off-flavours and yellowness (5: none; 4: slight; 3: moderate, limit of usability; 2: severe and 1: extreme). Bent and stem softening of the kailan-hybrid were evaluated with the same damage scale described above. Floret compactness of 'Parthenon' cv. was evaluated as 5: very compact; 4: compact; 3: slightly separate; 2: separate and 1: very separate. The basal stem pithiness of the kailan-hybrid was scored as 5: no pithiness; 4: pithiness within 5% surface area; 3: within 10% surface area; 2: within 25% surface area and 1: more than 25% surface area. Overall quality was assessed by using another five-point hedonic scale (5: excellent, 4: good, 3: fair, limit of usability, 2: poor and 1: extremely bad).

2.6. Physicochemical quality analysis

Floret colour, stem firmness, total soluble solids content (SSC), pH and titratable acidity (TA) were analysed according to Artés et al. (2001). All samples were analysed by triplicate on the processing day and after 6, 9, 12 and 15 days of shelf-life.

2.7. Statistical analysis

The experiment was a $2 \times 5 \times 3$ trifactorial design (atmosphere $\times$ storage time $\times$ storage temperature) subjected to an analysis of variance using Statgraphics Plus (version 5.1) software. When differences among treatments were significant ($p < 0.05$), the mean values were compared by the least significant difference multiple range test (LSD). Figures represent mean values ($n = 3 \pm \text{SD}$).

3. Results and discussion

3.1. Respiration rate, ethylene emission and gas analysis

For both the kailan-hybrid and 'Parthenon' cultivar., the higher the storage temperature the higher the respiration rate (RR), reaching initial values of 226, 272 and 315 $\text{nmol CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$ in the kailan-hybrid and 126, 158 and 203 $\text{nmol CO}_2 \text{ kg}^{-1} \text{ s}^{-1}$ in the 'Parthenon' at 2, 5 and 8 °C, respectively. The individual factors of storage temperature, and time, as well as their interaction, were significant for RR of both broccoli varieties, except the interaction for the kailan-hybrid (data not shown). The expected positive relationship between RR and temperature, agrees with similar values reported for broccoli (*Brassica rapa* var. *sylvestris*) florets stored at 3–20 °C (Torrieri, Perone, Cavella, & Masi, 2010). At higher storage temperature the metabolism of these FC products increased and consequently its RR, as it has been found, reaching the highest values at the highest temperatures. The use of a low storage temperature reduces the RR and the senescence of the FC product will be retarded, consequently extending the shelf-life. The kailan-hybrid recorded initial RR values approximately 40% higher than those of 'Parthenon' cv. regardless of the temperature (Fig. 2). The high RR of the kailan-hybrid will entail fast senescence processes which have to be slowed down with adequate postharvest techniques like MAP combined with chilled storage as it has been applied.

Once processed, the RR of both the kailan-hybrid and 'Parthenon' cultivar. decreased, reaching the lowest values after 6–8 days, and subsequently increasing from day 8 to the end of the shelf-life with a rate of around 35% for all samples (Fig. 2). The highest RR shown by both FC broccoli on the processing day may be understood since cutting and handling causes metabolic reactions that greatly increase the RR at this initial point, considering the minimal processing itself as an abiotic stress. After processing, and once the cold storage is applied, this maximum RR starts to decline since the metabolism of the product decreases. However, the FC

Fig. 2. Respiration rate of broccoli 'Parthenon' (●) and kailan-hybrid (○) stored up to 15 days at 2 °C (—), 5 °C (---) and 8 °C (···) ($n = 3 \pm \text{SD}$).

broccoli is ageing throughout its storage period, reaching after 8 days a stage where the low temperature is not able to slow down the incipient metabolic reactions associated with senescence, as it is reflected on the RR increase from day 8 until the end of shelf-life (Fig. 2). Similarly to ours results, Artés-Hernández, Robles, Gómez, Tomás-Callejas and Artés (2010) reported an RR increase after 8 days at 5 °C until the 13 days of conservation in FC watermelon. An increase in respiration rate during storage can be attributed either to a climacteric rise or to an increase in microbial metabolism, which can be not associated to visual symptoms.

In the same way to RR, a 1.3-fold higher C_2H_4 emission was found for the kailan-hybrid compared to 'Parthenon' cv. (Fig. 3) which was also significantly ($p < 0.05$) temperature-dependent for both broccoli varieties (data not shown). The factors storage temperature and time, unlike its interaction, were significant for C_2H_4 emission of both broccoli varieties (data not shown). In fact, increasing values of 5.6, 6.2 and 8.3 $\text{nmol C}_2\text{H}_4 \text{ kg}^{-1} \text{ s}^{-1}$ for the kailan-hybrid and 4.2, 4.6 and 6.0 $\text{nmol C}_2\text{H}_4 \text{ kg}^{-1} \text{ s}^{-1}$ for 'Parthenon' cv. at 2, 5 and 8 °C, respectively, were found. It is well known that C_2H_4 production of the FC products is highly influenced by the storage temperature. The higher kailan-hybrid C_2H_4 production than that of 'Parthenon' cv. shows, as above found for

Fig. 3. Ethylene emission of broccoli 'Parthenon' (●) and kailan-hybrid (○) stored up to 15 days at 2 °C (—), 5 °C (---) and 8 °C (···) ($n = 3 \pm \text{SD}$).

RR, this new FC broccoli as a high metabolism broccoli cv. Consequently, the kailan-hybrid shelf-life can potentially be extended by MAP as reported in this study. Similarly to RR, C_2H_4 production decreased after the processing stress until day 11, according to the above cited temperature influence. A decrease in the C_2H_4 production after the processing stress was also reported in broccoli florets stored at 5 °C (Baclayon & Matsui, 2008). As expected, the lowest C_2H_4 production was registered at 2 °C. After 11 days, reductions of 39 and 35%, compared to initial values, for the kailan-hybrid and 'Parthenon' cultivar, respectively were found. Moreover, and similarly to RR, a slight increase of C_2H_4 emission was registered after 11 days at 5 and 8 °C, in agreement with Bastrash, Makhoul, Castaigne, and Willemot (1993) which reported an C_2H_4 emission increment in broccoli florets air-stored at 4 °C. However, the interaction storage temperature-time did not show significant differences ($p < 0.05$) for both broccoli varieties (data not shown).

The atmosphere composition changes showed a similar pattern at all storage temperatures and for both broccoli cvs. The equilibrium gas partial pressures were reached after 7, 5 and 3 days at 2, 5 and 8 °C, respectively (Fig. 4), showing how the intensified initial metabolism due to higher temperatures resulted in an earlier attainment of this equilibrium. The factors storage temperature and time, as well as its interaction, were significant for atmosphere

composition, except the interaction for CO_2 in both broccoli varieties (data not shown). Similar results were found by Torrieri et al. (2010) in FC broccoli florets stored at 5 and 10 °C. The gas composition within packages at the steady state for both the kailan-hybrid and the 'Parthenon' cv. at 2, 5 and 8 °C were 5–7 kPa O_2 + 14–15 kPa CO_2 , 1.5–2.5 kPa O_2 + 15–16 kPa CO_2 and 1.2–1.6 kPa O_2 + 20–20.9 kPa CO_2 , respectively. This gas composition was similar to that recommended for broccoli florets by Gorny (2001, pp. 95–145), being these atmospheres, with high CO_2 and low O_2 , excellent coadjutants to low temperature and high RH, slowing down the senescence rate and extending the shelf-life of the FC broccoli. Furthermore, the atmosphere composition reached at 8 °C agree with that of 1% O_2 + 21% CO_2 recommended for the storage of broccoli florets, since at this MAP condition simultaneously aliphatic and indole glucosinolate levels and external appearance were kept and off-odour was prevented (Schreiner, Peters, & Krumbein, 2007).

3.2. Microbial analysis

The initial microbial counts were 2.8/3.9, 2.1/3.8, 1.5/3.3 and 2.5/3.3 log CFU g⁻¹ ('Parthenon'/kailan-hybrid) for mesophilic, psychrophilic, enterobacteria and yeasts counts, respectively, with undetectable moulds loads. These differences between microbial counts could be explained by the natural contamination of the plant material from the field, which was higher for the kailan-hybrid than for the 'Parthenon' cv. These values were lower than those found by Brackett (1989) in fresh-harvested conventional broccoli (6 CFU g⁻¹ for mesophilic). After washing and sanitising steps, reductions of 1.4–1.6-fold for mesophilic and 1.1 for psychrophilic counts were found (Fig. 5).

Microbial growth increased for both the kailan-hybrid and the 'Parthenon' cv. at all temperatures, with higher microbial proliferation recorded at higher storage temperatures (Fig. 5). These results confirm the low temperature management as a key factor for keeping microbial quality throughout the shelf-life of both FC broccoli. The best microbial quality was reached when MAP and low temperature storage were combined. In fact, 'Parthenon'/kailan-hybrid MAP samples at 8 °C showed 70/43, 88/10, 57/57 and 7/10% higher microbial counts for mesophilic, psychrophilic, enterobacteria and yeasts and moulds counts, respectively, than 'Parthenon'/kailan-hybrid MAP samples at 2 °C (Fig. 5). MAP samples always showed lower counts than air-stored samples (Fig. 5). The same trend was found earlier by Brackett (1989) and Mohd-Som et al. (1994) in broccoli florets stored at 1 and 8 °C, respectively. The low O_2 and high CO_2 levels reached at the steady state under passive MAP (Fig. 4) restricted the proliferation of most microorganisms, which commonly grow better under an air atmosphere.

Microbial counts were dominated mainly by mesophilic and psychrophilic groups with lower enterobacteria and yeasts and moulds counts in both the kailan-hybrid and the 'Parthenon' cv. samples. Enterobacteria and yeast and moulds groups can grow quite well in FC vegetables at refrigeration temperatures (Moreira, Roura, & Ponce, 2011). The low enterobacteria and yeasts and moulds growth found here for both the kailan-hybrid and the 'Parthenon' cv. could be explained by the competition of microbial populations, such as pectinolytic Gram negative microorganisms, which have a high growth rate at low temperature, being better adapted to develop on broccoli because of their pectinolytic enzymes (Jacxsens, Devlieghere, & Debevere, 2002).

The presence of *Salmonella* spp., *L. monocytogenes* and generic *E. coli* was also monitored. No colonies were detected for any treatment on the processing day, being safe according to the EU legislation (Regulation EC 1441/2007, 2007).

Fig. 4. Gas changes [CO_2 (●), O_2 (○)] within packages of broccoli 'Parthenon' (A) and kailan-hybrid (B) stored up to 15 days at 2 °C (—), 5 °C (---) and 8 °C (···) ($n = 3 \pm SD$).

Fig. 5. Changes in (A) mesophilic, (B) enterobacteria, (C) psychrophilic and (D) yeasts and moulds counts ($\log \text{CFU g}^{-1}$) of broccoli 'Parthenon' (first column) and kailan-hybrid (second column) stored in air (●) and under MAP (○) up to 15 days at 2 °C (—), 5 °C (---) and 8 °C (···) ($n = 3 \pm \text{SD}$).

3.3. Sensory analysis

The score for overall quality, yellowness, off-flavour, bent and stem turgidity are shown in Figs. 6 and 7. 'Parthenon' cv. showed no significant yellowing incidence for all treatments throughout shelf-life, except for air atmosphere at 8 °C which showed visual yellowing from day 12 reaching a score of 2 at the end of storage (Fig. 6). These results agree with those found by Olarte, Sanz, Echávarri, and Ayala (2009) which did not report yellowness in MAP broccoli florets stored at 10 and 4 °C for 7 and 15 days, respectively. The incipient yellowing, due to chlorophyll degradation, scored in our samples was greatly favoured by the higher storage temperature and air atmosphere. Samples MAP-stored at lower temperatures did not show yellowing during shelf-life, probably due to the MAP protective effect on the chlorophylls content (Barth, Kerbel, Broussard, & Schmidt, 1993). Similarly, the C₂H₄ emission reduction is influenced by MAP, since C₂H₄ highly contributes to the chlorophyll breakdown (Kasai, Kato, & Hyodo, 1996). However, compared to 'Parthenon' cv., air-stored kailan-hybrid samples showed an earlier yellowing, below the limit of usability from day 9 and 15 at 5 and 8 °C respectively, as a consequence of their higher C₂H₄ emission which leads to a faster chlorophyll degradation (Fig. 6).

Compactness of 'Parthenon' florets slightly declined after 6 days of storage, remaining quite stable up to 15 days, recording the air-

stored samples at 8 °C the lowest scores (data not shown), in agreement with results from Jacobsson, Nielsen, Sjöholm, and Wendin (2004). The high temperature induced water loss in the flower cells, favoured by the open packaging conditions of air storage, leading to an alteration of its structure and subsequent disaggregation with loss of compactness.

Stem softening was detected in the kailan-hybrid after 9 days of storage, being more pronounced in air-stored samples at 8 °C (data not shown). The combined air atmosphere and high temperature storage conditions may have increased the water loss suffered by the stem and the activity of some pectinases, which may have produced the breakdown of the FC product cell walls (Micheli, 2001) causing the observed stem softening.

Stem bent, which probably may be produced by an enzymatic degradation of stem cells structure, besides the weight loss suffered from treatments under air and high temperature storage, was significant from the sixth day of air storage at 5 °C and 8 °C (with higher intensity and severity), while MAP at these temperatures greatly lowered their severity (data not shown). The remaining treatments showed a slight curving after 9 days of storage, but this was not as extreme as in air treatments (Fig. 7). Considering stem bent, the only unmarketable kailan-hybrid samples after 15 days were those air-stored at 5 and 8 °C. Consequently, MAP and low temperature management will keep a good appearance for the FC kailan-hybrid, delaying the stem bent.

Fig. 6. Sensory scores for overall quality, off-flavours and yellowness of broccoli 'Parthenon' and kailan-hybrid stored up to 15 days in air (●) and under MAP (○) up to 15 days at 2 (—), 5 (---) and 8 °C (···) ($n = 7 \pm \text{SD}$).

Fig. 7. Visual appearance of kailan-hybrid after 15 days in air at 2, 5 and 8 °C (A, B and C) and under MAP at 2, 5 and 8 °C (D, E and F).

Pithiness developed on the stem basis of the kailan-hybrid samples after 9 days of air storage at 5 and, mainly, 8 °C. However, there was virtually no pithiness in 'Parthenon' samples throughout shelf-life (data not shown). In celery, this pithiness was associated with the breakdown of the cortical parenchyma, generating the aerenchyma with a white porous appearance, vacuolated consistency and big air spaces (Gómez & Artés, 2004). As the formation of the aerenchyma is commonly stimulated by C_2H_4 , the high C_2H_4 emission of air-stored samples at 5 and 8 °C could be probably the responsible for the pithiness on stem basis of these kailan-hybrid samples. As far as we know, no information about pithiness development in broccoli stems has been previously reported.

Throughout 15 days of MAP storage at 2 and 5 °C no off-flavours in kailan-hybrid samples were detected (Fig. 5). However, off-flavours were perceived after 9 and 12 days at 8 °C in air and in MAP-stored samples, respectively. In contrast, after 15 days 'Parthenon' samples showed off-flavours in all MAP treatments whichever temperature was assayed. This is probably due to a slight fermentation process associated to near-anaerobic storage conditions (Fig. 4). Throughout FC broccoli shelf-life, some degradative reactions of sulphur compounds may occur, leading to sulphur derived off-odours and off-flavours.

The excellent overall quality at harvest slightly decreases from the sixth day of storage in MAP conventional broccoli florets stored at 5 °C. Both broccoli cvs. air-stored at 8 °C showed a faster decline in overall quality, reaching scores below the usability limit after 12 days of storage. The kailan-hybrid was still marketable after 15 days when it was MAP-stored at 2 and 5 °C. Meanwhile, the 'Parthenon' cv. was still marketable after 15 days only when MAP-stored at 2 and 5 °C, and when air-stored at 2 °C (Fig. 6). The overall quality of air packaged 'Parthenon' cv. at 2 °C reached after 15 days newly manifests the more intense metabolic activity of FC kailan-hybrid underlining the importance of the combined MAP and low temperature for improving their shelf-life.

3.4. Physicochemical analysis

The kailan-hybrid showed lower SSC than the 'Parthenon' cv. with initial values of 6.2 and 7.6 °Brix, respectively. 'Parthenon' cv.

did not show any significant SSC change throughout storage (data not shown), in agreement with results from Artés et al. (2001) for conventional 'Shogun' broccoli florets MAP-stored for 7 days at 1.5 °C. Similarly to storage time, treatment and storage temperature factors, besides their double and triple interactions, did not show significant differences for SSC in 'Parthenon' cv. (data not shown). The exceptions were the air-stored samples at 8 °C, where SSC decreased from its initial value after 6 days, recording 7 °Brix after 15 days. At high temperature, such 8 °C is for broccoli, the metabolic reactions in the plant cells increase with the subsequent sugar consumption, reflected in the observed decrease of SSC. SSC of kailan-hybrid showed significant differences for temperature and storage time factors, meanwhile treatment and all corresponding interactions did not (data not shown). Similarly, no clear trend of SSC changes were found for the kailan-hybrid, although after 15 days lower values in air and MAP treatments at 8 °C with around 1.1-fold decrease were recorded (data not shown).

The kailan-hybrid and the 'Parthenon' cv. showed initial pH values of 6.1 and 5.9, respectively. Whichever temperature in air storage was assayed, no changes in pH values during storage were found (data not shown) in agreement with results reported by Artés et al. (2001). However, the pH showed significant changes for the individual factors temperature and storage time for both broccoli varieties (data not shown). MAP samples showed an increase in pH values, where the higher the temperature and storage duration the higher the pH (increases of 13% for 'Parthenon' cv. at 8 °C). This pH increase may possibly be due to initial slight fermentation processes induced by elevated CO_2 levels.

The kailan-hybrid showed slightly higher TA levels than the 'Parthenon' cv. with values around 0.17 and 0.15 g citric acid 100 mL^{-1} , respectively (data not shown). It was found that MAP treatments at 8 °C induced a significant decrease in TA from the sixth day of storage, reaching at day 12 decreases of 33 and 35% for 'Parthenon' cv. and kailan-hybrid, respectively. As expected, the pH increase under MAP, reported above, was highly related to the decrease in organic acids content inferred from the TA records. The SSC and TA parameters are very important to define the maturity and quality of any horticultural produce, since there is a minimum requirement for the SSC and maximum acidity for good taste.

According to the hereby reported data, an SSC/TA ratio of 36.5 and 50.7 could be considered as optimum for kailan-hybrid and 'Parthenon' cv., respectively.

In both broccoli cvs. at low and recommended temperatures for air and MAP treatments no colour changes during storage were found, confirming early findings from Artés et al. (2001). Colour is a very important quality attribute and the most obvious feature of broccoli postharvest senescence, being highly related to broccoli and floret yellowing. Storage under an air atmosphere and at relatively high temperature may accelerate yellowing, as it has been observed here by a °Hue decrease found for both broccoli cvs. air-stored at 8 °C after 12 days. This was in agreement with the colour assessment scores obtained from the sensory analysis (data not shown). An increase in the severity of yellowing due to high temperature during shelf-life has been previously reported in broccoli florets by Artés et al. (2001). Compared to values at harvest, the kailan-hybrid showed the earliest and highest °Hue decrease with 63% at the end of storage. This is probably due to its widely proved higher metabolic activity than that of 'Parthenon' cv. The kailan-hybrid stem firmness showed initial values around 31.2 N and no changes in any treatment were found throughout shelf-life (data not shown).

4. Conclusions

The new kailan-hybrid Bimi® broccoli showed a significantly higher metabolic activity than that of the conventional 'Parthenon' cv. MAP storage (balanced with N₂) at 2 °C (5–7 kPa O₂ + 14–15 kPa CO₂) or 5 °C (1.5–2.5 kPa O₂ + 15–16 kPa CO₂) provides great benefits for both broccoli cvs., reaching an acceptable sensory quality and safety for consumption after 15 days of shelf-life. In particular, these storage conditions reduced microbial growth, delayed undesirable changes in the main physical and chemical quality attributes, and inhibited or lowered sensory changes and disorders such as yellowness, pithiness, off-flavours, and stem softening and bent.

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CHAPTER II

Nutritional quality changes of fresh-cut kailan-hybrid broccoli throughout shelf-life

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2.1. INTRODUCTION

In the last few years there has been a growing interest in food components which may inhibit or interrupt the oxidation process and are able of counterbalancing free-radical activities that cause cell injuries leading to neoplastic lesions, inflammatory conditions or negative changes in blood vessels (Cieřlik *et al.*, 2006). Broccoli has revealed as a health-promoting vegetable produce highly rich in antioxidants such as phenolics, vitamins and other bioactive compounds (glucosinolates, total dietary fibre, minerals, folates, etc). The consumption of these bioactive compounds may prevent chronic disorders, such as some carcinogenic and cardiovascular pathologies (Jeffery *et al.*, 2003). The consumers' demand of this healthy vegetable may be enhanced, focussing the plant breeding companies in producing new broccoli cvs. with milder flavor than conventional ones, like the kailan-hybrid broccoli recently appeared into the market. Nowadays, the worldwide drive for a healthier lifestyle has led to an increasing demand of additives-free convenient fresh foods, with high nutritional value, including antioxidant and free-radical scavenging properties, ready-to-eat everywhere. FC plant products meet *all* requested properties and offer great advantages for consumers. However, the nutritional value may be affected during shelf-life and certain postharvest techniques should be implemented for keeping the quality of this vegetable (Artés *et al.*, 2009).

Polyphenolic compounds are very important constituents, because of their antioxidant capacity by chelating redox-active metal ions, inactivating lipid free-radical chains and preventing hydroperoxide conversion into reactive oxyradicals (Floegel *et al.*, 2011). Phenolic compounds and vitamin C are the major antioxidant sources of *Brassica* vegetables, containing a 75 % of the phenolic fraction antioxidant agents (Leja *et al.*, 2001). TAC has been reported to be positively correlated with the total phenolic content in many vegetables (Jacobo-Velázquez and Cisneros-Zevallos, 2009). In other cases, TAC was not correlated with the phenolic content playing other antioxidant factors major roles (Velioglu *et al.*, 1998). Dietary fibre, which consists of a variety of non-starch polysaccharides, is associated with the prevention and reduction of some diseases, such as diverticulitis and coronary heart diseases (Ramulu and Rao, 2003). The basic function of proteins in nutrition is to supply the adequate amount of the needed amino acids. Vegetable proteins represent a 3.5 % of the protein sources in the human diet. Broccoli has high protein content between 3 and 4 % (Friedman, 1996).

Essential minerals are necessary protective nutrients for the maintenance of the nutritional and the health status of the body (Lisiewska *et al.*, 2009). The minimal processing with slight physical treatments of FC vegetables appears not to induce significant losses of fibre, protein or mineral contents throughout normal shelf-life (Zyren *et al.*, 1983).

The aim of this work was to characterize the nutritional value of the FC kailan–hybrid broccoli compared to that of cv. Parthenon throughout its shelf-life. Minerals, total protein and dietary fibre contents were analysed in the different parts of kailan–hybrid broccoli and compared to that of cv. Parthenon, since there are no previous data about these bioactive compounds of this natural hybrid. The effects of three storage temperatures, under MAP and air, on the main health-promoting properties of both cvs. have been also studied.

2.2. MATERIALS AND METHODS

2.2.1. Plant material

About 15–18-cm-long kailan–hybrid broccoli with head diameter of 3–5 cm, and 17–20-cm-long conventional broccoli heads with 12–15 cm diameter, both at commercial ripening stage, were hand-harvested. The plant material was grown under open air cultivation in the same fields located in the Region of Murcia, in the southeast Mediterranean Spanish area. Broccoli was grown according to integrated pest management cultural practices. Immediately after harvesting, broccoli was pre-cooled with crushed ice and transported by car about 80 km to the Pilot Plant of the Postharvest and Refrigeration Research Group in the Universidad Politécnica de Cartagena, where it was stored at 1 °C and 90–95 % RH until next day.

2.2.2. Sample preparation, processing and storage conditions

Minimal processing and MAP were conducted in a disinfected cold room at 8 °C. All leaves were eliminated with a sharp knife. The kailan–hybrid broccoli was cut in about 15-cm-long spears and ‘Parthenon’ heads were divided into florets.

All plant material was then washed with chlorinated water (pH 6.5; 5 °C; 100 ppm free chlorine) for 2 min and rinsed with tap water at 5 °C for 1 min. Once drained,

120 g of sanitized plant material was placed into 1.5-L (kailan-hybrid) or 2-L ('Parthenon') rigid polypropylene (PP) trays (12 x 17 cm). These trays were thermally sealed on the top with an antimist 30- μ m thickness bioriented PP (BOPP) film (Plásticos del Segura S.L., Murcia, Spain) in order to generate a modified atmosphere (5–7 kPa O₂ + 14–15 kPa CO₂). The BOPP film permeability was 900 cm³ O₂ m⁻² day⁻¹ atm⁻¹ and 1100 cm³ CO₂ m⁻² day⁻¹ atm⁻¹ at 23 °C and 0 % RH (data provided by the supplier). As control under air conditions, a 30- μ m thickness macro-perforated (18 holes of 8.8 mm dm⁻²) BOPP film was used. Three replicates of one tray per each treatment and sampling time were prepared. Trays were kept at 2, 5 and 8 °C (90–95 % RH). Such temperatures were selected in order to simulate ideal, maximum recommended and mainly usual retail temperatures, respectively, throughout storage of broccoli in Europe (Artés *et al.*, 2001). Analyses were conducted on the processing day and after 6, 9, 12 and 15 days of storage in where, according to sensory quality, the product was in the limit of usability. No significant yellowing incidence was registered for any of the treatments throughout shelf-life except for air atmosphere at 8 °C, which showed visual yellowing from day 12 (data not shown).

2.2.3. Total dietary fibre and protein content

Total dietary fibre content of the whole plant (florete and stem), as well as of the stem, florets and immature flowers (hereinafter referred to as flowers), was assessed on the processing day by using the enzymatic-gravimetric method (AOAC, 1990). Protein content was determined according to the Kjeldahl method (AOAC, 1995) using a KjFlex Buchi distiller (Flawil, Switzerland) coupled with a titrator (702 SM Titritini Metrohm, Herisau, Switzerland) with HCl 0.1 N and a digester block (20 Selecta, Barcelona, Spain). All samples were analysed by triplicate.

2.2.4. Mineral content

The mineral content of the whole plant (florete and stem), stem, florets, and green flowers (in cv. Parthenon, the white flowers located in the inner side of the head were considered as another fraction) were analysed by X-ray fluorescence (XRF) according to Nielson *et al.* (1991), with slight modifications. A spectrometer S4 Pioneer (Bruker Corporation, Billerica, MA, USA), equipped with an Rh anticathode X-ray tube (20–60 kV, 5–150 mA, and 4 kW maximum), five analyzer crystals (LiF200, LiF220, Ge, PET,

and XS-55), sealed proportional counter for light elements detection and a scintillation counter for heavy elements was used. The recorded spectrum was evaluated by the fundamental parameters method using the Spectraplus software EVA 1.7. A standardless method was used owed to the lack of satisfactory certified reference materials with metal concentrations in the same range of the sample analysed. Values intensities were above 3 times the statistical background. Mineral content was expressed as g kg⁻¹ dw and mg kg⁻¹ dw for major minerals and trace elements, respectively. All samples were analysed by triplicate.

2.2.5. Phenolic compounds

2.2.5.1. Total phenolic content

Total phenolic content was analysed as described by Martínez–Hernández *et al.* (2011) based on Singleton and Rosi (1965). The absorbance of the samples was measured using a Multiscan plate reader (Tecan Infininte M200, Männedorf, Switzerland). Total phenolic content was expressed as ChAE in mg kg⁻¹ fw. All extracts were analysed by triplicate.

2.2.5.2. Extraction and determination of individual phenolic compounds

Individual phenolic compounds were analysed as described by Martínez–Hernández *et al.* (2011). The confirmation of the compound identity was achieved by HPLC–MS as described by Vallejo *et al.* (2003). An HPLC (Series 1100 Agilent Technologies, Waldbronn, Germany) equipped with a G1322A degasser, G1311A quaternary pump, G1313A autosampler, G1316A column heater, a G1315B photodiode array detector and a C18 (250 mm × 4.6 mm, 5 µm) column (Phenomenex, Torrance CA, USA) was used. Phenolic acids were quantified as chlorogenic acid (5–caffeoylquinic acid; Sigma, St Louis MO, USA) and sinapic acid derivatives as sinapic acid (Sigma, St Louis MO, USA). The calibration curves were made with at least six data points for each standard. The results were expressed as mg kg⁻¹ fw. All extracts were analysed by triplicate.

2.2.6. Total antioxidant capacity

The TAC was determined based on the evaluation of the free-radical scavenging capacity according to Brand-Williams *et al.* (1995) with slight modifications (Martínez-Hernández *et al.*, 2011). The absorbance of the samples was measured using a Multiscan plate reader (Tecan Infininte M200, Männedorf, Switzerland). Results were expressed as L-AA equivalent antioxidant capacity (AAEAC) in mg kg^{-1} fw. All measurements were made by triplicate.

2.2.7. Statistical analysis

For nutritional value data, the interaction among cv. and the analysed part of the broccoli was studied by conducting a bi-factorial analysis of variance (ANOVA) using the Statgraphics Plus (version 5.1) software. When differences among treatments were found, the mean values were compared by the least significant difference (LSD) multiple range test. Figures represent mean values ($n=3$) $\pm$ SD.

2.3. RESULTS AND DISCUSSION

2.3.1. Total dietary fibre and protein content

Generally, broccoli florets showed higher dietary fibre content than stems with 5.0 and 3.6 % for the kailan-hybrid and 2.2 and 2.0 % for broccoli cv. Parthenon, respectively (Table 1). The kailan-hybrid broccoli showed around 2-fold higher dietary fibre content than conventional cv. Parthenon. However, conventional broccoli registered 1.1-fold higher fibre concentration in green flowers than that found for the kailan-hybrid broccoli.

The total protein content of the kailan-hybrid florets was around 2.2-fold higher compared to cv. Parthenon with a value of 3 % (Table 1). Nevertheless, the total protein content of 'Parthenon' stems was higher than that showed by the kailan-hybrid broccoli with values of 1.6 and 1.0 %, respectively.

The total dietary fibre and protein contents were lower than those reported by Souci *et al.* (2000) in the conventional 'Plenck' broccoli and by Li *et al.* (2002) in an unreported cv. The differences in dietary fibre and protein contents among the two cvs. reported here may possibly reflect genetic factors (Sosa-Coronel *et al.*, 1976) and/or

preharvest conditions, such as excessive N fertilization or B deficiency that produce a decrease and an increase of fibre content, respectively (Petracek and Sams, 1987; Walters *et al.*, 1998). The different total dietary fibre and protein contents found in the broccoli parts might be attributed to their genetic-influenced histological differences (Pyee and Kolattukudy, 1994). However, the sample is too small to make this determination in the present study.

2.3.2. Mineral content

‘Parthenon’ florets showed higher concentrations of K, P, Na, Cl, Si and Al than the kailan-hybrid florets (Table 2). López-Berenguer *et al.* (2007) reported similar mineral content in the conventional cv. Nubia. In comparison to cv. Parthenon, the kailan-hybrid florets recorded higher contents of S, Ca, Mg, Fe, Sr, Mn, Zn and Cu. Ni concentration in the kailan-hybrid was 5.7-fold higher than the 1.7 mg kg⁻¹ dw reported by Kmiecik *et al.* (2007) in the conventional broccoli cv. Cymosa Duch. The conventional broccoli stems showed higher mineral content than those of the kailan-hybrid, recording values around 84 % higher for Mn and P. Similar Fe values were found for both broccoli cvs. The hereby found mineral profile differences among broccoli cvs. have been early studied in other plant sources (i. e., species, cvs., etc.). Farnham *et al.* (2000) reported mineral content differences up to 2-fold Ca among inrebds and commercial F1 broccoli hybrids, thereby demonstrating that the genetic differences among cvs. could contribute to the plant’s ability to acquire and sequester minerals from the soil through the non-specific xylem transportation mechanism (Grusak, 2002).

Higher Fe, Si, Mn, Zn, Ni and Cu levels in the kailan-hybrid florets than in stems were found, recording florets 52 % higher Fe values than stems. However, the kailan-hybrid stems showed a 37 and 73 % higher K and Na contents than florets, respectively. Nielson *et al.* (1991) reported similar mineral content for asparagus, but the kailan-hybrid broccoli showed around 1.4–1.5-fold higher contents of Mg, S, K and Ca. No data about the mineral content of the kailan-hybrid broccoli have been reported so far.

Green flowers of the kailan-hybrid broccoli also showed higher S, Ca, Mg, Mn and Cu levels than cv. Parthenon. Nevertheless, conventional broccoli registered Si, Na,

Fe and P values 3.8, 2.4, 1.4 and 1.1–fold higher than in the kailan–hybrid broccoli. Generally, inner white flowers of cv. Parthenon were found to have values around 1–1.2–fold higher compared to the outer green flowers for Na, K, Mg, P, Se, Cl, Cu and Zn. The floret cell tissues have higher rates of water loss due to their sun orientation and sensitive cell structure. This fact may produce the observed high mineral content of broccoli floret in contrast to stem, since minerals within the xylem sap are delivered non–selectively as columns of water, pulled up through the plant and accumulated preferentially in those tissues with high rates of water loss (Grusak, 2002).

2.3.3. Phenolic compounds

2.3.3.1. Total phenolic content

The cv. Parthenon registered 1.1–fold higher initial total phenolic content than the kailan–hybrid with 1,334 mg ChAE kg⁻¹ fw (Figure 1). Costa *et al.* (2006) found 54 % lower total phenolic content in conventional broccoli cv. Cicco compared to cv. Parthenon. This fact could be owed to the genetic variability and pre–harvest conditions (Jeffery *et al.*, 2003). The kailan–hybrid broccoli showed a high stem/inflorescence ratio (62.5 %, data not shown), since the stem is poorer than the floret in total phenolic content (42 % lower, data not shown). This reason could explain the lower total phenolic content of the whole kailan–hybrid compared to that of broccoli cv. Parthenon.

Throughout conservation, a general increase in total phenolic content for both broccoli cvs. was found, with increases ranging from 1.1–1.3–fold higher than values at harvest (Figure 1). Costa *et al.* (2006) reported a similar trend in the total phenolic increase for MAP broccoli cv. Cicco stored at 20 °C during 5 days. The accumulation of phenolic compounds during chilling storage may be promoted by the phenylalanine ammonia–lyase (PAL) activity. This enzyme may be activated by abiotic stresses, such as the wounding produced during the minimal processing, synthesizing the main phenolic compounds and new polyphenolic substances (Cisneros–Zevallos, 2003). In our experiment, MAP samples showed a 12 and 20 % higher total phenolic content than those air–stored for kailan–hybrid at 5 °C and broccoli cv. Parthenon at 8 °C, respectively. However, Cisneros–Zevallos (2003) found a decrease around 4 % in total phenolic content in conventional broccoli cv. Marathon stored under MAP after 21 days at 1 °C. Thus, the hereby presented data and those mentioned in previous reports

manifest how a storage temperature around 5–8 °C increases the total phenolic content of MAP kailan–hybrid and broccoli cv. Parthenon rather than a lower temperature. This observation might be due to the equilibrium between a high activity, favoured by these storage temperatures, of key enzymes involved in the secondary metabolic pathways, which affects the phytochemical accumulation, and the low phenolic deterioration achieved by such temperatures. Furthermore, MAP is considered as an abiotic stress itself (Serrano *et al.*, 2006), increasing the activity of the above–mentioned key enzymes of the secondary metabolic pathways.

2.3.3.2. Individual phenolic content

The phenolic profile found for both cvs. was very similar to that reported for the cv. Marathon (Vallejo *et al.*, 2003). The hydroxycinnamoyl acids identified were neochlorogenic acid, 1,2–diferuloylgentibiose, 1–sinapoyl–2–feruloylgentibiose, 1,2′–disinapoyl–2–feruloylgentibiose, chlorogenic acid, 1,2,2′–trisinapoylgentibiose, 1–sinapoyl–2,2′–diferuloylgentibiose and 1,2–disinapoylgentibiose.

Throughout shelf–life, in concordance with the total phenolic content, a general increase of individual phenolic compounds for the kailan–hybrid broccoli was found. Increases of individual phenolic compounds in the kailan–hybrid broccoli stored under air and MAP after 15 days at 2 and 8 °C are presented in Figure 2. Generally, the air–stored kailan–hybrid broccoli at 8 °C recorded the highest increases after 15 days, compared to values recorded on the processing day, with increases up to 3.2–fold higher in the case of the 1,2,2′–trisinapoylgentibiose. Furthermore, MAP–stored samples at 8 °C showed higher content of individual phenolics than those at 2 °C, according to the previous hypothesis mentioned for total phenolic content enhancement at this temperature under MAP.

2.3.4. Total antioxidant capacity

The 2,2–diphenyl–1–picrylhydrazyl (DPPH) assay has been widely used to characterize the TAC of *Brassica* spp. The kailan–hybrid broccoli showed higher TAC on the processing day than the conventional cv. (449 vs. 281 mg AAEC kg^{−1} fw, respectively) (Figure 3). These values, in contrast to the trend found for total phenolic content, can be explained by the vitamin C contribution (1.2–fold higher in the kailan–

hybrid than in the broccoli cv. Parthenon, data not shown). In this way, Velioglu *et al.* (1998) found several cases where the high antioxidant capacity was not correlated with the phenolic content in 28 examined plant products, probably playing other factors major roles as antioxidants. The kailan–hybrid florets and stems showed a TAC 28 % lower and 1.7–fold higher, respectively, than that of the whole broccoli (data not shown).

A decreasing TAC trend occurred throughout storage of the kailan–hybrid samples, in agreement with results reported for the conventional cv. Cicco (Costa *et al.*, 2006). MAP samples at 2 and 5 °C showed higher values than air–stored samples, reaching all MAP kailan–hybrid samples the lowest values after 12 days. In that stage, the TAC of MAP kailan–hybrid broccoli stored at 5 and 8 °C increased, reaching values 40 and 35 % higher than those at harvest, respectively. These values were higher than those found in air–stored samples at the same sampling day (Figure 3). In contrast, broccoli cv. Parthenon did not show a clear trend during the conservation period. Nevertheless, MAP–stored cv. Parthenon at 2, 5 and 8 °C registered 38, 24 and 101 % higher TAC values than air–stored samples after 15 days, respectively.

FIGURES AND TABLES

Figure 1. Total polyphenols changes of cv. Parthenon and the kailan–hybrid broccoli stored during 15 days in air and MAP at 2, 5 and 8 °C ($n=3 \pm \text{SD}$).

Figure 2. Individual caffeoyl–quinic, sinapic acid and feruloyl acid derivatives increases in the kailan–hybrid broccoli stored during 15 days in air and MAP at 2 and 8 °C ($n=3$).

Figure 3. Total antioxidant capacity changes of cv. Parthenon and the kailan–hybrid broccoli during 15 days in air and MAP at 2, 5 and 8 °C ($n=3 \pm \text{SD}$).

Table 1. Humidity, total protein and fibre contents of different fractions of cv. Parthenon and the kailan–hybrid broccoli after harvest ($n=3$).

Broccoli parts	Variety	Humidity (% fw)	Total dietary fibre content (% fw)	Total protein content ($N \times 6.25$) (% fw)
Whole	K	86.3	3.8	1.8
	P	81.5	3.0	2.8
Floret	K	88.4	5.0	3.0
	P	89.1	2.2	1.4
Stem	K	89.2	3.6	1.0
	P	81.5	2.0	1.6
Flowers	K	81.4	4.2	3.5
	P	86.3	4.8	2.9
Variety		(1.5)**	(0.2)***	(0.1)***
Part (B)		(3.0)***	(0.3)***	(0.2)***
A x B		(4.2)***	(0.4)***	(0.3)***

LSD values are in parentheses; K= kailan–hybrid; P= cv. Parthenon

** $p < 0.01$; *** $p < 0.001$

Table 2. Mineral content of the different fractions of cv. Parthenon and the kailan–hybrid broccoli after harvest (n=3).

Mineral nutrients ^a	Whole		Floret		Stem		Green flowers		White	Variety	Part (B)	A X B
	K	P	K	P	K	P	K	P	P			
P	8.0	11.1	10.0	11.1	6.4	7.6	10.1	11.5	11.9	(0.37)***	(0.53)***	(0.75)***
S	10.0	13.9	14.2	13.9	6.9	9.8	14.4	14.0	14.3	(0.53)***	(0.74)***	(1.05)***
Na	2.1	4.0	1.1	4.0	3.0	3.9	0.8	2.0	2.4	(0.09)***	(0.12)***	(0.18)***
K	31.2	40.9	26.6	40.9	36.6	75.4	26.5	40.0	42.3	(0.55)***	(0.78)***	(1.11)***
Ca	6.9	6.6	11.3	6.6	3.9	7.7	11.9	6.9	4.8	(0.28)***	(0.40)***	(0.56)***
Mg	2.7	3.2	3.4	3.2	1.9	2.9	3.2	3.1	3.1	(0.11)***	(0.15)***	(0.22)***
Cl	2.0	2.4	0.7	2.4	3.3	8.9	0.6	1.7	1.7	(0.28)***	(0.40)***	(0.56)***

Mineral traces found ^b

Fe	37.2	122.0	124.5	122.0	59.6	57.0	120.0	162.0	81.0	(13.7)*	(36.7)***	(52.0)***
Mn	40.2	43.8	71.3	43.8	16.0	19.0	86.0	65.3	44.7	(1.3)***	(1.9)***	(2.7)***
Zn	42.5	64.0	70.1	64.0	24.2	32.0	86.5	89.2	88.5	(3.46)***	(4.89)***	(6.92)***
Al	7.0	160.0	16.5	160.0	65.0	110.0	42.5	323.0	160.0	(45.4)***	(64.2)***	(90.8)***
Si	53.0	199.0	85.0	199.0	51.0	140.0	120.0	460.0	171.0	(35.1)***	(49.6)***	(70.1)***
Cu	5.0	5.0	6.3	5.0	3.5	5.0	7.0	6.0	6.0	(0.2)*	(0.5)***	(0.8)***
Ni	5.7	ND	8.6	ND	4.0	ND	11.5	ND	ND	(1.3)***	(1.8)***	(2.6)***
Br	3.0	ND	2.5	ND	8.6	11.0	3.0	ND	ND	(1.1)***	(1.6)***	(2.3)***
Sr	77.5	48.8	82.9	48.8	65.9	90.4	71.5	40.0	26.7	(2.8)***	(4.0)***	(5.6)***
Zr	2.7	ND	2.6	ND	2.1	2.4	2.7	ND	ND	(0.6)***	(0.8)***	(1.1)***

LSD values are in parentheses; K = kailan–hybrid; P = cv. Parthenon; ND = not detected; ^a (g kg⁻¹ dw); ^b (mg kg⁻¹ dw)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

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CHAPTER III

Moderate UV-C pretreatment as a quality enhancement tool in fresh-cut Bimi® broccoli

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ABSTRACT

The effects of several UV-C pre-treatments (1.5, 4.5, 9.0 and 15 kJ m⁻²) on changes in physiological, sensory and microbial quality and health promoting bioactive compounds over 19 days at 5 and 10 °C of fresh-cut Bimi® broccoli were studied. Non-irradiated samples were used as controls. Bimi® broccoli (*Brassica oleracea* Italica Group × Alboglabra Group) is characterised by a long stem with a small floret with a mild and sweeter flavor than conventional varieties well adapted for fresh-cut purposes. Low and moderate UV-C doses (1.5 and 4.5 kJ m⁻²) had inhibitory effects on natural microflora growth. In relation to sensory quality, all treatments resulted in a shelf-life of 19 and 13 days at 5 and 10 °C respectively with the exception of 15 kJ UV-C m⁻² treated samples which resulted in a shorter shelf-life. These doses immediately increased total polyphenols contents up to 25% after 19 days at 5 °C compared to the initial value. All the hydroxycinnamoyl acid derivatives were immediately increased after UV-C treatments, with values 4.8- and 4.5-fold higher for 4.5 and 9.0 kJ UV-C m⁻² treated samples respectively over the control. Changes in phenolic compounds were highly influenced by the storage temperature throughout shelf-life. Total antioxidant activity generally followed the same pattern: the higher the UV-C doses, the higher total antioxidant capacity values. Generally, UV-C slightly reduced initial total chlorophyll content but delayed its degradation throughout shelf-life. It is concluded that a pre-treatment of 4.5 kJ UV-C m⁻² is useful as a technique to improve epiphytic microbial quality and health promoting bioactive compounds of fresh-cut Bimi® broccoli.

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1. Introduction

Broccoli is an extremely valuable horticultural product, not only in economic terms but also for its excellent health claims. This brassica species has been described as a vegetable with high nutritional value owing to its exceptionally high levels of Zn, folic acid, antioxidants, glucosinolates, fiber, vitamin C and high antioxidant activity (Jeffery et al., 2003; Freshfel, 2006; Moreno et al., 2006). After harvest, overall quality of broccoli is greatly reduced due to several detrimental changes such as loss of green colour and sepal yellowing as a consequence of chlorophyll catabolism (Funamoto et al., 2002), tissue disruption, lipid peroxidation, protein degradation and the loss of antioxidant and health promoting bioactive compounds, which decreases nutritional value (Page et al., 2001).

New varieties of broccoli with less intense flavor than the conventional ones are appearing in the international market in order to increase their consumption. Bimi® broccoli, a new commercial broccoli variety (also called as tenderstem®, vellaverde®, broccolini®, asparation, inspiration, broccoletti or broccollette) is a hybrid between conventional broccoli (*Brassica oleracea*, Italica group) and Chinese broccoli (*B. oleracea*, Alboglabra group, also called kai-lan or Chinese kale). Bimi® looks like conventional broccoli with a long slender stem, but has a milder sweeter taste similar to green asparagus (Fig. 1).

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, antioxidant and free-radical scavenging properties, to be consumed both in the retail and food service sectors. In particular, fresh-cut fruit and vegetables offer great advantages for consumers, owing to their convenience and ready-to-use properties, although they provide an ideal medium for microbial development (Artés et al., 2009). UV-C treatments (0.5–20 kJ m⁻²) decrease microbial growth by inducing the formation of pyrimidine dimers that alter the DNA helix and block microbial cell replication (Nakajima et al., 2004). The effectiveness

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Fig. 1. Visual appearance and different morphological parts of Bimi® broccoli.

of UV-C seems to be independent of temperature in the range 5–37 °C, but it depends on the incident irradiation (Bintsis et al., 2000). It has been hypothesised that selected abiotic stress such as UV-C radiation could affect the secondary metabolism of fresh produce and could increase the synthesis of phytochemicals with nutraceutical activity (Cisneros-Zevallos, 2003).

The aim of the present work was to study the effect of four pre-packaging UV-C treatments and two storage temperatures on the main quality changes of fresh-cut Bimi® broccoli throughout shelf-life. To the best of our knowledge, no other studies on the postharvest behaviour of this broccoli hybrid have been published.

2. Materials and methods

2.1. Plant material

Bimi® broccoli (*B. oleracea* Italica Group × Alboglabra Group) was grown as a field crop in the Southeast Mediterranean coast by Campo de Lorca SCL (Murcia, Spain). Immediately after hand-harvesting, the broccoli was forced-air pre-cooled at 1 °C and then transported with top icing about 90 km to the Pilot Plant of the Technical University of Cartagena (UPCT), where it was stored in a cold room at 1 °C. The following morning the broccoli was processed.

2.2. Sample preparation, treatments and storage conditions

Plant material was minimally processed in a disinfected cold room at 8 °C. Broccoli was selected with a stem length between 15 and 18 cm, stem diameter 6–12 mm, 4 nodes per stem maximum, with no yellowing or damage and devoid of leaves. The raw material

was washed for 1 min with tap water at 5 °C to remove traces of soil and organic matter. The following UV-C radiation treatments were applied: 0 (Control), 1.5, 4.5, 9.0 and 15 kJ UV-C m⁻². UV-C exposure times ranged between 37 and 375 s. Such doses were selected based on previous reports and our preliminary experiments. The UV-C equipment has been earlier described (Artés-Hernández et al., 2010).

For passive modified atmosphere packaging (MAP), samples of about 200 g of broccoli per treatment were randomly placed in 1.2 L polypropylene (PP) baskets and thermally sealed on the top with a 50 µm microperforated bi-oriented PP film (BOPP) (Plásticos del Segura, Murcia, Spain). The O₂ and CO₂ transmission rates at 23 °C and 0% RH were similar with 11,000 cm³ m⁻² d⁻¹ atm⁻¹ (data provided by the supplier). Three replicates of one basket per treatment and MAP storage duration (processing day and after 7, 13, 16 and 19 days) were prepared and stored in dark cold rooms at 5 or 10 °C. The 5 °C temperature was selected as the maximum recommended during shelf-life for fresh-cut broccoli and 10 °C as an abuse temperature during storage, distribution and retail sale.

2.3. Analysis and determinations

2.3.1. Respiration rate and gas analysis within modified atmosphere packages

The respiration rate (RR) of broccoli was determined using a closed system. Three replicates of 80–90 g were placed within 750 mL glass jars at 5 or 10 °C up to 19 days. The increases in CO₂ 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 analyzed in a gas chromatograph (GC PerkinElmer Precisely Clarus 500, Massachusetts, USA). The GC was equipped with a thermal conductivity detector (90 °C), oven (temperature gradient from 40 to 90 °C), injector (150 °C) and with a Porapack Qs 80/100 (Barcelona, Spain) and Molecular Sieve 5A 45/60 (Barcelona, Spain) columns. Calibration of CO₂, O₂ and N₂ was done with known standards from gas cylinders (Air Liquid SA, Murcia, Spain). Three replicates were made for each treatment and evaluation period.

Throughout shelf-life gas composition (O₂ and CO₂) within packages was monitored. Headspace gas samples (1 mL) were withdrawn and analyzed in the GC described above from three replicates for each treatment and evaluation period.

2.3.2. Microbial analysis

To determine the microbial growth standard, enumeration methods were used. Three random samples were taken at each evaluation time. Samples of 20 g were homogenized in 180 mL of sterile peptone saline solution (pH 7) (Scharlau Chemie SA, Barcelona, Spain) for 1 min in a sterile stomacher bag (Model 400 Bags 6141, London, UK) using a masticator (Colwort Stomacher 400 Lab, Seward Medical, London, UK). For the enumeration of each microbial group (mesophilic, enterobacteria, psychrotrophic, yeasts and moulds), ten-fold dilutions series were prepared in 9 mL of sterile peptone saline solution. Mesophilic, enterobacteria and psychrotrophic were pour-plated and yeast and mould were spread-plated. The following media and incubation conditions were used: plate count modified agar (Scharlau Chemie, Barcelona, Spain) for mesophilic and psychrotrophic aerobic bacteria, incubated at 30 °C for 48 h and at 5 °C for 7 days respectively; violet red bile dextrose agar (Scharlau Chemie, Barcelona, Spain) for enterobacteria, incubated at 37 °C for 48 h and potato dextrose agar base (Scharlau Chemie, Barcelona, Spain) with oxytetracycline (100 mg L⁻¹) (Sigma Chemical Co., St Louis, MO, USA) for yeasts and moulds, incubated for 3–5 days at 22 °C. All microbial counts were reported as log colony forming units per gram of product (log CFU g⁻¹).

2.3.3. Sensory evaluation

Sensory quality was evaluated on the processing day and after 7, 13, 16 and 19 days of shelf-life by seven trained panelists aged 24–63. A five-point scale of damage incidence and severity was scored for off-odours, off-flavors, yellowness, bending and stem softening (5 = none; 4 = slight; 3 = moderate, limit of usability; 2 = severe and 1 = extreme). Overall quality was assessed by using another five-point hedonic scale of acceptance (5 = excellent, 4 = good, 3 = fair, limit of usability, 2 = poor and 1 = extremely bad).

2.3.4. Total phenolics content

For tissue preparation, broccoli samples were frozen in liquid N₂, ground with a mincer (IKA, A 11 basic, Berlin, Germany) at 12,700 × g for 10 s, and stored at –80 °C. Ground frozen samples of 0.5 g were placed in 5 mL glass bottles and 3 mL of methanol/water (7:3, v/v) was added. The extraction was carried out in an orbital shaker (Stuart, Staffordshire, UK) for 1 h at 200 × g in darkness inside a polystyrene box with an ice bed. Afterwards 2 mL of extracts were transferred in two 2 mL eppendorf tubes and centrifuged at 15,000 × g for 10 min at 4 °C. The supernatant was used as total phenolics and total antioxidant capacity extract for each sample. The amount of total phenolic compounds was determined based on Singleton and Rossi (1965) with slight modifications. A 19.2 µL aliquot of extract was placed on a 96 polystyrene flat-bottom 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 solution containing Na₂CO₃ (0.4%) and NaOH (2%) was added and the reaction was carried out for 1 h at room temperature in darkness, measuring the absorbance at 750 nm using a multiscan plate reader (Tecan Infinite M200, Männedorf, Switzerland). Total phenolic content was expressed as chlorogenic acid equivalents (ChAE) in mg kg^{–1} fresh weight (fw). All extracts were analyzed by triplicate.

2.3.5. Extraction and determination of individual phenolic compounds

The individual phenolic profile was analyzed based in Vallejo et al. (2003a) with slight modifications. Ground frozen (–80 °C) samples (4 g in triplicate) were homogenized three times in 70% methanol (12.5 mL each time) and the homogenate was filtered through a 4 layers cheesecloth. The combined fractions (37.5 mL) were evaporated under vacuum in a rotary evaporator (Heidolph VV2000, Kelheim, Germany) at 37 °C to approximately 1 mL and diluted to 4 mL with nanopure water. The diluted phenolic extraction (4 mL) was centrifuged in two 2 mL eppendorf tubes at 15,000 × g for 10 min at 4 °C. The supernatant was filtered through a 0.45 µm polyethersulphone filter and stored at –80 °C in amber vials until HPLC analysis.

Samples of 20 µL were analyzed using an HPLC (Series 1100 Agilent Technologies, Waldbronn, Germany) equipped with a G1322A degasser, G1311A quaternary pump, G1313A autosampler, G1316A column heater and G1315B photodiode array detector. The HPLC system was controlled by the software ChemStation Agilent, v. 08.03. The stationary phase was a Gemini NX (250 mm × 4.6 mm, 5 µm) C18 column (Phenomenex, Torrance CA, USA). The mobile phases were water/formic acid (95:5, v/v) (A) and methanol (B) with a flow rate of 1 mL min^{–1}. The linear mobile phase gradient started with 10% B to reach 15% B at 5 min, 30% B at 20 min, 50% B at 35 min and 90% B at 40 min. For column equilibration phase B was reduced to 10% in 4 min and maintained at this concentration for 10 min. Chromatograms were recorded at 320 nm. Phenolic acids were quantified as chlorogenic acid (5-caffeoylquinic acid; Sigma, St Louis, MO, USA) and sinapic acid derivatives (Sigma, St Louis, MO, USA). The calibration curves were made with at least six data points

for each standard. The results were expressed as mg kg^{–1} sample fw.

HPLC/MS analyses to identify the phenolic compounds were carried out according to Vallejo et al. (2002). The HPLC system was a Waters Alliance 2695 (binary pump and photodiode array detector 2996) (Waters, Mildford, USA), under the same chromatographic conditions for HPLC analyses described above. MS was performed using a Micromass ZQ4000 with double quadrupole analyzer, photomultiplier detector and equipped with an electrospray ionisation system (with steel capilar 3000 V). As nebulising gas N₂ was used. The extractor voltage was programmed at 5 V and RF voltage at 0.2 V. Source and desolvation temperatures were set at 100 and 297 °C, respectively. Desolvation flow was kept at 10 L min^{–1} and gas flow at 0.3 L min^{–1}. Data treatment was carried out with Waters Alliance 2695 Separations Module unit software Rev. 2.04. Mass spectrometry data were acquired in the negative ionisation mode from *m/z* 100 up to *m/z* 1000.

2.3.6. Total antioxidant activity

The total antioxidant activity was determined based on the evaluation of the free radical scavenging capacity according to Brand-Williams et al. (1995). A solution of 0.7 mM 2,2-diphenyl-1-picrylhydrazil radical (DPPH) in methanol was prepared 2 h before 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 96 polystyrene flat-bottom well plate (Greiner Bio-one, Frickenhausen, Germany) and 194 µL of DPPH absorbance adjusted solution was added. The reaction was carried out for 30 min at room temperature in darkness and the absorbance at 515 nm was measured using a multiscan plate reader (Tecan Infinite M200, Männedorf, Switzerland). Results were expressed as ascorbic acid equivalent antioxidant capacity (AAEAC) per kg fw. All measurements were made in triplicate.

2.3.7. Chlorophyll content

The sample preparation for chlorophyll determination was conducted according to Smith and Benitez (1955). A 0.5 g ground frozen (–80 °C) sample was mixed with 9 mL of hexane and 15 mL of a mixture of methanol/acetone (1:2, v/v). The extraction was carried out for 4 h in darkness inside a polystyrene box with ice and shaken continuously at 200 × g with an orbital shaker (Stuart, Staffordshire, United Kingdom). After extraction, 25 mL of a solution 1 M NaCl was added, samples were shaken again in a vortex (Heidolph, Reax Control, Kelheim, Germany) and the upper of the three layers formed was used as chlorophyll and carotenoid extract. An aliquot of 1 mL was placed into a quartz cuvette (Hellma GmbH & Co., Müllheim, Germany) and the absorbance (A) at 662, 644 and 470 nm was measured using a UV–visible spectrophotometer (Hewlett Packard, model 8453, Columbia, USA). The equations developed by Wellburn (1994) were used to determine the individual levels of chlorophyll *a* ($C_a = 10.05 A_{662} - 0.766 A_{644}$), chlorophyll *b* ($C_b = 16.37 A_{644} - 3.14 A_{662}$), total chlorophyll amount ($C_a + C_b$) and total carotenoids [$C_{x+c} = (1000 A_{470} - 1.28 C_a - 56.7 C_b)/205$]. Chlorophyll and carotenoids contents were expressed as mg kg^{–1} fw. All measurements were made in triplicates.

2.3.8. Statistical analysis

The experiment was a 5 × 5 × 2 trifactorial design (UV-C pre-treatments × storage time × storage temperature) which was subjected to analysis of variance (ANOVA) using Statgraphics Plus (version 5.1) software. Mean values were subjected to the least significant difference test (LSD) at *P* < 0.05.

Fig. 2. Respiration rate of fresh-cut Bimi® broccoli pre-treated with several UV-C radiation doses and stored up to 19 days at 5 °C (A) and 10 °C (B). Data represent means of three replicates ($n = 3 \pm \text{SD}$).

3. Results and discussion

3.1. Respiration rates and gas analysis

After the initial stress induced by the minimal processing, the RR of control broccoli had equilibrated after 3–4 days of storage at around 20 and 30 mg CO₂ kg⁻¹ h⁻¹ at 5 and 10 °C respectively (Fig. 2). After UV-C treatment, an important initial stress was found and all UV-C treated samples registered higher RR than the control. UV-C increased CO₂ emissions throughout shelf-life, which was more related to the dose levels than in previous work in fresh-cut watermelon (Artés-Hernández et al., 2010). In contrast, Costa et al. (2006) reported no differences in RR of broccoli L. var. Italica heads immediately after 10 kJ UV-C m⁻² illumination compared with the control.

At both storage temperatures, the higher UV-C dose induced a higher RR, which was slightly higher at 10 °C than at 5 °C. However no initial differences were found after 4.5 and 9.0 kJ m⁻² at both temperatures, while 1.5 kJ m⁻² induced the lowest RR. All UV-C treated samples registered higher RR than control throughout the storage period. The general trend for RR was to decrease the initial value reached after the stress. The decreasing RR ratio of UV-C treated samples was similar in all cases except for 15 kJ m⁻² in

Fig. 3. Gas changes within packages of fresh-cut Bimi® broccoli treated with several UV-C doses and stored up to 19 days at 5 °C (A) and 10 °C (B). Data represent means of three replicates ($n = 3 \pm \text{SD}$).

which, after the first week of storage, a strong initial decrease was found. After 19 days, UV-C treated samples showed higher RR than control ones, ranging from 29 to 41 and 32 to 51 mg CO₂ kg⁻¹ h⁻¹ at 5 and 10 °C respectively.

The gas atmosphere changes throughout shelf-life within MAP packages showed similar patterns at both temperatures. The equilibrium gas partial pressures from the 4th until the 19th day of storage were 7–8 kPa O₂ + 10–11 kPa CO₂ and 3–4 kPa O₂ + 15–16 kPa CO₂ at 5 and 10 °C respectively. This gas composition was similar to that recommended for broccoli florets by Gorny (2001), being favourable, when combined with low temperature and high RH, for slowing the rate of senescence and extending storage life. Due to the physiological stress induced by the UV-C treatment, slight changes in the equilibrium gas partial pressures were found (Fig. 3).

3.2. Microbial analysis

Immediately after UV-C treatment, initial microbial counts were lowered. This effect was more marked for mesophilic and yeast and moulds counts (Fig. 4A and D respectively), with a reduction of around 1 log CFU g⁻¹ (for 4.5 and 9.0 kJ UV-C m⁻²). Moreover, enterobacteria and psychrophilic counts had slightly lower reductions (Fig. 4B and C respectively). These data agree with those from

Fig. 4. Mesophilic (A), enterobacteria (B), psychrophilic (C) and yeast and moulds (D) counts ($\log \text{CFU g}^{-1}$) of fresh-cut Bimi® broccoli treated with several UV-C doses and stored up to 19 days at 5°C and 10°C. Data represent means of three replicates ($n=3 \pm \text{SD}$).

Lemoine et al. (2007) who found an initial reduction of around 1 $\log \text{CFU g}^{-1}$ in broccoli L. var. Italica florets after the 8.0 kJ UV-C m^{-2} treatment. Doses of 1.5, 4.5 and 9.0 kJ UV-C m^{-2} induced a higher reduction of microbial growth than 15 kJ UV-C m^{-2} . Apparently,

high doses of UV-C would help the growth of some bacteria, probably owing to an increase in damage of superficial tissues that makes nutrients available for microbial growth, as reported in fresh-cut spinach (Artés-Hernández et al., 2009), and observed in the

Fig. 5. Sensory scores for overall quality, off-odours and stem softening of fresh-cut Bimi® broccoli treated with several UV-C doses and stored up to 19 days at 5 and 10 °C. Data represent means of seven replicates ($n = 7 \pm \text{SD}$).

sensory analysis. These initial differences between treatments were more or less retained during MAP storage where control samples showed the highest microbial growth while low to moderate doses of UV-C radiation induced the lowest. This fact was even more evident for psychrophilic counts where 9.0 and 15 kJ UV-C m^{-2} treated samples showed higher counts than the control.

At 10 °C the trend was quite similar, but with counts after 19 days significantly higher than those at 5 °C (Fig. 4), being more pronounced for enterobacteria counts with a 47% increase over the mean of all treatments kept at 5 °C. It is significant that at 10 °C, moulds and yeast counts decreased after UV-C treatments, maintaining constant levels throughout the storage period. This could be due to the pH of Bimi® broccoli being close to 7 and, consequently, less favourable for yeast and moulds growth than for bacteria.

3.3. Sensory evaluation

The sensory quality parameters affected the most were off-odours and stem softening (Fig. 5). Yellowness, off-flavours and

bending was almost not detected in any treatment at any sampling time (data not shown). During the first week of storage at 10 °C, slight to moderate unpleasant odours for 4.5, 9.0 and 15 kJ UV-C m^{-2} treated samples were detected (Fig. 5). After 13 days at 10 °C all treatments reached the limit of usability mainly due to the moderate unpleasant odours and stem softening, except for 15 kJ UV-C m^{-2} treatment which showed a maximum shelf-life of 12 days. However, at 5 °C all samples reached a shelf-life of 19 days with the exception again of the 15 kJ UV-C m^{-2} treatment, which registered a shorter shelf-life. This fact could be due to high UV-C provoking cell damage that could have helped microbial growth inducing softening.

3.4. Phenolic compounds

UV-C radiation caused an important initial stress on the broccoli cells, inducing an increase of up to 14% of the total phenolics content on the processing day compared to the initial value (1.377 mg ChAE kg^{-1} fw). All treatments followed the same behaviour in total

Fig. 6. Total polyphenols content changes of fresh-cut Bimi® broccoli treated with several UV-C doses and stored up to 19 days at 5 °C (A) and 10 °C (B). Data represent means of three replicates ($n = 3 \pm \text{SD}$).

polyphenols content throughout shelf-life at 5 °C, with a slight increase after 7 days of storage and then a decrease after 13 days followed by a high increase until the 19th day (Fig. 6A). This behaviour confirms recently reported results by Lemoine et al. (2007) in 8 kJ UV-C m⁻² treated broccoli L. var. Italica during 21 days at 4 °C. A good correlation was found between UV-C dose and total phenolics content after 19 days at 5 °C. The higher UV-C treated samples resulted in higher total phenolics contents, with

40, 33, 25, 22 and 10% increases for 15, 9.0, 4.5, 1.5 kJ UV-C m⁻² and control treatments respectively, with respect to the initial content. These different UV-induced responses could be explained by the specific enzyme activities involved in phenylpropanoid metabolism, including phenylalanine ammonia-lyase (PAL) which catalyzes the first committed step in the phenolic biosynthesis pathway, after which individual branch pathways make possible a range of phenylpropanoid secondary compounds (Schutte, 1992; Cisneros-Zevallos, 2003) as phenolics.

The high total phenolics content increase registered after 13 days at 5 °C occurred earlier in samples stored at 10 °C (Fig. 6B) probably due to the acceleration of the metabolism of broccoli at such higher temperature. Although the product exceeded the limit of usability according to sensory attributes, the highest increase (54%) in total phenolics content after 19 days at 10 °C was reached for 4.5 kJ UV-C m⁻² treated samples while increases in the remaining treatments ranged from 12 to 43% initial values (Fig. 6B). Lemoine et al. (2007) also reported higher total phenolics contents in broccoli florets after illumination with 8 kJ UV-C m⁻². Other research has shown enriched total phenolics contents after UV-C radiation in fresh and fresh-cut mangoes (González-Aguilar et al., 2007) and strawberries (Erkan et al., 2008). All these findings agree with the hypothesis of Cisneros-Zevallos (2003) that UV-C radiation can be considered an abiotic stress favourable for enhancing phytochemicals compounds.

Individual phenolic compounds of Bimi® broccoli are shown in Table 1. Hydroxycinnamoyl acid derivatives were identified by their chromatographic behaviour and UV spectra. HPLC/MS and chromatographic comparisons with external standards. The phenolic profile found in Bimi® broccoli was very similar to that described for other broccoli cultivars and Chinese broccoli (Vallejo et al., 2003a,b; Lin and Harnly, 2009). The hydroxycinnamoyl acids identified, from higher to lower content, were 1,2-diferuloylgentibiose, neochlorogenic acid, 1,2,2'-trisinapoylgentibiose, 1,2-disinapoylgentibiose, 1,2'-disinapoyl-2-feruloylgentibiose, chlorogenic acid, 1-sinapoyl-2,2'-diferuloylgentibiose and 1-sinapoyl-2-feruloylgentibiose. A characteristic phenolic profile chromatogram is presented in Fig. 7.

UV-C radiation generally increased the hydroxycinnamoyl acid derivatives content on the processing day (20 mg kg⁻¹ fw) just after illumination, with the exception of 15 kJ UV-C m⁻² which generally resulted in the same values as the controls. The highest increases were found for 4.5 and 9.0 kJ UV-C m⁻², 4.8- and 4.5-fold higher than the control respectively (Table 2). In our experiment, neochlorogenic acid had the highest increases on the processing day for the 4.5 and 9.0 kJ m⁻² UV-C treatments (9.0- and 11.5-fold higher with respect to the initial value respectively) followed by 1,2-diferuloylgentibiose (5.5- and 5.3-fold higher respectively), and 1,2-disinapoylgentibiose and 1,2'-disinapoyl-2-feruloylgentibiose, both with similar increases over the control (4.4- and 4.5-fold higher for 4.5 and 9.0 kJ m⁻² doses respectively).

Table 1
HPLC/DAD/MS analysis of phenolic compounds in Bimi® broccoli. Phenolic compounds numbered as in Table 1.

No.	Phenolic compound	HPLC retention time (min)	Max UV absorption HPLC/DAD (λ_{max})	Mass spectral fragments HPLC/MS (m/z)
<i>Caffeoyl-quinic derivatives acid</i>				
1	Neochlorogenic	12.2	327, 295 sh	353, 179
2	Chlorogenic acid	19.8	327, 295 sh	353, 179
<i>Sinapic acid derivatives</i>				
3	1,2-Disinapoylgentibiose	34.7	331	753, 529
4	1-Sinapoyl-2-feruloylgentibiose	35.1	330	723, 499
6	1,2,2'-Trisinapoylgentibiose	36.8	328	959, 735
7	1,2'-Disinapoyl-2-feruloylgentibiose	37.0	328	929, 705
8	1-Sinapoyl-2,2'-diferuloylgentibiose	38.0	320	899, 705
<i>Feruloyl acid derivatives</i>				
5	1,2-Diferuloylgentibiose	36.0	330.4	693, 499

sh, spectrum shoulder.

Table 2

Total and individual caffeoyl-quinic, sinapic acid and feruloyl acid derivatives changes in fresh-cut Bimi® broccoli treated with several UV-C doses and stored up to 19 days at 5 °C and 10 °C. Data represents means ($n = 3$).

Phenolic acid (mg kg ⁻¹ fw) and UV-C treatment	Time initial	After 7 days		After 13 days		After 16 days		After 19 days	
		5 °C	10 °C	5 °C	10 °C	5 °C	10 °C	5 °C	10 °C
<i>Neochlorogenic^a</i>									
Control	3.8	20.9	41.8	40.1	50.6	1.5	51.7	14.6	52.3
1.5 kJ m ⁻²	6.5	1.5	15.7	64.3	3.3	3.3	69.9	12.1	32.2
4.5 kJ m ⁻²	47.2	1.4	15.7	38.5	6.7	7.2	5.5	19.1	5.2
9.0 kJ m ⁻²	38.1	21.8	1.1	57.3	2.1	0.9	19.5	8.0	11.5
15 kJ m ⁻²	3.0	20.1	13.9	52.2	9.1	1.2	13.2	5.0	11.9
<i>Chlorogenic^b</i>									
Control	1.5	1.7	1.3	1.4	2.2	1.7	3.1	1.5	54.6
1.5 kJ m ⁻²	1.5	1.6	1.4	3.2	1.1	2.7	4.7	2.6	71.9
4.5 kJ m ⁻²	1.4	1.0	1.2	1.6	2.5	2.8	2.1	3.1	4.1
9.0 kJ m ⁻²	2.5	1.7	0.8	2.6	2.5	1.3	4.4	1.4	25.3
15 kJ m ⁻²	1.1	1.1	0.9	3.1	3.0	1.6	3.6	1.7	21.4
<i>1,2-Disinapoylgentibiose^c</i>									
Control	2.7	8.0	9.8	12.7	15.2	3.0	17.6	4.0	18.4
1.5 kJ m ⁻²	4.2	2.2	7.9	18.0	3.3	5.4	26.1	4.4	30.2
4.5 kJ m ⁻²	14.5	1.9	9.6	11.7	5.4	6.8	5.8	5.1	5.0
9.0 kJ m ⁻²	14.6	7.4	2.0	18.7	2.9	1.8	30.0	2.5	14.8
15 kJ m ⁻²	2.5	7.9	6.7	15.4	3.7	2.5	14.0	3.1	12.4
<i>1-Sinapoyl -2 feruloylgentibiose^d</i>									
Control	1.0	1.6	2.1	1.4	2.2	1.7	3.3	1.9	3.3
1.5 kJ m ⁻²	1.2	1.3	2.1	2.6	1.2	2.4	3.1	2.6	3.5
4.5 kJ m ⁻²	1.0	1.2	2.4	2.0	1.5	2.0	2.2	2.0	2.4
9.0 kJ m ⁻²	1.7	1.1	1.2	2.6	1.2	1.1	6.0	1.2	6.4
15 kJ m ⁻²	1.0	1.6	1.9	1.9	1.1	1.2	4.0	1.3	4.5
<i>1,2-Diferuloylgentibiose^e</i>									
Control	4.7	16.8	23.1	26.8	32.8	5.7	36.3	13.9	41.2
1.5 kJ m ⁻²	8.3	3.9	16.1	42.5	6.2	11.5	60.5	23.6	55.6
4.5 kJ m ⁻²	30.2	2.8	20.9	26.1	9.4	11.9	11.1	15.4	12.6
9.0 kJ m ⁻²	30.9	15.8	2.9	41.4	4.6	2.9	81.9	5.4	97.4
15 kJ m ⁻²	3.4	17.5	13.7	35.8	6.3	4.3	33.2	7.7	40.6
<i>1,2,2'-Trisinapoylgentibiose^f</i>									
Control	2.8	3.4	2.7	2.7	2.7	4.8	4.7	6.2	5.1
1.5 kJ m ⁻²	2.7	2.4	3.8	3.8	1.7	6.1	6.6	7.1	7.5
4.5 kJ m ⁻²	4.3	1.4	4.8	2.7	2.3	3.5	2.9	3.7	3.4
9.0 kJ m ⁻²	4.7	1.8	1.5	3.4	2.3	1.9	10.7	2.3	12.6
15 kJ m ⁻²	1.7	2.3	2.4	3.3	1.1	2.5	7.7	3.6	8.8
<i>1,2'-Disinapoyl-2-feruloylgentibiose^g</i>									
Control	2.4	7.7	11.4	12.3	14.2	2.2	17.0	11.0	19.3
1.5 kJ m ⁻²	4.0	1.8	8.1	22.0	2.7	4.8	31.5	13.8	34.4
4.5 kJ m ⁻²	13.0	2.3	8.4	17.1	3.5	5.6	5.1	11.2	8.3
9.0 kJ m ⁻²	13.3	7.4	1.5	20.6	1.9	1.2	27.9	6.3	30.1
15 kJ m ⁻²	1.9	8.5	6.3	17.4	2.4	1.5	14.7	2.1	17.8
<i>1-Sinapoyl-2,2'-diferuloylgentibiose^h</i>									
Control	1.2	2.7	3.9	3.2	4.3	1.8	5.1	2.3	5.3
1.5 kJ m ⁻²	1.7	1.4	3.6	5.6	1.7	3.3	10.4	4.8	12.0
4.5 kJ m ⁻²	3.7	1.7	6.3	3.3	4.2	3.5	3.6	3.6	5.7
9.0 kJ m ⁻²	4.1	2.9	2.1	5.9	3.3	1.5	13.9	2.6	12.8
15 kJ m ⁻²	1.1	3.3	3.5	5.8	2.1	1.9	5.4	2.6	6.0
<i>Totalⁱ</i>									
Control	20.0	62.8	96.1	100.6	124.2	22.5	138.8	55.4	199.5
1.5 kJ m ⁻²	30.1	16.0	58.6	162.2	21.2	39.4	212.8	71.0	247.4
4.5 kJ m ⁻²	115.3	13.7	69.4	102.9	35.4	43.3	38.3	63.1	46.5
9.0 kJ m ⁻²	109.9	59.9	13.1	152.4	20.9	12.5	194.3	29.8	211.0
15 kJ m ⁻²	15.8	62.4	49.4	134.9	28.8	16.7	95.9	27.1	123.4

^a SE = 12.2, LSD ($P \leq 0.05$) = 19.8.

^b SE = 1.6, LSD ($P \leq 0.05$) = 2.6.

^c SE = 4.9, LSD ($P \leq 0.05$) = 7.9.

^d SE = 0.6, LSD ($P \leq 0.05$) = 1.0.

^e SE = 9.2, LSD ($P \leq 0.05$) = 14.9.

^f SE = 1.3, LSD ($P \leq 0.05$) = 2.2.

^g SE = 5.1, LSD ($P \leq 0.05$) = 8.3.

^h SE = 1.5, LSD ($P \leq 0.05$) = 2.4.

ⁱ SE = 38.4, LSD ($P \leq 0.05$) = 62.1.

There was no consistent trend in the levels of the identified individual phenols throughout the storage period. Nevertheless the total hydroxycinnamoyl acid content was highly influenced by the storage temperature after 19 days. At 10 °C an increase of up to 11.3-fold, with respect to the initial content, in 1.5 kJ UV-C m⁻² pre-treated samples was found. Meanwhile only a 2.5-fold increase at 5 °C for the same treatment was found (Table 2). Sinapic acid

derivatives reached the highest increases after 19 days at 5 °C for 1.5 kJ UV-C treated samples which registered up to 4.8-fold higher content of 1,2'-disinapoyl-2-feruloylgentibiose with respect to the initial content. In the meantime increases induced by the same treatment after 19 days at 10 °C were 13-fold higher than initial values. Chlorogenic acid content was also highly influenced by the storage temperature with an increase after 19 days at 10 °C found

Fig. 7. HPLC chromatogram recorded at 320 nm of phenolic compounds present in Bimi® broccoli.

in 1.5 kJ UV-C m⁻² treated samples up to 48-fold compared with the 2-fold increase reached at 5 °C (Table 2).

As far as we know, no references on the influence of UV-C radiation on individual phenolic compounds in fresh-cut broccoli have been reported. In pak choi exposed 10 days before harvest to 0.35–0.4 W UV-B m⁻² and stored 20 days at 2 °C under 1.5–2.5 kPa O₂ + 5–6 kPa CO₂, increases of total hydroxycinnamoyl acid content from 3.42 (at harvest) to 4.95 mg g⁻¹ dry matter were found (Harbaum-Piayda et al., 2010).

3.5. Total antioxidant capacity

The same initial stress observed in total phenolics was also found for the total antioxidant capacity in the UV-C treated samples (Fig. 8). This positive correlation between phenols and antioxidants has been reported before (Cisneros-Zevallos, 2003; Harrison and Were, 2007; Jacobo-Velázquez and Cisneros-Zevallos, 2009).

As we observed, such an increase in antioxidant synthesis is higher in UV-C irradiated samples as a response to the free radicals generated during illumination that might act as stress signals and may trigger stress responses (Fan et al., 2003).

After 19 days at 5 °C (Fig. 8A), the higher radiation dose showed the highest total antioxidant content, approximately 1.5-fold higher than the initial content (92.5 mg AAEAC kg⁻¹ fw). Costa et al. (2006) and Lemoine et al. (2007) concluded also that the total antioxidant capacity of broccoli florets L. var. Italica increased after UV-C pre-treatments of 10 and 8 kJ UV-C m⁻² after 6 and 21 days at 4 °C respectively. The total antioxidant capacity generally showed higher values at 10 °C (Fig. 8B) rather than at 5 °C as was recorded for total phenolics contents.

3.6. Chlorophyll content

Generally, UV-C reduced, immediately after radiation, initial total chlorophyll content (chlorophyll *a* + chlorophyll *b*), with decreases ranging from 23% (for 4.5 kJ m⁻² pre-treated samples) to 31% (9.0 and 15 kJ m⁻² pre-treated ones), 1.5 kJ UV-C m⁻² treated samples retaining the same amount as the control (99.9 mg kg⁻¹ fw) (Table 3). This irreversible breakdown of chlorophyll, due to the effect of the UV-C irradiation (Stird and Porra, 1992), results in the appearance of a number of intermediate and final products (Hynninen, 1991). Our data are in agreement with those reported by Lemoine et al. (2007) who found an initial reduction of total chlorophyll content in broccoli L. var. Italica broccoli after treatment with 8 kJ UV-C m⁻². The reductions observed in our data were mainly associated with chlorophyll *b* (data not shown), the 4.5, 9.0 and 15 kJ UV-C m⁻² treatments resulting in higher decreases (50–60%) with respect to initial values (Table 3). Chlorophyll *a* represented from 54 to 68% of the total chlorophyll content (data not shown). The general trend is a decrease throughout

Fig. 8. Total antioxidant capacity changes of fresh-cut Bimi® broccoli treated with several UV-C doses and stored up to 19 days at 5 °C (A) and 10 °C (B). Data represent means of three replicates ($n = 3 \pm \text{SD}$).

storage, according to Costa et al. (2006) who reported reductions for chlorophyll *a* and *b* in broccoli florets L. var. Italica after 4 days at 20 °C. The UV-C pre-treated samples had lower decreases than controls, retaining the highest total chlorophyll content, in agreement with Costa et al. (2006) who found lower chlorophyll degradation in 10 kJ UV-C m⁻² pre-treated broccoli samples in the same conditions as described above.

Table 3

Total chlorophylls ($a+b$) and carotenoids content changes in fresh-cut Bimi® broccoli treated with several UV-C doses and stored up to 19 days at 5 °C and 10 °C. Data represents means ($n=3$).

Parameter/UV-C dose	Time initial	Day 7		Day 13		Day 16		Day 19	
		5 °C	10 °C	5 °C	10 °C	5 °C	10 °C	5 °C	10 °C
Total chlorophylls ^a (mg kg ⁻¹ fw)									
Control	99.9	89.5	83.8	70.0	70.7	63.8	64.2	52.2	77.3
1.5 kJ m ⁻²	99.8	87.7	76.2	86.7	78.5	65.2	84.2	68.3	90.5
4.5 kJ m ⁻²	70.1	78.5	70.1	62.6	71.1	58.9	70.2	72.2	76.2
9.0 kJ m ⁻²	77.0	70.9	80.1	73.2	74.3	81.7	65.2	70.2	73.6
15 kJ m ⁻²	68.9	83.2	61.6	87.9	72.2	84.7	78.4	80.9	70.2
Total carotenoids ^b (mg kg ⁻¹ fw)									
Control	8.3	7.5	5.1	8.2	8.0	6.5	8.5	6.2	8.4
1.5 kJ m ⁻²	7.8	7.9	6.5	9.4	8.0	8.1	8.1	7.5	8.6
4.5 kJ m ⁻²	6.6	7.3	8.4	6.8	9.9	7.4	9.6	7.9	10.9
9.0 kJ m ⁻²	7.8	6.7	7.0	9.2	9.8	7.9	8.4	8.2	7.5
15 kJ m ⁻²	7.8	7.9	6.0	9.5	8.2	8.2	9.4	9.0	7.8

^a SE = 7.2, LSD ($P \leq 0.05$) = 20.1.

^b SE = 0.7, LSD ($P \leq 0.05$) = 1.9.

3.7. Total carotenoids content

The total carotenoids content decreased from 6 to 20% just after UV-C illumination compared with control values (8.3 mg kg⁻¹ fw). Throughout shelf-life at both storage temperatures, the total carotenoids content remained constant, with the exception of the 4.5 kJ UV-C m⁻² pre-treated samples stored at 10 °C and the control at 5 °C where increases of 36% and decreases of 26% respectively were observed when compared with values on the processing day (Table 3).

4. Conclusions

Low and moderate UV-C illumination (1.5 and 4.5 kJ m⁻²) retarded the natural microflora growth of fresh-cut Bimi® broccoli, retaining sensory quality for up to 19 days at 5 °C and 13 days at 10 °C. Moreover, our results suggest that a moderate UV-C pre-treatment could be used as a tool to increase certain health promoting bioactive compounds such as polyphenol content (mainly the hydroxycinnamoyl acid derivatives content) and total antioxidant capacity.

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CHAPTER IV

Combination of electrolysed water, UV–C and superatmospheric O₂ packaging for improving fresh–cut broccoli quality

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ABSTRACT

The effects of neutral electrolysed water (NEW), ultraviolet light C (UV-C) and superatmospheric O₂ packaging (HO), single or combined, on the quality of fresh-cut kailan-hybrid broccoli for 19 days at 5 °C were studied. As controls, washing with water and sanitation with NaClO were both used. Electrolyte leakage, sensory, microbial and nutritional quality changes throughout shelf-life were studied. At day 15, the combined treatments achieved lower mesophilic and psychrophilic growth compared to the single ones. Single treatments produced higher ascorbate peroxidase (APX) reductions just after its application, while superoxide dismutase (SOD) showed the opposite behaviour. After 5 days at 5 °C, a great increase of APX and guaiacol peroxidase (GPX) activity was observed, NEW + UV-C + HO and HO-including treatments achieving the highest and the lowest APX increases, respectively. UV-C-including treatments produced the highest α-linolenic acid (ALA) decreases ranging 35–38% over control contents on the processing day. NEW-including treatments greatly reduced, throughout shelf-life, ALA and stearic acid (SA) content by 27–44% and 31–61%, respectively. Total phenolic content and antioxidant capacity (1415 mg ChAE kg⁻¹ fw and 287 mg AAE kg⁻¹ fw, respectively) remained quite constant during shelf-life. In general, the treatments and their possible combinations seem to be promising techniques to preserve, or even enhance, the quality of fresh-cut kailan-hybrid broccoli and, probably, other vegetables.

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1. Introduction

Kailan-hybrid is a natural hybrid between kailan or kailan (*Brassica oleracea*, Alboglabra group, also called Chinese broccoli or Chinese kale) and conventional broccoli (*B. oleracea*, Italica group). This kailan-hybrid broccoli has a long slender stem and a mild sweet taste with a completely edible portion (Martínez-Hernández et al., 2011, in press-a). The characteristics of this new kailan-hybrid broccoli make it a suitable product for the fresh-cut vegetables industry. However, the minimal processing steps required result in microbial proliferation. Washing with a solution of 50–150 mg L⁻¹ NaClO is a sanitising method widely used by the fresh-cut industry. However, it may be potentially harmful for humans and the environment (Hrudey, 2009). Thus, several alternative techniques, including electrolysed water (neutral – NEW – or acidic – AEW),

ultraviolet light C (UV-C) and superatmospheric O₂ packaging (HO), seem to be useful to preserve quality of horticultural products (Artés et al., 2009; Cefola et al., 2010). The application of combined sanitising treatments, according to the hurdle technology (Leistner, 2000), could have a synergistic effect leading to a better microbial reduction.

NEW does not affect the surface colour and visual appearance of fresh-cut produce. Furthermore, NEW is preferable to AEW, since a neutral pH is less aggressive for the processing equipment. NEW showed great sanitising effects on natural microflora and pathogenic bacteria in several fresh-cut vegetables, such as carrots, spinach, bell pepper, potato, cucumber, Japanese radish, lettuce and mizuna baby leaves, lowering microbial counts from 0.6 to 2.6 log units (Abadías et al., 2008; Rico et al., 2008; Tomás-Callejas et al., 2011). UV-C can change cell permeability in leafy vegetables. Subsequently, amino acid, carbohydrate and electrolyte leakage (EL) may increase, which can stimulate bacterial growth (Hollosy, 2002). EL has been proposed as an indirect measure of plant cell-membrane damage (Fan and Sokorai, 2005). Lemoine et al. (2007) reported a decrease of 0.6 log units in broccoli cv. Cicco after illumination with 8 kJ UV-C m⁻². Packaging under HO conditions can be used to decrease microbial growth in fresh-cut vegetables. Furthermore,

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this technique may keep sensory attributes, reducing enzymatic browning and preventing anaerobic fermentation and moisture and odour losses (Day, 2001). The antimicrobial effect of HO, contrary to the high CO₂ levels reached within packages throughout storage, is not well known yet.

The NEW, UV-C and HO sanitising technologies use non-sophisticated equipment that does not require high economic investments, and maintenance costs are relatively low. Furthermore, these technologies can be easily automated, which does not imply great difficulties for operators during processing in the industry. All of these factors present these innovative sanitising techniques as good alternatives to the conventional NaClO treatment, obtaining a final product with excellent final quality and reduced production costs.

The sanitising treatments, wounding, MAP and chilled storage involved during the processing and shelf-life of fresh-cut produce have been regarded as abiotic stresses (Jacobo-Velázquez et al., 2011). Such stresses may increase the accumulation of reactive oxygen species (ROS) in the plant cells. In order to mitigate the possible plant cell damage, the enzymatic and non-enzymatic antioxidant systems are activated. Broccoli is a brassica vegetable rich in polyphenols, which have been widely reported as non-enzymatic antioxidants. Other non-enzymatic antioxidants present in broccoli are vitamins C and E, folic acid and carotenoids. Members of the enzymatic antioxidant defence system include superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), ascorbate peroxidase (APX; EC 1.11.1.11), guaiacol peroxidase (GPX; EC 1.11.1.7) and glutathione reductase (GR; EC 1.6.4.2). The superoxide radical (O₂⁻) is dismutated to H₂O₂ by SOD, and CAT, APX and GPX metabolize H₂O₂ to H₂O (Kang and Saltveit, 2001). It has been suggested that lipid degradation and peroxidation are early events in the postharvest deterioration of broccoli (Zhuang et al., 1997). ALA is an n-3 polyunsaturated fatty acid (FA) mainly found in flaxseed, rapeseed, walnuts and broccoli, among other vegetables (Page et al., 2001). It has been reported that the importance of a balanced intake of n-6 and n-3 FA, with a ratio of 1–4/1, in order to achieve their health beneficial effects in the control of chronic diseases (Simopoulos, 1999). PA and SA have also been found in broccoli florets (Page et al., 2001).

The aim of this study was to investigate the effects of NEW, UV-C, HO and their double and triple combinations on the microbial, sensory, EL and nutritional quality changes of fresh-cut kailan-hybrid broccoli throughout shelf-life.

2. Materials and methods

2.1. Plant material

Kailan-hybrid broccoli (*B. oleracea* Italica group × Alboglabra group, cv. Bimi®) sized 15–18 cm long was hand-harvested in March from open air commercial cultivation in the southeast Mediterranean area of Spain (Lorca, Murcia). The broccoli was grown according to integrated pest management cultural practices. Immediately after harvesting, broccoli was pre-cooled with crushed ice and transported by car about 80 km to the Universidad Politécnica de Cartagena. It was stored at 1 °C and 90–95% RH until next day, when it was processed.

2.2. Sample preparation, minimal processing and storage conditions

Minimal processing was accomplished in a disinfected cold room (8 °C). The broccoli was carefully inspected, selecting those pieces free from defects and with a similar visual appearance. These pieces were cut into 15-cm-long spears. Small leaves were removed

with a sharp knife. All broccoli samples were washed for 1 min with tap water (4 °C) in order to remove any organic material. The following treatments were then applied:

- **Control:** After a draining step, samples were packaged, as subsequently described.
- **NaClO:** A standard industrial disinfection treatment with NaClO (100 mg L⁻¹; 5 °C; pH 6.5 ± 0.1) for 2 min was used. A ratio 300 g plant material/5 L disinfectant (w/v) was used. After treatment, the material was rinsed for 1 min with tap water (5 °C) and subsequently drained in a perforated basket for 1 min.
- **NEW:** The plant material was washed with NEW (100 mg L⁻¹ free Cl; 5 °C; pH 7 ± 0.1; oxidation–reduction potential (ORP) = +900 mV) produced by an Envirolite EL 400 equipment (Aquarioja, Madrid, Spain). Contact time, rinsing, draining and disinfectant (w/v) ratio were the same as described above.
- **UV-C:** The plant material was exposed to 6.0 kJ UV-C m⁻² light prior packaging. The UV-C dose was selected based on previous reports and our preliminary experiments with this hybrid (Martínez-Hernández et al., 2011).
- **HO:** The kailan-hybrid was packaged under active MAP with an initial 90-kPa O₂ partial pressure, which was obtained after flushing the O₂ within packages and sealing them at the top with the film described below.
- **Double (NEW + UV-C; UV-C + HO; NEW + HO) and triple (NEW + UV-C + HO) combinations** of the above described treatments were also applied.

After treatment, kailan-hybrid samples of 200 ± 10 g were placed in 1 L (or 2 L for HO treatments) rigid polypropylene (PP) baskets. The baskets were then heat-sealed on the top with a bioriented PP (BOPP) film (30 µm thickness). The O₂ and CO₂ transmission rates at 23 °C and 0% RH were similar with 1100 cm³ m⁻² d⁻¹ atm⁻¹ (data provided by the supplier Plásticos del Segura, Murcia, Spain). In the treatments including HO, a polyethylene terephthalate (PET):PP multilayer film (12:20-µm thickness) was used. The O₂ and CO₂ transmission rates at 23 °C and 0% RH were similar with 1.0–1.2 cm³ m⁻² d⁻¹ atm⁻¹ (data provided by the supplier Plásticos del Segura, Murcia, Spain). The product was stored in darkness at 5 °C. Five replicates of one tray per treatment and sampling time were prepared. Analyses were conducted on the processing day and after 5, 9, 15 and 19 days of shelf-life.

2.3. Respiration rate, ethylene emission and gas analysis within packages

The respiration rate (RR) and C₂H₄ emission were determined using a closed system with five replicates. O₂ and CO₂ partial pressures within packages were analysed according to Martínez-Hernández et al. (in press-b) using five replicates for each treatment and evaluation period.

2.4. Electrolyte leakage

EL was measured according to the method described by Fan and Sokorai (2005) with slight modifications. Briefly, 60 g of plant material was incubated at room temperature in 1 L glass bottles containing 850 mL of deionised water. Five bottles per treatment were prepared in order to have five independent replicates. An orbital shaker (SSL1, Stuart, Staffordshire, UK) at a speed of 100 rpm was used during incubation. Electrical conductivity of the bathing solution was measured with a conductivity meter (GLP32, Crison, Barcelona, Spain) after 1 min (C₁) and 60 min (C₆₀) of incubation. Then, the samples were autoclaved (121 °C) for 25 min and total conductivity (C_T) of bathing solution was measured after cooling.

EL was calculated using the equation: $E = (C_{60} - C_1) / C_T \times 100$ and expressed as %.

2.5. Microbial analysis

The natural microflora concerned with mesophilic, psychrophilic, enterobacteria and yeast and mould were analysed. Standard enumeration methods were used (Tomás-Callejas et al., 2011). All microbial counts were reported as $\log_{10}$ colony forming units per gram ($\log CFU g^{-1}$). *Salmonella* spp., *Listeria monocytogenes* and generic *Escherichia coli* were monitored according to the European legislation for fresh-cut vegetables (Regulation EC 1441/2007, 2007). Each one of the five replicates was analysed by duplicate. Data referring to sampling days 5 and 15 are supplied as supplementary data.

2.6. Sensory evaluation

Sensory analyses were performed according to international standards (ASTM STP 913, 1986). The panel consisted of seven assessors (three women/four men, aged 24–64 years) screened for sensory ability (colour, odour detection, texture and basic taste) according to Martínez-Hernández et al. (in press-b). A 5 point scale of damage incidence and severity was scored for off-colours, off-odours, stem softening and bent (5: none; 4: slight; 3: moderate, limit of usability; 2: severe; 1: extreme). The basal stem pithiness of the kailan-hybrid was scored as 5: no pithiness; 4: pithiness within 5% surface area; 3: within 10% surface area; 2: within 25% surface area; 1: more than 25% surface area. Flavour and overall quality were assessed using another 5 point hedonic scale of acceptance (5: excellent, 4: good, 3: fair, limit of usability, 2: poor; 1: extremely bad).

2.7. Antioxidant enzymes

Enzyme extraction was performed according to Kang and Saltveit (2001) with slight modifications. Ground frozen (-80°C) samples of 5 g were homogenised at 4°C in 20 mL of extraction buffer [50 mM potassium phosphate buffer (pH 7.0), 1% Triton X-100 and 7 mM 2-mercaptoethanol] for 1 h in the orbital shaker at 250 rpm. Then, the homogenate was centrifuged at $20,000 \times g$ for 20 min at 4°C and the supernatant was used as the crude extract for the APX, GPX and GR assays. For SOD and CAT analyses, 5 g of plant material were extracted, as described above, in 20 mL of a different extraction buffer [50 mM Tris–HCl Buffer (pH 7.5), 3 mM MgCl_2 and 1 mM EDTA]. All enzymes activities, except SOD, were estimated by the initial velocities method from the linear portion of the curves. Samples were read against water instead of the enzyme assay solution as blank. The estimated enzyme activity was expressed per gram of protein, which was determined by the protein-dye binding method (Kang and Saltveit, 2001). Each of the five replicates was analysed by triplicate.

APX activity was determined by its capability to oxidise ascorbate in the presence of H_2O_2 as previously described (Chen and Asada, 1989) with slight modifications. A polystyrene 96 well flat-bottom UV-Star plate (Greiner Bio-one, Frickenhausen, Germany) (also used for CAT assay) was properly loaded with 285 μL of 50 mM phosphate buffer (pH 7.0); 0.1 mM EDTA, 0.5 mM ascorbate and 1.54 mM H_2O_2 and 15 μL of the enzyme extract. The decrease in absorbance was monitored using a Multiscan plate reader (Tecan-Infinite M200, Tecan, Männedorf, Switzerland), as for the rest of the spectrophotometric measurements, at 240 nm every 5 min for 60 min. One unit of activity (U) of APX was defined as a decrease in absorbance of min^{-1} .

GPX activity was assayed according to Upadhyaya et al. (1985), with slight modifications. A 96 well flat-bottom polystyrene plate

(Greiner Bio-one, Frickenhausen, Germany), used for the rest of enzyme assays, was properly loaded with 166 μL of 50 mM phosphate buffer (pH 6.1), 66 μL of 1% H_2O_2 , 66 μL of 1% guaiacol and 1.3 μL of the enzyme extract. Samples were read against water as blank instead of the APX assay solution. The increase in absorbance at 420 nm was followed for 1 min. One GPX U was defined as a decrease in absorbance of s^{-1} .

GR activity was determined based on the oxidation of NADPH (Klapheck et al., 1990) with slight modifications. To the 285 μL -reaction mixture containing 0.1 M Tris–HCl (pH 8.0), 1 mM EDTA, 0.1 mM NADPH, 1 mM GSSG were added 15 μL of the enzyme extract at 30°C . The decrease in absorbance at 334 nm was monitored every 5 min during 60 min. One GR U was defined as a decrease in absorbance of min^{-1} .

SOD activity was assayed by its ability to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) described by the method of Dhindsa et al. (1981) with slight modifications. In total, 6 μL of the enzyme extract was added to the 351 μL reaction mixture containing 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 2 μM riboflavin and 0.1 mM EDTA. Riboflavin was added last and the plate was placed 30 cm below a light bank, consisting of two 7.5 W fluorescent lamps, for 20 min. A non-irradiated reaction mixture did not develop colour and was used as control. The absorbance by the reaction mixture was read at 560 nm at time 0 and 1 h. SOD activity was calculated per g of fresh weight (fw) and expressed as a percentage of the control.

CAT activity was determined by measuring the rate of disappearance of H_2O_2 using the method of Maehly and Chance (1959) with slight modifications. For the 343 μL reaction mixture containing 50 mM phosphate buffer (pH 7.4) and 13 μL of 1% H_2O_2 , 6 μL of the enzyme extract was added. The decrease in H_2O_2 was monitored as a decline in absorbance at 240 nm every 5 min for 1 h. One CAT U was defined as a decrease in absorbance of min^{-1} .

Enzyme data referring to sampling days 5 and 15 are supplied as supplementary data.

2.8. Fatty acids

FAs were analysed according to Page et al. (2001) with slight modifications. Briefly, ground frozen (-80°C) samples of 0.2 g were placed in 15 mL screw cap glass tubes. 0.15 M acetic acid (1 mL) and chloroform/methanol (1/2, v/v; 7.5 mL) were added. It was homogenised in the orbital shaker at 250 rpm for 10 min in darkness. Then, 2.25 mL of nanopure water were added and the phase separation was facilitated by low-speed centrifugation. The lower chloroform phase containing the FA was removed and subsequently evaporated under N_2 . FAs were quantified after transmethylation performed in 2.5% (v/v) sulphuric acid in anhydrous methanol (2 mL) and separated by GS–MS chromatography. A gas chromatograph (6890, Agilent Technologies, Palo Alto, USA) equipped with a Multi Purpose Samples (MPS2, Agilent Technologies, Palo Alto, USA) and MS detector (Inerta Mass Selective Detector 5975, Agilent Technologies, Palo Alto, USA) was used. Methylated FAs (FAMES) separation was achieved on a $60 \text{ m} \times 0.25 \text{ mm} \times 0.25\text{-}\mu\text{m}$ capillary column (DB-23, Agilent Technologies, Palo Alto, USA). The carrier gas was helium with a constant flow equal to 2.8 mL min^{-1} and pressure of 264.8 kPa. Injection was performed in splitless mode. The oven temperature was held at 50°C for 1 min after injection, then programmed to reach 235°C at 10 min and held at 235°C until 29.5 min. MS was in electronic impact mode (70 eV) with a mass range of 40–400 amu. Source and MS quad temperatures were 230 and 150°C , respectively. FAMES peaks were identified by their mass spectra compared to those in the data base NIST05a.L (National Institute for Standards and Technology) and to those of available authentic FAME standards (Sigma, USA). FAMES were expressed as micromoles per gram fw. Each one

of the five FAMES extracts corresponding to each replicate was analysed in the GS-MS by duplicate. Data referring to sampling days 5 and 15 are supplied as supplementary data.

2.9. Total phenolic content and antioxidant capacity

Total phenolic content was analysed as recently described (Martínez-Hernández et al., in press-a), based on the Singleton and Rossi (1965) method. Total phenolic content was expressed in milligrams per kilogram fw chlorogenic acid equivalent. Each one of the five replicates was analysed by triplicate. Data referring to sampling days 5 and 15 are supplied as supplementary data.

Total antioxidant capacity was determined based on the evaluation of the free radical scavenging capacity according to the DPPH assay (Brand-Williams et al., 1995) with modifications (Martínez-Hernández et al., in press-a). Total antioxidant capacity was expressed in milligrams per kilogram fw ascorbic acid equivalent antioxidant capacity. Each of the five replicates was analysed by triplicate. Data referring to sampling days 5 and 15 are supplied as supplementary data.

2.10. Statistical analysis

The experiment was a 9×5 two-factor (treatment $\times$ storage time) design subjected to an analysis of variance using Statgraphics Plus (version 5.1) software. When differences among treatments were significant, the mean values were compared by the least significant difference multiple range test (LSD, $p < 0.05$). Possible synergistic effects of the treatments combinations were discarded after analysing data with Limpel's formula ($E_c = X + Y - (XY/100)$) according to Richer (1987).

3. Results and discussion

3.1. Respiration rate, ethylene emission and gas composition within packages

The stress induced after harvesting and processing increased the respiration and C_2H_4 emission rates of sanitised kailan-hybrid compared to control samples on day 1 ($72.9 \text{ mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ and $0.6 \text{ } \mu\text{L C}_2\text{H}_4 \text{ kg}^{-1} \text{ h}^{-1}$, respectively) (Fig. 1A and B). The increment of respiration and C_2H_4 emission rates may lead to early deterioration/loss of compounds comparing to the control, as it was observed for fatty acids and total phenolics (described in the sections below). As expected, the combination of sanitising treatments induced higher RR/ C_2H_4 values than control, up to 3.1/2.5-fold for NEW+UV-C. Such increases were lower for the remaining treatments, ranging RR and C_2H_4 emission around 1–2-fold. After this initial stress, these rates decreased, achieving stable values after 3 days. It is worth indicating that the HO treatments showed a strong RR and C_2H_4 emission enhancement after 3 days of shelf-life, in a similar manner to several other fruit and vegetables reviewed by Kader and Ben-Yehoshua (2000). While HO treatment produced the highest increase, 6.8-fold after 5 days, its combination with UV-C greatly exacerbated this response. Consequently, RR values were approximately 18-fold higher than those in control samples after 6 days at 5°C . This could be explained by the fact that the UV-C-stressed plant cells could be more sensitive to the hyperoxia stress. The use of NEW in the combined NEW+HO produced a RR peak 8.6-fold higher than that of control samples, lately observed after 8 days of storage. Liao et al. (2007) hypothesised that the high ORP of electrolysed water treatments affects and damages the redox state of the glutathione disulphide–glutathione couple (GSSG/2GSH). Consequently, that relatively lower respiration rate may be a response to the stress caused by the O_2 -enriched MAP. A

Fig. 1. Respiration rate (A) and ethylene emission (B) of fresh-cut kailan-hybrid after single and combined sanitising treatments and stored up to 13 days at 5°C ($n = 5 \pm \text{SD}$).

similar C_2H_4 emission pattern was observed throughout storage of kailan-hybrid samples.

The gas partial pressure changes throughout shelf-life within MAP packages are shown in Fig. 2. The equilibrium gas partial pressures for the treatments without HO were reached after 2 days of storage with 7–13 kPa O_2 and 9–15 kPa CO_2 . This gas composition was similar to that recommended for the kailan-hybrid (Martínez-Hernández et al., 2011, in press-b). Due to the physiological stress induced, slight changes in these equilibrium gas partial pressures were observed. The samples packaged under HO registered initial values of 90 kPa O_2 . As expected, the O_2 levels decreased and CO_2 increased, owed to the respiratory activity of the plant material (Fig. 2). After 19 days of storage, gas partial pressures within packages were around 50 kPa O_2 + 25 kPa CO_2 .

3.2. Electrolyte leakage

The applied treatments did not produce EL changes on the processing day, except UV-C with a significant increase ($p < 0.05$) of 1.4-fold compared to control samples (0.43%) (Fig. 3). The UV radiation has been reported to produce cell membrane damage in broccoli and other vegetables (Fan and Sokorai, 2005). However, the combined treatment NEW+UV-C did not register such cell disorders, probably due to the additive effect. Contrary to this, Lemoine et al. (2007) did not find EL after UV-C exposure (8 kJ m^{-2}) in broccoli heads cv. Cicco. This fact may be explained by a higher sensibility of the plant cells located in the stem, which represents

Fig. 2. Gas changes within packages of fresh-cut kailan-hybrid after single and combined sanitising treatments stored up to 19 days at 5 °C ($n = 5 \pm \text{SD}$) (O_2 solid lines; CO_2 dashed lines). Balanced with N_2 .

for the kailan-hybrid plant a higher proportion in comparison with the small stem portion of conventional broccoli heads. During 9 days of shelf-life, EL slightly increased in all samples. However, EL of the HO treatments drastically rose after 9 days of storage. The triple combination produced the greatest EL (7-fold) and the single HO treatment the lowest (3.6-fold) compared to their correspondent values on the processing day. Previous literature showed EL reductions in many MAP vegetables under HO initial levels of 80–100 kPa (Allende et al., 2004), which allowed preservation of tissue and membrane integrity. In our previous experiments, kailan-hybrid samples stored in controlled atmospheres of 25 kPa CO_2 and 2 kPa O_2 did not show pH, titratable acidity or stem firmness changes for 27 days at 5 °C (data not published). This finding could explain the unaffected EL data during the first 9 days, since CO_2 levels were around 20–25 kPa. The CO_2 levels above 25 kPa reached after 9 days could produce acidification of the plant tissue, as previously reported Lange and Kader (1997), which may cause cell damage

Fig. 3. Electrolyte leakage of fresh-cut kailan-hybrid after single and combined sanitising treatments and stored up to 19 days at 5 °C ($n = 5 \pm \text{SD}$).

and the subsequently observed EL increase. The remaining treatments without HO registered lower EL increments than control samples at the end of the storage. The single UV-C treatment did not registered EL changes throughout shelf-life compared to its initial content. NEW + UV-C only showed a slight EL increase of 4% after 19 days.

According to the observed EL data, and similarly to NaClO, these treatments did not produce important damage into the cell structure integrity during the first 9 days of storage. However, important cell damage was detected in the HO treatments when the CO_2 concentrations within packages rose over 25 kPa.

3.3. Microbiological analysis

Before sanitising, the microbial count for total aerobic mesophiles (TAM) was 2.4 (Table 1), a lower value than the previous data reported for this hybrid ($3.6 \log \text{CFU g}^{-1}$) as well as for broccoli var. Olympia ($5.9 \log \text{CFU g}^{-1}$) (Martínez-Hernández et al., 2011; Olarte et al., 2009). These lower TAM levels may be attributed to both the fast precooling of the harvested produce and/or occasional rainfall prior to the harvest. Sanitising treatments resulted in an initial TAM reduction of 1.2–1.4 log, without differences

Table 1

Changes in mesophilic, psychrophilic, enterobacteriaceae and yeast and mould counts ($\log \text{CFU g}^{-1}$) of fresh-cut kailan-hybrid after single and combined sanitising treatments stored up to 19 days at 5 °C ($n = 5 \pm \text{SD}$). Different capital letter among each row denotes significant difference ($p < 0.05$). Different lower case letter within each column denotes significant difference ($p < 0.05$).

	Days at 5 °C		
	Processing day	9	19
Mesophilic ($\log \text{CFU g}^{-1}$)			
Control	$2.4 \pm 0.1^{\text{B}}$	$2.2 \pm 0.4^{\text{D}}$	$2.8 \pm 0.1^{\text{E}}$
NaClO	$1.0 \pm 0.0^{\text{B}}$	$1.5 \pm 0.3^{\text{A}}$	$1.0 \pm 0.0^{\text{B}}$
UV-C	$1.2 \pm 0.3^{\text{B}}$	$1.4 \pm 0.1^{\text{B}}$	$1.7 \pm 0.3^{\text{D}}$
NEW	$1.9 \pm 0.3^{\text{B}}$	$28.4 \pm 9.6^{\text{C}}$	$1.0 \pm 0.0^{\text{B}}$
HO	$1.4 \pm 0.4^{\text{B}}$	$1.9 \pm 0.2^{\text{A}}$	$1, 172 \pm 120^{\text{E}}$
UV-C + HO	$1.2 \pm 0.3^{\text{B}}$	$1.9 \pm 0.3^{\text{A}}$	$1.2 \pm 0.2^{\text{B}}$
NEW + HO	$0.50 \pm 0.05^{\text{A}}$	$1.4 \pm 0.3^{\text{A}}$	$1.1 \pm 0.0^{\text{B}}$
NEW + UV-C	$1.1 \pm 0.1^{\text{B}}$	$1.2 \pm 0.2^{\text{B}}$	$1.0 \pm 0.0^{\text{B}}$
NEW + UV-C + HO	$1.1 \pm 0.1^{\text{B}}$	$1.3 \pm 0.2^{\text{C}}$	$1.1 \pm 0.1^{\text{B}}$
Psychrophilic ($\log \text{CFU g}^{-1}$)			
Control	$2.3 \pm 0.2^{\text{B}}$	$4, 235 \pm 224^{\text{A}}$	$4.0 \pm 0.2^{\text{E}}$
NaClO	$1.0 \pm 0.0^{\text{B}}$	$1.6 \pm 0.4^{\text{A}}$	$1.1 \pm 0.2^{\text{B}}$
UV-C	$1.5 \pm 0.2^{\text{B}}$	$1.6 \pm 0.2^{\text{A}}$	$1.1 \pm 0.1^{\text{B}}$
NEW	$1.0 \pm 0.0^{\text{B}}$	$1.2 \pm 0.1^{\text{A}}$	$1.0 \pm 0.0^{\text{B}}$
HO	$2.4 \pm 0.2^{\text{B}}$	$1.7 \pm 0.4^{\text{A}}$	$1.2 \pm 0.2^{\text{B}}$
UV-C + HO	$1.5 \pm 0.2^{\text{B}}$	$1.7 \pm 0.4^{\text{A}}$	$1.0 \pm 0.0^{\text{B}}$
NEW + HO	$1.0 \pm 0.0^{\text{B}}$	$1.3 \pm 0.0^{\text{A}}$	$1.5 \pm 0.4^{\text{A}}$
NEW + UV-C	$1.1 \pm 0.2^{\text{B}}$	$1.2 \pm 0.2^{\text{C}}$	$1.8 \pm 0.4^{\text{D}}$
NEW + UV-C + HO	$1.1 \pm 0.2^{\text{B}}$	$1.6 \pm 0.4^{\text{A}}$	$1.1 \pm 0.1^{\text{B}}$
Enterobacteriaceae ($\log \text{CFU g}^{-1}$)			
Control	ND	$1.7 \pm 0.5^{\text{B}}$	$1.9 \pm 0.1^{\text{A}}$
NaClO	ND	ND	ND
UV-C	ND	ND	ND
NEW	ND	ND	ND
HO	ND	ND	ND
UV-C + HO	ND	ND	ND
NEW + HO	ND	ND	ND
NEW + UV-C	ND	ND	ND
NEW + UV-C + HO	ND	ND	ND
Yeasts and moulds ($\log \text{CFU g}^{-1}$)			
Control	$3.1 \pm 0.4^{\text{B}}$	$3.0 \pm 0.3^{\text{B}}$	$3.2 \pm 0.6^{\text{AB}}$
NaClO	ND	ND	$2.1 \pm 0.1^{\text{A}}$
UV-C	$2.9 \pm 0.3^{\text{A}}$	$2.4 \pm 0.7^{\text{C}}$	$3.12 \pm 30^{\text{C}}$
NEW	ND	ND	$2.4 \pm 0.4^{\text{A}}$
HO	ND	$2.7 \pm 0.3^{\text{A}}$	$2.7 \pm 0.4^{\text{A}}$
UV-C + HO	$2.9 \pm 0.3^{\text{A}}$	$2.6 \pm 0.4^{\text{B}}$	$2.8 \pm 0.3^{\text{A}}$
NEW + HO	ND	ND	ND
NEW + UV-C	ND	ND	ND
NEW + UV-C + HO	ND	ND	ND

among them, compared to control samples. These results agree with those reported by Abadías et al. (2008), where reductions of approximately 1 CFU log were found for fresh-cut iceberg lettuce and for a four seasons salad (iceberg lettuce, carrot and cabbage) sanitised with NEW (pH 8.6; ORP +722 mV; 52 mg L⁻¹ free Cl) for 3 min. Lower TAM counts were found in mizuna baby leaves treated with NEW (pH 7.0; ORP +800 mV; 100 mg L⁻¹ free Cl) for 2 min (Tomás-Callejas et al., 2011). A TAM reduction of approximately 0.7 log CFU g⁻¹ was reported by Lemoine et al. (2007) in broccoli cv. Cicco after UV-C radiation (8 kJ m⁻²). However, a lower UV-C dose (6 kJ m⁻²) hereby applied on the kailan-hybrid induced a better TAM reduction of 1.2 log. This greater effectiveness of UV-C light is also determined by the structure and topography of the product surface. Recently, Artés-Hernández et al. (2009) and Escalona et al. (2010) reported that low UV-C radiation doses were enough to reduce natural microflora in fresh-cut spinach. After 5 days at 5 °C, the HO treatments underwent a TAM growth among 4.0–4.6 log CFU, in comparison to their respective initial values. Such levels returned to initial content after 9 days at 5 °C and were kept quite stable during the shelf-life. Likewise, Amanatidou et al. (1999) observed stimulation of some bacterial groups under approximately 80 kPa at 8 °C. Tomás-Callejas et al. (2012) found a similar TAM peak in fresh-cut tatsoi baby leaves stored under O₂-enriched MAP after 4 days at 5 °C, although those higher initial O₂ concentrations (100 kPa) resulted in a smaller TAM increment. This microbial growth stimulation was suppressed in HO treatments after 9 days when CO₂ concentrations underwent over 20 kPa, as previously stated Amanatidou et al. (1999). The bacteriostatic effect of the combined treatments, compared to single ones, was registered after 15 days. In this way, the combined sanitising treatments reported 1.8–2.0 log CFU lower TAM loads than control samples on that day, without differences among them.

On the processing day, psychrophilic loads registered a decrease of 0.9 log CFU after UV-C radiation, while the rest of treatments only showed reductions ranging among 1.2–1.3, without differences among them, compared to control values (2.3 log CFU g⁻¹) (Table 1). Similarly to TAM counts, sanitising treatments showed a residual bacteriostatic effect throughout shelf-life of the kailan-hybrid. The great bacteriostatic effect of combined treatments on psychrophilic levels was also observed after 15 days of shelf-life. The combined treatments attained psychrophilic decreases of 2.0–2.1 log CFU, while the single treatments achieved lower reductions that ranged between 0.7 and 1.6 log CFU compared to control values on this day. Accordingly, the combination of UV-C radiation (4.5 kJ m⁻²) and enriched-O₂ MAP (100 kPa initially) achieved the best psychrophilic growth suppression in fresh-cut tatsoi baby leaves during 6 days at 5 °C (Tomás-Callejas et al., 2012).

Enterobacteriaceae counts were kept below the detection limit after sanitising treatments throughout shelf-life at 5 °C (Table 1). However, *enterobacteriaceae* loads of approximately 1.7 log CFU g⁻¹ were detected in control samples after 9 days, which remained quite stable during shelf-life. Similarly to TAM results, HO treatments produced an *enterobacteriaceae* growth peak in the fifth day of storage, according to previous data (Tomás-Callejas et al., 2011, 2012).

The initial yeast and mould counts (3.1 log CFU g⁻¹) were reduced after all sanitising treatments, except UV-C, until undetected levels on the processing day (Table 1). Likewise, Lemoine et al. (2007) reported no fungistatic effect in broccoli cv. Cicco after UV-C radiation, although a residual UV-C-induced fungistatic effect was observed during shelf-life. Throughout storage, yeast and mould levels of all kailan-hybrid samples slightly increased with values ranging among 2.1–3.4 log CFU g⁻¹ after 19 days.

The analysed sanitising methods can be regarded as good, eco-friendly, cheap and safe alternatives to conventional NaClO washing treatments. The combination of these innovative sanitising

treatments showed a residual microbicidal effect in kailan-hybrid after 15 days of storage at 5 °C.

3.4. Sensory evaluation

The treatments did not produce changes in the sensory quality on the processing day, nor during the first 9 days of storage (Fig. 4). Significant basal stem pithiness, off-colours and stem bending were not detected during the 19 days for any of the treated samples. All treated samples preserved turgor, without differences among them, except UV-C + HO, which was quite affected after 19 days at 5 °C. Generally, off-odours were lightly appreciated during the first 15 days of shelf-life, as previously observed in broccoli raab (Cefola et al., 2010). Nevertheless, only NaClO and NEW kept off-odours and flavour over the limit of usability after 19 days at 5 °C, since the high CO₂ concentration within HO packages induced some unpleasant flavours. Although, the triple treatment produced the highest EL increment after 19 days, these samples achieved good sensory scores compared to the rest of treatments. This fact indicates that the substances leaked during the shelf life of this kailan-hybrid did not greatly affect the studied sensory parameters. According to the overall quality scores, the combined treatments achieved an excellent shelf-life of 15 days at 5 °C, except for NaClO and NEW that was prolonged until 19 days. In this way, the use of combined sanitising treatments could satisfactorily be considered as an eco-sustainable sanitising technique alternative to NaClO washings, since they achieved the same good sensory quality for 15 days at 5 °C. However, further investigation may be conducted to optimise the O₂-enriched modified atmosphere packaging design, in order to keep appropriate CO₂ levels that do not limit the shelf-life of the product.

3.5. Antioxidant enzymes

To understand the effect of the studied sanitising treatments on the enzymatic antioxidant system, APX, GPX, GR, CAT and SOD activities were analysed immediately after processing and during shelf-life (Table 2). Generally, APX and SOD activities decreased after treatments, contrary to CAT, GR and GPX which did not register significant changes ($p < 0.05$). The single sanitising treatments NEW and UV-C produced higher APX activity reductions than the combined NEW + UV-C with 74–90 and 57%, respectively, compared to initial APX activity (2028 U g⁻¹ protein). Contrary to APX, the combined treatment NEW + UV-C produced the highest SOD activity enhancement with approximately 62% over initial levels. SOD activity only registered a reduction of 35–34% for the single treatments NEW and UV-C, without significant differences ($p < 0.05$) among them, in comparison to initial values (376 U g⁻¹ protein). EW has shown ROS scavenging properties, due to its APX, CAT and SOD-like activities (Shirahata et al., 1997). In this way, the reduced APX and SOD activities of the NEW-containing treatments may be due to the ROS-scavenging effect of the EW.

With regard to the enzyme activity throughout the refrigerated storage, sanitised samples registered a great APX activation after 5 days of storage, UV-C + HO and NEW + UV-C + HO having the highest increases at 23.3 and 12.5-fold, respectively. Since respiration rate is known as one of the sources of ROS, the high RR of UV-C + HO and NEW + UV-C + HO-treated samples after 5 days at 5 °C could produce high levels of H₂O₂, as has been previously reported in hyperoxia-treated carrots (Jacobo-Velázquez et al., 2011). Consequently, it triggered the detoxifying reaction by the increase in the APX activity. These enhanced APX levels returned to initial content levels on day 9, which were retained until the end of storage. The triple combination produced the greatest APX increase with 9921 U g⁻¹ protein after 5 days, contrary to HO which achieved the lowest with 2350 U g⁻¹ protein compared to their respective

Fig. 4. Sensory scores for off-odours, off-flavours, flavour, turgor, bent, hollowness and overall quality of fresh-cut kailan-hybrid after single and combined sanitising treatments stored up to 19 days at 5 °C ($n = 7$). The shaded areas correspond to sensory scores under the limit of usability.

values on the processing day. In this way, the higher the degree of treatment combination applied, the higher APX activity increase reached after 5 days. These results could be explained since sanitising treatments are interpreted as plant abiotic stresses, and their combination would produce higher levels of stress on the plant cell system, as previously reported by Jacobo-Velázquez et al. (2011). Likewise to APX, GPX activity showed similar increases after 5 days, ranging around 3.6- and 9.2-fold for HO and UV-C, respectively, in comparison to the initial value ($333 \text{ U g}^{-1} \text{ protein}$). Attending to SOD activity, it generally rose after 5 days of storage, showing the triple combination and NaClO the highest increases with 2.7- and 3.1-fold, respectively, compared to their corresponding values on the processing day. Such enhanced SOD activity after 5 days at 5 °C maintained similar levels until the end of storage. CAT activity was also augmented throughout shelf-life by approximately 1.2–1.5-fold after 9 days, later than what was observed for APX and GPX on day 5. This can be explained since CAT has affinity for H_2O_2 at mM ranges (reached later), contrary to the μM ranges where peroxidases act. These differences in substrate affinity might be explained since APX is responsible for finely modulating ROS to serve as signalling molecules, and CAT is in charge of removing ROS when toxic levels are attained (Jacobo-Velázquez et al., 2011).

CAT activity, similarly to APX and GPX, decreased after the observed increase, achieving similar levels to the processing day after 19 days. GR activity also rose during shelf-life as the other enzymes did, but the highest activations were not attained until day 19. In general, and similarly to the APX activity peak on day 5, the higher the degree of treatment combinations applied, the higher the GR activity increases on day 19. In this way, the triple combination registered the greatest increment with 8.3-fold, followed by the NEW-including double combinations NEW + UV-C and

NEW + HO by 7.1- and 5.8-fold, respectively, compared to their respective initial values. Meanwhile, the single UV-C treatment registered the lowest increment with 2.8-fold over its activity on the processing day.

In some cases the differences within treatments were lower than those due to the storage time. It could be probably due to the fact that 5 °C is a temperature rather above the recommended for broccoli storage. Probably differences between treatments would be easier detected at lower temperatures. However, 5 °C is the temperature normally used by the industry.

The applied sanitising treatments UV-C, NEW and HO have been widely reported to act as abiotic stresses in plants (Jacobo-Velázquez et al., 2011; Martínez-Hernández et al., 2011; Tomás-Callejas et al., 2011), which may increase the ROS levels of plant cell systems. Furthermore, the low storage temperature may cause a ROS increment (Karpinski et al., 2002). Other sources of ROS, such as NADPH oxidase, may be activated by the application of these sanitising treatments, consequently generating the superoxide radical (O_2^-). The observed activation of APX, GPX and SOD activities during storage is related to the need of the cell plant to eliminate the generated ROS by conversion to H_2O_2 by SOD, and lately to the innocuous H_2O by APX and GPX. Consequently, the reduced ROS levels reached with the enhanced detoxifying enzymes could contribute to tissue integrity in advanced stages of senescence.

3.6. Fatty acids

The major FAs found in kailan-hybrid material were ALA (C18:3 , $\Delta^{9,12,15}$; $8.5 \mu\text{mol g}^{-1} \text{ fw}$) and PA (C16:0 , $3.4 \mu\text{mol g}^{-1} \text{ fw}$) with low amounts of other C16 unsaturated FAs, mainly SA (C18:0 , $0.5 \mu\text{mol g}^{-1} \text{ fw}$). On the processing day, UV-C-including

Table 2

Enzymatic antioxidant content (APX, GPX, GR, SOD and CAT) of fresh-cut kailan-hybrid after single and combined sanitising treatments stored up to 19 days at 5 °C ($n=5 \pm \text{SD}$). Different capital letter among each row denotes significant difference ($p < 0.05$). Different lower case letter within each column denotes significant difference ($p < 0.05$).

Enzymes (units g ⁻¹ protein)	Days at 5 °C		
	Processing day	9	19
APX			
Control	2, 028 ± 364 ^{ab}	1, 123 ± 312 ^c	370 ± 83 ^d
NaClO	8.4 ± 0.2 ^a	326 ± 66 ^c	230 ± 99 ^{bc}
UV-C	356 ± 54 ^b	795 ± 108 ^c	461 ± 380 ^{ab}
NEW	204 ± 45 ^c	111 ± 26 ^f	424 ± 222 ^d
HO	536 ± 66 ^b	532 ± 145 ^b	312 ± 65 ^d
UV-C + HO	356 ± 54 ^b	307 ± 59 ^b	448 ± 106 ^d
NEW + HO	204 ± 55 ^b	1, 358 ± 862 ^c	358 ± 72 ^e
NEW + UV-C	863 ± 44 ^b	610 ± 44 ^c	472 ± 66 ^{de}
NEW + UV-C + HO	863 ± 44 ^b	1, 360 ± 38 ^{cd}	691 ± 39 ^e
GPX			
Control	333 ± 19 ^b	2, 180 ± 146 ^a	319 ± 25 ^a
NaClO	325 ± 32 ^b	4.2 ± 0.2 ^c	60.0 ± 34.0 ^b
UV-C	267 ± 24 ^b	481 ± 15 ^c	ND
NEW	287 ± 45 ^b	1, 210 ± 301 ^c	ND
HO	325 ± 32 ^b	559 ± 105 ^c	ND
UV-C + HO	267 ± 24 ^b	1, 087 ± 143 ^c	ND
NEW + HO	1.5 ± 0.1 ^c	2, 550 ± 278 ^a	0.4 ± 0.6 ^c
NEW + UV-C	342 ± 32 ^b	2.5 ± 0.3 ^{bc}	ND
NEW + UV-C + HO	342 ± 32 ^b	1.9 ± 0.3 ^c	28.4 ± 9.6 ^c
GR			
Control	624 ± 102 ^b	2, 022 ± 189 ^d	4, 496 ± 225 ^a
NaClO	643 ± 75 ^b	1, 307 ± 105 ^b	2, 306 ± 33 ^a
UV-C	525 ± 31 ^{abc}	1, 418 ± 60 ^a	1, 458 ± 139 ^a
NEW	375 ± 21 ^d	1, 578 ± 21 ^d	2, 064 ± 213 ^a
HO	643 ± 75 ^b	1, 255 ± 101 ^d	0.30 ± 0.05 ^{bc}
UV-C + HO	525 ± 31 ^{abc}	1, 728 ± 82 ^d	2, 417 ± 49 ^a
NEW + HO	375 ± 21 ^d	1, 595 ± 343 ^d	2, 171 ± 242 ^a
NEW + UV-C	521 ± 16 ^{bc}	1, 082 ± 63 ^e	3, 654 ± 513 ^a
NEW + UV-C + HO	521 ± 16 ^{bc}	2, 250 ± 192 ^d	4, 235 ± 224 ^a
CAT			
Control	221 ± 13 ^b	304 ± 7 ^c	214 ± 30 ^b
NaClO	207 ± 19 ^b	228 ± 18 ^a	233 ± 11 ^a
UV-C	139 ± 15 ^c	212 ± 20 ^c	156 ± 3 ^e
NEW	175 ± 19 ^b	243 ± 29 ^a	189 ± 22 ^d
HO	207 ± 19 ^b	244 ± 26 ^a	161 ± 3 ^e
UV-C + HO	139 ± 15 ^c	176 ± 16 ^c	144 ± 29 ^{ce}
NEW + HO	175 ± 19 ^b	234 ± 10 ^a	80.3 ± 5.2 ^d
NEW + UV-C	190 ± 15 ^b	224 ± 27 ^a	192 ± 21 ^d
NEW + UV-C + HO	190 ± 15 ^b	181 ± 26 ^a	137 ± 6 ^e
SOD			
Control	376 ± 12 ^b	377 ± 23 ^b	387 ± 45 ^b
NaClO	143 ± 7 ^{bc}	367 ± 33 ^b	399 ± 35 ^b
UV-C	261 ± 11 ^c	364 ± 36 ^b	434 ± 20 ^a
NEW	245 ± 11 ^b	431 ± 29 ^a	310 ± 20 ^d
HO	143 ± 7 ^{bc}	275 ± 26 ^d	37 ± 8 ^e
UV-C + HO	261 ± 11 ^c	338 ± 41 ^c	290 ± 12 ^e
NEW + HO	245 ± 11 ^b	319 ± 33 ^c	209 ± 9 ^d
NEW + UV-C	137 ± 5 ^{bc}	528 ± 17 ^a	358 ± 12 ^d
NEW + UV-C + HO	137 ± 5 ^{bc}	411 ± 32 ^f	319 ± 27 ^{cd}

treatments (single UV-C and combined NEW + UV-C), showed the highest ALA reductions with 35–38% compared to control values (Table 3). The single UV-C treatment also reduced SA in a 20%, being the only treatment affecting SA levels on the processing day when related to the control. Nasibi and M-Kalantari (2005) reported a similar lipid peroxidation after UV-C radiation (10.3 kJ m⁻²) of the brassica rapeseed with a 200% thiobarbituric acid reactive materials (a relatively stable end product of lipid and other macromolecular-derived peroxidation reactions) increment compared to untreated samples. It has been reported that UV causes oxidative stress in plants with the consequent generation of ROS (Mackerness, 2000). Consequently, the FAs degradation may be due the reaction of the hydroxyl radicals and singlet oxygen with them forming the lipid

Table 3

Fatty acids content of fresh-cut kailan-hybrid after single and combined sanitising treatments stored up to 19 days at 5 °C ($n=5 \pm \text{SD}$). Different capital letter among each row denotes significant difference ($p < 0.05$). Different lower case letter within each column denotes significant difference ($p < 0.05$).

Fatty acids (μmol g ⁻¹ fw)	Days at 5 °C		
	Processing day	9	19
Linolenic acid			
Control	8.4 ± 0.2 ^a	2.4 ± 0.2 ^c	3.0 ± 0.2 ^d
NaClO	7.2 ± 0.3 ^a	4.5 ± 0.3 ^c	4.0 ± 0.2 ^{cd}
UV-C	5.5 ± 0.5 ^a	3.0 ± 0.5 ^c	4.2 ± 0.1 ^e
NEW	6.8 ± 0.4 ^a	2.4 ± 0.2 ^c	3.8 ± 0.3 ^d
HO	7.2 ± 0.3 ^a	5.1 ± 0.2 ^c	5.8 ± 0.3 ^d
UV-C + HO	5.5 ± 0.5 ^a	4.2 ± 0.4 ^c	5.4 ± 0.4 ^{de}
NEW + HO	6.8 ± 0.4 ^a	4.8 ± 0.4 ^c	5.4 ± 0.1 ^d
NEW + UV-C	5.2 ± 0.4 ^b	5.3 ± 0.2 ^{bc}	3.5 ± 0.2 ^d
NEW + UV-C + HO	5.2 ± 0.4 ^b	3.1 ± 0.3 ^d	3.8 ± 0.1 ^{de}
Palmitic acid			
Control	3.4 ± 0.3 ^a	1.1 ± 0.3 ^c	1.4 ± 0.2 ^b
NaClO	3.1 ± 0.2 ^a	2.3 ± 0.4 ^c	2.0 ± 0.2 ^c
UV-C	2.7 ± 0.4 ^a	2.2 ± 0.3 ^{bc}	2.4 ± 0.2 ^d
NEW	2.2 ± 0.4 ^a	1.5 ± 0.1 ^c	2.0 ± 0.3 ^d
HO	3.1 ± 0.2 ^a	2.4 ± 0.2 ^c	2.7 ± 0.1 ^c
UV-C + HO	2.7 ± 0.4 ^a	2.1 ± 0.3 ^{bc}	2.5 ± 0.1 ^d
NEW + HO	2.2 ± 0.4 ^a	2.5 ± 0.3 ^{bc}	2.8 ± 0.2 ^{de}
NEW + UV-C	2.6 ± 0.2 ^a	2.5 ± 0.3 ^{bc}	1.8 ± 0.0 ^d
NEW + UV-C + HO	2.6 ± 0.2 ^a	1.4 ± 0.5 ^c	1.7 ± 0.2 ^d
Stearic acid			
Control	0.50 ± 0.03 ^a	0.25 ± 0.02 ^{bc}	0.20 ± 0.02 ^c
NaClO	0.50 ± 0.05 ^a	0.48 ± 0.02 ^a	0.37 ± 0.02 ^{cd}
UV-C	0.40 ± 0.06 ^a	0.26 ± 0.02 ^{cd}	0.40 ± 0.05 ^{de}
NEW	0.50 ± 0.02 ^a	0.31 ± 0.03 ^c	0.34 ± 0.02 ^{cd}
HO	0.57 ± 0.03 ^a	0.47 ± 0.04 ^{ab}	0.47 ± 0.02 ^{cd}
UV-C + HO	0.40 ± 0.06 ^a	0.37 ± 0.04 ^{bc}	0.35 ± 0.04 ^{de}
NEW + HO	0.50 ± 0.02 ^a	0.37 ± 0.03 ^c	0.47 ± 0.07 ^{cd}
NEW + UV-C	0.50 ± 0.05 ^a	0.47 ± 0.03 ^{bc}	0.19 ± 0.02 ^d
NEW + UV-C + HO	0.50 ± 0.05 ^a	0.26 ± 0.02 ^{cd}	0.32 ± 0.03 ^{de}

peroxy radicals and hydroperoxide (Hollosoy, 2002; Blokhina et al., 2003). The higher degradation of the unsaturated ALA observed may be found in the three double bonds of its structure, since this type of bond is one of the most light-absorbing groups of lipids. Moreover, the necessary photon energy to break these bonds is available only at wavelengths <254 nm, where the UV-C radiation (200–283 nm) is located (Schaich, 2005).

NEW and NaClO treatments also affected ALA and PA contents, although SA levels remained unchanged after NEW. NEW induced lower ALA reduction (20%) compared to UV-C-including treatments, although PA decrease was higher after NEW (34%) than irradiated samples. The observed FA decreases after NEW and NaClO may be due to their hypochlorous acid and hypochlorite ion contents, which can act as an organic and fat solvent, degrading FAs and transforming them into derived salts and glycerols (Estrela et al., 2002). According to these findings, UV-C radiation induced higher degradation for the unsaturated ALA, although the saturated PA was affected by NEW to a greater extent. On the processing day, the combination of both treatments did not produce a significant additive effect on the studied FAs of the kailan-hybrid.

FAs levels decreased throughout shelf-life, with control samples having the highest decrease of around 60% after 5 days, levels which were maintained until the end of storage. Page et al. (2001) found similar ALA reductions for broccoli heads (var. Italica) stored at 4 °C up to 18 days. Following the control samples, NaClO also showed high FAs decreases during storage of 45 and 29% for ALA and PA, respectively, compared to their levels on the processing day. Among the rest of the treatments, the NEW ones showed the highest reductions ranging around 27–44% and 31–61% for ALA and SA, respectively, compared to their values on the processing day. The UV-C and HO treatments achieved the lowest ALA decreases at 24

Table 4

Total phenolics content and total antioxidant capacity of fresh-cut kailan-hybrid after single and combined sanitising treatments stored up to 19 days at 5 °C ($n = 5 \pm \text{SD}$). Different capital letter among each row denotes significant difference ($p < 0.05$). Different lower case letter within each column denotes significant difference ($p < 0.05$).

	Days at 5 °C		
	Processing day	9	19
Total polyphenols (mg ChAE kg ⁻¹ fw)			
Control		1,305 ± 170 ^C	1,600 ± 167 ^A
NaClO		1,429 ± 87 ^C	1,655 ± 14 ^A
UV-C		1,226 ± 197 ^C	1,584 ± 135 ^A
NEW		1,439 ± 98 ^A	1,387 ± 128 ^{cd}
HO		1,307 ± 166 ^B	1,376 ± 67 ^C
UV-C + HO		1,097 ± 110 ^C	1,075 ± 57 ^C
NEW + HO		1,358 ± 145 ^C	1,451 ± 148 ^A
NEW + UV-C		1,105 ± 18 ^D	1,526 ± 62 ^A
NEW + UV-C + HO		1,144 ± 19 ^D	1,545 ± 124 ^B
Antioxidant capacity (mg AAE kg ⁻¹ fw)			
Control		570 ± 78 ^A	385 ± 17 ^C
NaClO		616 ± 12 ^A	435 ± 39 ^B
UV-C		494 ± 146 ^A	337 ± 39 ^C
NEW		422 ± 268 ^D	509 ± 10 ^A
HO		507 ± 20 ^A	308 ± 15 ^B
UV-C + HO		704 ± 35 ^C	353 ± 27 ^D
NEW + HO		417 ± 16 ^A	430 ± 90 ^A
NEW + UV-C		312 ± 14 ^C	402 ± 30 ^A
NEW + UV-C + HO		298 ± 45 ^B	389 ± 24 ^A

and 20%, respectively, while UV-C + HO did not register significant differences for 19 days at 5 °C.

According to these results, UV-C, HO and UV-C + HO achieved the best ALA and SA retentions, while PA levels only decreased after NaClO and for control samples. These high ALA retentions throughout storage of UV-C-treated samples probably were due to a lipoxygenase inactivation, owing to the application of this low wavelength radiation.

3.7. Total phenolic content

The total phenolic content of kailan-hybrid at harvest was 1415 mg ChAE kg⁻¹ fw for control samples (Table 4), similarly to previous data (Martínez-Hernández et al., 2011). No significant differences among treatments were found on the processing day or throughout the shelf-life. Lemoine et al. (2007) did not find total phenols changes after UV-C irradiation of broccoli (cv. Cicco) or during storage at 4 °C for 14 days. Furthermore, total phenols remained unchanged after AEW and NEW-treated mizuna baby leaves (Tomás-Callejas et al., 2011). Similarly, the use of single HO and combined with UV-C radiation (4.54 kJ m⁻²) had little effect on total phenolics in tatsoi (*Brassica rapa* var. rosularis) baby leaves stored at 5 °C for 11 days (Tomás-Callejas et al., 2012). However, different effects of HO on total phenolic levels may vary depending on the commodity, O₂ partial pressure, storage time and temperature (Zheng et al., 2007).

3.8. Total antioxidant capacity

On the processing day, total antioxidant capacity (TAC) showed the same behaviour as total phenolic content, since the initial values (287 mg AAE kg⁻¹ fw) did not register any marked differences between treatments (Table 4). These initial levels were higher than previous TAC data, analysed by DPPH method and reported by Martínez-Hernández et al. (2011) in the kailan-hybrid. Several factors like preharvest climatic conditions and cultural practices, maturity and harvesting methods could induce a higher initial

vitamin C content (Lee and Kader, 2000), contributing to the higher TAC observed. The registered TAC differences may be also due to the different analytical methods used, since FRAP has been reported to provide higher antioxidant capacity values than DPPH in broccoli (Miglio et al., 2008). The higher TAC obtained by FRAP, compared to DPPH method may be attributed to a difference in the ability of antioxidant compounds in the extracts to quench aqueous peroxyl radicals and to reduce the DPPH free radical and ferric iron (Thaipong et al., 2006).

Throughout storage, TAC increased progressively reaching maximum levels on day 9. The highest TAC increases were reported by UV-C + HO, NaClO and control samples with 2.4-, 2.1- and 2.0-fold, respectively, followed by UV-C and HO with 1.7-fold compared to initial values. However, the rest of NEW-including treatments only showed a TAC enhancement between 1.0- and 1.2-fold compared to initial values. These findings could be explained since NEW may be regarded as a sanitising treatment that attenuated the ROS generation, which has been reported in other fresh-cut products during shelf-life. Generally, TAC dropped after the observed enhancement, recording after 19 days similar values to those on the processing day. This TAC peak may be explained by the contribution of antioxidants other than phenolics, which did not show such behaviour as has been described in the previous section. Similar TAC peaks have been described after single UV-C, HO and NEW treatments in other brassica species (Tomás-Callejas et al., 2011, 2012). TAC and total phenolics results show how the studied sanitised treatments, or their combination, did not alter these health-promoting compounds of the fresh-cut kailan hybrid for a period of 19 days at 5 °C.

4. Conclusions

The treatments studied may be used as potential eco-friendly alternatives to the conventionally used NaClO, since similar quality retention potential was attained. In this way, these alternative treatments allow a final product with high quality and low production costs to be obtained. In some cases, a better natural microflora reduction was achieved when treatments were combined. NEW-washed produce showed a potential shelf-life of 19 days at 5 °C, as NaClO did. In that way, this treatment may be considered as the best alternative to NaClO. However, and with regard to nutritional quality, the combination of treatments registered the highest increases of some antioxidant enzymes and achieved good fatty acid retention. Generally, there were not significant differences among triple and double combinations. This study provides useful information for the fresh-cut industry about the microbicidal effects of treatment combination. Nevertheless, further investigations are required to better optimise conditions, other technologies, etc., in order to preserve the overall quality of this kailan-hybrid and probably that of other horticultural produce.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.postharvbio.2012.09.013>.

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Supplementary data**Table 1**

		DAYS at 5 °C				
		Processing day	5	9	15	19
Mesophilic (log CFU g ⁻¹)						
	Control	2.4 ± 0.1 ^{B_a}	2.0 ± 0.3 ^{C_b}	2.2 ± 0.4 ^{D_a}	3.1 ± 0.1 ^{A_a}	2.8 ± 0.1 ^{E_a}
	NaClO	1.0 ± 0.0 ^{B_b}	1.2 ± 0.3 ^{C_c}	1.5 ± 0.3 ^{A_b}	1.4 ± 0.4 ^{D_b}	1.0 ± 0.0 ^{B_b}
	UV-C	1.2 ± 0.3 ^{B_c}	1.4 ± 0.3 ^{C_d}	1.4 ± 0.1 ^{C_{bc}}	1.8 ± 0.3 ^{A_c}	1.7 ± 0.3 ^{D_c}
	NEW	1.1 ± 0.1 ^{B_{bc}}	1.4 ± 0.3 ^{C_d}	1.4 ± 0.1 ^{C_{bc}}	1.6 ± 0.2 ^{A_d}	1.0 ± 0.0 ^{D_b}
	HO	1.4 ± 0.4 ^{B_d}	5.6 ± 0.2 ^{A_a}	1.9 ± 0.2 ^{C_d}	1.9 ± 0.2 ^{C_c}	1.2 ± 0.3 ^{D_d}
	UV-C+HO	1.2 ± 0.3 ^{B_c}	5.8 ± 0.3 ^{A_e}	1.9 ± 0.3 ^{C_d}	1.1 ± 0.1 ^{D_e}	1.2 ± 0.2 ^{B_d}
	NEW+HO	1.1 ± 0.1 ^{B_{bc}}	5.1 ± 0.3 ^{A_f}	1.4 ± 0.3 ^{C_{be}}	1.1 ± 0.1 ^{B_e}	1.1 ± 0.0 ^{B_{bd}}
	NEW+UV-C	1.1 ± 0.1 ^{B_{bc}}	1.9 ± 0.3 ^{A_g}	1.2 ± 0.2 ^{B_f}	1.2 ± 0.4 ^{C_f}	1.0 ± 0.0 ^{C_b}
	NEW+UV-C+HO	1.1 ± 0.1 ^{B_{bc}}	2.0 ± 0.1 ^{A_b}	1.3 ± 0.2 ^{C_{cef}}	1.2 ± 0.3 ^{B_f}	1.1 ± 0.1 ^{B_b}
Psychrophilic (log CFU g ⁻¹)						
	Control	2.3 ± 0.2 ^{B_a}	2.1 ± 0.4 ^{C_a}	1.8 ± 0.1 ^{D_a}	3.2 ± 0.2 ^{A_a}	4.0 ± 0.2 ^{E_a}
	NaClO	1.0 ± 0.0 ^{B_b}	1.2 ± 0.3 ^{C_b}	1.6 ± 0.4 ^{A_b}	1.6 ± 0.4 ^{A_b}	1.1 ± 0.2 ^{B_{bc}}
	UV-C	1.5 ± 0.2 ^{B_c}	1.5 ± 0.4 ^{B_c}	1.6 ± 0.2 ^{C_b}	2.2 ± 0.2 ^{A_c}	1.1 ± 0.1 ^{D_{bd}}
	NEW	1.0 ± 0.0 ^{B_b}	1.1 ± 0.3 ^{C_{bd}}	1.2 ± 0.1 ^{A_c}	1.0 ± 0.0 ^{D_d}	1.0 ± 0.0 ^{D_b}
	HO	2.4 ± 0.2 ^{B_a}	1.9 ± 0.3 ^{C_e}	1.7 ± 0.4 ^{D_{bd}}	2.5 ± 0.4 ^{A_e}	1.2 ± 0.2 ^{E_{cd}}
	UV-C+HO	1.5 ± 0.2 ^{B_c}	1.2 ± 0.2 ^{C_b}	1.7 ± 0.4 ^{A_{ad}}	1.2 ± 0.3 ^{C_f}	1.0 ± 0.0 ^{D_b}
	NEW+HO	1.0 ± 0.0 ^{B_b}	1.0 ± 0.0 ^{D_d}	1.3 ± 0.0 ^{C_c}	1.1 ± 0.3 ^{D_{df}}	1.5 ± 0.4 ^{A_e}
	NEW+UV-C	1.1 ± 0.2 ^{B_b}	1.1 ± 0.1 ^{B_d}	1.2 ± 0.2 ^{C_c}	2.1 ± 0.4 ^{A_c}	1.8 ± 0.4 ^{D_f}
	NEW+UV-C+HO	1.1 ± 0.2 ^{B_b}	1.0 ± 0.0 ^{C_a}	1.6 ± 0.4 ^{A_b}	1.1 ± 0.2 ^{B_{df}}	1.1 ± 0.1 ^{B_{cd}}
Enterobacteriaceae (log CFU g ⁻¹)						
	Control	ND	ND	1.7 ± 0.5 ^{B_a}	1.7 ± 0.5 ^{B_a}	1.9 ± 0.1 ^{A_a}
	NaClO	ND	ND	ND	ND	ND
	UV-C	ND	ND	ND	ND	ND
	NEW	ND	ND	ND	ND	ND
	HO	ND	5.2 ± 0.2 ^{A_b}	ND	ND	ND
	UV-C+HO	ND	5.5 ± 0.5 ^{A_a}	ND	ND	ND
	NEW+HO	ND	4.9 ± 0.3 ^{A_c}	ND	ND	ND
	NEW+UV-C	ND	ND	ND	ND	ND
	NEW+UV-C+HO	ND	ND	ND	ND	ND
Yeasts and moulds (log CFU g ⁻¹)						
	Control	3.1 ± 0.4 ^{B_a}	2.6 ± 0.3 ^{C_a}	3.0 ± 0.3 ^{B_a}	3.4 ± 0.4 ^{A_a}	3.2 ± 0.6 ^{A_{AB}}
	NaClO	ND	ND	ND	2.1 ± 0.1 ^{A_b}	2.1 ± 0.1 ^{A_b}
	UV-C	2.9 ± 0.3 ^{A_a}	2.6 ± 0.4 ^{B_a}	2.4 ± 0.7 ^{C_b}	2.5 ± 0.3 ^{D_c}	2.3 ± 0.5 ^{C_b}
	NEW	ND	ND	ND	ND	2.4 ± 0.4 ^{A_{bc}}
	HO	ND	2.4 ± 0.3 ^{B_a}	2.7 ± 0.3 ^{A_{ab}}	2.1 ± 0.3 ^{C_b}	2.7 ± 0.4 ^{A_{cd}}
	UV-C+HO	2.9 ± 0.3 ^{A_a}	2.5 ± 0.3 ^{B_a}	2.6 ± 0.4 ^{B_{bc}}	2.6 ± 0.6 ^{B_{bc}}	2.8 ± 0.3 ^{A_{cd}}
	NEW+HO	ND	ND	ND	ND	ND
	NEW+UV-C	ND	ND	ND	ND	ND
	NEW+UV-C+HO	ND	ND	ND	ND	ND

Table 2

Enzymes (units g ⁻¹ protein)	DAYS at 5 °C				
	Processing day	5	9	15	19
APX					
Control	2,028 ± 364 ^{ab}	2,838 ± 26 ^a	1,123 ± 312 ^c	2,016 ± 255 ^a	370 ± 83 ^b
NaOCl	536 ± 66 ^b	3,932 ± 71 ^a	326 ± 66 ^c	651 ± 67 ^b	230 ± 99 ^c
UV-C	356 ± 54 ^b	4,406 ± 65 ^a	795 ± 108 ^c	1,440 ± 85 ^d	461 ± 380 ^b
NEW	204 ± 45 ^b	4,152 ± 152 ^a	111 ± 26 ^c	1,371 ± 115 ^d	424 ± 222 ^e
HO	536 ± 66 ^b	2,887 ± 92 ^a	532 ± 145 ^b	907 ± 99 ^c	312 ± 65 ^d
UV-C+HO	356 ± 54 ^b	6,809 ± 383 ^f	307 ± 59 ^b	1,564 ± 213 ^{cd}	448 ± 106 ^d
NEW+HO	204 ± 55 ^b	5,675 ± 204 ^g	1,358 ± 862 ^c	936 ± 34 ^d	358 ± 72 ^e
NEW+UV-C	863 ± 44 ^b	5,397 ± 510 ^h	610 ± 44 ^c	1,338 ± 249 ^d	472 ± 66 ^e
NEW+UV-C+HO	863 ± 44 ^b	10,785 ± 246 ^a	1,360 ± 38 ^{cd}	1,665 ± 140 ^d	691 ± 39 ^e
GPX					
Control	333 ± 19 ^a	1,867 ± 153 ^c	2,180 ± 146 ^a	104 ± 8 ^b	319 ± 25 ^a
NaClO	325 ± 32 ^a	1,634 ± 73 ^a	458 ± 76 ^c	27.1 ± 4.0 ^b	60.0 ± 34.0 ^b
UV-C	267 ± 24 ^a	2,454 ± 193 ^a	481 ± 15 ^{cd}	143 ± 9 ^{bd}	ND
NEW	287 ± 45 ^a	1,397 ± 71 ^a	1,210 ± 301 ^c	107 ± 13 ^{bd}	ND
HO	325 ± 32 ^a	1,157 ± 115 ^a	559 ± 105 ^c	161 ± 14 ^{bd}	ND
UV-C+HO	267 ± 24 ^a	1,834 ± 375 ^a	1,087 ± 143 ^c	22.7 ± 3.4 ^b	ND
NEW+HO	287 ± 45 ^a	1,551 ± 124 ^c	2,550 ± 278 ^a	204 ± 21 ^{cd}	0.4 ± 0.6 ^c
NEW+UV-C	342 ± 32 ^a	1,473 ± 47 ^{ag}	518 ± 145 ^{cd}	45.9 ± 4.1 ^b	ND
NEW+UV-C+HO	342 ± 32 ^a	1,456 ± 229 ^d	914 ± 158 ^f	246 ± 10 ^{ac}	28.4 ± 9.6 ^c
GR					
Control	624 ± 102 ^b	463 ± 124 ^c	2,022 ± 189 ^b	1,172 ± 120 ^e	4,496 ± 225 ^a
NaClO	643 ± 75 ^{ab}	1,883 ± 488 ^a	1,307 ± 105 ^d	1,316 ± 25 ^a	2,306 ± 33 ^a
UV-C	525 ± 31 ^{abc}	919 ± 194 ^c	1,418 ± 60 ^a	1,020 ± 29 ^d	1,458 ± 139 ^a
NEW	375 ± 21 ^b	999 ± 172 ^{cd}	1,578 ± 21 ^d	1,124 ± 60 ^{bcd}	2,064 ± 213 ^d
HO	643 ± 75 ^{abc}	1,407 ± 144 ^c	1,255 ± 101 ^{de}	1,305 ± 121 ^{de}	3,127 ± 323 ^a
UV-C+HO	525 ± 31 ^{ac}	1,208 ± 160 ^f	1,728 ± 82 ^f	1,189 ± 68 ^{bde}	2,417 ± 49 ^a
NEW+HO	375 ± 21 ^b	1,025 ± 101 ^{cd}	1,595 ± 343 ^d	1,421 ± 129 ^e	2,171 ± 242 ^d
NEW+UV-C	512 ± 16 ^{bc}	1,089 ± 119 ^{df}	1,082 ± 63 ^c	1,035 ± 26 ^{cd}	3,654 ± 513 ^d
NEW+UV-C+HO	512 ± 16 ^{bc}	1,683 ± 670 ^g	2,250 ± 192 ^d	1,346 ± 100 ^{de}	4,235 ± 224 ^a
CAT					
Control	221 ± 13 ^a	317 ± 17 ^a	304 ± 7 ^a	212 ± 16 ^b	214 ± 30 ^b
NaClO	207 ± 19 ^b	206 ± 11 ^b	228 ± 18 ^a	202 ± 23 ^b	233 ± 11 ^a
UV-C	139 ± 15 ^b	189 ± 17 ^c	212 ± 20 ^d	245 ± 26 ^a	156 ± 3 ^e
NEW	175 ± 19 ^b	171 ± 25 ^b	243 ± 29 ^d	222 ± 3 ^{ab}	189 ± 22 ^d
HO	207 ± 19 ^b	143 ± 6 ^c	244 ± 26 ^d	74.2 ± 4.8 ^d	161 ± 3 ^e
UV-C+HO	139 ± 15 ^b	201 ± 41 ^{bc}	176 ± 16 ^f	79.8 ± 13.0 ^d	144 ± 29 ^{ce}
NEW+HO	175 ± 19 ^{da}	155 ± 13 ^c	234 ± 10 ^{bd}	77.3 ± 6.5 ^d	80.3 ± 5.2 ^f
NEW+UV-C	190 ± 15 ^b	148 ± 14 ^c	224 ± 27 ^{bc}	206 ± 7 ^b	192 ± 21 ^d
NEW+UV-C+HO	190 ± 15 ^b	183 ± 22 ^{cd}	181 ± 26 ^a	134 ± 13 ^e	137 ± 6 ^e
SOD					
Control	376 ± 12 ^a	652 ± 24 ^a	377 ± 23 ^b	312 ± 30 ^c	387 ± 45 ^b
NaClO	143 ± 7 ^b	437 ± 13 ^a	367 ± 33 ^c	381 ± 26 ^c	399 ± 35 ^b
UV-C	261 ± 11 ^b	390 ± 57 ^c	364 ± 36 ^d	392 ± 30 ^c	434 ± 20 ^a
NEW	245 ± 11 ^b	279 ± 37 ^d	431 ± 29 ^a	311 ± 43 ^b	310 ± 20 ^c
HO	143 ± 7 ^b	198 ± 33 ^c	272 ± 26 ^d	422 ± 39 ^a	337 ± 8 ^e
UV-C+HO	261 ± 11 ^{cd}	360 ± 2 ^f	338 ± 41 ^e	188 ± 58 ^d	290 ± 12 ^e
NEW+HO	245 ± 11 ^a	169 ± 33 ^b	319 ± 33 ^e	207 ± 25 ^d	209 ± 9 ^f
NEW+UV-C	137 ± 5 ^{bd}	267 ± 6 ^d	528 ± 17 ^a	276 ± 13 ^e	358 ± 12 ^d
NEW+UV-C+HO	137 ± 5 ^{bd}	376 ± 31 ^{cf}	411 ± 32 ^f	246 ± 5 ^f	319 ± 27 ^e

Table 3

Fatty Acids ($\mu\text{mol g}^{-1}$ fw)	DAYS at 5 °C				
	Processing day	5	9	15	19
Linolenic acid					
Control	8.4 ± 0.2^A	3.2 ± 0.4^B	2.4 ± 0.2^C	3.3 ± 0.2^B	3.0 ± 0.2^B
NaClO	7.2 ± 0.3^A	5.5 ± 0.5^B	4.5 ± 0.3^C	3.4 ± 0.2^D	$4.0 \pm 0.2^{E_{cd}}$
UV-C	5.5 ± 0.5^A	3.7 ± 0.2^B	3.0 ± 0.5^C	3.9 ± 0.4^D	4.2 ± 0.1^E
NEW	6.8 ± 0.4^A	3.2 ± 0.3^B	2.4 ± 0.2^C	3.8 ± 0.2^D	3.8 ± 0.3^B
HO	7.2 ± 0.3^A	6.3 ± 0.3^B	5.1 ± 0.2^C	5.8 ± 0.3^D	5.8 ± 0.3^D
UV-C+HO	5.5 ± 0.5^A	5.3 ± 0.3^B	4.2 ± 0.4^C	5.1 ± 0.3^D	5.4 ± 0.4^{AB}
NEW+HO	6.8 ± 0.4^A	6.0 ± 0.2^B	4.8 ± 0.4^C	4.8 ± 0.2^C	5.4 ± 0.1^D
NEW+UV-C	5.2 ± 0.4^B	5.4 ± 0.1^A	5.3 ± 0.2^B	4.2 ± 0.2^C	3.5 ± 0.2^D
NEW+UV-C+HO	5.2 ± 0.4^B	4.9 ± 0.2^B	3.1 ± 0.3^C	3.4 ± 0.2^D	$3.8 \pm 0.1^{E_{de}}$
Palmitic acid					
Control	3.4 ± 0.3^A	1.5 ± 0.2^B	1.1 ± 0.3^C	1.4 ± 0.2^B	1.4 ± 0.2^B
NaClO	3.1 ± 0.2^A	$2.0 \pm 0.2^{B_{cd}}$	2.3 ± 0.4^C	1.9 ± 0.3^B	2.2 ± 0.2^C
UV-C	2.7 ± 0.4^A	2.1 ± 0.4^B	$2.2 \pm 0.3^{B_{cd}}$	$1.9 \pm 0.3^{C_{cd}}$	2.4 ± 0.2^D
NEW	2.2 ± 0.4^A	1.9 ± 0.0^B	1.5 ± 0.1^C	$2.1 \pm 0.2^{D_{cd}}$	2.0 ± 0.3^D
HO	3.1 ± 0.2^A	2.8 ± 0.2^B	2.4 ± 0.2^C	2.4 ± 0.0^C	2.7 ± 0.1^D
UV-C+HO	2.7 ± 0.4^A	2.8 ± 0.4^A	2.1 ± 0.2^C	2.5 ± 0.3^C	$2.5 \pm 0.1^{C_{de}}$
NEW+HO	2.2 ± 0.4^B	2.5 ± 0.3^C	$2.5 \pm 0.3^{C_{ac}}$	2.4 ± 0.1^C	$2.8 \pm 0.2^{A_{de}}$
NEW+UV-C	2.6 ± 0.2^A	2.5 ± 0.4^B	$2.5 \pm 0.3^{B_{ac}}$	2.1 ± 0.2^C	1.8 ± 0.0^D
NEW+UV-C+HO	2.6 ± 0.2^A	2.4 ± 0.1^B	1.4 ± 0.5^C	1.5 ± 0.1^C	1.7 ± 0.2^D
Stearic acid					
Control	0.50 ± 0.03^A	0.26 ± 0.05^B	$0.25 \pm 0.02^{B_{bc}}$	0.22 ± 0.03^C	0.20 ± 0.02^C
NaClO	0.50 ± 0.05^A	$0.47 \pm 0.03^{A_{cd}}$	0.48 ± 0.02^A	$0.28 \pm 0.03^{B_{c}}$	$0.37 \pm 0.02^{C_{cde}}$
UV-C	0.40 ± 0.06^A	0.30 ± 0.05^B	$0.26 \pm 0.02^{C_{bd}}$	$0.28 \pm 0.03^{B_{c}}$	0.40 ± 0.05^A
NEW	0.50 ± 0.02^A	0.35 ± 0.06^B	0.31 ± 0.03^C	$0.26 \pm 0.02^{B_{bc}}$	$0.34 \pm 0.02^{B_{def}}$
HO	0.57 ± 0.03^A	0.45 ± 0.03^B	0.47 ± 0.04^B	0.45 ± 0.01^B	0.47 ± 0.02^B
UV-C+HO	0.40 ± 0.06^A	0.40 ± 0.02^A	0.37 ± 0.04^{AB}	0.36 ± 0.05^B	$0.35 \pm 0.04^{B_{eg}}$
NEW+HO	$0.50 \pm 0.02^{A_{bd}}$	$0.50 \pm 0.05^{A_{ach}}$	0.37 ± 0.03^B	0.38 ± 0.03^B	0.47 ± 0.07^A
NEW+UV-C	$0.50 \pm 0.05^{B_{bd}}$	0.53 ± 0.02^A	0.47 ± 0.03^B	0.35 ± 0.03^C	0.19 ± 0.02^D
NEW+UV-C+HO	$0.50 \pm 0.05^{A_{bd}}$	$0.43 \pm 0.06^{B_{dg}}$	$0.26 \pm 0.02^{C_{cd}}$	0.23 ± 0.05^D	$0.32 \pm 0.03^{E_{fg}}$

Table 4

		DAYS at 5 °C			
	Processing day	5	9	15	19
Total polyphenols					
(mg ChAE kg ⁻¹ fw)					
Control	1,415 ± 166 ^{B_a}	1,575 ± 50 ^{A_a}	1,305 ± 170 ^{C_b}	1,430 ± 66 ^{B_a}	1,600 ± 167 ^{A_a}
NaClO	1,351 ± 124 ^{B_{ab}}	1,308 ± 89 ^{B_b}	1,429 ± 87 ^{C_{ad}}	1,197 ± 74 ^{D_{bc}}	1,655 ± 14 ^{A_{ab}}
UV-C	1,295 ± 77 ^{B_b}	1,442 ± 112 ^{D_c}	1,226 ± 197 ^{C_c}	1,267 ± 58 ^{B_{cde}}	1,584 ± 135 ^{B_b}
NEW	1,298 ± 60 ^{B_b}	1,217 ± 54 ^{C_d}	1,439 ± 98 ^{A_a}	1,305 ± 64 ^{D_{df}}	1,387 ± 128 ^{A_{cd}}
HO	1,351 ± 124 ^{B_{ab}}	1,458 ± 47 ^{A_c}	1,307 ± 166 ^{B_b}	1,244 ± 66 ^{D_bdg}	1,376 ± 67 ^{C_c}
UV-C+HO	1,295 ± 77 ^{B_b}	1,407 ± 104 ^{B_d}	1,097 ± 110 ^{C_e}	1,386 ± 30 ^{A_a}	1,075 ± 57 ^{C_e}
NEW+HO	1,298 ± 60 ^{B_b}	1,299 ± 87 ^{B_b}	1,358 ± 145 ^{C_{bd}}	1,296 ± 82 ^{B_{efg}}	1,451 ± 148 ^{A_d}
NEW+UV-C	1,324 ± 65 ^{B_b}	1,416 ± 55 ^{C_c}	1,105 ± 18 ^{D_e}	1,144 ± 76 ^{D_e}	1,526 ± 62 ^{B_b}
NEW+UV-C+HO	1,324 ± 65 ^{B_b}	1,241 ± 74 ^{C_{bd}}	1,144 ± 19 ^{D_e}	1,425 ± 20 ^{E_a}	1,545 ± 124 ^{B_b}
Antioxidant capacity					
(mg AAE kg ⁻¹ fw)					
Control	287 ± 53 ^{B_b}	387 ± 17 ^{C_b}	570 ± 78 ^{A_b}	238 ± 29 ^{D_b}	385 ± 17 ^{C_b}
NaClO	293 ± 41 ^{B_b}	470 ± 48 ^{C_a}	616 ± 12 ^{A_c}	283 ± 29 ^{B_c}	435 ± 39 ^{D_c}
UV-C	294 ± 45 ^{B_b}	307 ± 54 ^{B_c}	494 ± 146 ^{A_d}	283 ± 38 ^{B_c}	377 ± 39 ^{C_{def}}
NEW	346 ± 87 ^{B_a}	193 ± 26 ^{C_d}	422 ± 268 ^{D_b}	432 ± 24 ^{D_a}	509 ± 10 ^{A_a}
HO	293 ± 41 ^{B_b}	473 ± 45 ^{C_a}	507 ± 20 ^{A_d}	284 ± 27 ^{B_c}	308 ± 15 ^{B_g}
UV-C+HO	294 ± 45 ^{B_b}	738 ± 14 ^{A_e}	704 ± 35 ^{C_a}	374 ± 56 ^{D_d}	353 ± 27 ^{D_d}
NEW+HO	346 ± 87 ^{B_a}	308 ± 22 ^{C_c}	417 ± 16 ^{A_e}	308 ± 30 ^{C_c}	430 ± 90 ^{A_c}
NEW+UV-C	290 ± 44 ^{B_bCD}	270 ± 12 ^{B_f}	312 ± 14 ^{C_f}	298 ± 17 ^{D_c}	402 ± 30 ^{A_{ceh}}
NEW+UV-C+HO	290 ± 44 ^{B_b}	365 ± 13 ^{A_b}	298 ± 45 ^{B_g}	384 ± 96 ^{A_d}	389 ± 24 ^{A_{fh}}

CHAPTER V

Innovative cooking techniques for improving the overall quality of a kailan-hybrid broccoli

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Innovative Cooking Techniques for Improving the Overall Quality of a Kailan-Hybrid Broccoli

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Abstract The microbial, physical, sensory and nutritional quality of boiled (vacuum and conventional), steamed, pressure cooked, *sous vide*, microwaved (*sous vide* and conventional), deep-fried (vacuum and conventional) and grilled kailan-hybrid broccoli (Bimi®) after cooking was studied. *Sous vide* microwaving greatly decreased microbial counts, achieving very low psychrophilic and enterobacteria counts (1.1 and 0.2 log CFU g⁻¹, respectively). Vacuum boiling and *sous vide* reduced the stem broccoli firmness by approximately 54–58 %, reaching a pleasant and moderate softening. *Sous vide*, grilling and steaming induced the lowest stem colour changes. Generally, all cooking treatments showed a good overall sensory quality. The total phenolic

content (1,148 mg CAE kg⁻¹ fw) usually increased after cooking, with microwave and grilled treatments registering the highest increases up to 2-fold. Commonly, the total antioxidant capacity (296.6 mg AAE kg⁻¹ fw) increased after cooking by *sous vide*, microwaving and frying treatments registering the highest increments, by approximately 3.6-fold. Generally, the cooking process reduced the initial vitamin C content, with vacuum and conventional boiling showing the lowest and highest losses with 27 and 62 %, respectively, while vacuum deep frying preserved the initial value (1,737 mg kg⁻¹ fw). As a main conclusion, the studied grilling and vacuum-based cooking treatments resulted in better microbial quality, colour, stem firmness and sensory quality than the remaining ones. This maintained or even improved the total antioxidant content of the new kailan-hybrid broccoli studied.

Keywords *Brassica oleracea* Italica group × Alboglabra group · Vacuum-cooking · Microwave · Grilled · Stem firmness · Health-promoting compounds

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Introduction

Bimi® (also commercially called Tenderstem®, Vellaverde®, Broccolini®, etc.) is a particular natural hybrid between kailan (*Brassica oleracea*, Alboglabra group, also called Chinese broccoli or Chinese kale) and conventional broccoli (*B. oleracea*, Italica group) (Martínez-Hernández et al. 2011). Broccoli is highly rich in bioactive compounds such as phenolics, vitamins, glucosinolates, flavonoids, folates and minerals (Moreno et al. 2006). These antioxidant and anti-inflammatory properties provide preventive benefits against cardiovascular and carcinogenic pathologies (Jeffery et al. 2003; Podsędek 2007; Bidchol et al. 2011). After

minimal processing and cooking treatments, the levels of health-promoting bioactive compounds can be altered (Moreno et al. 2006; Artés et al. 2009; López et al. 2010; Mamatha et al. 2012; Vadivambal and Jayas 2010; Cruz et al. 2011; Szymanek 2011). Among other factors, the nutritional quality changes after cooking have been reported to be due to thermal degradation and leakage in the cooking fluids (Lee and Kader 2000).

Boiling, steaming, pressure cooking, deep frying, baking and microwaving have been the most common conventional cooking methods used. Among conventional cooking methods, microwaving and steaming have been reported to increase flavour and texture in broccoli compared to the boiling method (Warthesen et al. 1984; Sanjuan et al. 2000; Nunn et al. 2006). Broccoli cv. Shogun *sous vide* cooked showed better flavour and a pleasant softness, compared to boiling and steaming treatments (Petersen 1993). However, deep-fried broccoli exhibited the lowest degree of softening compared to boiling and steaming (Miglio et al. 2008). Nevertheless, Johnson and Driskell (1987) and Schnepf and Driskell (1994) reported no differences in the texture scores for broccoli prepared by boiling, boiling-in-bag, steaming and microwaving, probably due to the different cooking times applied. In regard to colour, pressure cooked green leafy vegetables gave inferior colour scores compared to boiled and microwave-cooked samples (Kala and Prakash 2004). Furthermore, microwaving was the best cooking method to maintain the broccoli colour compared to boiling and steaming treatments (Pellegrini et al. 2010). Besides the desired sensory properties reached with cooking methods, these thermal procedures also imply great microbial reductions. In such manner, Sebastiá et al. (2010) reported total plate counts of $2.7 \log \text{CFU g}^{-1}$ in broccoli after *sous vide* treatment.

Conventional cooking methods, such as steaming, boiling, microwaving, stir frying, deep frying and grilling, have been reported to affect bioactive compounds such as phenolic, antioxidants and vitamin C, amongst others, in broccoli and other vegetables (Moreno et al. 2006, 2007; Galgano et al. 2007; López-Berenguer et al. 2007; Podsedek 2007; Rungapamestry et al. 2008; Jiménez-Monreal et al. 2009; Roy et al. 2009; Dini et al. 2010; Wolosiak et al. 2010). Conventional microwaving and pressure-cooking achieved more than 90 % of vitamin C retention in broccoli cv. Italica, while conventional boiling and steaming caused significant losses of 34 and 22 %, respectively (Galgano et al. 2007). On the other hand, Vallejo et al. (2002) reported vitamin C losses around 40 % after microwaving of broccoli cv. Italica, while steaming had minimal effects on vitamin C content. These differences may be found among the different cooking times and temperatures applied during the thermal processing. The nutritional quality after stir frying of vegetables may be considerably influenced by the type of oil used (Moreno et al. 2007).

Vacuum-based cooking treatments such as vacuum boiling, vacuum deep-frying, *sous vide* and *sous vide microwaving* may be innovative combined techniques with high potential application on an industrial scale. Furthermore, they can provide benefits in microbiological, nutritional, physicochemical and sensorial quality compared to conventional cooking methods, since the partial vacuum generated allows cooking at lower temperatures. The low oxygen atmosphere during vacuum-based cooking methods may minimise the enzymatic and non-enzymatic aerobic degradative reactions; these usually occur during conventional cooking procedures and affect the quality of the cooked product. The *sous vide* process is an extension of the conventional cook-chill method that includes vacuum packaging prior to the heat treatment (Church and Parsons 1993). *Sous vide microwaving* treatment, firstly reported in this work, uses microwave heating instead of water in a heating bath as in *sous vide*.

There is scarce information reporting the effects of vacuum-based cooking methods on physical, sensory, microbial and nutritional quality of fruits and vegetables. Stea et al. (2006) did not find differences in percentage folate retention after *sous vide* of broccoli. The highest vitamin C, B₆ and folacin retentions (97–100 %) in broccoli cv. Shogun were achieved after *sous vide*, followed by steaming (83–100 %), and boiling (45–46 %) (Petersen 1993). Da Silva and Moreira (2008) reported 60 % higher anthocyanin content for blue potato chips and 18, 19 and 51 % higher total carotenoids content for beans, mango chips and sweet potato chips, respectively, after vacuum deep-frying. Furthermore, total vitamin C decreased and total phenolics increased for higher vacuum-deep frying time and temperature for pineapple chips (Pérez-Tinoco et al. 2008). The reported nutritional benefits after vacuum-based cooking procedures may be due to the low boiling temperature of water for vacuum boiling or oil for vacuum frying (Shyu et al. 1998) and to the low O₂ cooking atmosphere, which induced the retention of the nutritional compounds (Petersen 1993; Werlein 1998; Stea et al. 2006) and enhanced product colour (Nunes and Moreira 2009). *Sous vide* and *sous vide microwaving* allow the product to be cooked on its own juice, sealing in the flavour and aroma (Petersen 1993). Vacuum frying yields less oil absorption (taste less greasy) and has a higher natural colour and flavour retention (Da Silva and Moreira 2008).

The effects of vacuum boiling, vacuum deep frying and *sous vide microwaving* on the health-promoting compounds and sensory and microbial quality of broccoli have not been studied. The effects of conventional cooking treatments on the kailan-hybrid broccoli have not been reported yet either. Therefore, the main objective of this work was to study the effects of innovative vacuum-based cooking methods (boiling, *sous vide*, deep frying and *sous vide microwaving*) compared to the effects of conventional ones (boiling, steaming, grilling, microwaving, deep frying and pressured

cooking) on the main quality attributes of a new kailan-hybrid broccoli.

Material and Methods

Plant Material

Kailan-hybrid broccoli (*B. oleracea* Italica Group $\times$ Alboglabra Group, cv. Bimi[®]) sized 15–18 cm long was hand-harvested in February from open air commercial cultivation parcels at the Campo de Lorca S.C.L. farm located in the southeast Mediterranean area of Spain (Lorca, Murcia). The broccoli was grown according to integrated pest management cultural practices. Immediately after harvesting, broccoli pieces were crushed, ice pre-cooled and transported by car about 80 km to the Pilot Plant of the Technical University of Cartagena, where it was stored at 1 °C and 90–95 % RH.

Sample Preparation and Cooking Treatments

Minimal processing was accomplished on the following day in a disinfected cold room at 8 °C. Broccoli was carefully inspected, with those pieces free from defects and with a similar visual appearance selected. These pieces were cut in approximately 15-cm-long spears, and small leaves were removed with a sharp knife. Cut pieces were washed with chlorinated water (5 °C; 100 ppm NaClO; pH 6.5) for 2 min and rinsed with tap water (5 °C) for 1 min.

The following nine cooking treatments were applied to the sanitised and drained broccoli pieces: boiling (vacuum and conventional), steaming, pressure cooking, *sous vide*, microwaving (*sous vide* and conventional), deep frying (vacuum and conventional) and grilling. The parameters used for all the cooking processes were selected after testing several temperature/time combinations, according to preliminary works conducted with the assessment of technologists from the Centro Tecnológico Nacional de la Conserva y la Alimentación (Molina de Segura, Murcia, Spain) and professional cooking instructors at the Centro de Cualificación Turística (Murcia, Spain). Raw, sanitised broccoli was used as control. Leakage of bioactive compounds in the cooking water after boiling treatments was analysed. Five treatment series for each cooking method were performed to have independent replicates for each cooking method. The applied cooking treatments were as follows:

- *Conventional boiling*: Broccoli was boiled in an industrial open pot (25 L) with boiling water (100 °C) for 3.5 min at a 5:15 broccoli/water ratio.
- *Vacuum boiling*: Broccoli pieces were placed in a stainless steel basket suspended above the cooking water ($w/v=0.5:5$)

filled into a Gastrovac[®] (International Cooking Concepts, Barcelona, Spain). Subsequently, the Gastrovac device lid was closed and vacuum pressure (−0.9 bar) was applied until reaching boiling point (at 54 °C) of the cooking water. Then, the basket was submerged by an externally actuated hermetic mechanism and vacuum boiled for 7 min.

- *Steaming*: Broccoli pieces were cooked for 5 min with water vapour at 100 °C in an industrial autoclave with the pressure valve opened in order to steam at low pressure (0.2 bar).
- *Pressure cooking*: Broccoli pieces carefully placed in several layers on a stainless steel basket were cooked for 2 min in the same industrial autoclave under 0.1 MPa vapour pressure.
- *Sous vide*: About 175 $\pm$ 5 g broccoli pieces were placed within sterile vacuum polypropylene (PP) bags (20 $\times$ 25 cm) and vacuum-packaged (97 % vacuum) in a vacuum chamber (V821, Sammic, Guipúzcoa, Spain). Vacuum-packaged broccoli pieces were placed in several layers of a stainless steel basket. When the cooking water of the autoclave reached 90 °C, the basket was submerged for 15 min. After *sous vide* cooking, the broccoli was rapidly cooled down in a water-ice bath at 0 °C.
- *Conventional microwaving*: About 130 g broccoli pieces were placed inside the sterile vacuum PP bags and cooked for 2.5 min in an industrial microwave (MWB-17AE, Fagor Industrial S. Coop., Valencia, Spain) at 900 W. Immediately after cooking, PP bags were sealed under a injected N₂ atmosphere by using the above-cited vacuum chamber.
- *Sous vide microwaving*: About 130 g broccoli pieces were vacuum packaged (97 %) in PP bags and cooked in the above described microwave for 2.5 min at 900 W. Cooling was made similarly to in *sous vide* treatment.
- *Grilling*: Broccoli pieces were cooked in an industrial grill (CGF9-120, Fagor Industrial S. Coop., Valencia, Spain) for 3 min at 280–300 °C (1 min per side and an additional minute turning constantly over the cooking plate).
- *Conventional deep frying*: Broccoli pieces (w/v of 0.4:3) were deep fried in extra virgin (0.2° acidity) olive oil (Oro Líquido, Coato, Totana, Spain) at 110 °C for 3 min in the Gastrovac device with the lid opened.
- *Vacuum deep frying*: Broccoli pieces (w/v of 0.4:3) were introduced in a stainless steel basket suspended above the extra virgin olive oil previously filled into the Gastrovac device. Subsequently, the Gastrovac was closed and vacuum pressure (−0.8 bar) applied, boiling the oil at 82 °C. Then, similarly to vacuum boiling, the broccoli pieces were submerged into the boiling oil and vacuum deep fried for 3 min.

Immediately after boiling, deep frying, grilling, steaming and pressure cooking treatments, about 200 g of warm broccoli pieces were placed into sterile PP trays (1 L, 4-cm depth) and vacuum-packaged (-0.5 bar) under N_2 atmosphere, using an automatic packaging device with gas injection (Efaman, Efabind S.L., Murcia, Spain). The packaged cooked broccoli was rapidly cooled down with a water-ice bath ($0\text{ }^{\circ}\text{C}$).

In order to avoid possible recontamination of the product throughout elaboration and packaging steps, good manufacturing practices were applied. After microbiological aliquots were taken, samples of broccoli were frozen with liquid N_2 and stored at $-80\text{ }^{\circ}\text{C}$ until further analysis.

Analysis and Determinations

Microbial Analysis

To determine the mesophilic, psychrophilic, enterobacteria, total anaerobes and yeast and mould growth, standard enumeration methods were used (Tomás-Callejas et al. 2011). Five random samples were taken on each treatment. Samples of 20 g were homogenised in 180 mL of sterile peptone saline solution (pH 7; Scharlau Chemie SA, Barcelona, Spain) for 1 min in a sterile stomacher bag (model 400 Bags 6141, London, UK) using a masticator (Colwort Stomacher 400 Lab, Seward Medical, London, UK). For the enumeration of each microbial group, 10-fold dilution series were prepared in 9 mL of sterile peptone saline solution. Mesophilic, enterobacteria, psychrotrophic and anaerobes were pour plated, and yeast and mould were spread plated. The following media and incubation conditions were used: plate count modified agar (PCA) (Scharlau Chemie, Barcelona, Spain) for mesophilic and psychrotrophic aerobic bacteria, incubated at $30\text{ }^{\circ}\text{C}$ for 48 h and at $5\text{ }^{\circ}\text{C}$ for 7 days, respectively; violet red bile dextrose agar (Scharlau Chemie, Barcelona, Spain) for enterobacteria, incubated at $37\text{ }^{\circ}\text{C}$ for 48 h; and potato dextrose agar base (Scharlau Chemie, Barcelona, Spain) with oxytetracycline (100 mg L^{-1} ; Sigma Chemical Co., St Louis, MO, USA) for yeasts and moulds, incubated for 3–5 days at $22\text{ }^{\circ}\text{C}$. Total anaerobic bacteria were analysed with PCA and incubated at $30\text{ }^{\circ}\text{C}$ for 48 h (Braconnier et al. 2003) in anaerobic conditions in a 5-L anaerobic jar (Gas-Pak150, BD, New Jersey, USA), devoid of O_2 by means of a commercial O_2 scrubber (Oxoid, Hants, England) and an anaerobic colour indicator (Oxoid, Hants, England). All microbial counts were reported as log colony forming units per gram of product ($\log\text{ CFU g}^{-1}$). Each of the five replicates was analysed by duplicate. The presence of *Salmonella* spp., *Listeria monocytogenes* and generic *Escherichia coli* was

monitored according to the European legislation for this kind of plant products (Regulation EC 1441/2007 2007).

Colour and Firmness Analysis

Floret and stem colour, and stem firmness (shear force) were analysed as quality attributes. Colour was determined using a colorimeter (Minolta CR-300 series, Ramsey, NJ, USA), calibrated with a white reference plate (light source C), 2° observer and 8 mm of viewing aperture. Measurements were recorded using standard tristimulus parameters (L^* , a^* , b^*) of the CIE Lab system on three equidistant points of each replicate. Hue angle (H°) and total colour difference (TCD) were calculated according to equations previously described (Artés et al. 2001; Ren et al. 2006). The three colour readings were averaged for each piece, and the mean measurement of five pieces from each replicate sample was recorded. The stem firmness was determined with a texture analyser (Ibertest eLib-5-W, Madrid, Spain) by the shear force (N) required to penetrate perpendicularly the stem (1-cm distance from the floret base) with a blade (1-mm thickness, 62-mm length) at 15 mm min^{-1} speed. Softening percentage was calculated according to Miglio et al. (2008).

Sensory Evaluation

Sensory analyses were performed according to international standards (ASTM STP 913 1986). The panel consisted of seven assessors (three women/four men, aged 24–64 years) screened for sensory ability (colour vision, odour detection, texture and basic taste) according to Bett (2002), as well as for the ability to communicate sensory descriptions of products (ISO 8586–1 1993). A 5-point hedonic scale was used for colour, odour, texture (chewiness), juiciness, flavour, visual dehydration and overall quality (Table 1).

Cryo-Scanning Electron Microscope

Possible cell damage due to vacuum packaging was studied. The analysis by cryo-scanning electron microscopy (cryo-SEM) of samples with high water content saves time compared to critical point drying technique. Samples were frozen and conditioned in a PolarPrep 2000 Cryo Transfer System (Quorum Technologies, East Sussex, UK), where the temperature was reduced by evacuating from boiling point ($-196\text{ }^{\circ}\text{C}$) to approximately $-210\text{ }^{\circ}\text{C}$, followed by a Au/Pd (5 nm) covering of the frozen sample. The metallic frozen sample was inserted with a matching cylindrical device attached to it and immediately placed on a pre-cooled sample stage. This chamber was attached to a SEM

Table 1 Sensory analysis scale used for the analyses

Attributes	Scores				
	5	4	3 ^a	2	1
Colour	Dark green	Light green	Green-ocher	Ocher	Ocher-brown
Texture (chewiness)	Very soft	Soft	Slightly soft	Slightly firm	Very firm
Juiciness	Very juicy	Juicy	Slightly juicy	Slightly juiceless	Very juiceless
Odor	Excellent	Good	Fair	Slightly unpleasant	Unpleasant
Flavor	Excellent	Good	Fair	Poor	Extremely bad
Visual dehydration	None	Slight	Moderate	Severe	Extreme
Overall quality	Excellent	Good	Fair	Poor	Extremely bad

^aLimit of usability

(S-3500N, Hitachi, Ratingen, Germany). Samples were inspected at 100× and 170× magnification.

Total Phenolic Content

Total phenolic content was analysed as recently described (Martínez-Hernández et al. 2011), based on the Singleton and Rossi (1965) method. The absorbance of samples was measured at 750 nm using a Multiscan plate reader (Tecan Infinite M200, Männedorf, Switzerland). Total phenolic content was expressed in milligrams per kilogram fresh weight (fw) chlorogenic acid equivalent. Each of the five replicates was analysed by triplicate.

Total Antioxidant Capacity

Total antioxidant capacity was determined based on the evaluation of the free radical scavenging capacity according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Brand-Williams et al. 1995), using the method described by Martínez-Hernández et al. (2011). The reducing ability of hydrophilic and lyophilic antioxidants towards DPPH was measured by monitoring the absorbance decrease at 517 nm using the above-cited Multiscan plate reader. The results were expressed in milligrams per kilogram fw ascorbic acid equivalent antioxidant capacity. Each of the five replicates was analysed by triplicate.

Vitamin C Measurements

The ascorbic (AA) and dehydroascorbic (DHA) acids were measured according to the methods of Zapata and Dufour (1992) and Vallejo et al. (2002) with slight modifications. Briefly, ground frozen samples of 5 g were placed into a 25-mL Falcon tube (protected from light with aluminium foil), and 10 mL of 0.1 M citric acid, 0.05 % EDTA, 4 mM sodium fluoride, and 5 % MeOH cold buffer were added. The mixture, placed on ice bed, was homogenised (Ultraturrax T25 basic, IKA, Berlin, Germany) for 10 s. Then, the extract was filtrated through four-layer cheesecloth and pH adjusted to 2.35–2.4 with 6 N NaOH. Afterwards, the extract was purified by solid phase extraction

(Sep-Pak cartridges C18, Waters, Dublin, Ireland) and filtered through a 0.45-µm polyether sulphone filter. DHA derivatisation was carried out mixing 750 µL of vitamin C extract with 250 µL of 7.7 M 1,2-phenylenediamine in a HPLC amber vial. The mixture was allowed to react for 37 min at room temperature. Immediately after derivatisation, 20 µL were injected on a Gemini NX (250 mm×4.6 mm, 5 µm) C18 column (Phenomenex, Torrance CA, USA), using an HPLC (Series 1100 Agilent Technologies, Waldbronn, Germany) equipped with a G1322A degasser, G1311A quaternary pump, G1313A autosampler, G1316A column heater and G1315B photodiode array detector. The HPLC system was controlled by the software ChemStation Agilent, v. 08.03. The mobile phase was 5 mM hexadecyl trimethyl ammonium bromide, 50 mM KH₂PO₄ and 5 % methanol (pH=4.59) with isocratic flow of 1.8 mL min⁻¹. Chromatograms were recorded for 14 min at 261 nm (AA, Rt=6.8 min) and 348 nm (DHA, Rt=3.7 min). AA and DHA were quantified using commercial standards (Sigma, St Louis, MO, USA). Calibration curves were made with at least six data points for each standard. Results were expressed as milligrams per kilogram fw. Each of the five replicates was analysed by triplicate.

Statistical Analysis

The experiment was a one-factor (treatment) design. Data was analysed using comparison among treatments carried *proc ANOVA* function and Duncan's multiple comparisons of means in Statistical Analyses System 9.1 (SAS Institute, Cary, NC, USA). Statistical significance was established when $p < 0.05$. All data were previously evaluated for normality and homogeneity of variance using the *proc univariate* function of SAS.

Results and Discussion

Microbial Analysis

As expected, cooking reduced mesophilic, psychrophilic, enterobacteria and mould and yeast initial counts (3.7, 3.2,

3.1 and 2.9 log CFU g⁻¹, respectively). According to microbial quality, the most effective cooking method was pressure cooking, where no growth was detected and enterobacteria counts were below 1 log CFU g⁻¹ (Table 2). The next effective treatment was *sous vide* microwaving, which recorded 1.1 and 0.2 log CFU g⁻¹ for psychrophilic and enterobacteria, respectively. Conventional microwaving reached lower microbial decreases than *sous vide* microwaving, probably because it was packed before cooking, which reduced a possible microbial cross-contamination, compared to conventional microwaving that was packaged after cooking. Similarly to *sous vide* microwaving, *sous vide* pre-packaging also reduced the microbial counts (Table 2).

In relation to mesophilic growth inhibition, the most effective cooking methods were pressure cooking, *sous vide* microwaving and vacuum deep frying, followed by conventional deep frying, *sous vide*, steaming, conventional microwaving, vacuum boiling, conventional boiling and grilling, with reductions of 93, 90, 88, 75, 62, 55 and 35 %, respectively. Regarding kailan-hybrid broccoli mesophilic counts (0.37 log CFU g⁻¹) cooked under *sous vide* (90 °C for 20 min), Sebastia et al. (2010) reported higher counts (2.69 log CFU g⁻¹) in conventional broccoli florets cooked with a milder *sous vide* (70 °C for 15–20 min). This indicates the importance of an adequate time–temperature combination to ensure microbial safety in *sous vide* cooked products, being this binomial well described by the Code of Practice for *Sous Vide* Advisory Committee (1991).

Sous vide microwaving, steaming and *sous vide* samples reached decreases of 57, 55 and 49 %, respectively, over initial psychrophilic counts. Thus, this great psychrophilic reduction may be good enough for extending the chilled storage period of the cooked kailan hybrid.

High temperature (pressure cooking, grilling and deep frying) and pre-packaging treatments (*sous vide* and *sous vide* microwaving) appeared to be, generally, the most effective methods to reduce initial enterobacteria levels (below 1 log CFU g⁻¹), while the remaining treatments reached lower reductions (around 50 %) compared to initial counts. All cooking treatments greatly reduced initial counts of moulds and yeasts until undetected levels (Table 1), except for conventional boiling, which reached 1 log CFU g⁻¹. Generally, cooking treatments drastically reduced total anaerobes initial counts (2.7 log CFU g⁻¹) until undetected levels (data not shown). *Salmonella* spp., *L. monocytogenes* and generic *E. coli* were not detected for any cooking treatment, neither in control samples, in accordance with the EU safety legislation (Regulation EC 1441/2007 2007).

The present study indicates that cooking under reduced oxygen concentrations reached better microbial reductions when anaerobic conditions were applied to different cooking treatments maintained at the same temperature and time parameters. Increased pressure during cooking greatly dropped microbial levels as previously reported (Arroyo et al. 1999). Furthermore, longer cooking times and higher temperatures achieved better microbial reductions as previously reported in Carlin et al. (2000).

Stem Firmness

The broccoli stem shear force and the softening after cooking are shown in Table 3. Pressure cooking, steaming, and boiling induced the highest decreases with 83, 76 and 71 %, respectively, compared to raw samples (46.1 N). Similarly, Miglio et al. (2008) reported the highest stem softening of conventional broccoli (var. *botrytis caput* L.) after boiling and steaming

Table 2 Mesophilic, psychrophilic, enterobacteria and moulds and yeasts counts (log CFU g⁻¹) of kailan-hybrid broccoli after vacuum-based and conventional cooking treatments

Treatment	Mesophilic	Psychrophilic	Enterobacteria	Yeasts and moulds
Raw	3.7±0.5 ^a	3.2±0.9 ^a	3.1±0.2 ^a	2.9±0.9 ^a
Conventional boiling	1.7±0.9 ^{bc}	1.8±1.1 ^{bc}	0.5±0.7 ^b	0.9±1.2 ^b
Vacuum boiling	1.4±0.1 ^c	0.5±0.6 ^d	0.7±1.0 ^b	ND
Steaming	0.5±0.1 ^d	0.6±0.8 ^d	0.6±0.9 ^b	ND
Pressure cooking	ND	ND	0.3±0.0 ^b	ND
<i>Sous vide</i>	0.4±0.2 ^d	0.7±0.9 ^d	0.2±0.4 ^b	ND
Microwaving	0.9±0.4 ^{cd}	0.4±0.5 ^d	0.7±0.7 ^b	ND
<i>Sous vide</i> -microwaving	ND	1.1±0.7 ^{cd}	0.2±0.4 ^b	ND
Grilling	2.4±0.4 ^b	2.1±0.6 ^b	ND	ND
Conventional deep frying	0.3±0.6 ^d	ND	0.4±0.5 ^b	ND
Vacuum deep frying	ND	0.2±0.4 ^d	0.8±0.7 ^b	ND

Different lower-case letters denote significant difference ($p < 0.05$, $n = 5 \pm \text{SD}$). Different letters within each column denotes significant difference ($p < 0.05$). Values are mean ($n = 5 \pm \text{SD}$)

ND not detected

Table 3 Steam firmness (shear force) (*N*) of kailan-hybrid broccoli after vacuum-based and conventional cooking treatments

Treatment	Stem firmness: shear force (<i>N</i>)	Softening (%)
Raw	46.1±4.9 ^{ab}	—
Conventional boiling	13.3±1.2 ^c	71.1
Vacuum boiling	21.3±2.7 ^d	53.8
Steaming	11.3±1.2 ^{ef}	75.5
Pressure cooking	7.8±1.2 ^f	83.1
<i>Sous vide</i>	19.3±3.5 ^d	58.1
Microwaving	44.0±5.1 ^a	4.6
<i>Sous vide</i> -microwaving	40.0±6.0 ^c	13.2
Grilling	42.0±5.2 ^{bc}	8.9
Conventional deep frying	40.7±4.6 ^c	11.7
Vacuum deep frying	40.0±4.6 ^c	13.2

Different lower-case letters denote significant difference ($p < 0.05$, $n = 5 \pm \text{SD}$). Different letters within each column denote significant difference ($p < 0.05$). Values are mean ($n = 5 \pm \text{SD}$)

with values around 84 and 82 %, respectively. The high temperatures used by pressure cooking and steaming treatments may induce high cell-wall disruption, affecting the stem firmness and softening. Additionally to the high cooking temperature effect over stem firmness, conventional boiling promotes an osmosis-based cell fluids lixiviation resulting in loss of firmness. In fact, Sanjuan et al. (2000) reported that water blanching (100 °C) of conventional broccoli stems (var. Italica Plenck L.) severely affected firmness. However, vacuum boiling and *sous vide* samples registered intermediate reductions (around 54 and 58 %) compared to raw samples. The low cooking temperatures of these two vacuum treatments avoided severe cell wall disruption. Furthermore, the mild temperature of vacuum boiling could activate the pectin methylesterase enzyme (Gormley and Tansey 2012), although the applied binomial temperature/time of *sous vide* could be enough to inactivate this enzyme. Grilling, deep frying (conventional and vacuum) and *sous vide* microwaving registered the lowest shear force decreases (around 9–13 % stem softening) over uncooked samples, without differences among them, obtaining a cooked broccoli with a slight and pleasant crispy texture. After microwaving, stem firmness did not register a significant decrease ($p > 0.05$), probably due to the short cooking time applied.

Consequently, kailan hybrid could be cooked according to the stem firmness preference of the consumer, using pressure cooking, steaming and boiling methods for a softer cooked product, or vacuum boiling and *sous vide* for a less soft product. Subsequent cold storage of cooked kailan hybrid affects its firmness due to the cell membranes and walls disruption as it has been observed in previous unreported

experiments in our laboratory (data not shown). According to the hereby reported intermediate stem softening after vacuum boiling and *sous vide*, fifth range industry may use these vacuum-based cooking methods in order to avoid severe softening of the cooked product throughout commercial life.

Colour Measurements

Initial colour of stem/floret were $L^* = 50.8/38.9$ and $H^o = 120.9/127.6$ (Table 4). After deep frying, broccoli registered an L^* increase of 1.0- to 1.3-fold over raw samples, resulting in a lighter green colour. The other treatments reduced L^* values by 38–46 % compared to uncooked samples, which showed darker green colour. Generally, after cooking, H^o increased in all treatments (from 0.91- to 1.04-fold), except for *sous vide* and grilling that showed H^o reductions around 13–18 % and 16–19 %, respectively, for stem floret. This is in accordance with the significant differences found in colour scores of the sensory analysis (Table 5). Florets registered 1.0- to 1.4-fold higher TCD values than stems, which show the high sensitivity of the kailan-hybrid florets to thermal treatments. Conventional and vacuum deep frying samples showed a high stem TCD of 42.6 and 38.6, respectively, achieving vacuum deep frying better colour retention than conventional deep-frying treatment. This result agrees with that found in vacuum deep-fried sweet potato, green bean, mango and blue potato (Da Silva and Moreira 2008), where panellists overwhelmingly preferred these vacuum deep-fried products instead of the conventional deep frying method. Contrary to this, *sous vide*, grilling and steaming induced the lowest TCD with stem values of 27.0, 26.4 and 28.8, respectively, while the remaining treatments registered stem TCD values ranging from 31.2 to 36.2. The samples directly vacuum-cooked (boiling and deep frying) achieved significant lower colour differences compared to the corresponding conventional treatments. This finding highlights the benefits of applying vacuum cooking in order to obtain better colour properties. Generally, deep-frying treatments achieved poor colour retention, while the rest of the treatments produced good colour retention with better results for pressure cooking, steaming, boiling and grilling treatments.

Sensory Analysis

Colour, odour, texture, juiciness, flavour, visual dehydration and overall visual appearance sensory scores are shown in Table 5. Attending to the colour scores, panellists observed a lighter green colour on the vacuum-based treatments *sous vide*, and vacuum deep frying, and for conventional deep fried product, while conventional boiling, grilling and microwaving enhanced the raw broccoli green colour. According to stem shear force data (Table 3), cooking treatments induced stem softening, improving chewiness,

Table 4 Colour parameters (L , H° and TCD) of kailan-hybrid broccoli after vacuum-based and conventional cooking treatments

	Stem				Floret			
	a	L	H°	TCD	a	L	H°	TCD
Raw	-19.9±0.6 ^{bc}	50.8±1.7 ^c	120.9±0.6 ^d	—	-12.5±1.0 ^e	38.9±1.9 ^b	127.6±1.6 ^d	—
Conventional boiling	-17.9±1.5 ^{cd}	40.3±1.6 ^g	122.8±0.9 ^{cd}	32.5±2.0 ^{cd}	-17.1±1.0 ^{bc}	28.8±2.2 ^d	127.3±1.4 ^{cd}	44.5±0.3 ^{bc}
Vacuum boiling	-16.9±1.3 ^d	46.0±0.9 ^d	126.0±1.6 ^b	31.2±1.9 ^{de}	-15.0±1.2 ^d	32.6±2.3 ^c	127.5±1.6 ^b	41.8±0.3 ^d
Steaming	-13.7±0.5 ^e	40.8±1.0 ^{fg}	116.1±1.9 ^e	28.8±0.9 ^{ef}	-9.9±1.5 ^f	28.8±2.3 ^d	113.5±1.4 ^e	38.7±0.9 ^e
Pressure cooking	-16.0±2.0 ^d	43.±2.0 ^e	116.7±1.5 ^e	32.8±3.5 ^{cd}	-9.8±1.2 ^f	25.7±3.2 ^e	116.5±2.6 ^e	41.4±2.5 ^d
<i>Sous vide</i>	-8.8±0.8 ^f	43.7±3.1 ^e	105.3±1.0 ^f	27.0±1.8 ^f	-5.1±0.4 ^h	30.3±0.9 ^d	104.9±0.8 ^f	35.4±0.6 ^f
Microwaving	-20.6±2.4 ^{ab}	38.7±0.7 ^{gh}	123.6±2.8 ^{bc}	36.2±2.5 ^b	-15.9±1.8 ^{cd}	20.4±1.2 ^f	120.3±2.7 ^{bc}	47.4±1.1 ^a
<i>Sous vide</i> -microwaving	-19.4±2.0 ^{bc}	37.9±2.2 ^h	121.8±3.5 ^{cd}	35.3±1.8 ^{bc}	-12.7±2.0 ^e	22.4±1.2 ^f	131.4±2.9 ^{cd}	46.4±1.4 ^{ab}
Grilling	-6.9±1.4 ^f	42.7±1.6 ^{fe}	101.6±2.1 ^g	26.4±1.7 ^f	-7.4±2.0 ^g	26.4±2.1 ^e	102.9±2.9 ^g	36.8±0.9 ^{ef}
Conventional deep frying	-22.2±1.7 ^a	58.2±1.1 ^b	128.7±1.6 ^a	41.4±2.6 ^a	-20.1±1.6 ^a	40.2±2.7 ^b	130.4±1.6 ^a	42.6±0.8 ^{cd}
Vacuum deep frying	-19.4±1.5 ^{bc}	62.0±1.2 ^a	124.4±1.6 ^{bc}	41.3±2.6 ^a	-17.8±1.4 ^b	50.8±3.0 ^a	124.7±1.6 ^{bc}	38.6±0.9 ^e

Different lower-case letters denote significant difference ($p < 0.05$, $n = 5 \pm \text{SD}$). Different letters within each column denotes significant difference ($p < 0.05$). Values are mean ($n = 5 \pm \text{SD}$)

especially conventional boiling, pressure cooking, steaming and *sous vide*, which were scored around 4.5. According to our results, Petersen (1993) reported that conventional broccoli var. Shogun was scored softer after *sous vide* compared to boiling treatment. Werlein (1998) also reported better texture scores in carrots type ‘Calenberger Feldfrisch’ (grade 2) after *sous vide* treatment (98 °C for 20 min) compared to conventional boiling (12 min). *Sous vide*, *sous vide* microwaving and pressure cooking induced higher juiciness, probably due to the protecting effect of *sous vide* packaging, which kept all the broccoli juice (Vlok 1998). Pressure-cooked samples turned juicier due to the relatively high pressure atmosphere, which kept a high humidity favoured by the cooking vapour. According to flavour, no great

differences among treatments were detected, being pleasant for all panellists. Conventional boiling and grilling reached the lowest scores (3.8 and 4.0, respectively). High visual dehydration was detected only for grilled samples, owing to the high cooking temperature combined with the cell injuries produced by turning the product on the grill plate during cooking, hence enhancing evaporation and lixiviation of plant cells fluids with the consequent dehydration. Contrary to grilled samples, a moderate visual dehydration was detected in conventional boiling, microwaving and deep frying treatments due to the high cooking temperatures and, particularly for conventional boiling, the plant fluids leaching to the cooking water. Nevertheless, no visual dehydration was detected for vacuum-based treatments. All

Table 5 Sensory scores for colour, odour, texture (chewiness), juiciness, flavour, visual dehydration and overall quality of kailan-hybrid broccoli after vacuum-based and conventional cooking treatments

Treatment	Colour	Odour	Texture (chewiness)	Juiciness	Flavour	Visual dehydration	Overall quality
Raw	4.1±0.7 ^b	5.0±0.0 ^a	2.5±0.7 ^c	4.0±0.0 ^{cd}	5.0±0.0 ^a	5.0±0.0 ^a	5.0±0.0 ^a
Conventional boiling	4.8±0.5 ^a	5.0±0.0 ^a	4.1±0.4 ^b	4.1±0.5 ^{cd}	3.8±0.5 ^c	4.5±0.2 ^b	4.0±0.3 ^d
Vacuum boiling	4.2±0.4 ^b	4.9±0.3 ^a	3.3±0.3 ^c	4.0±0.4 ^{cd}	4.6±0.3 ^{ab}	5.0±0.0 ^a	4.4±0.3 ^{bcd}
Steaming	4.0±0.4 ^b	4.6±0.3 ^b	4.5±0.2 ^{ab}	4.4±0.3 ^{bc}	4.1±0.3 ^{bcd}	5.0±0.0 ^a	4.3±0.4 ^{bcd}
Pressure cooking	4.1±0.3 ^b	5.0±0.0 ^a	5.0±0.0 ^{ab}	4.8±0.3 ^{ab}	4.6±0.5 ^{ab}	5.0±0.0 ^a	4.3±0.3 ^{cd}
<i>Sous vide</i>	3.5±0.4 ^{bc}	5.0±0.0 ^a	4.3±0.5 ^b	5.0±0.0 ^{ab}	4.3±0.3 ^{bcd}	5.0±0.0 ^a	4.1±0.3 ^d
Microwaving	4.5±0.3 ^a	5.0±0.0 ^a	2.6±0.5 ^{de}	3.6±0.5 ^d	4.4±0.1 ^{bcd}	4.3±0.3 ^b	4.6±0.3 ^{bcd}
<i>Sous vide</i> -microwaving	4.0±0.4 ^b	5.0±0.0 ^a	3.1±0.3 ^b	4.7±0.0 ^{ab}	3.9±0.5 ^{de}	5.0±0.3 ^a	4.8±0.3 ^{ab}
Grilling	4.5±0.3 ^a	4.5±0.0 ^b	3.2±0.4 ^{cd}	2.5±0.4 ^f	4.0±0.4 ^{cde}	2.1±0.3 ^c	3.4±0.3 ^e
Conventional deep frying	3.0±0.4 ^c	5.0±0.0 ^a	3.0±0.4 ^{cde}	3.1±0.3 ^c	4.4±0.3 ^{bcd}	4.6±0.3 ^b	4.1±0.3 ^d
Vacuum deep frying	3.6±0.3 ^c	5.0±0.0 ^a	2.8±0.3 ^{cde}	2.6±0.3 ^f	4.5±0.0 ^{abc}	5.0±0.0 ^a	4.4±0.1 ^{bcd}

Different lower-case letters denote significant difference ($p < 0.05$, $n = 5 \pm \text{SD}$). Different letters within each column denote significant difference ($p < 0.05$). Values are mean ($n = 5 \pm \text{SD}$)

cooking treatments, except grilling, showed a very good overall quality, scoring *sous vide* microwaving an excellent overall quality score.

Total Phenolic Contents

Generally, all cooking treatments increased the total phenolic content when compared to raw product (1,148.4 mg CAE kg⁻¹ fw), except for conventional and vacuum boiling, which had a lower content due to water lixiviation (Fig. 1). These nutritional increases after cooking may be found at microscopic levels. The cryo-SEM technique provides corroborative evidence of the thermal produced in cooked vegetables as early reported Young and Schadel (1993). Cooking causes the cell membrane and wall ruptures in the plant tissue, as shown in Fig. 2. Epidermal cells were ruptured (Fig. 2d) due to thermal treatment, when compared to intact epidermal cells of uncooked plant tissue (Fig. 2c). These microstructural ruptures in proximal areas and the pressure differences originated at cellular level by the thermal processing could form great epidermal depressions, as it is observed in the scratch found (Fig. 2b). The increase in total phenols could be primarily due to the above discussed cellular tissue disruption, releasing phytochemicals from the insoluble portion of the broccoli. This increases the pool of phenolics, making them more accessible in the extraction procedure (Harrison and Harris 1985; Schweiggert et al. 2006; Ornelas-Paz et al. 2010). This cellular tissue disruption may be higher as the temperature and cooking time increase (Young and Schadel 1993). Furthermore, the

breakdown of supramolecular structures produced by the thermal treatments releases the phenolic sugar glycosidic bounds, which react better with the Folin–Ciocalteu reagent (Bunea et al. 2008). Cooking can also inactivate the polyphenol oxidase enzyme during heating, leading to the inhibition of possible fast polyphenolics degradation (Chuah et al. 2008).

Microwaved and grilled samples showed the highest total phenolic increases approximately 2-fold higher over raw product values, while the remaining treatments increased up to about 1.8-fold without differences among them. This total phenolic enhancement in microwaved and grilled broccoli may be due to the low cooking time and to temperatures reached in inner plant parts (Vadivambal and Jayas 2010), not as high as the temperatures attained in the case of pressure cooking and boiling treatments. The high cooking temperatures used by deep-frying treatments may greatly induce high cell-wall disruption and consequently a higher phenolics extraction, as it is observed with the high total phenolics content (1.8-fold) of these methods compared to raw levels. Furthermore, the short cooking time of these frying treatments could avoid severe thermal degradation of phenolics compounds. The vacuum applied (97 %) in *sous vide* and *sous vide* microwaving produced cell damages, as it is observed in Fig. 2. This negative pressure forces the lixiviation of phenolic compounds. On the other hand, the small increases induced by steaming could be due to the high temperature of the vapour and to a possible small lixiviation of phenolic compounds in the condensing vapour.

Fig. 1 Total phenolics content of kailan-hybrid broccoli after vacuum-based and conventional cooking treatments. Different lower-case (edible part) and capital letters (cooking fluid) denote significant difference ($p < 0.05$, $n = 5 \pm \text{SD}$)

Fig. 2 Superficial and cross-section cryo-scanning electron microscopy photographs (100 $\times$ and 170 $\times$) of kailan-hybrid broccoli before (a, b) and after (c, d) *sous vide* cooking and packaging. E Epidermis detail; X epidermal scratch; S stoma

The low dehydration observed in broccoli samples (Table 5) was not closely correlated to the total phenolic increases according to data reported by Ormelas-Paz et al. (2010), suggesting that, besides dehydration, other factors could be involved in such increases.

Similar increases of total phenolic content after conventional boiling, steaming and microwaving have been found in conventional broccoli (Yamaguchi et al. 2001; Turkmen et al. 2005). These authors attributed these increases to the liberation of a great amount of antioxidant components, due to thermal destruction of cell walls and subcellular compartments, and/or thermal inactivation of oxidative enzymes. Total phenolic increments after cooking have also been reported in other vegetables such as pepper, squash, green bean, pea, leek, spinach (Turkmen et al. 2005), sweet corn (Dewanto and Wu 2002) and Mexican pepper (Ormelas-Paz et al. 2010). Frying process produced water loss, but using olive oil, the polyphenols and α -tocopherol content increased in potato, green pepper, zucchini and eggplant (Kalogeropoulos et al. 2007).

On the other hand, conventional and vacuum boiling induced a decrease (around 40 and 17 %, respectively) in initial total phenolic content. Such decreases were due to the lixiviation phenomenon and dissolution of the phenolic compounds into the cooking water (Zhang and Hamauzu 2004; Bunea et al. 2008; Chuah et al. 2008; Ormelas-Paz et al. 2010); this boiling water containing around 36 % of the initial phenolic content. The lower temperature (54 °C) used

in the vacuum boiling could be the reason for a lower cell disruption (Fig. 2) and consequently a lower lixiviation rate.

Vitamin C

The initial total vitamin C content (1,736.6 mg kg⁻¹ fw) generally decreased after all cooking methods, except for frying (Fig. 3). Moreno et al. (2007) found that vitamin C of conventional Nubia broccoli did not decrease after stir-frying for 3 min and 3 s in extra virgin olive oil at 125–140 °C. Vitamin C content dropped between 40 and 80 % when using soy, peanut, safflower and refined olive oil. The observed ascorbic acid retention after stir-frying with extra virgin olive oil may be found in the oil quality and composition. These findings prompt future studies on the effects of different stir-frying treatments (different oil temperatures compared with oil quality and composition) on vitamin C composition.

Conventional and vacuum boiling samples registered the highest and the lowest vitamin C reductions by 0.6- and 0.3-fold compared to the uncooked control, registering conventional and vacuum boiling waters 0.1- and 0.2-fold of raw broccoli vitamin C content. However, in terms of AA content, conventional boiling showed 3.7-fold higher amount than vacuum boiling. DHA content of vacuum boiling was 1,014.6 mg kg⁻¹ fw compared to conventional boiling that did not show significant quantities ($p>0.05$; Fig. 3). This high DHA content of vacuum boiling samples was due to

Fig. 3 Vitamin C (ascorbic and dehydroascorbic acids) content of kailan-hybrid broccoli after vacuum-based and conventional cooking treatments. Different lower-case (edible part) and capital letters (cooking fluid) denote significant difference ($p < 0.05$, $n = 5 \pm \text{SD}$) among vitamin C content (ascorbic and dehydroascorbic acid)

the high AA to DHA enzymatic conversion allowed by the low temperature (54 °C) of this treatment and also influenced by the great DHA instability (especially at low acid conditions of broccoli; Munyaka et al. 2010). Meanwhile, the absence of DHA in conventional boiling may be due to two main reasons: the inactivation of the AA- to DHA-catalysing enzymes produced by the high temperature of conventional boiling (Munyaka et al. 2010), and the easy and rapidly undergoing of DHA hydrolytic cleavage to form 2,3-DKG, a compound without antiscorbutic activity (Davey et al. 2000). Grilled broccoli registered a 47 % total vitamin C decrease compared to raw broccoli. The short grilling time probably did not reach very high temperatures at the centre of the broccoli stems allowing the enzymatic conversion of AA to DHA, such as ascorbate oxidase, plus the non-enzymatic conversions (Rojas and Gershenson 1997; Davey et al. 2000; Yamaguchi et al. 2001). The remaining treatments registered reductions from 33 to 40 % compared to initial values, in agreement with reports for conventional broccoli (Petersen 1993; Vallejo et al. 2002; Zhang and Hamauzu 2004; Galgano et al. 2007; López-Berenguer et al. 2007).

Total Antioxidant Capacity

The initial total antioxidant capacity ($296.6 \text{ mg AAE kg}^{-1} \text{ fw}$) increased after cooking in all treatments, except for vacuum boiling (Fig. 4). *Sous vide*, microwaving and frying treatments induced the highest increases with about 3.6-fold, followed by grilling, steaming and pressure cooking with 2.8-fold higher total antioxidant capacity than raw broccoli. This enhancement may be explained by different

ways (Jiménez-Monreal et al. 2009): (1) liberation of high amounts of antioxidant components due to the thermal destruction of cell walls and sub-cellular compartments, (2) production of strong radical-scavenging antioxidants by thermal chemical reaction, (3) suppression of the oxidation capacity of antioxidants by thermal inactivation of oxidative enzymes and/or (4) production of new non-nutrient antioxidants or formation of novel compounds such as Maillard reaction products with antioxidant activity.

An increase in antioxidant capacity of 21 % over raw broccoli values after conventional boiling and microwaving (600 W for 300 s) using a product weight/cooking water volume ratio of 1:20 for both treatments has been found (Zhang and Hamauzu 2004). Similar increases have also been reported after conventional boiling, steaming or microwaving of celery, pepper, squash, green bean, leek and asparagus (Turkmen et al. 2005; Sun et al. 2007; Jiménez-Monreal, et al. 2009). The high increase after *sous vide* cooking found in the kailan-hybrid broccoli may be partially favoured by the better retention due to the mild temperature used and the applied vacuum within packages that drastically reduced the O_2 levels. These would help the degradation of antioxidant compounds as phenolics and vitamin C (Ahn et al. 2005). The better bioactive compound retention in microwaved samples (Yamaguchi et al. 2001) and the short cooking time used for them allowed to reach the high antioxidant capacity found here. Furthermore, the high total antioxidant capacity in fried samples may be due to the low lexiviation into the cooking oil of hydrophilic compounds with antioxidant activity, such as vitamin C. The high temperatures reached for steaming and pressure cooking treatments influenced the lower antioxidant capacity increases registered in these samples.

Fig. 4 Total antioxidant capacity of kailan-hybrid broccoli after vacuum-based and conventional cooking treatments. Different lower-case (edible part) and capital letters (cooking fluid) denote significant difference ($p < 0.05$, $n = 5 \pm$ SD)

Among boiling treatments, the conventional boiling showed the lowest antioxidant capacity increase by 2.3-fold and registering the cooking water $14.2 \text{ mg AAE } 100 \text{ g}^{-1} \text{ fw}$ due to the hydrophilic antioxidant compounds lixiviated. Vacuum boiling showed a 23 % decrease below raw product content. The applied vacuum for this treatment highly forced the lixiviation of antioxidant compounds, registering the boiling water 1.3-fold total antioxidant capacity over the raw broccoli content.

The antioxidant capacity enhancement after the cooking of many vegetables has been correlated to phenolics content increase (Dewanto and Wu 2002; Dewanto et al. 2002;

Kurilich et al. 2002; Dini et al. 2010). However, this interpretation does not consider others factors such as (1) the phenolic profiles differences, which can be qualitative (type of phenolics present) and quantitative (relative amounts of phenolics); (2) genotype; (3) maturity stage; and/or (4) postharvest storage conditions (Jacobo-Velázquez and Cisneros-Zevallos 2009). Alternatively, other studies showed an increase in the total antioxidant activity with a lesser or unchanged total phenolics content (Dewanto et al. 2002; Roy et al. 2009).

This work showed a total antioxidant capacity better correlated to total phenolics ($R^2 = 0.80$) than to vitamin C content ($R^2 = 0.53$) in kailan hybrid (Fig. 5).

Fig. 5 Correlations between total phenolics content and total antioxidant capacity of kailan-hybrid broccoli after vacuum-based and conventional cooking treatments

Conclusions

Vacuum-based cooking treatments generally induced higher microbial reductions (mainly *sous vide* microwaving) on the new kailan-hybrid broccoli compared to conventional cooking. Moreover, vacuum-based cooking showed better sensory scores (mainly *sous vide* microwaving) and allowed to reach a moderate softening of the broccoli stem accompanied by a pleasant juiciness with a slight and nice crispness. Cooking treatments, in general, increased the total phenolic content and the correlated total antioxidant capacity, showing that microwaved samples have the highest increments. However, there was not an upward trend related to increased total phenolic content and antioxidant capacity among conventional and vacuum-based treatments. In contrast, the total vitamin C content after cooking decreased, showing that vacuum boiling has the best retention, compared to conventional boiling which showed the lowest content. As a main conclusion, the studied vacuum-based cooking treatments generally showed better microbial, physical and sensory quality, preserving, or even improving, antioxidant content compared to conventional methods. On the other hand, grilling was one of the most effective methods to avoid undesirable changes in stem firmness, enhancing green colour, increasing total phenolics content and antioxidant capacity, and reducing enterobacteria counts, even though a high visual dehydration was reached.

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CHAPTER VI

Quality changes after vacuum-based and conventional industrial cooking of kailan-hybrid broccoli throughout retail cold storage.

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ABSTRACT

The microbial, physical, sensory and nutritional quality changes of kailan-hybrid broccoli after industrial boiling, steaming, *sous vide* (SV), microwaving (MW), SV–MW and grilling throughout 45 days at 4 °C were studied. Boiling, SV–MW and MW induced the highest total colour differences. Boiling and steaming produced the greatest stem softening. Based on the overall sensory quality, the commercial life was established in 45 days, except grilling (14 days) and SV (21 days). Apparently, cooking increased the total phenolic content up to 2.0 and 1.7-fold for grilling and MW, respectively, owing to a better extraction. SV–MW, SV and MW produced the highest total antioxidant capacity increases around 5.4–4.7-fold, contrary to the low enhancements of boiling and grilling (2.9-fold). The best chlorophylls retention was attained by boiling. The total carotenoids content was enhanced up to 1.5–2-fold. Conclusively, these treatments generally showed an excellent microbial reduction and nutritional quality, which, in some cases, was enhanced after 45 days.

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1. Introduction

Broccoli is a health-promoting horticultural produce highly rich in phenolics, carotenoids (principally lutein and β -carotene), glucosinolates (largely glucoraphanin), folates and vitamins (C, A, E, etc.), among other compounds (Podszędek, 2007). Bimi® is a new kailan-hybrid broccoli composed by a long slender stem and florets, which has a mild sweeter taste and it is completely eatable (Martínez-Hernández, Artés-Hernández, Gómez, & Artés, 2012). The degree of greenness, due to chlorophyll pigments, is one of the major quality indicators for vegetable products (Turkmen, Poyrazoglu, Sari, & Velioglu, 2006). Cooking of such Brassica vegetables is necessary prior to its consumption, which implies nutritional, sensory and physicochemical changes (Patras, Tiwari, & Brunton, 2011). The heating process implied by cooking reduces the potentially harmful microbial populations, depending on the vegetable type, temperature and treatment time. Physical properties of vegetables, mainly texture and colour, are also greatly influenced by heat treatments. Domestic cooking methods have

been reported to increase the initial total phenolic content and antioxidant capacity of kailan-hybrid and conventional broccoli in a range of 1.2–2.0-fold and 1.2–3.6-fold, respectively, probably because the extractable part of these molecules was enhanced by a partial destructure of the vegetable tissues when cooking (Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008; Turkmen, Ferda, & Velioglu, 2005; Zhang & Hamaizu, 2004). Similarly, total carotenoids content enhancement after cooking of Brassica vegetables has been reported, as previously reviewed Podszędek (2007), showing boiled broccoli and green cabbage an increase of 31% of total carotenoids.

All previous studies of cooked Brassica vegetables refer to domestic cooking, which implies a limited shelf life of a few days in the domestic refrigerator. However, industrial cooking techniques imply an automated cooking procedure, which allows producing high quantities of product in aseptic conditions in order to achieve a long retail cold storage of several weeks.

However, no studies on the nutritional profile, microbial, sensory and physical changes in broccoli after industrial cooking methods, or their evolution throughout the subsequent retail cold storage, have been reported yet. Aspects like the microbial and sensory quality, colour, stem shear force, total phenolics, total antioxidant capacity, chlorophylls and total carotenoids of the new kailan-hybrid broccoli after seven industrial cooking methods and throughout 45 days of retail cold storage are reported here.

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2. Material and methods

2.1. Plant material

Bimi® (*Brassica oleracea* Italica Group × Alboglabra Group, cv.) of 15–18 cm long (Martínez-Hernández et al., 2012) was hand-harvested in March in open air cultivation parcels of the Campo de Lorca S.C.L. (Lorca, Murcia, Spain). Plant material was grown according to integrated pest management cultural practices. Immediately after harvesting, kailan-hybrid was crushed ice pre-cooled and transported by car about 90 km to the Pilot Plant of the Universidad Politécnica de Cartagena, where it was stored at 1 °C and 90–95% RH.

2.2. Sample preparation and cooking treatments

Plant material was inspected in a disinfected cold room (8 °C). The sound pieces were cut in approximately 15-cm-long spears. Kailan-hybrid was subsequently sanitised with chlorinated water (2 min; 5 °C; 100 mg L⁻¹ NaClO; pH 6.5), and rinsed with tap water (1 min; 5 °C). The following seven industrial cooking treatments were applied to the sanitised plant material (hereinafter called 'raw'):

- Boiling: Kailan-hybrid was boiled in an open pot (25 L capacity) with boiling water (100 °C) for 3.5 min. The broccoli weight/cooking water volume rate (w/v) was 5:15.
- Low pressure (LP) steaming: Plant material was cooked (5 min) with water vapour at 100 °C in an industrial autoclave with the pressure valve opened (0.02 MPa).
- High pressure (HP) steaming: Kailan-hybrid was placed on several layers of a steel stainless basket and steam cooked (2 min) in the industrial autoclave (0.1 MPa pressure).
- SV: Around 175 ± 5 g kailan-hybrid was placed within sterile vacuum polypropylene (PP) bags (20 × 25 cm) and vacuum-packaged (−98.2 kPa) with a vacuum chamber (V821, Sammic, Guipúzcoa, Spain). After packaging, vacuum-packaged kailan-hybrid was properly placed on different layers of a stainless steel basket. When autoclave cooking water reached 90 °C, the basket was submerged for 15 min. After cooking, the SV kailan-hybrid was rapidly cooled down in a water-ice bath (0 °C).
- MW: Sterile PP vacuum bags with 130 ± 5 g kailan-hybrid were placed in an industrial microwave (MWB-17AE, Fagor Industrial S.Coop., Valencia, Spain) and treated (900 W) for 2.5 min. Bags were sealed under N₂ atmosphere using the above-mentioned vacuum chamber.
- SV–MW: About 130 ± 5 g plant material was vacuum-packaged (−98.2 kPa) in PP bags and microwaved (900 W) for 2.5 min. Cooling was similar to SV.
- Grilling: Samples were grilled in an industrial grill (CGF9-120, Fagor Industrial, Valencia, Spain) for 3 min at 280–300 °C (1 min per side and an additional minute turning constantly over the cooking plate).

The parameters used for all the cooking processes were selected after preliminary studies testing several temperature/time combinations. After boiling, the solubilised bioactive compounds of the cooking water were analysed. Immediately after boiling, grilling and steaming treatments, about 200 g of warm kailan-hybrid were placed into sterile PP trays (1 L, 4-cm depth) and vacuum-packaged (−50 kPa) under 100 kPa N₂ atmosphere, using an automatic packaging device with gas injection (Efaman, Efabind S.L., Murcia, Spain). The packaged product was rapidly cooled down (0 °C) into a water-ice bath. In order to have independent replicates, five

treatment series were performed for each cooking method. Good manufacturing practices were applied throughout processing. Other samples were frozen with liquid N₂ and stored (−80 °C) until further analysis (n = 5).

2.3. Analysis and determinations

2.3.1. Microbial analysis

To determine the mesophilic, psychrophilic, enterobacteria, total anaerobes and yeast and mould growth, standard enumeration methods were used (Martínez-Hernández et al., 2012). All microbial counts were reported as log₁₀ colony forming units per gram of product (log CFU g⁻¹). Each of the five replicates was analysed by duplicate.

2.3.2. Physical quality analysis

Florets and stem colour was analysed using a Minolta colorimeter (CR-300 series, Ramsey, NJ, USA). *a**, *b**, Hue angle (*H*°) and total colour difference (TCD) (Ren, Tu, Pan, & Chen, 2006) were monitored. Readings were averaged for each piece, and the mean of five pieces from each sample was calculated. The stem firmness was determined with a texture analyser (Ibortest eLib-5-W, Madrid, Spain) by the maximum shear force (N) required to penetrate perpendicularly the stem (1 cm-distance from the floret base) with a blade (1-mm thickness, 62-mm length) at 15 mm min⁻¹ speed. Softening percentage was calculated according to Miglio et al. (2008).

2.3.3. Sensory evaluation

Sensory analyses were performed according to Martínez-Hernández et al. (2012). The panel consisted of seven assessors (three females/four males, aged from 24 to 64 years). A 5-point hedonic scale was scored for off-colours, off-odours and visual dehydration (1: none; 2: slight; 3: moderate, limit of usability; 4: severe and 5: extreme), stem softening (5: very soft, 4: soft, 3: slightly soft, 2: slightly firm and 1: very firm), and for flavour and overall quality (5: excellent, 4: good, 3: fair, limit of usability, 2: poor and 1: extremely bad).

2.3.4. Total phenolic content

Total phenolic content was analysed as described (Martínez-Hernández, Gómez, Pradas, Artés, & Artés-Hernández, 2011), based on the Singleton and Rossi (1965) method. The absorbance of samples was measured using a Multiscan plate reader (Tecan Infinite M200, Männedorf, Switzerland). Total phenolic content was expressed as mg chlorogenic acid equivalent kg⁻¹ fresh weight (fw). Each of the five replicates was analysed by triplicate.

2.3.5. Total antioxidant capacity

The total antioxidant capacity was determined by the free radical scavenging capacity (DPPH) (Brand-Williams, Cuvelier, & Berset, 1995), with some modifications previously reported by Martínez-Hernández et al. (2011), and by the ferric reducing antioxidant power (FRAP) (Benzie & Strain, 1999). The absorbance of samples was measured using the same device as for total phenolic content. Results were expressed as mg ascorbic acid equivalent antioxidant capacity kg⁻¹ fw. Each of the five replicates was analysed by triplicate.

2.3.6. Total chlorophylls and carotenoids content

Sample preparation for chlorophylls determination was conducted according to Martínez-Hernández et al. (2011), using a UV–visible spectrophotometer (8453, Hewlett Packard, Columbia, USA). The equations developed by Wellburn (1994) were used. Chlorophylls and total carotenoids contents were expressed as mg kg⁻¹ fw. Each of the five replicates was analysed by triplicate.

2.4. Statistical analysis

The experiment was an 8×6 two-factor (cooking treatments $\times$ storage time) design. Data was subjected to analysis of variance (ANOVA) using Statgraphics Plus (version 5.1). Mean values were subjected to the least significant difference test (LSD) at $P < 0.05$.

3. Results and discussion

3.1. Microbial analysis

The sanitising chlorinated washing dropped the initial mesophilic, psychrophilic, enterobacteria, anaerobes (data not shown) and yeast and mould counts ($5.0, 5.1, 3.7, 2.7$ and $3.0 \log \text{CFU g}^{-1}$, respectively) in $2.6, 1.4, 1.3$ and $1.0 \log$ units for yeasts and moulds, psychrophilic, mesophilic and enterobacteria, respectively (Table 1). Generally, no microbial counts were registered (detection limit of 10CFU g^{-1}) immediately after cooking treatments, except MW and LP steaming with 0.4 and $0.3 \log$ mesophilic CFU g^{-1} , respectively, and $0.3 \log$ psychrophilic CFU g^{-1} for SV–MW. However, grilled samples showed the highest mesophilic and psychrophilic counts with 3.2 and $2.8 \log \text{CFU g}^{-1}$ on the processing day, respectively. The relatively small decrease in grilled samples might be caused by the short cooking time and the great temperature inhomogeneity. The mesophilic and psychrophilic counts of cooked and uncooked samples increased throughout retail storage. SV–MW, MW and boiled samples registered the highest mesophilic and psychrophilic increases with 6.0 – 6.9 and 5.9 – $5.7 \log$, respectively, compared to their initial counts. Intermediate mesophilic and psychrophilic growth rate was achieved by SV, LP steaming and raw samples with 5.1 – 5.3 and 4.0 – 3.4 , respectively, after 45 days at 4°C comparing their initial values. Grilling showed the lowest mesophilic and psychrophilic increases with 1.9 and $2.5 \log$ units, respectively. Enterobacteria counts in cooked samples were not detected after 31 days of storage. Grilled, SV–MW, MW and HP steamed samples reached enterobacteria counts ranging among 1.8 – $3.4 \log \text{CFU g}^{-1}$ after 45 days. Anaerobes and yeast and mould counts (data not shown) of cooked samples were not detected, whereas yeast and mould counts of raw samples increased progressively reaching $4.4 \log \text{CFU g}^{-1}$ after 45 days. *Salmonella* spp., *Listeria monocytogenes* and generic *Escherichia coli* were not detected for any cooking treatment, accomplishing the EU safety legislation (Regulation EC 1441/2007, 2007) throughout storage.

3.2. Physical quality analysis

Initial colour values of floret/stem were $a^* = -12.6/-19.9$ and $H^* = 127.7/120.9$, respectively (Table 2). Grilling produced the highest a^* value increment of stems (71%). SV produced the highest a^* value increase of florets (60%), while boiling registered the highest a^* value decrease (36%). After cooking, any treatment produced significant H^* changes compared to raw samples, except grilling and SV with florets–stems reductions of 11–19% and 13–16%, respectively. Generally, cooked samples did not show significant changes on a^* and H^* values throughout cold storage, while these colour parameters dropped during shelf life for uncooked broccoli around 30% (florets and stems) and 16% (just florets), respectively. Attending to TCD values, MW, SV–MW and boiling produced the highest TCD at the processing day, with levels around 36.7 – 31.6 and 39.7 – 42.0 for florets and stems, respectively. MW, SV–MW and grilled samples registered the lowest TCD values after 45 days, being in the range of 17.2 – 18.4 for florets, compared to their values on the processing day. Boiling showed the highest TCD value with 28.3 for florets at the end of commercial life.

Table 1

Mesophilic, psychrophilic, enterobacteria and yeast and mould counts ($\log \text{CFU g}^{-1}$) of kailan-hybrid broccoli after cooking treatments and throughout storage up to 45 days at 4°C . Data represent means of five replicates ($n = 5 \pm \text{SD}$).

Microbial group	Days						
	0	7	14	21	31	38	45
Mesophilic^a							
Unsanitized raw	5.0	5.8	6.6	7.2	7.8	7.9	9.3
Raw	3.7	4.1	5.0	7.3	7.4	7.7	8.3
Boiling	ND	2.7	3.3	4.2	4.5	5.5	6.6
LP steaming	0.3	2.1	1.8	2.4	2.9	2.9	5.6
HP steaming	ND	2.1	2.1	2.5	3.4	3.5	4.4
Sous vide	ND	1.6	2.1	2.7	3.8	3.8	5.1
Microwaving	0.4	2.5	2.3	3.3	5.2	5.1	6.4
Sous vide–MW	0.0	1.9	2.6	4.9	6.3	6.2	6.9
Grilling	3.2	2.5	2.1	3.2	4.3	4.8	5.1
Psychrophilic^b							
Unsanitized raw	5.1	5.8	6.6	7.2	7.6	7.9	8.3
Raw	3.7	3.7	5.4	7.4	7.5	7.7	8.2
Boiling	ND	ND	0.9	2.1	3.7	4.9	5.9
LP steaming	ND	ND	ND	1.1	0.5	0.5	3.4
HP steaming	ND	ND	ND	ND	0.6	1.0	2.2
Sous vide	ND	ND	ND	ND	0.8	1.2	4.0
Microwaving	ND	0.3	1.0	1.5	4.2	4.2	5.7
Sous vide–MW	0.3	1.1	2.4	3.2	4.0	4.6	6.1
Grilling	2.8	1.3	1.6	3.1	4.1	5.1	5.3
Enterobacteria^c							
Unsanitized raw	3.7	4.5	5.3	5.5	4.6	5.2	4.3
Raw	2.7	3.5	4.9	5.9	6.4	6.7	6.6
Boiling	ND	ND	ND	ND	1.2	0.2	0.3
LP steaming	ND	ND	ND	ND	ND	0.5	0.8
HP steaming	ND	ND	ND	ND	ND	ND	3.4
Sous vide	ND	ND	ND	ND	ND	0.2	0.7
Microwaving	ND	ND	ND	ND	ND	ND	1.9
Sous vide–MW	ND	ND	ND	ND	ND	ND	2.0
Grilling	ND	ND	ND	ND	ND	ND	1.8
Yeast and mould^d							
Unsanitized raw	3.0	2.9	2.2	4.1	4.3	3.2	4.8
Raw	0.4	0.5	1.3	2.8	3.5	3.8	4.4
Boiling	ND	ND	ND	ND	ND	ND	ND
LP steaming	ND	ND	ND	ND	ND	ND	2.0
HP steaming	ND	ND	ND	ND	ND	ND	ND
Sous vide	ND	ND	ND	ND	ND	ND	ND
Microwaving	ND	ND	ND	ND	ND	ND	ND
Sous vide–MW	ND	ND	ND	ND	ND	ND	2.0
Grilling	ND	ND	ND	ND	ND	ND	ND

^a SE = 0.56, LSD ($P \leq 0.05$) = 1.57.

^b SE = 0.37, LSD ($P \leq 0.05$) = 1.04.

^c SE = 0.11, LSD ($P \leq 0.05$) = 0.32.

^d SE = 0.14, LSD ($P \leq 0.05$) = 0.39.

Meanwhile, stems registered TCD values ranging from 21.7 to 28.5 on day 45.

Steaming and boiling registered the greatest stem softening with 82.5 – 75.3 and 65.7% , respectively, on the processing day compared to initial stem firmness (36.5 N) (Table 3). The high temperature of steaming probably induced a strong cell-wall disruption, affecting the stem structure. In addition to the high temperature, boiling promotes an osmosis-based cell fluids lixivation with the resultant firmness loss. However, SV and SV–MW achieved intermediate decreases on shear force values of approximately 49 and 53% compared to raw samples on the processing day. The low cooking temperature and the vacuum packaging probably avoided severe cell-wall disruption, as it was shown above for boiled and steamed samples. After cooking, grilled and MW samples did not registered significant shear force decreases compared with raw samples, probably due to the short cooking time, obtaining a product with a slight and pleasant crispy texture.

Table 2

Colour measurements (a^* , H^* , TCD) of kailan-hybrid broccoli after industrial cooking treatments and throughout storage up to 45 days at 4 °C. Data represent means of seven replicates ($n = 7 \pm SD$).

		Initial		Day 7		Day 14		Day 21		Day 31		Day 45	
		Floret	Stem	Floret	Stem	Floret	Stem	Floret	Stem	Floret	Stem	Floret	Stem
Raw	a^*	−12.6	−19.9	−11.2	−20.1	−10.9	−19.3	−10.7	−19.2	−9.3	−16.9	−7.0	−17.0
	H^* ^b	127.7	120.9	123.4	122.6	125.7	122.5	121.3	121.8	122.3	124.0	107.0	125.5
Boiling	a	−17.2	−18.3	−18.3	−16.5	−18.9	−16.4	−16.9	−12.9	−17.0	−11.9	−16.8	−9.2
	H^*	127.0	122.8	118.9	117.7	122.7	117.4	118.6	112.1	118.5	110.1	117.5	105.9
	TCD	32.2	39.7	34.5	37.4	34.4	35.8	31.1	31.3	30.7	30.4	28.3	28.1
LP steaming	a	−9.7	−14.4	−9.7	−12.0	−8.2	−10.1	−6.2	−9.1	−5.9	−6.3	−4.6	−5.4
	H^*	113.0	116.5	108.0	110.4	105.0	107.4	101.6	105.1	100.9	101.5	97.4	99.6
	TCD	25.4	35.7	26.0	32.7	26.6	29.5	22.4	28.2	21.5	24.4	20.7	24.1
HP steaming	a	−10.3	−16.0	−10.7	−13.5	−8.8	−11.3	−8.2	−10.6	−7.2	−7.8	−6.4	−6.9
	H^*	116.4	117.1	109.1	112.3	105.9	108.9	103.4	106.8	101.8	102.6	100.0	101.4
	TCD	26.5	36.8	27.1	34.6	26.2	30.7	25.2	30.7	24.7	27.6	24.9	26.3
Sous vide	a	−5.1	−8.3	−5.7	−8.4	−5.0	−7.8	−4.9	−7.3	−4.4	−6.4	−4.5	−6.2
	H^*	104.7	104.9	101.1	103.3	99.7	103.2	100.5	102.3	99.0	100.6	97.8	100.7
	TCD	20.0	29.4	21.0	29.1	20.8	27.2	18.1	26.9	18.4	25.7	21.2	25.3
Microwaving	a	−16.7	−20.2	−12.5	−15.6	−14.6	−16.6	−9.5	−11.2	−11.0	−11.1	−5.4	−8.5
	H^*	120.3	123.1	109.5	116.0	114.2	118.8	105.5	109.1	110.1	109.6	100.9	105.3
	TCD	36.7	42.0	29.6	36.4	32.4	36.0	26.1	29.9	25.2	29.4	17.2	27.5
Sous vide–MW	a	−13.4	−18.3	−13.2	−14.6	−14.6	−18.1	−10.3	−14.1	−6.8	−13.7	−7.6	−9.9
	H^*	127.8	121.6	116.1	116.1	116.4	121.0	108.8	113.5	128.3	114.7	103.6	107.6
	TCD	31.6	40.4	28.5	35.8	30.3	37.4	23.9	32.7	20.4	31.3	18.4	28.5
Grilling	a	−7.7	−5.8	−4.9	−6.1	−3.3	−6.2	−3.0	−5.6	−14.1	−4.0	−2.4	−3.3
	H^*	103.6	101.7	98.0	101.2	95.7	101.3	95.9	100.2	126.9	98.0	94.3	96.8
	TCD	28.7	28.9	24.4	26.5	22.7	25.7	19.5	24.5	26.0	22.0	18.2	21.7

^a SE = 0.6, LSD ($P \leq 0.05$) = 1.6.

^b SE = 2.3, LSD ($P \leq 0.05$) = 6.4.

Throughout commercial life, no significant stem softening changes were registered.

3.3. Sensory evaluation

Generally, off-colours slightly appeared throughout shelf life remaining always within the limit of usability (LU) (Fig. 1). Boiled samples did not show off-colours throughout storage. A similar pattern was observed for off-odours. Nevertheless, raw and grilled samples registered off-odours scores above the LU after 21 and 31 days at 4 °C, respectively. Cooking treatments reduced the initial stem fibrousness except grilling, which increased it up to 2.7-fold on the processing day. The stem fibrousness of grilled samples decreased after 14 days, remaining within the LU until the end of storage. Stem fibrousness of uncooked samples was kept below the

LU after 21 days of cold storage, while the remaining samples scores were within this limit. MW showed the highest stem fibrousness increase after 45 days with 2.6-fold over its values on the processing day. The high stem fibrousness after grilling was in accordance to the moderate visual dehydration detected (Fig. 1), due to the high temperature reached. Similarly to stem fibrousness, visual dehydration of grilled samples decreased throughout storage, probably owed to a softening produced by the cell-wall degradation attributed to the microbial spoilage reported in Table 1. Among the studied cooking treatments, grilled samples registered the lowest flavour score on the processing day, while MW and SV–MW were scored as the best cooking methods. Flavour was slightly lost throughout storage, reaching raw and grilled samples values below the LU after 14 days, and after 21 days for SV treated samples. The remaining treatments preserved flavour scores within the LU during shelf life. MW and SV–MW registered the best overall quality on the processing day. The overall quality dropped throughout commercial life, showing raw samples scores below the LU after 14 days, probably due to the enzymatic reactions and the observed microbial spoilage. Overall quality of grilled samples also dropped below the LU after 21 days, while the remaining treatments were still above that limit after 45 days. According to the aforementioned sensory results, the commercial life of the kailan-hybrid stored at 4 °C was established in 14, 21 and 45 days for grilling, SV and the remaining treatments, respectively.

3.4. Total phenolic content

The total phenolic content of raw samples (1413.9 mg ChAE kg^{−1} fw) generally increased immediately after cooking, except

Table 3

Steam firmness (shear force) (N) of kailan-hybrid broccoli after industrial cooking treatments and throughout storage up to 45 days at 4 °C. Data represent means of five replicates ($n = 5 \pm SD$).

	Initial	Day 7	Day 14	Day 21	Day 31	Day 45
Raw	36.5	47.3	40.8	43.1	48.6	41.0
Boiling	12.5	12.3	13.1	16.3	9.6	12.9
LP steaming	9.0	7.9	9.1	6.6	8.3	10.1
HP steaming	6.4	7.5	6.5	5.3	4.8	5.4
Sous vide	18.7	20.9	20.2	15.0	15.5	20.0
Microwaving	38.9	25.1	24.8	17.6	27.4	43.1
Sous vide–MW	17.1	10.4	13.6	12.1	15.7	12.6
Grilling	40.1	23.8	35.3	26.6	25.8	27.9

SE = 2.1, LSD ($P \leq 0.05$) = 5.8.

Fig. 1. Sensory scores for off-colours (A), off-odours (B), fibrousness (C), visual dehydration (D), flavour (E) and overall quality (F) of kailan-hybrid broccoli after cooking treatments and throughout storage up to 45 days at 4 °C. Dotted line represents the limit of usability (3). (○) Control, (★) boiling, (▼) LP steaming, (▲) HP steaming, (●) sous vide, (■) microwaving, (◆) sous vide-MW and (×) grilling. Data represent means of seven replicates ($n = 7 \pm \text{SD}$).

boiled samples which showed a decrease (Fig. 2). This total phenolic content enhancement could be attributed to the cell membrane and wall disruption produced by the thermal processing, making the pool of phenolics more accessible for extraction. Furthermore, the supra-molecular structures breakdown produced by cooking would release the phenolic sugar glycosidic bounds, which react better with the Folin–Ciocalteu reagent (Bunea et al., 2008). Heating is also able to inactivate the polyphenol oxidase enzyme, leading to the inhibition of possible fast polyphenols degradation (Chuah et al., 2008). Grilling and MW treatments showed the highest total phenolics increments, with 2.0 and 1.7-fold higher than raw levels on the processing day, respectively. Grilling produces burnt tissue areas (desirable aspect for this cooking method), but the formed compounds could react with the Folin–Ciocalteu reagent, erroneously increasing the total phenolic content. The remaining cooking treatments produced lower increases with approximately 1.4–1.5-fold over uncooked samples on the

processing day, without significant differences among them. For HP and LP steaming, the scarce total phenolic enhancement may be owed to the high cooking temperatures and the poor lixiviation, since the condensed cooking vapour did not favour it. In contrast, the low enhancement found in SV samples could be due to the long cooking time, combined with the applied vacuum (−98.2 kPa). This vacuum may produce cell damage, forcing this negative pressure the lixiviation of phenolic compounds. Increases of total phenolic content have been reported in conventional broccoli after boiling, LP steaming and MW (Turkmen et al., 2005). This fact suggests that such increases may be due to other factors besides dehydration. Contrary to the remaining treatments, boiling dropped total phenolic content around 40% on the processing day. This phenolics loss is owed to the lixiviation phenomenon and dissolution of such compounds into the cooking water, as previously reported Zhang and Hamauzu (2004), registering the boiling water 483.8 mg ChAE leached per each kg of cooked kailan-

Fig. 2. Total phenolic content of kailan-hybrid broccoli after cooking treatments and throughout storage up to 45 days at 4 °C. (○) Control, (●) boiling, (▼) LP steaming, (▲) HP steaming, (●) sous vide, (■) microwaving, (◆) sous vide–MW and (×) grilling. Data represent means of five replicates ($n = 5 \pm \text{SD}$).

hybrid. Throughout storage, total phenolic content of cooked samples remained quite stable or registered a slight decrease (Fig. 2). On the other hand, those values of uncooked samples increased during commercial life, reaching after 45 days 2.3-fold higher values compared to the processing day. This phenolic enhancement on raw samples may be explained by the phenolic biosynthesis pathway, after which individual branch makes possible a range of phenylpropanoid secondary compounds as phenolics (Cisneros-Zevallos, 2003). In that pathway, specific enzymes present in the phenylpropanoid metabolism, as phenylalanine ammoniolyase, are involved. They could be thermally inactivated by the hereby-applied cooking methods, avoiding the increase of phenolic compounds by these biosynthesis pathways.

3.5. Total antioxidant capacity

Cooking increased the initial total antioxidant capacity in relation to uncooked samples (Table 4). FRAP values were consistently higher than DPPH values (Table 4), without differences among DPPH values of cooked samples on the processing day. Antioxidant compounds, such as polyphenols, may be more efficient reducing agents for ferric iron, but some may not scavenge the DPPH free radical so efficiently due to steric hindrance (Dini, Tenore, & Dini, 2010). The similar values of DPPH and FRAP of boiled and grilled samples may be owed to their reported high total carotenoids content, since these compounds have a spectra that overlaps DPPH at 515 nm (Dini et al., 2010). On the processing day, the highest total antioxidant content increments (FRAP) were found in SV–MW, SV and MW samples, with increases of 5.4–4.7-fold over uncooked samples. These increments are most likely due to the better antioxidants extraction, favoured by the thermal destruction of cell-wall and sub-cellular compartments for the microwaving method and the long cooking time of SV. The aforementioned antioxidant capacity enhancement agrees with findings in conventional broccoli by Zhang and Hamauzu (2004). They found an antioxidant capacity increase of 21% (both treatments) after boiling and MW (600 W) for 300 s, with a w/v of 1/20. Boiled and grilled samples registered the lowest FRAP values increases with 2.9-fold compared to raw samples, due to the antioxidant compounds leaching into the cooking water, which amounted 158.6 mg AAE kg^{-1} fw, together with the high cooking temperatures of grilling. Throughout storage, total antioxidant capacity losses were found

Table 4

Total antioxidant capacity of kailan-hybrid broccoli after industrial cooking treatments and throughout storage up to 45 days at 4 °C. Data represent means of five replicates ($n = 5 \pm \text{SD}$).

Treatment		Days of storage					
		0	7	14	21	31	45
Raw	DPPH ^a	307.8	452.5	637.1	767.1	696.1	783.1
	FRAP ^b	280.7	774.75	985.7	273.7	1525.7	1501.1
Boiling	DPPH ^a	742.1	590.3	486.4	471.6	361.2	489.6
	FRAP ^b	825.8	728.2	617.4	524.8	428.9	500.96
LP steaming	DPPH ^a	869.3	897.0	655.2	467.7	429.5	366.1
	FRAP ^b	988.0	866.3	642.0	531.6	675.9	402.4
HP steaming	DPPH ^a	868.6	704.0	559.0	509.2	355.8	374.6
	FRAP ^b	1020.2	984.2	797.4	598.1	646.5	345.0
Sous vide	DPPH ^a	902.4	954.2	765.3	785.5	804.9	720.6
	FRAP ^b	1495.4	964.4	977.6	1301.7	1071.9	810.0
Microwaving	DPPH ^a	905.3	943.4	769.0	713.0	890.4	781.1
	FRAP ^b	1328.5	817.6	1015.5	1046.3	1204.4	1019.0
Sous vide–MW	DPPH ^a	905.3	825.6	581.2	524.6	366.0	396.9
	FRAP ^b	1509.8	648.2	851.6	875.0	693.7	422.8
Grilling	DPPH ^a	837.4	668.1	616.0	487.6	591.0	497.2
	FRAP ^b	822.1	1016.7	482.0	478.7	796.3	518.4

^a SE = 36.2, LSD ($P \leq 0.05$) = 101.7.

^b SE = 51.8, LSD ($P \leq 0.05$) = 145.5.

for all cooking treatments, in contrast to the 5.3-fold enhancement of uncooked samples found after 45 days, always compared to its initial value. MW achieved the lowest total antioxidant degradation throughout storage (39%), while SV–MW and steaming (LP and HP) registered the highest total antioxidant degradation around 60–70% compared to their initial values. The hereby-studied total antioxidant capacity of kailan-hybrid broccoli was well correlated with the total phenolic content on the processing day ($R^2 = 0.86$), but not so much with the total ascorbic acid content ($R^2 = 0.77$) (data not shown). Grilling was excluded in the R^2 calculations of total phenolic content, since its Folin–Ciocalteu assay result did not reflect its real total phenolic content as previously it was discussed. The observed high correlation between total phenolics and total antioxidant capacity on the processing day was also followed throughout its commercial life.

3.6. Total chlorophylls and carotenoids content

Kailan-hybrid broccoli showed a chlorophyll a and b initial content of 68.5 and 49.3 mg kg^{-1} fw, respectively (Table 5), confirming previous data (Martínez-Hernández et al., 2011). As expected, cooking treatments reduced the initial chlorophyll a and b contents, whose concentrations also decreased throughout storage. The chlorophylls reduction after cooking was very probably due to the irreversible breakdown of chlorophyll a and b into their major derivatives pheophytin a and b, respectively, owing to the susceptibility of chlorophylls to heat treatments, among other processing methods (Turkmen et al., 2006). Boiling showed the best chlorophyll a and b retention with 89 and 78%, respectively, closely followed by SV–MW and MW with 84–78 and 66.3–58% retention, respectively, comparing to the uncooked samples. SV induced the highest chlorophylls degradation with only 62 and 39% retention for chlorophyll a and b, respectively. These chlorophylls retention differences may be due to variations in the cooking conditions (e.g. time and medium) and the lower organics acids

Table 5

Total chlorophylls and carotenoids content of kailan-hybrid broccoli after industrial cooking treatments and throughout storage up to 45 days at 4 °C. Data represent means of five replicates ($n = 5 \pm \text{SD}$).

Treatment	Days					
	0	7	14	21	31	45
Total chlorophylls^a						
Raw	117.7	108.3	102.5	94.7	86.6	75.0
Boiling	99.4	93.1	90.0	85.2	81.6	78.5
LP steaming	75.8	69.8	69.1	65.8	62.5	63.9
HP steaming	74.6	68.6	65.7	64.2	53.2	55.3
Sous vide	62.0	56.5	58.1	50.6	43.7	44.2
Microwaving	81.4	77.4	76.9	72.3	67.1	60.6
Sous vide–MW	89.8	82.8	83.3	79.0	75.3	51.8
Grilling	68.2	57.9	57.4	55.2	49.3	32.5
Chlorophyll a^b						
Raw	68.5	65.8	63.3	56.6	53.6	45.2
Boiling	60.7	58.3	57.3	55.5	51.7	51.0
LP steaming	49.5	47.0	47.8	45.7	45.2	46.3
HP steaming	49.9	46.7	45.7	45.1	39.7	40.6
Sous vide	42.6	37.5	35.7	34.8	33.6	33.3
Microwaving	53.0	51.6	50.5	48.8	45.8	42.1
Sous vide–MW	57.1	54.0	54.8	52.7	50.7	36.2
Grilling	43.9	36.8	35.6	36.1	32.4	22.4
Chlorophyll b^c						
Raw	49.3	42.5	39.2	38.0	33.0	29.8
Boiling	38.7	34.8	32.7	29.7	30.0	27.5
LP steaming	26.4	22.8	21.3	20.0	17.3	17.6
HP steaming	24.6	21.9	19.9	19.1	13.5	14.7
Sous vide	19.4	19.0	18.9	15.8	10.2	10.9
Microwaving	28.4	25.8	26.4	23.5	21.3	18.5
Sous vide–MW	32.7	28.8	28.5	26.3	24.6	15.6
Grilling	24.3	21.1	21.8	19.1	16.8	10.1
Total carotenoids^d						
Raw	8.0	9.6	14.3	14.0	13.3	16.2
Boiling	15.4	15.8	15.2	15.5	14.2	14.8
LP steaming	13.6	13.8	15.0	14.3	13.0	16.2
HP steaming	12.8	14.2	15.6	12.6	12.8	14.3
Sous vide	15.1	15.2	16.9	17.1	13.8	13.5
Microwaving	14.9	15.4	15.8	15.4	13.8	15.1
Sous vide–MW	12.2	15.4	17.0	13.8	13.8	12.9
Grilling	15.6	16.7	16.5	16.0	13.7	17.9

^a SE = 4.9, LSD ($P \leq 0.05$) = 13.6.

^b SE = 2.6, LSD ($P \leq 0.05$) = 7.4.

^c SE = 2.6, LSD ($P \leq 0.05$) = 7.3.

^d SE = 0.5, LSD ($P \leq 0.05$) = 1.4.

levels released from plant tissue matrix after MW (Turkmen et al., 2006). Cooking produced lower chlorophyll b retention compared to chlorophyll a (Table 5), probably due to a greater susceptibility to pheophytinization of this kailan-hybrid as it was found for cooked peas (Turkmen et al., 2006). Cooked samples showed higher chlorophylls retention than untreated during storage compared to their initial levels, except SV–MW and grilling. The higher or lower chlorophylls retention may be due to the complete or incomplete thermal inactivation of chlorophyllases, respectively, which are responsible for chlorophylls degradation.

Cooking enhanced (similarly to total phenolics) the total carotenoids content around 1.5–2-fold, compared to the initial value ($8.0 \text{ mg kg}^{-1} \text{ fw}$), without significant differences among treatments (Table 5). The total carotenoids content of raw samples showed an increase throughout storage, recording the same levels as cooked samples after 14 days, and remaining invariable until the end of storage. On day 45, grilled and SV–MW samples showed the highest and lowest total carotenoids content with 17.9 and $12.9 \text{ mg kg}^{-1} \text{ fw}$, respectively. Since lutein is the major carotenoid in broccoli (Miglio et al., 2008), the aforementioned total carotenoids enhancement after cooking may be due to its lutein levels,

which have been reported to be increased after cooking contrary to the rest of carotenoids (Zhang & Hamauzu, 2004). This lutein enhancement after cooking has been attributed to its great thermostability, as compared to its hydro carbonated carotenoid counterparts and most likely as a result of soluble solids loss into the cooking medium, inactivation of carotenoid-oxidizing enzymes and/or increased extraction efficiency due to disruption of carotenoid–protein complexes (Valduga et al., 2009).

4. Conclusions

An excellent microbial reduction was achieved after all cooking treatments (except grilling) keeping the mesophilic counts below $7 \log \text{ CFU g}^{-1}$ after 45 days. Based on the overall sensory quality, the commercial life was established in 45 days at 4 °C, except for grilled (14 days) and SV (21 days) samples. Generally, cooking treatments induced an enrichment of health-promoting compounds, showing MW, SV–MW and LP steaming the best chlorophylls retention and total phenolic (apparently) and antioxidant capacity enhancement. For those reasons, such industrial cooking methods could be recommended as a tool for keeping the quality of the fifth range kailan-hybrid broccoli processing. However, further studies should be implemented to optimise the time-temperature binomial and new treatments should be tested.

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CHAPTER VII

Induced changes in bioactive compounds of kailan-hybrid broccoli after innovative processing and storage

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ABSTRACT

The glucosinolates, sulforaphane, vitamin C and lutein content after several industrial cooking methods on the new kailan-hybrid broccoli and their changes during storage for 45 days at 4 °C were studied. Boiling and *sous vide* induced the highest glucosinolate loss (80%), while low pressure (LP) steaming, microwaving (MW) and *sous vide*-MW showed the lowest (40%) loss. Glucoraphanin was the most thermostable glucosinolate. Throughout their commercial life, microwaved and grilled samples showed a decrease in total glucosinolates. Generally, myrosinase activity was completely inhibited after cooking with undetected sulforaphane contents. The initial total vitamin C dropped by up to 58% after cooking and progressively decreased during storage, with *sous vide*-MW (92%) and microwaving (21%) showing the highest and lowest decrements, respectively. LP steaming and microwaving were the best industrial cooking methods for maintaining the glucosinolate and vitamin C contents, and enhancing up to 7.5-fold the initial lutein content.

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1. Introduction

Healthier diets, safety and convenience are targets correlated by current consumers to high nutritional and sensory quality plant products. In the last few years, innovative and attractive ready-to-use plant products, which satisfy the consumer demand, are appearing. Brassicaceae is a vegetable family that is highly appreciated due to their bioactive compounds and which commonly requires minimum cooking procedures (Manchali, Murthy, & Patil, 2012). However, its specific flavour and sulphur odours led the breeding companies to find new varieties with milder organoleptic characteristics. Recently a

natural kailan-hybrid broccoli (*Brassica oleracea Italica* Group × Alboglabra Group), commercially known as Bimi[®], Tenderstem[®], Vellaverde[®], Broccolini[®], asparation, inspiration, broccoletti or broccoletta, has been developed. This kailan-hybrid has a long slender stem that is completely edible, with a mild sweeter taste and less intense flavour than conventional broccoli cultivars (Martínez-Hernández, Gómez, Artés, & Artés-Hernández, 2011). The kailan-hybrid broccoli is widely consumed in northern European countries, the USA, Brazil and Australia, among others. United Kingdom is among the main consumers of this kailan-hybrid in Europe with 3000 tons commercialized in 2011, 20% more than the

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previous year. In the past 12 months, kailan-hybrid household penetration has experienced a 14% increase and purchase frequency has also increased by over 8% (Fresh Plaza, 2012).

Broccoli is a high-value and seasonal commodity. It is one of the most important vegetables produced in the Mediterranean coastal area of Spain (Artés, Vallejo, & Martínez, 2001). Its commercial interest has increased owed to its considerable relevance as a healthy vegetable, mainly due to its claimed anticancer, antioxidant, anti-inflammatory and cardiovascular diseases preventing properties (Losso & Truax, 2009; Manchali et al., 2012; Mirmiran, Bahadoran, Hosseinpahan, Keyzad, & Azizi, 2012; Yeh & Yen, 2009). This is because broccoli is rich in several health promoting compounds like glucosinolates, vitamins (mainly vitamin C), carotenoids (comprising lutein, the 46–77% over total) and phenolics, among others (Nunn, Giraud, Parkhurst, Hamouz, & Driskell, 2006; Pellegrini et al., 2010; Shahidi & Ho, 2003; Traka & Mithen, 2009). Tissue disruption, such as that caused by chewing or cutting, allows glucosinolates to come into contact with myrosinase (β -thioglucoside glucosyltransferase EC 3.2.3.1), which causes rapid hydrolysis to form glucose and a range of intermediates, such as isothiocyanates, thiocyanates and nitriles (Jones, Frisina, Winkler, Imsic, & Tomkins, 2010). Glucoraphanin, the main glucosinolate found in broccoli, forms the isothiocyanate sulforaphane (1-isothiocyanato-4-methylsulphanylbutane), depending on reducing conditions (such as acidic pH, ferrous ions, ascorbate) and/or the action of the epithiospecific protein (ESP) the sulforaphane nitrile, which has less potent effect on Phase I and II enzymes than sulforaphane nitrile (Jones et al., 2010).

The ready meals segment is an increasing sector in the fifth range industry. In Spain, the vegetables-based ready meals registered a consumption of 1.08 kg capita⁻¹ year⁻¹. From these vegetables-based ready meals, the sautéed (stir frying) dishes represent 30.1% over the total turnover (Sainz, Fálder, Vera, & Martín-Cerdeño, 2008).

The effects of cooking by boiling, LP and high pressure (HP) steaming and microwaving treatments on the aforementioned bioactive compounds content and on their bioavailability have been reported in conventional broccoli cvs. (Galgano, Favati, Caruso, Pietrafesa, & Natella, 2007; Jones et al., 2010; Miglio, Chiavaro, Visconti, Fogliano, & Pellegrini, 2008; Petersen, 1993; Zhang & Hamaizu, 2004). Nevertheless, these studies focused on cooking methods as domestic processes with no information about industrial cooking treatments, which involve an additional step of chilled storage for several weeks. Furthermore, to the best of our knowledge, no studies on the effects of *sous vide*, *sous vide*-microwaving (*sous vide*-MW) and grilling on glucosinolate, endogenous sulforaphane, vitamin C and lutein contents of broccoli have been reported yet, neither have their changes throughout the subsequent commercial chilled storage. In addition, most of these works studied the cooking effects on glucosinolate, but very limited information exists about the isothiocyanate levels, particularly sulforaphane, which is the biologically active form in humans.

The aim of the current work was to identify the bioactive compounds changes (glucosinolate, sulforaphane, vitamin C and lutein) on the new kailan-hybrid broccoli after the innova-

tive *sous vide*, *sous vide*-MW and grilling, compared to those of conventional cooking methods. These induced changes of the health-promoting compounds were studied after industrial treatments and throughout the subsequent chilling storage.

2. Material and methods

2.1. Plant material

Kailan-hybrid broccoli (*Brassica oleracea* Italica Group $\times$ Alboglabra Group) of 15–18 cm long (Martínez-Hernández, Artés-Hernández, Gómez, & Artés, 2013) was hand-harvested in March in open air cultivation parcels of the Campo de Lorca S.C.L. farm (Lorca, Murcia). Broccoli was grown according to integrated pest management cultural practices. Immediately after harvesting, broccoli was crushed ice pre-cooled and transported by car about 90 km to the Pilot Plant of the Universidad Politécnica de Cartagena, where it was stored at 1 °C and 90–95% RH.

2.2. Samples preparation and cooking treatments

Minimal processing was accomplished on the following day in a disinfected cold (8 °C) room. Broccoli was carefully inspected, selecting those free from defects and with a similar visual appearance. The plant material was cut in about 15-cm-long spears and leaves were eliminated using a sharp knife. Then, the cut broccoli was washed with chilled chlorinated water (2 min; 5 °C; 100 mg L⁻¹ NaClO; pH 6.5) and rinsed with chilled tap water (1 min; 5 °C). The following seven industrial cooking treatments were applied to the sanitised plant material (hereinafter called 'raw'):

- **Boiling:** broccoli was boiled in an open pot (25 L capacity) with boiling water (100 °C) for 3.5 min. The broccoli weight/cooking water volume rate (*w/v*) was 5/15.
- **LP steaming:** broccoli was treated for 5 min with water vapour at 100 °C in an industrial autoclave with the pressure valve opened in order to cook at low pressure steaming (0.02 MPa).
- **HP steaming:** the kailan-hybrid was placed on several layers of a steel stainless basket and steam cooked (2 min) in the industrial autoclave (0.1 MPa pressure).
- **Sous vide:** around 175 $\pm$ 5 g broccoli was placed within sterile vacuum polypropylene (PP) bags (20 $\times$ 25 cm) and vacuum-packaged (-98.2 kPa) with a vacuum chamber (V821, Sammic, Guipúzcoa, Spain). After packaging, vacuum-packaged broccoli was placed on different layers of a stainless steel basket. When the autoclave cooking water reached 90 °C the basket was submerged for 15 min. After cooking, the *sous vide* broccoli was rapidly cooled down to about 20 °C in a water-ice bath (0 °C).
- **Microwaving:** about 130 g broccoli was placed inside sterile PP special vacuum bags and treated for 2.5 min in an industrial microwave (MWB-17AE, Fagor Industrial, Valencia, Spain) at 900 W. After microwaving, PP bags were sealed under N₂ atmosphere using the above-mentioned vacuum chamber.

- *Sous vide*-MW: about 130 g broccoli was vacuum-packaged (−98.2 kPa) in PP bags and microwaved for 2.5 min at 900 W. Cooling was performed in a similar way as for the *sous vide* treatment.
- *Grilling*: samples were grilled in an industrial grill (CGF9-120, Fagor Industrial, Valencia, Spain) for 3 min at 280–300 °C (1 min per side and 1 additional min turning constantly over the cooking plate).

The parameters used for all the cooking processes were selected following preliminary studies testing several temperature/time combinations. After boiling, the solubilised bioactive compounds of the cooking water were analysed. Immediately after boiling, grilling and steaming treatments, about 200 g of warm kailan-hybrid was placed into sterile PP trays (1 L, 4-cm depth) and vacuum-packaged (−50 kPa) under 100 kPa N₂ atmosphere, using an automatic packaging device with gas injection (Efaman, Efabind S.L., Murcia, Spain). The packaged product was rapidly cooled down (0 °C) in a water-ice bath. In order to have independent replicates, five treatment series were performed for each cooking method. Good manufacturing practices were applied throughout processing. Further microbial analysis (data not shown) revealed very low counts (mesophilic, psychrophilic, enterobacteriaceae, yeasts and moulds) after cooking treatments. A subsequent microbial increase throughout cold storage was found, but only reaching intermediate values around 4–6 log CFU g^{−1} after 45 days at 4 °C, reporting an absence of *Escherichia coli*, *Salmonella* spp. and *Lysteria monocytogenes*, indicating that the sample were safe according to EU legislation (Regulation EC 1441/2007, 2007). Finally, other samples were frozen with liquid N₂ and stored (−80 °C) until further analysis (*n* = 5).

2.3. Analysis and determinations

2.3.1. Glucosinolates

The glucosinolate content was analysed according to the method described by Francisco et al. (2009). Briefly, 50 mg of freeze-dried powder was extracted in 1.5 mL 70% MeOH at 70 °C for 30 min in a shaking water bath. Then, the samples were centrifuged (13,000g, 15 min, 4 °C), supernatants were collected and methanol was completely removed using a rotary evaporator under vacuum at 37 °C. The obtained dry material was dissolved again in 1 mL of ultrapure water, filtered through a 0.20 µm syringe PTFE filter and stored in vials at −80 °C.

Samples of 20 µL were analysed using an HPLC (Series 1100 Agilent Technologies, Waldbronn, Germany) equipped with a G1322A degasser, G1311A quaternary pump, G1313A autosampler, G1316A column heater and G1315B photodiode array detector. The HPLC system was controlled by the software ChemStation Agilent, v. 08.03. A C18 (250 mm × 4.6 mm, 5 µm) Gemini NX column (Phenomenex, Torrance CA, USA) was used as stationary phase. 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,

99% B at 50 min and 0% B at 55–65 min. Readings were recorded at 227 nm. Glucosinolates were quantified with sinigrin (Sigma, St Louis MO, USA). The results were expressed as mg g^{−1} dry weight (dw). All measurements were made by quintuplicate.

The LC/UV-PAD/ESI-MSn system used was a Waters Alliance 2695 (binary pump and photodiode array detector 2996) (Waters, Mildford, USA), under the same chromatographic conditions for the above-described HPLC analyses. MS was performed using a Micromass ZQ4000 with a double quadrupole analyser, photomultiplier detector and equipped with an electrospray ionisation system with the same setting parameters as described by Francisco et al. (2009).

2.3.2. Endogenous sulforaphane

The endogenous sulforaphane content was analysed according to methods described by Bertelli, Plessi, Braghiroli, and Monzani (1998), and Campas-Baypoli, Sánchez-Machado, Bueno-Solano, Ramírez-Wong, and López-Cervantes (2010). Briefly, 0.25 g of freeze-dried sample was suspended in 5 mL of acidic water (0.1 M HCl; pH 6.0) at 45 °C for 2.5 h in a shaking water bath. After glucoraphanin conversion to sulforaphane by natural myrosinase present in the plant material, 20 mL of dichloromethane were added to the mixture followed by sonication for 1 min. Anhydrous sodium sulphate (6.5 g) was added, filtrated through filter paper (Whatman No. 41) and the eluent was collected. The filtered solid residue was washed twice with 3 mL of dichloromethane and the three eluted portions were collected together. A solid phase extraction (SPE) with Strata SI-1 silica gel 3-mL disposable columns was done. After SPE, the purified sulforaphane extract was evaporated to dryness with a vacuum oven at 45 °C for 2 h. Later, the residue was dissolved in 2 mL of dichloromethane, sonicated for 30 s and filtered through a 0.45-µm PTFE membrane filter. The same column was used as for the glucosinolates analysis. HPLC analyses were carried out with 30/70 acetonitrile/water isocratic elution and flow rate of 0.6 mL min^{−1}. The detector wavelength was fixed at 202 nm. Under these conditions, the retention time of sulforaphane was found to be 8.6 min. Sulforaphane peak identification was assessed by comparing to a sulforaphane standard (Sigma, St. Louis MO, USA). Calibration curves for quantification were made with at least six data points from 200 to 0.5 µM and results were expressed as mg g^{−1} dw. All measurements were made by quintuplicate.

2.3.3. Vitamin C measurements

The ascorbic and dehydroascorbic acids (AA and DHA) were measured according to the method of Zapata and Dufour (1992) with slight modifications. Briefly, ground frozen samples of 5 g were placed into a 25-mL Falcon tubes (protected from light with aluminium foil) and 10 mL of 0.1 M citric acid, 0.05% EDTA, 4 mM sodium fluoride, 5% MeOH cold buffer were added. The mixture, placed on ice, was homogenised in a homogeniser (Ultraturrax T25 basic, IKA, Berlin, Germany) for 10 s. After homogenisation, the extract was filtrated through a 4-layer cheesecloth and the pH was adjusted to 2.35–2.40 with 6 M NaOH. Then, the extract was purified by SPE (Sep-Pak cartridges C18, Waters, Dublin, Ireland) and filtered through a 0.45 µm PTFE membrane filter. DHA derivati-

sation was carried out by mixing 750 μL of vitamin C extract with 250 μL of 7.7 M 1,2-phenylenediamine in a HPLC amber vial. The mixture was allowed to react for 37 min at room temperature. Immediately after derivatisation, 20 μL was injected on the Gemini NX column (the same as the glucosinolates analysis) using a HPLC (Series 1100 Agilent Technologies, Waldbronn, Germany) equipped as mentioned before. The mobile phase was 5 mM hexadecyl trimethyl ammonium bromide, 50 mM KH_2PO_4 and 5% methanol (pH 4.59) with an isocratic flow of 1.8 mL min^{-1} . Chromatograms were recorded for 14 min at 261 nm (AA, $R_t = 6.8$ min) and 348 nm (DHA, $R_t = 3.7$ min). AA and DHA were quantified using commercial standards (Sigma, St. Louis MO, USA). Calibration curves for quantification were made with at least six data points from 3 to 0.1 mM and 3 to 0.01 mM for AA and DHA, respectively. Results were expressed as g kg^{-1} fresh weight (fw). All measurements were made by quintuplicate.

2.3.4. Lutein content

The lutein extraction was conducted according to Martínez-Hernández et al. (2011). Briefly, a 0.5-g ground frozen (-80°C) sample was mixed with 9 mL of hexane and 15 mL of a mixture of methanol/acetone (1/2 v/v). The extraction was carried out for 4 h in darkness inside a polystyrene box with an ice bed and continuously shaken at 200g with an orbital shaker (Stuart, Staffordshire, United Kingdom). After extraction, 25 mL of a 1 M NaCl solution was added, samples were vortex and the upper of the three formed layers was used as the lutein extract. Chromatographic separation and quantification of lutein was followed by Zhang and Hamauzu (2004) method. Briefly, 20 μL of the carotenoids extraction was loaded onto the Gemini NX column (the same as the glucosinolates analysis) at 40°C in a HPLC (Series 1100 Agilent Technologies, Waldbronn, Germany) as described before. The mobile phase consisted of a mixture of (A) acetonitrile/water (90/10) and (B) ethyl acetate with a gradient elution of 0% B at 0 min, 50% B at 20 min, and 50% B at 35 min with a 1.0 mL min^{-1} flow rate. Chromatograms were recorded at 450 nm. Lutein was quantified using a commercial lutein standard (Sigma, St Louis MO, USA). A calibration curve was made with at least six data points from 0.06 to 1 mM and results were expressed as mg kg^{-1} fw. All measurements were made by quintuplicate.

2.4. Statistical analysis

The experiment was an 8×7 two-factor (treatments $\times$ storage time) design. Data was subjected to analysis of variance (ANOVA) using Statgraphics Plus (version 5.1). Mean values were subjected to the least significant difference test (LSD) at $p < 0.05$. Significant differences ($p < 0.05$) among mean values were commented.

3. Results and discussion

3.1. Glucosinolates

The individual glucosinolates of the kailan-hybrid broccoli were identified by their chromatographic behaviour, UV

spectra and HPLC/MS (Table 1). A characteristic glucosinolate chromatogram of this broccoli hybrid is presented in Fig. 1. The main glucosinolates found in the total edible fraction of raw kailan-hybrid (stem and florets) were glucobrassicin (39.0% relative abundance), glucoraphanin (29.2%), 4-methoxyglucobrassicin (13.4%) and glucobrassicinapin (13.8%), followed by lower proportions of neoglucobrassicin (4.6%), gluconasturtiin (3.6%), 4-hydroxyglucobrassicin (1.9%) and gluconapin (0.5%; Table 2). The kailan-hybrid florets had 7.66 mg g^{-1} dw total glucosinolate content, which was 2.7-fold higher than in stems. Glucobrassicin registered 3.41 mg g^{-1} dw in florets, which was 7.4-fold higher than in stems, whereas the remaining individual glucosinolates showed between 1- and 3-fold higher content in florets than in stems (Table 2). Meanwhile, the complete kailan-hybrid pieces (stem plus floret) recorded intermediate levels (12.73 and 4.76 mg g^{-1} dw for total glucosinolate and glucobrassicin contents, respectively). Since glucosinolates have a prominent function in plant defence, their accumulation is higher in reproductive organs, such as florets, in order to protect them against herbivores and pathogen attacks (Fahey, Zalcmann, & Talalay, 2001). The hereby firstly reported kailan-hybrid glucosinolate profile revealed greater (between 1.7- and 9.3-fold higher) glucobrassicin content in their total edible fraction and florets (4.76 and 3.41 mg g^{-1} dw, respectively) than that reported in florets from different conventional broccoli cvs. (Jones et al., 2010; Miglio et al., 2008; Pellegrini et al., 2010) and kailan (*B. oleraceae* Alboglabra group) (Hanson, Yang, Chang, Ledesma, & Ledesma, 2011).

After cooking treatments, the kailan-hybrid (hereinafter referred to as the complete edible fraction) glucosinolates concentration significantly ($p < 0.05$) declined, as shown in Table 3, with thermal degradation being the most important cause, producing the correspondent isothiocyanates and/or nitriles as products (Jones et al., 2010; Rosa, Heaney, Fenwick, & Portas, 1997). The major glucosinolates, glucobrassicin, glucoraphanin, glucobrassicinapin, 4-methoxyglucobrassicin and neoglucobrassicin, are shown in Table 3, since the remaining minor glucosinolates were undetectable after cooking. Boiling and *sous vide* induced the largest total glucosinolates losses (77–80%), as reported in conventional broccoli after domestic boiling (Jones et al., 2010; Pellegrini et al., 2010). LP steaming, *sous vide*-MW and microwaving appeared to minimise total glucosinolate losses, reaching the lowest decreases (56–60% over raw values) on the processing day. Results for LP steaming agree with those found by Rosa et al. (1997). This low glucosinolate decrement after LP steaming was due to their slower migration to cooking water because of the lack of direct contact of the vegetable with water, and therefore a lower disruption of their structure. However, kailan-hybrid samples were submerged in water for boiling, registering a significant ($p < 0.05$) higher boiling water glucosinolate content than in the condensed water from LP steaming, with 33.5 and 22.5 mg total glucosinolates, respectively, leached into the cooking liquid per 100 g fw vegetable cooked (data not shown). The high glucosinolate decline during boiling may be due to the high evaporation of the water containing the leached glucosinolates, which could be important in the boiled kailan-hybrid since the boiling pot was not covered during the treatment. The hypothesis of the better

Table 1 – HPLC/DAD/MS analysis for individual glucosinolates of kailan-hybrid broccoli.

No.	Trivial name	Systematic name	Rt (min)	HPLC/DAD (λ_{max})	[M–H] [–]
1	Glucoraphanin	4-Methylsulphinylbutyl	5.9	226	436
2	Gluconapin	3-Butenyl	11.1	225	372
3	4-Hydroxyglucobrassicin	4-Hydroxy-3-indolylmethyl	13.2	222, 248, 280	463
4	Glucobrassicinapin	4-Pentenyl	14.0	225	386
5	Glucobrassicin	3-Indolylmethyl	16.8	222, 280	447
6	Gluconasturtiin	2-Phenylethyl	19.1	221	422
7	4-Methoxyglucobrassicin	4-Methoxy-3-indolylmethyl	19.7	222, 246, 278	
8	Neoglucobrassicin	N-Methoxy-3-indolylmethyl	22	223	477

glucosinolate retention owing to high myrosinase inactivation could be discarded by the study of Rungapamestry, Duncan, Fuller, and Ratcliffe (2006), which found unaffected myrosinase activity in cabbage after steaming owing to the short cooking time (2 min). However, Ludikhuyze, Ooms, Weemaes, and Hendrickx (1999), Ludikhuyze, Rodrigo, and Hendrickx (2000) reported lower myrosinase thermostability in broccoli after longer steaming treatments, in accordance with our results shown in the endogenous sulforaphane section. As described above, the great total glucosinolate decrease in the kailan-hybrid after *sous vide* could be due to several causes: (1) the long cooking time produced the thermal degradation of glucosinolates, which may be more exposed to heat since the applied vacuum during *sous vide* packaging favoured their extraction from plant cells; (2) the degradation by myrosinase, the activity of which may be favoured by the milder cooking temperature (compared to the other treatments) until complete inactivation was achieved, as shown in the next section; and/or (3) an incomplete ESP inactivation which led to a high production of nitriles (Jones et al., 2010). On the other hand, microwaving seemed to induce a lower glucosinolate decline since myrosinase activity, which has been related to glucosinolate degradation, is highly affected after microwaving, as reported in cabbage, where myrosinase was inactivated after 2 min at 750 W (Rungapamestry et al., 2006) and 5 min at 900 W (Verkerk & Dekker, 2004). However, steaming for 2 min did not decrease myrosinase activity in cabbage (Rungapamestry et al., 2006).

The latest findings may be understood since the internal temperatures of broccoli rise faster during microwaving, reaching 70 °C after 50 s, compared to 110 s for boiling and 150 s for steaming (Jones et al., 2010). HP steaming and grilling registered intermediate total glucosinolate decrements of 71–73%, without significant differences among them ($p < 0.05$) (Table 3). The glucosinolate degradation in the grilled samples may be due to the high cooking temperature, since glucosinolates are degraded above 100 °C (Oerlemans, Barret, Suades, Verkerk, & Dekker, 2006). The total glucosinolate decline after HP steaming hereby found could be due to a high plant cell disruption after treatment, which released more glucosinolates for thermal degradation, as well as the enhanced myrosinase activity by high pressure, since myrosinase activity did not increase at normal atmospheric pressure (Van Eylen, Oey, Hendrickx, & Van Loey, 2008). The last finding corroborates the low glucosinolate decreases of LP steaming in the kailan-hybrid.

The aliphatic glucosinolate glucoraphanin recorded a higher thermostability after cooking (Table 3), with lower decreases (40–67% over raw content of 3.47 mg g^{–1} dw), than indol glucosinolates (glucobrassicinapin, glucobrassicin, 4-methoxyglucobrassicin, neoglucobrassicin). This greater thermolability of indole glucosinolates compared to aliphatic ones in the kailan-hybrid agrees with findings in conventional broccoli (Oerlemans et al., 2006). Among indol glucosinolates, glucobrassicinapin and neoglucobrassicin were the most thermolabile with decreases of 80–98% and 85–92%, respectively,

Fig. 1 – HPLC chromatogram recorded at 227 nm of identified glucosinolates in kailan-hybrid broccoli. Numbered as Table 1.

Table 2 – Individual glucosinolates content (mg g⁻¹ dw) of floret, stems and whole kailan-hybrid broccoli (n = 5 ± SD). Different letter among each row denotes significant difference (p < 0.05).

	Glucosinolate content (mg g ⁻¹ dw)		
	Florets	Stems	Whole
Glucobrassicin (I)	3.41 ± 0.04 ^b	0.46 ± 0.00 ^c	4.76 ± 0.02 ^a
Glucoraphanin (AP)	1.53 ± 0.00 ^b	1.14 ± 0.00 ^c	3.47 ± 0.00 ^a
Glucobrassicinapin (I)	0.71 ± 0.15 ^b	0.24 ± 0.05 ^c	1.45 ± 0.12 ^a
4-Methoxyglucobrassicin (I)	1.40 ± 0.01 ^b	0.52 ± 0.01 ^c	1.74 ± 0.01 ^a
Neoglucobrassicin (I)	0.26 ± 0.01 ^b	0.20 ± 0.01 ^c	0.60 ± 0.01 ^a
Gluconasturtiin (AR)	0.23 ± 0.05 ^b	0.13 ± 0.03 ^c	0.41 ± 0.04 ^a
4-Hydroxyglucobrassicin (I)	0.09 ± 0.01 ^c	0.12 ± 0.01 ^b	0.25 ± 0.01 ^a
Gluconapin (AK)	0.02 ± 0.00 ^b	0.02 ± 0.00 ^b	0.05 ± 0.00 ^a
TOTAL	7.66 ± 0.15 ^b	2.82 ± 0.05 ^c	12.73 ± 0.16 ^a

compared to raw samples. The best glucoraphanin retention of 60–46% was reached by LP steaming, microwaving, *sous vide*-MW and HP steaming, without any significant ($p < 0.05$) differences among the techniques. Grilling, *sous vide* and boiling induced the greatest significant ($p < 0.05$) glucoraphanin reductions with 82% over the raw kailan-hybrid. LP steaming, microwaving and *sous vide*-MW, showed the highest significant ($p < 0.05$) glucobrassicin retention (around 54%), while boiling and grilling induced the greatest decrements (78–77%) compared to raw samples. After cooking, glucobrassicinapin and 4-methoxyglucobrassicin showed significant ($p < 0.05$) decreases of 79–98% and 68–95%, respectively, compared to raw samples, with no significant ($p < 0.05$) differences among treatments. On the other hand, neoglucobrassicin did not record any noticeable degradation after cooking.

Attending to glucosinolate changes during chilled storage of cooked kailan-hybrid, compared to values on the processing day, almost all of the samples suffered significant ($p < 0.05$) total glucosinolate increases after 45 days. Only the microwaved and grilled samples showed significant ($p < 0.05$) glucosinolate decreases of 32% and 38%, respectively. Boiled samples showed a significant ($p < 0.05$) glucosinolate enhancement of 66% over its initial level, mainly due to the 50% increase registered by glucoraphanin (Table 3). Since uncooked samples were packaged under an N₂ atmosphere, in order to compare data in the same conditions as cooked samples, the CO₂ production of the raw samples during storage may produce cell damage and enzymatic degradation of glucosinolates, as glucosinolate degradation was previously found in 'Marathon' broccoli heads stored at 10 °C under 20% CO₂ for 7 days (Hansen, Moller, Sorensen, & de-Trejo, 1995). Among the cooked samples, only microwaved and grilled kailan-hybrid registered glucobrassicin reductions of 54% and 65%, respectively, at the end of storage period, related to their levels on the processing day. However, some glucosinolates showed significant ($p < 0.05$) increases followed by a decline during storage (e.g. glucoraphanin of *sous vide* samples, 4-methoxyglucobrassicin of control samples and glucobrassicin of *sous vide*-MW, *sous vide* and LP steamed samples at day 31). These increases of the glucosinolate contents during cold storage have been attributed to an enhanced synthesis or a release of bound compounds, as previously reported Hansen, Moller, Sorensen, and de-Trejo (1995) in broccoli florets. The subsequent decreases are a consequence of the senescence processes. The variability of this specific

behaviour among treatments can be due to the different release of bound compounds. This release of bound compounds may be influenced by the different thermal disruptions of the plant material depending of the cooking treatments applied.

3.2. Endogenous sulforaphane

The control kailan-hybrid had 154.6 mg kg⁻¹ dw sulforaphane content after endogenous myrosinase activity (endogenous sulforaphane). This value was greater than data of 19.5, 49.3 and 51.9 mg kg⁻¹ dw reported in conventional broccoli florets of 'Marathon', 'Calabrese' and Boster™ cvs., respectively (Bertelli et al., 1998; Jones et al., 2010). However, Campas-Baypoli et al. (2009) found 246.2 mg kg⁻¹ dw endogenous sulforaphane content in whole (floret and stem) broccoli. These differences among studies may be due to myrosinase activity, which is highly affected by extrinsic and intrinsic factors, such as plant maturity, cultivar type, cultivation practices, and laboratory (extraction, purification and measurement) techniques. No endogenous sulforaphane was detected after cooking treatments, except for grilled samples which showed only 0.23 mg kg⁻¹ dw. As previously reported by Jones et al. (2010), a level of sulforaphane nitrile production still occurred after cooking methods. The hereby-presented results agree with complete myrosinase inactivation after cooking reported by Jones et al. (2010), who found 90–99% decreases after boiling, LP steaming and microwaving florets of conventional 'Marathon' and Boster™ broccoli. Throughout chilled storage no endogenous sulforaphane increases were found (data not shown).

3.3. Vitamin C

The influence of different cooking treatments on the AA and DHA levels of kailan-hybrid and their changes throughout cold storage are shown in Table 4. A total vitamin C (AA + DHA) of 1.7 g kg⁻¹ fw was found in raw samples (with 13.7% of DHA). These levels were higher than the ranges of 0.4–1.2 g kg⁻¹ fw and 5.4–10.9%, respectively, reported for conventional broccoli cvs. (Galgano et al., 2007; Munyaka, Oey, Van Loey, & Hendrickx, 2010). The aforementioned higher DHA proportion compared to conventional broccoli cvs. from literature might be attributed to the low stem proportion related to the total edible part of kailan-hybrid, since florets have higher ascorbic acid oxidase and ascorbate per-

Table 3 – Individual glucosinolates content (mg g⁻¹ dw) of kailan-hybrid broccoli after different cooking treatments and stored up to 45 days at 4 °C (n = 5 ± SD). Different capital letter among each row denotes significant difference (p < 0.05). Different lower case letter within each column denotes significant difference (p < 0.05).

	Storage time at 4 °C (days)		14	21	31	38	45
	0	7					
Glucobrassicin (mg g⁻¹ dw)							
Control	4.76 ± 0.02 ^A	1.62 ± 1.12 ^B	0.64 ± 0.11 ^D	0.35 ± 0.11 ^E	0.98 ± 0.22 ^C	0.69 ± 0.13 ^D	0.63 ± 0.04 ^D
Boiling	1.06 ± 0.27 ^B	1.31 ± 0.66 ^{AB}	1.17 ± 0.12 ^{AB}	0.81 ± 0.21 ^C	0.76 ± 0.11 ^C	0.79 ± 0.12 ^C	1.31 ± 0.19 ^A
LP steaming	2.59 ± 0.15 ^B	2.66 ± 0.15 ^A	2.32 ± 0.22 ^B	1.57 ± 0.61 ^C	2.51 ± 0.57 ^{AC}	1.82 ± 0.16 ^D	2.39 ± 0.36 ^{BC}
HP steaming	1.52 ± 0.94 ^C	1.56 ± 1.06 ^{BCDE}	2.10 ± 1.25 ^A	1.75 ± 0.21 ^{BC}	1.39 ± 1.75 ^{CE}	1.49 ± 0.47 ^{CD}	1.81 ± 0.04 ^B
Sous vide	1.37 ± 0.17 ^C	1.20 ± 0.39 ^E	1.47 ± 0.86 ^{BC}	0.93 ± 0.09 ^D	2.05 ± 0.69 ^A	0.53 ± 0.09 ^E	0.87 ± 0.02 ^D
Microwaving	2.07 ± 0.73 ^C	2.13 ± 1.19 ^C	2.83 ± 1.18 ^{AB}	2.84 ± 0.84 ^A	1.70 ± 0.21 ^D	1.24 ± 0.48 ^E	0.95 ± 0.02 ^D
Sous vide-MW	2.28 ± 0.49 ^{BC}	2.34 ± 0.51 ^B	1.99 ± 1.08 ^D	1.59 ± 0.44 ^{BC}	2.76 ± 0.32 ^A	1.47 ± 0.06 ^E	1.99 ± 0.55 ^D
Grilled	1.11 ± 0.38 ^{CF}	1.14 ± 0.39 ^A	1.30 ± 0.52 ^{AE}	0.48 ± 0.04 ^D	0.83 ± 0.06 ^E	0.66 ± 0.02 ^E	0.39 ± 0.01 ^F
Glucoraphanin (mg g⁻¹ dw)							
Control	3.47 ± 0.00 ^A	1.56 ± 0.65 ^B	0.74 ± 0.00 ^D	0.34 ± 0.07 ^E	0.83 ± 0.02 ^D	1.01 ± 0.04 ^C	0.78 ± 0.19 ^D
Boiling	1.15 ± 0.13 ^B	1.18 ± 0.14 ^B	0.95 ± 0.24 ^C	0.81 ± 0.22 ^C	0.73 ± 0.01 ^{CD}	0.64 ± 0.14 ^{DE}	1.73 ± 0.26 ^C
LP steaming	2.07 ± 0.83 ^{BC}	2.12 ± 0.32 ^{AB}	1.90 ± 0.16 ^{DE}	1.77 ± 0.77 ^E	1.94 ± 0.23 ^{CD}	1.51 ± 0.76 ^E	2.16 ± 0.25 ^A
HP steaming	1.59 ± 0.74 ^{BDE}	1.63 ± 0.77 ^{BD}	1.67 ± 0.36 ^{ABCDE}	1.72 ± 0.00 ^{AB}	1.45 ± 1.31 ^A	1.48 ± 0.19 ^{DE}	1.80 ± 0.02 ^A
Sous vide	1.21 ± 0.17 ^B	1.24 ± 0.18 ^B	1.28 ± 0.18 ^{BC}	0.67 ± 0.08 ^D	1.65 ± 0.38 ^A	0.46 ± 0.05 ^{CD}	1.37 ± 0.24 ^E
Microwaving	1.86 ± 0.75 ^A	1.90 ± 0.68 ^A	1.96 ± 0.18 ^A	1.85 ± 0.55 ^{AB}	0.87 ± 0.14 ^E	1.08 ± 0.44 ^B	1.30 ± 0.07 ^C
Sous vide-MW	1.85 ± 0.74 ^{AD}	1.89 ± 0.43 ^C	1.91 ± 0.03 ^{AB}	1.13 ± 0.26 ^B	1.59 ± 0.65 ^{BC}	1.14 ± 0.06 ^E	1.99 ± 0.55 ^A
Grilled	0.61 ± 0.19 ^{AB}	0.67 ± 0.17 ^A	0.54 ± 0.28 ^{BC}	0.66 ± 0.01 ^A	0.45 ± 0.04 ^B	0.40 ± 0.05 ^D	0.49 ± 0.03 ^B
Glucobrassicinapin (mg g⁻¹ dw)							
Control	1.45 ± 0.12 ^C	0.52 ± 0.02 ^E	0.39 ± 0.00 ^F	1.03 ± 0.22 ^E	2.28 ± 0.26 ^A	2.27 ± 0.19 ^A	1.99 ± 1.05 ^B
Boiling	0.03 ± 0.00 ^C	0.03 ± 0.01 ^C	0.03 ± 0.00 ^C	0.07 ± 0.01 ^C	0.12 ± 0.01 ^B	0.13 ± 0.01 ^{AB}	0.17 ± 0.01 ^A
LP steaming	0.13 ± 0.01 ^{BC}	0.13 ± 0.05 ^{BC}	0.11 ± 0.02 ^C	0.12 ± 0.05 ^C	0.17 ± 0.05 ^B	0.15 ± 0.10 ^{BC}	0.23 ± 0.01 ^A
HP steaming	0.09 ± 0.04 ^{CD}	0.09 ± 0.04 ^{CD}	0.13 ± 0.03 ^{BC}	0.12 ± 0.01 ^{BC}	0.10 ± 0.08 ^{BCD}	0.14 ± 0.04 ^{AB}	0.18 ± 0.01 ^A
Sous vide	0.12 ± 0.01 ^{BCD}	0.12 ± 0.01 ^{BCD}	0.14 ± 0.03 ^{AB}	0.17 ± 0.01 ^A	0.16 ± 0.03 ^A	0.10 ± 0.02 ^{BD}	0.18 ± 0.03 ^{DE}
Microwaving	0.27 ± 0.01 ^C	0.27 ± 0.08 ^C	0.32 ± 0.05 ^B	0.42 ± 0.01 ^B	0.07 ± 0.02 ^D	0.20 ± 0.02 ^D	0.31 ± 0.02 ^C
Sous vide-MW	0.29 ± 0.10 ^{BCD}	0.29 ± 0.10 ^{CE}	0.28 ± 0.02 ^{CD}	0.33 ± 0.08 ^A	0.32 ± 0.05 ^{ABC}	0.25 ± 0.02 ^{DE}	0.33 ± 0.07 ^{AB}
Grilled	0.31 ± 0.05 ^D	0.31 ± 0.05 ^D	0.42 ± 0.22 ^A	0.41 ± 0.04 ^B	0.33 ± 0.02 ^D	0.46 ± 0.01 ^B	0.53 ± 0.01 ^B
4-Methoxyglucobrassicin (mg g⁻¹ dw)							
Control	1.74 ± 0.01 ^B	0.69 ± 0.03 ^D	0.49 ± 0.00 ^E	1.03 ± 0.30 ^C	2.60 ± 0.29 ^A	0.06 ± 0.02 ^F	0.05 ± 0.02 ^F
Boiling	0.07 ± 0.01 ^{AB}	0.07 ± 0.03 ^{AB}	0.10 ± 0.01 ^A	0.03 ± 0.01 ^B	0.05 ± 0.01 ^{AB}	0.04 ± 0.01 ^B	0.09 ± 0.01 ^{AB}
LP steaming	0.25 ± 0.04 ^C	0.25 ± 0.12 ^C	0.40 ± 0.03 ^B	0.10 ± 0.05 ^D	0.59 ± 0.12 ^{DE}	0.15 ± 0.01 ^{AB}	0.25 ± 0.03 ^C
HP steaming	0.17 ± 0.07 ^{CD}	0.20 ± 0.08 ^C	0.48 ± 0.26 ^{DE}	0.01 ± 0.00 ^F	0.63 ± 0.39 ^{ACD}	0.17 ± 0.06 ^{CD}	0.21 ± 0.00 ^{ABC}
Sous vide	0.21 ± 0.03 ^{CD}	0.21 ± 0.03 ^C	0.22 ± 0.05 ^B	0.15 ± 0.02 ^{BC}	0.55 ± 0.16 ^A	0.06 ± 0.02 ^{CD}	0.17 ± 0.03 ^{CD}
Microwaving	0.48 ± 0.14 ^B	0.49 ± 0.14 ^B	0.56 ± 0.30 ^A	0.10 ± 0.01 ^E	0.33 ± 0.01 ^D	0.06 ± 0.01 ^{CD}	0.09 ± 0.01 ^{EF}
Sous vide-MW	0.53 ± 0.19 ^C	0.43 ± 0.30 ^{CD}	0.77 ± 0.23 ^B	0.04 ± 0.01 ^{DEF}	0.93 ± 0.18 ^A	0.12 ± 0.01 ^{ABCD}	0.26 ± 0.04 ^E
Grilled	0.55 ± 0.10 ^B	0.56 ± 0.10 ^B	0.68 ± 0.35 ^B	0.22 ± 0.08 ^B	0.69 ± 0.06 ^C	0.07 ± 0.00 ^D	0.28 ± 0.02 ^A
Neoglucobrassicin (mg g⁻¹ dw)							
Control	0.60 ± 0.01 ^A	0.28 ± 0.10 ^B	0.28 ± 0.01 ^B	0.16 ± 0.02 ^C	0.29 ± 0.10 ^B	0.08 ± 0.01 ^D	0.30 ± 0.03 ^B
Boiling	0.05 ± 0.01 ^D	0.33 ± 0.20 ^{BC}	0.27 ± 0.14 ^C	0.36 ± 0.05 ^B	0.33 ± 0.01 ^{BC}	0.33 ± 0.03 ^{BC}	0.65 ± 0.06 ^{BC}
LP steaming	0.08 ± 0.01 ^E	0.43 ± 0.20 ^{BC}	0.32 ± 0.21 ^D	0.41 ± 0.20 ^C	0.52 ± 0.14 ^B	0.59 ± 0.03 ^B	0.72 ± 0.03 ^{AB}
HP steaming	0.07 ± 0.03 ^F	0.37 ± 0.29 ^{CD}	0.43 ± 0.27 ^B	0.33 ± 0.01 ^{CD}	0.27 ± 0.18 ^D	0.63 ± 0.04 ^A	0.60 ± 0.01 ^C
Sous vide	0.07 ± 0.02 ^F	0.26 ± 0.15 ^C	0.24 ± 0.15 ^{CD}	0.17 ± 0.02 ^D	0.34 ± 0.09 ^B	0.24 ± 0.02 ^{CD}	0.42 ± 0.01 ^A
Microwaving	0.09 ± 0.01 ^F	0.46 ± 0.25 ^{AB}	0.62 ± 0.35 ^B	0.65 ± 0.20 ^A	0.37 ± 0.04 ^D	0.47 ± 0.07 ^B	0.59 ± 0.03 ^{CB}
Sous vide-MW	0.06 ± 0.00 ^F	0.54 ± 0.36 ^C	0.45 ± 0.37 ^D	0.41 ± 0.06 ^D	0.61 ± 0.19 ^B	0.60 ± 0.03 ^{BC}	0.75 ± 0.15 ^A
Grilled	0.70 ± 0.15 ^A	0.17 ± 0.07 ^{CD}	0.22 ± 0.12 ^C	0.30 ± 0.03 ^B	0.10 ± 0.08 ^D	0.36 ± 0.01 ^B	0.29 ± 0.01 ^B
Total							
Control	12.02 ± 0.08 ^A	4.77 ± 0.52 ^D	2.52 ± 0.20 ^E	2.88 ± 0.62 ^E	6.97 ± 0.65 ^B	4.15 ± 0.41 ^B	5.70 ± 0.19 ^C
Boiling	1.95 ± 0.35 ^F	2.38 ± 0.31 ^B	2.73 ± 0.30 ^C	2.26 ± 0.22 ^{CD}	2.13 ± 0.09 ^{CD}	1.92 ± 0.27 ^D	4.19 ± 0.11 ^A
LP steaming	4.68 ± 0.13 ^C	5.28 ± 0.08 ^B	4.98 ± 0.10 ^C	4.03 ± 0.91 ^D	6.01 ± 0.43 ^B	4.27 ± 0.33 ^{CD}	5.74 ± 0.35 ^B
HP steaming	4.11 ± 1.19 ^D	4.53 ± 1.16 ^{CD}	5.45 ± 1.04 ^A	2.87 ± 0.28 ^A	4.17 ± 2.55 ^A	4.08 ± 0.60 ^C	4.61 ± 0.06 ^B
Sous vide	2.97 ± 0.21 ^{CF}	3.12 ± 0.26 ^C	4.17 ± 0.56 ^B	4.47 ± 1.00 ^D	5.28 ± 0.24 ^C	1.41 ± 0.19 ^F	2.99 ± 0.25 ^{BC}
Microwaving	5.49 ± 1.40 ^C	6.22 ± 1.39 ^B	7.66 ± 0.37 ^A	5.06 ± 0.41 ^B	3.19 ± 0.35 ^D	3.05 ± 0.95 ^D	3.24 ± 0.11 ^F
Sous vide-MW	4.96 ± 0.57 ^{BC}	5.50 ± 0.76 ^{AB}	6.23 ± 0.40 ^B	3.20 ± 0.16 ^E	6.26 ± 0.47 ^A	3.53 ± 0.13 ^D	6.05 ± 0.48 ^B
Grilled	3.76 ± 0.504 ^{AE}	3.36 ± 0.46 ^E	3.14 ± 0.97 ^B	2.13 ± 0.15 ^{CD}	2.41 ± 0.18 ^{BC}	1.94 ± 0.04 ^D	1.98 ± 0.07 ^{CD}

oxidase activities by 7.5 and 4.4 (data not shown) times, respectively (Munyaka et al., 2010). The total vitamin C

losses after cooking reached between a 32% and 58% compared to control samples on the processing day. Cooking

treatments produce matrix disruption, bringing into contact AA and the enzymes ascorbic acid oxidase and peroxidase, hence facilitating the oxidation of AA into DHA (Lee & Kader, 2000). Grilling and boiling induced the highest significant ($p < 0.05$) AA decreases (58–55% over uncooked samples). Raw samples significantly ($p < 0.05$) showed between 91- and 2.4-fold higher DHA levels than all cooked samples (except grilled), corroborating the high DHA thermolability found in conventional broccoli (Munyaka et al., 2010). During boiling treatment, AA/DHA were leached to the cooking water in a rate of 0.2/0.01 g per kg fw of cooked sample, with 0.7/0.02 g kg⁻¹ fw remaining in the edible part. This result indicates that, since DHA is more unstable (especially at low pH typical of broccoli) than AA (Deutsch, 2000), it was rapidly and irreversibly hydrolysed to other compounds such as 2,3-diketogulonic acid (without antiscorbutic activity). Furthermore, the AA degradation could be favoured by the aqueous medium of boiling cooking. Grilled samples significantly ($p < 0.05$) showed 19.2-fold higher DHA than boiled samples, which indicates a high enzymatic oxidation of AA to DHA. This result could be attributed to an incomplete enzymatic inactivation owed to the short cooking time, with the oxidising enzymes remaining active in the inner parts of the kailan-hybrid stem. The rest of the cooked treatments showed AA decreases between 32% and 39%, without

differences among them, as found by Galgano et al. (2007), Petersen (1993), and Zhang and Hamauzu (2004). *Sous vide* and *sous vide*-MW showed the highest AA levels after cooking, probably due to the low O₂ atmosphere generated after vacuum packaging, which protected the AA from oxidation. *Sous vide* showed the lowest DHA level with 0.01 mg kg⁻¹ fw, apart from boiling due to the above-explained leaching to the cooking water, owing to a higher degradation of this unstable AA.

With regard to storage period, AA levels significantly ($p < 0.05$) decreased in all cooked samples reaching a steady-state level from day 21 to 31, while DHA increased due to the AA oxidation. After 45 days of storage, the order for total vitamin C decreases (compared to their respective values at the processing day) was *sous vide*-MW (91.5%) > LP steaming > HP steaming > grilling (the latter four treatments without significant differences among them) > boiling > *sous vide* > microwaving (21.1%). From the aforementioned decreases, microwaving and *sous vide* induced the best vitamin C retentions, without any significant ($p < 0.05$) differences between them. This result could be explained by the short cooking time, whereas vacuum packaging of *sous vide* and *sous vide*-MW probably forced intracellular liquids leakage and matrix disruption, resulting in an easier contact between AA and oxidative enzymes. After 45 days at 4 °C,

Table 4 – Vitamin C (AA and DHA) content (g kg⁻¹ fw) of kailan-hybrid broccoli after different cooking treatments and stored up to 45 days at 4 °C (n = 5 ± SD). Different capital letter among each row denotes significant difference ($p < 0.05$). Different lower case letter within each column denotes significant difference ($p < 0.05$).

	Storage time at 4 °C (days)						
	0	7	14	21	31	38	45
AA (g kg⁻¹ fw)							
Control	1.47 ± 0.03 ^A	1.27 ± 0.04 ^B	0.96 ± 0.05 ^B	0.90 ± 0.04 ^B	1.00 ± 0.10 ^{AB}	1.01 ± 0.12 ^{AB}	0.92 ± 0.05 ^B
Boiling	0.66 ± 0.08 ^A	0.57 ± 0.02 ^{AB}	0.36 ± 0.08 ^{AB}	0.14 ± 0.03 ^B	0.13 ± 0.01 ^B	0.14 ± 0.01 ^B	0.19 ± 0.08 ^{AB}
LP steaming	0.98 ± 0.05 ^A	0.62 ± 0.03 ^{AB}	0.36 ± 0.03 ^{BC}	0.16 ± 0.01 ^{BC}	0.22 ± 0.04 ^{BC}	0.15 ± 0.01 ^{BC}	0.10 ± 0.01 ^C
HP steaming	0.99 ± 0.05 ^A	0.63 ± 0.01 ^{AB}	0.30 ± 0.06 ^{BC}	0.17 ± 0.02 ^{BC}	0.16 ± 0.05 ^{BC}	0.12 ± 0.02 ^C	0.10 ± 0.01 ^C
<i>Sous vide</i>	1.10 ± 0.024 ^{AB}	0.98 ± 0.05 ^{AB}	0.86 ± 0.10 ^{AB}	0.82 ± 0.10 ^{AB}	0.80 ± 0.15 ^{AB}	0.72 ± 0.04 ^{AB}	0.56 ± 0.10 ^B
Microwaving	0.98 ± 0.14 ^{AB}	0.87 ± 0.13 ^{AB}	0.93 ± 0.19 ^A	0.88 ± 0.01 ^A	0.86 ± 0.05 ^A	0.86 ± 0.07 ^A	0.83 ± 0.15 ^A
<i>Sous vide</i> -MW	1.15 ± 0.17 ^A	0.46 ± 0.5 ^B	0.30 ± 0.09 ^B	0.15 ± 0.03 ^B	0.20 ± 0.06 ^B	0.14 ± 0.01 ^B	0.10 ± 0.01 ^B
Grilled	0.62 ± 0.03 ^B	0.44 ± 0.07 ^B	0.25 ± 0.13 ^C	0.12 ± 0.01 ^B	0.17 ± 0.02 ^C	0.14 ± 0.02 ^B	0.12 ± 0.03 ^B
DHA (g kg⁻¹ fw)							
Control	0.23 ± 0.03 ^A	0.12 ± 0.03 ^A	0.10 ± 0.05 ^A	0.10 ± 0.05 ^A	0.15 ± 0.05 ^A	0.11 ± 0.02 ^A	0.14 ± 0.04 ^A
Boiling	0.00 ± 0.00 ^B	0.01 ± 0.00 ^A	0.02 ± 0.01 ^A	0.08 ± 0.00 ^A	0.05 ± 0.03 ^A	0.04 ± 0.01 ^A	0.01 ± 0.00 ^A
LP steaming	0.05 ± 0.01 ^A	0.08 ± 0.01 ^A	0.11 ± 0.01 ^A	0.10 ± 0.00 ^A	0.06 ± 0.02 ^A	0.05 ± 0.01 ^A	0.00 ± 0.00 ^A
HP steaming	0.06 ± 0.01 ^A	0.08 ± 0.00 ^A	0.09 ± 0.02 ^A	0.09 ± 0.00 ^A	0.07 ± 0.02 ^A	0.04 ± 0.00 ^A	0.00 ± 0.00 ^A
<i>Sous vide</i>	0.01 ± 0.01 ^A	0.05 ± 0.03 ^A	0.02 ± 0.01 ^A	0.07 ± 0.01 ^A	0.06 ± 0.00 ^A	0.05 ± 0.00 ^A	0.03 ± 0.03 ^A
Microwaving	0.10 ± 0.01 ^A	0.02 ± 0.00 ^A	0.02 ± 0.00 ^A	0.08 ± 0.01 ^A	0.10 ± 0.04 ^A	0.07 ± 0.01 ^A	0.02 ± 0.02 ^A
<i>Sous vide</i> -MW	0.01 ± 0.014 ^B	0.08 ± 0.01 ^A	0.04 ± 0.04 ^A	0.05 ± 0.01 ^A	0.06 ± 0.02 ^A	0.05 ± 0.00 ^A	0.00 ± 0.00 ^A
Grilled	0.30 ± 0.03 ^A	0.20 ± 0.01 ^{AB}	0.08 ± 0.02 ^{AB}	0.07 ± 0.02 ^{AB}	0.06 ± 0.02 ^{AB}	0.04 ± 0.00 ^B	0.03 ± 0.02 ^B
AA ± DHA (g kg⁻¹ fw)							
Control	1.70 ± 0.09 ^A	1.39 ± 0.05 ^{AB}	1.06 ± 0.14 ^B	1.00 ± 0.12 ^B	1.15 ± 0.18 ^B	1.08 ± 0.11 ^B	1.06 ± 0.02 ^B
Boiling	0.67 ± 0.08 ^A	0.58 ± 0.02 ^A	0.38 ± 0.08 ^{AB}	0.22 ± 0.04 ^A	0.18 ± 0.03 ^A	0.18 ± 0.01 ^A	0.20 ± 0.12 ^B
LP steaming	1.03 ± 0.05 ^A	0.70 ± 0.03 ^{AB}	0.47 ± 0.02 ^{BC}	0.26 ± 0.01 ^{BC}	0.28 ± 0.03 ^{BC}	0.19 ± 0.02 ^{BC}	0.10 ± 0.00 ^C
HP steaming	1.05 ± 0.06 ^A	0.70 ± 0.01 ^{AB}	0.40 ± 0.07 ^{BC}	0.26 ± 0.02 ^{BC}	0.23 ± 0.08 ^{BC}	0.14 ± 0.04 ^C	0.10 ± 0.01 ^C
<i>Sous vide</i>	1.10 ± 0.02 ^A	1.02 ± 0.05 ^A	0.88 ± 0.09 ^{AB}	0.89 ± 0.10 ^A	0.86 ± 0.05 ^A	0.74 ± 0.02 ^A	0.59 ± 0.06 ^A
Microwaving	1.07 ± 0.11 ^A	0.89 ± 0.13 ^{AB}	0.95 ± 0.17 ^{AB}	0.95 ± 0.02 ^A	0.96 ± 0.09 ^A	0.90 ± 0.05 ^A	0.84 ± 0.10 ^A
<i>Sous vide</i> -MW	1.16 ± 0.16 ^A	0.54 ± 0.05 ^B	0.34 ± 0.05 ^B	0.21 ± 0.04 ^B	0.26 ± 0.09 ^B	0.17 ± 0.02 ^B	0.10 ± 0.01 ^B
Grilled	0.93 ± 0.02 ^A	0.64 ± 0.02 ^{AB}	0.33 ± 0.07 ^C	0.18 ± 0.03 ^B	0.23 ± 0.02 ^B	0.17 ± 0.01 ^B	0.15 ± 0.04 ^B

Fig. 2 – Lutein content ($\text{mg kg}^{-1} \text{fw}$) of kailan-hybrid after different cooking treatments and stored up to 45 days at 4°C ($n = 5 \pm \text{SD}$). Different capital letter denotes significant differences between treatments ($p < 0.05$).

raw samples showed a significant ($p < 0.05$) 37.7% AA reduction, with better AA retention than that of around 50% found in conventional broccoli (var. *Italica*, cv. Cicco) after 21 days at 4°C (Lemoine, Civello, Martínez, & Chaves, 2007).

3.4. Lutein content

Kailan-hybrid showed $14.8 \text{ mg kg}^{-1} \text{fw}$ of lutein, which was within the range of $5.4\text{--}28.3 \text{ mg kg}^{-1} \text{fw}$ reported for conventional broccoli cvs. (Fernández-León, Fernández-León, Lozano, Ayuso, & González-Gómez, 2012; Miglio et al., 2008; Nunn et al., 2006; Pellegrini et al., 2010). However, kailan showed higher contents with $38.9\text{--}58.5 \text{ mg kg}^{-1} \text{fw}$ (wet-dry season trials), as reported by Hanson et al. (2011). Lutein has been shown as the main carotenoid in conventional broccoli and in kailan, followed by β -carotene, recording among 1.3- to 1.6-times higher content than the latter (Hanson et al., 2011; Miglio et al., 2008; Nunn et al., 2006; Pellegrini et al., 2010). The studied kailan-hybrid showed 1.6-fold higher lutein content than β -carotene (data not shown), which was similar to the aforementioned reported ranges.

After cooking, lutein content significantly ($p < 0.05$) increased between 4.8- and 7.5-fold compared to the raw samples on the processing day (Fig. 2). Similar increases have been found in microwaved, steamed, blanched, boiled and induction-boiled conventional broccoli (Nunn et al., 2006; Updike & Schwartz, 2003; Zhang & Hamauzu, 2004). Such a

lutein increment after cooking was attributed to its greater thermostability, as compared to its hydrocarbon carotenoid counterparts (Henry, Catignani, & Schwartz, 1998) and most likely due to a soluble solids loss into the cooking medium, inactivation of carotenoid-oxidizing enzymes (Baloch, Buckle, & Edwards, 1977) and/or increased extraction efficiency due to disruption of the carotenoid-protein complexes (Siriamornpun, Kaisoon, & Meeso, 2012). On the processing day, boiling and sous vide-MW showed 7.4 and 7.1-fold significant ($p < 0.05$) higher lutein contents than uncooked samples, respectively (Fig. 2). The latter finding was probably a result of the above-mentioned greater extraction favoured by a higher cell disruption produced by the boiling water (for boiling) and to the vacuum-forced lixiviation (for sous vide-MW). After grilling and sous vide, lutein levels rose by 4.8- and 5.2-fold (without significant ($p < 0.05$) differences between them), respectively, compared to raw samples on the processing day. The latter lowest lutein enhancements after sous vide treatment may be owed to the long cooking time. However, the low lutein enhancements after grilling may be due to the high temperatures besides the leaching to the cooking oil favoured by the lipophilic properties of lutein. After 7 days of chilled storage, lutein content significantly ($p < 0.05$) decreased around 10% in all of the cooked samples except sous vide and sous vide-MW, which registered the lowest and highest reductions with 4.4% and 20.1%, respectively, compared to their respective values on the processing day. After 14 days, the lutein content of all cooked samples drastically dropped reaching the same levels as control samples, which did not

register any significant ($p < 0.05$) differences after the processing day, keeping all cooked and raw samples these basal levels after 45 days at 4 °C. This high lutein decrease after 14 days may be due to a reactivation of the carotenoid oxidising enzymes, whose activity was attenuated after cooking and chilled storage.

4. Conclusions

Among the high glucosinolate content of kailan-hybrid, glucobrassicin showed the highest levels (39% of total glucosinolates). The innovative industrial cooking methods hereby studied on broccoli revealed that LP steaming and microwaving are the best for keeping glucosinolates and total vitamin C contents after cooking and subsequent commercial chilled storage. On the other hand, grilled samples recorded the highest losses due to the remaining myrosinase activity, indirectly registered by endogenous sulforaphane, which was undetected in the remaining cooked samples. All of the cooking treatments highly increased lutein levels, with LP steaming and microwaving registering the highest increases of approximately 7-fold compared to uncooked samples, although a great lutein decrease was found after 14 days at 4 °C.

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CHAPTER VIII

Kailan-hybrid: A *brassica* vegetable with high chemopreventive properties. Human metabolism and excretion

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8.1. INTRODUCTION

Epidemiological data show that consumption of *Brassica* vegetables has been associated to a reduced incidence of several types of cancer (Steinmetz and Potter, 1991; Block *et al.*, 1992). Cruciferous vegetables contain glucosinolates, thioglycoside conjugates of ITC, which might be responsible for this health-promoting effect (Hwang and Lee, 2006). Among conventional broccoli varieties and kailan, glucoraphanin, the glucosinolate of sulforaphane (4-methylsulfinylbutyl ITC), has been reported as the major glucosinolate (Harris and Jeffery, 2008). The cancer-protective effect of ITC in animals has been attributed to their inhibitory action on Phase I enzymes responsible for bioactivation of carcinogens and their activity as inducers of Phase II detoxification enzymes (Hecht, 2000, Fahey *et al.*, 2002; Shibata *et al.*, 2010). Similar effects on Phase I and Phase II enzymes have been observed in humans, where large quantities of cruciferous vegetables were included in the diet (Conaway *et al.*, 2000).

Myrosinase is the enzyme which hydrolyzes glucosinolates into ITC and other breakdown products. In the intact plant cells of *Brassica* vegetables, myrosinase is largely stored as myrosin grains in the vacuoles of particular idioblasts, called myrosin cells, but it has also been reported in protein bodies or vacuoles (Andréasson *et al.*, 2001). Glucosinolates are stored in adjacent but separate S-cells (Koroleva *et al.*, 2000). When the plant cells are disrupted, after processing or chewing, the glucosinolate conversion into ITC is activated. Cooking procedures lead thermal inactivation of myrosinase. The glucosinolates that are not hydrolyzed may be converted to ITC, in a lesser extent, by the thioglucosidase activity of the human gut but are likely not absorbed intact (Krul *et al.*, 2002). After absorption, ITC are metabolised via the mercapturic acid pathway to the N-acetylcystein conjugate of ITC and excreted into the urine as their corresponding mercapturic acids (Mennicke *et al.*, 1988). Mercapturic acids excreted in urine can be used as a biomarker for the uptake of ITC and thus the intake of glucosinolates (Ye *et al.*, 2002). Previous reports indicate how individual ITC metabolites can be reliably quantified and used as a biomarker for exposure to individual glucosinolates (Vermeulen *et al.*, 2006, 2008). However, precaution must be taken when assuming that the excreted ITC conjugates are equivalent to the consumed amounts of ITC since ITC conjugates are unstable and rapidly dissociate back to ITC

(Kristensen *et al.*, 2007). According to this, the total ITC excretion has been used as a biomarker of overall ITC exposure in the present study.

Depending of the cooking method and conditions used, different grades of the disruption of the vegetable matrix can be achieved affecting the ITC absorption after ingestion of the vegetable. Vegetable matrix has been regarded as a key factor determining the bioavailability of ITC with different data reported in several vegetables and condiments (Vermeulen *et al.*, 2008). Kailan–hybrid broccoli is a particular natural hybrid between kailan (*B. oleracea*, Alboglabra group, also called Chinese broccoli or Chinese kale) and conventional broccoli (*B. oleracea*, Italica group). This kailan–hybrid broccoli has a long slender stem and a mild sweeter taste with a completely edible portion, both raw or cooked (Martínez–Hernández *et al.*, 2011).

This study shows, for the first time, the human absorption and excretion kinetics of glucosinolates of the kailan–hybrid broccoli, which provides useful data about the real uptake of these anti–cancer compounds. Furthermore, the effect of microwave cooking on the related kinetics of these compounds was also studied by a cross–over design.

8.2. METHODOLOGY

8.2.1. Human subjects

Seven healthy subjects (four men and three women, randomly S1 to S7) with a body mass index (BMI) between 19 and 28, aged 22–50 years–old, were recruited for the clinical study belonging from the Instituto de Biotecnología Vegetal (Cartagena, Spain) and the Hospital General Universitario Reina Sofía (Murcia, Spain). Subjects were screened by questionnaire and interview. Exclusion criteria were tobacco smoking, use of regular medications, had taken antibiotics for 4 weeks immediately preceding the study, history of kidney stones, glucose–6–phosphate dehydrogenase deficiency, diabetes mellitus, bleeding disorders, or family history of iron overload/hemochromatosis. Food pattern questionnaires previously supplied to all subjects revealed that all of them regularly followed a Mediterranean diet rich in fruit and vegetables prior to the study. Good health was confirmed by medical history, physical examination and clinical laboratory analysis of urine and blood (complete

blood count and liver function markers including serum glutamic–pyruvic transaminase and bilirubin test).

Written informed consent was obtained from enrolled subjects. The experiment was approved by the Clinical Research Ethical Committee of the Hospital General Universitario Reina Sofía according to the Spanish legislation for human studies (RD 223/2004, 2004), which accomplished the European legislation (Regulation EC 2001/20, 2001) and confirmed by the Bioethical Commission of the UPCT. The study was conducted during April 2011 at the Nutrition Unit of the Hospital General Universitario Reina Sofía.

8.2.2. Study design

Subjects followed a glucosinolate–free diet for one week prior each of the two intervention days. Therefore, vegetables and fruit were completely excluded.

The average daily nutritional composition was distributed in 21% proteins, 34% lipids and 45% carbohydrates with an energetic value of 2,223 kcal day⁻¹. Although the diet composition was set by the Nutrition Unit, the food amount was freely chosen by each subject. Only dairy products were restricted to a cup of milk per day in order to control vitamin A intake so minimizing interference with the chemical determinations. Water intake was established in an average of 1.5 L day⁻¹, as a way to homogenize dilution levels for every subject. Volunteers refrained from drinking artificial (carbonated, etc.) and alcoholic drinks during the designed diet. No medication was allowed during the study without informing the person responsible for the diet, except paracetamol.

On the morning of each intervention day, according to an open cross–over trial, subjects consumed 200 g of raw or microwaved kailan–hybrid broccoli. Volunteers were fasted for eight hours before the kailan–hybrid broccoli consumption.

8.2.3. Diets

Kailan–hybrid broccoli (*B. oleracea* Italica Group x Alboglabra Group) sized 15–18–cm–long was hand–harvested in April from open air commercial cultivation parcels located in the southeast Mediterranean area of Spain (Lorca, Murcia). The broccoli was

grown according to integrated pest management cultural practices. Immediately after harvesting, broccoli pieces were pre-cooled on crushed ice and transported by car to the Hospital General Universitario Reina Sofia, where it was stored at 1 °C and 90–95% RH. On each intervention day, 200–g servings of raw or microwaved (700W for 4 min) kailan–hybrid broccoli were immediately served to the subjects at 10 AM. MW was chosen as the best cooking technique for this kailan–hybrid broccoli as we previously studied (Martínez–Hernández *et al.*, 2012). Four of them consumed the raw samples and the other three the microwaved ones. The same procedure was followed during the remaining intervention day, with three eating raw samples and four the cooked ones. Samples were taken on both intervention days and frozen at –80 °C for later analysis of food composition.

8.2.4. Blood and urine collection

The study subjects were admitted to the Nutrition Unit of the Hospital General Universitario Reina Sofia at 8 AM. Thirty minutes before administration, an intravenous catheter was inserted. After the vegetable consumption, fourteen–ml blood samples were drawn at 14 time points (–0.10, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 h). Blood was immediately transferred from the syringe to a 15–ml conical polypropylene centrifuge tube (containing 107.5 µL of 400 L^{–1} disodium EDTA) and gently mixed. In order to analyze the plasma ITC equivalents, the plasma fraction was obtained according to Ye *et al.* (2002).

Spot urine samples were collected at 10 different time intervals (–0.25–(–0.17), 0–0.5, 0.5–1, 1–1.5, 1.5–2, 2–3, 3–4, 4–6, 6–8 and 8–10) after the kailan–hybrid broccoli consumption in order to analyze the urinary ITC equivalents. Spot urine samples were immediately frozen at –80 °C until further analysis. Furthermore, urine samples were collected during 24 h in a flask from 10 to 34 h after the broccoli consumption. The 24–h urine flask was kept refrigerated (4 °C) until finishing the collection time and then it was frozen at –80 °C until further analysis.

8.2.5. Analysis

The total ITC equivalents in kailan–hybrid broccoli, urine and plasma samples were determined according to the cyclocondensation assay (Ye *et al.*, 2002). In this

assay, the ITC react quantitatively with the vicinal sulfhydryl groups of 1,2-benzenedithiol to give rise to a cyclic condensation product (1,3-benzenodithiole-2-thione). The extraction of total ITC equivalents in plant samples and urine samples were conducted according to Kristensen *et al.* (2007). The total ITC equivalents in plasma samples were analyzed following Ye *et al.* (2002) with the poly-ethylene glycol protein-precipitation method. The separation of the cyclocondensation product of plant, urine and plasma samples was conducted according to Ye *et al.* (2002) with slight modifications. Briefly, a 50- μ L aliquot of supernatant extract was automatically injected onto a Gemini NX (250 mm $\times$ 4.6 mm, 5 μ m) C18 column (Phenomenex, Torrance CA, USA) using a Shimadzu UPLC LC-30AD system (Shimadzu Corporation, USA Manufacturing INC, Canby OP, USA) equipped with a degasser DGU-20A, autosampler SIL-30AC, column oven CTO-10AS, communications module CMB-20A and diode array detector SPD-20A. The mobile phase consisted of 80 % methanol/20 % water (by volume) with isocratic flow of 2 mL min⁻¹. Chromatograms were recorded at 365 nm and the 1,3-benzenodithiole-2-thione was eluted at 4.8 min. Quantification of the 1,3-benzenodithiole-2-thione was conducted according to Kristensen *et al.* (2007) using a commercial standard (Specs, Delft, The Netherlands). All measurements were made by duplicate.

8.2.6. Statistical design

Data are expressed as the mean value of indicated numbers of measurements $\pm$ SD. Coefficient of variation (CV) was also determined. The statistical significance of the difference between the urine recovery data after consumption of raw *vs.* cooked kailan-hybrid was calculated with a two-sided paired Student's t-test.

8.3. RESULTS

8.3.1. Study subjects and diets

Seven healthy subjects (three female and four male) students and hospital workers, with mean age of 36 $\pm$ 14 years and BMI of 23.5 $\pm$ 1.9 were recruited for the clinical portion of the study. The subject compliance with the protocol was excellent. Results of a complete analysis of blood count and liver function markers (enzymes) showed normal ranges for all subjects.

The total ITC equivalents of the raw/microwaved cooked kailan–hybrid broccoli servings (200 g), measured after glucosinolates conversion to ITC by purified myrosinase, were 8.2/6.3 μmol on average for both days of intervention (9.9/8.1 and 6.4/4.4 μmol , respectively).

8.3.2. Urinary excretion of total ITC equivalents

The total ITC equivalents excreted in the urine samples collected at different time points within the first 10 hours after ingestion of raw and microwaved kailan–hybrid broccoli are illustrated in Figure 1. The average cumulative excretion of total ITC equivalents in urine during the 34 h after consumption of raw and microwaved kailan–hybrid broccoli was 3.3 ± 1.0 and 0.8 ± 0.5 μmol , respectively (Figure 2). All excretion curves have a relatively smooth and gradual rise, except for subject S–7, that showed a great excretion increase six hours after consumption of microwaved kailan–hybrid broccoli. The maximum excretion of urinary ITC equivalents in subjects that ingested raw kailan–hybrid broccoli was observed within two to three hours, followed by a minor peak between 4 to 6 hours mainly observed for subjects S–2, S–3 and S–7. Related to subjects that ingested microwaved kailan–hybrid broccoli, the maximum ITC equivalents peak was found after 4 to 8 hours. Summarizing, the highest urinary ITC equivalents excretion rate was observed during the 0–4 h interval after ingestion of raw kailan–hybrid broccoli with 49.0 ± 7.0 %, over the total cumulative ITC equivalents excretion (34 h), while the consumption of the raw vegetable registered levels of 36.0 ± 8.1 and 14.4 ± 8.0 % in the intervals 4–10 and 12–34 h, respectively (data not shown). The microwaved kailan–hybrid broccoli showed the highest urinary ITC equivalents excretion rate in the interval 4–10 h with 62.1 ± 4.8 %, while the intervals 0–4 and 10–34 h registered levels of 10.3 ± 5.9 and 27.4 ± 8.2 %, respectively.

The excretion of the total amount of ITC ingested with raw kailan–hybrid broccoli (79.1 ± 18.8 %) was 2.5–fold higher than that from microwaved broccoli (32.0 ± 13.1 %), which is statistically significant ($p = 0.003$).

8.3.3. Analysis of plasma for total ITC

Mean ITC equivalents in plasma for all subjects after consumption of raw and microwaved kailan–hybrid broccoli are shown in Figure 3. Subjects who ingested raw product showed higher ITC equivalents levels in plasma than those who ingested the

microwaved vegetable. The individual ITC peaks in plasma samples after consumption of microwaved product were rather small, with two less relevant peaks when compared with raw material. Contrary to such findings, the ITC equivalents in plasma of subjects that ingested raw broccoli showed definite peaks between 1 to 3 hours after consumption. However, the mean ITC equivalents among subjects did not show marked differences with the mean baseline.

8.4. DISCUSSION

Results of human studies concerning the intake and absorption of nutritive and bioactive compounds by the human body usually depends on several factors, such as regular diet patterns, lifestyles, different vegetable varieties and cooking procedures, human genetic background, variation in gut bacteria strains, etc., that makes difficult to interrelate data among different studies. Absorption kinetics of ITC among several *Brassica* vegetables have shown variable results due to the fact that the vegetable matrix plays a major role in the absorption of these compounds (Vermeulen *et al.*, 2008). Our study shows innovative and useful data concerning to the absorption and excretion kinetics of ITC after ingestion of raw or microwaved kailan–hybrid broccoli.

The ITC content of raw and microwaved kailan–hybrid broccoli did not show significant differences, after the glucosinolate conversion catalyzed by the plant myrosinase and the additional myrosinase added during the ITC assay of the plant tissue. Consequently, MW could be regarded as a mild cooking technique, as previously described for other mild cooking methods like steaming (Conaway *et al.*, 2000).

The mercapturic acid pathway is considered the major route of ITC elimination, considering defecation, exhalation and perspiration as minor routes of ITC excretion (Vermeulen *et al.*, 2008). This study showed partial urinary ITC recovery of raw and microwaved kailan–hybrid broccoli with 79.1 and 32.0 %, respectively. This ITC recovery in urine, lower than 100 %, has been previously described for many cruciferous vegetables such as garden cress, water cress, broccoli sprouts, mustard, cabbage, etc. (Mennicke *et al.*, 1988; Chung *et al.*, 1992; Shapiro *et al.*, 1998; Getahun and Chung, 1999; Shapiro *et al.*, 2001; Rouzaud *et al.*, 2004). These relatively low recoveries could be explained by an incomplete glucosinolate conversion to ITC, alternative ITC–excretion routes, incomplete ITC absorption from the gut or ITC

metabolization into non-ITC metabolites (Vermeulen *et al.*, 2008). The hereby studied ITC absorption of the kailan-hybrid was 2 times higher for raw and 3 to 9 times higher for cooked samples than values previously reported in conventional broccoli varieties (Conaway *et al.*, 2000; Vermeulen *et al.*, 2006; Vermeulen *et al.*, 2008). However, the ITC absorption of the microwaved kailan-hybrid did not show differences with the steamed conventional broccoli reported by Kristensen *et al.* (2007), probably due to the short cooking time used in both cases (4 and 2 min, respectively) in comparison with other studies with lower ITC absorption rates. The vegetable matrix has been regarded as an important factor determining the ITC absorption, as found Vermeulen *et al.* (2006) after studying the excretion in urine of ITC mercapturic acids following the consumption of several cruciferous vegetables and condiments. In this way, the histological differences among kailan-hybrid and conventional broccoli varieties could be the reason for the higher absorption of these chemopreventive compounds in the studied kailan-hybrid. On the other hand, the aforementioned publications which used conventional broccoli also included other foods at the time of the vegetable ingestion, which would tend to dilute myrosinase activity and reduce the glucosinolates conversion to ITC.

The wide variations in the ITC absorption observed in some cases among individuals, and according to CV values, could be due to several factors as: (1) differences in individuals patterns through mastication and processing in the small intestine, whereby myrosinase is released from the broccoli cells; (2) the amount and variation in colonic bacteria strains with myrosinase activity among individuals (Shapiro *et al.*, 1998); and (3) the polymorphism between individuals of glutathione S-transferases, which catalyzes the initial conjugation of ITC with GSH in the mercapturic acid pathway (London *et al.*, 2000; Wu *et al.*, 2009). MW reduced the ITC absorption 2.5 times compared to uncooked kailan-hybrid broccoli. These results corroborate that myrosinase plays a crucial role in the conversion of glucosinolates to ITC. The thermal myrosinase inactivation after cooking reduces the glucosinolates hydrolysis and the absorption in the human body and later ITC excretion to a great extent. The reduction of ITC absorption could decrease the great health benefits of these sulphur compounds.

The higher ITC urinary excretion rates during the first four hours in subjects that ingested raw kailan-hybrid broccoli is most likely due to absorption of ITC in the small intestine (Wu *et al.*, 2009). The second peak in excretion within the 4–8 h period is

presumably attributed to a ITC absorption in the colon. Consequently, the delayed ITC excretion peak in subjects that ingested cooked kailan–hybrid broccoli in the 4–8 h interval is more likely due to the activity of the colonic bacteria with myrosinase activity, since the plant myrosinase was inactivated during the cooking process and the ITC absorption in the small intestine was probably minor. Similar ITC absorption kinetics after consumption of steamed conventional broccoli were reported (Kristensen *et al.*, 2007).

The cyclocondensation method used to determine the ITC content in plasma is subjected to several confounding factors, which could possibly account for some of the differences observed in the plasma ITC levels of this study. ITC are quickly bound to plasma proteins among other components, such as glutathione and cellular sulfhydryls. During the plasma ITC extraction, proteins were precipitated and eliminated to avoid interferences during the HPLC analyses, removing also the ITC bound to proteins and consequently decreasing the total ITC content. Besides, products of hydrolysis of indole–containing glucosinolates and glucosinolates containing β –hydroxyalkenyl do not react in the cyclocondensation reaction. Some dithiocarbamates, thiourea, and xanthenes will also react weakly with 1,2–benzenedithiol and, when present, could have contributed to the background levels detected in the assays for total ITC (Conaway *et al.*, 2000).

FIGURES

Figure 1. Urinary excretion of ITC equivalents after consumption of 200 g of raw or microwaved kailan-hybrid broccoli for each subject.

Figure 2. Cumulative urinary excretion of ITC equivalents through 34 h after consumption of 200 g of raw and microwaved kailan-hybrid broccoli for each subject.

Figure 3. Mean values of plasma total ITC equivalents after consumption of 200 g of raw and microwaved kailan–hybrid broccoli. Error bars represent SD for all individuals at that time point.

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CONCLUSIONS

The general conclusions of the current Ph. D. Thesis are as follows:

1. A new kailan–hybrid broccoli has been characterized and its postharvest behaviour was compared to that of the conventional cv. Parthenon, one of the most widely cultivated broccoli cv. in Spain. The kailan–hybrid broccoli showed a significantly higher metabolic activity than that of the cv. Parthenon MAP storage at 2 °C (5–7 kPa O₂ + 14–15 kPa CO₂) or 5 °C (1.5–2.5 kPa O₂ + 15–16 kPa CO₂) provides great benefits for both broccoli cvs., reaching an acceptable sensory quality and safety for consumption after 15 days of shelf–life. In particular, these storage conditions reduced microbial growth, delayed undesirable changes in the main physicochemical quality attributes and inhibited or lowered undesirable sensory changes and disorders (yellowness, pithiness, off–flavours, stem softening and stem bent).
2. The phenolic profile of both studied broccoli cvs. is similar to that reported for other *B. oleracea* cvs. Although cv. Parthenon showed higher initial total phenolic content than the kailan–hybrid, which was followed by an increase throughout shelf–life, the TAC at harvest of the kailan–hybrid was higher than that of the cv. Parthenon. It may be explained by the vitamin C contribution which was 1.2–fold higher in the kailan–hybrid than in the cv. Parthenon. The florets of the kailan–hybrid broccoli showed higher total dietary fibre, protein and phenolic contents, TAC and many important dietary minerals such as Ca, Mg, Fe and Zn than those of the cv. Parthenon. However, the whole kailan–hybrid edible portion registered lower levels owed to its high stem/inflorescence ratio.
3. Low and moderate UV–C radiation (1.5 and 4.5 kJ m^{−2}) retarded the natural microflora growth of FC kailan–hybrid broccoli, keeping sensory quality for up to 19 days at 5 °C and 13 days at 10 °C. Moreover, the results suggest that this moderate UV–C pretreatment could be used as a tool to increase certain health–promoting compounds content such as polyphenols (mainly the hydroxycinnamoyl acid derivatives) and TAC.

4. The studied emerging eco-friendly sanitising treatments may be used as potential alternatives to the conventionally used NaClO, since similar efficacy and quality retention potential was attained. In some cases, a higher natural microflora reduction was achieved when innovative sanitising treatments were combined. NEW-washed produce showed a potential shelf-life of 19 days at 5°C, as NaClO did. In that way, this treatment may be considered as the best alternative to NaClO. However, and attending to nutritional bioactive compounds, the combination of treatments registered the highest increases of some antioxidant enzymes and achieved good fatty acids retention. Generally, there were not significant differences among double and triple combinations. Even though, further investigations are required to better optimise conditions, other sustainable techniques, etc., in order to preserve the overall quality of this kailan-hybrid broccoli and probably that of other horticultural produce.
5. The studied vacuum-based cooking treatments generally induced better microbial, physical and sensory quality, and kept, or even improved, TAC compared to conventional cooking methods. On the other hand, grilling was one of the most effective methods to avoid undesirable changes in stem firmness, enhancing green colour, increasing total phenolic content and TAC, and reducing enterobacteria counts, even though a high visual dehydration was reached.
6. After all cooking treatments (except grilling), an excellent microbial reduction (with mesophilic counts below 7 log CFU g⁻¹ after 45 days) was achieved. Based on the overall sensory quality, the commercial life was established in 45 days at 4 °C, except for grilled (14 days) and *sous vide*-treated (21 days) samples. Generally, cooking treatments induced an enrichment of health-promoting compounds, showing MW, *sous vide*-MW and LP steaming the best chlorophylls retention and total phenolic (apparently) and TAC enhancement. For those reasons, such industrial cooking methods could be recommended as a tool for keeping the quality of the fifth range kailan-hybrid broccoli. However, further studies should be implemented to optimise the time-temperature binomial and new treatments should be tested.

7. Among the high glucosinolate content of kailan–hybrid broccoli, glucobrassicin showed the highest levels (39 % of total glucosinolates). The innovative industrial cooking methods hereby studied on broccoli revealed that LP steaming and MW are the best ones for keeping glucosinolates and total vitamin C contents after cooking and subsequent commercial chilled storage. On the other hand, grilled samples recorded the highest losses due to the remaining myrosinase activity, indirectly registered by endogenous sulforaphane, which was undetected in the remaining cooked samples. All of the cooking treatments highly increased lutein levels, with LP steaming and MW registering the highest increases of about 7–fold compared to uncooked samples, although a great lutein decrease was found after 14 days at 4 °C.
8. The kailan–hybrid broccoli showed a great ITC absorption, which presents this new broccoli cv. as a vegetable with high potential chemopreventive properties. However, further human studies comparing this kailan–hybrid broccoli with conventional cvs. must be conducted to corroborate this great ITC absorption. Furthermore, cooking kailan–hybrid broccoli by MW reduced the ITC absorption in the human subjects by inactivation of myrosinase. The information hereby reported may serve as a guideline for the evaluation of potential cancer chemopreventive effects of dietary consumption of kailan–hybrid broccoli by humans.

SCIENTIFIC PUBLICATIONS FROM THE PH. D. THESIS

Original papers published in peer-reviewed journals of the ISI, Institute for Scientific Information.

- Martínez-Hernández, G.B., Gómez, P., Pradas, I., Artés, F., Artés-Hernández, F. 2011. Moderate UV-C pretreatment as a quality enhancement tool in fresh-cut Bimi[®] broccoli. *Postharvest Biol. Technol.* 62, 327–337.
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